SYNTHETIC APPROACHES TO STEROIDAL BISQUATERNARY AMMONIUM COMPOUNDS.

A Thesis submitted to the University of Glasgow

for the degree of

DOCTOR OF PHILOSOPHY

in the

Faculty of Medicine

by

MOHAMMED ALAUDDIN B.Pharm. (Panjab)

November, 1962

Experimental Pharmacology
Institute of Physiology,
The University,
Glasgow, W.2.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

The convention for citing references employed in this thesis is that of the Journal of the Chemical Society.

Acknowledgements

I wish to thank Professor S. Alstead, C.B.E. and Professor R. C. Garry, F.R.S.E. for the opportunity to undertake this research and for their kind interest and encouragement. I wish also to thank Professor R. A. Raphael, F.R.S. for permitting free use to be made of facilities in the Department of Chemistry.

My sincere gratitude goes to Dr. M. Martin-Smith for suggesting the problem and for continued advice and encouragement throughout the work. I am greatly indebted to Dr. R. Hodges, Dr. K. H. Overton, Dr. W. Parker, Dr. N. J. McCorkindale, Dr. G. Nancollas and Dr. D. S. Payne of the Department of Chemistry, and to Mr. J. J. Lewis, Dr. T. C. Muir and Dr. S. T. Reid of the Experimental Pharmacology Division, Institute of Physiology, for helpful discussions.

I would like to thank Mr. J. M. L. Cameron, B.Sc. and his staff for the microanalyses, Dr. G. Eglinton and Mrs. F. Lawrie for infrared spectra, and Drs. R. J. Hamilton and C. J. W. Brooks for invaluable assistance with gas liquid chromatographic analyses. It is a pleasure to acknowledge the technical assistance of Miss Doreen Barclay, Miss June Galbraith, Miss Irene Wilson and Mr. John Thomson.

I am deeply indebted to British Dyewoods Ltd., who kindly undertook the grinding of the trunkwood of Acocanthera longiflora Stapf.

I am extremely grateful to the Pakistan Council of Scientific and Industrial Research for granting me leave with full benefits during the period of my study.

Finally I wish to gratefully acknowledge the tenure of a Colombo Plan Fellowship for the period 1958-1962 which was administered by the British Council, whose kindly consideration at all times has been deeply appreciated.

SUMMARY

This thesis is divided into four sections with the addition of two appendices. The first section gives a brief summary of the theoretical justifications for undertaking the research and includes an account of the role of acetylcholine at the neuromuscular synapse and an account of the receptor theory of neuromuscular blockade. The classification of neuromuscular blocking agents is then discussed and a survey given of the two point, multi-point and one point attachment theories. These considerations are followed by a discussion of rigid bisquaternary molecules leading to an outline of the factors influencing the choice of the compounds whose syntheses were to be attempted and an account of the synthetic routes proposed.

The second section of the thesis is a report of the preparation and reduction of a number of methanesulphonates derived from hydroxy 5β- and 5α-cholanes. These studies were deemed necessary in view of the poor yields of p-toluene-sulphonates which were encountered in certain instances and indeed it was found that utilisation of methanesulphonyl chloride rather than the more commonly employed p-toluenesulphonyl chloride afforded superior yields of sulphonate esters. In the course of this work it was shown that the material previously

reported as 3α,12α-dihydroxy-5β-cholane was in fact a mixture of this compound with 12α,24-dihydroxy-5β-cholane. Selectivity of sulphonation of the hydroxyl groups at positions 3,7,12 and 24 of the cholane skeleton is discussed. Lithium aluminium hydride reduction of the sulphonate esters was shown to hydrogenolyse the sulphonyloxy group when it is in the equatorial configuration and to generate the parent alcohol when the sulphonyloxy group has the axial configuration. Gas liquid chromatographic analyses of certain complex mixtures of steroidal hydrocarbons are reported.

The third section of the thesis reports the results of a study of the reductions under a variety of conditions, of 12-oximino-5β-cholane and 12-oximino-5α-cholane. In all instances only the 12α-amino compound was obtained and the assignments of configuration are rigorously proved. All attempts to obtain 12β-amino compounds by nucleophilic displacement reactions were unsuccessful.

The fourth section of the thesis records attempts to synthesise various steroidal bisquaternary ammonium compounds.

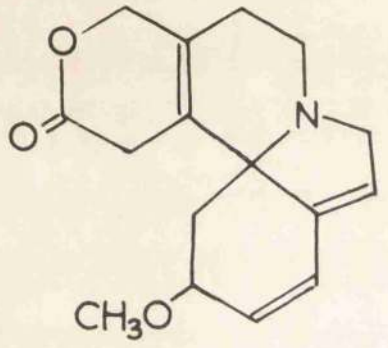
Appendix I consists of the reprints of a review article entitled, 'Biological Activity in Steroids Possessing Nitrogen Atoms', published in two parts and prepared as a general background with which to place the present work in perspective. An addendum

indicates progress in the field since the review was published and points out several errata in the review.

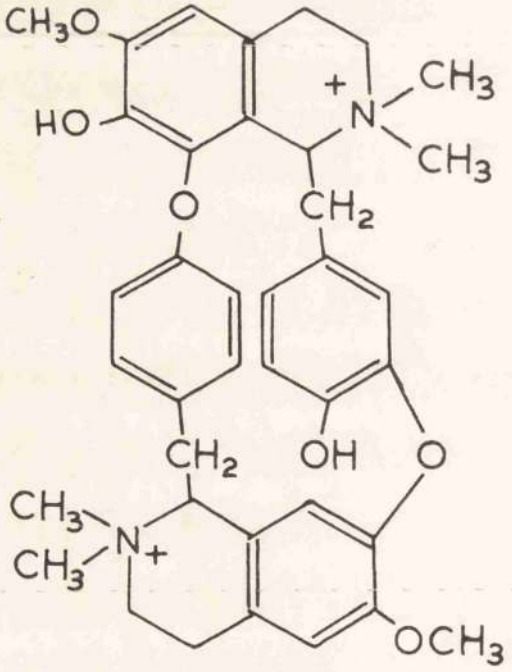
Appendix II describes an attempt to isolate acovenoside A, which would have served as a suitable material for the preparation of 1-amino steroids, from the trunk wood of Acocanthera longiflora Stapf. However although present in the seeds and bark of this tree, acovenoside A was not present in the wood. Another hitherto unreported cardenolide glycoside was shown to be present but its constitution was not established.

INDEX OF CONTENTS

INTRODUCTION



I β. Erythroidine



$2X^{=}$

II (+) Tubocurarine

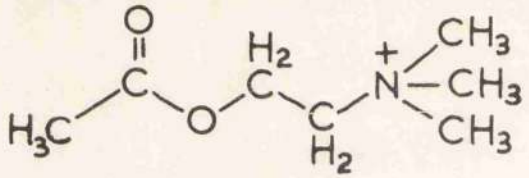
In 1850 Pelouze and Bernard[1] demonstrated, for the first time, that blockade of the normal physiological events at the neuromuscular synapse was responsible for the characteristic paralysis of skeletal muscle induced by curare. About twenty years later Crum Brown and Fraser[2] showed that muscle relaxant activity was associated with many chemically diverse quaternary alkaloidal salts, even though the corresponding tertiary bases had pharmacological properties vastly different from one another. The association between muscle relaxant activity and the quaternary ammonium function has thus long been recognised and subsequent chemical and pharmacological investigations, particularly with synthetic compounds, have served to strengthen this generalisation, although there are several important exceptions. For example the tertiary base β-erythroidine (I) exhibits greater neuromuscular blocking activity than its corresponding metho-salts[3].

The successful isolation and chemical characterisation of tubocurarine[4] (II), the active principle of the South American arrow poison "tube curare", coupled with the development of methods for its clinical use as an adjunct to surgery[5] (where its muscle relaxant properties permit the use of lower doses of anaesthetic with consequent gain in safety margin) provided a new impetus to the study of muscle relaxants. This work has resulted in the synthesis and pharmacological investigation of a large number of structurally diverse quaternary ammonium compounds, although comparatively few of these have seen clinical use. In particular, a great deal of attention has been paid to bis, tris and

tetra-quaternary ammonium compounds.[6] The voluminous literature concerning these studies does not permit a detailed coverage to be given in this thesis, but a number of extensive reviews[7] have recently been published on the subject to which reference may be made. The following discussion considers only those aspects of the published literature pertaining to attempts to correlate neuromuscular blocking activity with chemical structure and in particular with the interonium distance in polyonium compounds.

SECTION I

THEORETICAL CONSIDERATIONS



III Acetylcholine

The role of Acetylcholine at the Neuromuscular Synapse

In order to place the studies of neuromuscular blocking agents in their correct perspective, it is first necessary to consider the physiological sequence of events at the neuromuscular synapse and especially the role played by the neurohormone involved - acetylcholine.

Perhaps one of the greatest single advances in the science of pharmacology was provided by the recognition that the conduction and translation of nerve impulses in the body involved the intercession of "chemical transmitters" or neurohormones. This had, as a result, the rational interpretation of the mode of action of many drugs in terms of their interference with the normal physiological history of these substances which are responsible for the transmission of nerve impulses from neuron to neuron, or from neuron to effector cell.
The existence of neurohormones was first suggested by Elliot[8] who postulated a substance "sympathin" (now identified as mixture of noradrenaline and adrenaline) to be responsible for the transmission of nerve impulses from the nerve terminals to the effector cells in tissues innervated by certain nerves belonging physiologically to the sympathetic nervous system. Such nerves are now termed "adrenergic nerves". Recognition of the existence of a second neurohormone, now known to be acetylcholine (III), followed as a result of the elegant studies of Hunt and Taveau[9], Dixon[10], Dale[11], Loewi[12] and Dale, Feldberg and Vogt[13]. This second neurohormone is involved in the transmission of nerve impulses from the motor nerves to voluntary muscle, from parasympathetic nerve endings to their

effector organs, from all autonomic preganglionic fibres to the post-ganglionic fibres, and from certain nerves belonging anatomically to the sympathetic nervous system to their corresponding effector cells. Such nerves are termed "cholinergic nerves".

Briefly the sequence of events at the neuromuscular junction (i.e. the area embracing the muscle end plate and the terminations of the somatic motor nerves) can be described as follows. A nerve impulse after travelling the length of the nerve fibre brings about the release of acetylcholine at the nerve terminals and the liberated neurohormone diffuses across the synaptic gap between the nerve endings and the muscle end plate where it is adsorbed on the latter and induces a brief depolarisation. This depolarisation, which is associated with rapid change in the permeability of the membrane to sodium and potassium ions[14] generates a wave of excitation propagating in both directions along the membrane of the muscle fibre and leads to the mechanical response, i.e. contraction. That there is no direct cytoplasmic continuity between nerve and muscle but merely a close association between the muscle fibre and the nerve endings[15], which are extensively branched, has been firmly established by histological studies[16].

The actual process of liberation of acetylcholine is neither fully known nor easily investigated[17] but it is known that discrete quanta of acetylcholine insufficient in themselves to induce a mechanical response are continually released even when the muscle is at rest[18] and that the nerve fibres are not the only source of acetylcholine[19]. Moreover,

the release of acetylcholine in quantities large enough to induce a response in the muscle fibre appears to be a multistage process[20]. Inorganic ions also play a role but again this is not fully understood[21]. The acetylcholine within the nerve fibre is synthesised through the acetylation of choline by acetylcoenzyme A[22] in the presence of choline acetylase[23] and it is then stored in an inactive protein-bound form in the fibre pending release[24]. Actually the existence of vesicles, which are believed to store the bound acetylcholine, has been established in the terminal portions of the motor nerves by means of electron microscope studies[25]. Once the liberated acetylcholine has depolarised the muscle end-plate which shows a high sensitivity towards this agent[26], it is then rapidly destroyed through hydrolysis to choline and acetate by the enzyme acetylcholinestrase[27], which is present in very high concentrations at the neuromuscular synapse[28]; this permits repolarisation of the end plate. It is of considerable interest, that it is only the external surface of the muscle end plate which is sensitive to acetylcholine. Microinjection of acetylcholine into the muscle itself fails to induce contraction[29].

The Receptor Theory of Neuromuscular Blockade

The fact that the external surface of the muscle end plate is so extremely sensitive to acetylcholine has given rise to the concept that incorporated in it are certain receptors for this neurohormone. These are considered also capable of occupation by the molecules of neuromuscular blocking agents which thus exert their action by competing with

the acetylcholine for access to the receptors.

Ehrlich[30] was the first to formally postulate the existence of receptors as a general concept applicable to the explanation of drug action. He defined a receptor as "that combining group of the protoplasmic molecule to which a foreign group, when introduced, attaches itself". The lock and key analogy of Fischer[31] and the concept of a "receptive substance" proposed by Langley[32] and Lucas[33] are further examples of early receptor theories. In the course of time these original ideas have undergone considerable modification but the receptor theory of drug action is still in vogue to-day although little is known of the intimate nature of the majority of receptors whose very existence is still inferential.

Despite attempts to present detailed pictorial representations of various receptors[34] there would appear to be several advantages in accepting a rather generalised concept of the receptor such as that presented by Van Rossum and Ariëns[35]. These workers visualise drug-receptor interaction as being essentially an interaction of fields of force originating in the drug molecule and in the receptor itself. The ability of a drug to evoke a physiological response is then considered to depend upon two basic factors - the "affinity" and the "intrinsic activity" which by mathematical treatment can be shown to be represented by rational constants[36]. The affinity of a drug may be defined as its ability to enter into complex formation with a given receptor and is inversely proportional to the dissociation constant of the drug-receptor

complex[36]. The intrinsic activity, on the other hand, is a measure of the power of the drug-receptor complex to evoke a positive biological response[35] and arises as a proportionality constant relating the biological effect to the number of occupied receptors. Electrostatic and Van der Waals forces are assumed to play the dominant role in the formation of the drug-receptor complex and the general interaction is considered to determine the affinity of the drug for the receptor[35]. At the same time, certain specific interactions within the general field of force determine the "intrinsic activity" of the drug. Thus, for drugs showing an affinity for the acetylcholine receptors at the muscle end plate, those with a high intrinsic activity will mimic the action of acetylcholine whilst those with a low or zero intrinsic activity will antagonise the action of acetylcholine, i.e. function as antimetabolites. The concept thus accommodates two extreme types of blockade of the acetylcholine receptors at the muscle end plate. In one, drugs with high affinity and very low intrinsic activity will block the action of acetylcholine without any depolarisation, whilst in the other drugs possessing high affinity and high intrinsic activity will block the action of acetylcholine but themselves produce a depolarisation. Representatives of both types are indeed known, as well as a number of drugs showing intermediate properties.

Occupation of the receptors normally occupied by acetylcholine molecules is, however, by no means the only way in which interference with the normal function of acetylcholine at the neuromuscular junction

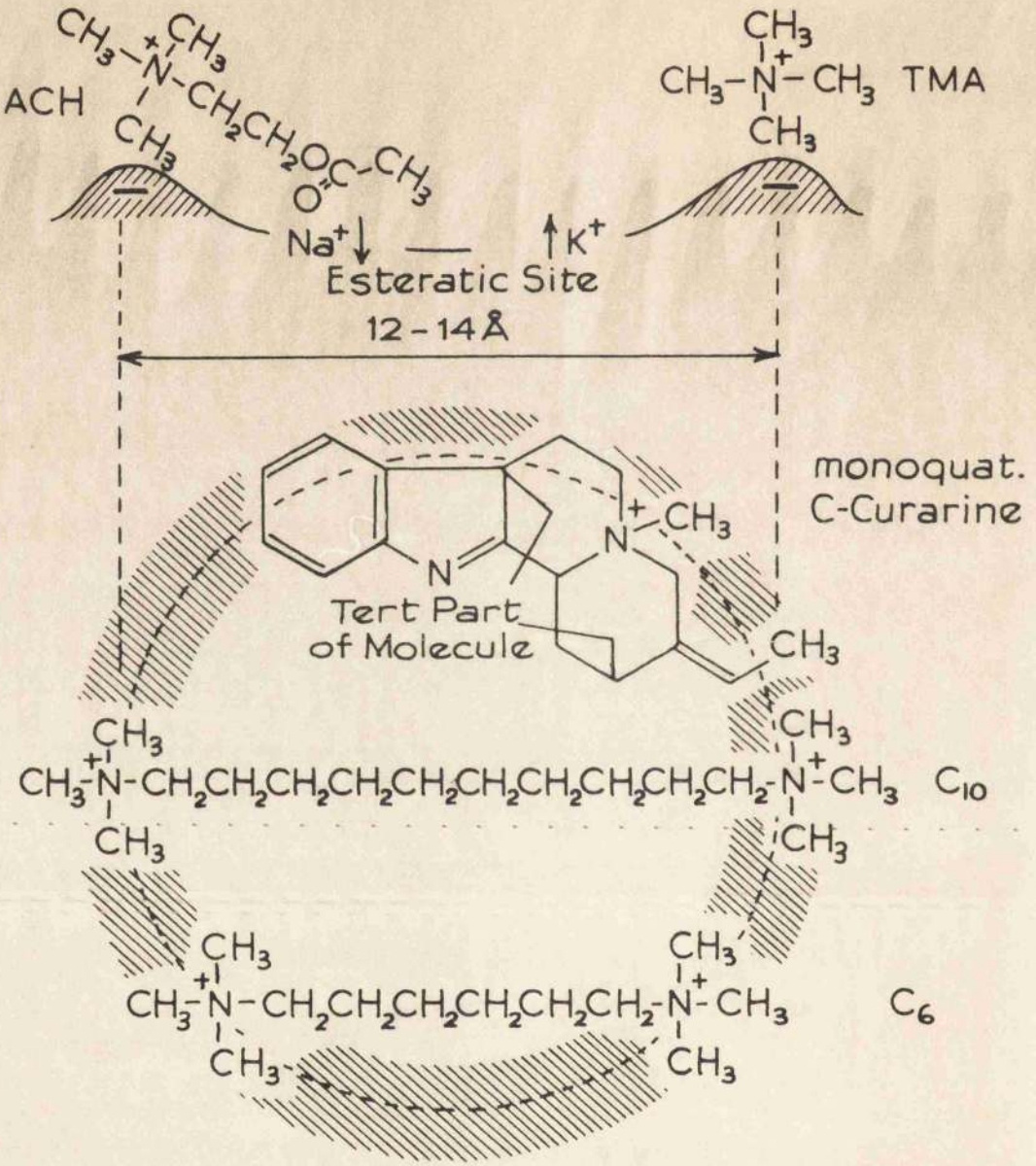


Fig. 1.

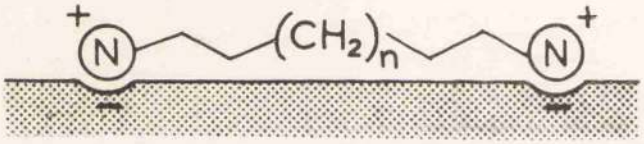
Pictorial representation of the acetylcholine receptor at the muscle end-plate as given by Waser in "Curare and Curare-like Agents" ed., Bovet, Bovet-Nitti and Marini-Betollo, Amsterdam, Elsevier, 1959, p.227. A further description of the receptor has been given by Waser, Arch. Ges. Physiol., 1962, 274, 431.

may be brought about. Thus drugs such as the hemicholiniums are believed to act by blocking the synthesis of acetylcholine[37], compounds such as botulinum toxin are believed to act by blocking the release of acetylcholine from its bound form[38] and drugs such as neostigmine prevent the destruction of acetylcholine by cholinesterase[39] whilst prodeconium is believed to act at receptors other than those competitively occupied by drugs such as decamethonium and tubocurarine[40].

A detailed pictorial representation of the intimate nature of the acetylcholine receptor at the muscle end plate has, however, been given by Waser[41] and this is shown in Fig. 1. Experimental evidence, to support the view that the receptor is a protein, has been provided by Nachmansohnand his associates[42], and attempts have been made to achieve isolation of this protein by means of complex formation with radioactive muscle relaxants. Indeed after the earlier isolation of a strong but unspecified complex formed between the muscle relaxant gallamine and a mucopolysaccharide[43] more refined techniques have recently led to claims for the successful isolation and purification of a protein material[44] which showed many of the characteristics of the in vivo receptor substance.

Classification of Neuromuscular Blocking Agents

It has now been established that the various muscle relaxant drugs do not act by identical mechanisms[45], although at one time all such drugs were grouped together regardless of their site or mechanism of action. In recent years classification of muscle relaxants has been attempted both in terms of their mode of action and in terms of their chemical



IV

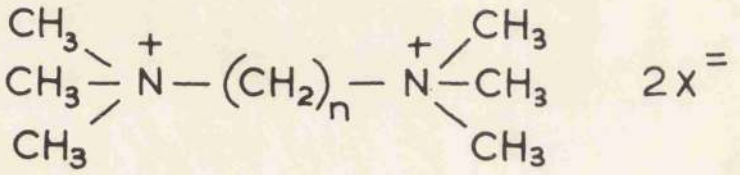
structure. Thus Paton and Zaimis[46] classified neuromuscular blocking agents as competitors (e.g. tubocurarine) and depolarisers (e.g. decamethonium), but the recognition of compounds showing a "two phase" block[47] and the recognition of species variation in the mechanism of action of certain compounds[48] have rendered this classification somewhat unsatisfactory. Bovet[49] attempted to base his classification on the dimensions of the relaxant molecules and termed those muscle relaxants having long thin molecules, "leptocurares" and those with thick molecules, "pachycurares". Broadly the leptocurares corresponded to the depolarisers and the pachycurares to the competitors but there were several striking anomalies[50]. The basis of classification of muscle relaxant drugs suggested by Ariëns et al[51] which utilises the nature of the dose response curve obtained from experiments using the frog rectus abdominis preparation appears to be more satisfactory in so far as it is not rigid and allows for the variabilities in the biological response towards the muscle relaxants under different conditions[52].

Two Point Attachment Theory

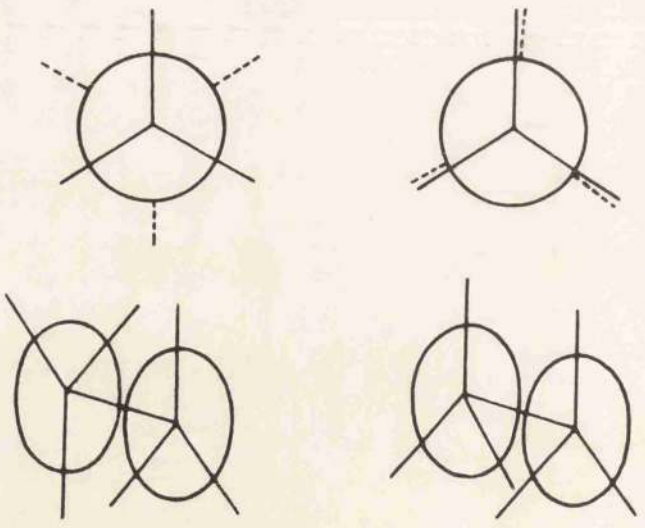
Since bisquaternary ammonium compounds are in general, much more potent neuromuscular blocking agents than monoquaternary compounds, greater attention has been focussed upon the synthesis and pharmacological studies of the former[53]. Paton and Zaimis[54] have advanced a "two point attachment" theory to explain the high activity of such compounds as decamethonium. In this, it is postulated that bisquaternary compounds interact simultaneously with two anionic sites in the receptor (IV)

giving rise to the concept of a "pharmacologically bivalent receptor"[55]. Many experimental attempts to define more closely the exact nature of these "bivalent" receptors have been made, and it has been possible to show that not only does the potency vary with change in interonium distance in the bisquaternary compounds, but also that the mode of action can change. This is well illustrated by reference to the pharmacological properties of the polymethylene bisammonium compounds[56]. Thus decamethonium is a powerful neuromuscular blocking agent, but lower homologues possess mixed ganglion blocking and neuromuscular blocking properties until the chain length is reduced to six carbon atoms when hexamethonium, a potent ganglion blocking agent with weak non-depolarising muscle relaxant properties is obtained. Reduction in the chain length to below six carbon atoms or an increase to above ten carbon atoms results in a gradual loss of both muscle relaxant and ganglion blocking properties. Factors such as the bulk of the substituents on the nitrogen atoms and the presence of ether oxygen or other functional groups in the molecule are known to exert considerable influence on the pharmacological properties of muscle relaxants but the greatest attention has been paid to attempts to correlate the type and degree of activity with interonium distance. Unfortunately nearly all such studies have been conducted with non-rigid molecules capable of existing in an infinite number of conformations, thus denying unequivocal determination of the exact distance between the anionic sites of the receptor.

Consideration of the homologous series of polymethylene bisquaternary

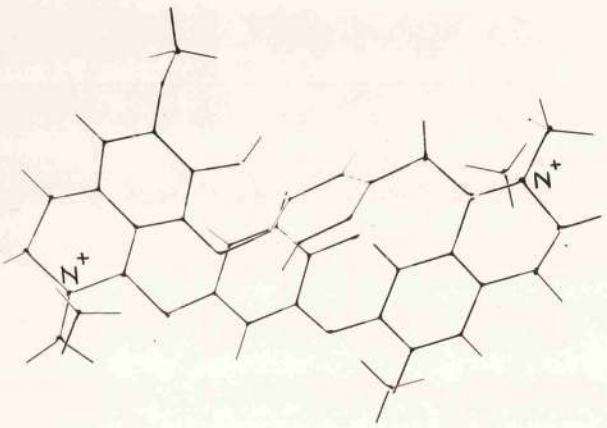


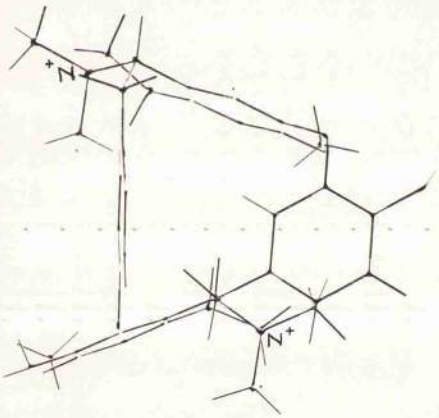
∇ Polymethylene bismethonium compound *(General formula)*

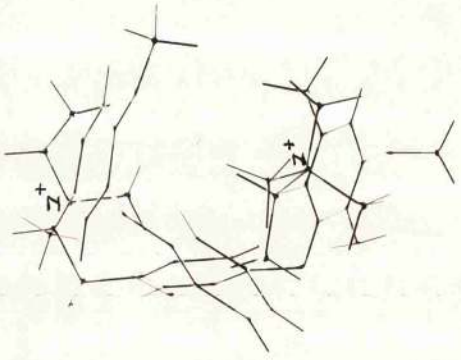


Staggered Eclipsed

VI

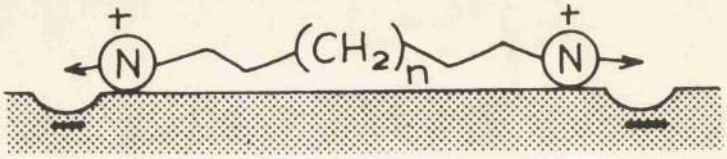




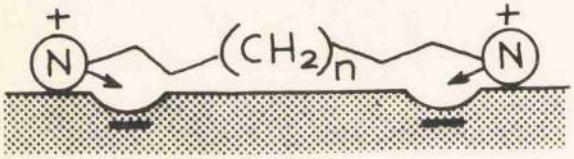


VII

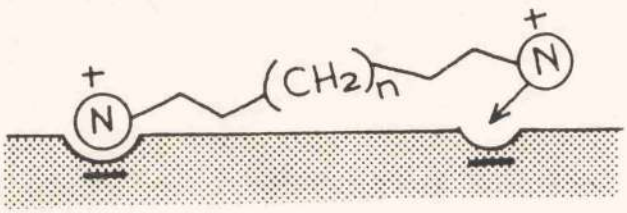
ammonium compounds (V) led to the observation that[54] for optimum activity the interonium distance should be about 14Å. This figure was based on the fact that the maximum neuromuscular blocking activity displayed within the series was shown by decamethonium (V, n = 10) and on the assumption that the interonium methylene chain of this compound would remain fully extended during complex formation with no eclipsed interactions (VI) within the chain. Moreover, it was claimed that the value of 14Å would also accommodate tubocurarine despite the difference in the mode of action of the two compounds. It is to be noted, however, that examination of Dreiding models shows that tubocurarine also possesses a flexible molecule which allows considerable folding and gives interonium distances ranging from about 6Å to a maximum of 12Å (VII). There is no a priori reason why either decamethonium or tubocurarine should react with the receptor in its thermodynamically most stable conformation. The fact that tubocurarine does show weak ganglion blocking activity[57] in addition to its muscle relaxant properties could be taken as supporting evidence for the existence of conformational changes within biological systems. Indeed Burger[58] considered the possibility that the polymethylene chain of decamethonium might not necessarily be in its most extended conformation but nevertheless concluded that it probably was, due to mutual repulsion between the two positively charged nitrogen atoms. He thus neglected the opposing influence of entropy which would tend to create the coexistence of numerous conformations within a given population of bisquaternary molecules. Burger further considered the



a



b



c

Fig. 2.

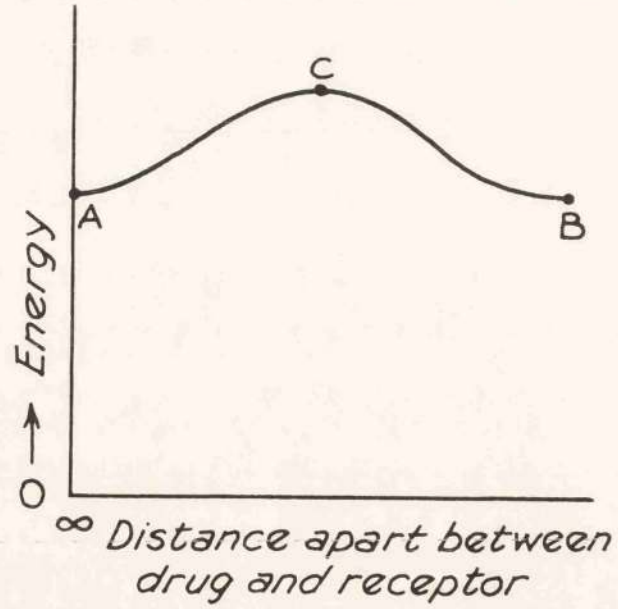
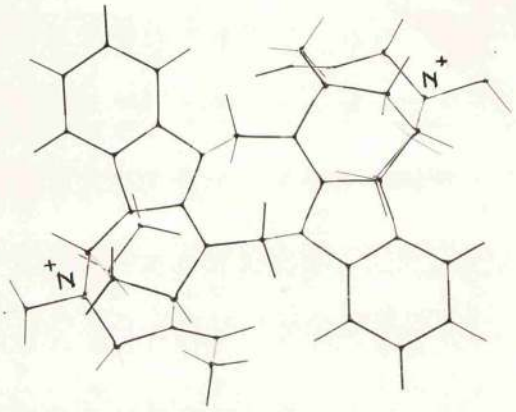
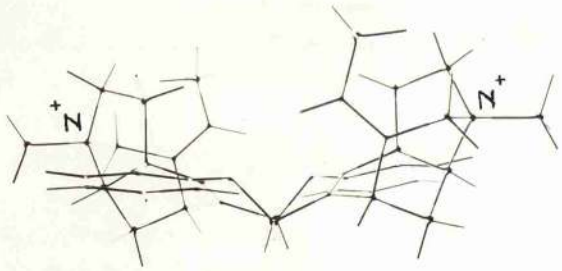


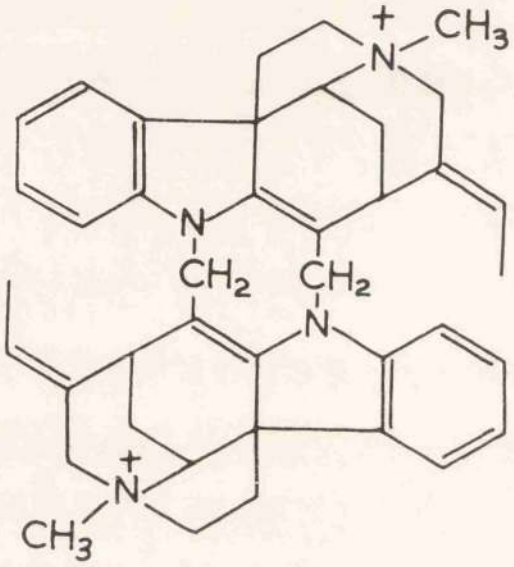
Fig. 3.

molecules of the bisquaternary compound to be spread in a monomolecular layer over the end plate (a contention supported by the experimental work of Waser[59] using ^{14}C labelled calabashcurarine) with the positive onium groups assuming as close a juxtaposition as possible to the anionic groups of the receptor. This would lead to the most extended conformation only if the anionic heads of the receptors are separated by a distance of 14Å or more (Fig. 2a). Should the distance be less than 14Å, buckling of the polymethylene chain from its most thermodynamically stable extended conformation would be expected to follow as a result of the diminished repulsion between the onium centres consequent upon the attraction of each for the anionic sites in the receptors (Fig. 2b). Alternatively, when one cationic charge is wholly or partially neutralised by one receptor anion, intramolecular repulsion between the onium groups in the polymethylene compound disappears (or at least is greatly reduced) allowing the other onium centre to move closer to the first (Fig. 2c). Again although little is known concerning the exact nature of the forces involved in drug-receptor interaction, it would seem safe to assume (by analogy with known physical and chemical processes) that energy must be supplied in order to give rise to drug receptor complex formation. Thus an increase in energy can be considered necessary in order to pass from the system, "drug molecule and receptor" (A - Fig. 3), to the system, "drug-receptor complex" (B - Fig. 3) via point C on the energy diagram, and one way in which this energy could be supplied would be for the drug molecule to leave its thermodynamically stable conformation. Hence



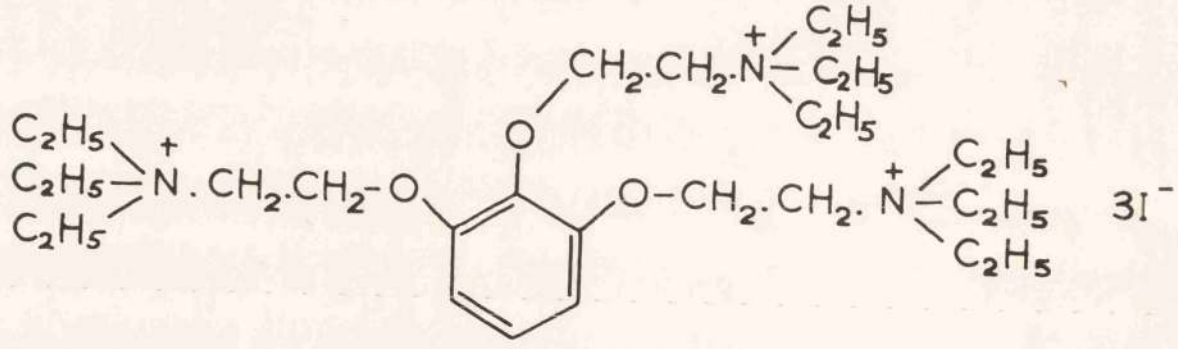


VIII a



2x=

VIII Toxiferine I



IX Gallamine Triethiodide

arguments based on the thermodynamically most stable conformation of the isolated drug molecule have no necessary validity when applied to deductions concerning the nature of the receptor.

There are actually a number of experimental observations which also controvert the postulated interonium distance of 14Å. Thus cyclo-octadecane-1,10-bis (trimethyl ammonium) iodide,[60] gallamine[61] and hexamethonium[56] as well as several polymethylene bis (tropinium halides)[62] with a maximal interonium distance of approximately 9Å display high muscle relaxant activity. Furthermore, Lewis et al[63] have recently shown that potent neuromuscular blocking properties are present in a series of polyonium compounds having interonium distances lower than the 14Å value and they have also suggested 9Å as the optimum distance between recurring anionic receptor sites. This value is in close agreement with the interonium distance in the fully rigid toxiferine I[64] (VIII) which has been calculated to be 9.7Å[62].

Multi-point Attachment

The high muscle relaxant activity displayed by certain bisquaternary compounds inspired the preparation of a number of tris, tetra, penta and hexa ammonium compounds in the belief that whereas the bisquaternaries would span two anionic sites, suitable polyonium derivatives would be capable of interacting with several anionic sites, thus producing more potent compounds. Of the compounds so prepared, none has shown any truly significant increase in potency over the bisquaternaries, however. Gallamine, a trisonium compound (IX), has been employed clinically

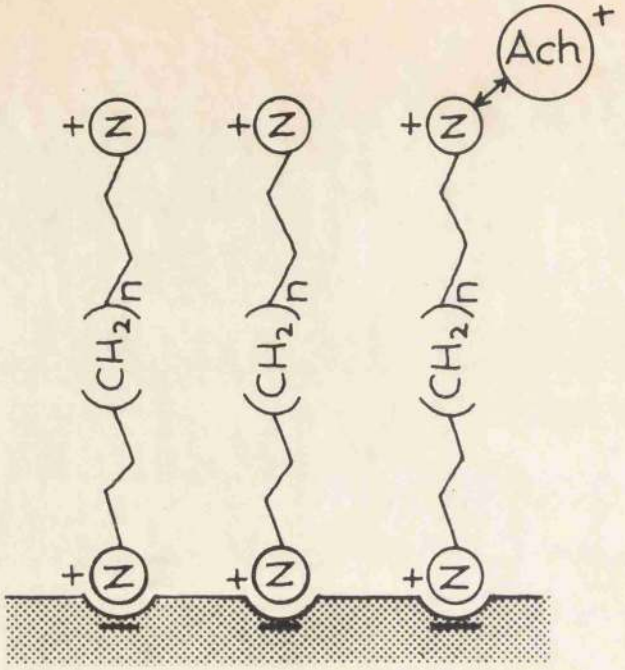


Fig. 4.

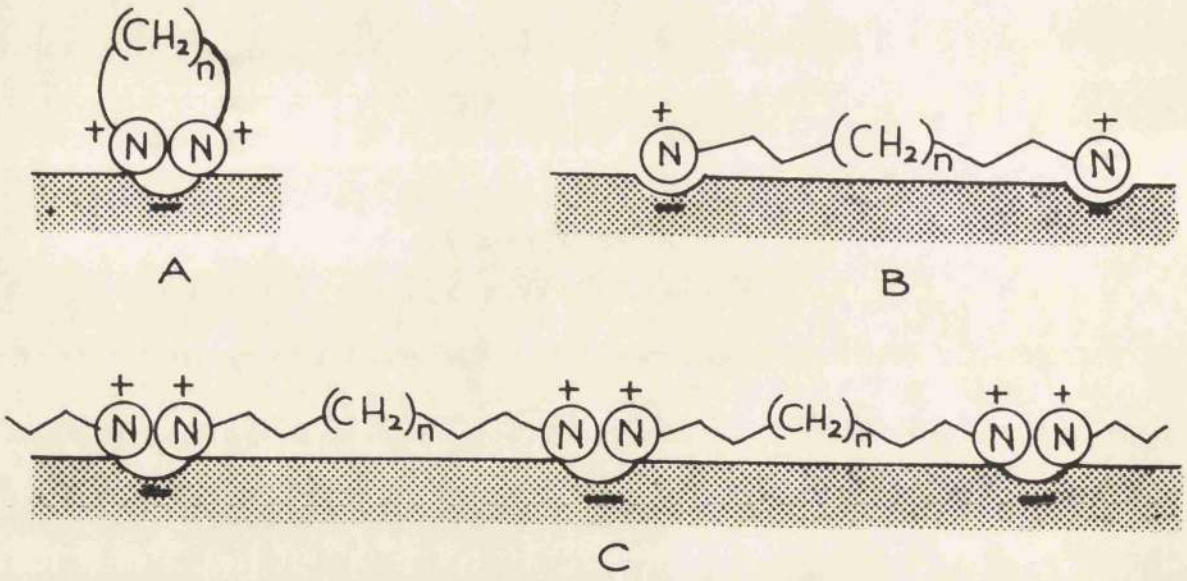


Fig. 5.

because of its freedom from side effects[65], but in fact it is only one-fifth (weight basis) as potent as tubocurarine[66]. It has been suggested that the central ammonium chain in gallamine acts as a stabiliser to keep the other two ammonium groups at a certain optimum distance apart (9Å)[67].

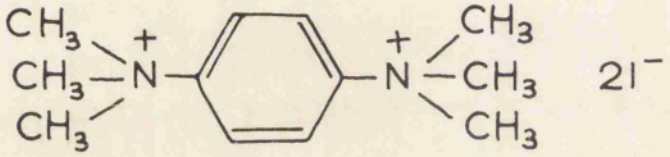
One Point Attachment Theory

Although the two point attachment theory has never been totally refuted, some pharmacologists believe that it has been overstressed. Loewe and Harvey[68] have postulated a one-point attachment theory, according to which only one cationic head of the drug molecule interacts with the receptor whilst the bulk of the molecule shields the receptor with the second cationic group exerting a repulsion against incoming acetylcholine molecules (Fig. 4). This theory, which is called the "adumbration theory" has been extended to ganglion blocking agents by Fakstorp and others[69]. Again, conductimetric experiments have shown the extreme stability of the ion pair involving a single anion and a bisquaternary ammonium cation in aqueous solutions[70] which raises the possibility that the receptor complex could be of type A rather than type B (Fig. 5). It is also possible that the receptor complex could be of type C.

Rigid Bisquaternary Molecules

In view of the ambiguities inherent in all attempts to deduce information concerning the anionic centres of the receptor site from conformationally non-rigid molecules as just outlined, it appeared an attractive proposition to prepare a number of conformationally rigid bisquaternary ammonium salts each having a different interonium distance. Although the receptor could conceivably be non-rigid and adjust itself to suit the demand of a given rigid molecule (the so called "induced fit" theory[71]) demonstration of activity in one rigid bisquaternary salt and absence of activity in another with a different inter-group distance would represent a great advance. Very few such compounds[73, 74] have so far been investigated for muscle relaxant activity and there is no report of a systematic approach towards this problem. Toxiferine I and the related calabash curare alkaloids would appear to be the only such naturally occurring rigid molecules studied.

Moreover, it was considered that the preparation of a series of rigid bisquaternary salts would be of interest from another point of view as Gill[72] has proposed that completely rigid molecules might prove inactive due to variability in the receptors and the need for a certain degree of flexibility in the drug molecule to ensure that an appreciable percentage of the available receptors can be occupied. This generalisation which rests on the absence of ganglion blocking activity in such compounds as the completely rigid NNNN′N′ tetramethyl p-phenylenediamine



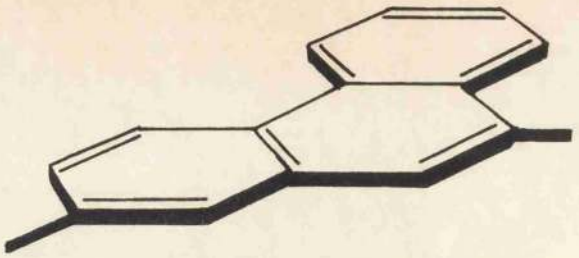
X NNN′N′ Tetramethyl-p-phenylene-diamine dimethiodide

dimethiodide (X)[73] and some furan derivatives still retaining a limited degree of rotational flexibility[74] certainly requires further substantiation. There would appear to be no a priori reason why some receptors (such as those involved in ganglion blockade) should not accept rigid molecules whilst others (such as those concerned in the action of the steroid hormones) obviously can accept the rigid oestrone or testosterone molecules, and so the possibility that the rigid molecules selected by Gill have the wrong interonium distance cannot be overlooked.

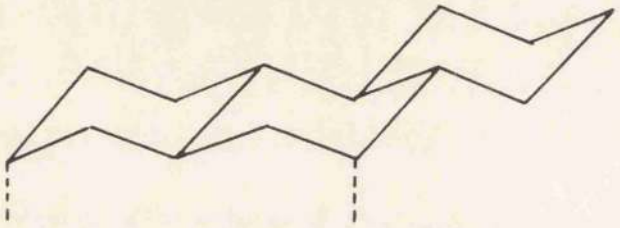
The present project to synthesise rigid bis-trimethylammonium compounds with varying interonium distances was therefore undertaken in the hope that pharmacological evaluation of such compounds might more closely define the nature of the distance between recurring anionic receptors. The studies were confined to the trimethylammonium compounds so as to maintain uniformity of the cationic heads and on account of steric considerations in the molecules concerned (vide infra).

Approach to the Selection of New Rigid Bis-Trimethylammonium Compounds

The first problem in such a project was the selection of a suitable molecular framework on which to base the rigidly held onium groups. Such a framework had to provide several sites at which to place the quaternary nitrogen functions in order to afford a series of compounds with different fixed interonium distances, and at the same time it had to be readily available. The choice therefore logically fell on larger polycyclic systems as these not only fulfilled the requirements but also provided for larger inter-group distances than were possible in mono

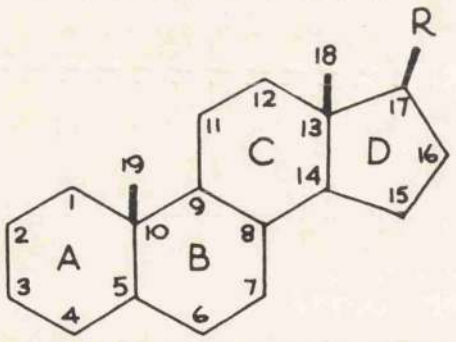


(a) Phenanthrene



(b) Perhydrophenanthrene

XI



R = C-17 Side Chain

XII

General Steroid Skeleton
Cyclopentanoperhydrophenanthrene

or bicyclic systems. Aromatic systems were rejected on the grounds that the substituents would be held in the plane of the flat molecule (XIa) and it was felt that if the receptor was not itself planar, suitable steric fit might not be achieved. On the other hand, were the ammonium groups to radiate away from the plane of the molecule, as is possible in alicyclic systems(XIb), the probability of their being able to interact with the receptor would be enhanced. Apart from steric considerations, incorporation of aromatic characteristics was felt to be undesirable, because of modification of the chemical properties of the substituents. For example aromatic amines are weak bases and phenols are acidic. Pharmacological evaluation of aromatic quaternary ammonium compounds also indicates that such compounds show weak muscle relaxant activity[75].

The ultimate choice fell on the steroid nucleus (XII) which was considered a suitable structure for the following reasons:

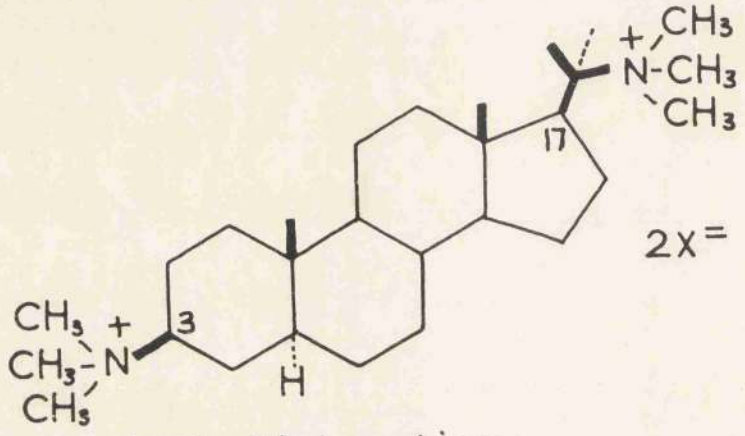
a) Being composed of four rigidly interlocked rings, it provided a uniform skeleton for the synthesis of several bis-trimethylammonium compounds of varying interonium distances.

b) The chemistry of the steroids has been thoroughly studied providing a large body of background knowledge which would be expected to reduce greatly the amount of preliminary investigations necessary.

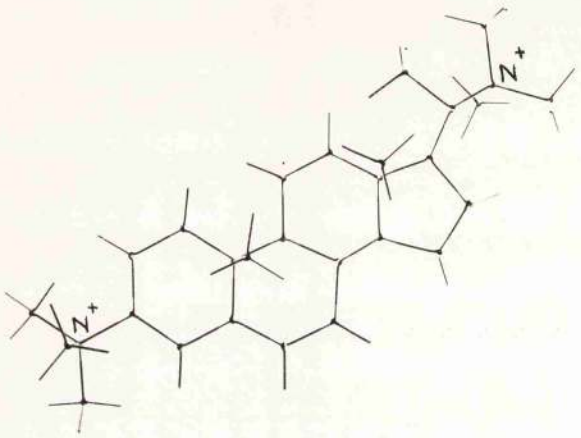
c) Several steroidal derivatives are readily available as starting materials.

d) Conformational analysis has been well applied to this group of compounds.

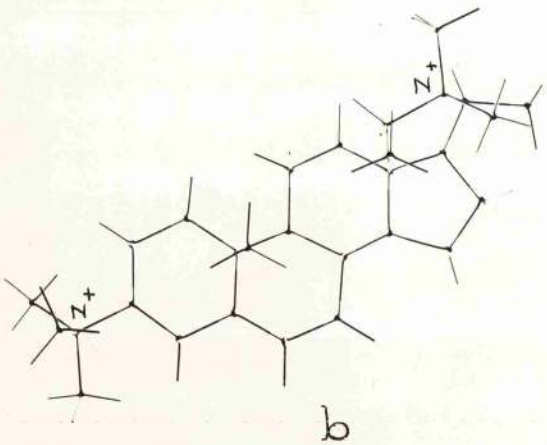
e) The utilisation of the steroid skeleton was regarded as favourable from the biological point of view since the coupling to this predominantly lipid soluble nucleus would be expected to confer upon the trimethylammonium groups, a certain degree of lipid solubility and perhaps also more favourable adsorption properties. Since the plasma proteins are of such a nature as to readily bind cholesterol, it was further conceivable that such steroidal compounds could use an existing transport mechanism although it must be admitted that binding to the blood proteins might produce sensitisation effects. These considerations are inherent in the "supporting moiety theory"[76] which contends that the molecules of pharmacologically active substances consist of a radical moiety, determining the type of activity displayed, and a supporting moiety conferring affinity for the site of action. Cavallini and his colleagues[77] who formally stated this theory, employed several nitrogenous steroids in their early experiments. The actual compounds included the β-diethylaminoethyl ethers of oestrone, testosterone and 3α-hydroxy-17-oxoandrost-5-ene and the bis-β-diethylaminoethyl ethers of oestradiol and 3,17-dihydroxyandrost-5-ene[78]. The combination of a "stripped down" drug molecule[79] or radical moiety (diethylaminoethanol) and the steroidal supporting moiety produced drugs with potent coronary vasodilator properties[80, 77] whilst the bisquaternary derivatives from the two diether compounds showed curare-like properties[81, 77]. The quaternary salts also showed in vitro anticholinestrase activity[82]. Quaternary salts derived from the monodiethylaminoethyl ethers exhibited ganglion blocking activity[77].



XIII Malouetine



a

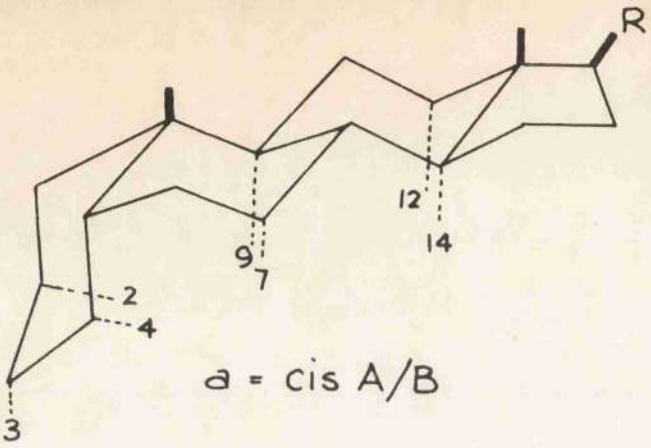


b

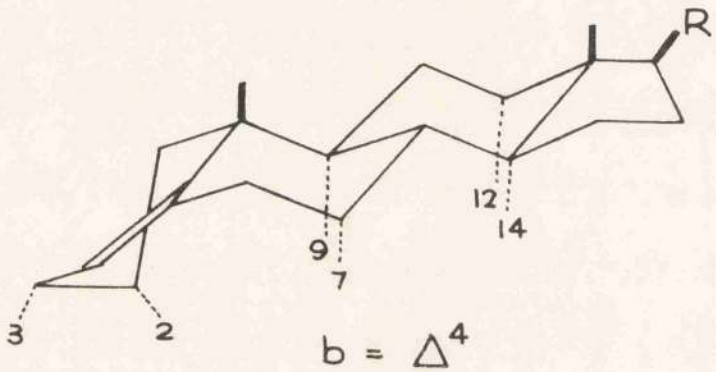
XIII

The present project received further encouragement from the reports of the potent neuromuscular blocking properties displayed by the recently characterised practically rigid bisquaternary steroidal alkaloid malouetine (XIII), which occurs in Malouetia bequaertiana[83]. This compound has been shown, in preliminary experiments, to possess competitive neuromuscular blocking potency quantitatively similar to that of (+)-tubocurarine whilst being only one third as toxic[84], and so malouetine or structurally related drugs could conceivably offer alternatives to tubocurarine as adjuncts to surgery. The remaining three possible isomers of malouetine involving the configuration of the nitrogen atoms, namely the bisquaternary bases in which the nitrogen atoms are in the 3β-20β-, 3α-20α- and 3α-20β-configuration have been prepared synthetically[85] though the potency of these compounds as muscle relaxants has not been reported. Although the molecule of malouetine is not completely rigid, due to rotation about the C-17, C-20 bond, measurements using Dreiding models (XIIIa and b) show that the range of variation does not exceed ca 1.5Å from the maximum possible interonium distance of ca 11.5Å.

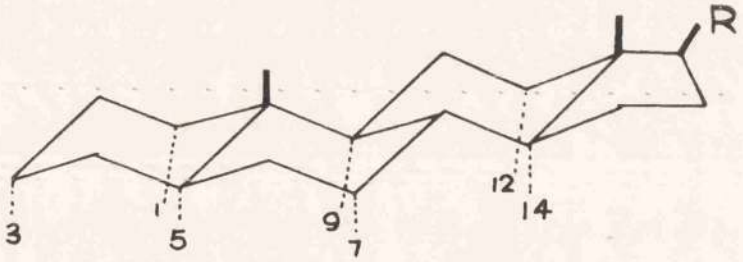
Apart from interest in the bisquaternary steroidal compounds, it was also conceivable that the primary and tertiary steroidal amines, which would be intermediates in the syntheses of the bisquaternary compounds, themselves might well be of pharmacological interest. Pronounced pharmacological activity is characteristic of many nitrogenous steroids but there had been no comprehensive treatment of the subject



a = cis A/B

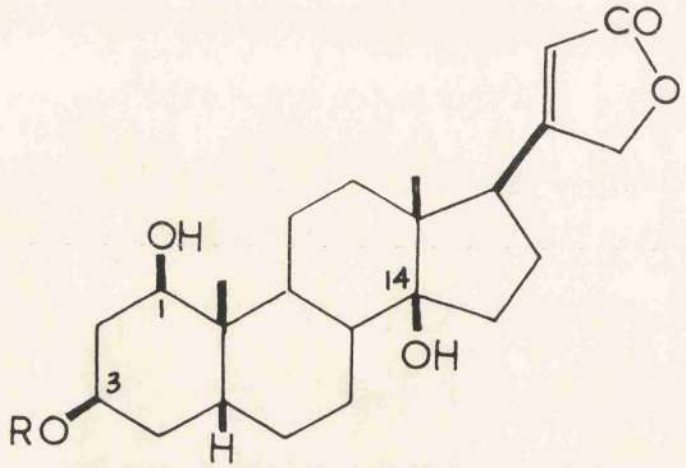


b = Δ^4



c = trans A/B

XIV



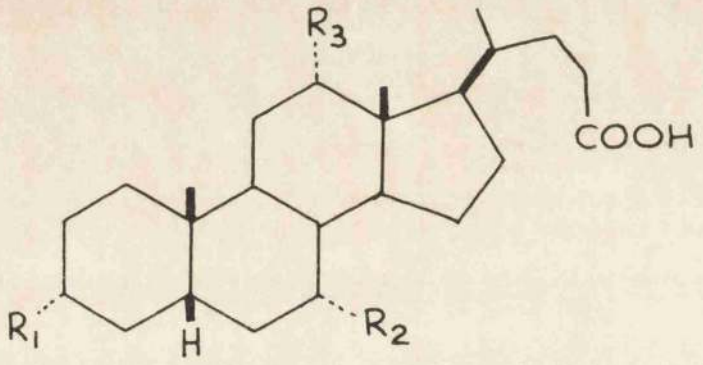
R = Acovenose ($C_1H_{12}O_5$)

XV Acovenoside A.

at the time the work described in this thesis was undertaken. Accordingly the biological activity found in nitrogenous steroids was reviewed in connection with the present work[85A]. Reprints are included as appendix I.

The conformational formulae of three steroidal systems (XIV) illustrate those positions in the molecule where substituents project below the plane of the molecule on the α face, and it was decided to utilise certain of these positions in order to avoid steric interference from the β-oriented angular methyl groups at C-10 and C-13 and the side chain at C-17. As it is difficult to introduce substituents at the tertiary carbon atoms C-5, C-9 and C-14 attention was directed in the first instance towards securing substitution at C-1, C-3, C-7 and C-12.

Steroids bearing suitable substituents at C-1 which would permit the introduction of a nitrogen function at this position are not readily available but Acovenoside A (XV)[86], a cardiac glycoside occurring in Acocanthera longiflora Stapf.[87], appeared to be a convenient starting material for the preparation of C-1 amino steroids. An attempt was therefore made to secure this compound from the trunk wood of this plant which was available in large quantities. However, examination revealed that this material, unlike the seeds, wood bark and root bark[87], did not contain detectable quantities of Acovenoside A although other known "chloroform insoluble" glycosides[87] were present in high percentage. In addition the extract also yielded a steroidal glycoside in about 0.05% yield, which appeared to be a new compound belonging to the



Cholic Acid : $R_1 = R_2 = R_3 = OH$

Deoxycholic Acid: $R_1 = R_3 = OH$, $R_2 = H$

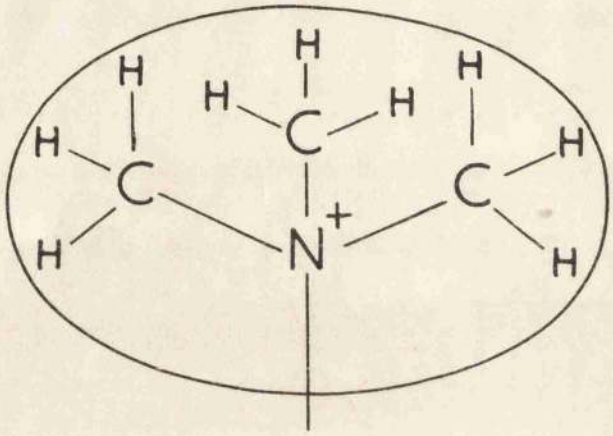
Chenodeoxycholic Acid: $R_1 = R_2 = OH$, $R_3 = H$.

XVI

"chloroform soluble" fraction. As Acovenside A was not readily available, this work was not pursued further. A brief account of it appears in appendix II.

The readily available bile acids (XVI) offered an obvious choice of starting material as they provided a means for facile attack at C-3, C-7 and C-12. These compounds possess a cis A/B ring junction but conversion into the trans A/B series via Δ^4 compounds is a well established procedure. Measurements on Dreiding models of the distances between the centres of the substituents in the α positions at C-3, C-7 and C-12 show that the values are approximately as follows:-

	α-Substituents on:	Intergroup Distance
A/B cis steroids	C-3, C-7	4.5Å
	C-7, C-12	4.2Å
	C-3, C-12	5.8Å
A/B trans steroids	C-3, C-7	5.1Å
	C-7, C-12	4.2Å
	C-3, C-12	6.8Å
Δ^4- steroids	C-3, C-7	5.0Å
	C-7, C-12	4.1Å
	C-3, C-12	6.8Å



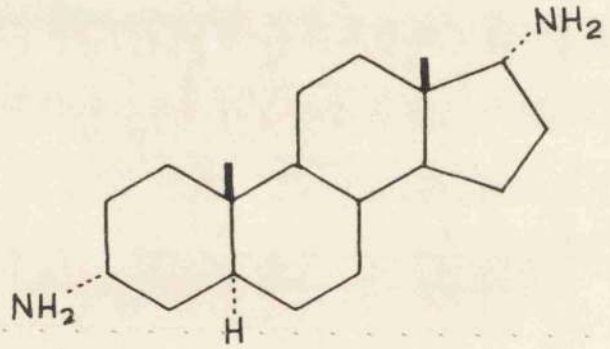
Trimethylammonium group

Fig. 6

Since the long diameter of the ovoid envelope bounding the trimethylamino group as measured from catalin models is ca 6Å (Fig. 6) it is clear that in addition to the charge repulsions between pairs of trimethylammonium substituents in the 3α-, 7α- and 12α- positions, severe steric interactions between such pairs of substituents will occur in all the above compounds except the 3,12 bisquaternaries. It is to be emphasised, however, that these steric interactions can be lessened through suitable geometrical adaptations of the trimethylammonium groups themselves and through certain changes in the geometry of the steroid nucleus* via bond angle distortions and so consideration of models alone is no sure guide to the possible existence of a molecule. For example in the present work construction of a catalin model of 12α-trimethylammonium-5β-cholane was difficult due to non-bonded interactions with the axial hydrogen atoms on C-9 and C-14, yet the compound was successfully prepared.

Nevertheless, consideration of models led to the conclusion that steric hindrance was particularly unfavourable in all compounds showing an interonium distance of 5Å units or less and so compounds of this type were not considered further. It was also noted that in the cis A/B series it was not possible to construct a model incorporating a trimethylammonium group in the axial configuration at C-7. In addition to the normal 1, 3 non-bonded interactions with the axial

* Cf the work of Ourisson and his colleagues in the dipterocarpol series[88].



3α, 17α - diaminoandrostane

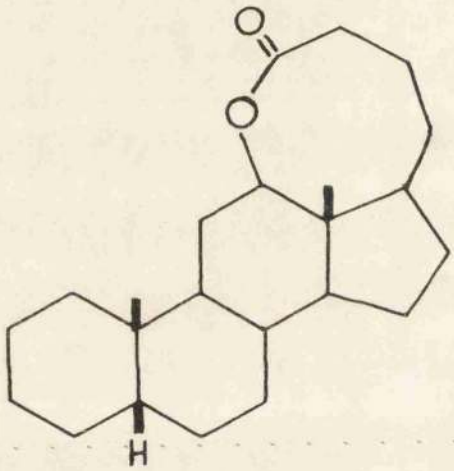
XVII

hydrogen atoms on C-9 and C-14, the 7α position in cis A/B steroids suffers strong steric interaction from C-4. Preparation of ammonium salts possessing N-alkyl substituents higher than methyl would be expected to be extremely difficult.

Attention was therefore directed in the first instance towards the syntheses of the 3α, 12α-bistrimethylammonium derivatives of the 5α and 5β series utilising desoxycholic acid as starting material, although attempts were also made to prepare 3α, 7α-bistrimethylammonium-cholestane from 7-ketocholesteryl acetate in order to ascertain if a rigid bisquaternary salt of interonium distance as low as 5.1Å was indeed capable of synthesis.

Attention was also directed towards the synthesis of 3α, 17α-diaminoandrostane (XVII) as the interonium distance of 9.5Å in this compound falls very close to that of toxiferine I and so it would be expected to yield extremely valuable information, after pharmacological study, concerning the importance of interonium distance.

