DESIGN AND SYNTHESIS OF 5-HYDROXYTRYPTAMINE ANALOGUES

THE PHOTOCHEMISTRY OF CERTAIN STEROIDS

A thesis submitted to the
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Convention for citing references

The conventions used in this thesis are those of the Journal of the Chemical Society (London). Where journal abbreviations do not appear in the "Handbook for Chemical Society Authors," or in recent publications of the Chemical Society, resort has been made to Chemical Abstracts.

SUMMARY

Design and Synthesis of 5-Hydroxytryptamine Analogues.- The primary processes of drug absorption, distribution, and metabolism are outlined, and their possible influence upon attempted correlations between chemical structure and pharmacological action emphasised. Theories concerning the mode of action of drugs and in particular the role of the receptor theory are reviewed. The general pharmacological and physiological properties of 5-hydroxytryptamine and related compounds are also discussed. Current views concerning the role of endogenous 5-hydroxytryptamine and the nature of the 5-hydroxytryptamine receptor are included.

Certain previously reported compounds which may be regarded as "less flexible" analogues of 5-hydroxytryptamine and tryptamine are noted and attempts to prepare other similarly "rigid" analogues are described. Such compounds should prove useful in investigations aimed at determining the structural requirements of the 5-hydroxytryptamine receptor. Routes to 4- and 5-amino-1,3,4,5-tetrahydrobenz[c,d]indole and the corresponding 6-hydroxy derivatives, 3-amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole, and 3-amino-7-hydroxy-1,2,3,4-tetrahydrocyclopent[b]indole are investigated, and successful syntheses are recorded.

The Photochemistry of Certain Steroids.- The transformations by which light-sensitive compounds are modified under the influence of ultraviolet light are briefly outlined according to the chromophore responsible for the initial excitation; examples of such transformations are drawn, wherever possible, from previously reported light-induced reactions of pharmacologically and physiologically active molecules.

The photochemistry of cortisone acetate, 11-ketoprogesterone and progesterone in ethanol is reported; the major products isolated from these reactions were the corresponding 5α-dihydro

steroids resulting from photochemically-induced reduction of the $C_{(4,5)}$ double bond. The photochemistry of certain 3-substituted 6-nitrocholest-5-enes is also reported; 3β-chloro-, 3β-acetoxy- and 3β-trifluoroacetoxy-6-nitrocholest-5-enes in ethanol gave, as the major product, cholest-4-ene-3,6-dione-3-oxime. The 3β-hydroxy derivative (6-nitrocholesterol), however, gave no oxime but 3β-hydroxycholest-4-en-6-one and 6β-nitrocholest-4-en-3β-ol as major products. The mechanism of formation of these photo-products is discussed; the nature of the product would appear to depend upon the solvent employed for the irradiation, the $C_{(3)}$ substituent, and the wavelength of the radiation employed.

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THE PHOTOCHEMISTRY OF CERTAIN STEROIDS

INTRODUCTION

The ability of certain chemical substances to exert a variety of effects upon biological systems has been recognised since the beginning of recorded history.[1] In the broadest sense these substances are defined as drugs by the pharmacologist, although this term has various other connotations: to the clinician a drug would be described as a substance having a beneficial effect on man while to the layman the term is generally regarded as describing a substance of addiction. In this thesis a drug is defined, not in its therapeutic sense, but more generally as a chemical substance which, in small quantities, induces an effect upon a biological system.

One of the fundamental aims of the pharmacologist and the medicinal chemist is to seek an explanation of drug action at a molecular level and hence to develop general theories accounting for these actions. However, as yet little is known concerning the relationship between the structure and function at cellular level of the macromolecules such as proteins, lipoproteins, carbohydrates and nucleic acids which form the elements of a biological system and must be involved in drug action. Until such information is available progress will understandably be slow and even then there still remain the inherent difficulties associated with the high complexity of the biological system and the polyfunctional structure of many of the drugs under examination. There is a distinct possibility that other factors, as yet unappreciated, also influence drug action and thereby hamper investigations aimed at correlating the response induced by a drug with its physical and chemical properties.

In any attempt to explain the mechanism of drug action on complex biological systems certain factors must be taken into

consideration; these include the route of administration, absorption by and distribution throughout the body, and the rate of metabolism of the drug. These processes, although not having any ultimate significance in terms of the molecular interaction of the drug with the biological system, can profoundly influence the activity of the drug.

It is now generally accepted that in order to exert its biological effect a drug must gain access to the biophase - that region surrounding the site at which it is believed to act. It is essential therefore that the absorption, distribution and rate of metabolism should be favourable if the drug is to be transported, in an active form, to these sites of action in a concentration sufficient to initiate the processes which are responsible for the observed effect.

DRUG TRANSFERENCE AND METABOLISM

The absorption and distribution of the drug involves its passage across various body membranes; the processes by which this occurs may be grouped into two classes, dependent upon whether the membrane acts in a passive or an active manner.

Passive transfer is characterised by an inert membrane, the rate of drug transfer being proportional to the concentration gradient existing across it. A relatively simple model for this system has been proposed by Danielli and Harvey;[2] the membrane, they suggest, is comprised of a bimolecular lipid layer containing water filled pores and covered with a monomolecular protein layer on each side. More complex structures have also been proposed.[3] Lipid soluble molecules pass through the membrane at a rate proportional to their lipid-water partition coefficient while lipid insoluble molecules simply diffuse through pores at a rate dependent upon their molecular weights, smaller molecules passing more rapidly than larger ones. The buccal, gastric and

intestinal mucosa act as lipoidal barriers,[4] most drugs crossing these by a simple non-ionic diffusion process.

Increased absorption of a poorly absorbed drug is sometimes accomplished by a chemical modification of the drug which increases the lipid-water partition coefficient or decreases ionization. However, the application of such modifications is limited, and the problem is further complicated by the interdependence of the various other factors involved in drug action: the modified drug will almost certainly possess an entirely novel set of physical and chemical parameters which, although allowing increased absorption, may render the drug inactive or prevent it from even reaching its site of action. An interesting extension of drug modification is that described by Harper[5] as drug latentiation in which the drug is chemically modified to confer upon its derivative specific absorption and distribution properties. Regeneration of the active species from administered modified material occurs within the body after the desired absorption and distribution.

Drugs may also traverse membranes by a filtration process[6] when a hydrostatic or osmotic pressure gradient exists across the membrane. Under the influence of the pressure gradient water flows through the membrane pores carrying with it any molecule having dimensions smaller than the pore size.

Some large lipid-insoluble molecules are also known to penetrate certain membranes; to explain this, and also the movement of certain species against a concentration or ionic gradient, the concept of active or specialised transport was introduced. The transfer of a drug from one side of a membrane to the other by this process is thought to involve a complex, comprised of a membrane component (carrier) and the drug molecule, which traverses the membrane and decomposes on reaching the other side to give the unchanged drug

molecule and carrier. The latter may then be enzymatically destroyed, migrate back to its original side of the membrane to repeat the process, or in some instances complex with another molecule which it transports to the first side of the membrane.[7] Such transport systems are present in the gastro-intestinal mucosa for the active absorption of certain natural substrates and provide a means of drug absorption, provided the drug has a similar structure to that of the natural substrate. Thus foreign sugars similar to glucose are absorbed by the saccharide transport process[8] and 5-fluorouracil is absorbed by the natural uracil and thymine transport systems.[9] Active transport processes also operate at many other membrane surfaces: the secretion of hydrogen ions in the stomach[10] and the loss of sodium ions from nerve and muscle cells[11] are both undoubtedly active processes.

There is also evidence that uptake of both large and small molecular weight substances, which might otherwise be excluded from the cell, occurs by yet another mechanism, termed pinocytosis.[12] As a result of invagination the cell membrane engulfs droplets containing the molecules to be absorbed which then pass into the cell interior. The process is similar to phagocytosis in which bacteria are engulfed by amoeboid cells.

Drugs which act upon the central nervous system penetrate the series of membranes which protect the brain and cerebrospinal fluid from foreign molecules; these have become known as the blood-brain and blood-cerebrospinal-fluid barriers, both exhibiting lipid boundary characteristics. The selectivity of these barriers to various molecules has been extensively studied, and the work summarised in a number of recent reviews.[13] Lipid solubility has been shown to be the dominant factor; highly ionized molecules with low lipid solubility penetrate these membranes only slowly, if at all. Certain other substances, however, such as tryptophan,

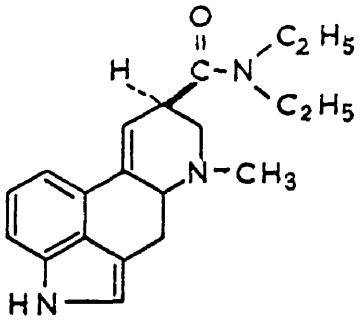
which are not particularly lipid soluble, are thought to enter the brain by active transport processes.[14] In contrast to their passage into the brain, the egress of drugs from the cerebrospinal fluid does not appear to be so dependent upon lipid solubility. Filtration and active transport processes have been proposed to explain this effect.[15]

Although the blood-brain and blood-cerebrospinal-fluid barriers have similar permeability properties to foreign molecules, their anatomical structures are quite different. The blood-cerebrospinal-fluid barrier is thought to consist largely of the epithelium of the choroid plexuses, while the blood-brain barrier appears to be either the brain capillary wall or its surrounding layer of glial cells.

The stability of the drug to metabolic breakdown is of equal importance to its effective transport in influencing the concentration of active material in the biophase, and it is this concentration which is of prime importance in determining the magnitude and duration of the response. The metabolic processes tend to protect the body from toxic compounds by modifying foreign molecules to metabolites, which are generally less active and more easily excreted. Their capacity to fulfil this function, however, is limited; some drugs are not detoxified while certain others are converted into even more toxic products.[16]

Metabolism, or biotransformation, involves one or more enzymes belonging to the six major classifications recognised by the International Union of Biochemistry in 1964.[17] These are numbered as follows: 1 - oxidoreductases (enzymes capable of oxidation or reduction); 2 - transferases (enzymes capable of methylation, acetylation etc.); 3 - hydrolases; 4 - lysases (enzymes capable of removing groups from a substrate by a non-hydrolytic process); 5 - isomerases; 6 - ligases (or synthetases). The process often,

though not always, consists of two phases; the first may be oxidation, reduction or hydrolysis, or a combination of these, followed by the second phase involving enzymes of groups two or six. Conjugation by ligases consists of combination of the first phase product with an amino acid or carbohydrate. Thus, lysergic acid diethylamide (I) in the rat is first hydroxylated, probably in the 12-position, and then conjugated to give the more soluble β-glucuronide (II; R= $-\overline{CH(CHOH)_3CH-COOH}^{O}$) of hydroxylysergic acid and the β-glucuronide (III; R= $-\overline{CH(CHOH)_3CH-COOH}^{O}$) of hydroxyisolysergic acid. These metabolites have been found in the bile excretion.[18]



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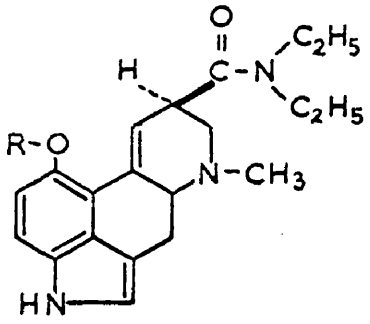

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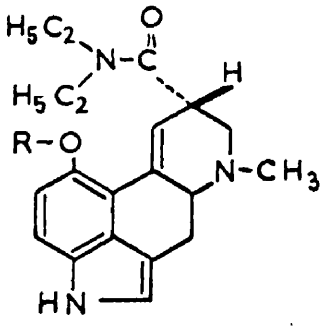


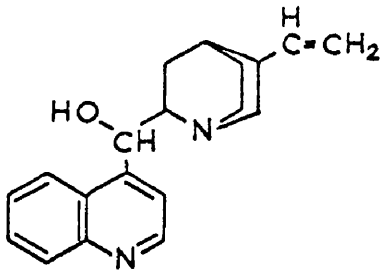
III

Oxidation is the most common initial metabolic process; alcohols are oxidised to aldehydes and acids, hydrocarbon moieties are hydroxylated and cyclic structures oxidatively cleaved. Other oxidation reactions include oxidative deamination, dehalogenation and dealkylation, while some compounds are oxidised as far as carbon dioxide. Reductions are less common; aldehydes and ketones are reduced in the body to alcohols, nitro-compounds to amines, often via hydroxylamine intermediates, but the saturation of carbon-carbon double bonds appears confined to those compounds in which this function is conjugated, generally with a carbonyl group. Hydrolysis is a commonly observed process during metabolism, esters and amides foreign to the body being cleaved by enzymes of

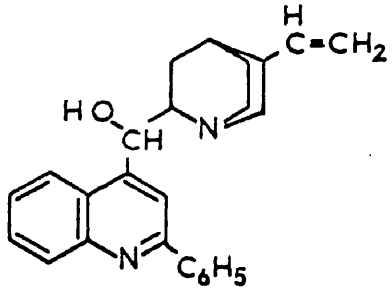
the blood plasma and cells. Hydrolysis is often responsible for the reactivation, by removal of the protecting group, of certain drugs modified to increase absorption or facilitate distribution (see page 3).