In the course of the above synthetic work detailed investigations on methanesulphonate formation and on the configuration of the amino group at C-12 in the cholane series became necessary and the undertaking of this work precluded the extension of studies to other compounds with interonium distances greater than 9.5Å. Suitable compounds possessing such large interonium distances could be prepared by the addition of a pre ring A through application of the Robinson-Mannich reaction[89] to suitably substituted steroidal 3-ketones by analogy with the work of Ourisson's school[90].



Lactone

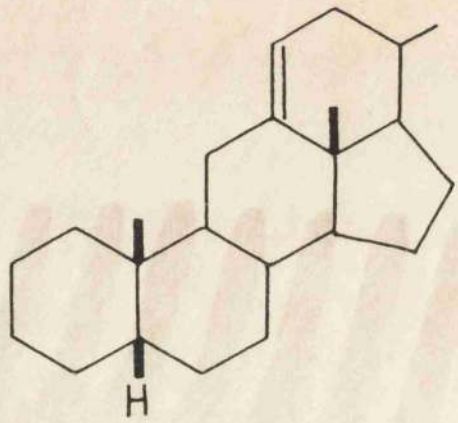
XVIII

Routes to the Bistrimethylammonium Steroids

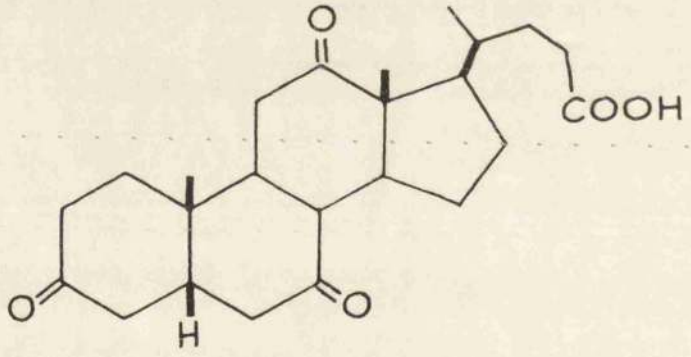
As mentioned in the earlier part of this thesis, the readily available bile acids appeared to be promising starting materials for the proposed syntheses of the rigid bisquaternary compounds. In the first instance it was considered highly desirable to achieve total elimination of any functional group in the side chain. Apart from the likelihood of interference with certain of the necessary chemical conversions at the substituted nuclear centres, there was the strong possibility that a side chain substituent might itself show an affinity for groups in the receptor and so prevent the acquisition of unambiguous information concerning interonium distances alone on biological evaluation. Intramolecular hydrogen bond formation would also be expected to occur between certain substituents at C-12 and C-24, again introducing intangible factors into the interpretation of the biological results.

The presence of a side chain in the 17β position *per se* was not considered disadvantageous for like the angular methyl groups in the β-positions at C-10 and C-13, it would be so oriented as to leave the α-face of the molecule with its appended axial nitrogen functions free from steric interference to drug-receptor interaction.

Direct decarboxylation in the bile acid series would be expected to be difficult owing to the already established facile nuclear dehydration[91] at the high temperatures employed, or to intramolecular reaction between the side chain and C-12, leading to lactone formation (XVIII) where there is a hydroxyl group present at C-12[92] or to condensation to form

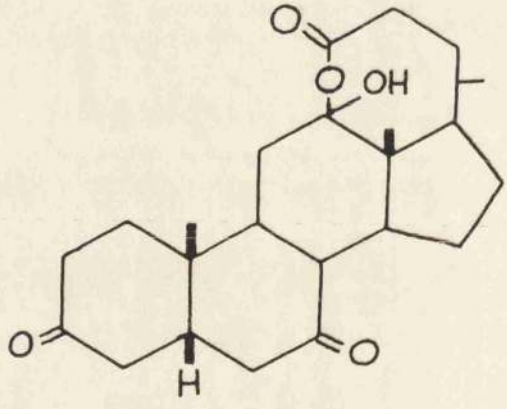


XIX



Dehydrocholic acid

XX



Lactol

XXI

derivatives of the cholanthrene ring system (XIX), where the 12-substituent is a keto group[93]. Actually there would appear to be no reports in the literature of successful simple decarboxylations of substituted cholanic acids by the standard procedures employing metal salts and soda lime, or copper powder and quinoline.

Preliminary studies undertaken in connection with this thesis showed that decarboxylation did not take place when dehydrocholic acid (XX) was heated with copper powder in quinoline. In addition to the recovery of starting material, a neutral compound was also obtained in good yield. This compound analysed for $C_{24}H_{34}O_5$ and showed a characteristic large ring lactone absorption in the infra red at 1735 cm^{-1} in addition to six membered ring ketone absorption at 1700 cm^{-1} and broad OH stretching from ca 3400-2650 cm^{-1}. It was readily converted into the parent acid on treatment with hot alcoholic alkali and also formed the trioxime of dehydrocholic acid on treatment with excess of hydroxylamine under basic conditions. These observations indicate that the neutral product was the lactol (XXI) resulting from cyclisation between the carboxylic group of the side chain and the C-12 oxo group. Presumably the reaction conditions were not sufficiently vigorous to give a cholanthrene derivative by loss of carbon dioxide and water.

A number of indirect methods for the removal of the carboxylic function in the bile acid series have, however, been reported in the literature. These include application[94] of the mixed Kolbe synthesis[95]

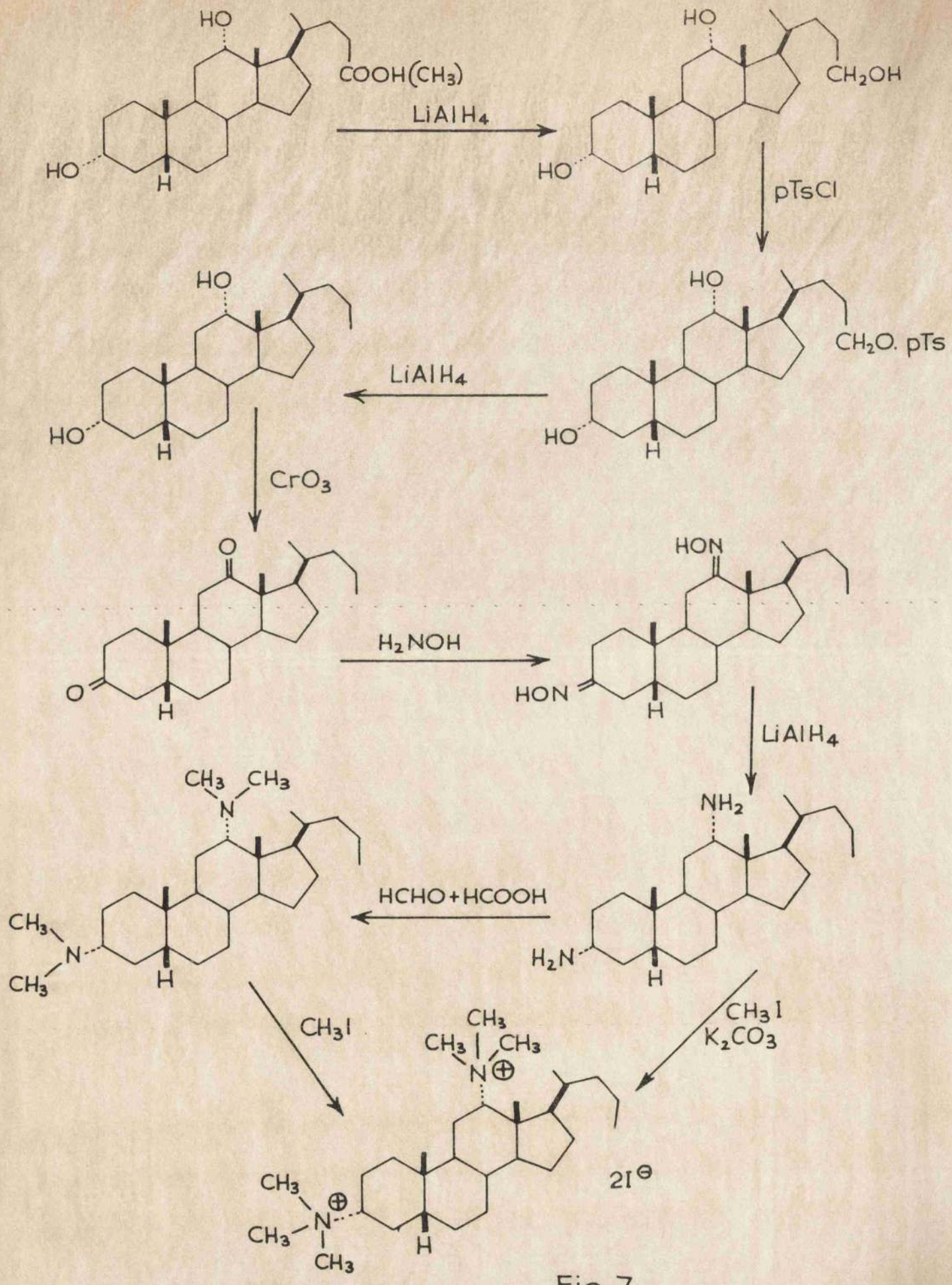

HO
COOH(CH3)
HO
H
LiAlH4
HO
CH2OH
HO
H
pTsCl
HO
CH2O. pTs
HO
H
LiAlH4
HO
H
CrO3
O
O
H
H2NOH
HON
HON
H
LiAlH4
CH3 CH3
N
CH3
N
CH3
H
HCHO+HCOOH
NH2
H2N
H
CH3I
CH3I
K2CO3
CH3
CH3 CH3
N⊕
CH3
N⊕
CH3
CH3
H
2I⊖

Fig. 7.

which affords an extension of the side chain, application[96] of the Hunsdiecker reaction[97], application[98] of the Curtius reaction[99] to the derived acid azide followed by reductive deamination, and reduction of the carboxylic function to the corresponding primary alcohol[100] followed by reductive elimination of the derived p-toluenesulphonate[101].

The last mentioned procedure appeared the most attractive as it could be utilised in conjunction with selective attack at the nuclear centres thus reducing the number of stages, in the chemical transformations, to a minimum.

After removal of the side chain function via the sulphonate ester, it was planned to employ various combinations of selective esterifications[102], oxidations[103] and reductions[104] already established in the steroid series, to yield diols with different combinations of the hydroxyl groups on two of the possible C-3, C-7 and C-12 positions. These in turn were to be converted into the corresponding diketones and thence into the dioximes, on which stereoselective reduction techniques were to be used in order to give the required axial primary diamines. Finally, methylation and quaternisation were to be employed to give the bisquaternary salts.

An outline of the application of these procedures to the preparation of 3α, 12α-bis-dimethylamino-5β-cholane bis-methiodide is illustrated in Fig. 7 to indicate the general approach.

Preparation of the bisquaternaries of the 5α-series was to be achieved by utilising the well established 4-bromination of 3-oxo-5β-steroids[105] followed by dehydrobromination to the corresponding Δ^4

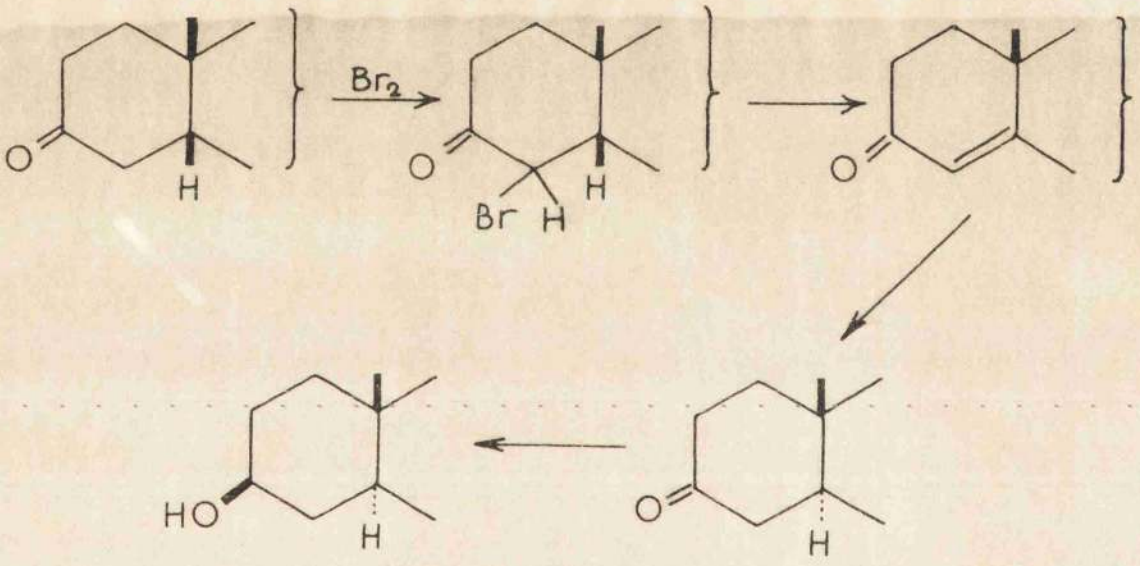
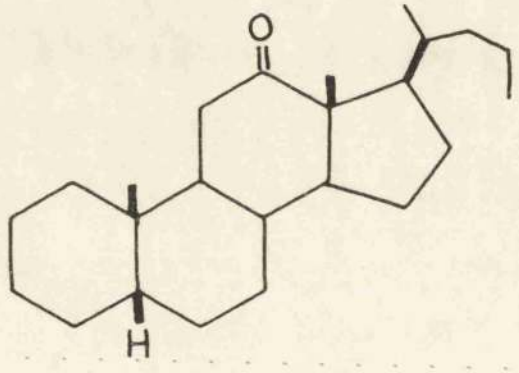


Fig. 8.

compound by means of collidine[106] or other methods[107]. Reduction of the 3-oxo-4-ene by means of an exact equivalent of lithium in liquid ammonia would then afford the 3-oxo-5α-steroid[108] and with an excess of lithium in liquid ammonia would afford the 3β-hydroxy-5α-steroid[109] (Fig. 8).

Preliminary investigation of oxime formation with dehydrocholic acid [110] followed by the application of several different reduction procedures served only to confirm the difficulties, already reported in the literature[111], in obtaining the triamine in a pure state. The previously reported procedure for the preparation of the trioxime using methanol as solvent[110] was found to be unsatisfactory, probably on account of precipitation of the highly insoluble dioximes during the reaction. However, use of a higher boiling alcohol or tetra hydrofuran as solvent gave a satisfactory yield of the trioxime. Lithium aluminium hydride in ether/tetrahydrofuran, even on prolonged refluxing, failed to reduce any of the oximino groups of trioximino-methyl dehydrocholate. The only group attacked was the ester group yielding a C-24-hydroxy compound. In view of the reports in the literature that oximino groups at C-3 and C-7 in the steroid series are readily reduced by lithium aluminium hydride[112], it appeared that the hydroxyl group at C-24 was complexing with the reagent, preventing further attack on the oximino groups through unfavourable solubility. Catalytic hydrogenation, on the other hand, proceeded extremely slowly and incompletely. The partially hydrogenated "mixture" after chromatography



12-oxo-5β-cholane

XXII

gave no pure crystalline material nor would it yield crystalline salts with acids.

In view of the difficulties encountered in these preliminary experiments, it was deemed necessary to undertake a separate investigation of the 12-oximino function since it appeared to be this group which was responsible for the incomplete catalytic reductions. The formation and reductions of oximes at C-3 and C-7 had already been studied in detail[113] and considerable information concerning these compounds and their derivatives was available. On the other hand very little attention had been paid to the 12-oximino function[114]. In order to study the 12-position without interference from other substituents, a procedure affording 12-oxo-5β-cholane (XXII) in good yield via 12α-hydroxy-5β-cholane was evolved and in the course of this work it was found that methanesulphonate esters afforded a more acceptable route than the more commonly employed p-toluenesulphonate esters. Accordingly a survey of selectivity of methanesulphonate formation and the behaviour of the esters on lithium aluminium hydride reduction was undertaken. The results of these studies are reported in Section II of the thesis.

The information obtained on reduction of 12-oximino-5β-cholane was of sufficient interest to warrant study of 12-oximino-5α-cholane. The studies on both of these compounds are reported in Section III of the thesis.

The studies directed towards bistrimethylammonium salt formation form the substance of Section IV.

EXPERIMENTAL

Lactol of Dehydrocholic Acid (XXI): Commercial dehydrocholic acid (XX) (4.0 g.) in freshly distilled quinoline (40 ml.) was heated under nitrogen with copper bronze powder (1.0 g.) for 3 hr. at 190° to 230° C. The reaction mixture was cooled under nitrogen overnight and filtered. Addition of excess of hydrochloric acid (6N) to the filterate gave a precipitate which was taken into chloroform, washed with water and the organic solvent removed under reduced pressure. Digestion with ether left a noncrystalline solid, which was fractionally recrystallised from methanol, yielding unchanged dehydrocholic acid as the least soluble fraction. The more soluble fraction on further crystallisation from methanol gave needles of the lactol (1.0g, 25%), m.p. 136-142°, $[\alpha]_D^{20}$ + 21 ± 2 (2.1% $CHCl_3$). (Found: C, 71.22; H, 8.53. $C_{24}H_{34}O_5$ requires: C, 71.64; H, 8.45%). Infrared in nujol mull showed hydroxyl absorption (3400 - 2650 cm^{-1}) ketonic absorption (1700 cm^{-1}) and lactol carbonyl (1735 cm^{-1}).

Refluxing the lactol (0.24 g.) in methanol (10 mls.) with hydroxylamine hydrochloride (0.14 g.) and sodium acetate (0.3 g.) in the presence of water (1 ml.) for 6 hr., converted the lactol into the trioxime of dehydrocholic acid, m.p. 265-272 (decomp.) (reported 273-274 (decomp.))[115]. (Found: C, 63.95; H, 8.66; N, 8.88. Calculated for $C_{24}H_{37}O_5N_3$: C, 64.14; H, 8.90; N, 9.35%).

Hydrolysis of the lactol with hot alcoholic sodium hydroxide gave dehydrocholic acid m.p. 234-236, and mixed m.p. 234-237.

Reduction with lithium aluminium hydride in tetrahydrofuran gave 3,7,12-trioximino-24-hydroxy-5β-cholane, crystallised from methanol, plates m.p. 260-270 (decomp.), $[\alpha]_D^{20}$ + 40 ± 2 (2.0% $CHCl_3$ + EtOH 4:1). (Found: C, 65.87; H, 9.38; N, 8.96. $C_{24}H_{39}O_4N_3$ requires C, 66.35; H, 9.20; N, 9.67%). The same compound resulted from the action of lithium aluminium hydride (6-7 equivalents) in tetrahydrofuran on the methyl ester of the trioxime of dehydrocholic acid m.p. 265-275 (decomp.) mixed m.p. 265-275 (decomp.).

Attempts to further reduce purified 3,7,12-trioximino-24-hydroxy-5β-cholane with lithium aluminium hydride proved unsuccessful and the unchanged trioxime was recovered in virtually quantitative yield.

Trioxime of Dehydrocholic acid Methyl ester: Dehydrocholic acid was converted into its methyl ester by the method of Borsche[110] m.p. 244-47 (reported[110] 241-242). Alternatively the action of diazo-methane in methanol/ether afforded the same compound. It was found convenient to modify the previously reported[110] procedure for conversion of the ester into the trioxime.

Methyldehydrocholate (0.5 g.) in ethanol (35 mls.) was refluxed with hydroxylamine hydrochloride (0.31 g.) and sodium acetate (0.65 g.) in the presence of water (3.5 mls.) for 6 hr. After standing overnight the product had separated as plates. Recrystallisation from ethanol gave 0.5 g. (90%) m.p. 258-260 (decomp.), reported[110] 265-266 (Found: C, 64.94; H, 8.69; N, 9.27. Calculated for $C_{25}H_{39}O_5N_3$: C, 65.03; H, 8.51; N, 9.13%).

Repeated attempts at catalytic hydrogenation employing a platinum oxide catalyst in acetic acid failed to yield a homogenous product. The maximum uptake of hydrogen observed in these experiments was ca 5 moles in 80 hr. (theory requires 6 moles for reduction of the three oximino groups).

S E C T I O N I I

METHANESULPHONATES OF HYDROXYCHOLANES

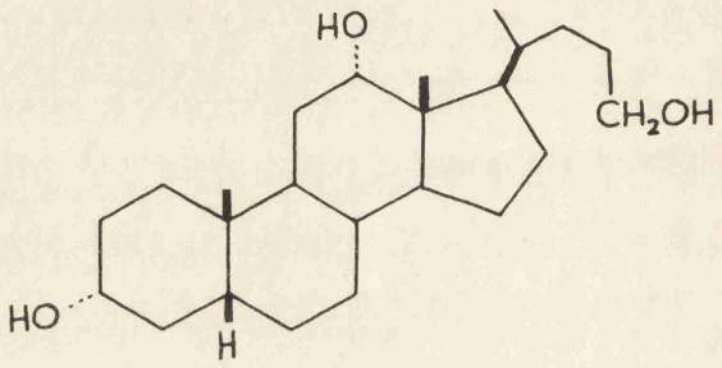
As outlined in Section I of this thesis, it was necessary to achieve selective removal of the functional groups in order to utilise the commonly available bile acids as starting materials for the syntheses of the desired bistrimethylammonium steroidal bases. Initially, this was attempted by selective p-toluenesulphonic ester formation followed by lithium aluminium hydride reduction, but the poor yield of the p-toluenesulphonates obtained in certain instances made it highly desirable to evolve a more satisfactory procedure. The reason for the low yield of the p-toluenesulphonates was not immediately apparent, but in view of the work of Leanza et al[116] and Madaev and Babanova[117] who showed that pyridinium salt formation occurred on treatment of certain hydroxy-steroids with p-toluenesulphonyl chloride in pyridine, it would seem likely that a similar reaction was occurring in the cholane series. Such pyridinium salts would be expected to be water soluble and so be readily lost in the aqueous layer during the working up of the reaction mixture.

It was decided to substitute methanesulphonate formation for p-toluenesulphonate formation, the decision being based on two main considerations. Firstly, the rate of reaction of hydroxy compounds in pyridine with methanesulphonyl chloride is well known to be much faster than that with p-toluenesulphonyl chloride - the former proceeding readily at 0°C whilst the latter often requires prolonged treatment at elevated temperatures[118]. Secondly, the results of solvolysis rate studies have indicated that the p-toluenesulphonate anion is a better

leaving group than the methanesulphonate anion in S_N displacements[119]. Although rates of solvolysis depend upon the dielectric constant and the molar volume of the solvent[120] and while the rate studies were for water not pyridine, it was reasoned that the steroidal methanesulphonates would be less likely to suffer nucleophilic attack by pyridine to give pyridinium salts than their p-toluenesulphonate analogues.

Accordingly a thorough investigation of methanesulphonate formation in the hydroxycholane series was undertaken and the results of these studies as well as comparisons with p-toluenesulphonate formation are described in this section of the thesis.

Previous studies of methanesulphonate formation at the 3- and 6-positions of the steroid nucleus[121] had given rise to the generalisation[122] that methanesulphonate formation was less selective than p-toluenesulphonate formation, but for the compounds studied in the present work it was found that not only were the methanesulphonates formed in better yield than the corresponding p-toluenesulphonates but also that a comparable selectivity of attack existed for both reagents. In nearly all cases, the sulphonate esters did not crystallise readily and so they were characterised by means of infrared analysis and by the crystalline products obtained after reduction with lithium aluminium hydride. Similar difficulties in obtaining crystalline p-toluenesulphonates and acetates of certain hydroxycholane derivatives have been reported by Blickenstaff and Chang[123]. These authors also quote other examples of such difficulties reported elsewhere in the literature.



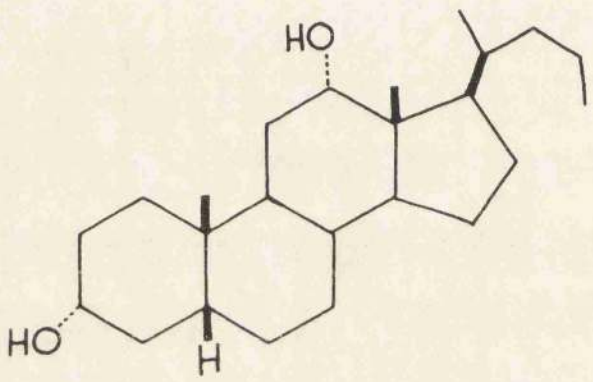
XXIII

As expected, all the compounds studied which possessed a primary alcoholic group in the C-24 position (formed by the reduction of the bile acid carboxylic group by lithium aluminium hydride[124, 125]) reacted readily with one equivalent of methanesulphonyl chloride in pyridine at 0°C but only in those compounds lacking an equatorial hydroxyl group in the C-3 position, was attack confined to the C-24 position. In both the 5α- and 5β-cholane series the equatorial secondary hydroxyl group at C-3 was virtually as reactive as the primary hydroxyl group at C-24. Thus 3α,12α,24-trihydroxy-5β-cholane (XXIII), prepared in comparable yield by the action of lithium aluminium hydride on either desoxycholic acid or methyl desoxycholate, on treatment with up to 1.5 equivalents of methanesulphonyl chloride in pyridine at 0°C, failed to give complete esterification at C-24. Proof of this was afforded as follows.

Treatment of the crude mixture of methanesulphonates with lithium aluminium hydride gave an ether-soluble mixture which, after chromatography over alumina, was resolved into three fractions. The two smaller fractions were identified as 12α-hydroxy-5β-cholane (ca 8% of total product) and 3α,12α,24-trihydroxy-5β-cholane (ca 40% of total product) whilst the main fraction (ca 52%) had the properties of a diol. However, oxidation of this diol fraction with chromic oxide furnished a mixture 12-oxo-5β-cholanic acid and 3,12-dioxo-5β-cholane in the ratio of 2:3, thus proving it to be a mixture of 2 parts 12α,24-dihydroxy-5β-cholane to 3 parts 3α,12α-dihydroxy-5β-cholane, which was not separable by

chromatography over alumina. The formation of the three compounds 12α-hydroxy-, 3α,12α-dihydroxy- and 12α,24-dihydroxy-5β-cholane thus shows that selective attack at the primary hydroxyl group on C-24 over the secondary hydroxyl group in the equatorial position on C-3 does not occur with methanesulphonyl chloride. The recovery of 3α,12α,24-trihydroxy-5β-cholane does not necessarily support the same argument, as this compound could have been regenerated by O-S cleavage rather than C-O cleavage of 3α,12α-dihydroxy-24-methanesulphonyloxy-5β-cholane by the lithium aluminium hydride. However, such preferential O-S cleavage would not be expected for a sulphonate of a primary alcohol although it is well known to occur with sulphonates of phenols and certain secondary alcohols[126]. Indeed, the present work demonstrated that O-S cleavage was typical of sulphonates of steroidal secondary alcohols where the hydroxyl group was in the axial configuration - vide infra.

Actually the properties of the 5β-cholane 3α,12α-diol and 12α,24-diol mixture corresponded closely to those assigned to 3α,12α-dihydroxy-5β-cholane by Blickenstaff and Chang[123] who prepared their sample by the action of p-toluenesulphonyl chloride on 3α,12α,24-trihydroxy-5α-cholane followed by treatment with lithium aluminium hydride. The extreme similarity between their method and that utilising methanesulphonate formation made it seem highly probable that their so called "3α,12α-dihydroxy-5β-cholane" was also in fact a mixture of 3α,12α-dihydroxy-5β-cholane and 12α,24-dihydroxy-5β-cholane in the proportion



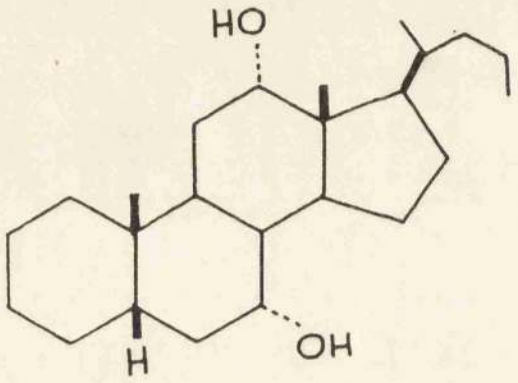
XXXII

of 3:2. Indeed, repetition of the Blickenstaff and Chang procedure gave identical results to that employing methanesulphonyl chloride. Once again 12α-hydroxy-5β-cholane, 3α,12α,24-trihydroxy-5β-cholane and the mixture of 3α,12α- and 12α,24-dihydroxy-5β-cholane were obtained when the crude p-toluenesulphonate mixture was treated with lithium aluminium hydride and chromatographed over alumina - the diol fraction, as before, yielded 3,12-dioxo-5β-cholane and 12-oxo-5β-cholanic acid in the ratio of 3:2, after oxidation with chromic oxide. It is therefore apparent that both methanesulphonyl chloride and p-toluenesulphonyl chloride show an identical lack of selectivity and that both the 3α-hydroxyl group and the 24-hydroxyl group show high reactivity.

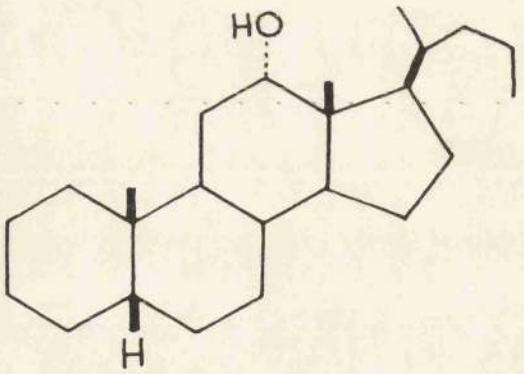
3α,12α-Dihydroxy-5β-cholane (XXXII) was obtained by catalytic hydrogenation of 3,12-dioxo-cholane obtained from the above oxidations (compare the catalytic reduction of dehydrocholic acid to cholic acid[127]).

Authentic 12α,24-dihydroxy-5β-cholane was prepared by lithium aluminium hydride reduction of 3α-methanesulphonyloxy-12α-hydroxy-5β-methylcholanate. The same compound was also obtainable from 3α-p-toluenesulphonyloxy-12α-hydroxy-5β-methylcholanate (prepared as described previously[128]) by reduction with lithium aluminium hydride.

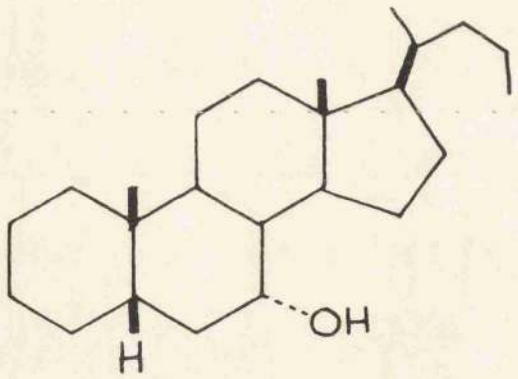
A similar lack of preferential methanesulphonation at the hydroxyl group on C-24 over the hydroxyl group on C-3 was observed in the case of 3α,7α,24-trihydroxy-5β-cholane, which was obtained by lithium aluminium hydride reduction of chenodeoxycholic acid. Once again the diol fraction of the crude product resulting from lithium aluminium hydride reduction



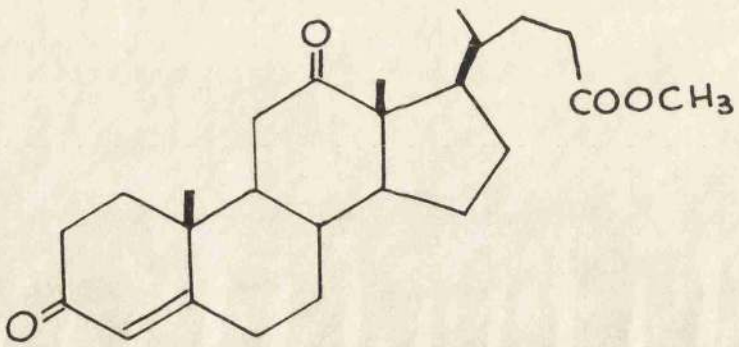
XXIV



XXV



XXVI



XXVII

of the unpurified methanesulphonates, after oxidation gave acid and neutral components although in this case the 7-keto acid and 3,7-diketone were not characterised further.

On the other hand, selective attack at both C-3 and C-24 over attack at either C-7 or C-12 by methanesulphonyl chloride was observed. Thus, treatment of 3α,7α,12α,24-tetrahydroxy, 3α,12α,24-trihydroxy-and 3α,7α,24-trihydroxy-5β-cholanes with exactly 2 moles of methanesulphonyl chloride in pyridine at 0°C followed by lithium aluminium hydride reduction of the noncrystalline dimethanesulphonates gave rise to 7α,12α-dihydroxy-5β-cholane (XXIV), 12α-hydroxy-5β-cholane (XXV) and 7α-hydroxy-5β-cholane (XXVI) in virtually quantitative yield in each case.

Attempted partial hydrolysis of 3α,24-di-p-toluenesulphonyloxy-12α-hydroxy-5β-cholane by the procedure used successfully in the partial hydrolysis of 5-androstene-3β,17β-di-p-toluenesulphonyloxy-16-one to 5-androstene-3β-hydroxy-17β-p-toluenesulphonyloxy-16-one[129] failed to give selective hydrolysis as evidenced by the product obtained after reduction with lithium aluminium hydride. The hoped-for dihydroxy compound on oxidation with chromic oxide gave a mixture of neutral keto compound and keto-acid showing the compound to be in reality a mixture. Due to the failure of selective hydrolysis, these compounds were not considered further.

In order to investigate methanesulphonate formation in the hydroxy-5α-cholane series, methyl 3,12-dioxochol-4-ene-24-carboxylate (XXVII), prepared by elimination of the elements of hydrobromic acid from methyl

4-bromo-3,12-dioxocholanate, was treated with an excess of lithium in liquid ammonia in the presence of ethanol. The action of an exact equivalent of lithium in liquid ammonia on Δ^4-3-oxo-steroids is well known to yield the corresponding 3-ketone of the trans A/B series[130] whilst an excess of the metal in the presence of a proton donor gives the 3β-hydroxy-trans A/B steroids[131].

Considerable difficulty was encountered in preparing pure methyl 3,12-dioxochol-4-ene-24-carboxylate which must be attributable to side reactions during the formation of methyl-4-bromo-3,12-dioxocholanate. Although cis A/B steroids are well known to brominate predominantly in the 4 position[132] (unlike trans A/B steroids where bromination occurs at the 2 position[133]), it has been observed that a certain percentage of 2-bromination can occur in steroids of the cis A/B series[134] and such bromination in the 2 position may have occurred in the present case. Moreover, it has been observed[135] that treatment of 3-ketocholane derivatives with exactly one mole of bromine leads to the formation of a certain proportion of 2,4-dibrominated material with unreacted starting material left in the reaction mixture. Thus methyl 4-bromo-3,12-dioxocholanate contaminated with the 2-bromo isomer or with unreacted methyl 3,12-dioxocholanate would give rise to 5β-cholane derivatives as contaminants of the methyl 3,12-dioxochol-4-ene-24-carboxylate.

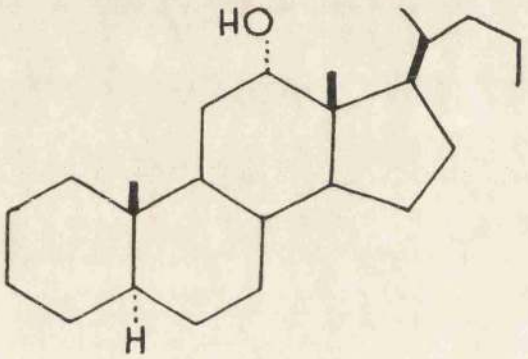
Gas liquid chromatography under standardised conditions failed to separate a synthetic mixture of methyl 3,12-dioxocholanate and methyl 3,12-dioxochol-4-en-24-carboxylate nor could separation be achieved on

alumina chromatography.

The lithium and liquid ammonia reduction product of the crude methyl 3,12-dioxochol-4-en-24-carboxylate certainly contained a percentage of 5β-cholane derivatives and these are ascribed to the initial presence of small amounts of 5β compounds contaminating the enone and not to cis A/B ring formation during the reduction itself.

Actually the mixture resulting from the lithium and liquid ammonia reduction of the crude methyl 3,12-dioxochol-4-en-24-carboxylate was not itself resolved into its components but treated with methanesulphonyl chloride (2 moles) followed by reduction of the total product with lithium aluminium hydride. Chromatography of the reduction product over alumina yielded three main fractions in the proportion of 3:5:2.

The first of these fractions proved to be a mixture of hydrocarbons (infrared spectrum) which was resolved into six components on gas liquid chromatography. The two major components, which together constituted ca 80% of the total hydrocarbon fraction, were identified as 5β-cholane (comparison with the retention time of an authentic specimen) and 5α-cholane (same difference in retention time as was found for the pairs of 5α and 5β 12-oxo-cholanes and 12α-hydroxy-cholanes in separate experiments). The four minor constituents of the hydrocarbon fraction were not identified but were concluded to be cholenes or jervicholenes arising from elimination reactions of the methanesulphonates. Jervicholenes would be expected to arise from eliminations involving the 12β-methanesulphonyloxy group[136]. The formation of the 5α-cholane could only have arisen from the formation



XXVIII

of 3β,12β,24-trihydroxy-5α-cholane during the lithium and liquid ammonia reduction since it was shown in a separate experiment (vide infra) that lithium aluminium hydride reduction of 12α-methanesulphonyloxy-5α-cholane led to an O-S cleavage regenerating 12α-hydroxy-5α-cholane.

That 3β,12α,24-trihydroxy-5α-cholane was also formed in the lithium and liquid ammonia reduction of methyl 3,12-dioxochol-4-en-24-carboxylate was demonstrated when the second fraction from the alumina chromatography of the methanesulphonate reduction product was shown to be 12α-hydroxy-5α-cholane (XXVIII) contaminated with a very small trace of 12α-hydroxy-5β-cholane. Identification was achieved by means of quantitative gas liquid chromatography and comparison with a specimen of authentic 12α-hydroxy-5α-cholane prepared from methyl 12α-acetoxy-3-oxochol-4-en-24-carboxylate as described below.

The third fraction from the alumina column was a mixture of diols which on oxidation with chromic oxide gave rise to equal quantities of 3,12-dioxo-5α-cholane and 12-oxo-5α-cholanic acid.

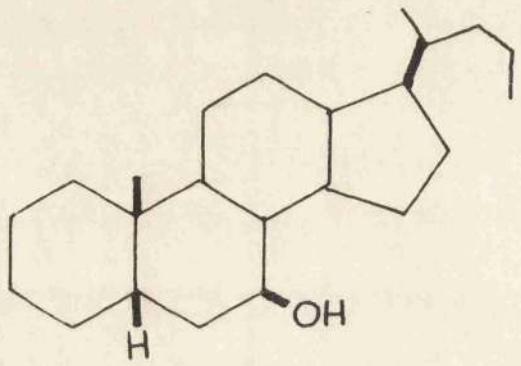
Treatment of the lithium and liquid ammonia reduction product of methyl 3,12-dioxochol-4-en-24-carboxylate with p-toluenesulphonyl chloride (2 moles) followed by lithium aluminium hydride reduction gave an identical mixture to that obtained by the methanesulphonate method.

Reduction of methyl 12α-acetoxy-3-oxo-chol-4-en-24-carboxylate (prepared by elimination of elements of hydrobromic acid from the 4-bromo derivative of methyl-3-oxo-12α-acetoxy-5β-cholanate[137]) with excess of lithium in liquid ammonia, in the presence of ethanol, followed by treatment

with two moles of methanesulphonyl chloride in pyridine at 0°C, and subsequent reduction of noncrystalline dimethanesulphonate with lithium aluminium hydride, gave 12α-hydroxy-5α-cholane in virtually quantitative yield. The same compound resulted in overall lower yield when p-toluenesulphonyl chloride was employed at room temperature in place of the methanesulphonyl chloride in the above procedure.

In view of the complexity of the above experiments, all of which clearly show the non-selectivity of attack by both methanesulphonyl chloride and p-toluenesulphonyl chloride, sulphonation studies were conducted separately at positions 7 and 12 of the cholane nucleus.

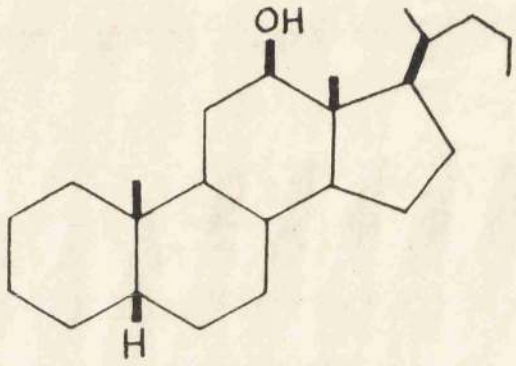
7α-hydroxy-5β-cholane, prepared by lithium aluminium hydride reduction of 3α,24-dimethanesulphonyloxy-7α-hydroxy-5β-cholane, failed to react with p-toluenesulphonyl chloride in pyridine even on prolonged treatment at 37-40°C. It was however readily converted into 7α-methanesulphonyloxy-5β-cholane, in quantitative yield, on treatment with methanesulphonyl chloride in pyridine at 0°C. The noncrystalline sulphonic ester readily underwent elimination on treatment with cold methanol, affording a crystalline mixture of unsaturated hydrocarbons (tetranitromethane test) which showed the presence of two components in equal quantities on application of gas liquid chromatography. This material would appear to be a mixture of $\triangle^{7}$-5β-cholene and its rearranged $\triangle^{8(14)}$ isomer. Such a rearrangement of a double bond from the 7-8 to the 8-14 position, in the steroid nucleus, is known to be particularly facile[138]. The freshly prepared noncrystalline 7α-methanesulphonyloxy-



XXIX

5β-cholane, on reduction with lithium aluminium hydride, regenerated 7α-hydroxy-5β-cholane in virtually quantitative yield proving O-S cleavage. 7β-Hydroxy-5β-cholane (XXIX), obtainable in 85% yield on reduction of 7-oxo-5β-cholane by sodium and ethanol, required fairly vigorous conditions before complete esterification occurred with p-toluenesulphonyl chloride (16 hours at 37-40°C). On the other hand methanesulphonate formation took place readily at 0°C. Although neither 7β-sulphonate ester could be obtained in crystalline form, both were stable in alcoholic solutions and both, on reduction with lithium aluminium hydride, gave pure 5β-cholane showing C-O cleavage.

12α-hydroxy-5β-cholane was found not to react with p-toluenesulphonyl chloride, when the reaction was carried out at 37-40°C for 36 hours. This observation is in agreement with the work of Von Euw and Reichstein[118] who showed that p-toluenesulphonate formation at the 12α position in the cholane series required incubation at 30°C for 4-6 days. p-Toluenesulphonation of 12α-hydroxy-5β-cholane occurred when the reaction was carried out at 60-65° for 2-3 hours, but the low melting, semicrystalline product still indicated the presence of free 12α-hydroxy-5β-cholane (infrared). On the other hand 12α-methanesulphonyloxy-5β-cholane was readily formed at 0°C in pyridine. The crystalline methanesulphonate was stable at room temperature but decomposed on heating to 60°C in vacuo yielding a mixture of noncrystalline unsaturated hydrocarbons (tetranitromethane test). Gas liquid chromatography showed very little material corresponding in retention time to Δ^{11}-5β-cholene. The major components



XXX

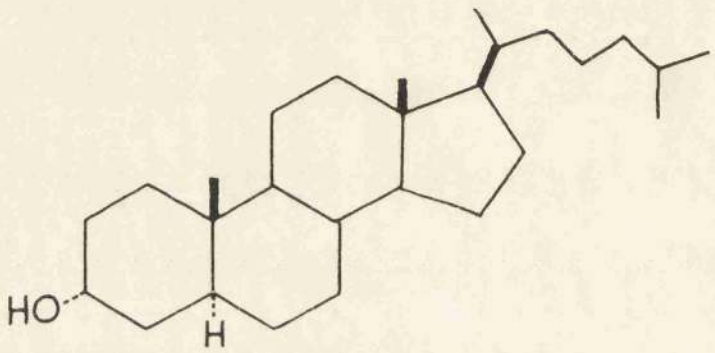
had lower retention times than Δ^{11}-5β-cholene. Presumably Δ^{11}-5β-cholene was formed initially and in the presence of the eliminated methanesulphonic acid underwent rearrangement. Indeed treatment of authentic Δ^{11}-5β-cholene with p-toluenesulphonic acid in benzene under refluxing conditions gave a mixture of five components as shown by gas liquid chromatography. Application of the same conditions for the formation of 7β-p-toluene-sulphonyloxy-5β-cholane to 12β-hydroxy-5β-cholane (XXX) (prepared by the reduction of 12-oxo-5β-cholane by lithium aluminium hydride (60-80%) or sodium/ethanol (50%)) gave incomplete sulphonate formation (infrared), indicating the greater reactivity of the hydroxyl function in the 7β-position than in the 12β-position. Lithium aluminium hydride reduction of this partially esterified product yielded a mixture of 5β-cholane and 12β-hydroxy-5β-cholane in the ratio of 2:1. Methanesulphonyl chloride in pyridine at 0°C readily converted 12β-hydroxy-5β-cholane into the crystalline 12β-methanesulphonyloxy-5β-cholane. This compound proved to be very unstable although it could be stored as a solution in nonpolar solvents for comparatively long periods without any signs of decomposition. A solvent-free sample decomposed on keeping at room temperature for 48-72 hours or on simple evacuation on the water pump for 30-40 minutes, yielding a colourless unsaturated noncrystalline hydrocarbon mixture (infrared and tetranitromethane test). Gas liquid chromatography showed very little material corresponding in retention time to Δ^{11}-5β-cholene. The main component had a lower retention time than Δ^{11}-5β-cholene. Boiling the 12β-methanesulphonate in ethanol afforded a mixture of products

which could be divided into petroleum ether-soluble and petroleum ether-insoluble (water soluble) fractions. The petroleum ether-soluble material was a colourless gum which showed a complex pattern in the infrared spectrum with absorption peaks indicating a hydroxyl group (3500 cm^{-1}), a carbonyl peak (1700 cm^{-1}) and a double bond (1650 cm^{-1}). Chromatography over alumina yielded an oily unsaturated hydrocarbon (infrared and tetranitromethane test). Gas liquid chromatography showed the presence of three components one of which was identified as Δ^{11}-5β-cholene.

The presence of both hydroxyl and carbonyl absorption was not due to the presence of 12β-hydroxy-5β-cholane in admixture with 12-oxo-5β-cholane, as these two compounds would be separable on chromatography since for 5β-cholanes the order of elution is hydrocarbon, 12-oxo compound, 12α-hydroxy compound and finally 12β-hydroxy compound (See pages 68,99).

Lithium aluminium hydride reduction of 12α-methanesulphonyloxy-5β-cholane and 12α-p-toluenesulphonyloxy-5β-cholane (partially esterified) regenerated 12α-hydroxy-5β-cholane in virtually quantitative yield showing O-S cleavage, whilst the reduction of freshly prepared 12β-methanesulphonyloxy-5β-cholane and 12β-p-toluenesulphonyloxy-5β-cholane (discussed earlier), like the equatorial sulphonate esters at C-3 and C-7, gave 5β-cholane, showing exclusive C-O cleavage.

7α,12α-Dihydroxy-5β-cholane (prepared by lithium aluminium hydride reduction of 3α,24-dimethanesulphonyloxy-7α,12α-dihydroxy-5β-cholane), on treatment with one mole of methanesulphonyl chloride in pyridine at



XXXI

0°C, gave a noncrystalline solid whose infrared spectrum showed the characteristic absorption of a methanesulphonate group (875-930 cm^{-1}, 1160 cm^{-1} and 1320 cm^{-1}) and also a sharp peak at 3570 cm^{-1} similar to that observed in the spectrum of 12α-hydroxy-5β-cholane. However, reduction with lithium hydride quantitatively regenerated 7α,12α-dihydroxy-5β-cholane, again showing O-S cleavage.

12α-Hydroxy-5α-cholane also failed to yield any p-toluenesulphonate ester on treatment with p-toluenesulphonyl chloride in pyridine at 37-40°C for 36 hours. However, it readily formed the crystalline 12α-methanesulphonyloxy-5α-cholane in pyridine at 0°C. Like the axial sulphonate esters at C-7 and C-12 in the 5β-series, this ester regenerated the alcohol on reduction with lithium aluminium hydride in quantitative yield.

In order to study the lithium aluminium hydride reduction of the axial sulphonate group at C-3 of steroids, 3α-methanesulphonyloxy-cholestane was prepared by treatment of epicholestanol (XXXI) with methanesulphonyl chloride in pyridine at 0°C. The crystalline methanesulphonate showed complete esterification (infrared). Lithium aluminium hydride reduction afforded a mixture of epicholestanol (66%) and cholestane (33%) easily separable on grade I neutral alumina. Epicholestanol and cholestane were characterised by melting point and by comparison of their infrared spectra with those reported in the literature[139]. This dual O-S and C-O cleavage has an analogy in the report that steroidal 6β(axial)-p-toluenesulphonates on lithium aluminium hydride reduction afford a

mixture of the 6β-hydroxy compound and the 6-methylene compound, with the former predominating[140].

EXPERIMENTAL

All m.p.'s were recorded on a Kofler block and are uncorrected. All $[\alpha]_D$'s are in $CHCl_3$ unless otherwise stated.

Light petroleum refers to petroleum ether of b.p. 40-60°C. Pyridine was dried over NaOH and distilled before use. Commercial anhydrous ether was dried over sodium before use. Tetrahydrofuran was distilled and left over NaOH being redistilled just prior to use. Acetic acid refers to glacial AnalaR acetic acid. Para-toluenesulphonyl chloride was recrystallised from light petroleum, taken up twice in benzene and the benzene evaporated under reduced pressure. Alumina is Brockmann unless otherwise stated.

Gas liquid chromatography was performed using 0.5% apiezon ApL on celite at ca 200°C on a Pye Argon instrument unless otherwise stated.

The following describe typical reaction conditions for given preparations although in many cases several runs were performed.

3α,12α,24-Trihydroxy-5β-Cholane (XXIII), was prepared in 92% yield by lithium aluminium hydride reduction in refluxing tetrahydrofuran of deoxycholic acid[124] or its methyl ester m.p. 120-127° (reported 107-114°[101], 106-118°[124b], 123°[124a], 165°, 185°[125]), $[\alpha]_D$ + 43° (c = 2.0) (reported[101] + 59° (ethanol)).

Formation of the Mixture of 12α-Hydroxy-5β-Cholane, 3α,12α-Dihydroxy-5β-Cholane and 12α,24-Dihydroxy-5β-Cholane from 3α,12α,24-Trihydroxy-5β-Cholane.

(a) To an ice cold solution of 3α,12α,24-trihydroxy-5β-cholane (2.0 g.) in pyridine (5 ml.) was added, dropwise, methanesulphonyl chloride (0.62 ml. 1.5 equiv.), and the reaction mixture left at 0°C for 30 min. At the end of this time ice was added, the mixture acidified with ice-cold 6N HCl and extracted with ether. The ether layer was washed (water) and dried (anhydrous sodium sulphate). The crude gum resulting on removal of the solvent was taken up in ether (100 ml.) and refluxed with a slurry of lithium aluminium hydride (4 g.) in ether (100 ml.) for 15 hr. After destroying the excess of reagent with moist ether and acidifying with HCl, the ether layer was taken to dryness under reduced pressure to yield a crude mixture of hydroxy-5β-cholanes (ca 1.6 g.). This material was incorporated in alumina (5 g.) by permitting a concentrated ethereal solution to evaporate over alumina powder, and the resulting dry mixture placed over a column of alumina (grade V 30 g.). Light petroleum eluted 12α-hydroxy-5β-cholane (115 mg. 8%) m.p. 104-106°C (reported[101] 100.9-103.3°), $[\alpha]_D^{19}$ + 40° (c = 2.0) (reported[101] + 41.0° (Found: C, 83.54; H, 12.33, calculated for $C_{24}H_{42}O$: C, 83.21; H, 12.24%). Ether/light petroleum (2:3) eluted material which on further investigation, proved to be a mixture of the 3α,12α-diol and the 12α,24-diol (0.8 g. 52%), the mixture showed a double m.p. 110: 170-172° (reported[101] 171.2-172.2° and 109-120°: 168-169.5°). Found: C, 79.59;

H, 11.57, calculated for $C_{24}H_{42}O_2$: C, 79.50; H, 11.68%). Finally, elution with 5% ethanol in ether afforded unchanged 3α,12α,24-trihydroxy-5β-cholane (0.62 g. 40%) m.p. and mixed m.p. 120-128°C.

Oxidation of the mixture of 3α,12α-dihydroxy-5β-cholane and 12α,24-dihydroxy-5β-cholane: The diol mixture (0.75 g.) was dissolved in acetic acid (20 ml.) and, with efficient cooling, treated with a solution of chromic oxide (0.6 g.) in acetic acid (15 ml.) containing water (2 ml.). After 16 hr. at room temperature, the mixture was treated with methanol to decompose the excess of chromic oxide. The oxidation product was precipitated by diluting the reaction mixture with water, extracted with ether and the ether layer washed (water). The ethereal layer was then extracted with 1.5N sodium hydroxide solution, washed (water) and dried (anhydrous sodium sulphate). On removing the solvent white plates of 3,12-dioxo-5β-cholane (0.44 g. 60%) resulted. Recrystallisation from ether gave m.p. 145-47°C, $[\alpha]_D^{24}$ + 103.6° (c = 1.9), (Found: C, 80.27; H, 10.50, $C_{24}H_{38}O_2$ requires C, 80.44; H, 10.61%). The aqueous alkaline washings on acidification with dilute hydrochloric acid, afforded a white semicrystalline precipitate of 12-oxo-5β-cholanic acid (0.30 g. 40%), m.p. 185-189°C (reported[141] 187°) $[\alpha]_D$ + 100° (c = 1.9).

(b) In a separate experiment p-toluenesulphonyl chloride was used in place of methanesulphonyl chloride following the conditions of Blickenstaff and Chang[101]. Results identical to the experiment using methanesulphonyl chloride were obtained. That the 3α,12α-dihydroxy-5β-cholane and the 12α,24-dihydroxy-5β-cholane were formed in the same

proportion as before was shown by chromic oxide oxidation of the diol fraction resulting after chromatography over alumina (gr. V), when 3,12-dioxo-5β-cholane and 12-oxo-cholanic acid were obtained in the ratio 3:2.

3α,12α-Dihydroxy-5β-Cholane (XXXII). 3,12-Dioxo-5β-cholane (50 mg.) was added to prereduced platinum oxide (25 mg.) in acetic acid (7 ml.) and the mixture was shaken for 6 hr. under hydrogen at atmospheric pressure. The catalyst was removed by filtration and the solid residue remaining after removal of the solvent (the infrared spectrum showed strong hydroxyl absorption at ca 3500 cm^{-1} and no carbonyl absorption), was chromatographed over alumina (gr. I Woelm, neutral, 7 g.). Ethanol/ether (1:99) eluted 3α,12α-dihydroxy-5β-cholane (40 mg.), which was crystallised with a minimal quantity of ethyl acetate. m.p. 174-175°C, $[\alpha]_D^{20}$ + 34.2° (c = 0.85). Gas liquid chromatography employing 1% SE-30 column at 225°C indicated only one component with the expected retention time of a C_{24} diol to be present.

12α,24-Dihydroxy-5β-Cholane: (a) Methyl-deoxycholate[142] (1 g.) on treatment with methanesulphonyl chloride (1.05 mole) in pyridine (3 ml.) at 0°C for 16 hr., yielded methyl 3α-methanesulphonyloxy-12α-hydroxy-cholanate (1.05 g.) after the normal isolation procedure. The product did not crystallise and was subjected to reduction with excess of lithium aluminium hydride in refluxing ether for 16 hr. The crude reduction product (m.p. 117-120°C) was chromatographed over alumina (gr. I, 25g.).

Benzene (250 ml.) eluted 12α,24-dihydroxy-5β-cholane which was recrystallised from light petroleum (850 mg.), m.p. 118-120°C, $[\alpha]_D^{19}$ + 39.5° (c = 2.1). (Found, C, 79.93; H, 11.48, $C_{24}H_{42}O_2$ requires C, 79.56; H, 11.60%).

(b) Methyl-3α-p-toluenesulphonyloxy-12α-hydroxy-cholanate (5 g.) (prepared according to the reported[143] method in ca 60% yield) was reduced with an excess of lithium aluminium hydride in refluxing ether for 16 hr. to yield crude 12α,24-dihydroxy-5β-cholane (3.2 g.) which on purification by chromatography over alumina (gr. I) gave 3 g. of pure product m.p. 118-120°C.

3α,7α,24-Trihydroxy-5β-Cholane*: Chenodeoxycholic acid was prepared by Wolff Kishner reduction of methyl 3α,7α-diacetoxy-12-oxo-cholanate[144] in 60% yield, m.p. 140-144°C (reported[144] 140-142°). This compound (4.7 g.) was then refluxed with lithium aluminium hydride (3 g.) in ether (150 ml.) for 12 hr. Working up of the reaction mixture in the normal way gave a noncrystalline solid (4.6 g.) which was chromatographed over alumina (gr. V 60 g.). Chloroform (1,000 ml.) eluted 3α,7α,24-trihydroxy-5β-cholane as a gum which on seeding became crystalline (4.4 g.) double m.p. 82-84°: 150°C, $[\alpha]_D^{24}$ + 14.5° (c = 2.1). (Found: C, 76.10; H,10.36, $C_{24}H_{42}O_3$ requires C, 76.19; H, 11.11%).

* This compound has been reported[125] as a noncrystalline solid obtained by the reduction of chenodeoxycholic acid with sodium in ethanol.

Formation of the Mixture of 7α-Hydroxy-5β-Cholane, 3α,7α-Dihydroxy-5β-Cholane and 7α,24-Dihydroxy-5β-Cholane from 3α,7α,24-Trihydroxy-5β-Cholane:

(a) 3α,7α,24-Trihydroxy-5β-cholane (300 mg.) dissolved in pyridine (2 ml.) was treated with methanesulphonyl chloride (1.5 mole) at 0°C for 20 min. Working up the reaction mixture in the usual way gave an ether soluble colourless gum (315 mg.). The crude methanesulphonates were reduced with an excess of lithium aluminium hydride in refluxing ether for 10 hr. and the mixture of hydroxycholanes obtained by the normal isolation procedure was chromatographed over alumina (gr. V 30 g.) to yield three fractions: light petroleum eluted 7α-hydroxy-5β-cholane (30 mg. 10%). After recrystallisation from methanol had m.p. 86-88°C $[\alpha]_D^{20}$ + 19° (c = 2.1). (Found: C, 83.56; H, 12.36, $C_{24}H_{42}O$ requires C, 83.21; H, 12.24%). Ethanol/ether(1:20) eluted what proved to be a mixture of 3α,7α-dihydroxy-5β-cholane and 7α,24-dihydroxy-5β-cholane (163 mg. 54%), recrystallised from ethylacetate, needles m.p. 89-94°C. Ethanol/ether (1:4) eluted unchanged 3α,7α,24-trihydroxy-5β-cholane (90 mg. 30%) double m.p. 80-82°: 148-150°C.

Oxidation of the diol mixture with chromic oxide in acetic acid gave, after washing the ethereal solution of the product with 2N sodium hydroxide (aqueous), a ketone (carbonyl absorption in the infrared spectrum at 1700 cm^{-1}) (92 mg. 61%) m.p. 92-94°. The alkaline washings on treatment with 2N HCl gave 7-oxo-cholanic acid (absorption at 1700 cm^{-1} for carbonyl group, at 1730 cm^{-1} for carboxylic C = O and between 2900-3300 cm^{-1} for carboxylic OH stretching): (55 mg. 36%),

m.p. 146-150°C (reported[145] 149-150°).

(b) An identical mixture of products resulted from 3α,7α,24-trihydroxy-5β-cholane when p-toluenesulphonyl chloride was substituted for methanesulphonyl chloride in the above experiment and the reaction carried out at room temperature for 30 min.

3α,7α,12α,24-Tetrahydroxy-5β-Cholane, was prepared by lithium aluminium hydride reduction of cholic acid (in tetrahydrofuran) or its methyl ester (in ether) in 80-85% yield, m.p. 234-235°C, (reported[124,125] 231-233) $[\alpha]_D^{19}$ + 39° (c = 2, ethanol) (reported[124] + 37.9°).

7α,12α-Dihydroxy-5β-Cholane (XXIV): (a) A solution of 3α,7α,12α,24-tetrahydroxy-5β-cholane (1.5 g.) in pyridine (7 ml.) was treated with methanesulphonyl chloride (2.1 mole) at 0°C for 16 hr. On working up the reaction mixture a noncrystalline solid (1.7 g.) was obtained, which on reduction with an excess of lithium aluminium hydride in refluxing ether (12 hr.) and concentrating the ethereal filtrate, obtained after destruction of the unreacted hydride, gave 7α,12α-dihydroxy-5β-cholane, needles (1.3 g.) m.p. 203-204°C, $[\alpha]_D^{24}$ + 25° (c = 2.0). (Found: C, 79.56; H, 11.36. $C_{24}H_{42}O_2$ requires C, 79.50; H, 11.69%).

(b) The tetra-ol on treatment with p-toluenesulphonyl chloride (2.2 mole) in pyridine at room temperature (16 hr.) gave the gummy 3α,24-di-p-toluenesulphonyloxy-5β-cholane in 50% yield. Lithium aluminium hydride reduction of the crude di-p-toluenesulphonate (3 g.), under the usual conditions, gave pure 7α,12α-dihydroxy-5β-cholane (1.35 g.).

(c) Methyl-3α-p-toluenesulphonyloxy-7α,12α-dihydroxy-cholanate[143] (5 g.) on reduction with excess of lithium aluminium hydride in refluxing ether (200 ml.) gave crude triol (3.1 g.). Chromatography over alumina (gr. I, 60 g.) using ethyl acetate/benzene (7:3) as eluant gave pure 7α,12α,24-trihydroxy-5β-cholane (2.9 g.) m.p. 198-200°C, $[\alpha]_D^{22}$ + 26.7° (c = 2.0). (Found: C, 76.54; H, 10.86, $C_{24}H_{42}O_3$ requires C, 76.2; H, 11.11%).

7α,12α,24-Trihydroxy-5β-cholane (0.9 g.) on treatment with p-toluenesulphonyl chloride (1.2 mole) in pyridine (4 ml.) at room temperature (16 hr.) gave 7α,12α-dihydroxy-24-p-toluenesulphonyloxy-5β-cholane, as needles from ether (1 g.) m.p. 126-128°C, $[\alpha]_D^{22}$ + 15° (c = 1.0). (Found: C, 69.97; H, 9.01; $C_{31}H_{47}O_5S$ requires C, 69.92; H, 9.02%). This p-toluene sulphonate (0.5 g.) on reduction with excess of lithium aluminium hydride in refluxing ether gave 7α,12α-dihydroxy-5β-cholane (0.30 g.) m.p. 199-200°C.

(d) 7α,12α-Dihydroxy-5β-cholane was also prepared via lithium aluminium hydride reduction of the noncrystalline 7α,12α-dihydroxy-24-methanesulphonyloxy-5β-cholane - yield 85%.