The metabolite from the first phase and the species of the animal determine which type of conjugation reaction is involved in the second phase: phenylacetic acid[19] is conjugated in man with glutamine whereas in most other animals, except in the hen, it conjugates with glycerine and glucuronic acid. In the hen ornithine is the conjugating agent.

Metabolic breakdown of a drug is initiated by enzymatic attack. Substitution at, or close to, the centres known to be involved in these reactions has been employed in certain instances to produce drugs having an increased resistance to metabolic decomposition. Partial protection of cinchonine (IV), which is metabolised by



IV



V

oxidation initially at the 2'-position, has been achieved by 2'-phenylation;[20] 2'-phenylcinchonine (V) has twice the antiplasmodial activity of quinine.

STRUCTURAL SPECIFICITY

The study of drug action has led to the division of compounds exhibiting biological activity into two general classes:[21] those whose biological activity is profoundly altered by minor

changes in their chemical structure are referred to as structurally specific drugs, and those which possess widely diverse chemical structures but induce similar biological responses, as structurally non-specific drugs. It has been suggested that these two classes, in their ideal form, represent two extremes lying at either end of a broad spectrum of drug activity,[22] so that in practice one can envisage all drugs as possessing both structurally specific and non-specific properties to varying degrees and a drug will be classed according to which of these properties predominate.

Structurally specific drugs are generally regarded as acting at a particular cellular reaction site which has been termed the receptor. Structurally non-specific drugs, however, are thought to act rather more indiscriminately, certain physical properties appearing to be more important than their precise chemical structure.

This view was inherent in the work of Clark[23] who introduced evidence for two general types of drug action by estimating the possible cell surface covered by a number of substances. His results showed that certain drugs were able to induce an effect with low cell surface coverage while it appeared that others required a complete, or near complete, monolayer over the cell surface. Clark proposed therefore that certain drugs act by some physical, or physicochemical process while others affect only certain areas of the cells.

Structurally non-specific drugs.

The biological activity associated with structurally non-specific drugs is primarily dependent upon their possessing favourable physical properties. The most important group of drugs which are regarded as acting structurally non-specifically are the general anaesthetics and much research in this field has been directed towards elucidating their mode of action. Attempts have

been made to correlate this activity with vapour pressure,[24] boiling point,[25] distribution coefficient between aqueous and non-aqueous phases,[26] and ability to lower surface tension.[27] More recently the ability to form clathrate crystals,[28] or to induce a higher degree of order in the surrounding water molecules[29] has been proposed to account for their pharmacological properties.

The physico-chemical theories currently of importance may be divided into two groups: those concerned with distribution between lipid and aqueous phases, first introduced by Meyer and Overton,[26] and those based upon the ability of the drug to order the surrounding water molecules of the aqueous phase, proposed independently by Pauling[28] and Miller.[29] Support for the aqueous phase theories is furnished by evidence that xenon acts as a general anaesthetic;[30] this gas, although chemically very unreactive does, like many other gaseous anaesthetics form clathrate crystals.

The original Meyer-Overton hypothesis has been modified by Ferguson,[31] and Brink and Postemak[32] who substituted activities for concentrations, and extended by Mullins[33] who suggested molecular size to be a criterion for activity.

The more recent theories of Pauling and Miller extend the description of drug action; they attempt to describe the mechanism by which the drug initiates the processes which lead to anaesthesia rather than merely correlating the activity with some physical property of the drug. Pauling envisages the formation of gas hydrate microcrystals in the "encephalonic fluid" stabilised by the inclusion of electrically charged protein side chains and ions within the cage-like structure. The resulting restriction on the movement of the charged species in the "encephalonic fluid," it is suggested, causes the failure of certain central processes which

lead to anaesthesia.

Miller's theory of anaesthesia is essentially the same as that of Pauling but eliminates the thermodynamic unfavourability of the hydrate microcrystal; the phase surrounding the gas molecule is therefore described as "more ordered water" rather than a true crystal. The build up of ordered water, or "ice-cover," as it is termed, at a membrane surface, is thought to affect the nerve conduction and transmission resulting in anaesthesia.

Discrimination between the lipid solubility and "ice-crystal" theories is difficult as any correlation between anaesthetic activity and a property dependent upon the intermolecular interaction energy between the anaesthetic agent and other molecules would give equally satisfactory results.[28]

However, more recent work using fluorinated alkanes,[34] which in solution exhibit large positive deviations from ideal solubility behaviour, is claimed to provide no evidence of a physico-chemical nature that the aqueous phase of the central nervous system is the site of action of these anaesthetics. The critical phase is considered to be non-aqueous.

Structurally specific drugs.

Structurally specific drugs are generally considered as complexing with certain cellular receptors as a direct consequence of certain structural and chemical features of both the drug and its receptor. The agonist is thought to have a complementary structure to that of the receptor, and owing to the relatively weak forces which are generally involved a very close fit between drug and receptor is essential for complex formation. The drug receptor has been described by Schueler[35] as "a pattern R of forces of diverse origin forming a part of some biological system and having roughly the same

dimensions as a certain pattern M of forces represented by the drug molecule, such that between patterns M and R a relationship of complementarity for interaction exists." This definition reflects the lack of detailed information available concerning the nature of receptors. Although there have been claims for the isolation of certain receptors,[36] these have not been fully substantiated.

Direct examination of receptors is not possible at this time, and conclusions concerning their structures are frequently deduced indirectly from structure-action studies of the drugs which are believed to complex with these receptors. Such studies have led to the pictorial representation of the nicotinic[37] and muscarinic[38] receptors for acetylcholine, the analgesic receptor,[39] and the receptors for adrenaline,[40] thyroid hormones,[41] 5-hydroxytryptamine[42] and certain steroids.[43]

The primary interaction between the drug and the cell is at the external cell surface and although this surface acts as the receptor for only certain drugs, a knowledge of its chemical nature is important in any attempt to elucidate drug-receptor interactions. Drugs which act intracellularly are thought to do so by interfering in some way with enzyme activity.

Structural studies on certain cells[44] indicate that their external surface consists of a matrix of protein molecules linked with mucopolysaccharide molecules. The protein matrix presents a variable surface of possible bonding sites: non-polar areas consisting of aliphatic side chains and aromatic nuclei, while hydroxyl groups, amides, unionized carboxylic acids and amines comprise the polar area of the surface capable of hydrogen bonding. Protonated amino groups and negatively charged dissociated acidic functions of the protein material also present possible binding sites. The mucopolysaccharides, however, present a more uniform

surface of negatively charged groups surrounded by solvated hydroxyl groups.

Drug receptor bonds are generally weak giving a transient, easily reversed complex. Certain drugs, however, form the considerably stronger covalent bonds with the tissues, resulting in the formation of complexes which are only slowly reversed in the body. Such drugs include the β-haloalkylamine adrenergic blocking agents,[45] the fluorophosphate anticholinesterases,[46] and the alkylating anticancer drugs.[47] Contributions to the total binding energy of the more common easily reversed complexes are thought to arise from hydrophobic bonds, van der Waals forces, electrostatic attractions and hydrogen bonding.

The energy associated with hydrophobic bonding,[48] which arises from the transfer of a non-polar molecule, or part of a molecule, from a polar phase to a non-polar environment, is thought to account for a significant fraction of the total binding energy of certain molecules to their receptors. The energy gain is thought to originate from the entropy change associated with the dissolution of ordered water molecules which takes place when the drug is transfered from the aqueous phase to the less polar environment of the receptor.

Van der Waals forces, due to their low magnitude and high distance specificity, are generally regarded as a secondary component of the total binding energy between the neutral hydrocarbon portion of the molecule and the receptor. When a very close fit between the drug and its receptor is achieved, however, these forces do appear to become more significant, particularly for larger molecules such as long-chain fatty acids and steroids.[49]

Electrostatic interactions between oppositely charged groups

on the drug and the receptor also form a significant contribution to the stability of the complex. Ion-ion interactions are the strongest and therefore the most important, but, in decreasing interaction magnitude, ion-dipole, dipole-dipole, and ion-induced dipole interactions also contribute to the total binding energy. Association of oppositely charged ions (ion-pair formation) does not tend to occur in aqueous solutions of high dilution as the high dielectric constant of the water and the high degree of solvation of the ions make it energetically unfavourable. Macromolecules, such as proteins, however, alter this simple situation by reducing the dielectric constant in the immediate vicinity of their surface making ion-pair formation more favourable.[50]

The contribution made by hydrogen bonding to the stability of the complex appears to be in dispute.[51] Although this form of bonding is almost certainly important in some drug-receptor interactions, its contribution may not be as significant as has been earlier suggested. In general, groups which are capable of hydrogen bonding will be solvated and exchange of these hydrogen bonds with water for those with the receptor material will result in only a small, if any, energy gain. This is illustrated by the weak binding between polyhydroxy compounds, such as carbohydrates, and protein material.[52]

The receptor.

The concept of a drug receptor was first introduced by Erlich,[53] and is inherent in the work of Langley[54] and Lucas,[55] and the classic lock and key analogy advanced by Fischer[56] to explain enzyme specificity.

Important quantitative studies of drug action in relation to the receptor theory were made by Clark[23] who proposed a simple model based on the laws of the Langmuir adsorption isotherm which made the fundamental assumption that the response of a tissue was

proportional to the fraction of the receptors occupied by the drug. Gaddum[57] extended this theory to account for the effects of specific antagonists. It was later observed that certain drugs, which themselves induce a small response, were capable of blocking the effects of other agonists. It has also been shown that the maximum response induced by different drugs varies. These observations indicate that the fraction of receptors occupied by the drug is not the only criterion which determines the magnitude of the response. To overcome these difficulties Clark's original theory was modified by Ariens,[58] who introduced the term intrinsic activity, and by Stephenson,[59] the term efficacy. Thus two parameters govern drug action: the affinity of the drug for the receptor, and the intrinsic activity, or efficacy, which measures the ability of the drug to elicit a response.

Ariens defined intrinsic activity as a "substance-constant determining the effect per unit pharmacon-receptor complex." Thus he originally retained Clark's assumption that the response was proportional to the fraction of receptors occupied, but extended the theory so that a complex formed by a receptor with one agonist might differ from the complex with another agonist in its ability to contribute to the response. More recently, however, this theory has been modified still further so that a maximum response could be obtained without assuming total occupancy of the receptors (see also Nickerson[60]). To give a more pictorial meaning to this parameter, Ariens[61] has drawn an analogy between intrinsic activity and the rate constant of the rate-limiting step of an enzyme reaction. It has also been proposed that the intrinsic activity may represent the fraction of effective collisions between the drug and its receptor.[62] The term efficacy differed from intrinsic activity in that it did not retain the assumption that the response is proportional to the fraction of the receptors occupied. Stephenson

postulated that provided a molecule possessed a high efficacy, a maximum response could be obtained with only a fraction of the receptors occupied. Efficacy is inversly proportional to the number of receptors that have to be activated in order to induce a certain stimulus and promote a certain effect. Although the efficacy and intrinsic activity are essentially the same, the quantitative differences in the two approaches arise from differences in the mathematical assumptions concerning the relationship between the stimulus and the response it induces. Furchgott[63] has proposed the hybrid parameter intrinsic efficacy to measure the ability of a drug to initiate a response.

Recent reviews by Mackay have summarised and compared the various mathematical analyses of drug-receptor interactions,[64] and Burgen[65] has attempted to define a theoretical basis for drug receptor kinetics.

A different approach to the problem of drug-receptor interactions has been made by Paton[66] who proposed an analysis of drug action based on the assumption that the response to an agonist is proportional, not to the fraction of receptors occupied, but to the rate of formation and decomposition of the drug-receptor complex. Thus the effect is the result of a number of quantal events rather than that of a persisting drug-receptor complex. The concept that the drug is only effective at the moment of encounter with the receptor was first introduced by Croxatto and Huidobro.[67] An agonist is described as a substance which promotes a high rate of complex formation and dissociation, while an antagonist forms a complex with the receptor which dissociates only slowly. The partial agonist-receptor interaction represents an intermediate state.

One of the attractions of the rate theory, as it is termed, is

the way in which it accounts for many of the observations concerning drug action which appear to lack all significance in terms of the occupation theories. Such observations include the existence of spare receptors; although an appendage to the occupation theories, these are a necessity in explaining drug action in terms of the rate theory. The non-existence of persistent stimulants, quickly reversible potent antagonists, or compounds possessing both a high affinity and high intrinsic activity are also accounted for by the rate theory, but remain as "extras" to the occupation theories.

In mathematical terms the two types of approach take a similar form; the stimulation and intrinsic activity terms of Ariens' basic equation describing drug action being replaced by the rate of association and the rate of dissociation of the drug-receptor complex respectively. Due to the similar predictions concerning the situation at receptor level at equilibrium, discrimination between the two theories can only be attempted under non-equilibrium conditions, that is at the onset and offset of action. In contrast to the occupation theories, which predict a smoothly rising response to the agonist, the rate theory predicts fade of the initial response as receptor occupancy increases. The problems associated with the detection of fade, however, are many; in fact, it may not be observed at all if the response is delayed due to the nature of the tissue, or due to a low rate of diffusion of the drug to the receptor. The situation is further complicated by the necessity of an appreciable receptor occupancy before fade becomes detectable. For these reasons, although Paton has obtained positive evidence for fade, no definite conclusions can be drawn concerning the general validity of the rate theory. Even Paton's evidence has been questioned by Furchgott,[68] who suggests that other factors, such as an unspecific

non-competetive antagonism by the drug after the stimulatory action, may be responsible for the observed fade in the response.