12α-Hydroxy-5β-Cholane (XXV): (a) On treatment of a solution of 3α,12α,24-trihydroxy-5β-cholane (2.5 g.) in pyridine (10 ml.) with methanesulphonyl chloride (2.15 mole) at 0°C for 16 hr., the gummy

3α,24-dimethanesulphonyloxy-12α-hydroxy-5β-cholane (2.7 g.) was formed. Lithium aluminium hydride reduction of the crude dimethanesulphonate in refluxing ether (16 hr.) afforded 12α-hydroxy-5β-cholane (2.2 g.) m.p. 95-100°C. Chromatography over alumina (gr. III 30 g.) using light petroleum as eluant gave pure 12α-hydroxy-5β-cholane (2.1 g.) m.p. 104-106°C (reported[101] 100.8-103.3°).

(b) 3α,12α,24-Trihydroxy-5β-cholane (2.33 g.) on treatment with p-toluenesulphonyl chloride (2.2 mole) in pyridine (10 ml.) at room temperature (16 hr.) gave the noncrystalline 3α,24-di-p-toluenesulphonyloxy-12α-hydroxy-5β-cholane (2.56 g.). Reduction with an excess of lithium aluminium hydride in refluxing ether (12 hr.) yielded crude 12α-hydroxy-5β-cholane which after chromatographic purification over alumina (gr. III 30 g.) gave pure material (1.2 g.) of m.p. 103-106°C.

(c) 12α,24-Dihydroxy-5β-cholane was prepared by lithium aluminium hydride reduction of methyl-3α-p-toluenesulphonyloxy-12α-hydroxy-cholanate[143], m.p. 118-120°C, $[\alpha]_D^{19}$ + 39.5° (c = 2.1). (Found: C, 79.93; H, 11.48, $C_{24}H_{42}O_2$ requires C, 79.56; H, 11.60%). This diol (2 g.) on treatment with methanesulphonyl chloride (1.1 mole) in pyridine (6ml.) at 0°C for 15 hr. gave the gummy 12α-hydroxy-24-methanesulphonyloxy-5β-cholane (2.2 g.) which on reduction with lithium aluminium hydride in refluxing ether (16 hr.) afforded 12α-hydroxy-5β-cholane (1.85 g.) m.p. 100-105°C.

7α-Hydroxy-5β-Cholane (XXVI): (a) 3α,7α,24-trihydroxy-5β-cholane (1.0 g.) dissolved in pyridine (4 ml.) was treated with methanesulphonyl chloride (2.1 mole) at 0°C for 16 hr. to yield the noncrystalline 3α,24-dimethanesulphonyloxy-7α-hydroxy-5β-cholane (1.1 g.). Lithium aluminium hydride reduction of the dimethanesulphonate in refluxing ether (16 hr.) gave a gum (800 mg.) which after chromatography over alumina (gr. III 20 g.) using light petroleum as eluant gave crystalline 7α-hydroxy-5β-cholane (785 mg.) m.p. 86-88°C, $[\alpha]_D^{20}$ + 19° (c = 2.1). (Found: C, 83.64; H, 11.89, $C_{24}H_{42}O$ requires C, 83.21; H, 12.24%).

(b) 3α,7α,24-Trihydroxy-5β-cholane (1.46 g.) on treatment with p-toluenesulphonyl chloride (2.2 moles) in pyridine (7 ml.) at room temperature (16 hr.) gave 3α,24-di-p-toluenesulphonyloxy-7α-hydroxy-5β-cholane as a gum. Reduction with lithium aluminium hydride employing the usual conditions and subsequent purification of the product on an alumina column (gr. III 30 g.) gave 7α-hydroxy-5β-cholane (850 mg.) m.p. 81-86°C.

(c) 7α-Hydroxy-cholanic acid (7.0 g.) (obtained by Wolff Kishner reduction of methyl 3,12,-dioxo-7α-acetoxy-cholanate[144a]) was refluxed with lithium aluminium hydride (3 g.) in ether (120 ml.) for 16 hr. to give 7α,24-dihydroxy-5β-cholane (6.4 g.). Purification on a column of alumina (gr. V 60 g.) using ether as eluant gave pure 7α,24-dihydroxy-5β-cholane (5.6 g.) m.p. 86-90, $[\alpha]_D^{24}$ + 7.5° (c = 2.0). (Found: C, 79.80; H, 12.0, $C_{24}H_{42}O_2$ requires C, 79.56; H, 11.68%).

7α,24-Dihydroxy-5β-cholane (4.4 g.) on treatment with methane-

sulphonyl chloride (1.1 mole) in pyridine (15 ml.) at 0°C for 16 hr. gave the gummy 7α-hydroxy-24-methanesulphonyloxy-5β-cholane (4.4 g.) which on reduction with lithium aluminium hydride in refluxing ether followed by chromatography on alumina in the usual way gave pure 7α-hydroxy-5β-cholane (3.8 g.) m.p. 80-85°C.

Attempted Selective Hydrolysis of 3α,24-di-Para-toluenesulphonyloxy-12α-Hydroxy-5β-Cholane to 3α,12α-Dihydroxy-24-Para-toluenesulphonyloxy-5β-Cholane. The method employed for the selective hydrolysis of the 3β-p-toluenesulphonate group in 5-androsten-3β,17β-di-p-toluenesulphonyloxy-16-one[146] when applied to 3α,24-di-p-toluenesulphonyloxy-12α-hydroxy-5β-cholane, failed to give selective hydrolysis. The crude hydrolysis product was reduced with lithium aluminium hydride in refluxing ether for 16 hr. The resulting material (0.5 g.) was then chromatographed over alumina (gr. I Woelm, neutral, 30 g.). Ether/light petroleum (1:9) and pure ether fractions eluted 12α-hydroxy-5β-cholane (180 mg. m.p. 104-106°C). Ethanol/ether (1:49) eluted 231 mg. of a material which on oxidation with chromic oxide in acetic acid at room temperature yielded a mixture of a neutral ketone and a keto acid (infrared spectrum) which were not characterised further.

Methyl 3,12-Dioxochol-4-en-24-Carboxylate (XXVII): A solution of methyl-3,12-dioxo-cholanate (prepared by chromic oxide oxidation of methyl deoxycholate[147] in acetic acid at room temperature for 16 hr.) (18.3 g.) in acetic acid (200 ml.) containing a drop of 48% aqueous

hydrobromic acid was titrated with bromine (8.2 g.: 1 mole) dissolved in acetic acid (120 ml.). After the addition of the bromine solution was complete, the mixture was stirred for 15 min. and diluted to large volume with water to yield a solid (22.0 g.). Recrystallisation from ether gave needles of methyl-4-bromo-3,12-dioxochol-24-carboxylate (20 g.) m.p. 156-160°C $[\alpha]_D$ + 114.6° (c = 1.3).

The crude crystalline 4-bromo compound on taking up in ether left behind a crystalline material (rods), sparingly soluble in ether (ca 2%) m.p. 169°C. This product would appear to be a 2,4-dibromo compound - cf. refs. 134, 148.

The methyl-4-bromo-3,12-dioxochol-24-carboxylate was highly soluble in ether and there was not any improvement in melting point after repeated crystallisations from ethanol. (Found: C, 62.36; H, 7.68, $C_{25}H_{38}O_4$ Br requires C, 62.24; H, 7.88%).

The methyl-4-bromo-3,12-dioxochol-24-carboxylate (1.95 g.) was dissolved in freshly distilled collidine* (60 ml.) and the solution refluxed under nitrogen for 35 min. After cooling under nitrogen the crystalline collidine hydrobromide was removed by filtration. The dark coloured filtrate was distilled in vacuo and the residue was chromatographed over alumina (gr. III, 100 g.) using ethyl acetate as solvent to yield methyl-3,12-dioxochol-4-en-24-carboxylate (12 g.) m.p. 130°C $[\alpha]_D$ + 136.6° (c = 2.1). U.V. spectrum in ethanol λ max = 236 mμ

* A parallel experiment using pyridine (2 hr.) gave a lower yield of dehydrobrominated product.

(ϵ = 13110). (Found: C, 74.27; H, 8.67: $C_{25}H_{36}O_4$ requires C, 75.00; H, 9.00%).

Mixture of 3β,12α,24-Trihydroxy-5α-Cholane and 3β,12β,24-Trihydroxy-5α-Cholane: Methyl-3,12-dioxochol-4-en-24-carboxylate (2 g.) was dissolved in a mixture of ethanol and dioxane (20 ml. and 10 ml.) and diluted with ether (200 ml.). Liquid ammonia (50-60 mls.) was mixed with the ethereal solution in a flask fitted with an efficient mechanical stirrer. While the reaction mixture was continuously stirred, small slices of lithium metal (total ca 5 g.) were slowly added during a period of 3 hr., replenishing the ammonia as necessary. Excess of lithium was decomposed with ammonium chloride and the ammonia was allowed to distill at room temperature overnight. The residual mixture was treated with 4N HCl and the ethereal layer separated, washed (water), dried (anhydrous sodium sulphate) and distilled in vacuo to yield a white solid (1.4 g.) which readily crystallised on trituration with ether containing traces of ethanol m.p. 175-185°C. (Infrared analysis indicated the complete reduction of the keto and ester groups and showed no double bond absorption). (Found: C, 76.40; H, 10.69, $C_{24}H_{42}O_3$ requires C, 76.19; H, 11.11%).

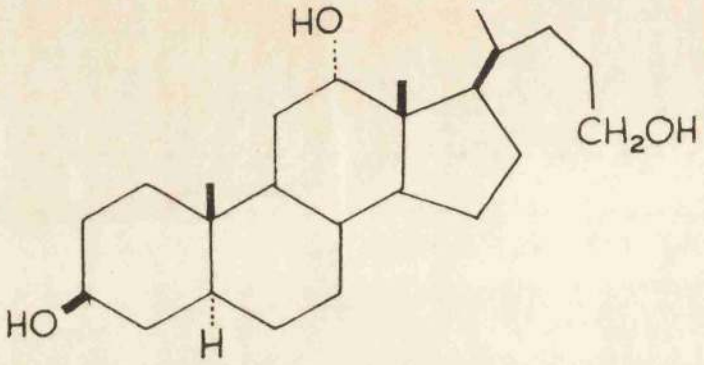
The product which appeared homogeneous on chromatography over alumina was concluded to be a mixture of epimeric 12-hydroxy compounds by comparison with authentic 3β,12α,24-trihydroxy-5α-cholane (p. 62) and from the results of the experiments described immediately below.

Treatment of the Mixture of 3β,12α,24- and 3β,12β,24-Trihydroxy-5α-Cholanes with Methanesulphonyl Chloride and Subsequent Reduction with Lithium Aluminium Hydride: (a) The mixture of trihydroxy-5α-cholanes (700 mg.) dissolved in pyridine (8 ml.) was treated with methanesulphonyl chloride (2.1 mole) at -5°C for 12 hr. Working up the reaction mixture in the usual manner gave a cream coloured gummy mixture of methanesulphonates (725 mg.). Lithium aluminium hydride reduction of this crude material in refluxing ether/dioxane (9:1, 50 ml.) for 12 hr. gave 680 mg. of a gum. Treatment with light petroleum left a residue (30 mg.) which proved to be the starting triol mixture as shown by m.p. (175-186°C) and infrared spectrum. The light petroleum extracts (650 mg.) on chromatography over alumina (gr. III 40 g.) gave three fractions. Light petroleum (100 ml.) eluted a gummy hydrocarbon fraction (infrared spectrum) (145 mg.). Gas liquid chromatography on a column of 1% QF 1 on gas chrome P resolved this hydrocarbon fraction into six components. The two main components representing 80% of the total material were identified as 5β-cholane (comparison with authentic sample) and 5α-cholane (same difference in retention time as found for the pairs of 12-oxo- and 12α-hydroxy-5α and 5β-cholanes in separate experiments). The four minor components were not identified but were concluded to be cholenes or jervicholenes. Elution with 10% and 20% ether in light petroleum (200 ml. and 100 ml.) gave 12α-hydroxy-5α-cholane (XXVIII) (300 mg.), m.p. 125-127°C, $[\alpha]_D^{20}$ + 40° (c = 2.0). (Found: C, 83.08; H, 11.54 $C_{24}H_{42}O$ requires C, 83.21; H, 12.24%). This product was characterised

by comparison with authentic 12α-hydroxy-5α-cholane, the preparation of which is described later. Pure ethyl acetate eluted a material, which on further investigation proved to be a mixture of 3,12 and 12,24-dihydroxy-5α-cholanes (130 mg.).

This diol mixture (60 mg.) on oxidation with chromic oxide in acetic acid afforded a crystalline solid (50 mg.) which was separated into neutral and acidic components by extracting the ethereal solution of the oxidation product with dilute aqueous sodium hydroxide. The neutral product was identified as 3,12-dioxo-5α-cholane (25 mg.), m.p. 178-180°C, $[\alpha]_D^{24}$ + 107.4° (c = 1.9). (Found: C, 80.25; H, 10.47 $C_{24}H_{38}O_2$ requires C, 80.44; H, 10.61%). The acidic product was concluded to be 12-oxo-5α-cholanic acid (25 mg.) m.p. 183-186, $[\alpha]_D^{20}$ + 108° (c = 2.0). (Found: C, 76.79; H, 9.05, $C_{24}H_{38}O_3$ requires C, 77.00; H, 10.01%).

(b) Treatment of the mixture of 3β,12α,24- and 3β,12β,24-trihydroxy-5α-cholanes (700 mg.) with p-toluenesulphonyl chloride (2.1 mole) in pyridine at room temperature for 16 hr. followed by the reduction of the crude product (750 mg.) with lithium aluminium hydride gave, after chromatography over alumina (gr. III, 40 g.), analogous products to those obtained in the methanesulphonate case. These were (i) mixture of hydrocarbons (130 mg.), (ii) 12α-hydroxy-5α-cholane (245 mg.) and (iii) mixture of 3,12- and 12,24-dihydroxy-5α-cholanes (105 mg.).



XXXIII

3β,12α,24-Trihydroxy-5α-Cholane (XXXIII): Methyl-12α-acetoxy-3-oxochol-4-en-24-carboxylate (prepared by the procedure of Reichstein et al[149] m.p. 133-134°C (reported[149] 132-134°), $[\alpha]_D^{20}$ + 112° (c = 1.0 acetone) (reported[149] + 114.2°) (10.0 g.) was subjected to reduction with excess of lithium in liquid ammonia in presence of ethanol as described for methyl 3,12-dioxochol-4-en-24-carboxylate (p. 57). The crude reduction product gave crystalline 3β,12α,24-trihydroxy-5α-cholane (4.23 g.) from ethyl acetate, m.p. 186-188°C, $[\alpha]_D^{22}$ + 33.8° (c = 2.1). (Found: C, 76.25; H, 10.94, $C_{24}H_{42}O_3$ requires C, 76.19; H, 11.11%).

The mother liquor on chromatography over alumina (gr. III, 40 g.) using ethyl acetate as eluant gave a further 1.52 g. of the product.

12α-Hydroxy-5α-Cholane (XXVIII): (a) 3β,12α,24-Trihydroxy-5α-cholane (1.64 g.) was treated with methanesulphonyl chloride (2.05 mole) in pyridine (8 ml.) for 12 hr. The noncrystalline 3β,24-dimethanesulphonyloxy-12α-hydroxy-5α-cholane, on reduction with an excess of lithium aluminium hydride in refluxing ether gave the crude 12α-hydroxy-5α-cholane (1.3 g.). Chromatographic purification over alumina (gr. III, 30 g.) afforded pure product (1.2 g.), eluted with ether in light petroleum (1:9 and 1:4), m.p. 126-127°C, $[\alpha]_D^{20}$ + 40° (c = 2.0). (Found: C, 83.1; H, 11.8, $C_{24}H_{42}O$ requires C, 83.21; H, 12.24%).

(b) 3β,12α,24-Trihydroxy-5α-cholane (1.2 g.) was converted into 3β-24-di-p-toluenesulphonyloxy-12α-hydroxy-5α-cholane by treatment with p-toluenesulphonyl chloride (2.1 mole) in the usual manner to yield

the non-crystalline di-p-toluenesulphonate (1.35 g.). Lithium aluminium hydride reduction in refluxing ether followed by chromatographic purification over alumina (grade III 30 g.) gave 12α-hydroxy-5α-cholane (0.6 g.), m.p. 125-127°C.

Attempted formation of 7α-Para-Toluenesulphonyloxy-5β-Cholane: A solution of 7α-hydroxy-5β-cholane (100 mg.) in pyridine (3 ml.) was treated with p-toluenesulphonyl chloride (1.2 moles) dissolved in pyridine (1 ml.) and the mixture incubated at 37°-40° for 16 hr. On working up the reaction mixture in the usual way 7α-hydroxy-5β-cholane (95 mg.) was obtained, m.p. and mixed m.p. 84-88° (infrared spectrum identical with that of 7α-hydroxy-5β-cholane).

7α-Methanesulphonyloxy-5β-Cholane: 7α-Hydroxy-5β-cholane (100 mg.) was dissolved in pyridine (2 ml.) and treated with methanesulphonyl chloride (1.2 mole) at 0°C for 10 hr. On working up the reaction mixture in the usual way the gummy 7α-methanesulphonyloxy-5β-cholane (105 mg.) was obtained (infrared spectrum indicated complete esterification).

The non-crystalline 7α-methanesulphonate on taking up in methanol (5 ml.) deposited white crystalline material (80 mg.) mixture of plates and rods, m.p. plates 73-76°C and rods 90-94°C. (Found: C, 87.24; H, 12.19. $C_{24}H_{40}$ requires C, 87.80; H, 12.19%).

This material did not show the presence of any functional groups in the infrared spectrum and gave a positive test with tetranitromethane. Gas liquid chromatography indicated two components to be present in equal

quantities. These had retention times very close to each other as well as to that of other C_{24} steroidal hydrocarbons (vide infra).

A freshly prepared specimen of 7α-methanesulphonyloxy-5β-cholane (104 mg.), on reduction with lithium aluminium hydride in refluxing ether gave pure 7α-hydroxy-5β-cholane (92 mg.), m.p. 80-87°C. The infrared spectrum was identical with that of an authentic specimen.

7-Oxo-5β-Cholane: 7α-Hydroxy-5β-cholane (360 mg.) dissolved in acetic acid (5 ml.) was treated with a solution of potassium dichromate (300 mg.) dissolved in water (0.4 ml.). The mixture was allowed to stand for 16 hr. On slow dilution with water crystalline 7-oxo-5β-cholane was precipitated. This was filtered, repeatedly washed with water and dried giving 364 mg. of m.p. 104-109°C. Recrystallisation from methanol gave plates m.p. 108-109°C, $[\alpha]_D^{20}$ - 50° (c = 2.0). The infrared spectrum showed strong carbonyl absorption (1700 cm^{-1}) and no hydroxyl absorption. The constitution follows from its conversion into the epimeric 7-hydroxy-5β-cholanes (see below).

Oxidation of 7α-hydroxy-5β-cholane with chromic oxide in acetic acid in the usual way also gave the same 7-oxo-5β-cholane, in slightly lower yield.

7β-Hydroxy-5β-Cholane (XXIX): 7-Oxo-5β-cholane (200 mg.) dissolved in boiling ethanol (10 ml.) was treated with sliced sodium metal (800 mg.) during a period of 1.5 hr. and allowed to cool. Dilution of the reaction mixture with water gave an oily ether soluble material (193 mg.).

Chromatography over alumina (gr. III, 15 g.) using light petroleum as solvent (15 ml.) gave 7α-hydroxy-5β-cholane (20 mg.) m.p. 80-86°C. Elution with ether/light petroleum (1:1) gave pure 7β-hydroxy-5β-cholane (170 mg.) as a gum which resisted all attempts at crystallisation, $[\alpha]_D^{20}$ + 42.1° (c = 1.9). The material showed strong hydrogen bonded hydroxyl absorption in the infra red at 3500 cm^{-1} (cf. sharp band in the 7α-hydroxy epimer at 3600 cm^{-1}) in nujol.

7β-Methanesulphonyloxy-5β-Cholane: 7β-Hydroxy-5β-cholane (100 mg.) dissolved in pyridine (2.5 ml.) was treated with methanesulphonyl chloride (1.2 mole) at 0°C for 12 hr. Working up the reaction mixture gave the non-crystalline 7β-methanesulphonyloxy-5β-cholane (106 mg.) (infrared spectrum showed complete conversion of the 7β-hydroxyl group into the methanesulphonate group). The material did not decompose on treatment with methanol.

Reduction of the 7β-methanesulphonate ester with lithium aluminium hydride in refluxing ether (16 hr.) and subsequent purification by chromatography over alumina (gr. III, 10 g.) gave 5β-cholane (80 mg.) m.p. and mixed m.p. 85-87°C. The infrared spectrum was identical with that of authentic 5β-cholane*. Gas liquid chromatography further confirmed the identity.

* 5β-cholane was prepared by Wolff-Kishner reduction of 12-keto-5β-cholane, following the method of Huang Minlon[150] for reduction of steroidal ketones, (yield 80%) m.p. 86-88°C (reported[151] 89-90°). Found: C, 86.63; H, 12.86; Calculated for $C_{24}H_{42}$: C, 87.27; H, 12.73%).

7β-Para-toluenesulphonyloxy-5β-Cholane: 7β-Hydroxy-5β-cholane (100 mg.) dissolved in pyridine (3 ml.) was treated with p-toluenesulphonyl chloride (1.2 mole) and the mixture was incubated at 37-40°C for 16 hr. Working up of the reaction mixture in the usual way gave the non-crystalline 7β-p-toluenesulphonyloxy-5β-cholane (108 mg.) (infrared spectrum showed complete esterification). Further proof of complete esterification was obtained as follows. The 7β-p-toluenesulphonate was treated with lithium aluminium hydride in refluxing ether for 16 hr. The crude reduction product on purification by chromatography over alumina (gr. I, 15 g.) gave 5β-cholane (70 mg.) m.p. and mixed m.p. 86-88°C. No 7β-hydroxy-5β-cholane was obtained.

Attempted Formation of 12α-Para-Toluenesulphonyloxy-5β-Cholane: 12α-Hydroxy-5β-cholane (100 g.) was treated with p-toluenesulphonyl chloride (1.2 mole) in pyridine (3 ml.). The mixture, after initial incubation at 60-65°C for 2.5 hr., was kept at 28-30°C for 16 hr. The solid reaction product showed only partial esterification (infrared spectrum showing both 12α-hydroxyl group and p-toluenesulphonate group bands). Reduction of this product with lithium aluminium hydride in refluxing ether afforded solely 12α-hydroxy-5β-cholane (65 mg.) m.p. 100-106°C.

12α-Methanesulphonyloxy-5β-Cholane: 12α-Hydroxy-5β-cholane (1 g.) was treated with methanesulphonyl chloride (1.1 mole) in pyridine at 0°C for 16 hr. On working up the reaction mixture a gum was obtained which

eventually crystallised after 18 hr. (1.2 g.). Recrystallisation from ethanol gave pure 12α-methanesulphonyloxy-5β-cholane (1.05 g.) m.p. 113-114°D, $[\alpha]_D^{19}$ + 40° (c = 2.0). (Found: C, 70.86; H, 10.43, $C_{25}H_{44}O_3S$ requires C, 70.80; H, 10.38%).

Pure 12α-methanesulphonyloxy-5β-cholane on heating at 60° in vacuo decomposed into a colourless gum leaving behind a dark water soluble material. The gum gave intense yellow colour with tetranitromethane and there were no functional peaks in the infrared spectrum. Gas liquid chromatography showed very little component corresponding to $\triangle^{11}$-5β-cholene, which increased in peak height on admixture with authentic $\triangle^{11}$-5β-cholene, (vide infra). The major component had a lower retention time than $\triangle^{11}$-5β-cholene. A pure sample of $\triangle^{11}$-5β-cholene when refluxed in benzene with p-toluenesulphonic acid for 1 hr. gave a non-crystalline product. Gas liquid chromatography of this product showed that a substantial amount of the $\triangle^{11}$-5β-cholene was rearranged to give four other components with lower retention times than that of $\triangle^{11}$-5β-cholene.

Reduction of 12α-methanesulphonyloxy-5β-cholane (100 mg.) with lithium aluminium hydride in refluxing ether gave 12α-hydroxy-5β-cholane (89 mg.).

12-Oxo-5β-Cholane: 12α-Hydroxy-5β-cholane (500 mg.) dissolved in acetic acid (3 ml.) was treated with a solution of chromic oxide (200 mg.) in acetic acid/water (97:3) mixture (5 ml.) at room temperature and allowed to stand for 14 hr. The crystalline 12-oxo-5β-cholane was removed by

filtration and the residue repeatedly washed with water and dried (380 mg.) m.p. 118-120°C (reported[152] 115-117°), $[\alpha]_D^{20}$ + 89° (c = 2.0) (reported[152] + 89.4°).

12β-Hydroxy-5β-Cholane (XXX): (a) 12-Oxo-5β-cholane (500 mg.) dissolved in ether (70 ml.) was slowly dropped into a refluxing slurry of lithium aluminium hydride (500 mg.) in ether (50 ml.). The reaction mixture was refluxed for 16 hr. and the excess of hydride decomposed with moist ether. The ethereal filtrate from the reaction mixture gave a gum (510 mg.) showing no carbonyl absorption in the infrared spectrum. The crude mixture of epimeric 12-hydroxy-5β-cholanes was resolved by chromatography over a column of alumina (grade III, 20 g.) using light petroleum. 12α-Hydroxy-5β-cholane (50 mg.) was quickly eluted with 20 mls. of solvent. After an intermediate fraction (10 mg.), light petroleum (400 ml.) eluted 12β-hydroxy-5β-cholane (386 mg.) m.p. 93-95°C (reported[152] 91-92°), $[\alpha]_D^{19}$ + 33.4° (c = 2.2) (reported[152] + 45°).

(b) Reduction of 12-oxo-5β-cholane (500 mg.) with sodium metal in boiling ethanol (as described for the reduction of 7-oxo-5β-cholane) gave a mixture of epimeric 12-hydroxy-5β-cholanes (340 mg.) which on chromatography over alumina (gr. III, 20 g.) using light petroleum as eluent gave 12α-hydroxy-5β-cholane (130 mg.) and 12β-hydroxy-5β-cholane (127 mg.).

12β-Methanesulphonyloxy-5β-cholane: 12β-Hydroxy-5β-cholane (350 mg.) was treated with methanesulphonyl chloride (1.1 mole) in pyridine (2 ml.)

at 0°C for 16 hr. The resultant gummy 12β-methanesulphonyloxy-5β-cholane (380 mg.) resisted all attempts at crystallisation, $[\alpha]_D^{20}$ + 39.1° (c = 1.84). This product was stable if stored as a solution in non-hydroxylic solvents for indefinite periods (infrared spectrum).

A solvent-free specimen decomposed on standing at room temperature for 48-72 hr., while another fresh specimen decomposed when evacuated on a water-pump at room temperature for 30-40 min. Both these decomposition products could be separated into a light petroleum-soluble colourless gum and a water soluble dark paste. The light petroleum-soluble gum gave an intense yellow colour on treatment with tetranitromethane but did not show any absorption for a double bond in the infrared spectrum (tetra-substituted?) nor for any other functional groups. Gas liquid chromatography showed very little material corresponding to Δ^{11}-5β-cholene. The main component had a lower retention time than that of Δ^{11}-5β-cholene.

Attempted crystallisation of a fresh specimen of the 12β-methane-sulphonate from hot ethanol also caused decomposition of the product into a dark gum. Extraction with light petroleum gave a colourless gum leaving behind a dark tarry matter (water soluble). The petroleum-soluble gum showed a complex pattern in its infrared spectrum with absorption peaks indicating a free hydroxyl group (3500 cm^{-1}), a keto peak (1700 cm^{-1}) and a peak at 1650 cm^{-1} (double bond?). Chromatography over alumina (gr. I, 10 g.) eluted an oily unsaturated hydrocarbon which gave an intense yellow colour with tetranitromethane and showed no other functional groups in the infrared spectrum. Gas liquid chromatography showed the presence of three main

components, one of which proved to be Δ^{11}-5β-cholene.

Reduction of freshly prepared 12β-methanesulphonate (100 mg.) with lithium aluminium hydride in refluxing ether gave a colourless gum, which on chromatography over alumina (gr. I Woelm, neutral 10 g.) using light petroleum as solvent gave 5β-cholane (70 mg.), m.p. and mixed m.p. 85-87°C. No 12β-hydroxy-5β-cholane was obtained.

12β-Para-Toluenesulphonyloxy-5β-Cholane: 12β-Hydroxy-5β-cholane (100 mg.) was treated with p-toluenesulphonyl chloride (1.2 mole) in pyridine (2.5 ml.) and the mixture incubated at 37-40°C for 16 hr. The resultant product (solid 100 mg.) showed partial esterification (absorption bands for both the free hydroxyl group and p-toluenesulphonate group). Reduction with lithium aluminium hydride in refluxing ether (16 hr.) gave a gum (75 mg.). Chromatography over alumina (grade I, 10 g.) using light petroleum eluted 5β-cholane (41 mg.) m.p. and mixed m.p. 85-87°C. Ether/light petroleum (1:4) eluted unreacted 12β-hydroxy-5β-cholane (23 mg.) m.p. and mixed m.p. 102-106°C.

Attempted Formation of 7α-Methanesulphonyloxy-12α-Hydroxy-5β-Cholane: 7α,12α-Dihydroxy-5β-cholane (200 mg.) was treated with methanesulphonyl chloride (1 mole) in pyridine (4 ml.) at 0°C for 16 hr. The product (210 mg. solid) showed a sharp absorption in the infrared spectrum at 3400 cm^{-1} showing the presence of a free hydroxyl group (in the cholane series, axial diols do not give sharp peaks). Reduction of this sulphonate ester with lithium aluminium hydride in refluxing ether

regenerated 7α,12α-dihydroxy-5β-cholane (190 mg.).

Attempted Formation of 12α-Para-toluenesulphonyloxy-5α-Cholane: 12α-Hydroxy-5α-cholane (100 mg.) dissolved in pyridine (2 ml.) was incubated with p-toluenesulphonyl chloride (1.2 mole) at 37-40°C for 16 hr. Working up the reaction mixture in the usual manner gave unchanged 12α-hydroxy-5α-cholane (90 mg.) m.p. and mixed m.p. 125-127°C.

12α-Methanesulphonyloxy-5α-Cholane: 12α-Hydroxy-5α-cholane (150 mg.) was treated with methanesulphonyl chloride (1.2 mole) in pyridine (2 ml.) at 0°C for 16 hr. The reaction product recrystallised from ethanol gave 12α-methanesulphonyloxy-5α-cholane (150 mg.), m.p. 103-105°C, $[\alpha]_D^{20}$ + 41.7° (c = 2.3). (Found: C, 71.08; H, 10.96. $C_{25}H_{44}O_3S$ requires C, 70.80; H, 10.38%).

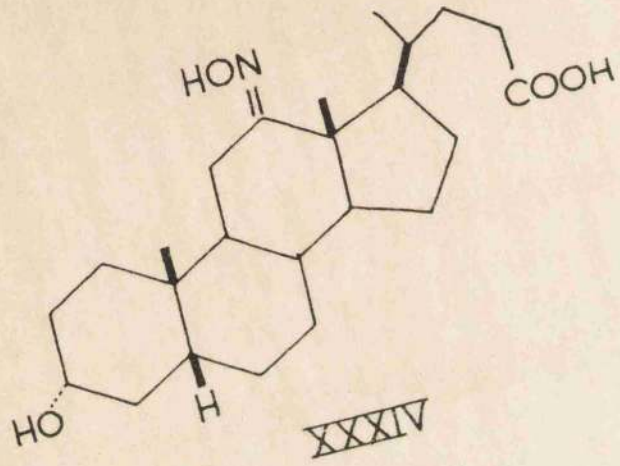
Reduction of the 12α-methanesulphonyloxy-5α-cholane (50 mg.) with lithium aluminium hydride in refluxing ether for 10 hr. gave pure 12α-hydroxy-5α-cholane (42 mg.) m.p. and mixed m.p. 123-125°C.

3α-Methanesulphonyloxy-Cholestane: Epicholestanol (XXXI) (15 mg.) dissolved in pyridine (0.5 ml.) was treated with methanesulphonyl chloride (0.04 ml.) at 0°C for 12 hr. On working up the reaction mixture in the usual manner solid 3α-methanesulphonyloxy-cholestane (17 mg.) was obtained, recrystallised from methanol, plates, m.p. 133°C. The infrared spectrum showed the absence of any free hydroxyl group and characteristic bands for the methanesulphonyl group.

Lithium aluminium hydride reduction of the 3α-methanesulphonyloxy-cholestane (12 mg.) in refluxing ether gave a crystalline material (ca 10 mg.). Chromatography of this reduction product over alumina (gr. I, Woelm, neutral, 5 g.) using light petroleum as solvent gave cholestane (ca 3 mg.) m.p. 77-79°C (reported[153] 80°). The infrared spectrum was identical with that of cholestane[139]. Ethanol/ether (1:19) eluted epicholestanol (ca 6 mg.) m.p. 176-180°C and mixed m.p. 177-180°C. The infrared spectrum was identical with that of authentic epicholestanol.

S E C T I O N I I I

CONFIGURATION OF 12-AMINO-CHOLANES



XXXIV

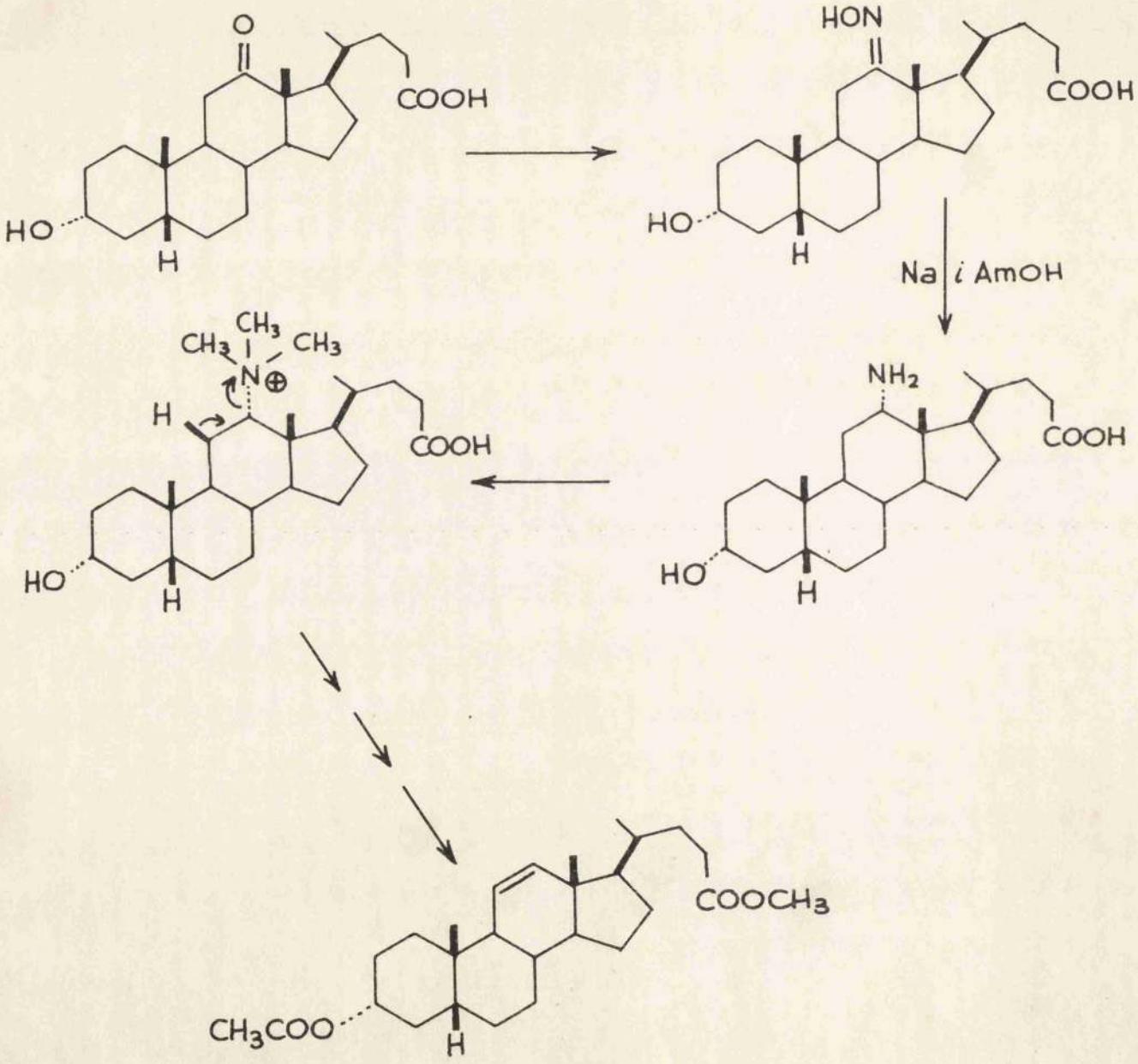
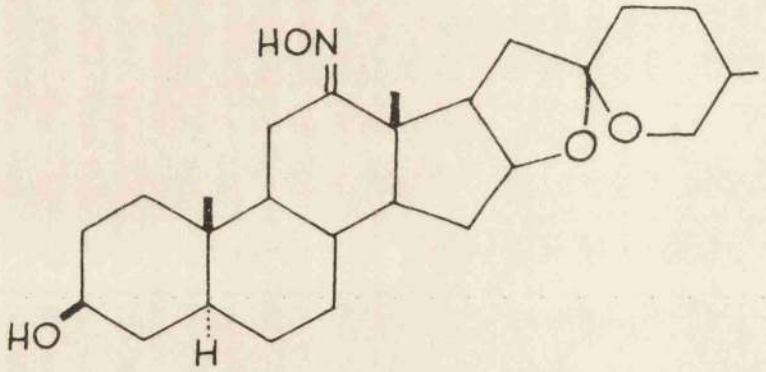

O
COOH
HO
H
HON
COOH
HO
H
Na | i AmOH
NH2
COOH
HO
H
CH3
CH3
CH3
N⊕
H
COOH
HO
H
COOCH3
CH3COO
H

Fig. 9.

As mentioned in Section I of this thesis, unsuccessful attempts to convert the trioxime of methyl dehydrocholate into the corresponding 3α,7α,12α-triamino compound focussed attention upon the need to examine conditions for the reduction of the steroidal 12-oximino function and to establish the configuration of the resulting 12-amino group. In contrast to the situation pertaining with 2-, 3-, 4-, 6-, 7- or 11-oximino steroids[154] where the steric course of reduction under a variety of conditions is firmly established, a survey of the literature (after elimination of studies reporting poorly characterised or impure specimens[155]) revealed what at first sight appeared to be conflicting results in the reduction of 12-oximino steroids.

Thus MacPhillamy and Scholtz[156] reduced the oxime of 3α-hydroxy-12-oxo-cholanic acid (XXXIV) by means of sodium metal and isoamyl alcohol to a 12-amino-3α-hydroxy cholanic acid in which the 12-amino function can be assigned the axial (α) configuration, since on quaternisation followed by Hofmann elimination[157] they obtained 3α-hydroxy-11-cholenic acid (Fig. 9). This was converted into methyl 3α-acetoxy-11-cholenate having identical physical properties with those reported for authentic material prepared by the pyrolysis of desoxycholic acid[158], although direct comparison of samples does not appear to have been made. The Hofmann elimination is well established as occurring by an E_2 mechanism[159] and as such requires that the quaternary ammonium groups and the proton on the β carbon atom, which are involved in the elimination, be trans and antiparallel[160]. The only way in which these requirements can be



XXXV

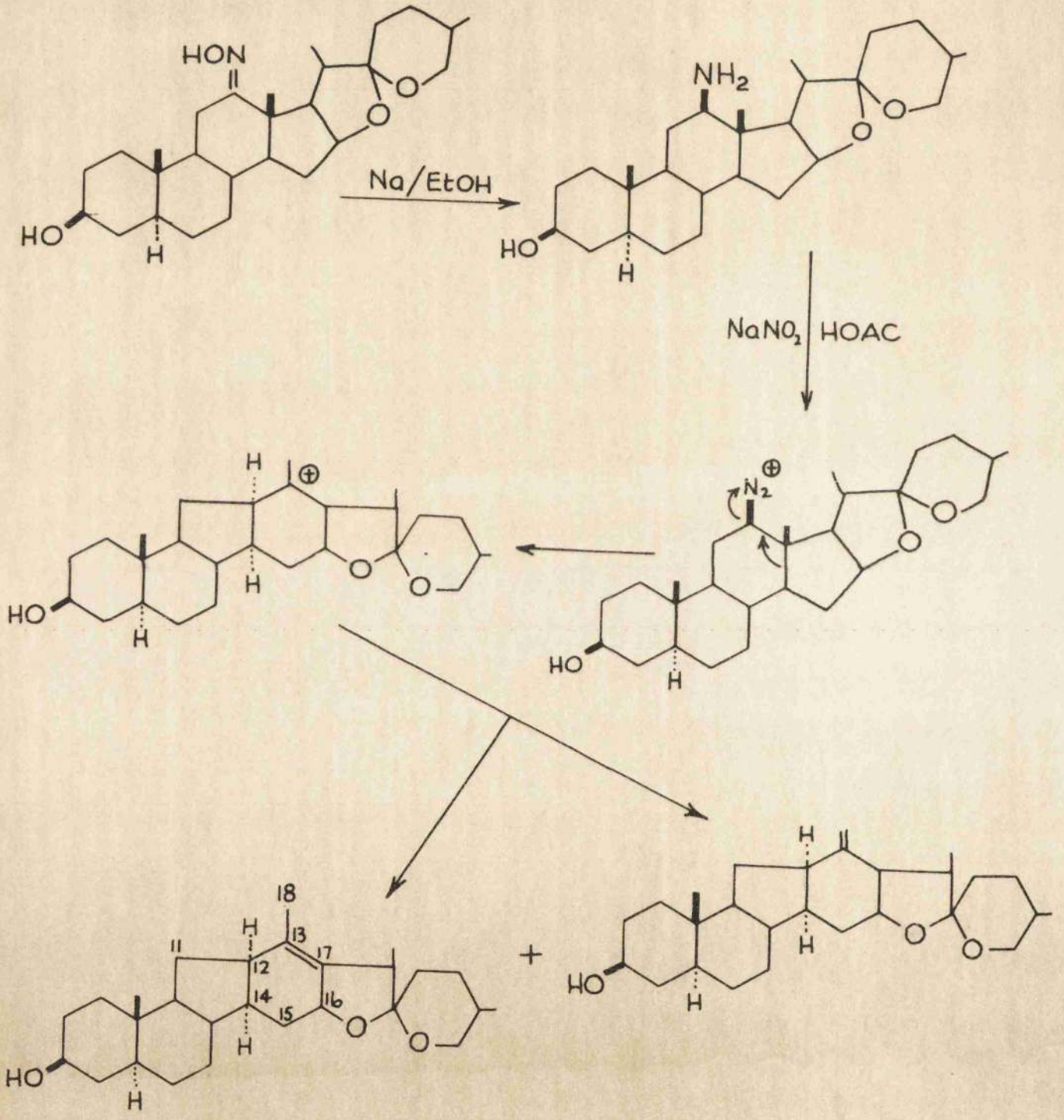
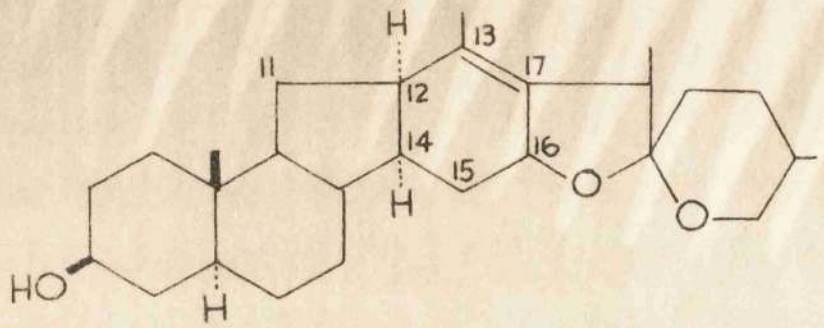

HON
HO
H
Na/EtOH
NH2
HO
H
NaNO2 | HOAC
N2
HO
H
H
HO
H
18
H
13
11
17
12
14
15
16
HO
H
+
H
HO
H

Fig. 10.

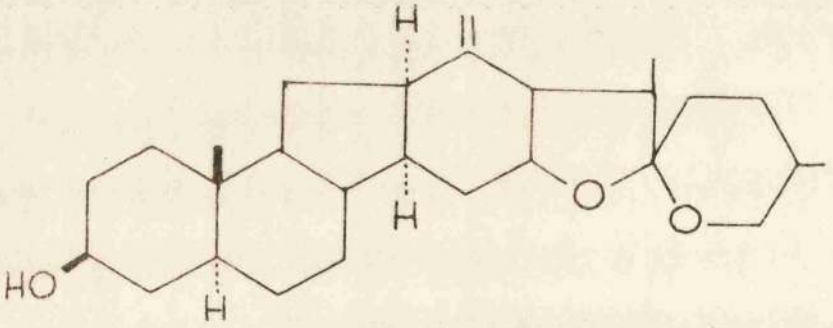
met by a hydrogen atom on C-11 and a quaternary ammonium function on C-12 is for the elimination to involve the 11β-hydrogen atom and a 12α-quaternary group. Hence the configuration of the amino group at C-12 following the reduction of the oxime must be α (axial). This requirement of trans and antiparallel elimination in the Hofmann degradation has been well illustrated in the steroid series by McKenna et al, who showed that axial quaternary amino functions at C-3[161] or C-6 (in 5α steroids)[162] gave rise to olefines under Hofmann conditions, whilst the corresponding equatorial quaternary ammonium compounds regenerated the corresponding tertiary bases.

On the other hand Waid and Taurins[163], without giving any structural proof, assigned the β (equatorial) configuration to the 12-amino group of the product resulting from the sodium metal and n-propanol or n-butanol reductions of 12-oximino-, 3,12-dioximino- and 7,12-dioximino-cholanic acids. The assignments were made on the argument that under equilibrating conditions the equatorial epimer would be formed.

Finally Anliker et al[164] reduced the oxime of hecogenin (XXXV) by means of sodium metal and propanol to a 12-aminospirostane derivative. In this compound the 12-amino function must have the equatorial (β) configuration as, on diazotisation, they obtained the characteristic mixture of jervi steroids[165] known to result from elimination of a 12β group with concurrent migration of the 13-14 bond of the steroid nucleus (Fig. 10). Since the conversion of the amino function into the diazonium group is well known to proceed with retention of configuration as it



XXXVI



XXXVII

involves an initial N-nitrosation followed by rearrangement of the nitrosamine to yield the diazonium ion[166] viz.

$$R - NH_2 \longrightarrow R - NH - N = O \xrightarrow{\Theta} R - N = N\,OH \downarrow R - N_2^{+} + OH^{-}$$

it therefore follows that the amino group must also have been in the 12β-configuration. The formation of the two products 3β-hydroxy-jervi-22α,5α-spirost-17(13)-ene (XXXVI) and 3β-hydroxy-jervi-22α,5α-spirost-13(18)-ene (XXXVII) in the ratio of ca 4:1 characteristic of such jervi rearrangements, arises from the alternate paths of proton elimination from C-17 or C-18 in the carbonium ion precursor.

The results of Anliker et al[164] and of MacPillamy and Scholz[156] thus appear at first glance to be directly contradictory as application of analogous reaction procedures (known normally to convert oximes and ketones into the thermodynamically most stable amine or alcohol[167]) to two different 12-oximino-steroids gave amines of opposite configurations for which the proof of structure in each case is convincing.

As a first step in the present work it therefore appeared logical to prepare the epimeric pair of 12-amino-5β-cholanes and apply both the diazotisation procedure and the Hofmann elimination reaction (after quaternisation) to each epimer in order to eliminate the possibility of any discrepancy between the two procedures as applied to the determinations of configuration of the two different 12-amino compounds reported in the literature. For instance it was conceivable, though not probable, that

a cis Hofmann elimination was occurring or that in the diazotisation reaction the migration of the 13-14 bond was not synchronous with the departure of molecular nitrogen from C-12, permitting the finite existence of a carbonium ion with consequent loss of stereochemical significance.

In actual fact it was not found possible to prepare both epimeric 12-amino-5β-cholanes. Application of a variety of reduction procedures to 12-oximino-5β-cholane, which was prepared in good yield from 12-oxo-5β-cholane (Section II) by the standard procedure, gave in all cases the same single epimer. No attempt was made in the present work to separate the cis and trans forms of this oxime or of any of the other oximes described below.

Reduction of 12-oximino-5β-cholane by means of sodium and boiling alcohol, afforded a crude low melting solid which was separated by means of alumina chromatography into a single 12-amino-5β-cholane and 12-oxo-5β-cholane. It would seem clear that the formation of the latter compound was not due to hydrolysis of the oxime during the reduction as the corresponding alcohol, not the oxo-compound, would have been isolated. A likely explanation is that incomplete reduction to the imine had occurred and that this intermediate was protected from further reduction through complex formation. In the working up of the reaction mixture, the liberated imine would then be hydrolysed to the ketone.

Treatment of 12-oximino-5β-cholane with lithium aluminium hydride in boiling ether afforded in 90% yield a single 12-amino-5β-cholane which proved to be identical with that obtained from the sodium and ethanol

reduction (mixed m.p., $[\alpha]_D$ and infrared spectrum). Not only was there no evidence of the formation of the epimeric amine but there was no evidence of the production of any 12-hydroxy-compound, which is of interest in view of the report[163] that the action of lithium aluminium hydride on 3,12-dioximino-cholanic acid leads to the formation of a cholane-3,12,24-triol.

Catalytic hydrogenation of 12-oximino-5β-cholane in acetic acid proceeded poorly, but in the presence of a trace of perchloric acid or at 100-110 atmospheres, a good yield resulted of the same single 12-amino-5β-cholane as was obtained by the other procedures.

Treatment of 12-oximino-5β-cholane with lithium metal and liquid ammonia yielded only a mixture of hydrocarbons (one of which proved to be 5β-cholane) and unreacted starting material, there being no detectable amount of amine in the product. Similarly attempts to convert 12-nitrimino-5β-cholane (prepared from 12-oximino-5β-cholane by the action of nitrous acid following the method of Schenck[168] for the synthesis of other 12-nitrimino-cholanes) into a 12-amino-5β-cholane were unsuccessful. Unlike 2-nitriminobornane, which liberates 2-iminobornane on treatment with 880 ammonia[169], 12-nitrimino-5β-cholane gave rise only to 12-oxo-cholane on treatment with 880 ammonia or with liquid ammonia, and no imine could be isolated. This is of significance in connection with the formation of 12-oxo-5β-cholane in the sodium and ethanol reduction of 12-oximino-5β-cholane already discussed (p. 76).

In connection with the formation of the same single 12-amino-5β-

cholane by the reduction procedures described above, it is of considerable significance that Shoppee *et al*[170] were able to obtain only the one 1-amino-5α-cholestane (to which no configuration was assigned) by catalytic hydrogenation, treatment with lithium aluminium hydride or sodium/alcohol reduction of 1-oximinocholestane. It would appear therefore that an analogous situation to that at C-12 in the cholane series pertains at C-1 in the cholestane series.

Configurational studies were then commenced on the 12-amino-5β-cholane, and these conclusively showed that it possessed the α ie axial configuration.

Thus deamination of 12-amino-5β-cholane with nitrous acid in 80% aqueous acetic acid (according to the method described by Anliker *et al*[164] for the deamination of 3β-hydroxy-12β-amino-22α,5α-spirostane) gave a neutral noncrystalline product, the infrared spectrum of which showed absorptions characteristic of a 12α-hydroxyl group (3540 cm^{-1}), a carbonyl group (1700 cm^{-1}) and of double bonds (1600 and 1630 cm^{-1}). Chromatographic fractionation of this product over alumina afforded a mixture of unsaturated hydrocarbons (the infra red showing double bond absorptions at 1600 and 1630 cm^{-1}), 12-oxo-5β-cholane and 12α-hydroxy-5β-cholane in the ratio of 2:3:5. Gas liquid chromatography of the hydrocarbon fraction indicated the presence of four components which could be divided into two pairs on the basis of their retention times. One component of the pair having the lower retention time, and which constituted *ca* 70% of the total, was identified as Δ^{11}-5β-cholene by

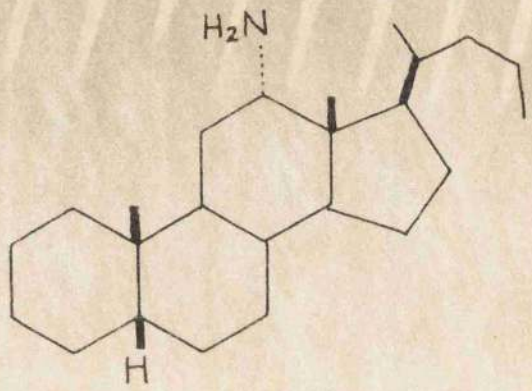
admixture with an authentic sample. The second pair which had retention times ca twice that of the first pair appeared to be a mixture of unsaturated jervi hydrocarbons. These could have arisen through loss of stereochemical significance consequent upon carbonium ion formation at C-12. That the components of each pair differed only in the position of the double bonds and not in basic skeleton followed from the results of catalytic hydrogenation of the hydrocarbon mixture. The resulting mixture of saturated compounds (infrared spectrum and negative tetranitromethane test) on gas liquid chromatography showed only two components - one corresponding to each of the pairs of the unsaturated compounds. The component with the lower retention time was identified as 5β-cholane by comparison with an authentic specimen.

The 12-oxo-5β-cholane was concluded to have arisen from 12α-hydroxy-5β-cholane under the reaction conditions, as it was demonstrated in a separate experiment that 12α-hydroxy-5β-cholane, on treatment with nitrous acid in 80% aqueous acetic acid, was partially oxidised to 12-oxo-5β-cholane. On the other hand 12β-hydroxy-5β-cholane formed an unstable nitrite ester under the same conditions and this readily regenerated 12β-hydroxy-5β-cholane in quantitative yield upon attempted recrystallisation from ethanol. Thus isolation of 12α-hydroxy-5β-cholane together with 12-oxo-5β-cholane from the reaction product in ca 80% combined yield with no trace of the 12β-epimer showed the 12-amino-5β-cholane to have the α (axial) configuration since replacement of the diazonium group by the hydroxyl group proceeds with retention of configuration[171].

Quaternisation of the 12-amino-5β-cholane proved difficult but was achieved on heating the N,N-dimethylamino-5β-cholane (prepared by adaptation of the formic acid/formaldehyde method used by Dodgson and Haworth[172] for the preparation of other steroidal tertiary amines) with methyl iodide in a sealed tube at 100°C for 5 hr. (60%). It has been established that the formation of the tertiary amine by the formic acid/formaldehyde method does not produce racemisation of the resultant dimethylamino group[162,173]. Application of the quaternisation procedure of Woodward et al[174], even after prolonged refluxing, gave low yields. Separation of the quaternary iodide from the tertiary amine was readily achieved by taking the tertiary amine into light petroleum after removal of the solvent from the alkaline reaction mixture.

Hofmann degradation of the quaternary base under the conditions described by MacPhillamy and Scholz[156] yielded an unsaturated hydrocarbon in ca 80% yield and a tertiary base (ca 10%) which was identified as 12α-dimethylamino-5β-cholane by comparison of $[\alpha]_D$'s and through mixed m.p. of its hydrochloride with authentic 12α-dimethylamino-5β-cholane hydrochloride.

The unsaturated hydrocarbon was concluded to be Δ^{11}-5β-cholene as the Hofmann degradation requires trans elimination[160]. Gas liquid chromatography indicated one component having a retention time very close to that of 5β-cholane. Catalytic hydrogenation afforded pure 5β-cholane, whose identify was proved by gas liquid chromatography employing authentic 5β-cholane. This formation of 5β-cholane showed that the Hofmann



12α - amino - 5β - cholane

elimination occurred without rearrangement of the carbon skeleton of the molecule.

The products obtained on deamination of the primary amine and on Hofmann degradation of the corresponding quaternary ammonium base therefore show conclusively that the configuration of the 12-amino group must be α (axial).

The position of the double bond in the compound designated Δ^{11}-5β-cholene was not rigorously proven in the present work. The obvious method to follow would involve epoxidation of the double bond followed by treatment of the product with (a) lithium aluminium hydride and (b) lithium aluminium hydride in the presence of aluminium chloride. These reagents are known[175] to open epoxides in the opposite senses and one of the resultant alcohols would have to correspond to a known 12-hydroxy-5β-cholane. As the Δ^{11} compound has already been shown to give 5β-cholane on reduction thus demonstrating that there has been no rearrangement, the above experiments would give conclusive proof that the double bond is in the 11-12 position.

Having thus established the fact that reductions of 12-oximino-5β-cholane, all lead to the sole formation of 12α-amino-5β-cholane, attempts were made to obtain the 12β-amine by procedures involving S_N2 attack on 12α-methanesulphonyloxy-5β-cholane. Treatment of this compound with sodium azide gave rise to the expected displacement but the resulting 12β-azide could not be converted into the desired amine by the accepted reduction procedures employing lithium aluminium hydride[176], sodium

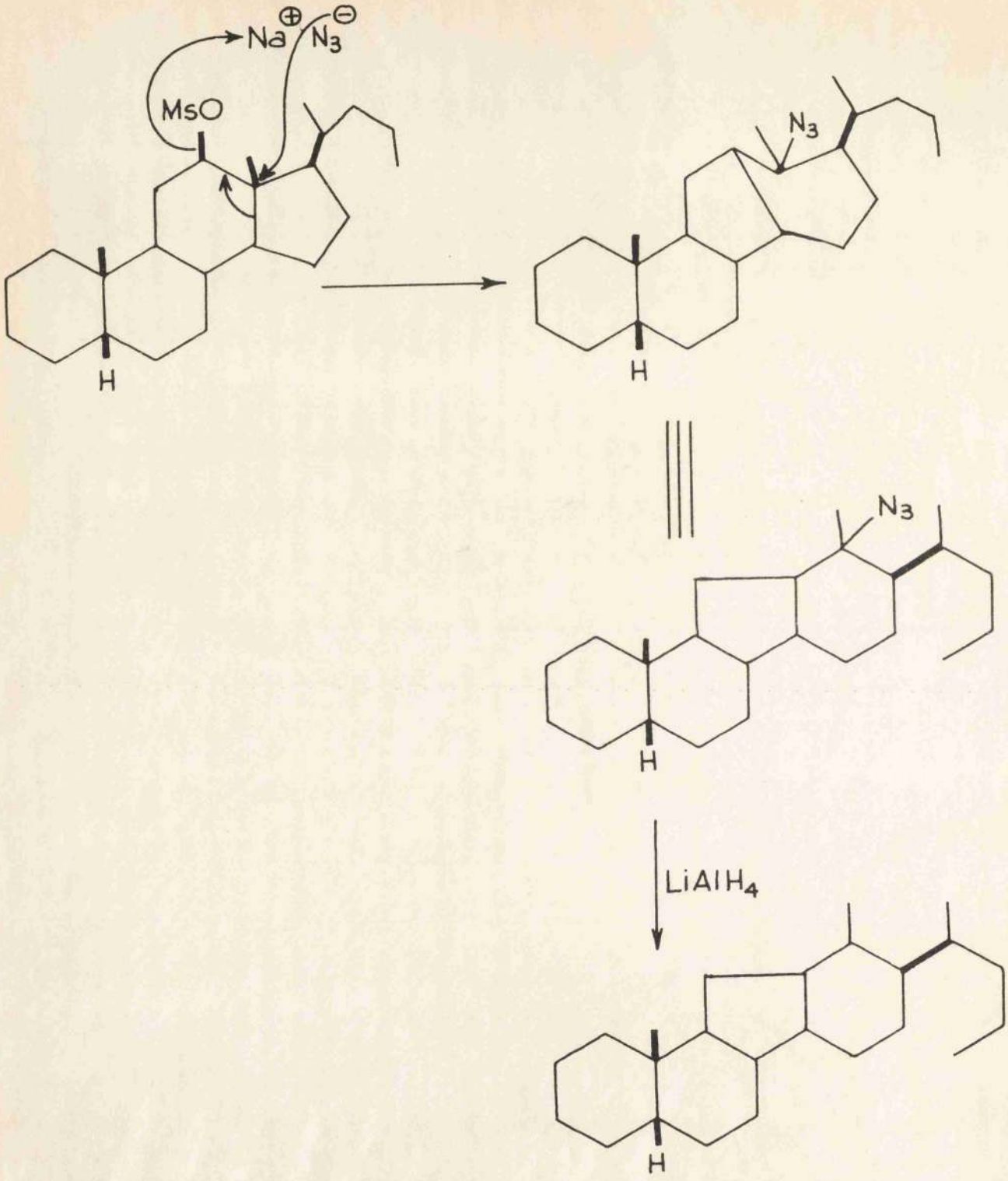


Fig. 10a.

hydrosulphite[177] or catalytic hydrogenation[178]. Instead, the first two methods yielded a noncrystalline unsaturated hydrocarbon mixture (tetranitromethane test) which after gas liquid chromatography was concluded to be an equiproportional mixture of Δ^{11}-5β-cholene and another compound with very close retention time to that of other C_{24} steroidal hydrocarbons, not belonging to the jervi series. Catalytic hydrogenation of the 12β-azide afforded a noncrystalline saturated hydrocarbon mixture (negative tetranitromethane test). Gas liquid chromatography showed that the product was a complex misture of five components, with 5β-cholane constituting ca 50% of the total product.

Similar formation of hydrocarbons was observed on reduction of 12α-azido-5β-cholane prepared by a displacement reaction on 12β-methanesulphonyloxy-5β-cholane. Once again no amine could be isolated.

The unsaturated hydrocarbon mixture obtained by lithium aluminium hydride reduction of the 12α-azide, on gas liquid chromatography was shown to consist of six components. The two main components, which constituted ca 80% of the total, were concluded to be Δ^{11}-5β-cholene and a jervi hydrocarbon, by comparison with the hydrocarbons obtained on diazotisation of 12α-amino-5β-cholane. The jervi hydrocarbon could have arisen through partial rearrangement during the 12α-azide formation. As the β-methanesulphonate group was leaving C-12 the 13-14 bond could migrate with synchronous attack by azide ion at C-13 instead of the normal S_N2 attack on C-12 (Fig. 10a) (cf. ref. 165).

The saturated hydrocarbons obtained on catalytic hydrogenation of

12α-azido-5β-cholane, gave two main components on gas liquid chromatography, corresponding, in retention time, to the 5β-cholane and jervi-cholane components of the hydrogenated hydrocarbons obtained from the diazotisation reaction (p.79).

The action of sodamide on 12α-methanesulphonyloxy-5β-cholane also failed to afford any 12β-amino compound. The sole products of the reaction were 12-oxo-5β-cholane and 12α-hydroxy-5β-cholane. It is of interest that the action of sodium iodide in acetone on 12α-methane-sulphonyloxy-5β-cholane gave an extremely unstable iodo compound, which on standing liberated free iodine and formed a mixture of hydrocarbons (gas liquid chromatography) giving a positive test for unsaturation with permanganate, bromine or tetranitromethane. One possible interpretation of the instability of this iodo derivative is that it is sterically unfavoured. Attempts to convert a freshly prepared sample of the 12β-iodo compound into 12α-amine by means of sodamide in xylene were unsuccessful.

Application of the Leuckart reaction[179] to 12-oxo-5β-cholane afforded a 12-formylamino-5β-cholane in good yield. Hydrolysis of this derivative yielded 12α-amino-5β-cholane (m.p. infrared spectrum) together with trace amounts of two unsaturated hydrocarbons in equal quantities (tetranitromethane test and gas liquid chromatography).