Another similar theory, the dissociation theory, has been tentatively suggested by Paton;[69] an agonist is envisaged as reacting with and destabilising the receptor with accompanying breakdown of the latter and the consequent expulsion of the agonist. This is in agreement with the suggestion by Gill[70] that acetylcholine acts by interfering with the stability of the helical structure of the protein constituting the cell membrane. Although this theory, like the rate theory, envisages stimulation as a quantal process it will be indistinguishable from the occupation theories by response observations because the stimulation is dependent upon the rate of dissociation which in turn is proportional to the occupancy.

A most significant addition to the theories of drug-receptor interaction is that proposed by Belleau[71,72] and based on enzymatic-substrate interactions. The theory, although ingenious and convincing, due to the lack of direct evidence cannot be considered as indisputably established. Nevertheless, it does attempt to describe the mechanism of drug action at a molecular level in structural terms, rather than making use of parameters such as intrinsic activity which appear to have little obvious physical significance.

The estimated energy change associated with the drug-receptor interaction led Belleau to the conclusion that on complex formation the receptor underwent a conformational change, or perturbation. Antagonists, he suggests, induce conformational changes which are non-specific in character and as a result do not produce a biological response. Agonists, on the other hand, induce a specific, or possibly a number of related specific,

conformational changes in the receptor material which are capable of initiating the processes which lead to the biological response. The specific conformational change is thought to convert the inactive receptor into a catalytically active state which is capable of modifying a substrate to a product which is essential for production of the response. Support for this thoery is furnished by recent information concerning the basic mechanism of enzyme activation[73] and by the work of Koshland[74] on the similar induced fit theory of enzyme-substrate interactions. Belleau's theory may be symbolised as follows: when a small molecule M_s reacts with the receptor material P it induces a conformational change in the protein and gives an "activated" complex $P''M_s$.

$$M_s + P \rightleftharpoons P''M_s$$

In this form P" is an effective catalyst for the conversion of a substrate A to a product B,

$$P''M_s + A \rightleftharpoons P''M_sA \longrightarrow P''M_s + B$$

B being required to initiate the response. If, however, a small molecule M_i is incapable of bringing about the favourable conformational perturbation of the receptor P but instead produces an unfavourable one, then a complex P^*M_i is formed which is incapable of catalysing the A into B reaction.

$$P + M_i \rightleftharpoons P^*M_i$$

$$P^*M_i + A \rightleftharpoons P^*M_iA \not\longrightarrow P^*M_i + B$$

Partial agonists are substances which are intermediate between M_i and M_s and hence produce an equilibrium mixture of the favourable and unfavourable perturbations. It has been pointed out by Triggle[75] that this perturbation treatment reconciles, in part, at

least, the treatments of drug action by Ariens and Paton. The rate of rearrangement of the protein-like receptor might be considered as determining the intrinsic activity of Ariens, or the rate of dissociation of the complex which is required by Paton's rate theory. If the rate theory is to be explained in this way it is necessary to assume that it is the rearrangement of the receptor which in fact expels the drug molecule.

The general theory outlined above has been extended to describe the nature of, and a possible mechanism of action at, the muscarinic receptor. Investigations were made into the binding characteristics of a series of alkyltrimethylammonium compounds with the active surface of the enzyme, acetylcholinesterase, as a model for the muscarinic receptor. These quaternary ammonium salts present, by elongation of the alkyl side chain, a series of compounds exhibiting the entire spectrum of drug action from agonist, via partial agonist to antagonist, and yet differ physico-chemically only in their hydrophobic character. The use of acetylcholinesterase as the model system was prompted by the similar chain-length dependent transitions which occur in the binding of these compounds with the enzyme and with the muscarinic receptor surface.

Entropy changes associated with enzyme-alkylammonium compound interaction were calculated; these were negative for complexes involving compounds possessing less than eight carbon atoms but became increasingly positive as more methylene groups were introduced into the side-chain. This transition indicates a difference in the degree of order between those complexes formed by the smaller molecules of the series and those formed by compounds having more than eight carbon atoms in the side-chain. This observation, along with the change in pharmacological activity which is known to occur at $C_{(8)}$, has led Belleau to suggest that lower members of the series overlap only with the regulating surface of the receptor and

induce P" (active) states, while those molecules with longer side chains overlap onto the periphery of this surface and induce P** (inactive) states.

The free energies (ΔF) of binding associated with the interaction of this series of compounds with acetylcholinesterase show two chain length dependent transitions, one at $C_{(4)}$ and the second at $C_{(8)}$. These values, when plotted against the number of side-chain carbon atoms (n) showed three separate linear free energy relationships (see fig. 1), indicating the existence of three distinct uniform binding surfaces.

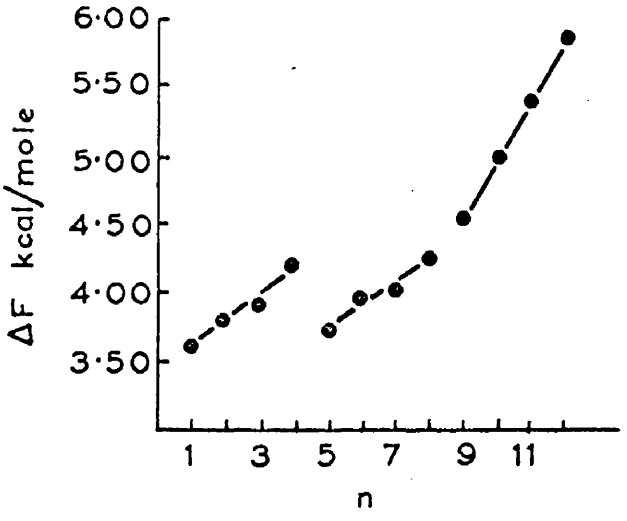


fig. 1

The cholinergic receptor is therefore described as consisting of three compartments, each being on a separate protein chain (see fig. 2). The first includes the anionic site and accommodates side chains up to four carbon atoms in length, the second includes the esteratic site and accommodates side-chain carbon atoms five to eight, and finally the third compartment which is not required for binding acetylcholine accommodates methylene groups in excess of eight. Overlap onto this results in blockade of the receptor. The difference between the nicotinic and muscarinic receptors is

envisaged as a difference in the polarity of the second compartment; the increased polarity in the nicotinic receptor prevents the effective binding of alkyltrimethylammonium compounds with alkyl side-chains of more than four carbon atoms.