In view of these unsuccessful attempts to secure 12β-amino-5β-cholane, a further possibility was to attempt the preparation of 12β-NN-dimethylamino-5β-cholane by an S_N2 displacement with dimethylamine on 12α-methane-sulphonyloxy-5β-cholane, as quaternisation of the dimethylamino compound

would at least permit of an investigation of the Hofmann elimination on the corresponding 12β-quaternary ammonium salt even although a study of the diazotisation of the corresponding primary amine would not be possible. This approach was not investigated, however, after careful consideration of steric interactions at C-12.

Examination of models shows that there are differences in the steric hindrance occurring at C-12 in the cholane series and the spirostane series. Thus in the latter, the oxygen bridge between C-16 and C-22 ensures that C-21 (the side chain methyl group) is held spatially well removed from C-12 and offers little hindrance to this last position. In the cholane series, on the other hand (with the same configuration at C-20, as both the bile acids and the spirostanes arise in nature from lanosterol[180] and so retain the same stereochemistry at C-20), it is not possible for the 12β position to be without steric hindrance from C-21 or C-22 without there being serious non-bonded interactions within the side chain or between the side chain and C-16. It must be assumed that the side chain will adopt a conformation in which a compromise is made between all possible non-bonded interactions, but in which there is greater steric hindrance at the 12β position than occurs in the spirostane series.

The shielding of the 12β position in the cholane (and by extension, the cholestane series) by the C-17 side chain can be regarded as a type of long range effect if such shielding is ascribed to reduction of non-bonded interactions involving C-16 and the side chain. Barton[181] has

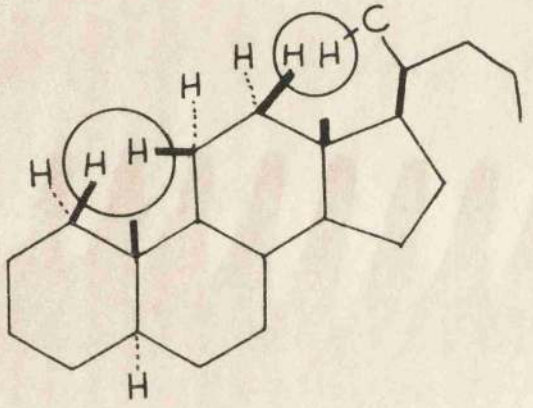


Fig. 11.

introduced the concept of long range effectsin a somewhat narrower sense to explain differences in reactivity between different steroids at the same position in the nucleus, but in the case of C-12, the long range effect actually reverses the normal relative stability of the epimers.

It is to be noted that a steric interaction similar to that just described for the 12β-position and the cholane side chain, also occurs between the 1β-position and C-11 in the steroid nucleus (Fig. 11) and it is therefore tempting to assign the axial (α) configuration to the 1-aminocholestane prepared by Shoppee et al[170], by analogy with the axial configuration of 12-amino-5β-cholane. It is to be noted, however, that reduction of 5α-cholestane-1-one with sodium and alcohol gives the 1β and 1α hydroxy compounds in the ratio of ca 2:1[182]. A similar steric interaction is found between C-19 and C-12 in the triterpene nucleus and it is therefore of considerable interest that sodium and alcohol reduction of certain 19-keto triterpenes is known to give the corresponding axial alcohol[183].

The experimentally observed fact that reduction under equilibrating conditions (sodium and alcohol) of 12-oximes in the spirostane series gives solely the equatorial amine whilst 12-oximes of the cholane series afford solely the axial amine would therefore be accommodated if the increased steric hindrance of the 12β position occurring in the cholanes were sufficiently great to actually render the axial configuration thermodynamically more stable than the equatorial configuration. Such a reversal of the normal stabilities was not envisaged by Waid and Taurins[163]

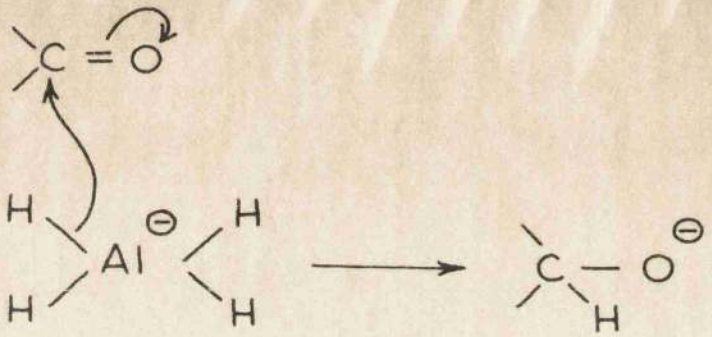


Fig. 12.

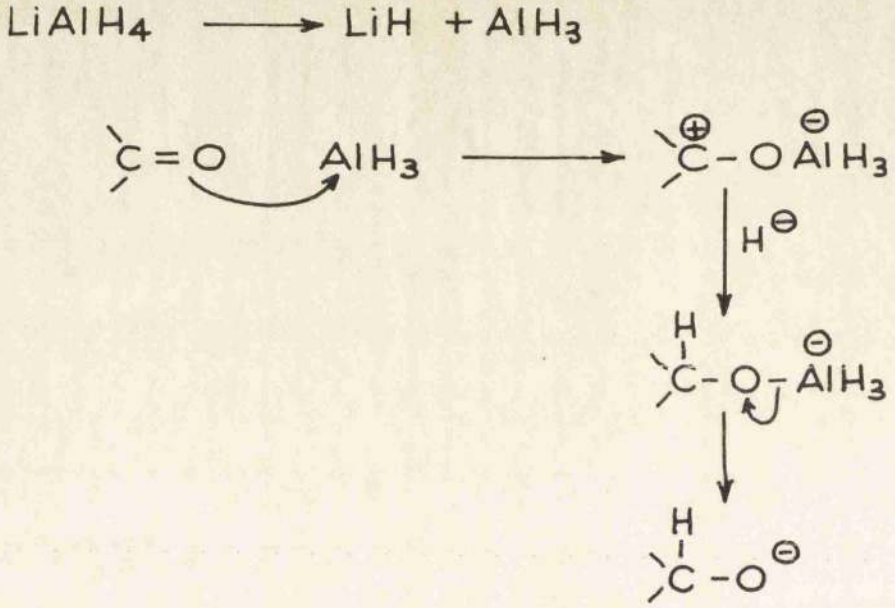
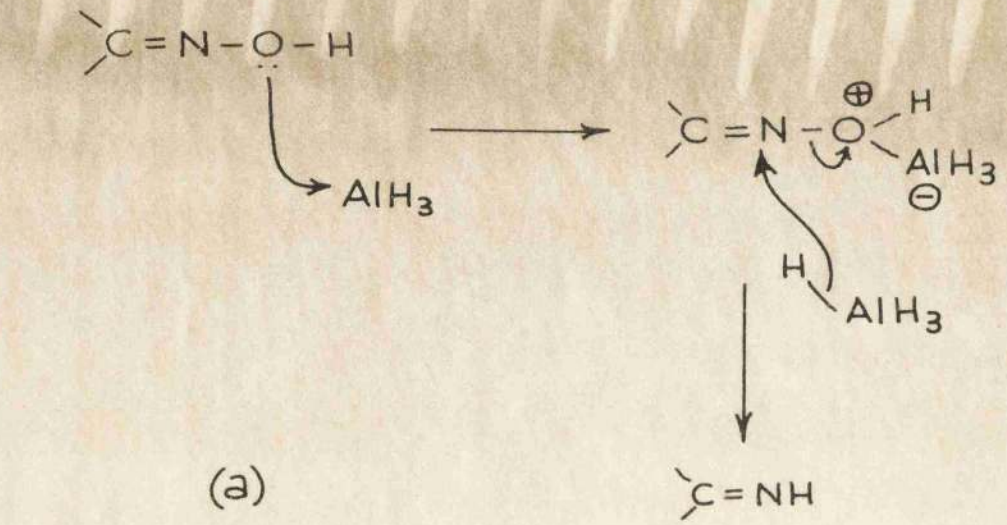


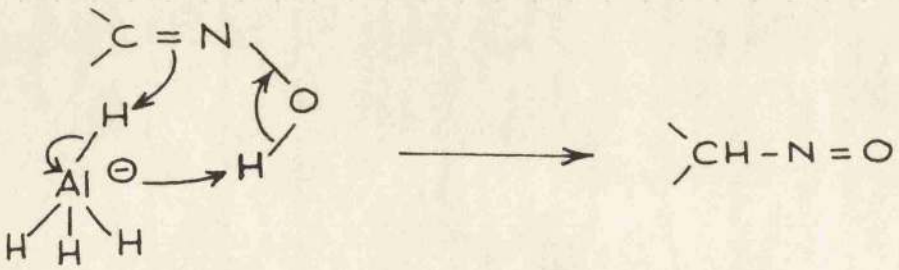
Fig. 13.

and so inversion of their assignments of configuration gives a completely rational picture of sodium and alcohol reduction of 12-oximino steroids.

The results obtained on lithium aluminium hydride reduction of 12-oximino-5β-cholane are somewhat more difficult to explain. With steroidal 3, 6 and 7-oximes, lithium aluminium hydride usually gives a mixture of epimers with the most hindered amine predominating[184], although instances are known where a single epimer only is formed[184, 185] or where the more stable epimer is formed[184]. In the case of complex metal hydride reductions of ketones, an explanation of the relative proportions of the epimeric alcohols formed has been advanced[186] on the grounds that two effects are operative. These are the ease of formation of the complex between the carbonyl group and the complex hydride (steric approach control) and the relative energies of formation of the products once the initial complex is produced (product development control). However, more recently certain anomalies, which could not be accommodated by this theory, have been eliminated by the postulate[187] that two distinct reaction pathways exist for the reduction of carbonyl groups by complex metal hydride. These are (i) a direct attack on the carbonyl carbon by the metal hydride anion (Fig. 12) giving a large proportion of the unstable alcohol owing to the preferred approach of the hydride ion from the less hindered side of the molecule; (ii) the formation of a complex between the Lewis acid formed by dissociation of the metal hydride and the carbonyl oxygen which adopts the most stable conformation and is then reduced (Fig. 13).



(a)



(b)

Fig. 14.

When different complex metal hydrides are considered, the second mode of attack will be dependent upon the chemical nature of the actual hydride employed as this will influence the dissociation of the complex metal hydride.

In the case of the oximino group the situation is much more complex than with the carbonyl group as further modes of reaction are possible which involve the OH function (Fig. 14) and so there is little point in attempting to interpret the formation of the 12-amines in terms of mechanisms. That the path shown in Fig. 14(a) may well be involved is perhaps indicated by the results of Waid and Taurins[163] who interpreted the conversion of a 12-oximino-cholanic acid into the corresponding 12-hydroxy compound as proceeding via the imine.

Examination of models shows that it is extremely difficult to introduce a trivalent atom such as nitrogen into the 12β position of the cholane nucleus although a divalent atom such as oxygen can be relatively easily accommodated, and it may well be this trivalent nature of the amino group which determines its α-orientation as lithium aluminium hydride reduction of 12-oxo-5β-cholane gives predominantly the 12β-hydroxy compound (XXIX) (p. 68).

The steric course of the catalytic hydrogenation of 12-oximino-5β-cholane cannot be used in support of the theoretical arguments since it is not fully understood exactly what factors determine the direction of hydrogenation. It is clear, however, that the relative orientations of the molecule undergoing reduction in relation to the catalyst surface

and in relation to the number of molecules of hydrogen available for reaction must both play important roles.

If the conclusion that the 12 axial position is thermodynamically more favoured than the 12 equatorial position in the cholane series is correct, further supporting evidence should be available from reduction studies of 12-keto steroids. Accordingly a careful study of the reductions of 12-oxo-5β-cholane was undertaken.

After this work had been completed a paper[188] appeared having important bearing on the results to be discussed below. The authors obtained results in excellent agreement with those of the present work, but they offered the explanation that neither metal in ammonia nor sodium in alcohol, reduction of ketones are thermodynamically controlled.

In the present work, sodium and ethanol reduction of 12-oxo-5β-cholane (p.68) afforded a mixture of 12α and 12β-hydroxy-5β-cholanes which were present in equal amounts as shown by alumina chromatography. The formation of both epimers in equal quantities in the cholane series is in striking contrast to the situation encountered on sodium and alcohol reduction of 3β-hydroxy-12-oxosolanid-5-ene which gives solely the 12β-hydroxy compound[189] indicating that whilst the equatorial position in the solanidane series is definitely favoured it is not so favoured in the cholane series. Similarly sodium and alcohol reduction of hecogenin gives a higher preponderance of the equatorial epimer[188]. Huffman et al[188] found that reduction of 12-oxo-5β-cholane with sodium and n-propanol gave 61% 12α-hydroxy-5β-cholane.

Lithium aluminium hydride reduction of 12-oxo-5β-cholane (p. 68) gave a mixture of epimers with the 12β-hydroxy compound predominating (60-80% of the product) as shown after alumina chromatography whilst lithium aluminium tritertiarybutoxy hydride gave a mixture of epimers with the 12α-hydroxy compound predominating (ca 80% of the product). As this last reagent is assumed to act by the mechanism shown in Fig. 14(b) (i.e. dissociation to lithium hydride and aluminium tritertiarybutoxide) with the bulky tritertiarybutoxy aluminium group adopting the less hindered position[187], these observations support the contention that the 12 axial position is less hindered than the 12-equatorial position in cholanes.

It is to be noted that lithium aluminium hydride reduction of hecogenin has been reported to give a mixture of 12α and 12β-hydroxy compounds[190], and that the 12-oxo-compound derived from polyporenic acid A (12α-OH) has been shown to regenerate polyporenic acid A on treatment with the same reagent[191].

Catalytic hydrogenation of 12-oxo-5β-cholane with platinum oxide in acetic acid in the absence of added hydrogen ions proceeded poorly and the only products isolated were 12α-hydroxy-5β-cholane and unchanged starting material. In the presence of added perchloric acid, hydrogen uptake was rapid yielding a mixture of 12α-hydroxy-5β-cholane (80%) and 5β-cholane (20%) which must be assumed to have arisen via dehydration of a proportion of the 12α-hydroxy-5β-cholane formed followed by saturation of the Δ^{11} double bond so introduced. In the case of 3β-hydroxy-12-

oxo-solanid-5-ene catalytic hydrogenation is known to give the 12β-epimer[189]. The situation with 3β-hydroxy-12-oxosolanid-5-ene is thus completely in line with that found for agavogenin[192], mannogenin[193] and mexogenin[193] where both sodium/alcohol reduction and catalytic hydrogenations give the same epimer (the 12β compound).

It may be noted, in passing, that originally the hydroxyl group at C-12 in the bile acids was assigned the β configuration following an erroneous interpretation of X-ray data[194] and much of the work before 1947[195] when reassignment was made[196] therefore needs careful re-interpretation. Even as late as 1956 a review[197] of the reduction of oxo-steroids erroneously shows the catalytic hydrogenation under acid conditions of 12-oxo-cholanes as yielding the 12β-hydroxy compound.

In the cis A/B steroids the 7α-position is sterically hindered by non-bonded interaction with C-4 and in this connection it is interesting that 7-oxo-5β-cholane on reduction with sodium and ethanol (p. 64) gave a mixture of 15% of the 7α-hydroxy compound and 85% of the 7β-hydroxy derivative. Lithium aluminium hydride reduction of the same ketone (p.110) afforded the α and β epimeric alcohols in the ratio of 19:1, whilst prolonged catalytic hydrogenation with platinum oxide in acetic acid with added perchloric acid present, gave 30% 5β-cholane, 35% 7α-hydroxy-5β-cholane and 35% unreacted starting material (cf. catalytic reduction of 12-oxo-5β-cholane p.110). The hydrocarbon presumably arose via hydrogenolysis or by dehydration followed by the reduction of the mixture of the olefins so formed. These results are also of

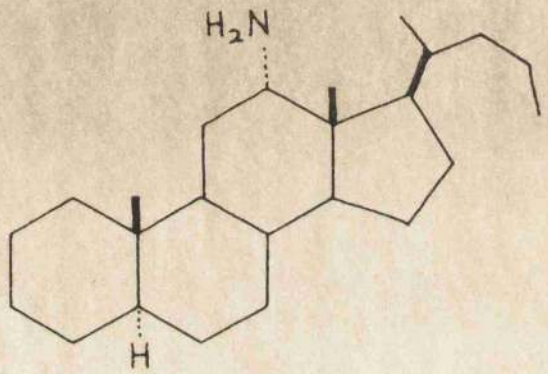
interest in connection with the known greater case of oxidation of the 7α-alcohol than the 12α-alcohol[198].

One other difference between compounds of the spirostane series and the naturally occurring cholane derivatives is that the former possess a trans A/B ring junction whilst the latter possess a cis A/B ring junction. This difference would also be expected to influence the thermodynamic stability of the 12α configuration, but closer examination shows that the 12α position is less favoured in the cis A/B series than in the trans A/B series (a 1, 3 non-bonded interaction between C-4 and the axial hydrogen atom on C-9 giving a greater butressing effect to the non-bonded interaction between the axial C-9 hydrogen and the 12α-position than is produced in the interaction of the 5α and 9α hydrogen atoms in the trans A/B series).

Nevertheless in order to adduce further evidence that the 12α-configuration is the preferred one in steroids possessing an acyclic side chain of more than two carbon atoms at C-17, it was decided to include the reduction of 12-oximino-5α-cholane in the present studies.

12-Oximino-5α-cholane was prepared from 12-oxo-5α-cholane (p.111) employing the usual procedure in ca 80% yield.

The reduction of 12-oximino-5α-cholane employing the usual procedures i.e. sodium ethanol, lithium aluminium hydride and catalytic hydrogenation in presence of added hydrogen ions gave rise to one single 12-amino-5α-cholane. Characterisation of the product proved difficult, the base being noncrystalline and affording an ether soluble hydrochloride. The noncrystalline base, after chromatography on grade I alumina, however,



12α-amino-5α-cholane

could be easily crystallised as its hydrochloride on treatment with ca 5% methanolic hydrochloric acid.

Diazotisation of 12-amino-5α-cholane (according to the method described by Anliker et al[164]) afforded a mixture of unsaturated hydrocarbons, 12-oxo-5α-cholane and 12α-hydroxy-5α-cholane, separable on chromatography over alumina in the usual manner. The unsaturated hydrocarbon fraction was smaller in proportion than that obtained on diazotisation of 12α-amino-5β-cholane. These observations prove that the 12-oximino group in the 5α series is also reduced solely to the α (axial) compound. Hence it was concluded that the nature of the A/B ring junction has no effect on the course of reductions at C-12.

The quaternisation of 12-amino-5α-cholane was more facile than that of 12α-amino-5β-cholane as under similar reaction conditions the conversion of 12-amino-5α-cholane to 12-dimethylammonium-5α-cholane methiodide was ca 80% while that of 12α-amino-5β-cholane was only 50%. This result would be expected on the argument that the trans A/B ring junction favours the axial substitution at C-12 (p. 91). Hofmann elimination proceeded as expected to give Δ^{11}-5α-cholene.

EXPERIMENTAL

12-Oximino-5β-Cholane: 12-Oxo-5β-cholane (p.67) (1.0 g.) in ethanol (20 ml.) was refluxed with a solution of hydroxylamine hydrochloride (0.33 g.) and sodium acetate (0.66 g.) in ethanol (20 ml.) containing water (1 ml.) for 6 hr. The reaction mixture was distilled *in vacuo* to one third of its volume and allowed to stand overnight. The inorganic material was removed by filtration, and the filtrate on evaporation at room temperature yielded 12-oximino-5β-cholane (0.925 g.), recrystallised from methanol, rods m.p. 141-142° C, $[\alpha]_D^{20}$ + 152° (c = 2.0). (Found: C, 80.04; H, 11.36; N, 4.32. $C_{24}H_{41}ON$ requires C, 80.22; H, 11.51; N, 3.90%).

Reduction of 12-Oximino-5β-Cholane with Sodium and Ethanol:
A boiling solution of 12-oximino-5β-cholane (1.0 g.) in ethanol (40 ml.) was treated with sliced sodium metal (4.0 g.) during a period of one hr. The cooled reaction mixture was diluted with water and the precipitated white semicrystalline solid was removed by filtration (925 mg.) m.p. 85-95°C. The infrared spectrum showed a carbonyl peak (1700 cm^{-1}) and an amino peak (1605 cm^{-1}). (a) The reduction product (530 mg.) was chromatographed over alumina (grade III, 30 g.) using light petroleum as eluant. Light petroleum (70 ml.) eluted 12-oxo-5β-cholane (103 mg.) m.p. and mixed m.p. 118-120°C. Subsequent elution with light petroleum (50 ml.) gave very little material. Ether (70 ml.) eluted 12α-amino-5β-cholane (414 mg.), recrystallised from methanol, m.p. 106-107°C,

$[\alpha]_D^{19}$ + 43.5° (c = 2.0). (Found: C, 83.42; H, 12.48; N, 4.21. $C_{24}H_{43}N$ requires C, 83.72; H, 12.31; N, 4.07%).

(b) The reduction product (100 mg.) dissolved in ether (10 ml.), was stirred with N HCl (10 ml.) resulting in the formation of a middle layer of solid 12α-amino-5β-cholane hydrochloride. The ethereal filtrate after removing the solvent gave crude 12-oxo-5β-cholane (ca 20 mg.) m.p. 116-119°C. The crystalline hydrochloride was combined with the residue obtained on distilling the aqueous layer in vacuo (80 mg.) and recrystallised from methanol giving 12α-amino-5β-cholane hydrochloride, needles, double m.p. 160: 205-207°C. Treatment of this hydrochloride with 2N sodium hydroxide followed by extraction with ether yielded the free base m.p. 100-104°C.

Reduction of 12-Oximino-5β-Cholane with Lithium Aluminium Hydride:
12-Oximino-5β-cholane (500 mg.), dissolved in ether (25 ml.) was treated with a slurry of lithium aluminium hydride (500 mg.) in ether (25 ml.) and the reaction mixture was refluxed for 16 hr. The excess of hydride was decomposed with moist ether and the ethereal filtrate therefrom, after removal of the solvent, gave white needles (461 mg.) (the infrared spectrum showed complete conversion of the oximino-group into the amino group, and the spectrum was identical with that of 12α-amino-5β-cholane obtained by the reduction with sodium and ethanol).

The crude base (350 mg.) on chromatography over alumina (grade III 20 g.) using ether as eluant gave pure 12α-amino-5β-cholane (340 mg.)

m.p. 105-107°C. Repeated attempts to fractionate the base into the possible epimeric products failed. The free base and its hydrochloride had physical constants (m.p. $[\alpha]_D$, and infrared spectra) identical with the 12α-amino-5β-cholane obtained on reduction of 12-oximino-5β-cholane with sodium and ethanol.

Catalytic Hydrogenation of 12-Oximino-5β-Cholane:

(a) 12-Oximino-5β-Cholane (200 mg.) was added to a pre-reduced suspension of platinum oxide (40 mg.) in acetic acid (10 ml.) containing a drop of perchloric acid, and the mixture was allowed to shake under hydrogen at room temperature and atmospheric pressure. The uptake of the calculated amount (2 moles) of hydrogen was complete within one hour but the shaking was continued for two hours more. The catalyst was removed by filtration and the filtrate was distilled *in vacuo* to yield a colourless solid. The infrared spectrum showed traces of oximino-compound to be present. Purification of the amino-compound was achieved by means of chromatography over alumina (grade I 20 g.) in the usual manner. Elution with ether/light petroleum (1:19) yielded 12α-amino-5β-cholane (160 mg.) m.p. and mixed m.p. 103-106°C (correct infrared spectrum) $[\alpha]_D^{19}$ + 43°(c = 1.9). Hydrochloride, needles from methanol, double m.p. and mixed m.p. 158-160°: 204-207°C. Elution with ether/light petroleum (2:3, 100 ml.) gave a noncrystalline gum (30 mg.) which contained both the 12-oximino-5β-cholane and the 12α-amino-5β-cholane (infrared spectrum).

Hydrolysis of this gum (30 mg.) in boiling ethanol (20 ml.) containing 6N sulphuric acid (5 ml.) for 16 hr. gave a solid consisting of a mixture of 12-oxo-5β-cholane and the sulphate of 12α-amino-5β-cholane (infrared spectrum). Treatment of this mixture with sodium hydroxide, followed by chromatography over alumina (grade I 5 g.) gave 12-oxo-5β-cholane (15 mg.) and 12α-amino-5β-cholane (10 mg.)

(b) Catalytic hydrogenation of 12-oximino-5β-cholane (1 g.) in acetic acid (40 ml.) containing platinum oxide (200 mg.) at 100-120 atmosphere at room temperature for 72 hr. gave a noncrystalline solid which was chromatographed over alumina (grade I 30 g.). Ether/light petroleum (1:19) (500 ml.) eluted 12α-amino-5β-cholane (650 mg.) m.p. and mixed m.p. 102-107°C. Ether/light petroleum (2:3) (500 ml.) eluted a mixture of 12α-amino-5β-cholane and 12-oximino-5β-cholane (300 mg.) as a gum (infrared spectrum). The 12α-amino-5β-cholane could be isolated from this mixture after acid hydrolysis of the oxime into the ketone as described in (a).

(c) Catalytic hydrogenation of 12-oximino-5β-cholane with platinum oxide in acetic acid at atmospheric pressure without the presence of perchloric acid proceeded very poorly. After prolonged shaking (48 hr.) the product still contained 12-oximino-5β-cholane as the major component as demonstrated by acid hydrolysis of the mixture. The amine could not be obtained in more than 15% yield.

Reduction of 12-Oximino-5β-Cholane with Lithium in Liquid Ammonia:
A suspension of 12-oximino-5β-cholane (100 mg.) in liquid ammonia (ca 15 ml.) was treated with thin slices of lithium metal (ca 500 mg.) during a period of one hr. while the reaction mixture was efficiently stirred. The ammonia was allowed to distil at room temperature overnight and the residue was extracted with light petroleum to yield a gum (96 mg.). Chromatography over alumina (grade III 30 g.) using light petroleum as eluant gave 5β-cholane (40 mg.), recrystallisation from methanol afforded needles (30 mg.) m.p. and mixed m.p. 88-91°C. (infrared spectrum identical with that of the 5β-cholane). Ethanol eluted unchanged 12-oximino-5β-cholane (46 mg.) m.p. and mixed m.p. 139-141°C (infrared spectrum identical with that of the authentic specimen).

12-Nitrimino-5β-Cholane: A solution of 12-oximino-5β-cholane (100 mg.) in acetic acid (25 ml.) containing crystalline sodium acetate (50 mg.) was treated dropwise with a solution of sodium nitrite (75 mg.) in water (0.5 ml.) in the cold. The turbid mixture was made homogeneous by the addition of more acetic acid (8 ml.) but soon a white crystalline material was thrown out of solution. After allowing the reaction mixture to stand at room temperature for 3 hr. the 12-nitrimino-5β-cholane was removed by filtration, washed with dilute acetic acid and dried (80 mg.) m.p. 120-122°C. Recrystallisation from methanol gave needles, m.p. 122-123°C, $[\alpha]_D^{19}$ + 143.3° (c = 2.0). (Found: C, 74.01; H, 10.23; N, 6.36. $C_{24}H_{40}O_2N_2$ requires C, 74.22; H, 10.31; N, 7.22%).

A solution of 12-nitrimino-5β-cholane (25 mg.) in ether (5 ml.) was stirred with 880 ammonia (ca 2 ml.) for 4 hr. On removing the solvent after washing (water), the gum was recrystallised from methanol to yield needles of 12-oxo-5β-cholane (20 mg.) m.p. and mixed m.p. 112-118°C. The ketone was further characterised by infrared analysis.

A solution of 12-nitrimino-5β-cholane (100 mg.) in ether (20 ml.) was treated with an excess of liquid ammonia for 12 hr. The residue was digested with light petroleum to yield a crystalline material (90 mg.) showing a carbonyl absorption (1700 cm^{-1}) and a band at 1630 cm^{-1}. Chromatographic fractionation of this product over alumina (grade I 10 g.) using light petroleum as eluant gave a hydrocarbon (infrared spectrum) (11 mg.): recrystallised from methanol, plates m.p. 90-91°C. Elution with ether/light petroleum (1:9) gave 12-oxo-5β-cholane (75 mg.), recrystallised from methanol, needles, m.p. and mixed m.p. 120-121°C (infrared spectrum identical with that of an authentic specimen).

Diazotisation of 12α-Amino-5β-Cholane: A solution of 12α-amino-5β-cholane (900 mg.) in 90% aqueous acetic acid (70 ml.) was treated with a solution of sodium nitrite (900 mg.) in 80% aqueous acetic acid (20 ml.) at 0°C. The mixture, which threw down a semicrystalline precipitate, was kept at 0°C with frequent stirring for 2 hr. After storing at 0°C for 24 hr. the temperature of the reaction mixture was raised slowly to 22°C when brown fumes were evolved. Dilution of the mixture with water yielded further solid material, which was extracted with light petroleum, washed

(water), and dried (anhydrous sodium sulphate). The solvent was removed in vacuo to yield a gum (860 mg.). The infrared spectrum of this gum showed absorption characteristic of the 12α-hydroxyl group (3570 cm^{-1}), a carbonyl group (1700 cm^{-1}) and double bonds (902, 1600 and 1630 cm^{-1}). The gum (800 mg.) was subjected to chromatography over alumina (grade I Woelm, neutral 45 g.). Light petroleum (20 ml.) eluted an unsaturated crystalline hydrocarbon fraction (158 mg.) (infrared spectrum and tetranitromethane test). Elution with ether/light petroleum (1:19, 80 ml.) gave 12-oxo-5β-cholane (225 mg.) m.p. and mixed m.p. 118-120°C (infrared spectrum correct). Progressively increasing concentrations of ether in light petroleum (300 ml.) slowly eluted 12α-hydroxy-5β-cholane (181 mg.) and ethanol/ether (1:49, 50 ml.) eluted the residual 12α-hydroxy-5β-cholane (180 mg.). Elution with higher concentrations of ethanol in ether yielded a very small quantity of gummy material.

Gas liquid chromatography of the unsaturated hydrocarbon fraction indicated the presence of four components, divisible into two pairs on the basis of their retention times. The pair having the lower retention times, and which were calculated to be ca 70% of the total material, had retention times very close to that of 5α and 5β cholanes. A specimen mixed with authentic Δ^{11}-5β-cholene, showed a sharp increase in the height of the first component belonging to the pair with lower retention times. The pair with the higher retention times were concluded to be jervicholenes. The infrared spectrum of the mixture of hydrocarbons showed absorption of moderate intensity at 902 cm^{-1} which is characteristic

of a vinylic methylene group. As no such function is possible in the unrearranged steroid nucleus but is present in one of the jervi hydrocarbons resulting from the jervi rearrangement, this absorption in the infrared gives good support for the conclusion that the two hydrocarbons with the high retention times are indeed jervicholenes.

The unsaturated hydrocarbon fractions, after complete (negative tetranitromethane test and infrared spectrum) hydrogenation in acetic acid with platinum oxide as catalyst (24 hr.) showed only two components on gas liquid chromatography, each component corresponding approximately, in retention time, to one pair of the unsaturated hydrocarbons. The component of lower retention time gave a significant increase in peak height on admixture with authentic 5β-cholane and showed a split peak on admixture with Δ^{11}-5β-cholene.

Action of Nitrous Acid on 12α-Hydroxy-5β-Cholane: A solution of 12α-hydroxy-5β-cholane (200 mg.) in 80% aqueous acetic acid (25 ml.) was treated with a solution of sodium nitrite (200 mg.) in 80% aqueous acetic acid (8 ml.) at 0°C and the mixture was allowed to stand at 0°C for 24hrs. Dilution of the reaction mixture with water yielded a light petroleum soluble gum which showed the presence of both a carbonyl group and the 12α-hydroxyl group in the infrared. Chromatographic separation over alumina (grade I, 25 g.) using light petroleum (300 ml.) as eluant gave 12-oxo-5β-cholane (56 mg.). Ether/light petroleum (1:1, 100 ml.), ether (100 ml.), and ethanol/ether (1:40, 50 ml.) eluted 12α-hydroxy-5β-cholane (135 mg.).

Action of Nitrous Acid on 12β-Hydroxy-5β-Cholane: 12β-Hydroxy-5β-cholane (100 mg.) was treated with sodium nitrite (100 mg.) exactly as described for 12α-hydroxy-5β-cholane. The product (108 mg.) readily crystallised on standing, m.p. 52-53°C. The infrared spectrum showed the absence of 12β-hydroxyl absorption but an absorption at 1630 cm^{-1} (nitrite ester). The product also gave a positive test for nitrogen on sodium fusion. Recrystallisation from hot ethanol quantitatively regenerated 12β-hydroxy-5β-cholane.

12α-Dimethylamino-5β-Cholane: A solution of 12α-amino-5β-cholane (200 mg.) dissolved in formic acid (4 ml.) was treated with a 36% aqueous solution of formaldehyde (4 ml.). The turbid mixture, which became homogeneous on warming, was refluxed for 15 hr. Removing the solvent in vacuo gave a solid, which turned oily on treatment with ammonia. Extraction with ether afforded the gummy 12α-dimethylamino-5β-cholane (no N-H absorption in the infrared spectrum). The tertiary base, which resisted crystallisation on repeated chromatography over alumina, gave a crystalline hydrobromide on treatment with 4.8% hydrobromic acid in ether/ethanol (3:7): recrystallised from ethanol, needles, m.p. 280-300°C (decomp.). (Found: C, 68,79; H, 10.58; N, 3.07. $C_{26}H_{47}N$ HBr requires C, 68.72; H, 10.35; N, 3.03%) $[\alpha]_D^{20}$ of the free base + 70.4° (c = 2.4).

12α-Dimethylamino-5β-Cholane Methiodide: (a) 12α-Dimethylamino-5β-cholane (300 mg.) dissolved in methyl iodide was sealed in a Carius tube and heated at 100°C for 6 hr. After cooling, the tube was broken and the methyl

iodide removed by evacuation of the amber coloured solution. Extraction of the residue with light petroleum removed unquaternised material (25 mg.). The light petroleum insoluble residue (296 mg.) was taken up in hot methanol (ca 20 ml.) and the solution was decolourised with activated charcoal. The methanolic filtrate was taken to dryness in vacuo. Recrystallisation from ethyl acetate gave colourless rods of 12α-dimethylamino-5β-cholane methiodide (combined crops 260 mg.) m.p. 290-300°C decomp. (Found: C, 62.97; H, 9.39; N, 2.88. $C_{27}H_{50}I\,N$ requires C, 63.03; H, 9.70; N, 2.71%).

(b) A solution of 12α-amino-5β-cholane (500 mg.) in anhydrous ethanol (25 ml.) was refluxed with methyl iodide (3 ml.) in the presence of anhydrous potassium carbonate (2 g.) for 48 hr. Fresh small quantities of methyl iodide (total 2 ml.) and anhydrous potassium carbonate (total 2 g.) were added during the period of reflux. The ethanolic filtrate from the reaction mixture was taken to dryness in vacuo and the residue was taken up in chloroform, the inorganic material was removed by filtration, and the chloroform was distilled in vacuo to yield a semi-solid material (640 mg.). Extraction of the semi-solid material with light petroleum yielded a white residue of 12α-dimethylamino-5β-cholane methiodide (270 mg.): recrystallisation from ethyl acetate m.p. and mixed m.p. 290-300°C (decomp.). The light petroleum extract after removal of the solvent in vacuo gave unquaternised 12α-dimethylamino-5β-cholane (361 mg.) (infrared spectrum and $[\alpha]_D$). Further 12α-dimethylamino-5β-cholane methiodide was obtained on recycling the tertiary base through the quaternisation procedure.

Hofmann Degradation of 12α-Dimethylamino-5β-Cholane Methiodide:

12α-Dimethylamino-5β-cholane methiodide (200 mg.) was thoroughly mixed with a paste of sodium hydroxide (1 g.) in water (1 ml.) in a pyrex tube, which was inserted into a metal bath at 90°C. The temperature was allowed to rise to 160°C over a period of 30 min. and maintained there for 30 min. when a strong smell of trimethylamine was detected. After allowing the reaction mixture to cool slowly, it was diluted with water and the oil which separated was taken into light petroleum. Washing the ethereal layer with 2N HCl gave the white crystalline 12α-dimethylamino-5β-cholane hydrochloride (20 mg.) (m.p. and mixed m.p. with authentic 12α-dimethylamino-5β-cholane hydrochloride, 217-220°C). The ethereal layer after removal of the solvent in vacuo gave an unsaturated hydrocarbon (tetranitromethane test and infrared spectrum). Recrystallisation of the hydrocarbon from ethanol yielded Δ^{11}-5β-cholene, rods m.p. 77-79°C.

Catalytic hydrogenation of the Δ^{11}-5β-cholene (40 mg.) in acetic acid (8 ml.) with platinum oxide (15 mg.) at room temperature and atmospheric pressure for 20 hr. gave a saturated product (negative tetranitromethane test). Recrystallisation from ethanol afforded 5β-cholane, needles, m.p. 87-89°C (reported[199] 89-90°).

Gas liquid chromatography of the Δ^{11}-5β-cholene showed one single component, while the 5β-cholane obtained on catalytic hydrogenation of the Δ^{11}-5β-cholene on admixture with authentic 5β-cholane also showed one component to be present.

12β-Azido-5β-Cholane: Finely powdered sodium azide (220 mg.) was added to a hot solution of 12α-methanesulphonyloxy-5β-cholane (220 mg.) (p.66) in ethanol (20 ml.) containing water (4 ml.), and the mixture was refluxed for 8 hr. The solvent was removed from the reaction mixture in vacuo and the residue was extracted with ether. The ethereal layer on removal of the solvent gave the gummy 12β-azido-5β-cholane (the infrared spectrum showed a strong absorption at 2075 cm^{-1} and no absorption for a methanesulphonyloxy group or a free hydroxyl group).

Attempted Formation of 12β-Amino-5β-Cholane by Reduction of 12β-Azido-5β-Cholane:

(a) 12β-Azido-5β-cholane (50 mg.) was refluxed with lithium aluminium hydride (ca 100 mg.) in ether (20 ml.) for 10 hr. Working up of the reaction mixture in the usual manner afforded a noncrystalline unsaturated hydrocarbon fraction (40 mg.) (tetranitromethane test and infrared spectrum). Chromatographic purification over alumina (grade I) gave a gum (35 mg.)

(b) A solution of 12β-azido-5β-cholane (200 mg.) in ethanol (10 ml.) was refluxed with a solution of sodium hydrosulphite (40 mg.) in 4N sodium hydroxide (4 ml.) for 2 hr. Dilution of the reaction mixture with water gave a gummy precipitate, which was extracted with ether, washed (water), and dried (anhydrous sodium sulphate). On removing the solvent in vacuo a gummy unsaturated hydrocarbon fraction was obtained (160 mg.) which resisted crystallisation. It gave a positive tetranitromethane test.

(c) 12β-Azido-5β-cholane (100 mg.) was hydrogenated at room temperature and atmospheric pressure in acetic acid (10 ml.) with platinum oxide (20 mg.) for 16 hr. The resultant hydrogenation product (80 mg.) was obtained as a gum showing no azide absorption in the infrared and a broad low absorption in the carbonyl region (1700 cm^{-1}). The material showed no basic properties and on chromatography over alumina (grade III, 10 g.) using ether as eluant gave a saturated hydrocarbon (46 mg.) (negative tetranitromethane test and infrared spectrum). No other fraction could be eluted with ether or ethanol/ether (1:4).

Separate gas liquid chromatography of the unsaturated hydrocarbons from procedures (a) and (b) showed in each case the presence of several components with two components accounting for ca 75% of the total. These major components had very close retention times and the component with the higher retention time showed a sharp increase in intensity on admixture with Δ^{11}-5β-cholene. Gas liquid chromatography of the saturated hydrocarbon obtained from procedure (c) showed five components, with one component predominant (50% of the total). This compound showed a sharp increase in intensity on admixture with 5β-cholane.

12α-Azido-5β-Cholane: Treatment of 12β-methanesulphonyloxy-5β-cholane (p. 68) with sodium azide in the same manner as described for the preparation of the 12β-azido-5β-cholane yielded 12α-azido-5β-cholane in comparable yield. This azido compound was also obtained as a gum and showed characteristic azide absorption at 2075 cm^{-1} in the infrared

spectrum.

Lithium aluminium hydride reduction of the 12α-azido-5β-cholane employing the usual procedure gave a neutral gum which gave a positive test for unsaturation with tetranitromethane. Gas liquid chromatography of this product showed six components to be present, with two representing ca 80% of the total material, having retention times similar to those of Δ^{11}-5β-cholene and the jervi hydrocarbons obtained by diazotisation of 12α-amino-5β-cholane (p. 98). The component with the lower retention time showed an increase in peak-height on admixture with Δ^{11}-5β-cholene.

Action of Sodamide on 12α-Methanesulphonyloxy-5β-Cholane: A solution of 12α-methanesulphonyloxy-5β-cholane (100 mg.) in toluene (10 ml.) was refluxed with sodamide (75 mg.) for 4.5 hr. The excess of sodamide was decomposed with water, and the ethereal layer separated, washed (water) and dried (anhydrous sodium sulphate). The residue obtained after removing the solvent in vacuo (80 mg.) was chromatographed over alumina (grade III, 10 g.). Ether/light petroleum (1:9) eluted 12-oxo-5β-cholane (30 mg.) (mixed m.p. and infrared spectrum). Ether/light petroleum(1:1) eluted 12α-hydroxy-5β-cholane (45 mg.) (m.p. and mixed m.p. and infrared spectrum).

12β-Iodo-5β-Cholane: A solution of 12α-methanesulphonyloxy-5β-cholane (200 mg.) in acetone (5 ml.) was heated with sodium iodide (200 mg.) in a sealed Carius tube at 100°C for 4 hr. After cooling, the tube was broken and the white crystalline material was removed by filtration.

The acetone filtrate was decolourised with charcoal and on removing the solvent *in vacuo* gave *12β-iodo-5β-cholane* as a colourless gum.

A solvent free specimen on keeping at room temperature, slowly liberated iodine. Extraction of the dark coloured material with light petroleum and removal of the solvent yielded an unsaturated hydrocarbon mixture (tetranitromethane test, bromine decolourisation test, potassium permanganate test and infrared spectrum). Gas liquid chromatography indicated that this gum was a mixture of several components.

A freshly prepared specimen of 12β-iodo-5β-cholane when treated with sodamide in toluene under reflux for 6 hr., gave a saturated noncrystalline hydrocarbon (infrared spectrum and a negative tetranitromethane test).

A solution of 12β-iodo-5β-cholane in ether, on standing at room temperature for several days, did not show any signs of decomposition.

Leuckart Reaction on 12-Oxo-5β-Cholane: A solution of 12-oxo-5β-cholane (250 mg.) in formamide (10 ml.) containing formic acid (0.25 ml.) was refluxed at *ca* 200°C for 5 hr. The dark reaction mixture on dilution with methanol gave a dark tarry precipitate, which was removed by filtration. The combined methanolic filtrates, after removal of the solvent *in vacuo* yielded a brown solid, which was repeatedly washed with water and dried (200 mg.). Crystallisation from methanol gave colourless rods of a formyl derivative of an amine, m.p. 210-220°C (infrared spectrum). This formyl derivative was not characterised

further and was directly hydrolysed to the free primary amine as described below.

The formyl derivative (100 mg.) dissolved in methanol (18 ml.) containing 36% aqueous hydrochloric acid (2 ml.) was refluxed for 2 hr. and then allowed to cool overnight. The deposited white needles were removed by filtration, washed (methanol) and dried, m.p. 76-82°C. This material gave a positive test with the tetranitromethane. Gas liquid chromatography showed the presence of two components in equal amounts and having very close retention times. The methanolic filtrate, on removing the solvent yielded crystalline 12α-amino-5β-cholane hydrochloride (61 mg.). Recrystallisation from methanol gave needles. The hydrochloride was treated with aqueous ammonia in the presence of ether and the ethereal layer containing the free base was washed (water) and dried (anhydrous sodium sulphate). On removal of the solvent, 12α-amino-5β-cholane was obtained, and recrystallised from methanol, m.p. and mixed m.p. 102-106°C (the infrared spectrum was identical with that of an authentic specimen).

Reduction of 12-Oxo-5β-Cholane with Lithium Aluminium Tri-tertiary-butoxy-hydride: A solution of 12-oxo-5β-cholane (100 mg.) in ether (10 ml.) was refluxed with a suspension of lithium aluminium tri-tertiarybutoxy-hydride in ether for 16 hr (the tri-tertiarybutoxyhydride was prepared by treating a suspension of lithium aluminium hydride in ether with three moles of tertiary butanol, the suspension being used directly for

the reduction). On working up the reduction mixture in the normal manner a crystalline solid (95 mg.) resulted. Chromatography over alumina (grade I, Woelm, neutral, 10 g.) with ether/light petroleum (1:4) as eluant gave 12α-hydroxy-5β-cholane (80 mg.) (identified by comparison with an authentic specimen). Ether eluted 12β-hydroxy-5β-cholane (15 mg.).

Catalytic Hydrogenation of 12-Oxo-5β-Cholane: A solution of 12-oxo-5β-cholane (300 mg.) in acetic acid (13 ml.) containing perchloric acid (0.1 ml.) was added to a suspension of prereduced platinum catalyst (50 mg.) in acetic acid. The mixture was allowed to shake under hydrogen at room temperature and atmospheric pressure. The calculated volume of hydrogen was taken up within 30 min. After 1 hr. the catalyst was removed by filtration. The filtrate was distilled to yield a gum (283 mg.) (the infrared spectrum was identical with that of 12α-hydroxy-5β-cholane). Chromatography of this product over alumina (grade V, 20 g.) using light petroleum as eluant (20 ml.) gave a noncrystalline saturated hydrocarbon (40 mg.) (infrared spectrum and negative tetranitromethane test). Ether/light petroleum (1:9) eluted 12α-hydroxy-5β-cholane (203 mg.). Further elution with ethyl acetate/ether (1:5) and ethyl acetate gave only trace amounts of noncrystalline material.

(b) In another experiment, employing the same conditions as above but without the presence of perchloric acid, the rate of hydrogenation was extremely slow. The reduction product isolated after 24 hr. shaking

under hydrogen still contained unreduced ketone (infrared spectrum). Chromatographic separation over alumina in the usual manner gave 12-oxo-5β-cholane and 12α-hydroxy-5β-cholane in the ratio of 3:4.

Reduction of 7-oxo-5β-Cholane with Lithium Aluminium Hydride: A solution of 7-oxo-5β-cholane (200 mg.) in ether (20 ml.) was refluxed with a slurry of lithium aluminium hydride (300 mg.) in ether (30 ml.) for 16 hr. Working up of the reaction mixture in the usual manner gave a gum (200 mg.) which was chromatographed over alumina (grade V, 20 g.). Ether/light petroleum (1:1, 100 ml.) eluted 7α-hydroxy-5β-cholane (165 mg.) m.p. and mixed m.p. 83-87°C. (The infrared spectrum was identical with that of an authentic specimen). Elution with ethylacetate/ether (1:1, 50 ml.) gave 7-β-hydroxy-5β-cholane (7 mg.) (identified by its infrared spectrum).

Catalytic Hydrogenation of 7-oxo-5β-Cholane: A solution of 7-oxo-5β-cholane (200 mg.) in acetic acid (5 ml.) was added to a prereduced suspension of platinum catalyst (40 mg.) in acetic acid (5 ml.) containing perchloric acid (0.1 ml.) and the mixture was allowed to shake under hydrogen at room temperature and atmospheric pressure. The uptake of hydrogen was extremely slow and the shaking was continued for 16 hr. During this time a crystalline material appeared in the reduction mixture, which was removed by filtration along with the catalyst. Extraction of the crystalline material from the catalyst with ether and subsequent removal of the solvent gave 5β-cholane (60 mg.) m.p. and mixed m.p. 86-88°C (the infrared spectrum was identical with that of authentic 5β-cholane).

Removal of the solvent *in vacuo* from the filtrate of the hydrogenation mixture gave a gum (150 mg.). Chromatographic separation of this gum over alumina (grade V, 20 g.) using light petroleum as eluant gave 7-oxo-5β-cholane (71 mg.) whilst ether/light petroleum (1:9) gave 7α-hydroxy-5β-cholane (68 mg.). Both these products were identified by comparison with authentic specimens.

12-Oximino-5α-Cholane: 12α-Hydroxy-5α-cholane (p. 60) (1 g.) on oxidation with chromic oxide in acetic acid as described for the oxidation of 12α-hydroxy-5β-cholane (p. 67) gave 12-oxo-5α-cholane (920 mg.) m.p. 164-165°C, $[\alpha]_D^{20}$ + 95.0° (c = 2.0). (The infrared spectrum showed strong absorption for the carbonyl group (1700 cm^{-1}) and total absence of hydroxyl absorption).

Treatment of a solution of 12-oxo-5α-cholane (900 mg.) in ethanol (160 ml.) with hydroxylamine hydrochloride (350 mg.) and sodium acetate (700 mg.) in water (3 ml.) in the same way as described for 12-oximino-5β-cholane yielded 12-oximino-5α-cholane (810 mg.) recrystallised from ethanol, needles, m.p. 90-91°C, $[\alpha]_D^{20}$ + 151.0° (c = 2.0). (Found: C, 80.32; H, 11.80; N, 4.06, $C_{24}H_{41}ON$ requires C, 80.22; H, 11.59; N, 3.90%).

12α-Amino-5α-Cholane: (a) A solution of 12-oximino-5α-cholane (500 mg.) in ether (30 ml.) was refluxed with a slurry of lithium aluminium hydride (ca 500 mg.) in ether (25 ml.) for 16 hr. After working up the reaction mixture in the usual manner the crude amine was obtained as a gum.

This gum (showing complete conversion of the oximino group into the amino group from its infrared spectrum) was chromatographed over alumina (grade I Woelm, neutral, 20 g.). Light petroleum (100 ml.) eluted a neutral gum (32 mg.). Ether/light petroleum (300 ml.) eluted 12α-amino-5α-cholane as a gum (390 mg.), $[\alpha]_D^{20}$ + 42° (c = 2.1). Treatment of the noncrystalline base with methanolic hydrochloric acid (5%) gave needles of 12α-amino-5α-cholane hydrochloride, recrystallised from methanol, m.p. 140°C (Found: C, 75.04; H, 11.01, $C_{24}H_{43}N$ HCl requires C, 75.40; H, 11.51%). The hydrochloride was soluble in ether.

Elution of the alumina column with ether (200 ml.) gave a gum (90 mg.) which contained further 12α-amino-5α-cholane, separable as the hydrochloride (24 mg.) after treatment of the crude gum with methanolic hydrochloric acid followed by extraction of the neutral component with light petroleum.

(b) A solution of 12-oximino-5α-cholane (500 mg.) in boiling ethanol (20 ml.) was treated with sliced sodium metal (3 g.) during a period of 1 hr. The resulting base was isolated as a gum (480 mg.). Chromatography over alumina (grade I Woelm, neutral 20 g.) using ether/light petroleum (1:3, 250 ml.) gave 12α-amino-5α-cholane as a gum (300 mg.), $[\alpha]_D^{20}$ + 41.5°: hydrochloride, needles from methanol, m.p. and mixed m.p. 138-140°C (the infrared spectra of both the base and its hydrochloride were identical with those of authentic specimens). Elution with ether (200 ml.) gave a gum (100 mg.) which showed the presence of the same

amino-compound in the infrared spectrum and which did not show any absorption from an oximino group.

(c) 12-Oximino-5α-cholane (200 mg.) dissolved in acetic acid (10 ml.) was added to a prereduced suspension of platinum catalyst (50 mg.) in acetic acid (3 ml.) containing perchloric acid (0.1 ml.). The mixture was shaken under hydrogen at room temperature and atmospheric pressure for 24 hr. Working up of the reaction mixture gave a gum (200 mg.) (the infrared spectrum of which showed absorption attributable to an oximino group and to an amino group). Chromatography over alumina (grade I Woelm, neutral, 15 g.) using ether/light petroleum (1:3, 150 ml.) eluted 12α-amino-5α-cholane (61 mg.) (identified as the free base and as the hydrochloride). Elution with ethanol/ether (1:9) gave unchanged 12-oximino-5α-cholane (85 mg.). No evidence was obtained for the formation of 12β-amino-5α-cholane.

Diazotisation of 12α-Amino-5α-Cholane: A solution of 12α-amino-5α-cholane (100 mg.) in acetic acid (8 ml.) was diluted with water (1 ml.). This mixture was treated with a solution of sodium nitrite (100 mg.) in 80% aqueous acetic acid (6 ml.) at 0°C, and the mixture was allowed to stand at 0°C for 20 hr. The temperature was then raised slowly to 22°C when the reaction mixture was diluted with water. The white precipitate was extracted with ether, and the ethereal extract washed with water and dried over anhydrous sodium sulphate. The gummy residue (80 mg.) obtained after removing the ether *in vacuo* showed absorption characteristic of

the 12α-hydroxyl group (3400 cm^{-1}), a carbonyl group (1700 cm^{-1}) and a double bond (1660 cm^{-1}). Chromatography over alumina (grade I, Woelm, neutral 30 g.) using light petroleum as eluant (250 ml.) gave an unsaturated hydrocarbon fraction (7 mg.). Ether/light petroleum (1:3, 100 ml.) yielded 12-oxo-5α-cholane (18 mg.) contaminated with some unsaturated compound (infrared spectrum). Ether (100 ml.) gave pure 12-oxo-5α-cholane (27 mg.). Ethanol/ether (1:19, 50 ml.) eluted 12α-hydroxy-5α-cholane (28 mg.) containing a trace of 12-oxo-5α-cholane. Both the 12-oxo-5α-cholane and the 12α-hydroxy-5α-cholane were characterised by means of mixed m.p. with authentic specimens and by the comparison of infrared spectra.

12α-Dimethylamino-5α-Cholane Methiodide and 12α-Dimethylamino-5α-Cholane: 12α-Amino-5α-cholane (117 mg.) was refluxed with methyl iodide (2.5 ml.) in anhydrous ethanol (15 ml.) containing anhydrous potassium carbonate (480 mg.) for 72 hr. Small quantities of methyl iodide (total 1.5 ml.) and potassium carbonate (total 200 mg.) were added to the reaction mixture during the refluxing period. Potassium carbonate was removed from the reaction mixture by filtration and the filtrate was distilled in vacuo. The residue was digested with chloroform to remove any inorganic material. The chloroform soluble material, after removing the solvent, was extracted with light petroleum. The light petroleum insoluble residue was recrystallised from ethanol, to yield plates of 12α-dimethylamino-5α-cholane-methiodide (101 mg.), m.p. 225-235°C decomp.

(Found: C, 63,74; H, 9.39; N, 2.50; $C_{27}H_{50}I N$ requires C, 63.03; H, 9.70; N, 2.71%). The light petroleum soluble gum (30 mg.) on treatment with a solution of hydrobromic acid in methanol (4.5%) gave white needles of 12α-dimethylamino-5α-cholane hydrobromide, recrystallised from methanol, needles, m.p. 190-205°C decomp. (Found: C, 68.79; H, 10.58; N, 3.07. $C_{26}H_{47}N$ HBr requires C, 68.72; H, 10.35; N, 3.09%).

Hofmann Degradation of 12α-Dimethylamino-5α-Cholane Methiodide: 12α-Dimethylamino-5α-cholane methiodide (15 mg.) was mixed with a paste of sodium hydroxide (400 mg.) in water (ca .5 ml.) in a pyrex tube, which was inserted in a metal bath at 90°C and the temperature raised to 160°C during a period of 10 min. The mixture was heated at 160°C for 30 min., during which time the mixture gave a strong smell of trimethylamine. After cooling, the reaction mixture was diluted with water and the precipitated material extracted with ether. The ethereal extract after washing with water, was extracted with 2N HCl. The acid extract on treatment with sodium hydroxide did not give any precipitate or turbidity. The ethereal layer on removing the solvent yielded a gummy hydrocarbon (7 mg.).

5α-Cholane: This compound was prepared by application of the Huang Minlon[150] procedure to 12-oxo-5α-cholane as described for the preparation of 5β-cholane from 12-oxo-5β-cholane (p. 65). 12-oxo-5α-cholane (100 mg.) afforded ca 60 mg. of 5α-cholane, recrystallised from ethanol, m.p. 68-70°C (the infrared spectrum showed absence of the carbonyl absorption).

SECTION IV

SYNTHESES OF STEROIDAL BISQUATERNARY SALTS.

In view of the considerations outlined on pp.16-23 (Section I) attention was concentrated on securing the syntheses of the three compounds 3α,7α-bis(dimethylamino)-5α-cholestane dimethiodide, 3α,12α-bis(dimethylamino)-5β-cholane dimethiodide and 3α,17α-bis(dimethylamino)-5α-androstane dimethiodide. These compounds which should have a favourable lipophilic-hydrophilic balance (believed to be a primary factor in determining neuromuscular blocking potency[200]) will be discussed in turn.

3α,7α-Bis(Dimethylamino)-5α-Cholestane Dimethiodide:

The attempted preparation of this compound appeared to be of some importance since models show the intergroup distance (as measured from the centres of the atoms concerned) between axial substituents on C-3 and C-7 in trans A/B steroids to be ca.5.1Å. whilst the effective radius of the trimethylammonium group is ca. 3Å. This would be expected to make 3α,7α-bis (dimethylamino)5α-cholestane dimethiodide subject to steric strain, in addition to unfavourable charge repulsions, and so the very possibility of the existence of the compound was questionable.

Difficulties in achieving complete quaternisation of non-rigid compounds of the type $-N-(CH_2)_n-X-(CH_2)_n-N-$ where n is less than 5 have been reported in the literature[201] although trisammonium compounds of this type where n = 2 or 3 have been prepared[202] In such compounds both steric strain and charge repulsions can of course be relieved by rotation about carbon-carbon bonds. It is

to be noted, however, that the low activity in polymethylene bisquaternary compounds $^{+}N\text{-}(CH_2)_n\text{-}N^{+}$ where n = 2,3 or 4 has been ascribed to onium group interaction.[203]

Attempted preparation of 3α,7α-diamino-5α-cholestane by reduction of 3,7-dioximino-5α-cholestane was limited to the catalytic hydrogenation procedure as sodium ethanol reduction would afford the 3β,7β-diamino compound whilst lithium aluminium hydride reduction would be expected to give a complex mixture of four compounds due to the formation of epimeric amines at both C-3 and C-7.

Catalytic hydrogenation using prereduced platinum oxide catalyst in acetic acid in the presence of perchloric acid failed to give complete reduction of 3,7-dioximino-5α-cholestane as evidenced by the appearance of oxime absorption at 3200, 1630 and 930-960 $cm.^{-1}$ in the infrared spectrum of the crude product. Application of alumina chromatography using a variety of grades of alumina resulted in the loss of almost all of the material which could not be eluted from the column. Presumably this lost material was in fact the desired 3α,7α-diamino compound which was chelating on the alumina. Similar chelation was observed[204] with 2-endo-hydroxy-3-endo-aminobornane which could not be eluted from alumina columns.

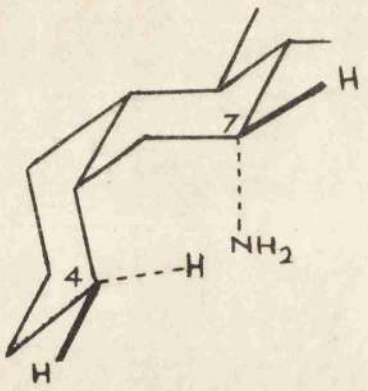


Fig. 15

All attempts to separate 3α,7α-diamino-5α-cholestane from unreacted or partially reduced 3,7-dioximino-5α-cholestane by means of bishydrochloride formation were unsuccessful.

It is to be noted that extreme difficulty was observed in the catalytic hydrogenation of 7-oximino-5β-cholane and in no case could any basic product be isolated. In this case, however, an amino group in the 7α-configuration would be expected to be greatly unfavoured sterically since the cis A/B ring junction introduces a non-bonded interaction between the 7α substituent and C-4 (Fig.15) - an interaction which is absent in 5α steroids. In this connection, it is to be noted, however, that it has been claimed[205] that 3α-hydroxy-7-oxo-5β-cholanic acid yields solely the 7α-hydroxy compound on reduction with sodium in butanol or ethanol, or on reduction with sodium borohydride, whilst reduction with sodium and propanol gave the 7β-epimer.

These observations would seem erroneous in view of the results obtained in Section II of this thesis where it was shown that 7-oxo-5β-cholane on treatment with sodium and ethanol gave *ca.* 85% of the β-hydroxy compound whilst reduction with lithium aluminium hydride gave 90% of the α-hydroxy compound.

An alternate route to 3α,7α-bis(dimethylamino)-5α-cholestane dimethiodide was then investigated. Thus 3β,7β-dihydroxy-5α-cholestane, prepared by the lithium liquid ammonia reduction of

7-oxo-cholesteryl acetate, was converted into the di-methanesulphonate ester and this latter compound was heated in a sealed tube with dimethylamine to achieve S_N2 displacement at both C-3 and C-7. Such nucleophilic displacements by dimethylamine with inversion of configuration have previously been performed at C-3 in steroids.[206] Heating 3β,7β-dimethanesulphonyloxy-5α-cholestane with dimethylamine in a sealed tube at 100°C for 5 hours failed to give nucleophilic displacement and unchanged starting material was reclaimed in 75% yield. However heating the dimethanesulphonate in ethanol-dimethylamine with added p-toluenesulphonic acid[206] in a sealed tube at 100°C for 20 hours afforded the bis-dimethylamino compound in good yield as a gum. It is possible that the reaction was not purely S_N2 at C-7 giving a mixture of epimers at this position, but the displacement at C-3 is known to proceed with inversion.[211] The gum showed no hydroxyl absorption between 3600-3100 $cm.^{-1}$ in the infrared and no absorption due to residual methanesulphonyloxy groups but gave prominent peaks at 2825 and 2780 $cm.^{-1}$ ($N-CH_3$ groups). It was insoluble in water but soluble in dilute hydrochloric acid.

Without further characterisation the gum was heated in a sealed tube with excess of methyl iodide at 100°C for 5 hours. The reaction mixture became very dark and the brown product obtained was divisible into a non-bitter water insoluble fraction and a smaller bitter water-soluble fraction which showed hydroxyl

absorption and evidence of double bond absorption in the infrared.

It was therefore concluded, in view of the ease of formation of 3α,12α-bis(dimethylamino)-5β-cholane dimethiodide on quaternisation of the corresponding diamine under the same conditions, that steric strain was indeed too great at an interonium distance of 5.1Å.or less to permit the existence of rigid bisquaternary salts and that all compounds listed on page 21 of section I which would have interonium distance of 5.1Å. or less would be incapable of preparation.

3α,12α-Bis(Dimethylamino)-5β-Cholane Dimethiodide:

Preparation of this compound was attempted from 3,12-dioximino-5β-cholane through reduction to the 3α,12α-diamino compound by lithium aluminium hydride. This reagent is known to give solely the 3α-amine from the 3-oxime in steroids possessing a cis A/B ring fusion[207] and it was also established (section III of this thesis) to give solely the 12α-amine from the 12-oxime in the cholane series.

The 3α,12α-diamino-5β-cholane resulting from the reduction could not be eluted from an alumina column on attempted chromatographic purification, and was directly subjected to vigorous quaternisation conditions, without intermediate conversion into the bis-dimethylamino compound, giving the water-soluble bitter-tasting bisquaternary compound in high yield.

3α,17α-Bis(Dimethylamino)-5α-Androstane Dimethiodide.