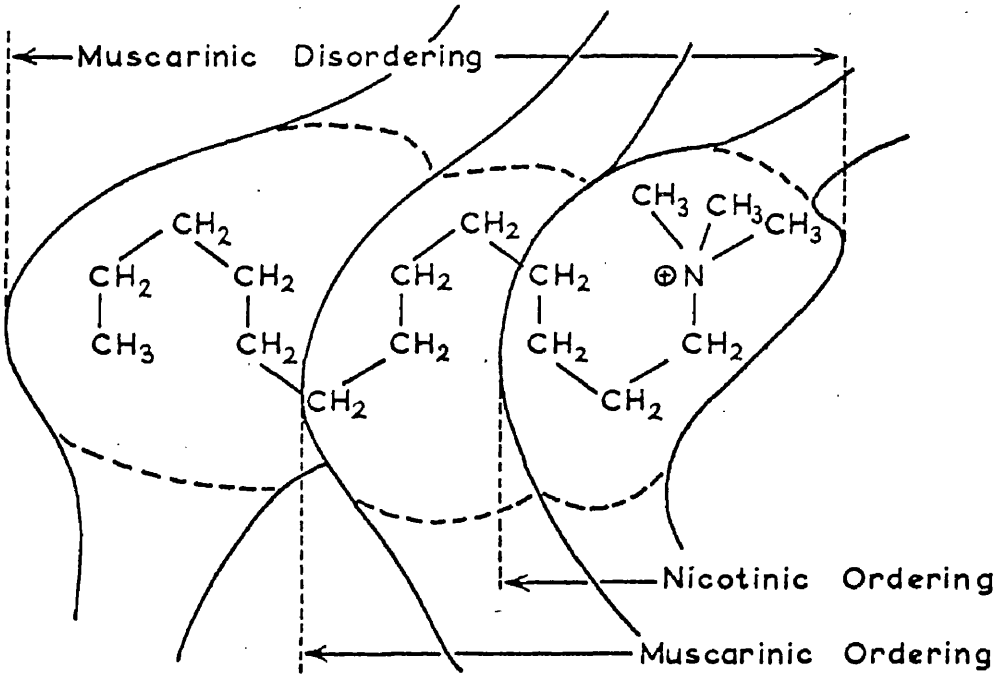
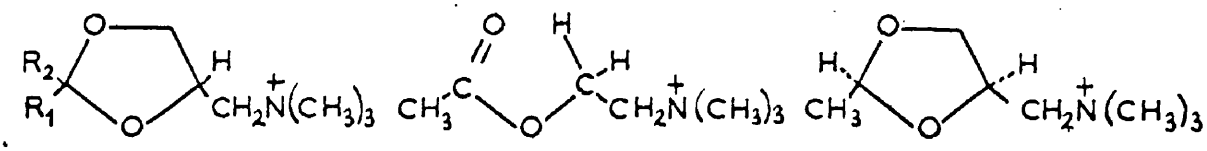


fig. 2

This three compartment hypothesis has been further elaborated to describe the 'uphill' transport of sodium and potassium ions.[72]

Maximum efficiency in the induction of the favourable perturbation (P") is thought to be reserved to those molecules which produce a true lock and key type fit with the receptor. This has been demonstrated by investigating the effects of a series of dioxolane quaternary ammonium compounds (VI), which represent fairly stereochemically rigid structures closely related to acetylcholine (VII), upon acetylcholinesterase and the muscarinic receptor.[76] The cholinergic receptor and acetylcholinesterase display similar patterns of stereospecificity towards the quaternary ammonium compounds. The L-(-) isomer of cis-2-methyl-4-trimethylammoniummethyl-1,3-dioxolane iodide (VIII) was the most

effective muscarinic stimulant and acetylcholinesterase inhibitor of the series. It is therefore postulated to represent the natural orientation of receptor- and enzyme-bound acetylcholine.



VI VII VIII

This isomer was found to possess ten times the affinity for acetylcholinesterase, and a factor of six times the potency of acetylcholine itself. From a consideration of the binding energies of these molecules to the receptor it appears that only in the particular instance of VIII is it necessary to evoke contributions from van der Waals bonding. This suggests that, although other members of the series possess some activity, only VIII, like the natural substrate acetylcholine, forms a lock and key type complex with the receptor. Hydrophobic bonding, it is suggested, is most widely operative for other molecules of the series which are incapable of lock and key type combination.

Whether the molecular perturbation theory accurately, or even partially, describes the elementary processes involved in drug-receptor interactions is not, as yet, fully established. Nevertheless it must be regarded as a significant development in receptor theory as it provides the first detailed account, based upon physico-chemical data, of the nature and operation of the receptive material at a molecular level. Conformational changes in the receptor under the influence of the drug are not unreasonable; it is unlikely that the receptor is a completely rigid entity with a fixed conformation. However, much of the significance, and value, of the study on the muscarinic receptor must hinge on the validity

of interpreting the drug-enzyme results in terms of drug-receptor interactions. The cholinergic receptor and acetylcholinesterase are known to have certain similar characteristics, but how far the analogy may be taken is in some dispute.[77]

Chemical transmission.

The concept of structural specificity has physiological application in the established view that in certain sites within the body the transmission of nervous impulses to the effector organ is achieved by the participation of chemical agents. The concept of chemical or neurohormonal transmission was first applied to the sympathetic branch of the autonomic nervous system, and has subsequently been extended to include all autonomic sites, peripheral voluntary neuro-effector junctions, and more recently, to the central nervous system. Of the compounds postulated to act as chemical transmitters acetylcholine and noradrenaline have been established beyond any reasonable doubt. Other substances which have been proposed to possess a transmitter role include histamine, gaba, substance P, and 5-hydroxytryptamine.

The possibility that 5-hydroxytryptamine may be a transmitter (see ref.99) accounts for much of the interest which has been shown in this substance since its first isolation. However, its role in the body is far from established and much controversy still exists concerning the likelihood of it being a neurohormone. The present situation is briefly outlined later (see p.27). Many excellent reviews[78] dealing with chemical transmission, and in particular the participation of acetylcholine and noradrenaline, have been published in recent years.