The attempted preparation of this compound appeared to be of some theoretical importance in view of the current trend towards a more rational basis for the design of new biologically active compounds. Application of the receptor theory of drug action and the theory of metabolite displacement has, to a certain degree, eliminated some of the purely empirical approach to the synthesis of new drugs capable of exerting specifically desired effects, and indeed application of the second theory has given rise to what has been termed[208] 'the revolution in pharmacology'. Moreover an inspired application of the receptor theory of drug action has recently led to the synthesis of a new class of anabolic steroids.[209] It was assumed that the receptors concerned with the androgenic and anabolic properties of the natural male hormones differ somewhat in their nature and accordingly attention was concentrated on securing an alteration of the inter-group distance between the substituents at C-3 and C-17 with an accompanying change in the nucleophilicity of the C-3 substituent. These considerations led logically to the preparation of pyrazolo and isoxazolo steroids which showed a very high anabolic to androgenic ratio.[209,210]

Since the completely rigid toxiferin I with its fixed interonium distance of 9.7 Å.[62,64] is a powerful neuromuscular

blocking agent as are other non-rigid bisquaternary compounds whose interonium distance is also believed to lie in this region,[60,61,62,63] it was of considerable interest that the rigid 3α,17α-bis(dimethylamino)-5α-androstane dimethiodide with an interonium distance of 9.5Å. be made available for pharmacological study.

The known steroids possessing nitrogen functions in the 3 and 17 positions viz. 3ξ,17ξ-diamino-5α-androstane,[211] 3ξ,17β-diaminoandrost-4-ene[212] and 3ξ,17ξ-bis(dimethylamino)-androstane[211] can safely be concluded to have the amino functions of unassigned stereochemistry in the β configuration as these amino groups resulted from reduction of the corresponding oximes under equilibrating conditions.

In order to obtain the 3 and 17 nitrogen functions in the α configuration, therefore, it was necessary to convert 3β,17β-dihydroxy-5α-androstane (prepared by the reduction of 3,17-dioxo-5α-androstane with sodium in ethanol) into the dimethanesulphonate ester, upon which the requisite nucleophilic displacements could be performed. Application of the normal procedure for preparation of methane-sulphonates (methanesulphonyl chloride in pyridine at 0°C for 16 hours) failed to give complete esterification as shown by the presence of hydroxyl stretching in the infrared spectrum of the product. However treatment of the diol with excess of methane-sulphonyl chloride in pyridine at room temperature for 12 hours gave

a satisfactory yield of the disulphonate ester as shown by the complete lack of OH absorption in the infrared spectrum of the crystalline product.

Nucleophilic displacement of the methanesulphonyloxy group in the 3β-position was not expected to involve any complications[206,211] but in view of the known[213] difficulties involved in effecting such reactions in the neopentyl type system at C-17, it was concluded that vigorous conditions would be needed in order to secure introduction of the 17α-dimethylamino group. Indeed heating the dimethanesulphonate of 3β,17β-dihydroxy-5α-androstane in ethanol/dimethylamine in the presence of p-toluenesulphonic acid, in a sealed tube at 100°C for 20 hours failed to effect displacement of both methanesulphonyloxy groups as evidenced by the infrared spectrum of the product (insoluble in aqueous hydrochloric acid) which showed both $N\text{-}\overset{C\text{-}H}{CH_3}$ stretching at 2750 $cm.^{-1}$ and sulphonate ester absorption at 960, 1170 and 1240 $cm.^{-1}$

Application of the recently introduced[214] method for effecting nucleophilic displacements of 17β substituents through the use of N-methylpyrrolidone/tert.-butyl alcohol (19:1) was therefore investigated. Heating 3β,17β-dimethanesulphonyloxy-5α-androstane with dimethylamine in N-methylpyrrolidone/tert.-butyl alcohol in a sealed tube for 60 hours at 100°C however still gave replacement of only one methanesulphonyloxy group (concluded to be

at the 3-position). A final attempt using added sodium acetate to promote a 'pull' mechanism was made. This also failed to effect the required displacement of both methanesulphonyloxy groups to afford the desired bis-dimethylamino compound. Infrared analysis and direct comparison of the product with the compound obtained in the previous experiment showed the formation of the same mono-dimethylamino derivative.

EXPERIMENTAL

3β,7β-Dihydroxy-5α-cholestane: A solution of 7-keto-cholesteryl acetate (1.0 g.) in a mixture of ether, dioxane and ethanol (30 ml. 30 ml. and 20 ml.) was placed in a round bottom flask fitted with an efficient stirrer and containing liquid ammonia (ca. 50 ml.). Sliced lithium metal (ca.3g.) was added to this mixture during a period of 2.5 hr. with constant stirring. The ammonia was allowed to distil at room temperature overnight. The residual material was taken up in dilute hydrochloric acid and the ethereal layer was repeatedly washed with water and dried. On removing the solvent in vacuo, the ethereal layer yielded crude 3β,7β-dihydroxy-5α-cholestane (0.8 g.), m.p. 145-153°. A solution of this material in ethyl acetate, on filtration through a column of alumina (grade III, 20 g.) gave pure diol (0.67 g.), m.p. 160-166°C (reported[215] 166°C). (the infrared spectrum showed complete reduction of the carbonyl functions of the starting material).

3,7-Dioxo-5α-cholestane: 3β,7β-Dihydroxy-5α-cholestane (200 mg.) dissolved in acetic acid (5 ml.) was treated with a solution of chromic oxide (125 mg.) in 96% aqueous acetic acid (3 ml.) and allowed to stand at room temperature for 16 hr. Dilution with water gave a white precipitate (180 mg.). Recrystallisation from methanol gave plates (140 mg.) of dioxo-cholestane, m.p. 188-190°C (reported[216] 190°).

3,7-Dioximino-5α-cholestane: 3,7-Dioxo-5α-cholestane (140 mg.) was refluxed with hydroxylamine hydrochloride (105 mg.) and sodium acetate (210 mg.) in ethanol (22ml.) containing water (0.5 ml.) for 6 hr. The reaction mixture was then concentrated by distillation *invacuo* to *ca.* 4 ml. and the crystalline material was collected by filtration (145 mg.) m.p. 255-265°C (decomp.). Recrystallisation from methanol gave pure 3,7-dioximino-5α-cholestane (130 mg.), m.p. 260-265°C (decomp.). (Found: C,75.06; H,10.57; N,6.70. $C_{27}H_{46}O_2N_2$ requires C,75.35; H,10.69; N,6.51%).

Attempted Catalytic Hydrogenation of 3,7-Dioximino-5α-cholestane: 3,7-Dioximino-5α-androstane (70 mg.) was shaken with a pre-reduced suspension of platinum catalyst (35 mg.) in acetic acid (12 ml.) containing a drop of perchloric acid under hydrogen at atmospheric pressure and room temperature for 48 hr. The uptake of hydrogen during this period was extremely slow but the theoretical volume was consumed. The catalyst was removed by filtration and the filtrate, on taking off the solvent *in vacuo* gave a colourless glass (ca. 80 mg.). This 'glass' was freely soluble in water and gave a copious precipitate on treatment with alkali. The precipitated base was extracted with ether, and on removal of the solvent a gum (61 mg.) resulted. This material showed absorption characteristic of the oximino group in the infrared spectrum and also a weak band characteristic of an amino group between 1650-1600 $cm.^{-1}$ The

gummy base formed a solid hydrochloride which could not be converted into a well defined crystalline form. The free base (50 mg.) on attempted chromatography over a column of alumina (grade V, 10 g.) could not be eluted.

7-Oximino-5β-cholane: A solution of 7-oxo-5β-cholane (500 mg.) in methanol (25 ml.) was treated with a solution of hydroxylamine hydrochloride (150 mg.) and sodium acetate (300 mg.) in water (1.5 ml.). After heating the mixture at 100°C for 5 min., it was allowed to stand at room temperature (24 hr.). Dilution of the reaction mixture with water (25 ml.) gave a white precipitate which was collected by filtration, washed with water and dried (500 mg.). A concentrated methanolic solution of this material deposited a white non-crystalline solid (405 mg.) m.p. 60-65°C. (Found: C,79.97; H,11.31; N,4.08. $C_{24}H_{41}ON$ requires C,80.22; H,11.51; N,3.90%).

Attempted Formation of 7α-Amino-5β-cholane: (a) A solution of 7-oximino-5β-cholane (100 mg.) in ether (15 ml.) was refluxed with a suspension of lithium aluminium hydride (ca. 200 mg.) in ether (10 ml.) for 16 hr. Working up of the reaction mixture afforded a neutral gum (85 mg.) which showed the absence of any functional groups in the infrared spectrum nor would it afford a hydrochloride.

No crystalline material resulted after chromatography over alumina. (b) 7-Oximino-5β-cholane (50 mg.), dissolved in acetic acid (5 ml.), was shaken under hydrogen with a suspension of prereduced platinum catalyst (20 mg.) in acetic acid (1.5 ml.) for 24 hr. at room temperature and atmospheric pressure. The uptake of hydrogen was extremely slow. Working up of the reaction mixture in the usual manner afforded a neutral gum which showed a broad shallow band between 1800-1700 $cm.^{-1}$ in the infrared spectrum. Repeated attempts to purify this product by chromatography over alumina were unsuccessful. No hydrochloride was formed on treatment with hydrochloric acid.

3β,7β-Dimethanesulphonyloxy-5α-cholestane: 3β,7β-Dihydroxy-5α-cholestane (300 mg.) was treated with methanesulphonyl chloride (2.5 mole) in pyridine (3 ml.) at 0°C for 16 hr. Dilution of the reaction mixture with ice and an excess of cold 3N HCl gave a white precipitate which was removed by filtration, washed (water) and dried (320 mg.). Recrystallisation from methanol or ether gave cubes of 3β,7β-dimethanesulphonyloxy-5α-cholestane (290 mg.) m.p. 146-147°C; $[\alpha]_D^{17}$ + 38.6° (c = 1.5). (Found: C,61.75; H,9.00, $C_{29}H_{52}O_6S_2$ requires: C,62.14; H,9.28%). Infrared analysis showed complete sulphonation of both the 3β and 7β-hydroxyl groups.

Action of Dimethylamine on 3β,7β-Dimethanesulphonyloxy-5α-cholestane: 3β, 7β-Dimethanesulphonyloxy-5α-cholestane (150 mg.) was heated with dimethylamine (2 ml.) and p-toluenesulphonic acid (40 mg.) dissolved in ethanol (2 ml.) in a sealed tube at 100°C for 20 hr. The reaction mixture was diluted to large volume with water, and the oil which separated was extracted with ether after addition of dilute ammonia to the aqueous layer. The ethereal extract after removing the solvent in vacuo afforded a gum (105 mg.). This gum was insoluble in water and formed a water soluble salt on treatment with dilute hydrochloric acid. The infrared spectrum showed no hydroxyl nor methanesulphonyloxy absorption but showed prominent peaks at 2825 and 2780 $cm.^{-1}$ (N-methyl groups). The compound was accordingly assumed to be 3α,7α-bis-dimethylamino-5α-cholestane.

Attempted Formation of 3α,7α-Bis-dimethylamino-5α-cholestane Dimethiodide: A solution of 3α,7α-bisdimethylamino-5α-cholestane (90 mg.) in anhydrous ethanol (2 ml.) was heated with methyl iodide (2 ml.) and anhydrous potassium carbonate (100 mg.) in a sealed tube at 100°C for 5 hr. During the time of heating the reaction mixture gradually developed an amber colour. The potassium carbonate was removed by filtration and the filtrate was distilled in vacuo to yield a coloured residue. Inorganic material was removed by dissolving the residue in chloroform followed by filtration. The

chloroform extract then afforded a dark reddish brown solid (88 mg.). This material on digestion with water gave a dark water insoluble residue (45 mg.). The frothy aqueous extract on removal of the solvent *in vacuo* afforded a yellow coloured bitter tasting solid (40 mg.) whose infrared spectrum showed hydroxyl and double bond (C=C) absorptions.

3,12-Dioximino-5β-cholane: 3,12-Dioxo-5β-cholane (200 mg.) was refluxed with hydroxylamine hydrochloride (120 mg.) and sodium acetate (240 mg.) in ethanol (15 ml.) containing water (1 ml.) for 6 hr. The reaction mixture was concentrated *in vacuo* to *ca*. 3 ml. Dilution with water gave a white precipitate of *3,12-dioximino-5β-cholane*, which was collected by filtration, washed with water and dried (220 mg.). This material showed complete conversion of the keto groups into oximino groups as evidenced by the infrared spectrum, but could not be obtained in crystalline form. (Found: C,73.30; H,10.09; N,7.29. $C_{24}H_{40}O_2N_2$ requires C,74.22; H,10.30; N,7.21%).

3α,12α-Diamino-5β-cholane: A solution of 3,12-dioximino-5β-cholane (211 mg.) in ether (30 ml.) was refluxed with a slurry of lithium aluminium hydride (500 mg.) in ether (25 ml.) for 24 hr. The reaction mixture was worked up in the usual manner to yield a gum (180 mg.). The infrared spectrum of this material showed complete

conversion of the oximino-groups into amino groups (strong absorption at 1570 cm.$^{-1}$ and N-H stretching between 2300-3200 cm.$^{-1}$). The base readily formed a solid hydrochloride on treatment with dilute hydrochloric acid, but this salt could not be obtained in crystalline form. Chromatography over alumina of a sample of the free noncrystalline base resulted in loss of about 80% of the material. The eluted 15% material was oily and would not form a hydrochloride. The noncrystalline 3α,12α-diamino-5β-cholane was accordingly used directly for quaternisation.

3α,12α-Bis-dimethylamino-5β-cholane Dimethiodide: A solution of 3α,12α-diamino-5β-cholane (100 mg.) in anhydrous ethanol (2 ml.) was heated with methyl iodide (2 ml.) and anhydrous potassium carbonate (100 mg.) in a sealed tube at 100°C for 5 hr. The potassium carbonate was removed by filtration and the pale yellow filtrate was distilled *in vacuo*. The residue was digested with chloroform and the inorganic material still present removed by filtration. Removal of the chloroform from the filtrate gave pale yellow 3α,12α-bisdimethylamino-5β-cholane dimethiodide (190 mg.). This quaternary compound was freely soluble in water giving a strongly bitter-tasting solution, which did not give any precipitate on treatment with ammonia or sodium hydroxide solution.

3β,17β-Dihydroxy-5α-Androstane: A solution of 3,17-dioxo-5α-androstane (1 g.) in boiling ethanol (50 ml.) was treated with sliced sodium metal (ca. 4 g.) under reflux during a period of 2 hr. The amber coloured reaction mixture was cooled and diluted with water, and the granular precipitate collected by filtration. Chromatography of this product which was free from carbonyl absorption (infrared spectrum) over alumina (grade III 20 g.) using ethyl acetate as eluant (90 ml.) gave 3β,17β-dihydroxy-5α-androstane (790 mg.), m.p. 159-163°C (reported[217] 164°). Elution with ethanol/ethyl acetate (1:4, 200 ml.) yielded more of the diol (30 mg.).

3β,17β-Dimethanesulphonyloxy-5α-Androstane: 3β,17β-Dihydroxy 5α-androstane (300 mg.), dissolved in pyridine (3 ml.) was treated with methanesulphonyl chloride (3 mole) at 0°C. and the mixture was allowed to stand at room temperature for 16 hr. Dilution of the dark brown reaction mixture with ice gave a cream coloured precipitate, which was collected by filtration after acidifying the reaction mixture with cold 4N HCl. The precipitate was taken up in ether and decolourised with charcoal. A concentrated solution of the dimethanesulphonate in ether afforded needles of 3β,17β-dimethanesulphonyloxy-5α-androstane (300 mg.), mp.149-150°C,

$[\alpha]_D^{17}$ 0° (c = 1.2) (intramolecularly compensated). (Found: C,56.20; H,7.86. $C_{21}H_{36}O_6S_2$ requires: C,56.25; H,8.03%). The infrared spectrum showed absorption characteristic of methanesulphonyloxy groups and the absence of free hydroxyl groups.

Attempted Formation of 3α,17α-Bis(dimethylamino)-5α-androstane:

(a) A solution of 3β,17β-dimethanesulphonyloxy-5α-androstane (150 mg.) in anhydrous ethanol (1.5 ml.) was heated with dimethylamine (1.5 ml.) in the presence of p-toluenesulphonic acid (ca. 40 mg.) in a sealed tube at 100°C for 20 hr. The mixture was diluted with water, basified with 4N sodium hydroxide and extracted with ether. The ethereal layer was washed (water) and dried (anhydrous sodium sulphate) and after removal of the solvent in vacuo afforded a crystalline material (115 mg.), recrystallised from methanol, m.p. 135-140°C. The infrared spectrum of this material showed absorption for $N\text{-}CH_{3\wedge}^{C\text{-}H}$ stretching at 2750 $cm.^{-1}$ as well as the usual sulphonate ester absorptions at 960, 1170 and 1240 $cm.^{-1}$.

The material was not soluble in cold dilute hydrochloric acid (although soluble in hot dilute hydrochloric acid), and the suspension in warm hydrochloric acid, when treated with an excess of 2N sodium hydroxide followed by extraction with ether, regenerated the same material (infrared spectrum and mixed m.p.)

(b) A solution of the 3β,17β-dimethanesulphonyloxy-5α-androstane

(130 mg.) in N-methyl pyrrolidone/t-butanol mixture (19:1 1.5 ml.) was heated with dimethylamine (1.5 ml.) in a sealed tube at 100°C for 60 hr. Working up of the reaction mixture as described in (a) afforded a crystalline compound (ca. 100 mg.) identical with the product obtained in (a) (infrared spectrum and mixed m.p.).

(c) A solution of the product obtained in (b) (60 mg.) in N-methyl pyrrolidone/t-butanol mixture (19:1, 1 ml.) was heated with dimethylamine (1.5 ml.) and anhydrous sodium acetate (30 mg.) in a sealed tube at 100°C for 26 hr. Workimg up of the reaction mixture yielded unchanged starting material (57 mg.) (infrared spectrum and mixed m.p.).

REFERENCES

1. Pelouze and Bernard, cited in Barlow, "Introduction to Chemical Pharmacology", John Wiley and Sons, New York, 1955, p.180

2. Crum Brown and Fraser, Trans. Roy. Soc. Edinburgh, 1969, 25, 151 and 693.

3. (a) Boekelheide, Grundon and Weinstock, J. Amer. Chem. Soc., 1952, 74, 1866.

(b) Unna and Greslin, J. Pharmacol., 1944, 80, 53; Unna, Kniazuk and Greslin, J. Pharmacol., 1944, 80, 39.

4. King, J. Chem. Soc., 1935, 1381; 1936, 1276; 1948, 265; Wintersteiner and Dutcher, Science, 1943, 97, 467; Dutcher, J. Amer. Chem. Soc., 1946, 68, 419.

5. Bennett, Amer. J. Psychiat., 1941, 97, 1040; Griffith and Johnson, Anaesthesiology, 1942, 3, 418; Cullen, Surgery, 1943, 14, 261.

6. Carey, Lewis, Stenlake and Williams, J. Pharm. Pharmacol., Supplement 1961, 13, 103T and earlier papers; Vanecek and Votava, Physiolog. Bohemosloven, 1955, 4, 220; Bovet, Depierre and Lestrange, C.R. Acad. Sci., Paris, 1947, 225, 74; Bovet, Depierre, Courvoisier and Lestrange, Arch. int. Pharmacodyn., 1949, 80, 172.

7. Bowman, Progress in Medicinal Chemistry, Vol. II, ed. Ellis and West, London, Butterworth, 1962, p.88; Nachmansohn, Structure and Function of Muscle, Vol. II, Biochemistry and Physiology, ed. Bourne, New York, London, Academic Press, 1960, p.99; Koelle, J. Pharm. Pharmacol., 1962, 14, 65; D'Arcy and Taylor, ibid,

1962, 14, 129 and 193; Waser, ibid, 1960, 12, 577;

8. Elliot, J. Physiol., 1905, 32, 401.

9. Hunt and Taveau, Brit. Med. J., 1906, 2, 1788.

10. Dixon, Med. Mag., 1907, 16, 454.

11. Dale, J. Pharmacol., 1914, 6, 147.

12. Loewi, Pflug. Arch. Ges. Physiol., 1921, 189, 239.

13. Dale, Feldberg and Vogt, J. Physiol., 1936, 86, 353.

14. Hodgkin, Biol. Rev., 1951, 26, 339; Nastuk, Ann. N.Y. Acad. Sci., 1959, 81, 317; Huxley, ibid, 1959, 81, 221.

15. Eccles, "The Neurophysiological basis of Mind", Oxford, Clarendon Press, 1953.

16. Katz, Rev. Mod. Phys., 1959, 31, 524; Eccles, "The Physiology of Nerve Cells". Baltimore. The John Hopkins Press, 1957, p.217.

17. Castillo and Katz, Progr. Biophys., 1956, 6, 121; Straughan, Brit. J. Pharmacol., 1960, 15, 417; Seite, Annee Biol., 1961, 37, 217 (Chem. Abstr., 56, 5241).

18. Fatt and Katz, J. Physiol., 1952, 117, 109.

19. Nachmansohn, "Chemical and Molecular Basis of Nerve Activity", New York, London: Academic Press, 1959.

20. Straughan, Brit. J. Pharmacol., 1960, 15, 417.

21. Nastuk, Ann. N.Y. Acad. Sci., 1959, 81, 317.

22. Nachmansohn, Ann. N.Y. Acad. Sci., 1946, 47, 395; Lipmann and Kaplan, J. Biol. Chem., 1946, 162, 743.

23. Hebb and Smallman, J. Physiol., 1956, 134, 385.

24. Nachmansohn, Nerve Impulse Transactions of the Third Conference, New York Josiah Macy Jnr. Foundation, 1952, p.15; Feldberg, Physiol. Rev., 1945, 25, 596; Feldberg, Brit. Med. Bull., 1950, 6, 312.

25. De Robertis and Bennett, J. Biophys. Biochem. Cytol., 1955, 1, 47; Palade, Anat. Rec., 1954, 118, 335; Palay, Anat. Rec., 1954, 118, 336.

26. Buchthal and Lindhard, Acta Physiol. Scand., 1942, 4, 136; Kuffler, J. Neurophysiol., 1943, 6, 99; 1945, 8, 77.

27. Nachmansohn and Rothenberg, Science, 1944, 100, 454; J. Biol. Chem., 1945, 158, 653; Augustinsson and Nachmansohn, Science, 1949, 110, 98.

28. Courteaux and Nachmansohn, Proc. Soc. Exp. Biol., N.Y., 1940, 43, 177; Courteaux, Intern. Rev. Cytol., 1955, 4, 335.

29. Del Castillo and Katz, J. Physiol., 1955, 128, 157; Progress in Biophysics and Biophysical Chemistry, 1956, 6, 121; Proc. Roy. Soc., 1957, B146, 339.

30. Ehrlich and Morgenroth, "Studies in Immunity", New York, Wiley, 1910, p.24, translated from an original paper by these authors in Klin. Wschr., 1910, 682, and cited by Albert in Selective Toxicity, London, Mathuen, 1960, 2nd ed., p.23.

31. Fischer, Ber. Dtsch. Chem. Ges., 1894, 27, 2985.

32. Langley, Proc. Roy. Soc., 1906, B78, 170.

33. Lucas, J. Physiol., 1907, 36, 113.

34. Beckett, J. Pharm. Pharmacol., 1956, 8, 848, 860, 874; Waser, in "Curare and Curare-like Agents", ed. Bovet, Bovet-Nitti and Marini-Bettolo, 1959, Amsterdam, Elsevier, p.219; Land, J. Pharmacol., 1951, 102, 219; Long and Lands, J. Pharmacol., 1957, 120, 40; Beckett, Harper, Clitherow and Lesser, Nature, Lond., 1961, 189, 671; Waser, Pflug. Arch. Ges. Physiol., 1962, 274, 431.

35. Van Rossum and Ariens, Experientia, 1957, 13, 161.

36. Ariëns, Arch. Int. Pharmacodyn., 1954, 99, 32.

37. Schueler, J. Pharmacol., 1955, 115, 127; Reitzel and Long, Arch. Int. Pharmacodyn., 1959, 119, 20; Gardner, Biochem. J., 1961, 81, 297.

38. Ambache and Lessin, J. Physiol., 1955, 127, 449.

39. Augustinsson and Nachmansohn, J. Biol. Chem., 1949, 179, 543; Nachmansohn, Rothenberg and Feld, J. Biol. Chem., 1948, 174, 247.

40. Van Rossum, Ariëns and Linssen, Biochem. Pharmacol., 1958, 1, 193.

41. Waser, "Curare and Curare-like Agents", ed. Bovet, Bovet-Nitti and Marini-Bettolo, Amsterdam, London, New York, Elsevier, 1959, p.227.

42. Altamirano, Coates, Grundfest and Nachmansohn, J. Gen. Physiol., 1953, 37, 91; Nachmansohn, Science, 1961, 134, 1962.

43. Chagas, "Curare and Curare-like Agents", ed. Bovet, Bovet-Nitti and Marini-Bettolo, Amsterdam, London, New York, Elsevier, 1959, 327.

44. Ehrenpreis, Science, 1959, 129, 1613; Fed. Proc., 1959, 18, 220; Biochim. Biophys. Acta, 1960, 44, 561; Nistratova and Turpaev, Biokhimiya, 1961, 26, 952 (Chem. Abstr., 1962, 56, 1860c).

45. Zaimis, "Curare and Curare-like Agents", ed. Bovet, Bovet-Nitti and Marini-Bettolo, Amsterdam, London, New York, Elsevier, 1959, p.191.

46. Paton, Ann. N.Y. Acad. Sci., 1951, 54, 347; Anaesthesia, 1953, 8, 151; Zaimis, J. Physiol., 1951, 112, 176; Nature, Lond., 1952, 170, 617; Paton and Zaimis, Pharmacol. Rev., 1952, 4, 219.

47. Jenden, Kamijo and Taylor, J. Pharmacol., 1951, 103, 348; Ibid, 1954, 111, 229; Jenden, J. Pharmacol., 1955, 114, 398; Sabawala and Dillon, Acta Anaesth. Scand., 1959, 3, 83; Brennan, Brit. J. Anaesth., 1956, 28, 159; Hodges and Foldes, Lancet, 1956, 2, 788.

48. Buttle and Zaimis, J. Pharm. Pharmacol., 1949, 1, 991; Zaimis, Nature, Lond., 1952, 170, 617; J. Physiol., 1953, 122, 238; Bigland, Goetzee, Maclagan and Zaimis, J. Physiol., 1958, 141, 425.

49. Bovet, Ann. N.Y. Acad. Sci., 1951, 54, 407.

50. Paton, Ann. N.Y. Acad. Sci., 1951, 54, 347; Zaimis, Nature, Lond., 1952, 170, 617.

51. Ariëns, Van Rossum and Simonis, Anzneimittel-Forsch, 1956, 6, 282, 611 and 737.

52. Paton, Proc. Roy. Soc., 1961, B154, 21.

53. Bovet and Bovet-Nitti, "Structure et Activite Pharmacodynamique des Medicaments du Systems Nerveux Vegetatif", Karger, Bale, 1948; Experientia, 1948, 4, 325; Bovet, Ann. N.Y. Acad. Sci., 1951, 54, 407; Barlow and Ing, Brit. J. Pharmacol., 1948, 3, 298; Nature, Lond., 1948, 161, 718; Paton and Zaimis, Nature, Lond., 1948, 161, 718 and 162, 810 and Brit. J. Pharmacol., 1949, 4, 381; Collier and Taylor, Nature, Lond., 1949, 164, 491.

54. Paton and Zaimis, Nature, Lond., 1948, 161, 718; Brit. J. Pharmacol., 1949, 4, 381.

55. Barlow, "Introduction to Chemical Pharmacology", New York, Wiley; Lond., Methuen, 1955, p.179 and 120.

56. Paton and Zaimis, Nature, Lond., 1948, 161, 718; Brit. J. Pharmacol., 1949, 4, 381; Barlow and Ing, Brit. J. Pharmacol., 1948, 3, 298; Nature, Lond., 1948, 161, 718.

57. Langley, J. Physiol., 1918, 52, 247.

58. Burger, "Medicinal Chemistry", ed. Burger, New York, London, Interscience, 2nd ed. 1960, p.499.

59. Waser, "Curare and Curare-like Agents", ed. Bovet, Bovet-Nitti and Marini-Bottolo, Amsterdam, London, New York, Elsevier, 1959, p.220; Waser, Schmid and Schmid, Arch. Intern. Pharmacodynamie, 1954, 96, 386.

60. Lüttringhaus, Kerp and Preugschas, Arzneimittel-Forsch., 1957, 7, 222; cited by Burger, "Medicinal Chemistry", New York, London, Interscience, 2nd ed. 1960, p.499.

61. Bovet, Courvoisier, Ducrot and Harclois, C.R. Acad. Sci. Paris, 1946, 223, 597; Bovet, Depierre and de Lestrange, C.R. Acad. Sci. Paris, 1947, 225, 74.

62. Haining and Johnston, Brit. J. Pharm. Chemotherap., 1962, 18, No. 2, 275, and references cited there.

63. Carey, Edwards, Lewis and Stenlake, J. Pharm. Pharmacol., Supplement, 1959, 11, 70T.

64. King, J. Chem. Soc., 1949, 3263.

65. Huguenard, 1948, cited by Goodman and Gilman, "The Pharmacological Basis of Therapeutics", New York, Macmillan, 1955, 2nd ed., p.616.

66. Goodman and Gilman, "The Pharmacological Basis of Therapeutics", New York, Macmillan, 1955, 2nd ed., p.616, and references cited there.

67. Riker and Wescoe, Ann. N.Y. Acad. Sci., 1951, 54, 373.

68. Loewe and Harvey, Arch. Exp. Path. Pharmak., 1952, 214, 214.

69. Fakstorp, Pedersen, Poulsen and Schilling, Acta Pharm. Fox. Kbh., 1957, 13, 52.

70. Cavallito and Gray, "Fortschritte der Arzneimittelforschung", ed. Jucker, Basel, Birkhauser, 1960, vol. 2, p.135.

71. Kashland, Proc. Nat. Acad. Sci. (Wash.), 1958, 44, 98.

72. Gill, Proc. Roy. Soc., 1959, B150, 381.

73. Wien and Mason, Brit. J. Pharmacol., 1953, 8, 306.

74. Gill and Ing, J. Chem. Soc., 1958, 4728.

75. Burger, "Medicinal Chemistry", ed. Burger, New York, London, Interscience, 2nd ed. 1960, p.497.

76. Cavallini, Farmaco, 1955, 10, 644.

77. Cavallini and Massarani, J. Med. Pharm. Chem., 1959, 1, 365.

78. Cavallini and Massarani, Boll. Soc. Ital. Biol. Sper., 1951, 27, 629.

79. Gero and Withrow, Nature, Lond., 1957, 180, 1354.

80. Cavallini and Massarani, Farm. Sci. e Tec (Pavia), 1951, 6, 291.

81. Cavallini, Ferrari, Mantegazza and Massarani, Farm. Sci. e Tec. (Pavia) 1951, 6, 815.

82. Cogni and Salvaneschi, Atti. Soc. Lombarda Sci. Med. e Biol., 1951, 7, 60; in Chem. Abstr., 1953, 47, 6459.

83. Janot, Laine and Goutarel, Ann. Pharm. Franc., 1960, 18, 673.

84. Quevauviller and Laine, Ann. Pharm. Franc., 1960, 18, 678.

85. Goutarel, Tetrahedron, 1961, 14, 126.

85A. Alauddin and Martin Smith, J. Pharm. Pharmacol., 1962, 14, 325, 469.

86. Veldsmann, J. S. Afr. Vet. Med. Ass., 1949, 20, 45; S. Afr. Ind. Chem., 1949, 144, 172, 217; Van Euv and Reichstein, Helv. Chim. Acta, 1950, 33, 485.

87. Bally Mohr and Reichstein, Helv. Chem. Acta, 1951, 34, 1740.

88. Hanna, Sandris and Ourisson, Bull. Soc. Chem. France, 1959, 1454.

89. Cornforth and Robinson, J. Chem. Soc., 1946, 676; Nature, 1947, 160, 737; Cardwell, Cornforth, Duff, Holtermann and Robinson, Chem. and Ind., 1951, 389; J. Chem. Soc., 1953, 361; du Feu, McQuillin and Robinson, J. Chem. Soc., 1937, 53; Robinson and Weygand, ibid, 1941, 386; Cornforth and Robinson, J. Chem. Soc., 1949, 1855.

90. Bloch, Crabbe, Kincl, Ourisson, Perez and Zderic, Bull. Soc. Chem. France, 1961, 559.

91. Wieland and Weil, Z. Physiol. Chem., 1913, 80, 287.

92. Wieland, Z. Physiol. Chem., 1925, 142, 191.

93. Wieland and Wiedersheim, Z. Physiol. Chem., 1930, 186, 229; Wieland and Dane, Z. Physiol. Chem., 1933, 219, 240; Cook and Haslewood, Chem. and Ind., 1933, 758; Fiezer and Newman, J. Amer. Chem. Soc., 1935, 57, 961.

94. Bergstrom and Krabisch, Acta Chem. Scand., 1957, 11, 1067; Bergstrom, Lindstedt and Sen, Acta Chem. Scand., 1957, 11, 1692; Kazuno, Moori, Sasaki, Kuroda and Mizuguchi, Proc. Japan Acad., 1952, 28, 416.

95. Kolbe, Ann, 1949, 69, 257.
Weedon, Quart. Revs. (London), 1952, 6, 380.

96. Brink, Clark and Wallis, J. Biol. Chem., 1946, 162, 695; Bergstrom, Rottenberg and Voltz, Acta Chem. Scand., 1953, 7, 481; Bergstrom and Paulo, Acta Chem. Scand., 1955, 9, 699; 1952, 35, 1286; Johnson and Ingham, Chem. Rev., 1956, 56, 219.

97. Hunsdiecker and Hunsdiecker, Chem. Ber., 1942, 75B, 291.

98. Caldwell, J. Amer. Chem. Soc., 1938, 60, 991.

99. Curtius, Chem. Ber., 1890, 23, 3023; J. Pract. Chem., 1894, 50, 275; Smith, Organic Reactions, ed. Adams, John Wiley, New York, 1946, III, p.337.

100. Wessely and Swoboda, Monatsch., 1951, 82, 437;

101. Blickenstaff and Chang, J. Amer. Chem. Soc., 1958, 80, 2726; 1959, 81, 2835.

102. Barnett and Reichstein, Helv. Chem. Acta, 1938, 21, 926; von Euw and Reichstein, Helv. Chem. Acta, 1946, 29, 654. Borsche, Chem. Ber., 1924, 57, 1620; Fieser, Rajagopalan, Wilson and Tishler, J. Amer. Chem. Soc., 1951, 73, 4133; Fieser and Rajagopanan, ibid, 1950, 72, 5530.

103. Barton and Cox, J. Chem. Soc., 1948, 783; Fieser and Rajagopalan, J. Amer. Chem. Soc., 1950, 72, 5530; Mobach, Meyer and Kendall, ibid, 1954, 76, 5800.

104. Huang Minlon, J. Amer. Chem. Soc., 1946, 68, 2487; ibid, 1949, 71, 3301; Kawai, Z. Physiol. Chem., 1933, 214, 71; Fieser and Rajagopalan, J. Amer. Chem. Soc., 1950, 72, 5534; Wieland and Sorge, Z. Physiol. Chem., 1919, 106, 190; Borsche, Chem. Ber., 1919, 52, 342 and 1353; Karrer and Asmis, Helv. Chem. Acta, 1951, 34, 1022; von Schmid and Karrer, ibid, 1949, 32, 1371.

105. Fieser and Fieser, Steroids, Reinhold, New York, Chapman and Hall, London, 1959, p.281; Burckhardt and Reichstein, Helv. Chem. Acta, 1942, 25, 821; Koechlin, Kritchevsky and Gallaghar, J. Biol. Chem., 1950, 184, 393; Butenandt and Wolff, Chem. Ber., 1935, 68B, 2091.

106. Inhoffen, Koelling and Nehring, Chem. Ber., 1952, 85, 89; Inhoffen, Koelling, Koch and Nebel, ibid, 1951, 84, 361.

107. Burckhardt and Reichstein, Helv. Chem. Acta, 1942, 25, 821; Butenandt and Wolff, Chem. Ber., 1935, 68B, 2091;

108. Barton, Ives and Thomas, Chem. and Ind., 1953, 1180; J. Chem. Soc., 1954, 903; Johnson, Bannister, Bloom, Kemp, Pappo, Rogier and Szmuszkovicz, J. Amer. Chem. Soc., 1953, 75, 2275; Bowers, Ringold and Dorfman, ibid, 1957, 79, 4556.

109. Johnson, Rogier, Szmuszkovicz, Hadler, Ackerman, Bhattacharya, Bloom, Stalmann, Clement, Bannister and Wynberg, J. Amer. Chem. Soc., 1956, 78, 6289; Johnson, Bannister, Bloom, Kemp, Pappo, Rogier and Szmuszkovicz, ibid, 1953, 75, 2275.

110. Borsche, Chem. Ber., 1919, 106, 190.

111. Hadacek and Duchoslav, Publs. Fac. Sci. Univ. Masaryk Cislo, 1954 357, 251; Waid and Taurius, Can. J. Chem., 1960, 38, 987.

112. Shoppee, Evans, Richards and Summers, J. Chem. Soc., 1956, 1649; Bird and Cookson, ibid, 1960, 2343.

113. Bird and Cookson, J. Chem. Soc., 1960, 2343; Shoppee, Cremlyn, Evans and Summers, ibid, 1957, 4364; Shoppee, Evans, Richards and Summers, ibid, 1956, 1649; Gent and McKenna, ibid, 1959, 137; Barnett, Ryman and Smith, ibid, 1946, 524; Hershberg, Oliveto and Rausser, Chem. and Ind., 1958, 1477.

114. Redel, Bouteville, Ganthier and Nguyen Huu Quy, Comp. Rend., 1949 229, 128; Same authors, Bull. Soc. Chim. France, 1949, 877; Waid and Taurius, Can. J. Chem., 1960, 38, 987; Hadacek and Duchoslav, Publs. Fac. Sci. Univ. Masaryk Cislo, 1954, 357, 251 (In C.A. 1955, 49, 14015).

115. Schenk, Z. Physiol. Chem., 1914, 89, 360.

116. Leanza, Conbere, Rogers and Pfister, J. Amer. Chem. Soc., 1954, 76, 1691.

117. Madaeva and Babanova, Zhur. Obshchei Khim., 1955, 25, 1950; Chem. Abstr., 19 , 50, 8700.

118. Von Euw and Reichstein, Helv. Chim. Acta, 1946, 29, 654.

119. Leffek, Llewellyn and Robertson, Can. J. Chem., 1960, 38, 1505; Llewellyn, Robertson and Scott, ibid, 1960, 38, 222 Leffek, Robertson and Sugamori, ibid, 1961, 39, 1989.

120. Laughton and Robertson, Can. J. Chem., 1956, 34, 1714.

121. Corbellini and Nathansohn, Gazz., 1956, 86, 1240; Reich and Lardon, Helv. Chim. Acta, 1946, 29, 671.

122. Loewenthal, Tetrahedron, 1959, 6, No.4, 269.

123. Blickenstaff and Chang, J. Amer. Chem. Soc., 1959, 81, 2835.

124(a). Wessely and Swoboda, Monatsh. 1951, 82, 437; (b) Spero, J. Amer. Chem. Soc., 1948, 70, 1907.

125. Matsumoto, J. Biochem. (Japan), 1955, 42, 207.

126. Schmid and Karrer, Helv. Chim. Acta, 1949, 32, 1371.

127. Borsche, Frank, Chem. Ber., 1926, 59, 1748; Borsche, Feske, Z.Physiol. Chem., 1928, 176, 109.

128. Barnett and Reichstein, Helv. Chim. Acta, 1938, 21, 926.

129. Huffman, Lott and Tilloston, J. Biol. Chem., 1956, 218, 565

130. Barton and Robinson, J. Chem. Soc., 1954, 3045; Bowers, Ringold and Dorfman, J. Amer. Chem. Soc., 1957, 79, 4556.

131. Johnson, Rogier, Szmuszkovicz, Hadler, Ackerman, Bhattacharya, Bloom, Stalmann, Clement, Bannister and Wynberg, J. Amer. Chem. Soc., 1956, 78, 6289; Johnson, Bannister, Bloom, Kamp, Rogers and Szmuszkovicz, ibid., 1953, 75, 2275.

132. Burckhardt and Reichstein, Helv. Chim. Acta, 1942, 25, 821; Butenandt and Wolff, Chem. Ber., 1935, 68B, 2091; Koechlin, Kritchevsky and Gallagher, J. Biol. Chem., 1950, 184, 393; Julian, Cochrane, Magnani and Karpel, J. Amer. Chem. Soc., 1956, 78, 3153; Fiezer and Fiezer, Steroids, Reinhold, New York, Chapman and Hall, London, 1959, p.282.

133. Fiezer and Fiezer, Steroids, Reinhold, New York, Chapman and Hall, London, 1959, p.282-283 and references cited there.

134. Inhoffen, Kalling, Koch and Nebel, Chem. Ber., 1951, 84, 361.

135. Koechlin, Kritchevsky and Gallagher, J. Biol. Chem., 1950, 184, 393.

136. Hirschman, Snoddy and Wendler, J. Amer. Chem. Soc., 1952, 74, 2693.

137. Burckhardt and Reichstein, Helv. Chim. Acta, 1942, 25, 821.

138. Fiezer and Fiezer, Steroids, Reinhold, New York, Chapman and Hall, London, 1959, p.258, 260.

139. Dobriner, Katzenellenbagen and Jones, Infrared Absorption Spectra of Steroids, An Atlas, Interscience, New York, London Vol.I, 20 and 38.

140. Karrer and Asmis, Helv. Chim. Acta, 1951, 34, 1022.

141. Wieland and Schlichting, Z. Physiol Chem., 1925, 150, 267

142. Morsan, Steiger and Reichstein, Helv. Chim. Acta, 1937, 20, 3.

143. Barnett and Reichstein, Helv. Chim. Acta, 1938, 21, 926.

144(a) Fieser and Rajagopalan, J. Amer. Chem. Soc., 1950, 72, 5530; (b) Berner, Lardon and Reichstein, Helv. Chim. Acta, 1947, 30, 1542; (c) Plattner, Heusser, ibid, 1944, 27, 748.

145. Iwasaki, Z. Physiol. Chem., 1936, 244, 181; Wieland and Dane, ibid, 1932, 210, 268.

146. Huffman, Lott and Tilloston, J. Biol. Chem., 1956, 218, 565.

147. Reichstein and Sorkin, Helv. Chim. Acta, 1942, 25, 797.

148. Koechlin, Kritchevsky and Gallaghar, J. Biol. Chem., 1950, 184, 393.

149. Burckhardt and Reichstein, Helv. Chim. Acta, 1942, 25, 821.

150. Huang Minlon, J. Amer.Chem. Soc., 1949, 71, 3301.

151. Kazuno, Moori, Sasaki, Kuroda and Mizuguchi, Proc. Japan Acad., 1952, 28, 416.

152. Huffman, Abraham and Bethea, J. Org. Chem., 1962, 27, 3383.

153. Fieser and Fieser, Steroids, Reinhold, New York, Chapman and Hall, London, 1959, p.28.

154. Shoppee, Cremlyn, Evans and Summers, J. Chem. Soc., 1957, 4364; Bird and Cookson, ibid, 1960, 2343; Shoppee, Richard and Summers, ibid, 1956, 1649; Gent and McKenna, ibid, 1959, 137;

Barnet, Ryman and Smith, ibid., 1946, 524; Hershberg, Oliveto and Rausser, Chem. and Ind., 1958, 1477.

155. Redel, Bouteville, Gauthier and Nguyen Huu Quy, Bull Soc. Chim. France, 1949, 877; Hadacek and Duchoslav, Publs. Fac. Sci. Univ. Masaryk Cislo, 1954, 357, 251 in Chem. Abstr. 1954, 49, 14015; James, Smith, Stacey and Webb, J. Chem. Soc., 1946, 665.

156. MacPhillamy and Scholz, J. Org. Chem., 1949, 14, 643.

157. Hofmann, Ber. Dtsch. Chem. Ges., 1881, 14, 494 and 659.

158. Press and Reichstein, Helv. Chim. Acta, 1942, 25, 878; Gallagher and Long, J. Biol. Chem., 1946, 162, 495.

159. Ingold, Structure and Mechanism in Organic Chemistry, Cornell University Press, Ithaca, N.Y. 1953, Chapter 8.

160. Dhar, Hughes, Ingold, Mandour, Maw and Woolf, J. Chem. Soc., 1948, 2093; cf. Barton, ibid, 1953, 1027.

161. Haworth, McKenna and Powell, J. Chem. Soc., 1953, 1110; Gent and McKenna, ibid., 1956, 573.

162. Gent and McKenna, J. Chem. Soc., 1959, 137.

163. Waid and Taurins, Canad. J. Chem., 1960, 38, 987.

164. Anliker, Rohr and Heusser, Helv. Chim. Acta, 1955, 38, 1171

165. Hirschmann, Snoddy, Hiskey and Wendler, J. Amer. Chem. Soc. 1954, 76, 4013; Hiskey, Hirschmann and Wendler, ibid, 1953, 75, 5135; Elks, Phillips, Taylor and Wyman, J. Chem. Soc. 1954, 1739.

166. Ingold, Structure and Mechanism in Organic Chemistry, Cornell University Press, Ithaca, N.Y., 1953, p. 397.

167. Barton, J. Chem. Soc., 1953, 1027.

168. Schenck, Ber. Dtsch. Chem. Ges., 1942, 75, 198; ibid., 1943, 76, 874; ibid., 1944, 77, 29 and 501: cf. Brooks, Evans, Green, Hunt, Long, Mooney and Wyman, J. Chem. Soc., 1958, 4614.

169. Houben and Pfankuch, Ber. Dtsch. Chem. Ges., 1927, 60, 586; Mahla and Tiemann, ibid, 1896, 29, 2807.

170. Shoppee, Roy and Goodrich, J. Chem. Soc., 1961, 1583.

171. Shoppee, Cremlyn, Evans and Summers, J. Chem. Soc., 1957, 4364; Evans and Summers, ibid, p.906, Shoppee, Evans and Summers, ibid, 97.

172. Dodgson and Haworth, J. Chem. Soc., 1952, 67.

173. Grob and Gagneus, Helv. Chim. Acta, 1957, 40, 130; McKenna and Slinger, J. Chem. Soc., 1958, 2759.

174. Woodward and Doering, J. Amer. Chem. Soc., 1945, 67, 860.

175. Eliel and Rerick, J. Amer. Chem. Soc., 1960, 82, 1362.

176. Boyer, J. Amer. Chem. Soc., 1951, 73, 5865.

177. Adams and Blomstrom, J. Amer. Chem. Soc., 1953, 75, 3405.

178. Vander Werf, Heisler and McEwen, J. Amer. Chem. Soc., 1954, 76, 1231; Bretschneider and Hormann, Monatsh, 1953, 84, 1021 and 1033; Bretschneider and Karpitschka, ibid, 1043.

179. cf. Sauers, J. Amer. Chem. Soc., 1958, 80, 4721.

180. Ruzicka, Proc. Chem. Soc., 1959,

181. Barton and Head, J. Chem. Soc., 1956, 932; Barton, Head and May, ibid, 1957, 935; Barton, McCapra, May and Thudium, ibid, 1960, 1297.

182. Henbest and Wilson, J. Chem. Soc., 1956, 3289.

183. Ames, Beton, Bowers, Halsall and Jones, J. Chem. Soc., 1954 1905; see too Barton and Morrison, Fortscht. Chem. Org. Naturst., 1961, 19, 165.

184. Pierce, Richards, Shoppee, Stephenson and Summers, J. Chem. Soc., 1955, 694; Shoppee, Evans, Richards and Summers, ibid, 1956, 1649.

185. Gent and McKenna, J. Chem. Soc., 1956, 573; ibid, 1959, 137.

186. Dauben, Fonken and Noyce, J. Amer. Chem. Soc., 1956, 78, 2579; Dauben, Blanz, Jiu and Micheli, ibid, 1956, 78, 3752.

187. Wheeler and Huffman, Experientia, 1960, 16, 516.

188. Huffman, Alabran and Bethea, J. Org. Chem., 1962, 27, 3381.

189. Locke and Pelletier, Chem. and Ind., 1956, 1049.

190. Hirschmann, Snoddy and Wendler, J. Amer. Chem. Soc., 1952, 74, 2693.

191. Halsall, Hodges and Jones, J. Chem. Soc., 1953, 3019.

192. Wagner, U.S. Patent 2,408,835, Oct. 8, 1946, in Chem. Abstr. 1947, 41, 1256.

193. Marker, Wagner, Ulshafer, Wittbecker, Goldsmith and Ruof, J. Amer. Chem. Soc., 1947, 69, 2167.

194. Giacomello, Gazz., 1939, 62, 790.

195. Koechlin and Reichstein, Helv. Chim. Acta, 1942, 25, 918; Sorkin and Reichstein, ibid, 1944, 27, 1631.

196. Shoppee, Chem. and Ind., 1947, 109.

197. Dauben, Blanz, James Jin and Micheli, J. Amer. Chem. Soc., 1956, 78, 3752.

198. Fieser and Fieser, Steroids, Reinhold, N.Y., Chapman and Hall, London, 1959, p.216.

199. Kazuno, Moori, Sasaki, Kuroda and Mizuguchi, Proc. Japan Acad., 1952, 28, 416

200. Cavallito, Gray and Spinner, J. Amer. Chem. Soc., 1954, 76, 1862.

201. Edwards, Lewis, Stenlake and Zoha, J. Pharm. Pharmacol., 1958, 10, 106T; 1957, 9, 1004.

202. Marxer and Miescher, Helv. Chim. Acta, 1951, 34, 924; Delaby, Damiens and Marquist, Compt. Rend., 1953, 236, 1976.

203. Gray and O'Dell, Nature, London, 1958, 181, 634.

204. Ahmad, M. Sc. Dissertation, University of Glasgow, 1962.

205. Kanazawa, Shimazaku, Sato and Hoshima, Nippon Kagaku Zasshi, 1955, 76, 297; in Chem. Abstr., 1957, 51, 17965.

206. Haworth, McKenna and Powell, J. Chem. Soc., 1953, 1110.

207. Shoppee, Evans, Richards and Summers, J. Chem. Soc., 1956, 1649.

208. Woolley in Fortschritte der Arzneimettelforschung, Vol.II (Ed. E. Jucker), Basel, Birkhouser, 1960, pp 613.

209. Clinton, Manson, Stonner, Neumann, Christiansen, Clarke, Ackerman, Page, Dean, Dickinson and Carabateas, J. Amer. Chem. Soc., 1961, 83, 1478.

210. Clinton, Manson, Stonner, Beyler, Potts and Arnold, J. Amer. Chem. Soc., 1959, 81, 1513; Clinton, Manson, Stonner, Christianson, Beyler, Potts and Arnold, J. Org. Chem., 1961, 26, 279; Arnold, Beyler and Potts, Proc. Soc. Exp. Biol. N.Y., 1959, 102, 184; Beyler, Potts and Burnham, Abstracts 1st International Congress of Endocrinology, 1960, 829; Potts, Beyler and Burnham, Proc. Soc.Exp. Biol. N.Y., 1960, 103, 383.

211. Dodgson and Haworth, J. Chem. Soc., 1952, 67.

212. Joska and Sorm, Coll. Czech. Chem. Comm., 1956, 21, 754.

213. Madaeva and Luri, Doklady Acad. Nauk, S.S.S.R., 1958, 84, 713, in Chem. Abstr., 1953, 47, 3326; Elks and Shoppee, J. Chem. Soc., 1953, 241.

214. Henbest and Jackson, J. Chem. Soc., 1962, 954.

215. Fieser and Fieser, Steroids, Reinhold, N.Y., Chapman and Hall, London, 1959, p.100.

216. Fieser and Rajagopalan, J. Amer. Chem. Soc., 1950, 72, 5530.

217. Ref. 215, p.519.

APPENDIX I

Reprinted from *The Journal of Pharmacy and Pharmacology*, 1962, **14**, pp. 325–349

REVIEW ARTICLE

BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS.

PART I. SYNTHETIC NITROGENOUS STEROIDS

BY M. ALAUDDIN,* B.Pharm. AND M. MARTIN-SMITH,† M.Sc., Ph.D.

Division of Experimental Pharmacology, Institute of Physiology, The University, Glasgow, W.2.

THE broad spectrum of biological activity found within the group and the multiplicity of actions displayed by certain individual members, make the steroids one of the most intriguing classes of biologically-active compounds. Structural modification studies, whose extent is unequalled in any other area of medicinal chemistry, have not only furnished so many steroidal derivatives that structure-action relationship studies are possible to a degree undreamt of in other fields, but they have also led to the introduction of several cheaper, safer, more specific and more potent therapeutic agents. Among the many steroidal derivatives now known, are a number of compounds which incorporate nitrogen atoms in their molecular structure, and it is the purpose of the present review to indicate the importance of these nitrogenous steroids and to evaluate, where possible, the influence of the nitrogen atoms upon the biological activity displayed. Certain aspects of the subject have been reviewed previously, particularly the pharmacology of the veratrum alkaloids—for more recent reviews see Abreu (1959), Hoobler and Dontas (1953), Krayer (1958), Stoll (1954). The earlier work is exhaustively reviewed by Krayer and Acheson (1946) who refer to previous review articles. A brief survey of the biological properties of nitrogenous steroids in general has also been published (Voigt and Kallistratos, 1957).

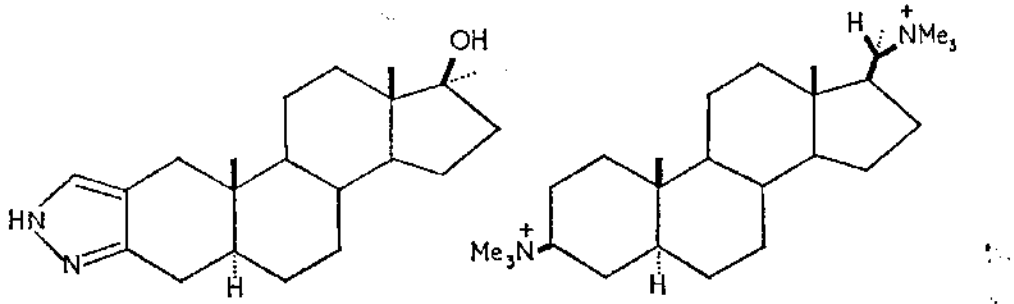
To set the field in perspective the present review will consider nitrogenous steroids in several contexts. Suitable examples, chosen mainly from the synthetic derivatives, will be used to illustrate how the group fits in with modern concepts of drug action and a brief survey will then be given of the biological properties of the rapidly expanding group of known steroidal alkaloids. With the large number of nitrogenous steroids now known, it is quite impossible to achieve a complete coverage of the pertinent literature, but a serious attempt has been made to make the survey as representative as possible.

Clinical Implications

At the present day clinical application of steroids possessing nitrogen atoms is very limited, although there are several indications that the full potentialities have not yet been realised. This is particularly true in the field of synthetic derivatives, as it is only within the past few years that

* Colombo Plan Fellow 1958-62.
† Present address: Department of Pharmacy, Royal College of Science and Technology, Glasgow, C.1.

the intensive search for modified steroidal hormones, showing high specificity or accentuating a minor or secondary biological characteristic of the natural analogue, has been extended to include more than a handful of nitrogenous derivatives. It does not therefore seem unreasonable to anticipate that among new nitrogenous steroids will be found compounds exhibiting clinically desirable carcinolytic properties, improved anabolic to androgenic ratios or superior lipodiatic to oestrogenic indices. Indeed certain steroidal [3, 2-c]-pyrazoles (Clinton and others, 1959, 1961b) and [2, 3-d]-isoxazoles (Clinton and others, 1961a) are already known to possess very favourable anabolic to androgenic ratios. Moreover, as these derivatives are active by the oral route and one representative, 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole (I), has shown

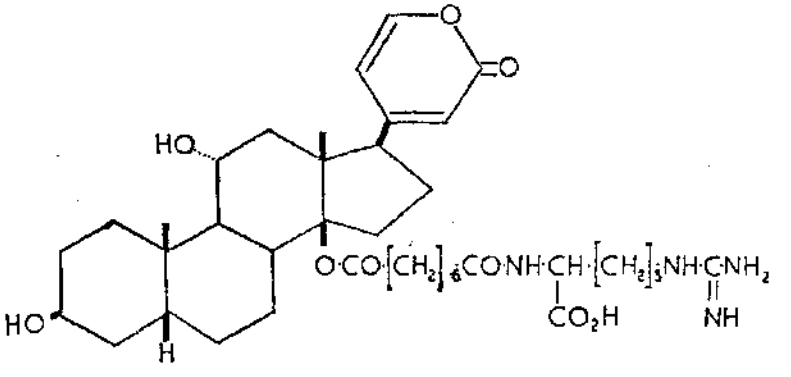


I II

promise on preliminary clinical trial (Howard and others, 1959), such compounds may well find a permanent place as therapeutic agents. Anabolic properties are also present in other nitrogenous steroids including 17β-hydroxy-17α,2′-dimethylandrostano-[3,2-b]-thiazole, certain *N*-substituted 2-aminomethylene-17α-methyldihydrotestosterones (Zderic and others, 1960) and various substituted 16α-aminopregnenes (Rhone-Poulenc, 1960).

The possibility of securing new therapeutic agents amongst nitrogenous steroids is by no means confined to synthetic derivatives, however, and naturally-occurring compounds may also have their role to play. Thus the recently characterised bisquaternary steroidal alkaloid, malouetine (II), which occurs in *Malouetia bequaertiana* (Janot, Lainé and Goutarel, 1960), has been shown in preliminary experiments to possess competitive neuromuscular-blocking potency equal to that of (+)-tubocurarine whilst being only one third as toxic (Quévauviller and Lainé, 1960) and so this compound or related drugs could conceivably offer alternatives to tubocurarine as an adjunct to surgery. The three possible isomers of malouetine involving the configurations of the nitrogen atoms, namely the diquaternary bases in which the nitrogen atoms are in the 3β-20β-, 3α-20α- and 3α-20β-configurations have been prepared synthetically (Goutarel, 1961) and it will be of some interest to learn of their relative potencies. Again, the steroidal alkaloid funtumidine, on the basis of motility tests in rats, has been claimed to possess tranquillizing properties comparable to those of reserpine (La Barre and Desmarez, 1959) and so alkaloids of this type may have a role to play in this area of clinical medicine.

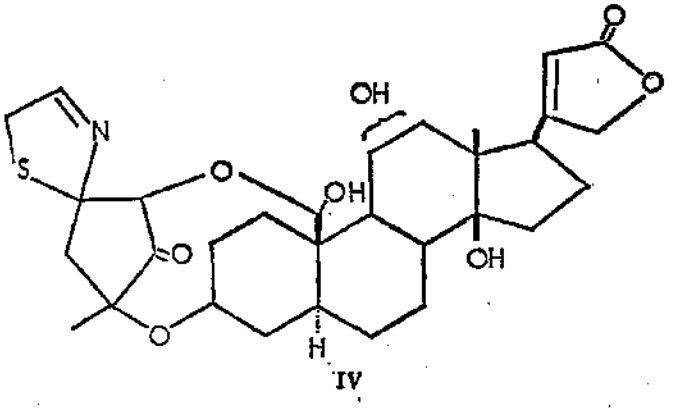
Certain clinically acceptable nitrogenous steroids are found in nature but are not used because cheaper or superior agents are available. Examples are the suberylarginine derivatives of the bufadienolides, such as bufotoxin and gamabufotoxin (III), which are constituents of the poison



III

secreted by the parotid glands of the toad, and which have similar activity to the plant cardenolide and scilladienolide glycosides employed clinically in the treatment of congestive heart failure. It is of some interest that the toxic principles secreted by the salamander are also nitrogenous steroid derivatives (Schöpf, 1961) although these compounds are of a different chemical type and are without potential clinical application.

Nitrogenous derivatives of cardenolides are also found in plants. Examples are uscharine (IV) (Hesse and Mix, 1959) and its dihydro-derivative voruscharine (Hesse and Ludwig, 1960) which occur in the latex



IV

of *Calotropis procera*. These compounds do not appear to have seen clinical trial but uscharine has been shown to have a potency 58 per cent of that of ouabain in the etherised cat (Chen, Bliss and Robbins, 1942).

The nitrogenous steroids which have seen the most extensive clinical application are the steroidal alkaloids of the protoveratrine type which produce a reflex fall in blood pressure through a generalised vasodilatation and fall in heart rate. Crude and complex mixtures of these alkaloids saw a certain amount of clinical use around the turn of the century, but their toxicity and unpleasant side effects brought them into disfavour. The use of the purified principles saw a resurgence of popularity several

years ago with the heightened interest in the problem of hypertension (see for example Meilman and Krayer, 1952; Meilman, 1959; Robson and Keele, 1956), especially as they produce vasodilatation in all peripheral circuits including the brain and kidneys, and are free from the disturbance of the postural reflexes produced by ganglion-blocking agents. Unfortunately these highly desirable physiological properties are more than offset by the narrow margin between the therapeutic and toxic doses and the fact that emesis nearly always occurs with therapeutic doses. Hence these alkaloids have been virtually eliminated from clinical use, although they may still find occasional application in the treatment of certain toxaemias of pregnancy (see for example Finnerty and Fuchs, 1953; Meilman, 1953; Krupp and others, 1956).

The fact that the protoveratrine group of alkaloids afford such a clinically desirable integrated response, and yet cannot be employed because of their side effects, has presented a most tantalising challenge to the medicinal chemist and numerous attempts have been made to prepare synthetic nitrogenous steroids retaining the hypotensive properties, but devoid of the side effects. So far these efforts have met with little success. Among the compounds prepared are numbered several cholylamine esters (Fieser and Wei-Yuan Huang, 1953), certain ternorcholanylthiazoles (Dodson, 1955a), various 16α-aminopregnenolones (Rhone-Poulenc, 1960; Gould and others, 1956), some aminoalkanol esters of 17α-hydroxy-3-ketoandrost-5-en-17β-carboxylic acid (Bloom, 1956) and two 17-imidazolylandrostenes (Sturtevant, 1958).

Other steroidal alkaloids have seen limited clinical trial in the treatment of conditions other than hypertension. For example the "Kurchi" alkaloids from various *Holarrhena* spp. have been employed in the treatment of amoebic dysentery, both free and in the form of bismuth iodide complexes (see for example Acton and Chopra, 1933; Tanguy, Robin and Raoult, 1948; Lavier, Crosnier and Merle, 1948) and *Solanum* alkaloids were once used in the treatment of asthma and neuralgia (Leclerc, 1938), but neither class is of any great value.

SYNTHETIC NITROGENOUS STEROIDS AND THEIR PLACE IN MODERN THEORY

The lack of knowledge of the principles by which biological activity is related to chemical structure has necessitated numerous tedious structural modification studies in which the medicinal chemist has sought to improve upon known drugs of proven efficacy, and many steroids possessing nitrogen atoms have played their role in this work. Usually the preparation of such compounds has been conducted on purely empirical grounds but occasionally it has followed from the application of theoretical concepts such as the receptor theory of drug action, the theory of metabolite displacement, the concept of bioisosterism or the supporting moiety theory. In the following sections examples will be given of nitrogenous steroids which have either been prepared from consideration of these concepts or which can be discussed in retrospect in terms of these concepts. It is felt that this treatment will place the compounds in their correct perspective and at the same time afford a comprehensive survey without giving rise

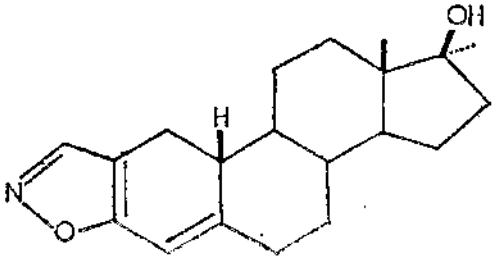
merely to a catalogue of the various synthetic nitrogenous steroids which have been studied biologically.