5-HYDROXYTRYPTAMINE

5-Hydroxytryptamine (5-HT, serotonin, enteramine) was first isolated by the extraction of mammalian gastro-intestinal mucosa and salivary glands of octopods by Erspamer.[79] Its structure consists of a 5-hydroxylated indole nucleus bearing, in the 3-position, a 2-aminoethyl side chain (XIII). The first chemical synthesis of 5-HT, by Hamlin and Fisher,[80] involved elaborating the side chain of 5-benzyloxygramine (X), obtained from 5-benzyloxyindole (IX) as shown in fig. 3. More recent and improved synthetic routes have been developed.[81]

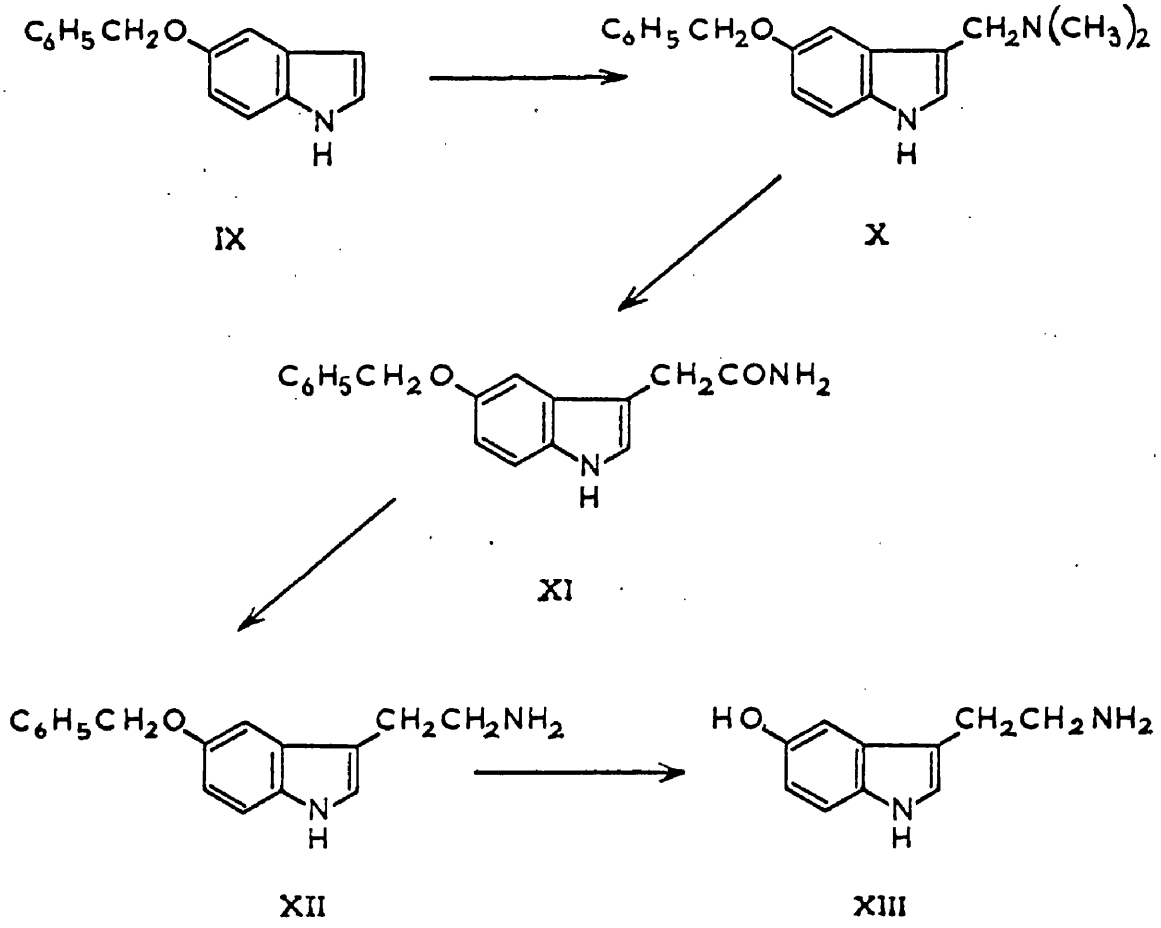


fig. 3

Since its first isolation 5-HT has attracted a great deal of

interest, mainly due to its association with the central nervous system, and much effort has been directed towards elucidating its significance in the body and in particular in the brain; this work has been summarised in a number of reviews.[82-84]

5-HT is widely distributed throughout the body, the most important localisations being in the enterochromaffin cells of the gastro-intestinal tract,[85] where it is synthesised from dietary L-tryptophan, in blood platelets,[86] and in the central nervous system. High concentrations have also been observed in the mast cells of mice and rats.[87] Lower concentrations, which possibly arise from platelet 5-HT, are found in the liver, lungs, kidneys, and in the spleen.

The presence of 5-HT in the central nervous system was first demonstrated by Gaddum and his co-workers,[88] and by Twarog and Page,[89] and since this time it has been shown to be present in all vertebrate brains, in a free and bound form. It is not evenly distributed throughout the brain but is concentrated in certain areas such as the hypothalamus and mid-brain. Although its origin is not completely resolved, most of the evidence indicates that 5-HT is synthesised *in situ* from L-tryptophan, which, unlike tryptamine, does cross the blood brain barrier (by an active transport process[14]). Enzymatic hydroxylation of the tryptophan (XIV) to 5-hydroxy-tryptophan (XV), followed by decarboxylation results in 5-HT (XIII). Its metabolic breakdown proceeds by various routes,[83] the most important of which involves first oxidation by monoamine oxidase to give 5-hydroxyindol-3-ylacetaldehyde (XVI), followed by further oxidation to the easily excreted 5-hydroxyindol-3-ylacetic acid (XVII), as shown in fig. 4. The rate of synthesis and metabolism is high; brain 5-HT has a half-life in the order of 10-30 minutes, measured using monoamine oxidase inhibitors,[90] and may be even less.[91]