Receptor Theory

Although very little is known of the intimate nature of the hypothetical "drug receptors" in the tissues, the receptor theory has proved an extremely useful aid to the medicinal chemist in his attempts to rationalise drug action in so far as it stresses the importance of the 3-dimensional geometrical shape and electronic distribution of the drug molecule. It was consideration of the receptor theory which led to the planned synthesis of the anabolic steroidal [3,2-c]-pyrazoles and [2,3-d]-isoxazoles, where it was assumed that the receptors concerned with the androgenic and anabolic properties of the natural male hormones differed in their nature (Clinton and others, 1961b). Attention was concentrated on securing an alteration of the intergroup distance between the substituents at C(3) and C(17), with an accompanying change in the nucleophilicity of the C(3) substituent, and satisfaction of these requirements led logically to the preparation of the pyrazole and isoxazole derivatives.

After oral administration 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole (I) proved to be some 30 times as potent as 17α-methyltestosterone in the rat nitrogen retention test, whilst it was only one quarter as androgenic in the ventral prostate weight gain test (Arnold, Beyler and Potts, 1959). In the levator ani muscle test in immature castrated male rats it proved to be twice as myotrophic when given by the oral route as 17α-methyltestosterone (Potts, Beyler and Burnham, 1960), and it also proved effective in reversing the catabolic actions of cortisone acetate in the same animals (Beyler, Potts and Burnham, 1960). Surprisingly, acylation of the pyrazole ring imparted some oestrogenicity to the pyrazole series and a 6α-methyl group decreased both the androgenic and anabolic properties (Clinton and others, 1961b).

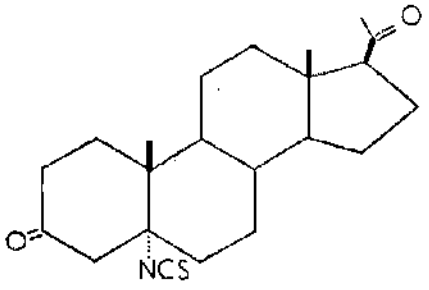
The isoxazole derivatives show broadly similar activity to the pyrazole compounds (Clinton and others, 1961a; Zderic and others, 1960) although one, 17β-hydroxy-17α-methyl-19-norandrost-4-eno-[2,3-d]-isoxazole (V), showed a conspicuous lack of specificity. Thus it exhibited progestational



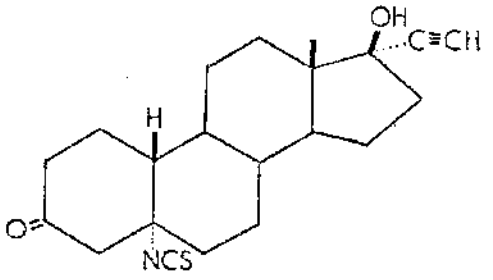
V

activity equal to progesterone on intramuscular administration and to ethisterone on oral administration, as well as showing anabolic, myotrophic, androgenic and oestrogenic properties (Clinton and others, 1961a).

In their visualisation of drug-receptor interaction Van Rossum and Ariens (1957) suggest that the "drug-receptor complex" is basically an interaction of fields of force originating in the drug molecule and in the tissue. Electrostatic and van der Waals forces play the dominant role, and it is postulated that certain specific interactions within the general field determine the intrinsic activity or ability of the drug to evoke the biological response. If this representation is correct then maintenance of the general interaction with concurrent variation in electron density at certain specific areas might be expected to produce large changes in intrinsic activity without appreciable changes in the affinity for the receptor. In the glucocorticoid field such a situation would appear to arise from the introduction of an electron-withdrawing substituent in the form of a 9α-fluorine atom which greatly enhances potency. The spectacular success of this introduction of a 9α-fluorine atom has logically led to investigations of the effect of introducing other electron-withdrawing groups at various positions in the steroid nucleus and among the compounds so prepared are several with groups containing nitrogen. Thus several 5α-, 7α-, 9α- and 11β-thiocyanato-steroid hormone derivatives have been synthesised (Kawasaki and Moscttig, 1959; Schaub and Weiss, 1961; Takeda, Kubota and Kawanami, 1960) and it was discovered that 3,20-dioxo-5α-thiocyanatopregnane (VI) and 17α-ethynyl-17β-hydroxy-3-oxo-5α-thiocyanato-19-norandrostane (VII) were approximately equal in



VI



VII

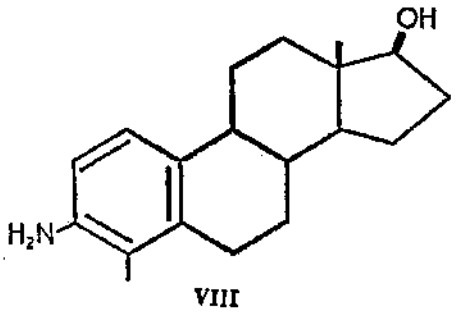
progestational activity to their parent compounds progesterone and 19-norethynyltestosterone respectively. Similarly the 4,5-dihydro-5α-thiocyanato-analogue of cortisone acetate showed comparable activity to cortisone acetate (Takeda, Kubota and Kawanami, 1960) although the 4,5-dihydro-5α-thiocyanato-derivative of hydrocortisone acetate showed little or no activity. Doubt is expressed, however, whether the thiocyanato-derivatives were themselves active in view of their ready reconversion into the parent hormone.

Other steroid hormone analogues with electron-withdrawing groups in the molecule include various 5- and 6-cyano-derivatives (Bowers, 1961; Bowers and others, 1959) and several 6-nitro compounds (Bowers, Ibáñez and Ringold, 1959; Bowers, Sánchez and Ringold, 1959). Of these, 6α-nitro-17α-acetoxyprogesterone was found in the Clauberg assay, oral route, to be 3–4 times as active as 17α-acetoxyprogesterone as a progestational agent (Bowers, Ibáñez and Ringold, 1959). On the other

hand both 6α- and 6β-nitrotestosterone were inactive as myotrophic, androgenic or gonadotrophin-suppressing agents in the parabiotic rat (Bowers, Sánchez and Ringold, 1959). The isomeric 6α- and 6β-nitroprogesterones exhibited less than one eighth the progestational activity of progesterone in the guinea-pig copulatory assay (Bowers, Sánchez and Ringold, 1959) and 21-nitroprogesterone was inactive (Bowers and Ringold, 1959).

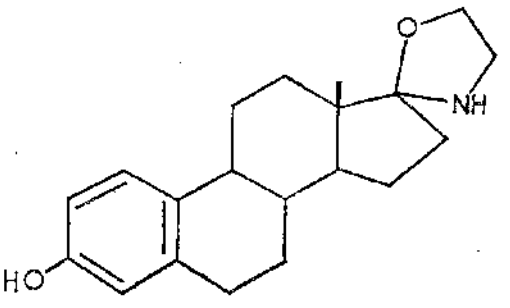
The 2-nitro-, 4-nitro- and 2,4-dinitro-derivatives of oestrone (Werbin and Holoway, 1956) and oestradiol (Patton, 1959b) are known, but like the 16-isonitroso-derivative of oestrone-3-methyl ether (Litvan and Robinson, 1938) they do not appear to have been tested biologically.

Various nitrogenous steroids, where the nitrogen forms part of an electron-donating group, have also been studied. In particular considerable attention has been paid to derivatives of oestrone and oestradiol. Amongst such compounds may be mentioned 2-amino-4-methyloestra-1,3,5(10)-trien-17β-ol (Dannenberg and others, 1960), 3-amino-4-methyloestra-1,3,5(10)-trien-17β-ol (VIII) (Dannenberg and others, 1959) and



VIII

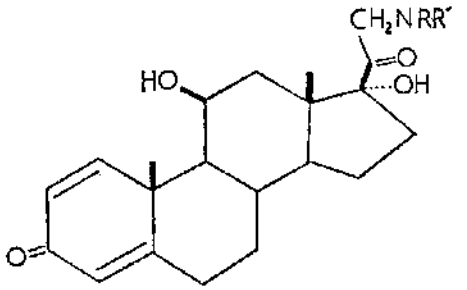
certain 2-dialkylaminomethyl derivatives (Patton, 1959a; 1960) which all proved devoid of activity. It is claimed however that a derivative of oestrone thought to possess the 17-spiro-oxazolidine structure IX exhibits an oral activity ten times that of oestrone (Hebo, 1951).



IX

Several synthetic steroids possessing the provitamin D 5,7-diene system with a terminal tertiary amino-function in the side chain have been prepared but on irradiation, only slight anti-rachitic activity was observable in the most favourable substance (Louw, Strating and Backer, 1955). The amides from which these amines were prepared were also inactive on irradiation.

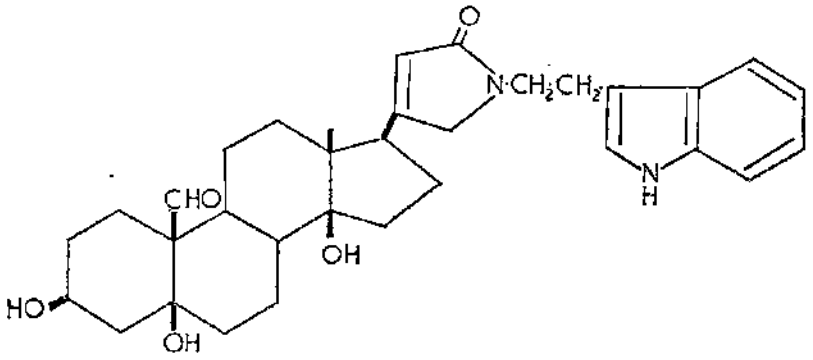
Other examples of the introduction of an electron-donating nitrogen function into the steroid side chain are afforded by a number of *N*-substituted 21-amino-11β,17α-dihydroxypregna-1,4-diene-3,20-dione compounds of type X, which have been shown to retain the glucocorticoid



X

activity of prednisolone from which they are derived, as evidenced by the results of liver glycogen accumulation and rat foot oedema tests (Tóth, Tuba and Szporny, 1961).

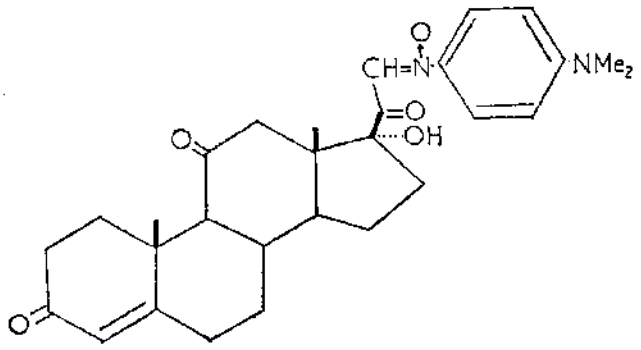
More complex structural modification studies involving nitrogen have been reported in the cardenolide field. Thus strophanthidin reacts with primary and secondary amines to form nitrogenous derivatives (Bembry, Elderfield and Krueger, 1960), one of which, tryptamine-strophanthidin, not only retains a typical digitalis-like action on the isolated papillary muscle of the cat (Greiner and Reilly, 1952), but unlike the glycosides of the strophanthus series, it is active by the oral route in man (Otto and others, 1953). Unfortunately, however, it often produces emesis. The structure originally assigned to this compound (Otto and others, 1953) has since been retracted (Bembry and others, 1960) and it is now believed to be that shown in XI. Other cardenolide derivatives which have been



XI

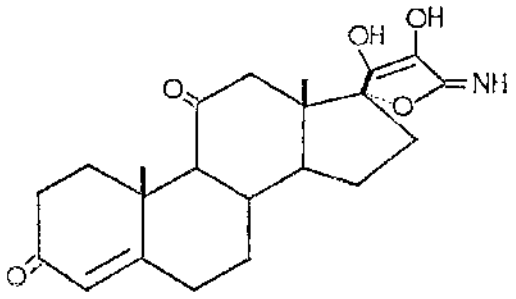
studied biologically include the 3-diethylaminoacetate and the 3-nicotinate of strophanthidin (Küssner, 1939; Steldt, Anderson and Chen, 1944). In the cat the diethylaminoacetate proved more potent than the parent aglycone, but the nicotinate was less active (Steldt and others, 1944).

Two *p*-dimethylaminophenylnitrones related to cortisone and hydrocortisone, viz. 17α-hydroxy-3,11,20-trioxopregn-4-en-21-*p*-dimethylaminophenylnitrone (XII) and the corresponding 11β-hydroxy compound,



XII

have been shown to retain glucocorticoid activity as demonstrated in the liver glycogen deposition assay (Leanza and others, 1954), but several 21-pyridinium salts derived from cortisone and hydrocortisone were inactive, as was the iminolactone XIII.



XIII

There are a number of references in the literature to the screening of various nitrogenous steroids only remotely related structurally to steroid hormones, and as might be expected, in most tests these compounds proved inactive, as, for example, the series of 24-amino-derivatives prepared from bile acids which showed no antirheumatic activity (Wessely and Swoboda, 1951). It is therefore a little surprising that certain substituted amino-alkanol esters of bile acids appear to exhibit some anti-inflammatory activity based on claims of their efficacy in the treatment of fibrositis and certain types of arthritis (Burtner, 1951).

Considerable attention has been devoted to structural modifications of the steroid nucleus itself and a number of these studies have been concerned with the introduction of nitrogen atoms into various ring positions. At the present time, in addition to several homoaza-steroids, aza-steroids are known in which each secondary carbon atom of the steroid nucleus, with the exceptions of C(1) and C(11), has been replaced by nitrogen (see for example Doorenbos and Mu Tsu Wu, 1961; Gut and Uskokovic, 1961; Knof, 1961; Kutney and Johnston, 1961; Jacobs and Brownfield, 1960; Shoppee and Krueger, 1961). Little work appears to have been published on the biological properties of these compounds so far, but they can be expected to provide interesting information in terms of the receptor theory with their regions of high electron density actually incorporated in the nucleus. Certain oxygenated 12a-aza-C-homo-steroids

have been reported (Mazur, 1957a,b) to inhibit the harmful deposition of liver glycogen occurring as an untoward effect in cortisone therapy and 4-aza-pregn-5-en-3,20-dione is claimed to exhibit marked anti-inflammatory activity in rats (Wildi, 1959). Weak androgenic properties and anti-oestrogenic activity are present in certain lactams belonging to the 4-aza-androstane series (Doorenbos and Huang, 1961). Several bis-dehydrodoisynolic acid analogues possessing the 1,2,3,4-tetrahydrobenz-[f]-isoquinoline nucleus were inactive as oestrogens and also failed to exhibit any androgenic or anti-inflammatory activity (Nelson and Hsi, 1961).

Nitrogenous Steroids as Antimetabolites

Where the affinity for a receptor fitted by a normal metabolite of a living organism is also present in a synthetic analogue of the metabolite, but the analogue exhibits a greatly reduced intrinsic activity, then the analogue is likely to function as an antagonist of the metabolite. Recently there has been an intense interest in the planned synthesis of antimetabolites, and this has been referred to as "the revolution in pharmacology" (Woolley, 1960). In the steroid field, several antimetabolites, such as the spirolactone antagonists of aldosterone (Atwater and others, 1961; Barter, 1960) are well established whilst other compounds, such as the halogenated analogues of the corticoid steroids (Fried, 1957) and the dihydrocardenolides (Cosmides, Muja and Carr, 1956) are believed to competitively antagonise their parent compounds. Yet nitrogen-containing steroids seem to have been little investigated as metabolite-displacing agents. Cholesterylamine, however, has been demonstrated to be a weak inhibitor of the use of cholesterol by the cockroach (Noland, 1954) and there are claims in the patent literature (Dodson, 1955b; Rorig, 1953) that certain nitrogenous steroids possess antihormonal activity. It is also possible that the antimicrobial activity displayed by various nitrogenous steroids may be due to antagonism towards steroidal metabolites of the organism, although it is clear that many of these compounds act by virtue of their surface-active properties (Stacey and Webb, 1947a). Such surface activity is widespread within the steroid field, and is particularly pronounced in the saponins, cardiac glycosides, various steroidal alkaloids, and the bile acids. In addition to conferring antimicrobial properties, the surface activity also confers haemolytic properties and it is therefore not unexpected that 3,6-diaminocholestane is a haemolytic agent (Stückradt, 1939).

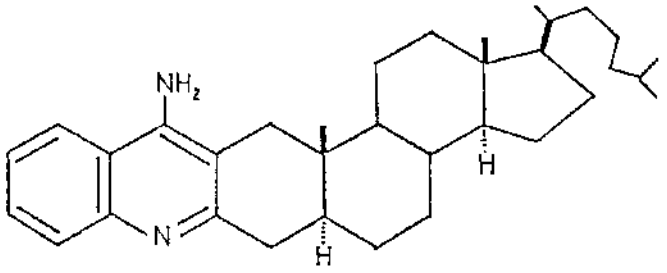
A large number of nitrogenous steroids have been tested for antibacterial and antifungal activity. These compounds cover a wide range of chemical complexity and include both synthetic compounds and alkaloids. Among the simpler synthetic compounds may be mentioned the epimeric 7-aminocholesterols (Barnett, Ryman and Smith, 1946a), various mono- and diaminocholestane derivatives with the nitrogen functions in the 3-, 6- or 7-positions (Barnett, Ryman and Smith, 1946b) and several hydroxylated 23-aminonorcholane derivatives, including 23-guanido-3,7,12- trihydroxynorcholane (James and others, 1946). The monoaminocholestane

derivatives showed some activity against Gram-positive organisms (Barnett and others, 1946a,b) and the potency is increased in the diamino-compounds which in addition, showed some activity against Gram-negative organisms (Barnett and others, 1946b).

Amino-steroids prepared from bile acids in which the nitrogen atom is attached directly to the steroid nucleus exhibit but weak antibacterial properties (Hilton, Jones and Westwood, 1955; Jones, Webb and Smith, 1949; Redel and others, 1951) but where the amino-group is in the side chain, the potency is higher (Hilton and Webb, 1951; Stacey and Webb, 1947b).

Among the more complex synthetic nitrogenous steroids which have been shown to exhibit antibacterial properties are a number of *N*-substituted 16-amino-derivatives (Schering, 1955) and 3,3-di(*N*-acetyl-*p*-aminophenylmercapto)-7,12-diketocholanic acid (Jones, Smith and Webb, 1948). Several *N*-substituted 22-aminobisnorcholanes and their derived quaternary methiodides were found to possess antifungal activity in tests with *Candida albicans* (Herzog, Payne and Hershberg, 1955), but the amides from which they were derived showed little or no activity. Various aminopregnane derivatives of varying chemical complexity also possess antimicrobial activity (Kull, Castellano and Mayer, 1953; Micheli and Bradsher, 1955). The anti-amoebal properties of the steroidal alkaloid conessine inspired the screening of several synthetic nitrogenous steroids (Dodgson and Haworth, 1952), but none was more active than conessine itself. Certain compounds of this type showed antibacterial activity of about one twentieth that of streptomycin (Joska, Černý and Šorm, 1954).

Of a series of quinolino-, indolo-, pyrrolo-, thiazolo- and triazafluoreno-steroids prepared as potential antimicrobial agents only one, XIV, was



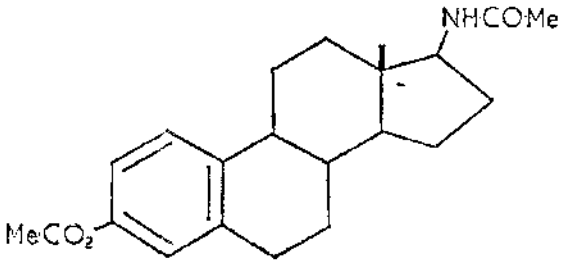
XIV

sufficiently soluble to be tested and it proved inactive (Antaki and Petrow, 1951). *N*-Phenyl-3β-cholestanamine and *N*-*p*-tolyl-3β-cholestanamine, which were prepared during a search for drugs effective in leprosy and tuberculosis, also proved to be inactive (Buu-Hoi and Cagniant, 1944).

Bioisosterism

Certain nitrogenous steroidal derivatives are of particular interest in terms of the concept of bioisosterism (Schatz, 1960; Friedman, 1951) which postulates that compounds in isosteric relationship should possess either similar or opposite biological activity. This behaviour can be rationalised in terms of the receptor theory of drug action since the great

similarity in chemical and physical properties shown by isosteres should ensure similar affinities for the same receptors, mimicry or antagonism then being determined by the intrinsic acitivities of the individual isosteres. In the extended definition of isosterism (Erlenmeyer, 1948), functional groups which are related by the Hydride Displacement Law of Grimm (1934 and earlier refs.) are considered to be isosteric and so such a relationship will pertain for steroids related by the substitution of the -NH-group for the -O- function. Unfortunately, however, this particular isosteric relationship is complicated by the greater willingness of the nitrogen atom to enter salt formation and so strictly comparable biological activities are not necessarily to be expected. At physiological pH the amino-steroids will be ionised and so may have difficulty in penetrating permeability barriers (Brodie and Hogben, 1957) and may not reach the receptor. Although most of the amino-isosteres of steroidal hormones which have so far been tested are inactive and lack the ability to antagonise their natural analogues, potent oestrogenic activity is present in 17β-acetamido-3-acetoxyoestra-1,3,5(10)-triene (XV) (Dannenberg, Scheurlen

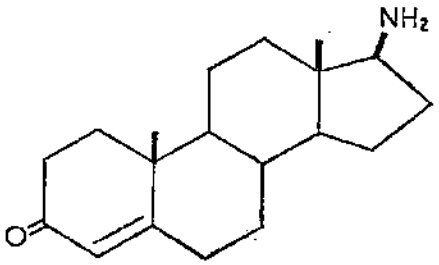


XV

and Simmer-Rühle, 1956) in which salt formation at the nitrogen atom is prevented by the amide function. The activity of this compound is also of interest in connection with the suggestion that oestrogenic activity is associated with the presence in the molecule of two groups capable of entering into hydrogen-bonding and which are held in a certain steric relationship (see for example Fisher, Keasling and Schueler, 1952; Macovski and Georescu, 1946; Oki, 1952). It has been further suggested that the distance between the two groups is a multiple of the "identity distance" (Long and Schueler, 1954) which is the distance between peptide links in a polypeptide chain.

The inactive oestrogen isosteres which have been reported are the 3-amino-isostere of equilenin (Bachmann and Dreiding, 1950) the 3-amino-, 17β-amino- and the 3,17β-diamino-isosteres of oestradiol and the 3-amino-isostere of oestrone (Hecker and Walk, 1960; Gold and Schwenk, 1959).

In the androgen series 3,17β-diaminoandrost-4-ene, 3-amino-17β-hydroxyandrost-4-ene and 3-oxo-17β-aminoandrost-4-ene have been reported to be devoid of male hormone activity (Joska and Šorm, 1956), but the analogue of testosterone, 17β-amino-3-oxo-androst-4-ene (XVI), although showing no activity in male rats, produced a pronounced increase in kidney weight and lesser increases in the liver and adrenal weights of female rats (Gaunt and others, 1954).



XVI

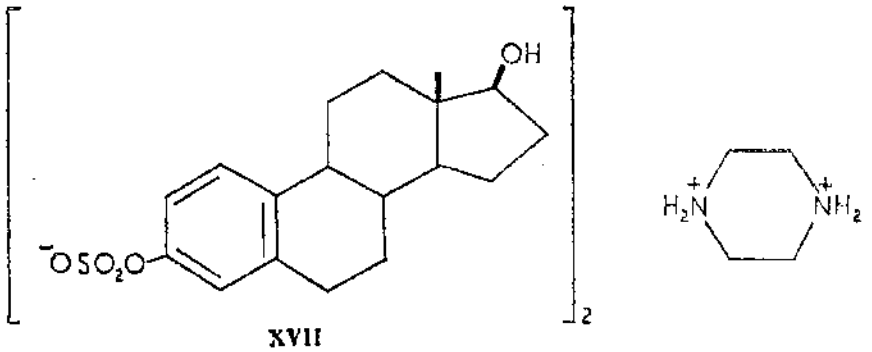
Replacement of the oxygen atom in the lactone ring of scillaren A by the-NH-group gave rise to a very sparingly soluble isostere which showed no cardiotonic activity when tested on the isolated guinea-pig auricle at a concentration of 10^{-6}M (Uhle and Schröter, 1961).

Drug Latentiation

Nitrogenous steroids have played a small but nevertheless significant role in drug latentiation (Harper, 1959) where a chemical derivative of an active drug is administered to overcome unfavourable rates of biotransformation or unfavourable solubility, distribution, transport and absorption characteristics—the active drug being regenerated *in vivo*. The steroidal moiety has sometimes functioned as the latentiating agent and at other times an active steroid has been latentiated.

Examples of the use of steroids as latentiating agents include the preparation of insoluble steroidal amine salts of penicillin capable of maintaining prolonged therapeutic concentrations of the antibiotic in the bloodstream (Coghill, Weston and MacCorquodale, 1950; Madinaveitia, 1955; Vaidya and Boyce, 1959) and the application of the cholesterol-6-sulphonate anion to yield an insoluble thiamine salt with which to enrich cereal flour (Mima, 1955). Another example is afforded by the choline salts of cholic acid and desoxycholic acid which exert actions typical of both moieties on the guinea-pig intestine (Meyer and McEwen, 1948). Attempted latentiation of 3-indolylacetic acid by the formation of steroid esters failed, however, to enhance parthenocarpic fruit induction in the tomato (Hofert and Sell, 1960).

Examples of nitrogenous moieties being used to latentiate biologically-active steroids are far more numerous. For instance much attention has been devoted to the preparation of amine salts of steroid hormone sulphate esters to increase the water solubility of the parent hormone. Among such compounds can be listed the ethylenediamine salts of the sulphate esters of oestrone, oestradiol, equilenin, androsterone and pregnenolone (Abbot, 1954a,b); the piperazine salts of the sulphate esters of oestradiol and equilenin (Hasbrouck, 1953); and the procaine (Deans and Scarrow, 1951), amphetamine (Grant, Glen and Barker, 1950) and 2-aminopyridine (Beall and Grant, 1952) salts of the sulphate esters of various steroidal oestrogens. Of these compounds piperazine oestradiol sulphate (XVII) has been used clinically. Dehydroandrosterone and androstenediol lose their biological activity if administered as dialkylamino sulphuric ester derivatives (Goisis and Polvani, 1955). Various quaternary ammonium

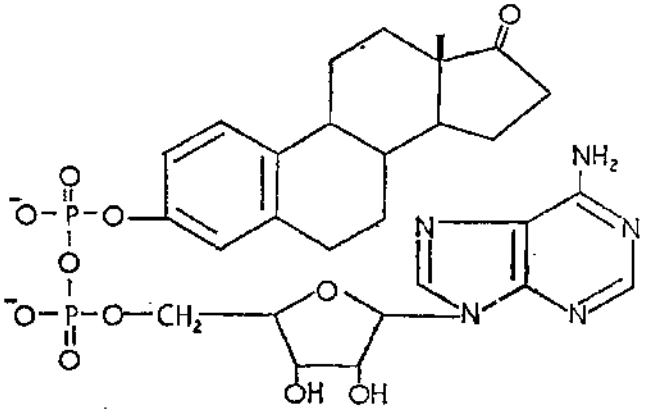


XVII

derivatives of hydrocortisone, prednisolone and dexamethasone retain activity (Mori and Nakagawa, 1961).

Latentiation of steroids may also be a natural phenomenon since amino-acid conjugates of steroidal hormones have been discovered in aqueous adrenal cortical extracts (Voigt and Schroeder, 1956 and earlier refs.) in urine (Eades, Pollack and King, 1954; Schneider and Frahm, 1955) in blood (Hudson and Lombardo, 1955) and in liver (Butenandt, 1956) and chorion-gonadotrophic extracts (Schneider and Frahm, 1956; Schneider and Birtel, 1956). These discoveries prompted Schroeder and Voigt (1958) to investigate the efficacy of glycyltestosterone in the survival test on adrenalectomised golden hamsters, but the compound was inactive and also devoid of androgenic properties (Overbeck, 1957). Nevertheless certain steroids in the form of amino-acid esters do appear to retain their activity (Organon, 1960), and latentiation of hydrocortisone by conversion to the more soluble diethylaminoacetate hydrochloride has been used in dermatology (Welsh, 1956; Kuhn, 1959). Prednisolone and dexamethasone have also been administered as their diethylaminoacetates (Dorner and Hohlweg, 1961; Zicha and others, 1960). The β-diethylaminoethyl ester of dehydrodesoxycholic acid was found to have one-quarter of the potency of dehydrocholic acid as a choleretic agent on a molar basis (Gunter and others, 1950).

Recently interest has been aroused in the preparation of steroidal nucleotides like XVIII (Oertel and Agashe, 1960; Riess and Ourisson, 1961) and in the preparation of the nicotinic esters of male, female and adrenocorticoid hormones (Weichsel and Zirm, 1961), the synthesis of



XVIII

the latter being inspired by the fact that favourable analgesic activity was retained in the bisnicotinic ester of morphine (Skursky, 1957).

An important example of drug latentiation in the steroid field is provided by the general anaesthetic hydroxydione which is the sodium salt of the hemisuccinate of 21-hydroxypregnane-3,20-dione. In this compound the hemisuccinate moiety confers increased water solubility on a predominantly lipid soluble molecule and rapid hydrolysis by the non-specific esterase of the serum regenerates the parent compound which then crosses the blood-brain barrier and quickly builds up an anaesthetically active concentration in the brain (Figdor and others, 1957; Jakoby and Tomkins, 1956). There would seem to be no *a priori* reason why other readily hydrolysed water-solublising groups could not be employed in place of the sodium hemisuccinate moiety and indeed a series of substituted aminoacetates, tertiary amine hemisuccinate salts and a substituted ammonium phosphate salt of 21-hydroxy-pregnane-3,20-dione have been tested for anaesthetic potency (Fidgor and others, 1957). Also included in the study were the hemisuccinates of two 3-spirothiazolidine derivatives of 21-hydroxy-20-oxo-5α-pregnane. The results showed that the nature of the solubilizing group could markedly influence the activity displayed. The aminoacetates exhibited high potency and high toxicity. Within the series there was much variation in the time of onset of anaesthesia.

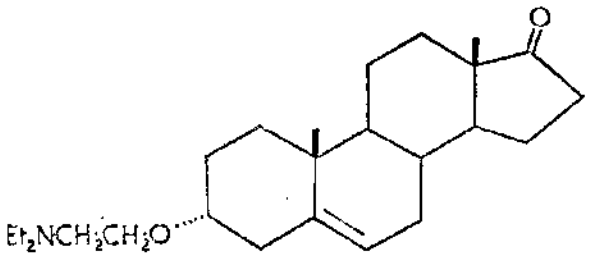
Although anaesthetic properties are associated with many steroids (Figdor and others, 1957; Selye, 1942) the phenomenon appears a structurally specific one as nuclear substitution profoundly alters activity (Figdor and others, 1957). Moreover if the action was simply an extension of the properties of the gaseous general anaesthetics whose activity can be related with their thermodynamic activities and hence with their concentration in the central nervous system (Ferguson, 1939), one would expect other predominantly lipid soluble molecules of intermediate molecular weight to form a connecting bridge between the simple anaesthetics on the one hand and the steroids on the other. The sodium hemisuccinates of representative mono- and di-terpenoids, however, proved inactive (Ahmad and others, 1961).

Little attention would appear to have been given to the possibility of improving the solubility, absorption and transport properties of nitrogenous drugs by forming inclusion compounds with desoxycholic acid, although it has long been recognised that various alkaloids do form choleic acids (Wieland and Sorge, 1916)—that is, inclusion compounds with desoxycholic acid. Inclusion compounds in which steroid hormone molecules are the entrapped species have, however, excited some interest and recently investigations of this kind of compound have been made with phenylurethane and hippuric acid as models for the study of the interaction of proteins with steroids (Dirscherl and Gerhards, 1961).

Supporting Moiety Theory

Nitrogenous steroids played an important part in the development of the supporting moiety theory which contends that the molecules of pharmacologically active substances consist of a radical moiety determining the

type of activity displayed and a supporting moiety conferring affinity for the site of action. Cavallini and his colleagues who were the first to formally state this theory (Cavallini, 1955; Cavallini and Massarani, 1959) employed such compounds in their early experiments. The actual compounds included the β-diethylaminoethyl ethers of oestrone, testosterone and 3α-hydroxy-17-oxoandrost-5-ene (XIX), and the bis β-diethyl-

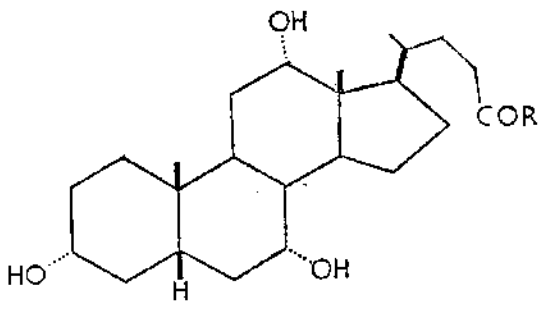


XIX

aminoethyl ethers of oestradiol and 3,17-dihydroxyandrost-5-ene (Cavallini and Massarani, 1951b). The combination of "stripped down" drug molecule (Gero and Withrow, 1957) or radical moiety (diethylaminoethanol) and the steroidal supporting moiety produced drugs with potent coronary vasodilatator properties (Cavallini and Massarani, 1951b, 1959) whilst the bisquaternary salts derived from the two di-ether compounds showed curare-like properties (Cavallini and Massarani, 1959; Cavallini and others, 1951). These quaternary salts also showed *in vitro* anticholinesterase activity (Cogni and Salvaneschi, 1951). Quaternary salts derived from the mono-diethylaminoethyl ethers exhibited ganglion-blocking activity (Cavallini and Massarani, 1959).

Coupling to the predominantly lipid soluble steroid nucleus would be expected to confer upon a radical moiety not only different solubility characteristics but perhaps also more favourable adsorption properties. Since the plasma proteins are of such a nature as to readily bind cholesterol, it is conceivable that such compounds could use an existing transport mechanism.

Application of these ideas was independently made by several groups of workers in the sulphonamide field, who attempted to overcome the unfavourable lipid solubility characteristics of this group of antibacterials by preparing sulphonamido derivatives of cholesterol (Lieb, 1947; Kwartler, 1948) or the bile acids (Redel and others, 1951; Haslewood, 1941). Sulphacholazine (XX,R = NH·NH·SO_2·C_6H_4-*p*-NH_2) was found



XX

to possess *in vitro* activity against streptococci and moreover on intravenous administration to rabbits it was demonstrated to gain access to the bile (Barber, Dible and Haslewood, 1943). It was unfortunate that the compound showed little activity against the coliform group of organisms. More recently steroidal 4-amino-2-methoxyphenyl ethers have been prepared with the object of using the bile acid transport system to bring schistosomicidal amines in contact with the adult schistosomes residing in the portal veins (Davis, 1962).

The N^1-hydroxycholanyl-*p*-aminophenylsulphonamides (e.g. XX,R = $NH{\cdot}SO_2{\cdot}C_6H_4$-*p*-$NH_2$) are claimed to exhibit antibacterial and antiviral properties (Berczeller, 1948) but where the sulphonamide moiety is attached directly to the steroid nucleus as, for example, at position 7, the compounds show little or no activity (Redel and others, 1951).

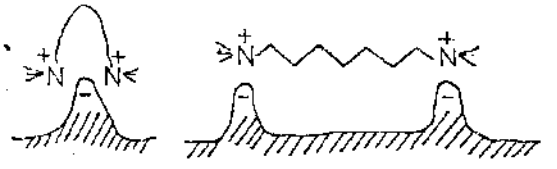
The rigid nucleus of steroids which possess a benzenoid ring A or a trans A/B ring junction is of interest as a supporting moiety from another point of view, since it can function as a skeletal framework upon which two or more radicals can be held in fixed spatial relationship to one another. Such a function for the steroid nucleus has been suggested to be involved in the activity displayed by the natural oestrogens where the oxygen functions at C(3) and C(17) are held at a rigid intergroup distance (see for example: Fisher, Keasling and Schueler, 1952; Macovski and Georescu, 1946; Oki, 1952). Other supporting moieties would be expected to be able to fulfil this role and in this may lie at least a part explanation for the potent oestrogenic properties displayed by such chemically diverse molecules as certain isoflavones, chlorotrianisene, the oestrogenolic acids and the oestrogenic stilbenes. Relatively little success has been achieved in securing alternative supporting moieties to replace the steroid nucleus in the androgenic and corticosteroid fields, although several attempts have been made (e.g. Clarke and Martini, 1959).

The hypothesis, first advanced by Paton and Zaimis (1949, 1951), that bisquaternary ammonium neuromuscular blocking agents interact by a two point attachment with anionic sites, normally involved in the physiological functioning of acetylcholine, has led to a number of attempts to define closely the actual interonium distance at the time of drug-receptor complex-formation. Unfortunately most compounds tested are conformationally non-rigid and so incapable of affording the desired information, as there is no reason to suppose that the thermodynamically most stable conformation of the isolated molecule is that actually adopted at the receptor. Whether various rigid bisquaternary ammonium salts in which the steroid nucleus functions as the supporting moiety will provide further information remains to be discovered. Although the receptor itself could conceivably be non-rigid, demonstration of activity in one rigid bisquaternary salt and absence of activity in another with a different interonium distance would represent a great advance.

Such steroidal bisquaternary ammonium salts will also be of interest in terms of Gill's hypothesis (1959) that completely rigid molecules should prove to be inactive due to variability in the receptors. This generalisation, which rests on the absence of ganglion-blocking activity in a limited

number of compounds, such as the completely rigid *NNN'N'*-tetramethyl-*p*-phenylenediamine dimethiodide (Wien and Mason, 1953) and certain virtually rigid furan derivatives still retaining a limited degree of rotational flexibility (Gill and Ing, 1958), certainly requires further substantiation. That there is a strong possibility that the molecules chosen do not possess a suitable interonium distance is indicated by the fact that other rigid molecules do indeed exhibit pronounced pharmacological activity. Examples are afforded by the natural oestrogens, testosterone, and the virtually rigid *cis* 1-hydroxy-2-trimethylammoniumcyclopentane, which shows marked depolarizing properties on the kitten phrenic nerve diaphragm (Standaert and Freiss, 1960).

Moreover, the two-point attachment theory is by no means universally accepted. Loewe and Harvey (1952) have postulated a one-point attachment theory in which the bulk of the molecule shields the receptor—the so-called "adumbration theory"—and their ideas have been extended by Fakstorp and others (1957). Again, conductimetric experiments have shown the extreme stability of the ion pair involving a single anion and a bisquaternary ammonium cation (Brody and Fuoss, 1956) which raises the possibility that the receptor-complex could be of type A, rather than type B (Cavallito and Gray, 1960).



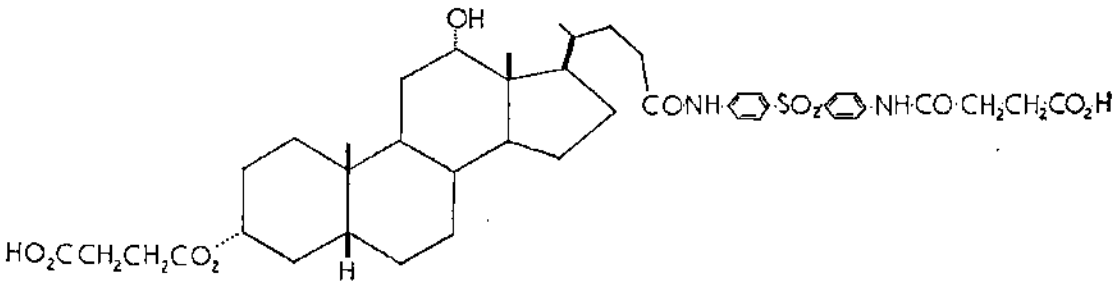
A B

In view of all these facts, steroidal bisquaternary ammonium salts may well prove to be of great importance in distinguishing between the various possibilities.

It is to be noted that malouetine (II) is not fully rigid, due to rotation about the C(17)—C(20) bond and to the possibility of chair to boat conformational isomerism in ring A, and these effects permit some variation in the interonium distance.

The discovery that marked antituberculous properties were present in various 4,4'-diaminodiphenylsulphone derivatives, thiosemicarbazones and hydrazones, resulted in the synthesis of many related compounds, including several steroidal derivatives in which the steroidal portion can be regarded as a supporting moiety. Some of these compounds proved to have a high activity, especially the bile acid amide derivatives of diaminodiphenylsulphone (Berczeller, 1949; 1958). One compound in this series, 4-(3-hemisuccinyldesoxycholylamino)-4'-hemisuccinylamino-diphenyl-sulphone (XXI) also proved to be an active inhibitor of the multiplication of the PR8 strain of influenza virus A in the chick embryo (Berczeller, 1958–59).

The hydrazones and isonicotinylhydrazones of testosterone, oestrone and dehydroandrosterone all proved active against the tubercle bacillus *in vitro* (Cavallini and others, 1952; Mantegazza and Tommasini, 1952)



XXI

as did the thiosemicarbazones of testosterone, progesterone and dehydroandrosterone (Mantegazza and Tommasini, 1951). These derivatives are virtually devoid of the physiological properties of the parent steroids (Cavallini and Massarani, 1951a; Ercoli, Koller and de Ruggièri, 1951). Dehydroisoandrosteryl-thiolpyrazinoate which was prepared as an analogue of the antituberculous ethylthiolpyrazinoate proved inactive (Kushner and others, 1955) as did the benzoylhydrazone of cholestenone (Offe, Siefken and Domagk, 1952). Cholesteryl *p*-nitrobenzoate, unlike certain other esters of *p*-nitrobenzoic acid was inactive against pneumococci (Mayer and Occhslin, 1939).

The promise shown by the nitrogen mustards as potential anti-cancer agents inspired the utilisation of the steroid nucleus as a supporting moiety in this field as well, and several mono- and bis-(2-chloroethyl)aminosteroids have been synthesised (Burstein and Ringold, 1961; Gensler and Sherman, 1958; Havranek and Doorenbos, 1960). Only three of these compounds appear to have been tested for anti-tumour activity, however, and these proved inactive (Havranek and Doorenbos, 1960). *Added in proof:* A more recent investigation has shown that antitumour activity is present in certain steroidal nitrogen mustards (Rao and Price, 1962).

The potent positive inotropic cardiac activity present in both the steroidal cardiac glycosides and the erythrophleum alkaloids led two groups of workers to prepare steroidal analogues of the latter, in which bile acids were used to replace the diterpenoid acids as supporting moieties (Ruzicka, Plattner and Engel, 1944; Uhle, Mitman and Krayer, 1956), but the new compounds were virtually inactive. It will be interesting to see whether steroidal esters of pyrrole -α-carboxylic acid will be prepared as analogues of the diterpenoid alkaloid ryanòdine (Valenta and others, 1962) which exhibits such a remarkable pharmacological action on muscle (Hillyard and Procita, 1959 and refs. cited).

Several groups of naturally-occurring nitrogenous steroids can probably be quoted as exemplifying the supporting moiety theory, although in some cases it is difficult to distinguish a supporting moiety function from a latentiation function. A good illustration of this situation is afforded by the taurine and glycine conjugates of the bile acids whose anions are the true bile salt anions. Another example is afforded by the readily hydrolysed veratrum ester alkaloids, such as protoveratrine, which is some 6,000 times as toxic on a molar basis as is its alkamine, protoverine (Krayer, Moe and Mendez, 1944).

It is also possible to consider the suberylarginine radical of the toad poisons as a supporting moiety, but the position with the cardiotonic steroids is particularly complex. It is generally held that the strong positive inotropic action is intimately linked with the unsaturated lactone function (Chen and Elderfield, 1940; Goodman and Gillman, 1955) but to regard this group as the active moicty is a gross oversimplification, as varying degrees of positive inotropic action are shown by the dihydrocardenolides (e.g. Jacobs and Hoffmann, 1927; Vick, Kahn and Acheson, 1957), the bile acids (e.g. Wakim, Essex and Mann, 1939), certain steroidal alkaloids (Benforado, 1957; Krayer, Moe and Mendez, 1944; Quévauviller and Blanpin, 1958), cortisone (Fleischhacker, 1956) and various other steroids (e.g. Abrams and Harris, 1951; Hajdu and Szent-Gyorgyi, 1952; Tanz and Kerby, 1961) none of which possess an unsaturated lactone. Indeed it is tempting to regard the lactone as a supporting moiety intensifying an activity associated with a hydroxylated steroid nucleus (Craig and Jacobs, 1943). A supporting moiety role can probably be assigned to the sugar residues of the cardiac glycosides since they have a marked influence on distribution and solubility properties and so affect the time of onset and duration of action (Chen, Henderson and Anderson, 1951; Keyl and Dragstedt, 1954). Although some scilladienolide genins show some potency in bioassays, the duration of action is transitory and so it is concluded that the sugar moieties play an indispensable role in determining the activity of the glycosides (Stoll, 1956). The effect of varying the number and nature of the sugar residues on the activity displayed has been summarised in several places (Chen, 1945; Fieser and Fieser, 1959; Oettel, 1947; Tamm, 1957).

The pharmacological actions of the suberylarginine conjugates of the bufadienolides were studied by Gessner (1926) and his work was followed by an elegant series of papers by K. K. Chen and his colleagues (e.g. Chen and Chen, 1934). The results of these and other studies (Arora, 1953; Chen, Anderson and Rose, 1952) would indicate that the compounds possess broadly similar pharmacological properties to the cardiac glycosides, but that the suberylarginine moiety does affect the rate of penetration into and removal from the myocardial tissue, thus producing differences of a quantitative nature.

Other Theoretical Interests of Nitrogenous Steroids

Several isolated examples are known in which nitrogenous steroids have played minor roles in studies designed to throw more light on the intimate nature of biological processes. One such instance involves the application of complex steroid derivatives in the study of artificial antigens (Grob and Goldberg, 1949).

The interesting hypothesis has put forward that certain steroids may interact with enzymes and other proteins by formation of spirothiazolidines since 3-oxo-steroids lacking a 4,5-double bond were shown to form such compounds under a variety of conditions with cysteine (Lieberman, 1946), but this suggestion is in need of further substantiation.

REFERENCES

Abbot (1954a). Brit. Patent 708541 in *Chem. Abstr.* (1955), **49**, 6326.
Abbot (1954b). U.S. Patent 2,666,066 in *Chem. Abstr.* (1954), **48**, 12816.
Abrams, W. B. and Harris, T. N. (1951). *Amer. Heart J.*, **42**, 867–883.
Abreu, B. E. (1959) in *Hypertension*, editor J. Moyer, pp. 327–332, Philadelphia: Saunders.
Acton, H. W. and Chopra, R. N. (1933). *Indian med. Gaz.*, **68**, 6.
Ahmad, K., Khatoom, T., Lewis, J. J. and Martin-Smith, M. (1961). Unpublished work.
Antaki, H. and Petrow, V. (1951). *J. chem. Soc.*, 901–904.
Arnold, A., Beyler, A. L. and Potts, G. O. (1959). *Proc. Soc. exp. Biol. N.Y.*, **102**, 184–187.
Arora, R. B. (1953). *J. Pharmacol.*, **108**, 26–32.
Atwater, N. W., Bible, R. H., Brown E. A., Burtner, R. H., Mihina, J. S., Nysted, L. N. and Sollman, P. B. (1961). *J. org. Chem.*, **26**, 3077–3083.
Bachmann, W. E. and Dreiding, A. S. (1950). *J. Amer. chem. Soc.*, **72**, 1,329–1,331.
Barber, M., Dible, J. H. and Halsewood, G. A. D. (1943). *Biochem. J.*, **37**, p. vi.
Barnett, J., Ryman, B. E. and Smith, F. (1946a). *J. chem. Soc.*, 524–526.
Barnett, J., Ryman, B. E. and Smith, F. (1946b). *Ibid.*, 528–530.
Barter, F. C. (1960). *The Clinical Use of Aldosterone Antagonists*, Springfield, Illinois; Thomas.
Beall, D. and Grant, G. A. (1952). U.S. Patent 2,581,350 in *Chem. Abstr.*, (1952), **46**, 7596.
Bembry, T. H., Elderfield, R. C. and Krueger, G. L. (1960). *J. org. Chem.*, **25**, 1,175–1,179.
Benforado, J. M. (1957). *J. Pharmacol.*, **120**, 412–425.
Berczeller, A. (1948). U.S. Patent 2,441,129 in *Chem. Abstr.*, (1948), **42**, 5,622.
Berczeller, A. (1949). U.S. Patent 2,485,253 in *Chem. Abstr.*, (1950), **44**, 2,575.
Berczeller, A. (1958). *Dis. of Chest*, **33**, 475–481.
Berczeller, A. (1958–59). *Antibiot. Ann.*, 88–94.
Beyler, A. L., Potts, G. O. and Burnham, D. F. (1960). *Abstracts 1st International Congress of Endocrinology*, 829–830.
Bloom, B. M. (1956). U.S. Patent 2,763,645 in *Chem. Abstr.*, (1957), **51**, 3,678.
Bowers, A. (1961). *J. org. Chem.*, **26**, 2043–2047.
Bowers, A., Denot, E., Sánchez, M. B., Sánchez-Hidalgo, L. M. and Ringold, H. J. (1959). *J. Amer. chem. Soc.*, **81**, 5233–5242.
Bowers, A., Ibáñez, L. C. and Ringold, H. J. (1959). *Ibid.*, **81**, 3707–3710.
Bowers, A. and Ringold, H. J. (1959). *Ibid.*, **81**, 3710–3712.
Bowers, A., Sánchez, M. B. and Ringold, H. J. (1959). *Ibid.*, **81**, 3702–3706.
Brodie, B. B. and Hogben, C. A. M. (1957). *J. Pharm. Pharmacol.*, **9**, 345–380.
Brody, O. V. and Fuoss, R. M. (1956). *J. phys. Chem.*, **60**, 156–160.
Burstein, S. H. and Ringold, H. J. (1961). *J. org. Chem.*, **26**, 3084–3086.
Burtner, R. B. (1951). U.S. Patent 2,562,351 in *Chem. Abstr* (1951), **45**, 9812.
Butenandt, A. (1956). Cited by Voigt and Kallistratos (1957).
Buu-Hoi, Ng. Ph. and Cagniant, P. (1944). *Ber. dtsch. chem. Ges.*, **77B**, 761–766.
Cavallini, G. (1955). *Farmaco*, **10**, 644.
Cavallini, G. and Massarani, E. (1951a). *Boll. Soc. ital. Biol. sper.*, **27**, 629–630.
Cavallini, G. and Massarani, E. (1951b). *Farm. sci. e tec. (Pavia)*, **6**, 291–299.
Cavallini, G. and Massarani, E. (1959). *J. med. pharm. Chem.*, **1**, 365–370.
Cavallini, G., Ferrari, W., Mantegazza, P. and Massarani, E. (1951). *Farm. sci. e tec. (Pavia)*, **6**, 815–825.
Cavallini, G., Massarani, E., Mazzuchi, F. and Ravenna, F. (1952). *Ibid.*, **7**, 397–404.
Cavallito, C. J. and Gray, A. P. (1960). In *Fortschritte der Arzneimittelforschung*, editor E. Jucker, Vol. 2, p. 135. Basel: Birkhauser.
Chen, K. K. (1945). *Ann. Rev. Physiol.*, **7**, 681.
Chen, K. K., Anderson, R. C. and Rose, C. L. (1952). *J. Pharmacol.*, **106**, 314–318.
Chen, K. K., Bliss, C. I. and Robbins, E. B. (1942). *Ibid.*, **74**, 223–234.
Chen, K. K. and Chen, A. L. (1934). *Arch. int. Pharmacodyn*, **47**, 297–317.
Chen, K. K. and Elderfield, R. C. (1940). *J. Pharmacol.*, **70**, 338–346.
Chen, K. K., Henderson, F. C. and Anderson, R. C. (1951). *Ibid.*, **103**, 420–430.
Clarke, R. L. and Martini, C. M. (1959). *J. Amer. chem. Soc.*, **81**, 5716–5724.
Clinton, R. O., Manson, A. J., Stonner, F. W., Beyler, A. L., Potts, G. O. and Arnold, A. (1959). *Ibid.*, **81**, 1513–1514.
Clinton, R. O., Manson, A. J., Stonner, F. W., Christiansen, R. G., Beyler, A. L., Potts, G. O. and Arnold, A. (1961a). *J. org. Chem.*, **26**, 279.

Clinton, R. O., Manson, A. J., Stonner, F. W., Neumann, H. C., Christiansen, R. G., Clarke, R. L., Ackerman, J. H., Page, D. F., Dean, J. W., Dickinson, W. B. and Carabateas, C. (1961b). *J. Amer. chem. Soc.*, **83**, 1478–1491.
Coghill, R. D., Weston, A. W. and MacCorquodale, D. W. (1950). U.S. Patent 2,519,112 in *Chem. Abstr.* (1951), **45**, 666.
Cogni, G. and Salvaneschi, S. (1951). *Atti. soc. lombarda. sci. med. e biol.*, **7**, 60–64, in *Chem. Abstr.* (1953), **47**, 6459.
Cosmides, G. J., Muja, T. S. and Carr, C. J. (1956). *J. Pharmacol.*, **118**, 286–295.
Craig, L. C. and Jacobs, W. A. (1943). *Science*, **97**, 122.
Dannenberg, H., Dannenberg-von Dresler, D. and Köhler, T. (1960). *Chem. Ber.*, **93**, 1989–1998.
Dannenberg, H., Doering, C. H. and Dannenberg-von Dresler, D. (1959). *Hoppe Seyl. Z.*, **317**, 174–181.
Dannenberg, H., Scheurlen, H. and Simmer-Rühle, I. (1956). *Liebigs Ann.*, **600**, 68–80.
Davis, M. (1962). *J. chem. Soc.*, 178–181.
Deans, S. A. V. and Scarrow, J. A. (1951). U.S. Patent 2,555,579, in *Chem. Abstr.* (1952), **46**, 1054.
Dirscherl, W. and Gerhards, E. (1961). *Liebigs Ann.*, **639**, 181–194.
Dodgson, D. P. and Haworth, R. D. (1952). *J. chem. Soc.*, 67–71.
Dodson, R. M. (1955a). U.S. Patent 2,705,232 in *Chem. Abstr.* (1956), **50**, 5793.
Dodson, R. M. (1955b). U.S. Patent 2,709,701 in *Chem. Abstr.* (1956), **50**, 5782.
Doorenbos, N. J. and Huang, C. L. (1961). *J. org. Chem.*, **26**, 4106–4108.
Doorenbos, N. J. and Mu Tsu Wu (1961). *Ibid.*, **26**, 2548–2549.
Dorner, G. and Hohlweg, W. (1961). *Z. exp. Med.*, **134**, 162.
Eades, C. H., Pollack, R. L. and King, J. S. (1954). *Fed. Proc.*, **13**, 201.
Ercoli, A., Koller, M. and de Ruggieri, P. (1951). *Farm. sci. e tec. (Pavia)*, **6**, 471–472, in *Chem. Abstr.* (1952), **46**, 8142.
Erlenmeyer, H. (1948). *Bull. Soc. Chim. biol., Paris*, **30**, 792–805.
Fakstorp, J., Pedersen, J. G. A., Poulsen, E. and Schilling, M. (1957). *Acta pharm. tox. Kbh.*, **13**, 52–58.
Ferguson, J. (1939). *Proc. roy. Soc.*, B **127**, 387–404.
Fieser, L. F. and Fieser, M. (1959). *Steroids*, pp. 800–808, New York: Reinhold.
Fieser, L. F. and Wei-Yuan Huang (1953). *J. Amer. chem. Soc.*, **75**, 6306–6307.
Figdor, S. K., Kodet, M. J., Bloom, B. M., Agnello, E. J., P'an, S. Y. and Laubach, G. D. (1957). *J. Pharmacol.*, **119**, 299–309.
Finnerty, F. A. and Fuchs, C. J. (1953). *Amer. J. Obst. Gynec.*, **66**, 830–841.
Fisher, A. L., Keasling, H. H. and Schueler, F. W. (1952). *Proc. Soc. exp. Biol., N.Y.*, **81**, 439–441.
Fleischhacker, H. (1956). *Wien. klin. Wschr.*, **68**, 989–992.
Fried, J. (1957). *Cancer*, **10**, 752–756.
Friedman, H. L. (1951). *Nat. res. Counc. Wash. Pub.*, **206**.
Gaunt, R., Leathem, J. H., Tuthill, C. H., Antonchak, N., Gilman, M. and Renzi, A. A. (1954). *Endocrinology*, **54**, 272–283.
Gensler, W. J. and Sherman, G. M. (1958). *J. org. Chem.*, **23**, 1227–1228.
Gero, A. and Withrow, C. L. (1957). *Nature, Lond.*, **180**, 1354–1355.
Gessner, O. (1926). *Arch. exp. Path. Pharmak.*, **118**, 325–357.
Gill, E. W. (1959). *Proc. roy. Soc.*, B**150**, 381–402.
Gill, E. W. and Ing, H. R. (1958). *J. chem. Soc.*, 4728–4731.
Goisis, M. and Polvani, F. (1955). *Biol. Latina*, **8**, 86–106.
Gold, A. M. and Schwenk, E. (1959). *J. Amer. chem. Soc.*, **81**, 2198–2200.
Goodman, L. S. and Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 672, New York: Macmillan.
Gould, D., Shapiro, E. L., Finckenor, L. E., Gruen, F. and Hershberg, E. B. (1956). *J. Amer. chem. Soc.*, **78**, 3158–3163.
Goutarel, R. (1961). *Tetrahedron*, **14**, 126–137.
Grant, G. A., Glen, W. L. and Barber, R. J. (1950). U.S. Patent 2,534,121 in *Chem. Abstr.* (1951), **45**, 3878.
Greiner, T. and Reilly, J. (1952). *Proc. Soc. exp. Biol., N.Y.*, **81**, 141–144.
Grimm, H. G. (1934). *Angew. Chem.*, **47**, 594–601.
Grob, C. A. and Goldberg, W. A. (1949). *Helv. chim. Acta*, **32**, 191–197.
Gunter, M. J., Kim, K. S., Magee, D. F., Ralston, H. and Ivy, A. C. (1950). *J. Pharmacol.*, **99**, 465–478.
Gut, M. and Uskoković, M. (1961). *J. org. Chem.*, **26**, 1943–1944.
Hajdu, S. and Szent-Gyorgyi, A. (1952). *Amer. J. Physiol.*, **168**, 171–175.
Harper, N. J. (1959). *J. med. pharm. Chem.*, **1**, 467–500.

Hasbrouck, R. B. (1953). U.S. Patent 2,642,427 in *Chem. Abstr.* (1954), **48**, 6474.
Haslewood, G. A. D. (1941). *Biochem. J.*, **35**, 1307–1310.
Havranek, R. E. and Doorenbos, N. J. (1960). *J. Amer. pharm. Ass. Sci. Ed.*, **49**, 328–329.
Hebo, H. (1951). U.S. Patent 2,557,655 in *Chem. Abstr.* (1952), **46**, 3094.
Hecker, E. and Walk, E. (1960). *Chem. Ber.*, **93**, 2928–2937.
Herzog, H. L., Payne, C. C. and Hershberg, E. B. (1955). *J. Amer. chem. Soc.*, **77**, 5324–5327.
Hesse, G. and Ludwig, G. (1960). *Liebigs Ann.*, **632**, 158–171.
Hesse, G. and Mix, K. (1959). *Ibid.*, **625**, 146–156.
Hillyard, I. W. and Procita, L. (1959). *J. Pharmacol.*, **127**, 22–28.
Hilton, M. L., Jones, A. S. and Westwood, J. R. B. (1955). *J. chem. Soc.*, 3449–3453.
Hilton, M. L. and Webb, M. (1951). *Ibid.*, 2767–2768.
Hofert, J. F. and Sell, H. M. (1960). *J. org. Chem.*, **25**, 1831–1833.
Hoobler, S. W. and Dontas, A. (1953). *Pharmacol. Rev.*, **5**, 151–157.
Howard, R. P., Norcia, L. N., Peter, J. A. and Furman, R. H. (1959). Cited by Clinton and others (1961b).
Hudson, P. B. and Lombardo, M. E. (1955). *J. clin. Endocr.*, **15**, 324–330.
Jacobs, T. L. and Brownfield, R. B. (1960). *J. Amer. chem. Soc.*, **82**, 4033–4039.
Jacobs, W. A. and Hoffmann, A. (1927). *J. biol. Chem.*, **74**, 787–794.
Jakoby, W. B. and Tomkins, G. (1956). *Science*, **123**, 940–941.
James, S. P., Smith, F., Stacey, M. and Webb, M. (1946). *J. chem. Soc.*, 665–670.
Janot, M. M., Lainé, F. and Goutarel, R. (1960). *Ann. pharm. franc.*, **18**, 673–677.
Jones, A. S., Smith, F. and Webb, M. (1948). *Nature, Lond.*, **162**, 857–858.
Jones, A. S., Webb, M. and Smith, F. (1949). *J. chem. Soc.*, 2164–2168.
Joska, J., Černý, V. and Šorm, F. (1954). *Coll. Trav. chim. Tchécosl.*, **19**, 551–558.
Joska, J. and Šorm, F. (1956). *Ibid.*, **21**, 754–760.
Kawasaki, T. and Mosettig, E. (1959). *J. org. Chem.*, **24**, 2071–2072.
Keyl, A. C. and Dragstedt, C. A. (1954). *J. Pharmacol.*, **110**, 411–414.
Knof, L. (1961). *Liebigs Ann.*, **642**, 194–199.
Krayer, O. (1958) in *Pharmacology in Medicine*, 2nd ed., editor V. A. Drill, pp. 515–524, New York: McGraw-Hill.
Krayer, O. and Acheson, G. H. (1946). *Physiol. Rev.*, **26**, 383–446.
Krayer, O., Moe, G. K. and Mendez, R. (1944). *J. Pharmacol.*, **82**, 167–186.
Krupp, P. J., Farris, C., Pierce, C. and Jacobs, A. (1956). *Amer. J. Obst. Gynec.*, **71**, 247–254.
Kuhn, B. H. (1959). *J. Amer. med. Women's Ass.*, **14**, 54–55.
Kull, F. C., Castellano, G. A. and Mayer, R. L. (1953). *J. invest. Dermatol.*, **21**, 227–228.
Kushner, S., Dalalian, H., Bach, F. L., Centola, D., Sanjurjo, J. L. and Williams, J. H. (1955). *J. Amer. chem. Soc.*, **77**, 1152–1155.
Küssner, W. E. (1939). *Merck's Jahrb.*, **53**, 45–51.
Kutney, J. P. and Johnston, R. A. (1961). *Chem. and Ind.*, 1713–1714.
Kwartler, C. E. (1948). Cited in Northey "*The Sulphonamides and Allied Compounds*" (1948) ref. No. 1503, New York: Reinhold.
La Barre, J. and Desmarez, J. J. (1959). *Arch. int. Pharmacodyn.*, **119**, 514–516.
Lavier, G., Crosnier, R. and Merle, F. (1948). *Bull. Soc. Path. exot.*, **41**, 548.
Leanza, W. J., Conbere, J. P., Rogers, E. F. and Pfister, K. (1954). *J. Amer. chem. Soc.*, **76**, 1691–1694.
Leclerc, H. (1938). *Presse méd.*, **46**, 480.
Lieb, H. (1947). *Monatshefte*, **77**, 324–332.
Lieberman, S. (1946). *Experientia*, **2**, 411–412.
Litvan, F. and Robinson, R. (1938). *J. chem. Soc.*, 1997–2001.
Loewe, S. and Harvey, S. C. (1952). *Arch. exp. Path. Pharmak.*, **214**, 214–226.
Long, J. P. and Schueler, F. W. (1954). *J. Amer. pharm. Ass., Sci. Ed.*, **43**, 79–86.
Louw, D. F., Strating, J. and Backer, H. J. (1955). *Rec. Trav. chim. Pays-Bas*, **74**, 1540–1554.
Macovski, E. and Georescu, J. (1946). *Bull. Sect. Sci. Acad. roumaine*, **28**, 645–667.
Madinaveitia, J. L. (1955). Brit. Patent 729,160 in *Chem. Abstr.* (1956), **50**, 2128.
Mantegazza, P. and Tommasini, R. (1951). *Boll. Soc. ital. Biol. sper.*, **27**, 631–633.
Mantegazza, P. and Tommasini, R. (1952). *Atti. soc. lombarda sci. med. e biol.*, **7**, 496–503.
Mayer, R. L. and Oechslin, C. (1939). *Arch. int. Pharmacodyn.*, **62**, 211–230.
Mazur, R. H. (1957a). U.S. Patent 2,806,028 in *Chem. Abstr.* (1958), **52**, 2102.
Mazur, R. H. (1957b). U.S. Patent 2,806,029 in *Chem. Abstr.* (1958), **52**, 2102.
Meilman, E. (1953). *J. clin. Invest.*, **32**, 80–89.

Meilman, E. (1959). in *Hypertension*, editor J. Moyer, pp. 395–399, Philidelphia: Saunders.
Meilman, E. and Krayer, O. (1952). *Circulation*, **6**, 212–221.
Meyer, A. E. and McEwan, J. P. (1948). *Amer. J. Physiol.*, **153**, 386–392.
Micheli, R. A. and Bradsher, C. K. (1955). *J. Amer. chem. Soc.*, **77**, 4788–4793.
Mima, H. (1955). Japanese Patent 8125('55) in *Chem. Abstr.*, (1957), **51**, 18387.
Mori, Y. and Nakagawa, T. (1961). *J. pharm. Soc., Japan*, **81**, 972–975.
Nelson, N. A. and Hsi, R. S. P. (1961). *J. org. Chem.*, **26**, 3086–3090.
Noland, J. L. (1954). *Arch. Biochem.*, **52**, 323–330.
Oertel, G. W. and Agashe, B. D. (1960). *Biochem. Biophys. Acta*, **45**, 1–8.
Oettel, H. (1947). *Pharmazie*, **2**, 385–388.
Offe, H. A., Siefken, W. and Domagk, G. (1952). *Z. Naturforsch.*, 7B, 446–462.
Oki, M. (1952). *J. chem. Soc., Japan*, **73**, 252–254. *in Chem. Abstr.* (1953), **47**, 3522.
Organon (1960). *Derwent Fine Chemicals Patent J.*, **188** [5], 3A No. 833,582.
Otto, H. L., Greiner, T., Gold, H., Palumbo, F., Warshaw, L., Kwit, N. T. and Chen, K. K. (1953). *J. Pharmacol.*, **107**, 225–231.
Overbeck, G. A. (1957). Cited by Voigt and Kallistratos (1957).
Paton, W. D. M. and Zaimis, E. J. (1949). *Brit. J. Pharmacol.*, **4**, 381–400.
Paton, W. D. M. and Zaimis, E. J. (1951), *Ibid.*, **6**, 155–168.
Patton, T. L. (1959a). *Chem. and Ind.*, 923–924.
Patton, T. L. (1959b). *J. org. Chem.*, **24**, 1795–1796.
Patton, T. L. (1960). *Ibid.*, **25**, 2148–2152.
Potts, G. O., Beyler, A. L. and Burnham, D. F. (1960). *Proc. Soc. exp. Biol., N.Y.*, **103**, 383–384.
Quévauviller, A. and Blanpin, O. (1958). *J. de Physiol.*, **50**, 1123–1127.
Quévauviller, A. and Lainé, F. (1960). *Ann. pharm. franç.*, **18**, 678–680.
Rao, G. V. and Price, C. C. (1962). *J. org. Chem.*, **27**, 205–210.
Redel, J., Bouteville, A., Gauthier, B. and Nguyen-Huu Quy, (1951). *Bull. Soc. chim. France*, 524–526.
Rhone-Poulenc (1960). *Derwent Fine Chemicals Patent J.*, **207**, [6], 3A, No. 847,445.
Riess, J. and Ourisson, G. (1961). *Bull. Soc. chim. France*, 1243–1244.
Robson, J. M. and Keele, C. A. (1956). *Recent Advances in Pharmacology*, pp. 82–86, 2nd ed., London: Churchill.
Rorig, K. (1953). U.S. Patent 2,664,423 in *Chem. Abstr.* (1955), **49**, 7608.
Ruzicka, L., Plattner, P. A. and Engel, B. G. (1944). *Helv. chim. Acta*, **27**, 1553–1560.
Schatz, V. B. (1960). In *Medicinal Chemistry*, editor A. Burger, 2nd ed., pp. 72–78, New York: Interscience.
Schaub, R. E. and Weiss, M. J. (1961). *J. org. Chem.*, **26**, 3915–3925.
Schering (1955). Brit. Patent 735,568 in *Chem. Abstr.* (1956), **50**, 7872.
Schneider, W. G. and Birtel, A. (1956). *Klin. Wschr.*, **34**, 1175–1178.
Schneider, W. G. and Frahm, H. (1955). *Acta endocr.*, **20**, 279–285.
Schneider, W. G. and Frahm, H. (1956). *Naturwiss.*, **43**, 61.
Schöpf, C. (1961). *Experientia*, **17**, 285–295.
Schroeder, W. and Voigt, K. D. (1958). *Acta endocr.*, **27**, 110–117.
Selye, H. (1942). *Endocrinology*, **30**, 437–453.
Shoppee, C. W. and Krueger, G. (1961). *J. chem. Soc.*, 3641–3655.
Skursky, J. (1957). *Wien. med. Wschr.*, **107**, 678–679.
Stacey, M. and Webb, M. (1947a). *Proc. roy. Soc.*, B **134**, 522–537,
Stacey, M. and Webb, M. (1947b). *Ibid.*, B**134**, 538–543.
Standaert, F. G. and Friess, S. L. (1960). *J. Pharmacol.*, **128**, 55–64.
Steldt, F. A., Anderson, R. C. and Chen, K. K. (1944). *Ibid.*, **82**, 98–102.
Stoll, A. (1954). *Gazz. chim. ital.*, **84**, 1190–1209.
Stoll, A. (1956). in *Medicinal Chemistry*, Vol. 2, editors, F. F. Blicke and C. M. Suter, p. 24, New York: Wiley.
Stückradt, H. (1939). *Arch. exp. Path. Pharmak.*, **191**, 362–368.
Sturtevant, F. M. (1958). *Proc. Soc. exp. Biol., N.Y.*, **97**, 619–621.
Takeda, K., Kubota, T. and Kawanami, J. (1960). *Pharm. Bull., Tokyo*, **8**, 615–620.
Tamm, C. (1957). *Fortschr. Chem. org. Naturstoffe*, **14**, 71–140.
Tanguy, F., Robin, C. and Raoult, A. (1948). *Méd. trop.*, **8**, 12.
Tanz, R. D. and Kerby, C. F. (1961). *J. Pharmacol.*, **131**, 56–64.
Tóth, J., Tuba, Z. and Szporny, L. (1961). *Nature, Lond.*, **191**, 607.
Uhle, F. C., Mitman, B. A. and Krayer, O. (1956). *J. Pharmacol.*, **116**, 444–449.
Uhle, F. C. and Schröter, H. (1961). *J. org. Chem.*, **26**, 4169–4171.
Vaidya, S. S. and Boyce, S. F. (1959). *Antibiot. Ann.*, **9**, 364–368.

Valenta, Z., Wiesner, K., Babin, D. R., Bögri, T., Forrest, T. P., Fried, F. and Reinshagen, H. (1962). *Experientia*, **18**, 111–113.
Van Rossum, J. M. and Ariens, E. J. (1957). *Ibid.*, **13**, 161–163.
Vick, R. L., Kahn, J.B. and Acheson, G. H. (1957). *J. Pharmacol.*, **121**, 330–339.
Voigt, K. D. and Kallistratos, G. (1957). *Endokrinologie*, **35**, 56–64.
Voigt, K. D. and Schroeder, W. (1956). *Acta endocr.*, **21**, 343–358.
Wakim, K. G., Essex, H. E. and Mann, F. C. (1939). *Amer. Heart J.*, **18**, 171–175
Weichsel, H. and Zirm, K. L. (1961). *Monatshefte*, **92**, 667–671.
Welsh, A. L. (1956). *Int. Rec. Med. gen. Pract. Clinics.*, **169**, 775–777.
Werbin, H. and Holoway, C. (1956). *J. biol. Chem.*, **223**, 651–660.
Wessely, F. and Swoboda, W. (1951). *Monatshefte*, **82**, 437–442.
Wieland, H. and Sorge, H. (1916). *Hoppe-Seyl, Z.*, **97**, 1–27.
Wien, R. and Mason, D. F. J. (1953). *Brit. J. Pharmacol.*, **8**, 306–314.
Wildi, B. S. (1959). U.S. Patent 2,897,202 in *Chem. Abstr.* (1960), **54**, 646.
Woolley, D. W. (1960). In *Fortschritte der Arzneimittelforschung*, Vol. II, (ed. E. Jucker), pp. 613–636, Basel: Birkhäuser.
Zderic, J. A., Halpern, O., Carpio, N., Ruiz, A., Limon, D. C., Magaña, L., Jiménez, H., Bowers, A. and Ringold, H. J. (1960). *Chem. and Ind.*, 1625–1626.
Zicha, L., Schmid, E., Scheiffarth, F., Graf, N. and Koschera, H. (1960). *Arzneimitt.-Forsch.*, **10**, 831–834.

Reprinted from *The Journal of Pharmacy and Pharmacology*, 1962, **14**, pp. 469–495

REVIEW ARTICLE

BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS

PART II. STEROIDAL ALKALOIDS‡

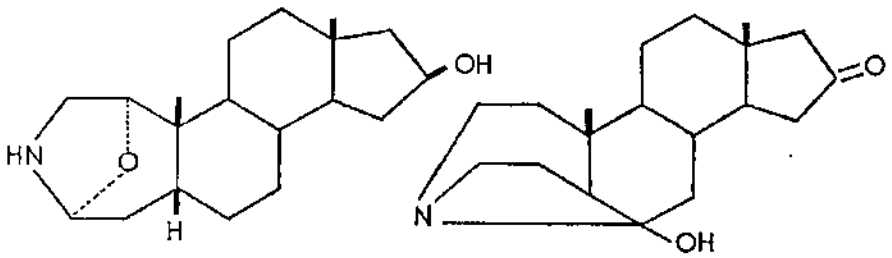
BY M. ALAUDDIN*, B.Pharm. AND M. MARTIN-SMITH†, M.Sc., Ph.D.

Division of Experimental Pharmacology, Institute of Physiology, The University, Glasgow, W.2

CHEMICALLY the steroidal alkaloids form a complex group, the individual members displaying much diversity in molecular structure (Fieser and Fieser, 1959; Goutarel, 1961; Jeger and Prelog, 1960; Morgan and Barltrop, 1958; Schöpf, 1961). Some occur unconjugated as the free alkamines in nature, but others occur as glycosides or esters. It has been customary to base classification of the group on botanical origin, but with the number of representatives now known, it is more convenient to consider them according to the nature of the skeleton of the alkamine. Such a classification in point of fact does not diverge too greatly from the botanical classification, but it should serve to give greater emphasis to possible structure-action relationships. Chemically, four main groups can be recognised.

3-Aza-A-homoandrostane derivatives.
Bases formally derived from the pregnane skeleton.
Bases formally derived from the unrearranged cholestane skeleton.
Bases possessing the "jervi" skeleton.

The first group is small and the only known representatives are the four salamander alkaloids samandarine (I), samandarone, samandaridine and cycloneosamandione (II) (Schöpf, 1961; Habermehl, 1962). Their pharmacological properties have been studied in detail by Gessner and his co-workers (1948 and earlier papers) who showed that these compounds exhibit analeptic activity, producing convulsions in mice and antagonising the narcotic effects of barbiturates, ethylurethane and tribromoethanol in salamander larvae and small fish.



I II

Their action on smooth muscle appears to be variable. Thus they produce vasoconstriction of the Löwen-Trendelenburg preparation of

*Colombo Plan Fellow, 1958–62.

†Present address: Department of Pharmacy, Royal College of Science and Technology, Glasgow, C.1.

‡Part I appeared on pp. 325–349 of June, 1962 issue.

isolated frog vessels, relax the carotid artery of calves, relax the guinea-pig uterus and antagonise the action of adrenaline on certain preparations. Intracutaneous injection in man produces pain and hyperaemia.

Bases Formally Derived from Pregnane

The alkaloids of this group occur in various plants belonging to the family Apocynaceae and are characterised by the possession of amino functions at C(3) or C(20), or at both positions. Usually the members

TABLE I

MONOACID BASES DERIVED FROM PREGNANE

3-AMINOPREGNANES

Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Funtumine $C_{21}H_{35}NO$	3α-Amino-20-oxo-5α-pregnane	126	+95	*Funtumia latifolia*	1
Funtumidine $C_{21}H_{37}NO$	3α-Amino-20α-hydroxy-5α-pregnane	182	+10	*F. latifolia*	1
Holamine $C_{21}H_{33}NO$	3α-Amino-20-oxo-pregn-5-ene	135	+23	*Holarrhena floribunda*	2
Holaphyllamine $C_{21}H_{33}NO$	3β-Amino-20-oxo-pregn-5-ene	260 as HCl salt	+33 as HCl salt	*H. floribunda*	2, 3
Holaphylline $C_{22}H_{35}NO$	3β-Methylamino-20-oxo-pregn-5-ene	128	+23	*H. floribunda*	3
Paravallarine $C_{23}H_{33}NO_2$	3β-Methylamino-20α-hydroxypregn-5-en-18-carboxylic acid lactone	181	−52	*Paravallaris microphylla*	4

20-AMINOPREGNANES

Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Funtuphyllamine A $C_{21}H_{37}NO$	20α-Amino-3β-hydroxy-5α-pregnane	173	+13	*Funtumia africana*	5
Funtuphyllamine B $C_{22}H_{39}NO$	20α-Methylamino-3β-hydroxy-5α-pregnane	214	+24	*F. africana* and *Malouetia bequaertiana*	5, 6
Funtuphyllamine C $C_{23}H_{41}NO$	20α-Dimethylamino-3β-hydroxy-5α-pregnane	172	+24	*F. africana*	5
Funtumafrine B $C_{22}H_{37}NO$	20α-Methylamino-3-oxo-5α-pregnane	160	+43	*F. africana*	5
Funtumafrine C $C_{23}H_{39}NO$	20α-Dimethylamino-3-oxo-5α-pregnane	174	+45	*F. africana* and *M. bequaertiana*	5
Gluco-alkaloid $C_{27}H_{45}NO_6$	20α-Amino-3β-hydroxy-pregn-5-ene-β-D-glucoside	—	—	*Conopharyngia pachysiphon*	7
Holafebrine $C_{21}H_{35}NO$	20α-Amino-3β-hydroxy-pregn-5-ene	177	−61	*Holarrhena febrifuga* and *Kibatalia arborea*	8a

ALKALOIDS NOT FULLY CHARACTERISED

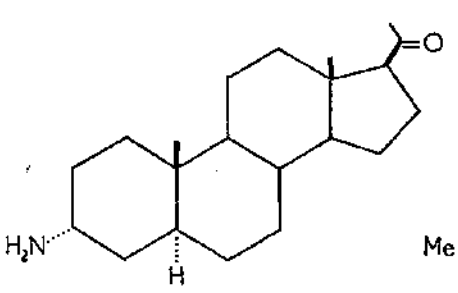
Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Holadysamine $C_{23}H_{37}NO$	—	173	−78	*Holarrhena antidysenterica*	8
Holadysine $C_{23}H_{37}NO$	—	120	−199	*H. antidysenterica*	8
Irehine $C_{23}H_{39}NO$	—	163	−30	*Funtumia elastica*	8
Latifoline $C_{28}H_{37}NO$	—	135	−4	*F. latifolia*	8*

1. Janot, Qui Khuong Huu and Goutarel (1959). 2. Janot, Cavé and Goutarel (1960). 3. Janot, Cavé and Goutarel (1959). 4. Le Men (1960). 5. Janot, Qui Khuong Huu and Goutarel 1960). 6. Janot, Lainé and Goutarel (1960). 7. Dickel, Lucas and MacPhillamy (1959). 8. Goutarel (1961). 8a. Janot and others (1962b)

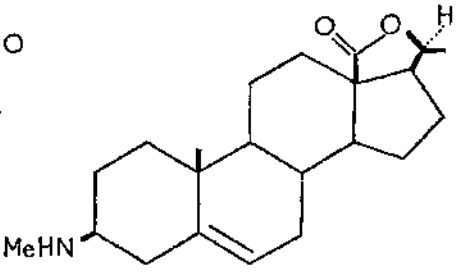
*Latifoline is now known to be the 3β-hydroxy compound corresponding to conamine (Table II) (Janot, Qui Khuong Huu and Goutarel, 1962).

of this group occur in nature as the free alkamine but at least one representative is found in glycosidic combination (Dickel, Lucas and MacPhillamy, 1959) and at least two others are found in the form of pyroterebate ester conjugates (Rostock and Seebeck, 1958).

The chemistry of the monoacid bases has recently been reviewed (Goutarel, 1961) and the known alkaloids belonging to this group are listed in Table I. Examples of the monoacid bases with the nitrogen atom in the 3-position are funtumine (III) (Janot, Qui Khuong Huu and Goutarel, 1959), and paravallarine (IV) (Le Men, 1960) which possesses a saturated lactone ring, and thus bears some structural resemblance to the dihydrocardenolides. A typical example of the monoacid bases possessing the nitrogen atom in the 20-position is afforded by funtuphyllamine A (V) (Janot, Qui Khuong Huu and Goutarel, 1960).

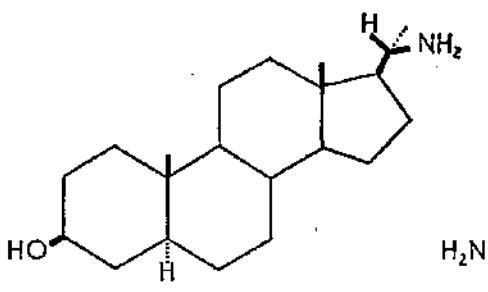


III

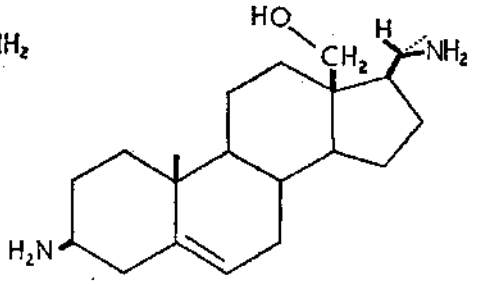


IV

The alkaloids containing two nitrogen atoms in the molecule can be subdivided into three main groups. Where the nitrogen atom on C(20) is not incorporated in a ring the alkaloids belong to the holarrhimine class, which is exemplified by holarrhimine itself (VI) (Černý, Lábler and Šorm, 1957). Where the nitrogen atom on C(20) forms a bridge to C(18) the conarrhimine and conkurchine groups result. In the conarrhimine group the nitrogen ring is fully saturated whilst in the conkurchine group the nitrogen ring possesses a double bond in the 17-20-position (Tschesche and Roy, 1956). The most extensively investigated alkaloids of the conarrhimine and conkurchine groups are conessine and conessidine respectively. The known diacid alkaloids of the pregnane group are listed in Table II.



V



VI

Two main pharmacological actions appear to be characteristic of the pregnane group of alkaloids. These are hypotensive activity and local

anaesthetic activity and both properties do not appear to be dependent upon either the number or the position of the nitrogen atoms. Thus hypotensive activity has been reported in kurchicine (Chopra, Gupta and Chopra, 1933) (later shown to be impure holarrhimine (Bertho, 1939)) and conessine (Bakhsh, 1936; Burn, 1915; Paris, 1938) as well as in funtumine, funtumidine and related alkaloids (Quévauviller and Blanpin, 1960, and earlier refs.) and in 20α-amino-3β-hydroxy-5-pregnene-βD-glucoside (Dickel and others, 1959). The activity of this last compound inspired the synthesis of several related glycosides (Lucas and others, 1960) and some of these synthetic compounds also exhibited hypotensive activity when administered intravenously to dogs, although like the parent alkaloid, they were inactive by the oral route. For similar reasons the 20-glucoside of funtumidine (glucofuntumidine) was prepared synthetically for pharmacological studies (Quévauviller and Blanpin, 1960).

More detailed studies have indicated that the hypotensive properties stem from direct actions on the heart and blood vessels. Conessine and holarrhimine, in the anaesthetised cat (Bakhsh, 1936; Burn, 1915) show a preliminary rise in blood pressure before a prolonged depression and this has been attributed to an initial stimulation of the smooth muscle of the blood vessels, followed by slowing and incoordination of the heart. Section of the vagi has no influence on the drop in blood pressure (Bakhsh, 1936; Chopra and others, 1933) although the magnitude of the fall is smaller in decerebrate cats (Chopra and others, 1933), indicating that the medullary centres are playing some rôle.

Conessine and holarrhimine have been shown to produce a dilatation of the splanchnic vessels but to contract the renal vessels (Bakhsh, 1936; Chopra and others, 1933) whilst funtumine and funtumidine have been shown to dilate both peripheral and coronary vessels (Quévauviller and Blanpin, 1958), and to exhibit a positive inotropic and negative chronotropic action on the isolated rabbit heart. Conessine and holarrhimine have been reported to stimulate intestinal and uterine contractions (Bakhsh, 1936; Chopra and others, 1933) but later work (Stephenson, 1948) has shown that conessine has a quinidine-like action and antagonises the action of acetylcholine on skeletal, cardiac and smooth muscle. In this connection it is interesting that funtumidine has been reported to slightly inhibit peristalsis of the dog intestine *in situ* (Quévauviller and Blanpin, 1958).

The local anaesthetic activity exhibited by the pregnane group of alkaloids (Burn, 1915; Chopra and others, 1933; Quévauviller and Blanpin, 1958) is in most compounds more pronounced than that of cocaine (Quévauviller and Blanpin, 1960; Stephenson, 1948; Stephenson and Dutta, 1948; Trevan and Boock, 1927) but as the compounds produce necrosis on injection (Stephenson, 1948; Stephenson and Dutta, 1948) they are without clinical value.

Other actions which have been shown to be present in the group include antipyretic activity (Quévauviller and Blanpin, 1960; Paris, 1938) and ability to potentiate barbiturate hypnosis (Quévauviller and Blanpin, 1960). Holarrhimine and conessine exert a direct narcotic effect on frogs, but

this action is absent in mammals (Bakhsh, 1936; Burn, 1915). Although funtumidine has been classed as a tranquilliser on the basis of its ability to depress motility in rats (La Barre and Desmarez, 1959) it is possible that the effect could be produced by a direct paralysis of the peripheral motor nerves rather than by a reserpine-like action. Conessine has been shown to inhibit certain enzymes (Chopra and others, 1927; Kaushiva and Ghatak, 1956) and holamine on intraperitoneal administration gives rise to Parkinsonian-like tremors (Quévauviller and Blanpin, 1960).

The structural similarity of the funtumia alkaloids to the steroid hormones inspired an investigation of these agents for hormonal activity (Blanpin and Quévauviller, 1960). The results showed that all the alkaloids studied were devoid of positive hormonal properties, but there were some indications that the bases exhibited a degree of antagonism towards a limited number of specific effects of the natural hormones.

Conessine has been termed a general protoplasmic poison since it exhibits marked toxicity towards various micro-organisms, especially protozoans (Bertho, 1944b; Chopra and others, 1927; Goyal, 1935; Henry and Brown, 1923; Paris, 1938). It appears to have little or no activity against the malaria parasite (Stephenson, 1948) or helminths (Janot and Cavier, 1949; Mackie and others, 1955) although it has been reported to show weak antituberculous properties (Lambin and Bernard, 1953; Meissner and Hesse, 1930). Its toxicity towards *Entamoeba dysenteriae* has led to a limited clinical use (see for example Acton and Chopra, 1933; Lavier, Crosnier and Merle, 1948; Tanguy, Robin and Raoult, 1948) particularly on the Indian subcontinent and there have been several studies (see for example Durieux, Trenous and Tanguy, 1948; Kaushiva, 1957; Muhlpfordt and Martinez-Silva, 1956; Piette, 1950) in which its efficacy has been compared to that of emetine. The results indicate that it is inferior to emetine as an amoebicide, but not such a potent emetic. Studies have also been made on the distribution and fixing of conessine in the monkey (Auffret and Tanguy, 1950) and on its rate of elimination in man, which is very slow (Pluchon and Pille, 1950). Several reviews concerning the clinical potentialities of conessine in the treatment of amoebiasis have been published (Duviau, 1953; Kerny, 1948; Leake, 1932) and the authors all agree that conessine is not a suitable drug.

Alkaloids Formally Derived from Cholestane

Members of this group have been isolated as the free alkamines or as glycosides of mono-, di-, tri- and tetrasaccharides but it is possible that at least some of the alkamines and lower glycosides are produced by the hydrolysis of higher glycosides during the isolation procedure. The group embraces steroidal alkaloids occurring in various *Solanum spp.* and at least three alkaloids occurring in *Veratrum spp.*, namely rubijervine (12β-hydroxysolanidine), isorubijervine (18-hydroxysolanidine) and isorubijervosine, which is the 3-glucoside of isorubijervine. These alkaloids are all characterised by a hexacyclic skeleton incorporating a piperidine

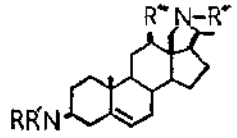
TABLE II

DIACID BASES DERIVED FROM PREGNANE

A. HOLARRHIMINE GROUP

Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Chonemorphine $C_{23}H_{40}N_2$	3β-Amino-20α-dimethylamino-5α-pregnane	145	+25	*Chonemorpha fragrans* *C. penangensis*	9
Holarrhidine $C_{21}H_{36}N_2O$	3α,20α-Diamino-18-hydroxy-pregn-5-ene	181–2	−23	*Holarrhena antidysenterica*	10
Holarrhimine (*syn* kurchicine) $C_{21}H_{38}N_2O$	3β,20α-Diamino-18-hydroxy-pregn-5-ene	183–6	−14·2	*H. antidysenterica*	11–13
Malouetine $C_{27}H_{52}N_2^{++}$	3β,20α-Bistrimethylammonium-5α-pregnane	264 (picrate)	+3 (chloride in water)	*Malouetia bequaertiana*	14
(3)-*N*-Methylholarrhimine $C_{22}H_{38}N_2O$	20α-Amino-18-hydroxy-3β-methylamino-pregn-5-ene	above 360 (dihydrochloride)	−28 (dihydrochloride in MeOH)	*H. antidysenterica*	15
(20)-*N*-Methylholarrhimine $C_{22}H_{38}N_2O$	3β-Amino-18-hydroxy-20α-methylamino-pregn-5-ene	163–164	−19	*H. antidysenterica*	15
NNN'N'-Tetramethylholarrhimine $C_{25}H_{44}N_2O$	3β,20α-Bis(dimethylamino)-18-hydroxy-pregn-5-ene	227–229	−35	*H. antidysenterica*	15, 16

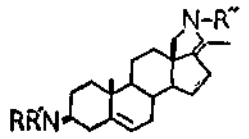
B. CONARRHIMINE GROUP



Alkaloid	R	R'	R''	R'''	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Conarrhimine $C_{21}H_{34}N_2$	H	H	H	H	Impure preparation	—	*H. antidysenterica*	13
Conimine $C_{22}H_{36}N_2$	Me	H	H	H	134	−30 (EtOH)	*H. antidysenterica*	17, 18
Conamine $C_{22}H_{36}N_2$	H	H	Me	H	130	−21	*H. antidysenterica*	13, 18
Conessimine $C_{23}H_{38}N_2$	Me	Me	H	H	100	−22·3	*H. antidysenterica*	19, 20
Isoconessimine $C_{23}H_{38}N_2$	Me	H	Me	H	92	+30 (EtOH)	*H. antidysenterica*	21
Conessine (*syn* wrightine) $C_{24}H_{40}N_2$	Me	Me	Me	H	125	−2	*Holarrhena spp.*	16, 22–24
Holarrhemine $C_{24}H_{40}N_2O$	Me	Me	Me	OH	198	−7	*H. congolensis*	25, 26
Holafrine $C_{29}H_{48}N_2O_2$	Me	Me	H	$OCOC_6H_5$	116–117	−19·1	*H. africana DC*	25
Holarrhetine $C_{30}H_{48}N_2O_2$	Me	Me	Me	$OCOC_6H_5$	74–75	−14·9	*H. africana DC*	25

TABLE II—*continued*

C. CONKURCHINE GROUP



Alkaloid	R	R′	R″	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Conkurchine $C_{21}H_{35}N_3$	H	H	H	152	−67·4	*H. antidysenterica*	27
Conessidine $C_{22}H_{36}N_2$	Me	H	H	123	−52·2	*H. antidysenterica*	18, 28
Trimethylconkurchine $C_{24}H_{38}N_2$	Me	Me	Me	125–127	+12·0	*H. antidysenterica*	18

D. ALKALOIDS INCOMPLETELY CHARACTERISED

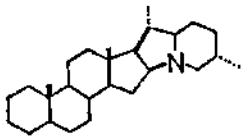
Alkaloid	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Base $C_{28}H_{34}N_2$	129·5	—	*H. antidysenterica*	29
Base $C_{23}H_{38}N_2$	87–88	—	*H. antidysenterica*	30
Conkurchinine $C_{26}H_{34}N_2$	161	−47 (EtOH)	*H. antidysenterica*	28
Holarrhessimine $C_{30}H_{36}N_2O$	160–164	−30	*H. antidysenterica*	20
Holarrhine $C_{30}H_{38}N_2O_2$	240	−17 (MeOH)	*H. antidysenterica*	19
α-Hydroxyconessine $C_{24}H_{40}N_2O$	—	−9 (EtOH)	*H. antidysenterica*	22
Kurchamine $C_{22}H_{36}N_2$	115–117	−16	*H. antidysenterica*	15
Kurchenine $C_{21}H_{32}N_2O_2$	335–336	−78 (H_2SO_4 in water)	*H. antidysenterica*	31
Kurchessine $C_{24}H_{40}N_2$	132–133	−36	*H. antidysenterica*	15
Kurchine (*syn* Curchine, nor-Conessine) $C_{23}H_{38}N_2$	75	−7	*H. antidysenterica*	12, 28, 32
Lettocine $C_{17}H_{25}NO_2$	350–352	—	*H. antidysenterica*	33
Malouphylline $C_{24}H_{40}N_2O_2$	259	−10	*M. bequaertiana*	34*

9. Janot and others (1962a). 10. Černý, Lábler and Šorm (1959). 11. Černý, Lábler and Šorm (1957). 12. Ghosh and Bose (1932). 13. Siddiqui (1936). 14. Janot, Lainé and Goutarel (1960). 15. Tschesche and Wiensz (1958). 16. Lábler and Černý (1959). 17. Siddiqui and Siddiqui (1934). 18. Tschesche and Roy (1956). 19. Siddiqui and Pillay (1932). 20. Tschesche and Petersen (1954). 21. Siddiqui (1935). 22. Bertho and Gotz (1958). 23. Favre and others (1953). 24. Siddiqui (1934). 25. Rostock and Seebeck (1958). 26. Uffer (1956). 27. Bertho (1951). 28. Bertho (1939). 29. Bertho (1947). 30. Bertho (1944a). 31. Bertho, von Schuckmann and Schönberger (1933). 32. Haworth (1932). 33. Peacock and Chowdhury (1935). 34. Goutarel (1961).

* The structure of malouphylline is now known to be 3β — acetamido-20α-dimethylamino-5α-pregnan-18-al (Janot, Lainé and Goutarel, 1962).

TABLE III

BASES DERIVED FROM SOLANIDANE



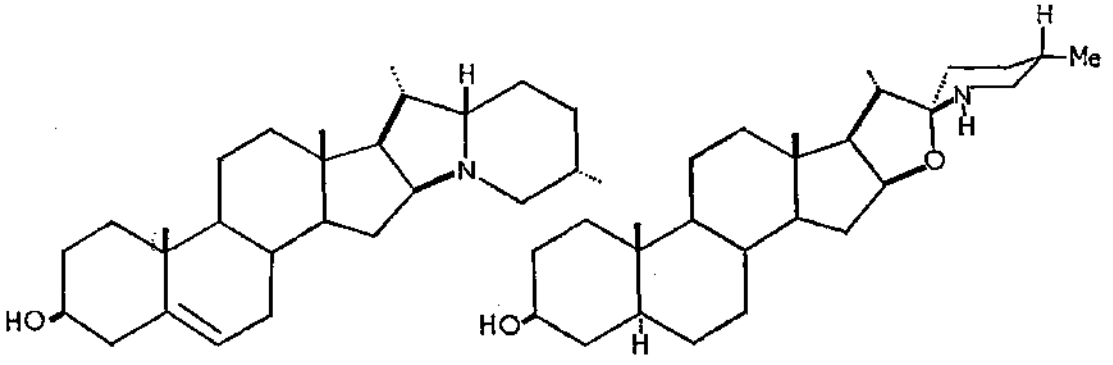
Alkamine	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids	m.p. °C.	$[\alpha]_D$ in pyridine	Sugar	Source	Refs.
Acetylleptinidine $C_{29}H_{45}NO_3$	3β-Hydroxy-x-acetoxy-solanid-5-ene	192–196	—	Leptine I $C_{47}H_{75}NO_{16}$	230	−85	Trisaccharide of 2 moles L-rhamnose and 1 mole D-glucose	*Solanum chacoense*	35
				Leptine II				,,	
				Leptine III				,,	
				Leptine IV				,,	
Demissidine $C_{27}H_{45}NO$	3β-Hydroxy-5α-solanidane	220–222	+21	Demissine (*syn* Solanine d) $C_{50}H_{83}NO_{20}$	305–308	−20	Branched tetrasaccharide of 1 mole D-xylose 1 mole D-galactose and 2 moles D-glucose (lycotetraose)	*S. demissum*	36, 37
Leptinidine $C_{27}H_{43}NO_3$	3β,-x-Dihydroxy-solanid-5-ene	247–248	−24	Leptinine I $C_{45}H_{73}NO_{15}$	230	−90	Trisaccharide of 1 mole D-glucose and 2 moles L-rhamnose	*S. chacoense*	35
				Leptinine II $C_{45}H_{73}NO_{16}$	225	−62	Trisaccharide of 1 mole D-galactose 1 mole L-rhamnose and 1 mole D-glucose	,,	35
				Leptinine III	—	—	—	,,	35
				Leptinine IV				,,	35
Rubijervine $C_{27}H_{43}NO_2$	3β,12α-Dihydroxysolanid-5-ene	242–243	+8					*Veratrum album*	38, 39
Isorubijervine $C_{27}H_{43}NO_2$	3β,18-Dihydroxysolanid-5-ene	235–237	+6·5 (EtOH)	Isorubijervosine $C_{33}H_{53}NO_7$	279–280	−20	D-Glucose	*V. viride* *V. album*	38, 40, 41

TABLE III—*continued*

Alkamine	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids	m.p. °C.	$[\alpha]_D$ in pyridine	Sugar	Source	Refs.
Solanidine (*syn* Solatubine Solanidine t) $C_{27}H_{43}NO$	3β-Hydroxysolanid-5-ene	219	−27	α-Chaconine $C_{45}H_{73}NO_{14}$	242	−85	α-L-Rhamnopyranosyl (1 → 2 glucose)-α-L-rhamnopyranosyl (1 → 4 glucose)-D-glucose	*Solanum tuberosum* *S. nigrum* *S. dulcamara* *S. chacoense*	42
				β-Chaconine $C_{39}H_{63}NO_{10}$	255	−61·5	α-L-Rhamnopyranosyl (1 → 4) D-glucose	"	42
				γ-Chaconine $C_{33}H_{53}NO_6$	243–244	−40	D-Glucose	"	42
				α-Solanine (*syn* Solanine Solanine t Solatunine) $C_{45}H_{73}NO_{15}$	285	−59	α-L-Rhamnopyranosyl (1 → 2 galatose)-β-D-glucopyranosyl (1 → 3)galactose) D-galactose	"	37, 43, 44
				β-Solanine $C_{33}H_{63}NO_{11}$	295	−31 (MeOH)	β-D-Glucopyranosyl (1 → 3) D-galactose	"	42
				γ-Solanine $C_{33}H_{53}NO_6$	240–250	−26 (MeOH)	D-Galactose	"	42
				Tetroside	—	—	Tetrasaccharide of 1 mole D-xylose 2 moles D-glucose 1 mole D-galactose	*S. acaulia*	45
				Solacauline $C_{42}H_{69}NO_{14}$	260–265	−30	Trisaccharide of 1 mole D-glucose and 2 moles D-xylose	*S. acaulia*	37
Soladulcamaridine $C_{27}H_{43}NO$	—	220–222	−78 (MeOH)	Soladulcamarine $C_{45}H_{79}NO_{17}$	193–197	—	Tetrasaccharide of 1 mole D-glucose 1 mole L-rhamnose 2 moles L-arabinose	*S. dulcamara*	46

35. Kuhn and Löw (1961 a,b). 36. Kuhn, Löw and Trischmann (1957). 37. Schreiber (1954). 38. Jacobs and Craig (1945). 39. Pelletier and Locke (1957). 40. Klohs and others (1953b). 41. Weisenborn and Burn (1953). 42. Kuhn, Löw and Trishmann (1955,b,c). 43. Kuhn, Löw and Trishmann (1955c). 44. Uhle and Jacobs (1945). 45. Schreiber (1957b). 46. Rasmussen and Boll (1958).

ring and they may be divided into two subclasses according to the immediate environment of the piperidino nitrogen atom. These subclasses are the solanidane and spirosolane groups and are exemplified by the alkamines solanidine (VII) and tomatidine (VIII) respectively. The known alkaloids belonging to the solanidane group are listed in Table III and those belonging to the spirosolane group are shown in Table IV.



VII VIII

The literature contains a number of references to the poisonous nature of various *Solanum spp.* and this can be attributed to the presence of cholestane-type alkaloids (see for example Griebel, 1923; Lowe, 1929; Rühl, 1951; Schowalter and Hartmann, 1924; Sirotina and Spirina, 1948). Potato sprouts and potatoes which have turned green through exposure above the ground develop a detectable amount of solanine and its aglycone solanidine and human consumption of such potatoes or potato shoots has led to a number of outbreaks of potato poisoning, several of which have been discussed by Willimott (1933). The most extensively investigated alkaloid of the cholestane group from a biological point of view is α-solanine (solanine), but it is to be noted that commercial samples of solanine have been shown to consist of 6 components (Kuhn, Löw and Trischmann, 1955b).

The cholestane group of alkaloids show certain similarities in their pharmacological properties to the pregnane group. Thus solanocapsine has been shown to slow the heart and induce incoordination by a direct action on cardiac muscle (Watt, Heimann and Epstein, 1932) and solanine, like conessine, has been shown to possess local anaesthetic properties (Weill, 1913). Rubijervine and several of its synthetic esters possess hypotensive properties (Poethke and Kuntze, 1958). Both solanine and solasonine induce haemolysis (de Lavergne and Kissel, 1935; Fischer, 1929; Macht, 1933) whilst solanine diminishes blood catalase (Levi, 1936), and inhibits non-specific cholinesterase (Pokrovskii, 1956). Solanine is also active as a mitotic poison (Danneberg and Schmähl, 1953) and has been shown to inhibit the oxygen uptake of mouse ascites tumour cells (Schmitz, 1951).

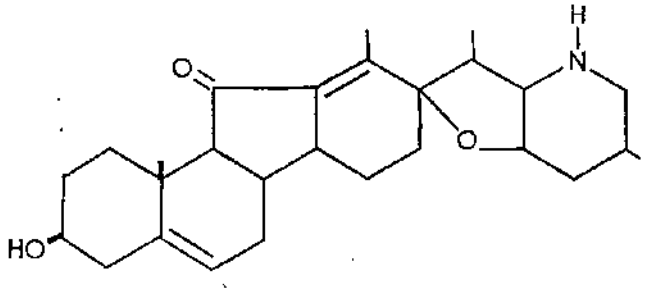
The discovery that extracts of tomato leaves exhibited antifungal and antibacterial activity led to the isolation of tomatine (Fontaine and

others, 1948) and similarly the observation that the leaves of *Solanum demissum* were resistant to the attacks of the larvae of the potato beetle *Leptinotarsa decemlineata* had as a result the isolation of demissine (Kuhn and Gauhe, 1947). Later work showed that other cholestane-type glycosidic alkaloids possessed the ability to prevent the ravages of the potato beetle and as tested on the leaves of solanum *tuberosum*, the order of potency was leptine I, then tomatine, then demissine, then α-solanine, and finally α-chaconine (Kuhn and Löw, 1961a). Independent work showed demissine to be more active than solacauline, which was more active than solanine (Shreiber, 1954). The insecticidal activity of various preparations of solanum steroidal alkaloids has, however, been shown to be but slight (Bergmann, Levinson and Mechoulam, 1958; Pollacci and Gallotti, 1940; Sievers and others, 1949).

Several studies have been devoted to the investigation of the antimicrobial properties of the group. Tomatine and several other alkaloids are antifungal (Chanussot, 1957; McKee, 1959; Sackman, Kern and Wiesman, 1959) and solanocapsine is claimed to possess *in vitro* activity against *Mycobacterium tuberculosis* (Boll and others, 1955–56). A number of synthetic solanine-type glycosides have been prepared, but they do not appear to have been investigated biologically (Schreiber, 1955).

Alkaloids possessing the "Jervi" Skeleton

The completely characterised alkaloids possessing the modified or "jervi" steroid skeleton in which ring C is 5-membered and ring D is 6-membered, are conveniently divided into two subclasses with the fritillaria alkaloids whose chemical constitution is as yet incompletely established, forming a third subclass. The first group consists of alkaloids whose alkamines possess a secondary nitrogen atom and contain only two or three atoms of oxygen. They occur in nature as the free alkamine or as D-glucosides and may be termed the "jerveratrum" alkaloids, as suggested by Fieser and Fieser (1959). Representative alkamines of this class are jervine (IX) and veratramine (X). The second subclass, which may be termed the "ceveratrum" group, consists of alkaloids whose alkamines are polyhydroxy tertiary bases possessing seven to nine atoms of oxygen and incorporating a quinolizidine ring system.

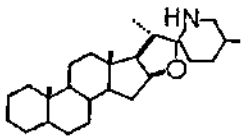


IX

The alkamine germine (XI) and the closely related alkamines, protoverine, veracevine and zygadenine possess a masked α-ketal system and

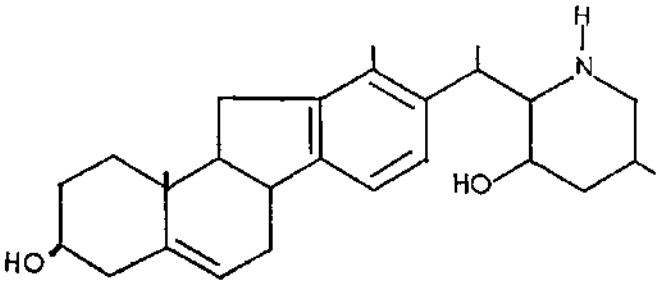
TABLE IV

DERIVATIVES OF SPIROSOLANE



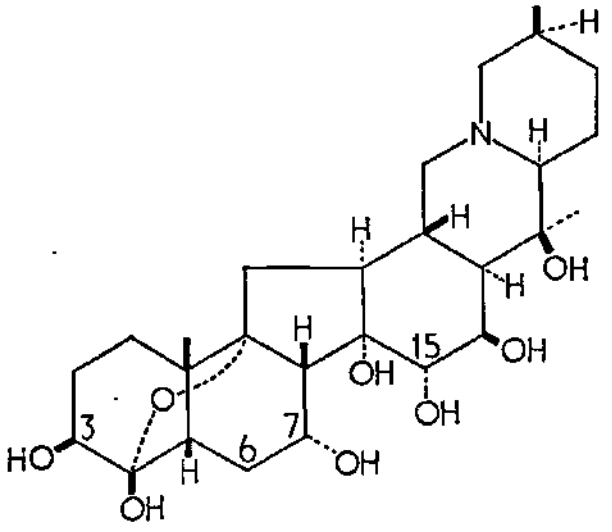
Alkamine	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids	m.p. °C.	$[\alpha]_D$ in pyridine	Sugar	Source	Refs.
Solasodine (*syn* Solanidine s Solancarpidine Purapuridine) $C_{27}H_{43}NO_2$	3β-Hydroxy-22a, 25-L-spirosol-5-ene	202	−80 (MeOH)	Solasodamine $C_{51}H_{83}NO_{20}$	298–302	−72 (MeOH)	L-Rhamnosido-L-rhamnosido-D-galactosido-D-glucose	*Solanum sodomeum* *S. auriculatum* *S. marginatum* *S. aviculare*	47
				Solasonine (*syn* Solanine s Solancarpine Purapurine γ-solanigrine) $C_{45}H_{73}NO_{16}$	301–303	−88	L-Rhamnosido-D-galactosido-D-glucose	*S. aviculare* *S. sodomeum* *S. xanthocarpum* *S. nodiflorum* *S. torvum* *S. laciniatum*	48–52
				Solamargine (*syn* δ-solanigrine) $C_{45}H_{73}NO_{15}$	301–310	−114	L-Rhamnosido-L-rhamnosido-D-glucose	*S. marginatum* *S. nigrum*	47, 48, 50, 52a
Tomatidine $C_{27}H_{45}NO_2$	5α,22b,25-L-Spirosolan-3β-ol	210–211	−8 (MeOH)	Tomatine $C_{50}H_{83}NO_{21}$	263–267	−19	Branched tetrasaccharide of 1 mole D-xylose 1 mole D-galactose and 2 moles D-glucose	*Lycopersicum pimpinellifolium* *L. esculentum* *L. peruvianum* *L. hirsutum*	52b, 53
				Trioside	—	—	Trisaccharide of 2 moles D-xylose and 1 mole D-glucose	*S. polyadenum*	52a
Solanocapsine $C_{27}H_{46}N_2O_2$	3α-Amino-x-hydroxy spirosolane	222	+25·5	—	—	—	—	*S. pseudocapsicum*	54
Solauricidine $C_{27}H_{43}NO_2$	—	219	−90 (MeOH)	Solauricine $C_{45}H_{73}NO_{16}$	270	—	L-Rhamnosido-D-galactosido-D-glucose	*S. auriculatum*	55
Solangustidine $C_{27}H_{45}NO_2$	—	amorphous (above 315 (HCl))	—	Solangustine $C_{38}H_{59}NO_7.H_2O$	235	—	D-Glucose	*S. angustifolium*	56
Soladulcidine $C_{27}H_{45}NO_2$	5α,22a,25-D-Spirosolan-3β-ol	206·5	−52·6	α-Soladulcine	—	—	—	*S. dulcamara*	57–59
				β-Soladulcine	—	—	—		
				γ-Soladulcine	—	—	—		
				Tetroside $C_{50}H_{83}NO_{21}$	268–270	−53	2 moles D-glucose, 1 mole D-galactose and 1 mole D-xylose	*S. dulcamara*	60
5,6-Dehydrotomatidine $C_{27}H_{43}NO_2$	3β-Hydroxy-22b, 25-L-spirosol-5-ene	206	−45	—	—	—	—	*S. tuberosum*	61

47. Briggs and Brooker (1958). 48. Boll (1958). 49. Briggs and Cambie (1958). 50. Kuhn, Löw and Trischmann (1955a). 51. Taylor (1958). 52. Uhle (1954). 52a. Schreiber (1957b). 52b. Fontaine and others (1948). 53. Kuhn, Löw and Trischmann (1957). 54. Boll and Lillevik (1959). 55. Briggs and Carroll (1942). 56. Tutin and Clewer (1914). 57. Briggs and O'Shea (1952). 58. Shreiber (1958). 59. Tuzson and Kiss (1957). 60. Alkemeyer and Sander (1959). 61. Shreiber (1957a).



X

undergo rearrangement with base. In veracevine, the rearrangement is particularly facile to yield first cevagenine and then cevine and for a time it was believed that cevine was the actual alkamine of the veracevine ester alkaloids. The ceveratrum alkaloids occur in various *Veratrum*, *Zygadenus* and *Schoenocaulon* species and are the agents responsible for the poisonous nature of these plants (Bealth and others, 1933; Reinhardt, 1909).



XI

The ceveratrum group have been isolated as the free alkamines or as mono-, di-, tri- or tetra- esters of various organic acids. Partial deacylation of the ester alkaloids occurs readily, however, and it is possible that some of the lower esters which have been isolated are in fact artefacts. It is the ceveratrum ester alkaloids, more particularly the tri- and tetra-esters which are the agents responsible for the hypotensive properties present in crude extracts of veratrum alkaloids. The known alkaloids of the ceveratrum group, together with the known jerveratrum alkaloids, are shown in Table V.

The jerveratrum alkaloids (both the alkamines and the glucosides) are characterised by an ability to antagonise the cardioaccelerator action of sympathetic nerve stimulation or of sympathomimetic amines (Krayer, 1952). This effect is thought to arise from a highly selective action upon the pacemaker of the heart and is not shown by adrenergic blocking agents. Accordingly the term "anti-accelerator agent" has been coined to describe

TABLE V

ALKALOIDS POSSESSING THE JERVI SKELETON

A. JERVERATRUM ALKALOIDS

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids (3-glucosides)	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Jervine $C_{27}H_{39}NO_3$	240–245	−167·5	Pseudojervine $C_{33}H_{49}NO_8$	300–301	−131	*Veratrum album* *V. viride* *Schoenocaulon officinale*	62–66
Veratramine $C_{27}H_{39}NO_2$	204–207	−71	Veratrosine $C_{33}H_{49}NO_7$	242–243 (decomp.)	−55 (EtOH/$CHCl_3$)	*V. album* *V. viride*	67–69

B. CEVERATRUM ALKALOIDS

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived ester alkaloids	Esterifying acids—position of substitution in brackets	m.p. °C.	$[\alpha]_D$ in pyridine	Source	Refs.
Germine $C_{27}H_{43}NO_6$	218–221	+4 (EtOH)	Germanitrine $C_{39}H_{59}NO_{11}$	Acetic (7): (−)-2-Methylbutyric (15): Angelic (3)	228–229	−61	*Veratrum fimbriatum*	70–72
			Germbudine $C_{37}H_{59}NO_{12}$	(−)-2-Methylbutyric (15): (+)-*threo*-2,3-Dihydroxy-2-methylbutyric (3)	160–164	−8	*V. viride*	73, 74
			Neogermbudine $C_{37}H_{59}NO_{12}$	(−)-2-Methylbutyric (15): (−)-*erythro*-2,3-Dihydroxy-2-methylbutyric (3)	149–152	−12	*V. album* *V. viride*	75, 76
			Germerine $C_{37}H_{59}NO_{11}$	(−)-2-Methylbutyric (3) (+)-2-Hydroxy-2-methylbutyric (15)	200–203	−14	*V. album* *V. viride* *V. nigrum*	77–81
			Germidine $C_{34}H_{53}NO_{10}$	Acetic (3) (−)-2-Methylbutyric (15)	230–231	−11	*V. viride* *Zygadenus venenosus*	78, 81, 82
			Isogermidine (*syn* neogermidine) $C_{34}H_{53}NO_{10}$	Acetic (7) (−)-2-Methylbutyric (15)	221–223	−63	*V. viride* *Z. paniculatus* *Z. venenosus*	78, 79, 81, 83
			Germinitrine $C_{39}H_{57}NO_{11}$	Acetic Angelic Tiglic	175–176	−36	*V. fimbriatum*	63, 70
			Germitetrine (*syn* germitetrine B) $C_{41}H_{63}NO_{14}$	Acetic (7): (−)-2-Methylbutyric (15): 2-Hydroxy-2-methyl-3-acetoxybutyric (3)	229–230	−74	*V. album*	75, 84, 85, 92
			Deacetylgermitetrine $C_{39}H_{61}NO_{13}$	(−)-2-Methylbutyric (15): 2-Hydroxy-2-methyl-3-acetoxybutyric (3)	143–149	−8	*V. album*	75, 76
			Germitrine $C_{39}H_{61}NO_{12}$	Acetic (7): (−)-2-Methylbutyric (3): (+)-2-Hydroxy-2-methylbutyric (15)	216–219	−69	*V. viride*	77, 78
			Neogermitrine $C_{34}H_{55}NO_{11}$	2 moles Acetic (3,7): (−)-2-Methylbutyric (15)	234–235	−78	*V. viride* *V. fimbriatum* *V. escholtzii* *Z. paniculatus* *Z. venenosus*	78, 82, 86, 92
			Protoveratridine $C_{33}H_{51}NO_9$	(−)-2-Methylbutyric (3)	272–273	−9	*V. album* *V. viride* *Z. venenosus*	78, 83

TABLE V—*continued*

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived ester alkaloids	Esterifying acids—position of substitution in brackets	m.p. °C.	$[\alpha]_D$ in pyridine	Source	Refs.
Protoverine $C_{27}H_{45}NO_9$	195–200	−11 (EtOH)	Escholerine $C_{41}H_{61}NO_{13}$	2 moles Acetic (6,7): (−)-2-Methylbutyric (15): Angelic (3)	235–236	−30	*V. escholtzii*	87, 88
			Deacetylprotoveratrine $C_{39}H_{61}NO_{13}$	Acetic (6): (−)-2-Methylbutyric (15): (+)-2-Hydroxy-2-methyl-butyric (3)	191–192	−15	*V. album*	76, 89
			Deacetylneoprotoveratrine (*syn* germbutrine deacetylprotoveratrine B protoveratetrine) $C_{39}H_{61}NO_{14}$	Acetic (6): (−)-2-Methylbutyric (15): (+)-*threo*-2,3-Dihydroxy-2-methylbutyric (3)	182–183	−9	*V. album* *V. viride*	73, 89, 90
			Protoveratrine A $C_{41}H_{63}NO_{14}$	2 moles Acetic (6,7): (−)-2-Methylbutyric (15): (+)-2-Hydroxy-2-methyl-butyric (3)	267–269	−40	*V. album* *V. viride* *Z. venenosus*	85, 91–93
			Protoveratrine B (*syn* neoprotoveratrine veratetrine) $C_{41}H_{63}NO_{15}$	2 moles Acetic (6,7): (−)-2-Methylbutyric (15): (+)-*threo*-2,3-Dihydroxy-2-methylbutyric	268–270	−37	*V. album* *V. viride* *Z. venenosus*	79, 85, 91, 93, 94
Neo-Sabadine $C_{27}H_{43}NO_9$	140–150	−33 (EtOH)	Sabadine $C_{29}H_{45}NO_9$	Acetic	120-140	−9·5 (EtOH)	*V. sabadilla*	95
Sabine $C_{27}H_{43-47}NO_7$	173–176	—	Sabatine $C_{29}H_{47-49}NO_8$	Acetic	256–258	—	*Schoenocaulon officinalie*	96

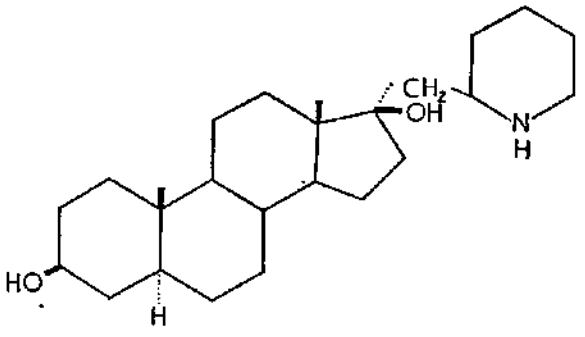
[*continued overleaf*

TABLE V—*continued*

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived ester alkaloids	Esterifying acids—position of substitution in brackets	m.p. °C.	$[\alpha]_D$ in pyridine	Source	Refs.
Veracevine (*syn* protocevine) $C_{27}H_{43}NO_8$	181–183	−33	Cevacine $C_{36}H_{45}NO_9$	Acetic (3)	205–207	−27 ($CHCl_3$)	*V. sabadilla*	97–100
			Cevadine (*syn* α-veratrine crystalline veratrine pure veratrine) $C_{32}H_{49}NO_9$	Angelic (3)	208–215	+6	*V. sabadilla* *V. viride*	101, 102
			Vanilloylveracevine $C_{35}H_{49}NO_{11}$	Vanillic (3)	256–257	+43	*V. sabadilla*	103
			Veratridine (*syn* amorphous veratrine) $C_{34}H_{51}NO_{11}$	Veratric (3)	160–180	−19 ($CHCl_3$)	*V. album* *V. sabadilla* *V. viride*	100, 101, 104
Zygadenine $C_{27}H_{43}NO_7$	201–204	−45	Angeloylzygadenine $C_{44}H_{69}NO_{11}$	Angelic (3)	222–224	−35 ($CHCl_3$)	*V. album*	105, 106
			Vanilloylzygadenine $C_{35}H_{49}NO_{10}$	Vanillic (3)	258–259	−27 ($CHCl_3$)	*Z. paniculatus* *Z. venenosus*	107
			Veratroylzygadenine $C_{39}H_{51}NO_{10}$	Veratric (3)	270–271	−27 ($CHCl_3$)	*V. album* *V. fimbriatum* *V. escholtzii* *V. nigrum* *Z. paniculatus* *Z. venenosus*	105, 108, 109
			Zygacine $C_{29}H_{45}NO_9$	Acetic (3)	Amorphous	−22 ($CHCl_3$)	*Z. paniculatus* *Z. venenosus*	110, 111

62. Jacobs and Craig (1944). 63. Klohs and others (1953a). 64. Okuda and Tsuda (1961). 65. Poethke (1938). 66. Tsukamoto and Kishimoto (1954). 67. Jacobs and Sato (1949). 68. Klohs and others (1953b). 69. Tamm and Wintersteiner (1952). 70. Klohs and others (1952b). 71. Kupchan and Afonso (1959). 72. Kupchan and Narayanan (1959). 73. Myers and others (1955). 74. Kupchan and Gruenfeld (1959b). 75. Kupchan and Ayres (1959). 76. Myers and others (1956). 77. Fried, White and Wintersteiner (1950). 78. Kupchan (1959a). 79. Myers and others (1952). 80. Poethke (1937). 81. Weisenborn and Bolger (1954). 82. Fried, Numerof and Coy (1952). 83. Kupchan and Deliwala (1952b). 84. Glen and others (1952). 85. Nash and Brooker (1953). 86. Klohs and others (1954). 87. Klohs and others (1952c). 88. Kupchan and others (1960). 89. Kupchan, Ayres and Hensler (1960). 90. Klohs and others (1953c). 91. Kupchan and Ayres (1960). 92. Kupchan and Deliwala (1953). 93. Stoll and Seebeck (1953a). 94. Klohs and others (1952a). 95. Möhrle and Auterhoff (1959). 96. Mitchner and Parks (1959). 97. Barton and others (1954). 98. Kupchan, Johnson and Rajagopalan (1959). 99. Kupchan and others (1953). 100. Vejdelek, Macek and Kahac (1956). 101. Ikawa and others (1945). 102. Kupchan and Afonso (1960). 103. Morgan and Barltrop (1958). 104. Blount (1935). 105. Kupchan (1959b). 106. Suzuki and others (1957). 107. Kupchan and Deliwala (1952a). 108. Klohs and others (1953a). 109. Stoll and Seebeck (1953b). 110. Kupchan, Lavie and Zonis (1955). 111. Shimizu (1958).

compounds exhibiting this particular pharmacological action (Krayer, 1950). Since antiaccelerator activity is absent in the tertiary veratrum alkaloids, it was concluded that such activity was associated with a secondary nitrogen atom incorporated in a piperidine ring (Krayer, Uhle and Ourisson, 1951) and attention was devoted to the preparation of synthetic steroids possessing a piperidine ring in the side chain (Krayer and Briggs, 1950; Uhle, 1951), among which were included several compounds prepared by cleavage of the ether link in derivatives of spirosolane. These compounds indeed proved active, but later work showed antiaccelerator activity to be present both in tertiary amino-steroids (Gould, Shapiro and Herschberg, 1954) and in secondary amino-steroids in which the nitrogen atom was not part of a piperidine ring (Gould and others, 1954; Margolin and others, 1954). These observations serve to emphasise the dangers of postulating structure-action relationships from a consideration of an inadequate number of compounds of insufficient chemical diversity. Quinidine was also shown to possess dual anti-accelerator and antifibrillatory activity and in this connection it is interesting that certain nitrogenous steroids (Gould and others, 1954; Robson and Trounce, 1955; Schallek and others, 1957) such as 17α-(2-piperidylmethyl)-3β,17β-dihydroxyandrostane (XII) and 16α-cyclohexylamino-3β-hydroxy-20-oxopregn-5-ene show quinidine-like properties.



XII

Detailed investigations have shown that veratramine decreases the oxygen consumption of atrial tissue without any initial augmentation of uptake (Reiter, 1950). In high doses veratramine produces excitation of the central nervous system (Krayer, 1949) whilst it is claimed that jervine in high doses produces hypotension in dogs (Wood, 1906).

Preparations of the ceveratrum ester alkaloids of varying purity have been employed medicinally from the time of the ancient Greek herbalists until the present day, the modern interest lying in their hypotensive properties, but as their pharmacology is covered in standard texts and has been extensively reviewed elsewhere (Krayer and Acheson, 1946; Stoll, 1954), a relatively brief summary will suffice in the present article. No attempt will be made to cover the literature pertaining to each individual ceveratrum ester.

Much of the earlier work was done with a preparation known as veratrine, which was first obtained by Pelletier and Caventou (1820),

but as this proved to be a complex mixture (Auterhoff, 1955; Blount, 1935), care must be taken in assessing this work owing to the great variation in potency exhibited by the alkamines and their esters (Krayer and Acheson, 1946; Krayer, Moe and Mendez, 1944). Other mixtures of alkaloids which have been employed in biological studies or medicinally, are cevadilline (also called sabadilline), cryptenamine (Kupchan and Gruenfield, 1959), protoveratrine, sabadine and sabatrine. As a broad generalisation it would appear that the alkamines are almost devoid of hypotensive activity, the naturally-occurring monoesters are feebly active, the diesters more active and the tri- and tetra-esters very active (Wintersteiner, 1953). In this connection it is to be noted that the highly active ester alkaloid germitetrine, although giving rise to four molecules of organic acids per molecule on hydrolysis, is really only a triester, as one of the esterifying acids is 2-hydroxy-2-methyl-3-acetoxybutyric acid (Kupchan and Ayres, 1959). Similar potency relationships have been found amongst synthetic esters of germine, where it was also discovered that several synthetic tetraesters were virtually inactive (Weisenborn and others, 1954).

More recent structure-action studies of a large number of synthetic esters of protoverine (Kupchan, Hensler and Weaver, 1961) have shown that esterification at positions 3 and 15 is necessary for high activity and that esterification at position 16 is accompanied by a profound loss of activity. Positions 6 and 7 need not be esterified for high activity. Esterification by a branched chain acid is advantageous at position 15, of no great import at position 3 and disadvantageous at position 7. Moreover, these relations were found to be broadly true for both the naturally-occurring and the previously prepared synthetic esters of germine (Weisenborn and others, 1954) whose structures were unknown at the time of the original experiments.

The pharmacological actions exhibited by the ceveratrum ester alkaloids are complex, making it difficult to analyse the exact contribution each makes to the total response, but there would now seem to be general agreement that in low doses they act by triggering reflex mechanisms.

The most pronounced pharmacological effect of the ceveratrum esters at therapeutic doses is the production of a rapid fall in arterial pressure, which is mediated by a reflex general vasodilatation, and a fall in heart rate. Thus they act in a unique fashion, differing from all other hypotensive agents. They are without any direct action on the blood vessels. The drop in blood pressure is also accompanied by respiratory depression. These effects have been demonstrated in various species of animals as well as in man and the experiments have indicated the existence of species differences (Rothlin and Cerletti, 1954), rodents being much more resistant to the hypotensive effect than man, the dog, or the cat.

The experimental evidence indicates that the afferent sensory receptors, upon which the ceveratrum esters act to produce the reflex fall in blood pressure, lie in the lungs and the heart (Heymans and Neil, 1958), with their afferent fibres lying in the vagi. Elicitation of the Benzold-Jarisch reflex (Aviado and Schmidt, 1955; Jarisch and Richter, 1939; von

Benzold and Hirt, 1867) as it is now known, however, is not the only reflex action produced by the ceveratrum esters, as vagotomised animals may still show a fall in blood pressure, indicating that other receptors are also involved (Heymans and Vleeschhouwer, 1950; Wang, Ngai and Grossman, 1955). The most important of these other receptors appear to be the baroreceptors situated in the region of the carotid bifurcation (see for example Aviado and others, 1955; Martini and Calliauw, 1955). Experiments with dogs would seem to indicate that stimulation of receptors within the nodose ganglion are responsible for the production of emesis (Borison and Fairbanks, 1952).

In higher concentrations the ceveratrum esters induce vasoconstriction and so exert a pressor effect. This action is mediated in part at least by the liberation of adrenaline from the adrenal medulla (Krayer, Moe and Mendez, 1944; Mendez and Montes, 1943).

At high doses the ceveratrum esters also produce changes in the electrophysiological state of nerve and muscle (see for example Shanes, 1958) which manifest themselves in the elicitation of a series of repetitive responses to a single stimulus—the so-called "veratrinic" response (see for example Acheson and Rosenbleuth, 1941; Gregor, 1904). It is thought that these electrophysiological changes result from alterations in the concentration of calcium ions on the cell membrane (Gordon and Welsh, 1948; Straub, 1954) with disruption of the normal ion transport mechanisms across the membrane (Shanes, 1952 and earlier papers; Straub, 1956) although muscle and nerve do not show the same changes in ionic migration when exposed to ceveratrum esters. Thus there is an increase of potassium ion efflux from nervous tissue (Shanes, 1952) and cardiac muscle (Lister and Lewis, 1959; Vick and Kahn, 1957) but no increase from skeletal muscle (Lister and Lewis, 1959). In view of the general assumption that ionic exchange occurs by similar mechanisms in all tissues (Davson and Danielli, 1952; Heilbrunn, 1956; Hodgkin, 1951) these facts are disturbing. It has also been shown that the ceveratrum esters do not promote potassium ion influx into potassium-poor cold-stored human erythrocytes (Kahn, Acheson and Cohen, 1955).

Whatever the detailed mechanism, it would appear safe to conclude that the ceveratrum esters interfere with the functioning of the cell membrane and it is probable that similar changes occur at the sensory afferent receptors (Jarisch and Zotterman, 1948) which would appear to have a far greater sensitivity than nerve or muscle cells.

Experimentally veratrine has been used to induce auricular arrhythmias in order to screen drugs for antifibrillatory activity (Scherf and Chick, 1951).

Other Actions of Ceveratrum Esters

Pronounced insecticidal activity is present in the ceveratrum group and dusts and extracts prepared from the seeds of *Schoenocaulon spp.* (sabadilla seed) have been tried as insecticides (see for example Anderson, 1945; Frazier, 1945; Filmer and Smith, 1946; Ikawa and others, 1945; Walton, 1946), over eighty publications dealing with their efficacy against

various insect species appearing in the years 1944–1956. These studies have also been extended to include ceveratrum alkaloid preparations from *Veratrum spp.* (see for example Fisher, 1940; Jaretzky and Janecke, 1940; Krupp, Lendle and Stapenhorst, 1952; Seiferle, Johns and Richardson, 1942). A particularly active preparation is produced by treating sabadilla seed with lime (Allen and Brunn, 1945; Walton, 1945). As is true for hypotensive activity, the alkamines appear virtually inactive as insecticides (Allen and others, 1945). In attempts to elucidate the exact mechanism by which the ceveratrum esters act upon the insect, several studies have been concerned with their effect on various enzyme systems (Collias, McShan and Lilly, 1952; Hartley and Brown, 1955).

Other studies have been concerned with their ability to induce mutations in *Drosophila funebris* (Tinyakov, 1947) and their ability to produce C-mitotic effects (Burroni, 1955).

TABLE VI

FRITILLARIA ALKALOIDS

Alkaloid	m.p. °C	$[\alpha]_D$ in $CHCl_3$	Refs.
Alginine $C_{28}H_{39}NO_3$	271–272	+108 (EtOH)	112
Amianthine $C_{27}H_{41}NO_2$	251–253	−87	113
Base $C_{27}H_{45}NO_3$	256	—	114
Beilupeimine $C_{27}H_{43}NO_3$	155–157	−53 (EtOH)	114
Chinpeimine $C_{27}H_{43}NO_2$	247–248	−21	114
Fritiminine	258–260	—	114
Imperoline $C_{27}H_{45}NO_3$	237–238	−8	115
Imperonine $C_{27}H_{43}NO_3$	239	−65	115
Peimidine $C_{27}H_{45}NO_3$	222	−74 (EtOH)	116
Peimine (*syn* Peimunine Verticine apo Verticine) $C_{27}H_{45}NO_3$	223–224	−26 (EtOH)	117–120
Peiminine (*syn* Peimiphine Peimitidine Verticilline Fritillarine) $C_{27}H_{43}NO_3$	212–213	−78	116, 120, 121
Peimissine $C_{27}H_{49}NO_4$	270	−51 (EtOH)	116
Sipeimine (*syn* Imperialine) $C_{27}H_{43}NO_3$	267	−36	122–124
Sonpeimine $C_{27}H_{45}NO_4$	256–258	—	114

112. Yunusov, Konovalova and Orekhov (1939). 113. Neuss (1953). 114. Chu and Loh (1956b). 115. Paul and Boit (1958). 116. Chou (1947). 117. Chou and Chu (1941). 118. Chu and Loh (1956a, b, and earlier papers). 119. Ito and others (1961). 120. Wu (1944). 121. Chi, Kao and Chang (1940). 122. Bauer and others (1958). 123. Boit (1954). 124. Chu and Loh (1955).

The Fritillaria Alkaloids

The fritillaria group on present indications would appear to possess the same skeleton as the ceveratrum group, but to possess only two or three hydroxyl groups (Chu and Loh, 1956b and earlier papers). The group deserves more research, especially on the inter-relations of the

alkaloids which are claimed to be individual entities. These alkaloids are listed in Table VI.

The pharmacology of the group is also in need of re-investigation with modern techniques. The Chinese drug pei-mu (Chou, 1954), from which many of the alkaloids have been isolated, was used quite irrationally for widely diverse conditions (Chi, Kao and Chang, 1936). Claims (Liu, Chang and Chang, 1936) that one of the alkaloids, peimine (also called peimunine) resembles atropine in its pharmacological properties seem surprising, as do claims of veratrine-like activity (Narumi, 1935), since these alkaloids do not possess the ester groups now known to be essential for this activity. On the other hand, reports (Chen, Chen and Chou, 1933; Narumi, 1936) that peimine and peiminine possess hypotensive properties and induce incoordination in the heart are more in line with the known activity of the pregnane group of alkaloids and may further illustrate the lack of dependence of these properties upon the position and configuration of the nitrogen atom in nitrogenous steroids. Additional evidence on this point would be provided by the reports (Zolotukhina, 1945) that alginine, the alkaloid from *Fritillaria sewerzowii*, possesses pronounced local anaesthetic activity, comparable to that of cocaine and exhibits vasodilatatory properties—if one assumes that the originally proposed molecular formula (Yunusov, Konovalova and Orekhov, 1939) is in error and that alginine does indeed have a cevane skeleton.

Amianthine, whose admission to the group is by no means certain, is obtained from the plant known as staggergrass or fly poison (*Amianthium muscaetoxicum*) (Neuss, 1953). This alkaloid has been shown to depress respiration and lower blood pressure and to be definitely without a typical veratrinic action on muscle (Alsberg, 1914).

There are a number of other alkaloids which, in all probability, belong to the steroid group, but their purity, identity or chemical constitution, are still unknown. Three such alkaloids which would appear to be pure entities are geralbine (Stoll and Seebeck, 1952), which is a C_{22} compound and raddeanine (Aslanov and Sadykov, 1956) and veratrobasine (Stoll, Stauffacher and Seebeck, 1955), which are C_{24} compounds. Raddeanine has been shown to stimulate the central nervous system of cats, rabbits and dogs in small doses, but in larger doses to be a depressant (Zolotukhina, 1944).

Conclusions

Despite the shortcomings of current theories of drug action and the lack of a simple correlation of chemical structure and biological activity, it is nevertheless clear from the foregoing account that much of the recent interest in the biological properties of nitrogenous steroids has a rational basis. Two examples may be quoted. The first is the application of the conclusions drawn from the receptor theory to the synthesis of the anabolic steroidal [3,2-c] pyrazoles and [2,3-d] isoxazoles, and the second is the synthesis of new drugs suggested by the supporting moiety theory. Both represent significant steps forward. Only within the last ten years

has interest developed in the synthetic nitrogenous steroids and, in view of the encouraging progress in the field, it would seem that even greater attention will be devoted to these compounds in the future. This is especially true now that aza-steroid hormone analogues have been synthesised (Zderic, Carpio and Limon, 1962) and with the interesting demonstration that the anabolic steroid 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole loses its ability to promote nitrogen retention on introduction of a double bond into the 4-position whilst it is converted into an oestrogenic compound showing no anabolic or androgenic properties when the 4,-6-diene system is introduced (Beyler, Potts and Arnold, 1961). With the recent discoveries that anabolic properties are present in a number of steroidal Schiff's bases (Irmscher, 1962) and that hypotensive properties are present in certain steroidal enamines (Clinton and others, 1962), it can be confidently predicted that nitrogenous steroids will play a further rôle in studies of drug action.

Moreover, the nitrogenous steroids as a group faithfully reflect the biological properties of the steroids in general, affording a broad spectrum of biological action and emphasizing changes in pharmacological properties with species, and routes of administration. Certain individual members, notably the ceveratrum ester alkaloids, display a variety of pharmacological actions which in themselves have led the pharmacologist to disentangle basic mechanisms of action, thus contributing to a better understanding of biological phenomena. Nitrogenous steroids are not unique in this respect, but they do present a happy choice with which to illustrate the slow but certain development of the theoretical aspects of pharmacology.

REFERENCES

Acheson, G. H. and Rosenbleuth, A. (1941). *Amer. J. Physiol.*, **133**, 736–751.
Acton, H. W. and Chopra, R. N. (1933). *Indian med. Gaz.*, **68**, 6.
Alkemeyer, M. and Sander, H. (1959). *Naturwissenschaften*, **46**, 207–208.
Allen, T. C. and Brunn, L. K. (1945). *J. econ. Ent.*, **38**, 291–293.
Allen, T. C., Link, K. P., Ikawa, M. and Brunn, L. K. (1945). *Ibid.*, **38**, 293–296.
Alsberg, C. L. (1914). *Science*, **39**, 958.
Anderson, R. F. (1945). *J. econ. Ent.*, **38**, 564–566.
Aslanov, K. A. and Sadykov, A. S. (1956). *J. gen. Chem. U.R.S.S.*, **26**, 623–627.
Auffret, C. and Tanguy, F. (1950). *Méd. trop.*, **10**, 530–536.
Auterhoff, H. (1955). *Arch. Pharm.*, **288**, 549–560.
Aviado, D. M., Cerletti, A., Li, T. H. and Schmidt, C. F. (1955). *J. Pharmacol.*, **115**, 329–338.
Aviado, D. M. and Schmidt, C. F. (1955). *Physiol. Rev.*, **35**, 247–300.
Bakhsh, I. (1936). *J. Pharmacol.*, **58**, 373–392.
Barton, D. H. R., Jeger, O., Prelog, V. and Woodward, R. B. (1954). *Experientia*, **10**, 81–90.
Bauer, S., Masler, L., Orszagh, S., Mekry, J. and Tomko, J. (1958). *Chem. Zvesti*, **12**, 584–586.
Bealth, O. A., Eppson, H. F., Draize, J. H. and Justice, R. S. (1933). *Wyo. Agr. Exp. Sta. Bull.*, **194**, 3–39.
Bergmann, E. D., Levinson, Z. H. and Mechoulam, R. (1958). *J. Insect. Physiol.*, **2**, 162–177.
Bertho, A. (1939). *Arch. Pharm.*, **277**, 237–257.
Bertho, A. (1944a). *Liebigs Annalen.*, **555**, 214–224.
Bertho, A. (1944b). *Arch. exp. Path. Pharmak.*, **203**, 41–46.
Bertho, A. (1947). *Liebigs Annalen*, **558**, 62–70.
Bertho, A. (1951). *Ibid.*, **573**, 210–219.

Bertho, A. and Gotz, M. (1958). *Ibid.*, **619**, 96–121.
Bertho, A., von Schuckmann, G. and Schönberger, W. (1933). *Ber. dtsch. chem. Ges.*, **66**, 786–790.
Beyler, A. L., Potts, G. O. and Arnold, A. (1961). *Endocrinology*, **68**, 987–995.
Blanpin, O. and Quévauviller, A. (1960). *Semaine des Hôpitaux Semaine Thérapeutique*, **36**, 909–912.
Blount, B. K. (1935). *J. chem. Soc.*, 122–125.
Boit, H. G. (1954). *Chem. Ber.*, **87**, 472–475.
Boll, P. M. (1958). *Acta chem. scand.*, **12**, 358.
Boll, P. M. and Lillevik, H. A. (1959). *Ibid.*, **13**, 2039–2046.
Boll, P. M., Lillevik, H. A., Gottshall, R. Y. and Lucas, E. H. (1955–56). *Antibiot. Ann.*, 255–259.
Borison, H. L. and Fairbanks, V. F. (1952). *J. Pharmacol.*, **105**, 317–325.
Briggs, L. H. and Brooker, E. G. (1958). *J. chem. Soc.*, 1419–1421.
Briggs, L. H. and Cambie, R. C. (1958). *Ibid.*, 1422–1425.
Briggs, L. H. and Carroll, J. J. (1942). *Ibid.*, 17–18.
Briggs, L. H. and O'Shea, T. (1952). *Ibid.*, 1654–1658.
Burn, J. H. (1915). *J. Pharmacol.*, **6**, 305–321.
Burroni, M. (1955). *Caryologia*, **7**, 87–97.
Černý, V., Lábler, L. and Šorm, F. (1957). *Coll. Trav. chim. Tchécosl.*, **22**, 76–84.
Černý, V., Lábler, L. and Šorm, F. (1959). *Ibid.*, **24**, 378–383.
Chanussot, P. (1957). *Anales. Asoc. quim. Arg.*, **45**, 113–120.
Chen, K. K., Ling Chen, A. and Chou, T. Q. (1933). *J. Amer. pharm. Ass. Sci. Ed.*, **22**, 638–641.
Chi, Y. F., Kao, Y. S. and Chang, K. J. (1936). *J. Amer. chem. Soc.*, **58**, 1306–1307.
Chi, Y. F., Kao, Y. S. and Chang, K. J. (1940). *Ibid.*, **62**, 2896–2897.
Chopra, R. N., Gupta, J. C., David, J. C. and Ghosh, S. (1927). *Ind. med. Gaz.*, **62**, 132–140.
Chopra, R. N., Gupta, J. C. and Chopra, G. S. (1933). *Ind. J. med. Res.*, **21**, 277–281.
Chou, T. Q. (1947). *J. Amer. pharm. Ass. Sci. Ed.*, **36**, 215–217.
Chou, T. Q. (1954). *Pharmazie*, **9**, 688–691.
Chou, T. Q. and Chu, T. T. (1941). *J. Amer. chem. Soc.*, **63**, 2936–2938.
Chu, T. T. and Loh, J. Y. (1955). *Acta chim. sinica*, **21**, 241.
Chu, T. T. and Loh, J. Y. (1956a). *Ibid.*, **22**, 210.
Chu, T. T. and Loh, J. Y. (1956b). *Ibid.*, **22**, 361.
Clinton, R. O., Manson, A. J., Stonner, F. W., Clarke, R. L., Jennings, K. F. and Shaw, P. E. (1962). *J. org. Chem.*, **27**, 1148–1154.
Collias, E. C., McShan, W. H. and Lilly, J. H. (1952). *J. cell. comp. Physiol.*, **40**, 507–527.
Danneberg, P. and Schmähl, D. (1953). *Arzneimitt.-Forsch.*, **3**, 151–161.
Davson, H. and Danielli, H. F. (1952). *The Permeability of Natural Membranes*, Cambridge: University Press.
de Lavergne, V. and Kissel, P. (1935). *C.R. Soc. biol., Paris*, **120**, 149–150.
Dickel, D., Lucas, R. and MacPhillamy, H. B. (1959). *J. Amer. chem. Soc.*, **81**, 3154–3155.
Durieux, C., Trenous, J. and Tanguy, F. (1948). *Méd. trop.*, **8**, 7–11.
Duvian, G. (1953). *J. Med., Bordeaux*, **130**, 44–51.
Favre, H., Haworth, R. D., McKenna, J., Powell, R. G. and Whitfield, G. H. (1953). *J. chem. Soc.*, 1115–1129.
Fieser, L. F. and Fieser, M. (1959). *Steroids*, pp. 847–895, New York: Reinhold.
Filmer, R. S. and Smith, C. L. (1946). *J. econ. Ent.*, **39**, 309–313.
Fischer, R. (1929). *Biochem. Z.*, **209**, 319–325.
Fisher, R. A. (1940). *J. econ. Ent.*, **33**, 728–734.
Fontaine, T. D., Irving, G. W., Ma, R. M., Poole, J. B. and Doolittle, S. P. (1948). *Arch. Biochem.*, **18**, 467–475.
Frazier, N. W. (1945). *J. econ. Ent.*, **38**, 720.
Fried, J., Numerof, P. and Coy, N. H. (1952). *J. Amer. chem. Soc.*, **74**, 3041–3046.
Fried, J., White, H. L. and Wintersteiner, O. (1950). *Ibid.*, **72**, 4621–4630.
Gessner, O. (1948). *Arch. exp. Path. Pharmak.*, **205**, 1–20.
Ghosh, S. and Bose, I. B. (1932). *Arch. Pharm.*, **270**, 100–108.
Glen, W. L., Myers, G. S., Barber, R., Morozovitch, P. and Grant, G. A. (1952). *Nature, Lond.*, **170**, 932.
Gordon, H. T. and Welsh, J. H. (1948). *J. cell. comp. Physiol.*, **31**, 395–419.
Gould, D., Shapiro, E. L. and Hershberg, E. B. (1954). *J. Amer. chem. Soc.*, **76**, 5567.

Goutarel, R. (1961). *Tetrahedron*, **14**, 126–137.
Goyal, R. K. (1935). *C.R. Soc. biol., Paris*, **120**, 296–297.
Gregor, A. (1904). *Pflugers Arch.*, **101**, 71.
Griebel, C. (1923). *Z. Nahr. Genussm.*, **45**, 175–183.
Habermehl, G. (1962). *Angew. Chem.*, **74**, 154.
Hartley, J. B. and Brown, A. W. A. (1955). *J. econ. Ent.*, **48**, 265–269.
Haworth, R. D. (1932). *J. chem. Soc.*, 631–634.
Heilbrunn, L. V. (1956). *The Dynamics of Living Protoplasm*, New York: Academic Press.
Henry, T. A. and Brown, H. C. (1923). *Trans. roy. Soc. trop. Med. Hyg.*, **17**, 61–71.
Heymans, C. and de Vleeschhouwer, G. (1950). *Arch. int. Pharmacodyn.*, **84**, 409–416.
Heymans, C. and Neil, O. (1958). *Reflexogenic Areas of the Cardiovascular System*, London: J. and A. Churchill.
Hodgkin, A. L. (1951). *Biol. Rev.*, **26**, 339–409.
Ikawa, M., Dicke, R. J., Allen, T. C. and Link, K. P. (1945). *J. biol. Chem.*, **159**, 517–524.
Irmscher, K. (1962). *Chem. Ber.*, **95**, 907–917.
Ito, S., Kato, M., Shibata, K. and Nozoe, T. (1961). *Chem. Pharm. Bull. Tokyo*, **9**, 253–255.
Jacobs, W. A. and Craig, L. C. (1944). *J. biol. Chem.*, **155**, 565–572.
Jacobs, W. A. and Craig, L. C. (1945). *Ibid.*, **160**, 555–565.
Jacobs, W. A. and Sato, Y. (1949). *Ibid.*, **181**, 55–65.
Janot, M. M., Cavé, A. and Goutarel, R. (1959). *Bull. Soc. chim. France*, 896–900.
Janot, M. M., Cavé, A. and Goutarel, R. (1960). *C.R. Acad. Sci., Paris*, **251**, 559–561.
Janot, M. M. and Cavier, R. (1949). *Ann. pharm. franç.*, **7**, 549–552.
Janot, M. M., Lainé, F. and Goutarel, R. (1960). *Ibid.*, **18**, 673–677.
Janot, M. M., Lainé, F. and Goutarel, R. (1962). *Bull. Soc. chim. France*, 648–651.
Janot, M. M., Lainé, F., Qui Khuong Huu and Goutarel, R. (1962a). *Ibid.*, 111–118.
Janot, M. M., Monseur, X., Conreur, C. and Goutarel, R. (1962b). *Ibid.*, 285–287.
Janot, M. M., Qui Khuong Huu and Goutarel, R. (1959). *C.R. Acad. Sci., Paris*, **248**, 982–984.
Janot, M. M., Qui Khuong Huu and Goutarel, R. (1960). *Ibid.*, **250**, 2445–2447.
Janot, M. M., Qui Khuong Huu and Goutarel, R. (1962). *Ibid.*, **254**, 1326–1328.
Jaretzky, R. and Janecke, H. (1940). *Arch. Pharm.*, **278**, 34–42.
Jarisch, A. and Richter, H. (1939). *Arch. exp. Path. Pharmak.*, **193**, 355–371.
Jarisch, A. and Zotterman, Y. (1948). *Acta physiol. scand.*, **16**, 31–51.
Jeger, O. and Prelog, V. (1960). In *The Alkaloids* (editor R. H. F. Manske), Vol. 7, pp. 319–417. New York: Academic Press.
Kahn, J. B., Acheson, G. H. and Cohen, S. B. (1955). *J. Pharmacol.*, **115**, 305–318.
Kaushiva, B. S. (1957). *J. sci. ind. Res. India*, **16C**, 210–214.
Kaushiva, B. S. and Ghatak, S. (1956). *Ibid.*, **15C**, 195–198.
Kerny, M. (1948). *Ann. pharm. franç.*, **6**, 534–539.
Klohs, M. W., Arons, R., Draper, M. D., Keller, F., Koster, S., Malesh, W. and Petracek, F. J. (1952a). *J. Amer. chem. Soc.*, **74**, 5107–5110.
Klohs, M. W., Draper, M. D., Keller, F., Koster, S., Malesh, W. and Petracek, F. J. (1952b). *Ibid.*, **74**, 4473–4474.
Klohs, M. W., Keller, F., Koster, S. and Malesh, W. (1952c). *Ibid.*, **74**, 1871.
Klohs, M. W., Draper, M. D., Keller, F., Koster, S., Malesh, W., and Petracek, F. J. (1953a). *Ibid.*, **75**, 4925–4927.
Klohs, M. W., Draper, M. D., Keller, F., Malesh, W. and Petracek, F. J. (1953b). *Ibid.*, **75**, 2133–2136.
Klohs, M. W., Draper, M. D., Keller, F., Malesh, W. and Petracek, F. J. (1953c). *Ibid.*, **75**, 3595–3596.
Klohs, M. W., Draper, M. D., Keller, F., Koster, S., Malesh, W. and Petracek, F. J. (1954). *Ibid.*, **76**, 1152–1153.
Krayer, O. (1949). *J. Pharmacol.*, **96**, 422–437.
Krayer, O. (1950). *Ibid.*, **98**, 427–436.
Krayer, O. (1952). *J. Mt. Sinai Hosp., N.Y.*, **19**, 53–69.
Krayer, O. and Acheson, G. H. (1946). *Physiol. Rev.*, **26**, 383–446.
Krayer, O. and Briggs, L. H. (1950). *Brit. J. Pharmacol.*, **5**, 517–525.
Krayer, O., Moe, G. K. and Mendez, R. (1944). *J. Pharmacol.*, **82**, 167–186.
Krayer, O., Uhle, F. C. and Ourisson, P. (1951). *Ibid.*, **102**, 261–268.
Krupp, H., Lendle, L. and Stapenhorst, K. (1952). *Arzneimitt. Forsch.*, **2**, 258–262.

Kuhn, R. and Gauhe, A. (1947). *Z. Naturforsch.*, **2b**, 407–409.
Kuhn, R. and Löw, I. (1961a). *Chem. Ber.*, **94**, 1088–1095.
Kuhn, R. and Löw, I. (1961b). *Ibid.*, **94**, 1096–1103.
Kuhn, R., Löw, I. and Trischmann, H. (1955a). *Ibid.*, **88**, 289–294.
Kuhn, R., Löw, I. and Trischmann, H. (1955b). *Ibid.*, **88**, 1492–1507.
Kuhn, R., Löw, I. and Trischmann, H. (1955c). *Ibid.*, **88**, 1690–1693.
Kuhn, R., Löw, I. and Trischmann, H. (1957). *Ibid.*, **90**, 203–218.
Kupchan, S. M. (1959a). *J. Amer. chem. Soc.*, **81**, 1921–1924.
Kupchan, S. M. (1959b). *Ibid.*, **81**, 1925–1928.
Kupchan, S. M. and Afonso, A. (1959). *J. Amer. pharm. Ass., Sci. Ed.*, **48**, 731–734.
Kupchan, S. M. and Afonso, A. (1960). *Ibid.*, **49**, 242–244.
Kupchan, S. M. and Ayres, C. I. (1959). *Ibid.*, **48**, 440–442.
Kupchan, S. M. and Ayres, C. I. (1960). *J. Amer. chem. Soc.*, **82**, 2252–2258.
Kupchan, S. M., Ayres, C. I. and Hensler, R. H. (1960). *Ibid.*, **82**, 2616–2620.
Kupchan, S. M., Ayres, C. I., Neeman, M., Hensler, R. H., Masamune, T. and Rajagopalan, S. (1960). *Ibid.*, **82**, 2242–2251.
Kupchan, S. M. and Deliwala, C. V. (1952a). *Ibid.*, **74**, 2382–2383.
Kupchan, S. M. and Deliwala, C. V. (1952b). *Ibid.*, **74**, 3202.
Kupchan, S. M. and Deliwala, C. V. (1953). *Ibid.*, **75**, 4671–4672.
Kupchan, S. M. and Gruenfeld, N. (1959a). *J. Amer. pharm. Ass., Sci. Ed.*, **48**, 727–730.
Kupchan, S. M. and Gruenfeld, N. (1959b). *Ibid.*, **48**, 737–739.
Kupchan, S. M., Hensler, R. H. and Weaver, L. C. (1961). *J. med. pharm. Chem.*, **3**, 129–155.
Kupchan, S. M., Johnson, W. S. and Rajagopalan, S. (1959). *Tetrahedron*, **7**, 47–61.
Kupchan, S. M., Lavie, D., Deliwala, C. V. and Andoh, B. Y. A. (1953). *J. Amer. chem. Soc.*, **75**, 5519–5524.
Kupchan, S. M., Lavie, D. and Zonis, R. D. (1955). *Ibid.*, **77**, 689–691.
Kupchan, S. M. and Narayanan, C. R. (1959). *Ibid.*, **81**, 1913–1921.
La Barre, J. and Desmarez, J. J. (1959). *Arch. int. Pharmacodyn.*, **119**, 514–516.
Lábler, L. and Černy, V. (1959). *Coll. Trav. chim. Tchécosl.*, **24**, 370–377.
Lambin, S. and Bernard, J. (1954). *C.R. Soc. biol., Paris*, **147**, 638–641.
Lavier, G., Crosnier, R. and Merle, F. (1948). *Bull. Soc. Path. exot.*, **41**, 548–553.
Leake, C. D. (1932). *J. Amer. med. Ass.*, **98**, 195–199.
Le Men, J. (1960). *Bull. Soc. chim. France*, 860–864.
Levi, A. (1936). *Arch. Farm. sper.*, **61**, 121–142.
Lister, R. E. and Lewis, J. J. (1959). *J. Pharm. Pharmacol.*, **11**, 185*T*–194*T*.
Liu, S. K., Chang, Y. T. and Chang, F. C. (1936). *Chinese med. J.*, **50**, 249–251.
Lowe, H. (1929). *Analyst*, **54**, 153–154.
Lucas, R. A., Dickel, D. F., Dziemian, R. L., Ceglowski, M. J., Hensle, B. L. and MacPhillamy, H. B. (1960). *J. Amer. chem. Soc.*, **82**, 5688–5693.
McKee, R. K. (1959). *J. gen. Microbiol.*, **20**, 686–696.
Macht, D. I. (1933). *Proc. Soc. exp. Biol., N.Y.*, **30**, 988–990.
Mackie, A., Steward, G. M., Cutler, A. A. and Misra, A. L. (1955). *Brit. J. Pharmacol.*, **10**, 7–11.
Margolin, S., Lu, G., Yelnosky, J. and Makovsky, A. (1954). *Science*, **120**, 576–577.
Martini, L. and Calliauw, L. (1955). *Arch. int. Pharmacodyn.*, **101**, 49–67.
Meissner, G. and Hesse, E. (1930). *Arch. exp. Path. Pharmak.*, **147**, 339–359.
Mendez, R. and Montes, G. (1943). *J. Pharmacol.*, **78**, 238–248.
Mitchner, H. and Parks, L. M. (1959). *J. Amer. pharm. Ass., Sci. Ed.*, **48**, 303–307.
Möhrle, H. and Auterhoff, H. (1959). *Arch. Pharm.*, **292**, 337–340.
Morgan, K. J. and Barltrop, J. A. (1958). *Quart. Rev.*, **12**, 34–60.
Muhlpfordt, H. and Martinez-Silva, R. (1956). *Z. Tropenmed. Parasitol.*, **7**, 211–219.
Myers, G. S., Glen, W. L., Morozovitch, P., Barber, R. and Grant, G. A. (1952). *J. Amer. chem. Soc.*, **74**, 3198–3199.
Myers, G. S., Glen, W. L., Morovitch, P., Barber, R., Papineau-Couture, G. and Grant, G. A. (1956). *Ibid.*, **78**, 1621–1624.
Myers, G. S., Morovitch, P., Glen, W. L., Barber, R., Papineau-Couture, G. and Grant, G. A. (1955). *Ibid.*, **77**, 3348–3353.
Narumi, Y. (1935). *Tôhoku J. exp. Med.*, **26**, 325–335.
Narumi, Y. (1936). *Ibid.*, **28**, 26–43.
Nash, H. A. and Brooker, R. M. (1953). *J. Amer. chem. Soc.*, **75**, 1942–1948.
Neuss, N. (1953). *Ibid.*, **75**, 2772–2773.
Okuda, S. and Tsuda, K. (1961). *Chem. Ind.*, 512.
Paris, R. (1938). *Bull. Sci. pharmacol.*, **45**, 453–457.

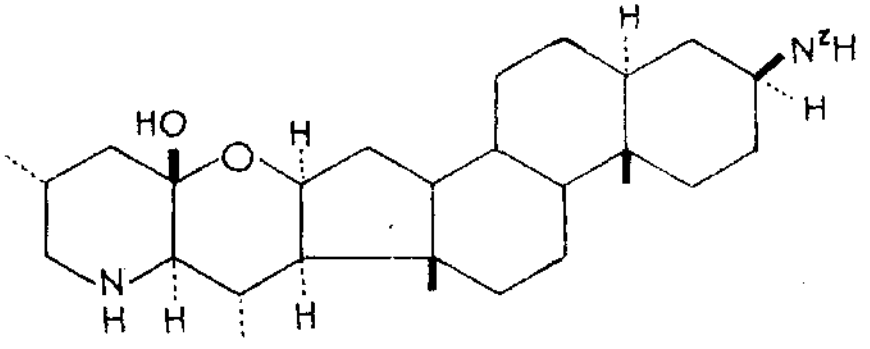
Paul, L. and Boit, H. G. (1958). *Chem. Ber.*, **91**, 1968–1970.
Peacock, D. H. and Chowdhury, J. C. (1935). *J. chem. Soc.*, 734–735.
Pelletier, P. J. and Caventou, J. B. (1820). *Ann. chim.*, [2], **14**, 60.
Pelletier, S. W. and Jacobs, W. A. (1952). *J. Amer. chem. Soc.*, **74**, 4218–4219.
Pelletier, S. W. and Locke, D. M. (1957). *Ibid.*, **79**, 4531–4538.
Piette, M. (1950). *Ann. pharm. franç.*, **8**, 460–462.
Pluchon, J. P. and Pille, G. (1950). *Ibid.*, **8**, 741–744.
Poethke, W. (1937). *Arch. Pharm.*, **275**, 571–599.
Poethke, W. (1938). *Ibid.*, **276**, 170–181.
Poethke, W. and Kuntze, M. (1958). *Planta Med.*, **6**, 92–94.
Pokrovskii, A. A. (1956). *Biokhimiya*, **21**, 683–688.
Pollacci, G. and Gallotti, M. (1940). *Boll. Soc. ital. Biol. sper.*, **15**, 328–330.
Quévauviller, A. and Blanpin, O. (1958). *J. de Physiol.*, **50**, 1123–1127.
Quévauviller, A. and Blanpin, O. (1960). *Semaine des Hôpitaux Semaine Thérapeutique*, **36**, 895–898.
Rasmussen, H. B. and Boll, P. M. (1958). *Acta Chem. scand.*, **12**, 802–806.
Reinhardt, L. (1909). *Munch. med. Wschr.*, **56**, 2056–2057.
Reiter, M. (1950). *J. Pharmacol.*, **99**, 132–139.
Robson, J. M. and Trounce, J. R. (1955). *J. Physiol.*, **129**, 10P–11P.
Rostock, H. and Seebeck, E. (1958). *Helv. chim. Acta*, **41**, 11–22.
Rothlin, E. and Cerletti, A. (1954). *Schweiz. med. Wschr.*, **84**, 137–142.
Rühl, R. (1951). *Arch. Pharm.*, **284**, 67–74.
Sackmann, W., Kern, H. and Wiesmann, E. (1959). *Schweiz. Z. allgem. Path. Bakteriol.*, **22**, 557–563.
Schallek, W., Zabransky, F. W., Jampolsky, L. M., Rehl, W. R. and Goldberg, M. W. (1957). *Proc. Soc. exp. Biol., N.Y.*, **95**, 433–437.
Scherf, D. and Chick, F. B. (1951). *Amer. Heart J.*, **42**, 212–225.
Schmitz, H. (1951). *Z. Krebsforsch.*, **57**, 463–480.
Schöpf, C. (1961). *Experientia*, **17**, 285–295.
Schowalter, E. and Hartmann, W. (1924). *Z. Nahr. Genussm.*, **47**, 251–257.
Schreiber, K. (1954). *Chem. Ber.*, **87**, 1007–1010.
Schreiber, K. (1955). *Angew. Chem.*, **67**, 127..
Schreiber, K. (1957a). *Ibid.*, **69**, 483.
Schreiber, K. (1957b). *Der Züchter*, **27**, 289.
Schreiber, K. (1958). *Planta Med.*, **6**, 94–97.
Seiferle, E. J., Johns, I. B. and Richardson, C. H. (1942). *J. econ. Ent.*, **35**, 35–44.
Shanes, A. M. (1952). *Ann. N.Y. Acad. Sci.*, **55**, 1–36.
Shanes, A. M. (1958). *Pharmacol. Rev.*, **10**, 165–273.
Shimizu, B. (1958). *J. pharm. Soc., Japan*, **78**, 443–444.
Siddiqui, S. (1934). *J. Indian chem. Soc.*, **11**, 283–291.
Siddiqui, S. (1935). *Proc. Indian Acad. Sci.*, **2A**, 426–437.
Siddiqui, S. (1936). *Ibid.*, **3A**, 249–256.
Siddiqui, S. and Pillay, P. P. (1932). *J. Indian chem. Soc.*, **9**, 553–563.
Siddiqui, S. and Siddiqui, R. H. (1934). *Ibid.*, **11**, 787–795.
Sievers, A. F., Archer, W. A., Moore, R. H. and McGovran, E. R. (1949). *J. econ. Ent.*, **42**, 549–551.
Sirotina, O. N. and Spirina, A. P. (1948). *Gigiena i Sanit.*, **13**, No. 10, 42–43.
Stephenson, R. P. (1948). *Brit. J. Pharmacol.*, **3**, 237–245.
Stephenson, R. P. and Dutta, N. K. (1948). *Ibid.*, **3**, 326–327.
Stoll, A. (1954). *Gazz. chim. ital.*, **84**, 1190–1209.
Stoll, A. and Seebeck, E. (1952). *J. Amer. chem. Soc.*, **74**, 4728–4729.
Stoll, A. and Seebeck, E. (1953a). *Helv. chim. Acta*, **36**, 718–723.
Stoll, A. and Seebeck, E. (1953b). *Ibid.*, **36**, 1570–1575.
Stoll, A., Stauffacher, D. and Seebeck, E. (1955). *Ibid.*, **38**, 1964–1976.
Straub, R. (1954). *Helv. Physiol. Acta*, **12**, C89–C92.
Straub, R. (1956). *Ibid.*, **14**, 1–28.
Suzuki, M., Shimizu, B., Murase, Y., Hayashi, R. and Sanpei, N. (1957). *J. pharm. Soc., Japan*, **77**, 1050.
Tamm, C. and Wintersteiner, O. (1952). *J. Amer. chem. Soc.*, **74**, 3842–3849.
Tanguy, F., Robin, C. and Raoult, A. (1948). *Med. trop.*, **8**, 12.
Taylor, D. A. H. (1958). *J. chem. Soc.*, 4216.
Tinyakov, G. G. (1947). *Doklady Akad. Nauk S.S.S.R.*, **58**, 307–310.
Trevan, J. W. and Boock, E. (1927). *Brit. J. exp. Path.*, **8**, 307–315.
Tschesche, R. and Petersen, R. (1954). *Chem. Ber.*, **87**, 1719–1725.
Tschesche, R. and Roy, A. C. (1956). *Ibid.*, **89**, 1288–1295.
Tschesche, R. and Wiensz, K. (1958). *Ibid.*, **91**, 1504–1511.

Tsukamoto, T. and Kishimoto, Y. (1954). *J. pharm. Soc., Japan*, **74**, 729–731.
Tutin, F. and Clewer, H. W. B. (1914). *J. chem. Soc.*, **105**, 559–576.
Tuzson, P. and Kiss, Z. (1957). *Acta chim. Acad. Sci. hungar.*, **12**, 31–34.
Uffer, A. (1956). *Helv. chim. Acta*, **39**, 1834–1843.
Uhle, F. C. (1951). *J. Amer. chem. Soc.*, **73**, 883.
Uhle, F. C. (1954). *Ibid.*, **76**, 4245–4246.
Uhle, F. C. and Jacobs, W. A. (1945). *J. biol. Chem.*, **160**, 243–248.
Vejdelek, Z. J., Macek, K. and Kahac, B. (1956). *Coll. Trav. chim. Tchécosl.*, **21**, 995–1002.
Vick, R. L. and Kahn, J. B. (1957). *J. Pharmacol.*, **121**, 389–401.
Von Bezold, A. and Hirt, L. (1867). *Untersuch. physiol. Lab., Wurzburg*, **1**, 73.
Walton, R. R. (1945). *J. econ. Ent.*, **38**, 713–714.
Walton, R. R. (1946). *Ibid.*, **39**, 273.
Wang, S. C., Ngai, S. H. and Grossman, R. G. (1955). *J. Pharmacol.*, **113**, 100–114.
Watt, J. M., Heimann, H. L. and Epstein, E. (1932). *Quart. J. Pharm.*, **5**, 649–656.
Weill, J. (1913). *C.R. Soc. biol., Paris*, **74**, 1014–1015.
Weisenborn, F. L. and Bolger, J. W. (1954). *J. Amer. chem. Soc.*, **76**, 5543–5544.
Weisenborn, F. L., Bolger, J. W., Rosen, D. B., Mann, L. T., Johnson, L. and Holmes, H. L. (1954). *Ibid.*, **76**, 1792–1795.
Weisenborn, F. L. and Burn, D. (1953). *Ibid.*, **75**, 259–262.
Willimott, S. G. (1933). *Analyst*, **58**, 431–439.
Wintersteiner, O. (1953). *Record. Chem. Prog.*, **14**, 19–34.
Wood, H. C. (1906). *J. Amer. med. Ass.*, **47**, 2061–2065.
Wu, Y. H. (1944). *J. Amer. chem. Soc.*, **66**, 1778–1780.
Yunusov, S. Y., Konovalova, R. A. and Orekhov, A. (1939). *J. gen. Chem., U.R.S.S.*, **9**, 1911.
Zderic, J. A., Carpio, H. and Limon, D. C. (1962). *J. org. Chem.*, 1125–1129.
Zolotukhina, E. S. (1944). *Farmakol. i Toksikol.*, **7**, No. 6, 51–58.
Zolotukhina, E. S. (1945). *Ibid.*, **8**, No. 6, 15–21.

APPENDIX I

Addendum

I



After publication of 'Biological Activity in Steroids Possessing Nitrogen Atoms' several important publications having bearing on the subject came to our notice. Foremost amongst these were the proceedings of a symposium on the chemistry and biochemistry of the solanum alkaloids[1] held in Berlin in 1959 and published in 1961, a copy of the publication being given to us by Dr. K. Schreiber to whom we are greatly indebted for this courtesy. At this symposium it was reported[2] that the alkaloid solasodamine (Table IV in review) is in fact solasonine monohydrate whilst the alkaloid solauricine is a mixture of solasonine and solamargine. The alkamine 'solauricidine' was concluded to be a complex formed solasodine and solasodine galactoside.[2]

Other papers delivered at the symposium included a report of the action of solanum alkaloids on the chemoreceptors of Leptinotarsa decemlineata Say,[3] a comprehensive review of the distribution of the solanum alkaloids[4] and a survey of the medicinal applications of the extra-European Solanum species, mainly amongst primitive peoples.[5]

Further publications which were overlooked in the review were those by Schreiber and Ripperger[6] in which the constitution of the alkamine solanocapsine (Table IV in review) was shown to be 3β-amino-22,26-imino-16β,23-oxido-5α,22α,23β,25α-cholestan-23-ol (I), thus being a deviation from the usual spiro structure.

In the field of synthetic nitrogenous steroids further thiazolo steroids[7] and some [2,3d] triazolo steroids[8] have been synthesised as analogues of the anabolic steroidal [3,2c] pyrazoles. Several [17,16c] pyrazolino steroids[9] have also been prepared as have several steroids possessing a quinoline ring system fused to ring D.[10] These latter compounds were prepared as potential antitumour agents in view of the earlier report[11] that another steroido-quinoline possessed haemolytic properties. Syntheses of a number of cyano steroid hormone derivatives have been reported[12] and lactam isosteres of the spiro-lactone aldosterone antagonists have been prepared.[13] Application of the α-amino-nitrile synthesis to the preparation of a number of 17-alkyl-17-dimethylamino steroids gave a new oestrogenic compound, 17β-N,N-dimethylamino-17α-methyl-3-methoxyoestra-1,3,5-triene.[14] Further work with steroidal nitrogen mustards has resulted in the synthesis of the 2-[bis(2-chloroethyl)-aminomethyl] derivative of oestrone.[15]

The great interest in new nitrogenous steroids as potential therapeutic agents may be expected to be maintained for some time. The successful development of photochemical methods for the synthesis of nitrogenous steroids[16] can be expected to widen interest still further, as will the demonstration that steroids can be amidated by microorganisms.[17]

References.

1. 'Chemie und Biochemie der Solanum-Alkaloide', Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin, Tagungsberichte Nr. 27, 1961.

2. Briggs, ref.1 p.37.

3. Sturckow, ref.1 p.17

4. Schreiber, Hammer, Ithal, Ripperger, Rudolph and Weissenborn, ref.1, p.47.

5. Stopp, ref. 1, p.255.

6. Schreiber and Ripperger, Experientia, 1960, 16, 536; Schreiber and Ripperger, Tetrahedron Letters, 1960, No.27, 9.

7. Doörenbos and Dorn, J. Pharm. Sci., 1962, 51, 414.

8. Nathansohn,Testa and Di Mola, Experientia, 1962, 18, 57.

9. Moore, Holton and Wittle, J. Amer. Chem. Soc., 1962, 84, 390.

10. Hassner and Haddadin, J. Org. Chem., 1962, 27, 1911.

11. Buu-Hoi and Cagniant, Ber. Dtsch. Chem. Ges., 1944, 77, 118.

12. Kissman, Hoffman and Weiss, J. Org. Chem., 1962, 27, 3168

13. Nysted and Burtner, J. Org. Chem., 1962, 27, 3175.

14. Lednicer and Babcock, J. Org. Chem., 1962, 27, 2541.

15. Pettit and Das Gupta, Chem. and Ind., 1962, 1016.

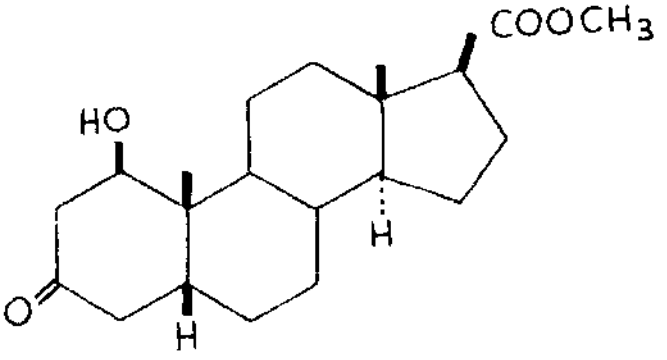
16. Barton and Beaton, J. Amer. Chem. Soc., 1960, 82, 2641; Barton, Beaton, Geller and Pechet, ibid., p.2640; Nussbaum, Carlon, Oliveto, Townley, Kabasakalion and Barton, ibid., p.2973; Nussbaum, Carlon, Oliveto, Townley, Kabasakalion

and Barton, Tetrahedron, 1962, 18, 373; Nussbaum and Robinson, ibid., 1962, 17, 35.

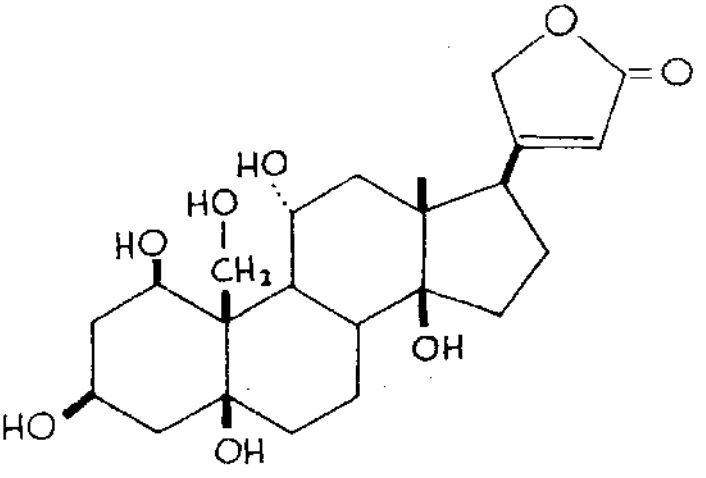
17. Smith, Marx, Mendelsohn, Foell and Goodman, J. Amer. Chem. Soc., 1962, 84, 1265.

APPENDIX II

Attempts to secure Acovenoside A from the trunk wood of Acocanthera longiflora Stapf



I



II

Preparation of 1-oxygenated steroids suitable for conversion into 1α-amino steroids is usually a somewhat tedious procedure[1] although microbiological methods of 1-hydroxylation are available[2] and recently a new chemical method for introducing an oxygen substituent at the 1-position has been developed.[3] In order, therefore, to secure a suitable starting material which would permit the inclusion of certain 1α-amino steroids in the study of steroidal bisquaternary ammonium salts, it was decided to ascertain whether the trunk wood of Acocanthera longiflora Stapf. would prove to be a suitable source of the 1-hydroxy compound acovenoside A (XV, p.20). This cardiac glycoside is known to be present in the seeds and bark[4] of Acocanthera longiflora, and it was felt that it might also be present in the trunk wood since extracts of the wood are used by natives of the drier and cooler regions of East Africa as potent arrow poisons. Large quantities of the trunk wood were kindly made available by Mr. J. J. Lewis. Application of the established[5] conversion of acovenosigenin A (the aglycone of acovenoside A) into 17β-carbomethoxy-1β-hydroxy-3-oxo-5β-androstane (I) would then give a compound capable of conversion (e.g. by application of the Hunsdiecker reaction[6]) into an androstane derivative with oxygen functions at C-1 and C-17.

Another possible favourable aglycone for this type of approach would be ouabagenin (II)[7] although the number of hydroxyl groups in this compound would give additional complications.

The trunk wood of Acocanthera longiflora Stapf. was finely ground for the following work by British Dyewoods Ltd., to whom the author wishes to express his sincere appreciation.

During preliminary investigations it was found that water was a suitable solvent for the large scale extraction of the total bitter glycosidic material from the wood. Subsequent extraction of the wood with ethanol or chloroform failed to achieve the isolation of any further cardiac glycosides (as evidenced by paper chromatography) and so it was concluded that all such compounds were extractable by the water. Ultraviolet spectrophotometric determinations indicated the presence of aromatic components in the ethanol and chloroform extracts. The aqueous extracts of the Acocanthera wood were very bitter in taste and showed effects on the isolated rabbit heart muscle preparation typical of the digitalis-like glycosides.* The U.V. spectra of the bitter principle in ethanol showed absorption $\lambda_{max.}$ = 214mμ.

---

* The author wishes to thank Mr. J. J. Lewis for arranging the biological testing of various materials during these investigations.

characteristic of the carenolide αβ-unsaturated lactone system.[8]

Application of the reported[4] procedure for partitioning the glycosidal components present in the seeds and bark of Acocanthera longiflora Stapf into 'chloroform soluble' and 'chloroform/ethanol' (2:1) soluble components failed to give acovenoside A which is readily isolated from the chloroform soluble fraction by direct crystallisation from methanol. It is conceivable that as the woody parts of plants in general contain comparatively low amounts of lipoid soluble substances, the wood under investigation would not yield as a high proportion of chloroform soluble glycosides as the seeds.

Extensive investigations led to an entirely satisfactory procedure for the fractionation of the glycosidal components of the wood of Acocanthera longiflora Stapf. by column chromatography. This is outlined below. The yields which are indicated in brackets are based on the total dry weight of the wood.

Extraction: The powdered wood of Acocanthera longiflora Stapf. (8 kg.) was soaked in water (18 l.) for three days at room temperature under a layer of xylene to prevent growth of moulds. The aqueous extract was removed by suction and the insoluble residue was successively extracted with water (10 l.) three times to yield 40 l. of total aqueous extract. The residual wood at

this stage was completely free from any bitter taste. The combined aqueous extracts were concentrated to a semi-solid (1 l.) in a climbing film evaporator by repeated recyclisation. The concentrated extract (1 l.) was diluted with methanol (3 l.) and the insoluble cream coloured non-bitter residue was removed by filtration. The methanolic filtrate was distilled *in vacuo* to yield a hygroscopic solid (320 g. 4%). The paper partition chromatography described by Miyatake *et al.*[9] for cardiac glycosides of *Digitalis purpurea* L., when applied to the crude extract of Acocanthera, showed the presence of four components. The paper chromatogram was developed using water-saturated methyl ethyl ketone as the moving phase and water-saturated chromatographic filter paper (Whatman) as the stationary phase. The chromatogram was developed for 8 hr. dried, sprayed with a solution of antimony trichloride (20% in $CHCl_3$), heated at 70°C for 3 min. and the components spotted under an ultraviolet lamp.

Separation of The Crude Glycosidic Components by Column Chromatography: The methanolic extract (320 g.) was incorporated in alumina (grade V, 600 g.) with the aid of methanol and dried *in vacuo* at 40°C. The dried 'alumina/extract' mixture was poured into a glass chromatographic column containing butanol.

Elution with butanol (2 l.) gave a cream coloured, slightly hygroscopic, strongly bitter crude glycoside (160 g. 2%) showing the presence of four substances on paper chromatography. The R_F values corresponded exactly to those of the four components of the crude extract.

Chromatographic Purification of The Glycosides: The crude glycosides obtained above (160 g.) were chromatographed over alumina (grade V 1kg.) using butanol saturated with water as solvent. The first fraction (400 ml. of eluant) gave an amber coloured viscous material which was freely soluble in chloroform. The next fraction (200 ml. of eluant) gave a semi-solid which was partially soluble in chloroform (total chloroform soluble fraction, 24 g. 0.3%). This fraction did not give any spots on paper chromatography, all the material moving with the solvent front. The last eluted fractions (1.5 l.) gave a white, non-hygroscopic, strongly bitter chloroform insoluble glycosidic component (64 g. 0.8%) showing four components on paper chromatography - identical with those from the crude glycosides. The chloroform soluble fraction did not yield any light petroleum soluble material.

Isolation of Cardenolide 'A': The chloroform soluble material (6 g.) was taken in methanol (20 ml.) and allowed to stand at

room temperature for 24 hr. The white needles, which separated, were removed by filtration (ca. 0.9 g.) m.p. 158-160°C. The mother liquor on standing at room temperature for 7 days gave 0.1 g. more of the same product, m.p. 158-160°C. (combined yield 1.0 g. 0.05%). Recrystallised from methanol, rods, m.p. 159-160°, $[\alpha]_D^{24}$ - 64.0° (C = 0.92 in acetone) (Found: C,62.16, 62.29; H,8.02, 8.65; Methoxyl, 4.70, 4.67. Calculated for Acovenoside A ($C_{30}H_{46}O_9$): C,65.45; H,8.36; Methoxyl, 5.45%) ($C_{30}H_{46}O_{11}$ requires: C,61.84; H,7.96%). $\lambda_{max.}$ 217mμ [calculated for $C_{30}H_{46}O_{11}$) ϵ = 18,600].

The melting point of acovenoside A is reported[4,10] as 223-225°C (from methanol) and double m.p. 160-163:230-232°C (from methanol/ether or from acetone/ether). No double m.p. was observed for the present product after repeated attempts employing various solvent systems for recrystallisation.

The mother liquor when left at room temperature in the open air, afforded several small crops of mixed crystalline materials over a period of 6 months which appeared to be mixtures (indistinct m.p.s).

Acetate of Cardenolide 'A': The glycoside of m.p. 159-160° (32 mg.) was dissolved in pyridine (0.5 ml.) and treated with acetic anhydride (0.3 ml.) at room temperature. After 40 hr. the mixture was diluted with water to yield white needles, m.p.

227-229°C.

Chloroform Insoluble Material: All attempts to obtain crystalline products from this material proved unsuccessful. A solution of the material (500 mg.) in pyridine (5 ml.)was treated with acetic anhydride (3 ml.) and allowed to stand at room temperature for 48 hr. The reaction mixture on dilution with water gave a cream coloured precipitate which was removed by filtration and recrystallised from ethanol, needles (420 mg.) m.p. 289-299°C decomp. (reported[4] for acolongifloroside-k-acetate 288-294°C decomp.).

Table I shows the proportion of total glycosides and glycosidic fractions, on a solubility basis, of the trunk wood compared with those of the root bark, the wood bark and the seeds as described by Reichstein et al.[4] The non-glycosidal, light petroleum soluble fraction is also included. Table II lists the cardiac glycosides isolated from Acocanthera species together with their m.p.s and $[\alpha]_D$s.

TABLE I.

Fractions	Trunk Wood	Wood Bark	Root Bark	Seeds
Chloroform soluble	0.3%	1.22%	1.16%	2.5%
Chloroform insoluble	0.8%	0.446%	0.52%	2.1%
Total glycosides	1.1%	1.67%	1.66%	4.6%
Acovenoside A	-	0.44%	0.22%	1.2%
Acolongifloroside k	(0.8%)	0.01%	not reported	0.6%
Light petroleum soluble	-	0.47%	0.32%	not reported

TABLE II

Cardiac Glycosides of Acocanthera Species.

	Glycoside	M.p. °C	$[\alpha]_D$	Refs.
1.	Acovenoside A	222-223	-64.8 (Dioxane)	10
2.	Acovenoside B	251-253	-71.4 (Dioxane)	10
3.	Acovenoside C	188-190	-63 (Methanol)	11
4.	Acolongifloraside E	257-260	-36 (Methanol)	4
5.	Acolongifloroside G	264-268	-24 (Methanol)	4
6.	Acolongifloraside H	230-235	-67 (Methanol)	4
7.	Acolongifloroside J	(158-160) (260-280)	-69.7 (Methanol)	4
8.	Acolongifloroside K	294-302 acetate	-54.1 (Methanol)	4
9.	Acofrioside L	248-253	-54.1 (Methanol)	12
10.	Acofrioside M	185-224	-54.5 (Methanol)	12
11	Ouabain	183-187	-44.3 (Methanol)	12

The paper chromatographic method of separation described by Miyatake et al.[9] for Digitalis glycosides was not satisfactory when applied to the chloroform soluble glycosides of Acocanthera longiflora, but it could be useful for the chloroform insoluble glycosides, although in the present work, the components shown to be present by the paper chromatography did not appear to be separable on column chromatography.

References Appendix II

1. See for example Shoppee, Roy and Goodrich, J. Chem. Soc., 1961 1583; Striebel and Tamm, Helv.chim. Acta, 1954, 37, 1094; Plattner, Furst and Els, ibid., 1954, 37, 1399; Tamm and Albrecht, ibid., 1960, 43, 768; Henbest and Wilson, J. Chem. Soc., 1956, 3289; Albrecht and Tamm, Helv. chim. Acta, 1957, 40, 2216.
2. Dodson, Kraychy, Nicholson and Mizuba, J. Org. Chem., 1962, 27, 3159; Dodson, Goldkamp and Muir, J. Amer. Chem. Soc., 1957, 79, 3921; ibid., 1960, 80, 4026; McAleer, Kozlowski, Stoudt and Chemerda, J. Org. Chem., 1958, 23, 508; Greenspan, Schaffner, Charney, Herzog and Hershberg, J. Amer. Chem. Soc., 1957, 79, 3922; Nussbaum, Carlon, Gould, Oliveto, Hershberg, Gilmore and Charney, ibid., 1959, 81, 5230.
3. Djerassi, Williams and Berkoz, J. Org. Chem., 1962, 27, 2205.
4. Bally, Mohr and Reichstein, Helv. chim. Acta, 1951, 34, 1740.
5. Schlegel, Tamm and Reichstein, Helv. chim. Acta, 1955, 38, 1013.
6. Hunsdiecker and Hunsdiecker, Ber., 1942, 75B, 291.
7. Turner and Meschino, J. Amer. Chem. Soc., 1956, 78, 5130.
8. Paist, Blout, Uhle and Elderfield, J. Org. Chem., 1941, 6, 273.
9. Miyatake, Okano, Hoji and Miki, Pharm. Bull. Pharm. Soc. Japan, 1957, 5, No.2, 157.
10. Euw and Reichstein, Helv. chim. Acta, 1950, 33, 485.
11. Mohr and Reichstein, Helv. chim. Acta, 1951, 34, 1239.
12. Muhr, Hunger and Reichstein, Helv. chim. Acta, 1954, 37, 403.

SYNTHETIC APPROACHES TO STEROIDAL BISQUATERNARY AMMONIUM COMPOUNDS

SUMMARY

of

A Thesis submitted to the University of Glasgow

for the degree of

Doctor of Philosophy

in the

Faculty of Medicine

by

MOHAMMED ALAUDDIN, B. Pharm.(Panjab)

November, 1962

Experimental Pharmacology,
Institute of Physiology,
The University of Glasgow.

SUMMARY

This thesis is divided into four sections with the addition of two appendices. The first section gives a brief summary of the theoretical justifications for undertaking the research and includes an account of the role of acetylcholine at the neuromuscular synapse and an account of the receptor theory of neuromuscular blockade. The classification of neuromuscular blocking agents is then discussed and a survey given of the two point, multi-point and one point attachment theories. These considerations are followed by a discussion of rigid bisquaternary molecules leading to an outline of the factors influencing the choice of the compounds whose syntheses were to be attempted and an account of the synthetic routes proposed.

The second section of the thesis is a report of the preparation and reduction of a number of methanesulphonates derived from hydroxy 5β- and 5α-cholanes. These studies were deemed necessary in view of the poor yields of p-toluenesulphonates which were encountered in certain instances and indeed it was found that utilisation of methanesulphonyl chloride rather than the more commonly employed p-toluenesulphonyl chloride afforded superior yields of sulphonate esters. In the course of this work it was shown that the material previously reported as 3α,12α-dihydroxy-5β-cholane was in fact a mixture of this compound with 12α,24-dihydroxy-5β-cholane. Selectivity of sulphonation of the hydroxyl groups at positions 3,7,12 and 24 of the cholane skeleton is discussed. Lithium aluminium hydride reduction of the sulphonate esters

was shown to hydrogenolyse the sulphonyloxy group when it is in the equatorial configuration and to generate the parent alcohol when the sulphonyloxy group has the axial configuration. Gas liquid chromatographic analyses of certain complex mixtures of steroidal hydrocarbons are reported.

The third section of the thesis reports the results of a study of the reductions under a variety of conditions, of 12-oximino-5β-cholane and 12-oximino-5α-cholane. In all instances only the 12α-amino compound was obtained and the assignments of configuration are rigorously proved. All attempts to obtain 12β-amino compounds by nucleophilic displacement reactions were unsuccessful.

The fourth section of the thesis records attempts to synthesise various steroidal bisquaternary ammonium compounds.

Appendix I consists of the reprints of a review article entitled, "Biological Activity in Steroids Possessing Nitrogen Atoms", published in two parts and prepared as a general background with which to place the present work in perspective. An addendum indicates progress in the field since the review was published and points out several errata in the review.

Appendix II describes an attempt to isolate acovenoside A, which would have served as a suitable material for the preparation of 1-amino steroids, from the trunk wood of Acocanthera longiflora Stapf. However although present in the seeds and bark of this tree, acovenoside A was not present in the wood. Another hitherto unreported cardenolide glycoside was shown to be present but its constitution was not establishe