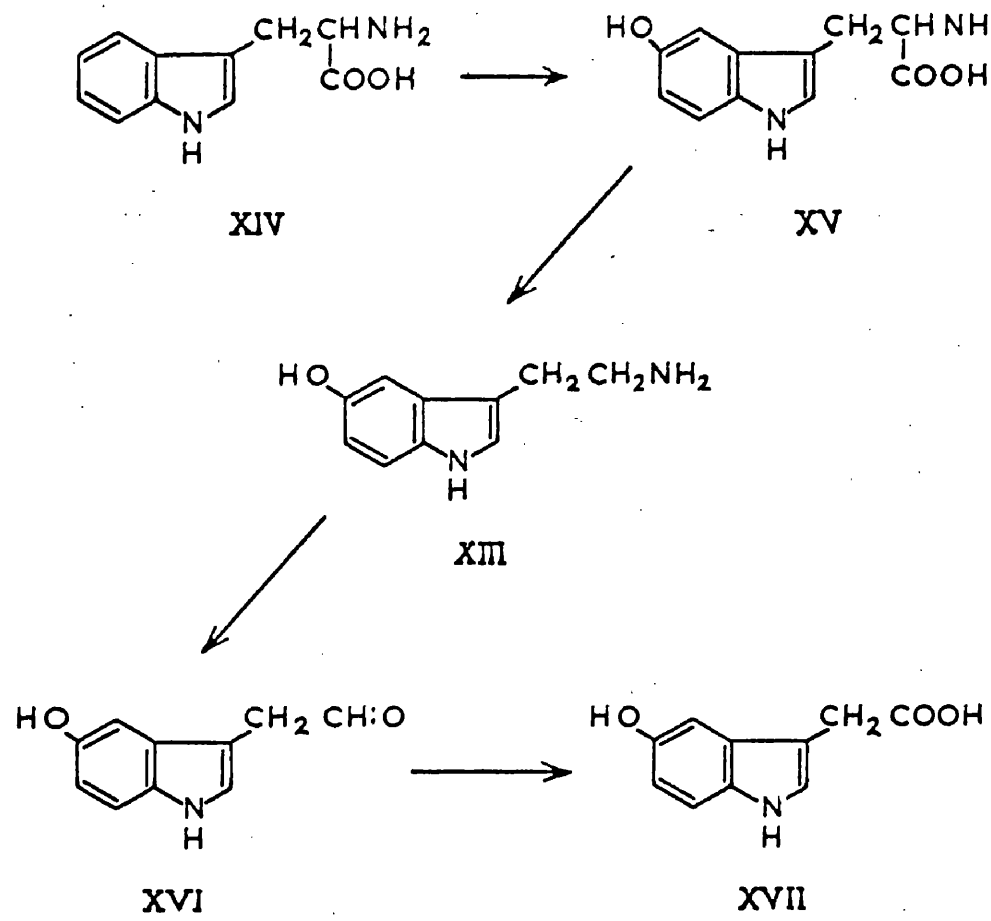


fig. 4

The general occurrence of 5-HT in the vertebrate brain led Woolley[92] and Gaddum[93] to conclude that 5-HT has a biological role in the central nervous system. Although a great deal of research has been directed towards elucidating the nature of this role, the exact significance of 5-HT in the brain is still unknown and much controversy exists. Many difficulties are associated with this problem: perhaps the most important are the impermeability of the blood brain barrier to 5-HT, and those attending the interpretation of "central effects" in the presence and indeed influence of the very marked peripheral effects. In an attempt to overcome the impermeability problem, the precursor, 5-hydroxytryptophan (5-HTP)

which does cross the blood brain barrier,[94] has been used in place of 5-HT, but the resulting effects must be viewed critically as 5-HTP is also decarboxylated in areas of the brain which do not contain 5-HT.[95]

Brodie and his co-workers,[96] and Marazzi and Hart,[97] have proposed that 5-HT is present in the central nervous system as a neurohormone. According to Brodie, 5-HT is a transmitting substance in the parasympathetic division of the central autonomic nervous system with noradrenaline as its counterpart in the sympathetic division. Marazzi and Hart, however, consider it to be an inhibitory synaptic neurohormone possessing an exclusively depressant role. Other investigators question these proposals: Iggo and Vogt,[98] for example, found no fundamental differences between the preganglionic sympathetic outflow of normal cats and that of cats in which the brain had been depleted of 5-HT. This observation would indicate that the normal level of 5-HT is not essential for efficient sympathetic discharge. The information available on chemical transmission in the central nervous system has been critically reviewed by Crossland[99] and it is apparent that the role of 5-HT in the brain is still open to question. More recent investigations, however, tend to support a transmission role for 5-HT; several authors[100] have reported that 5-HT is principally located in isolated nerve endings in the mammalian brain, and the electrophysiological studies of Gerschenfeld and Stefani[101] on the central neurones of the molluscan nervous system have led these authors to support the view that 5-HT acts as a central transmitter.

When given systemically, 5-HT produces sedation and nervous depression, although mixed effects of depression and excitation have also been recorded. The precise nature of the effect is dependent upon the species and the dose administered. Since 5-HT cannot cross the blood brain barrier, these central effects are thought to arise

from the action of 5-HT upon the blood vessels of the brain, either by vasoconstrictor action or an alteration in their permeability, or possibly by an indirect effect upon certain centres associated with the central nervous system but which are outside the blood brain barrier.[102] To bypass the blood brain barrier and to avoid the peripheral effects, 5-HT has been introduced directly into the brain tissue and into the lateral ventricles of experimental animals. The results are rather inconsistent depending largely upon species and area of administration; no general conclusions have been drawn.

Since Udenfriend and his co-workers[94,103] first demonstrated that the administration of 5-HTP resulted in an increased level of 5-HT in the brain, this precursor has been widely used for investigating the central effects of high 5-HT levels. At low doses 5-HTP generally decreases spontaneous activity and has a tranquillising effect, while at higher doses the effects vary considerably, generally excitation results but depressant effects have also been recorded.

5-HT levels may also be raised by the use of monoamine oxidase (MAO) inhibitors (e.g. iproniazid) which inactivate the enzyme initiating the metabolic breakdown of 5-HT.[104] The symptoms induced by 5-HTP are enhanced by pre-treatment with the MAO inhibitors but the relationship between the observed response, the concentration of 5-HT, and the use of MAO inhibitors is not a simple one. In certain circumstances, both 5-HTP and MAO inhibitors are necessary to induce a response, while alone they are ineffective. Green and Sawyer[105] have suggested that MAO inhibitors increase the penetration of 5-HTP into the brain and further propose that 5-HTP induced high levels of 5-HT are in some way different from those produced from endogenous precursors. The system is further complicated by the non-specific nature of the inhibitors, and the

lack of specificity of MAO itself.

The level of 5-HT in the brain and also in other tissues may be decreased by the administation of reserpine and certain related alkaloids.[106] The rate and extent of loss varies according to the species and tissue under investigation. Depletion of the tissue probably results from an interference with the binding sites rather than an effect upon the biosynthesis of 5-HT. In the central nervous system, reserpine acts predominantly as a tranquillising agent, and also interferes with the extrapyramidal motor functions. Whether these effects are a direct consequence of the decreased level of 5-HT is not known, as reserpine also decreases the levels of other biologically important brain amines[107] such as adrenaline, noradrenaline, and dopamine.

The peripheral effects of 5-HT are many and varied: its action upon blood pressure is neither purely hypotensive nor hypertensive, but like many of its peripheral effects is dependent upon dosage, route of administration, and the species and physiological condition of the animal. It increases capillary permeability[108] and has been reported to increase capillary resistance, it affects the function of the heart,[109] the kidneys (usually acting as an antidiuretic)[85] and induces bronchoconstriction.[110] It also excites afferent and efferent autonomic fibres, has a variety of effects upon enzyme systems and increases intestinal tone.[111] This effect upon the gastro-intestinal tract along with the naturally high level of 5-HT in the mucosa has led to the suggestion that 5-HT may be involved in peristalsis, although this is now open to question.[112]

Rocha e Silva and his co-workers,[113] studying the effect of 5-HT upon isolated guinea pig ileum, demonstrated the existence of tissue receptors which react preferentially with 5-HT. Subsequent work by Gaddum and Picarelli[114] led to the discovery of two distinct

5-HT receptors, one being a nervous element (M) which is blocked by morphine, methadone, atropine and cocaine, while the other, termed the D-receptor, is located in smooth muscle and is blocked by dibenzyline and ergot derivatives. More recent work[115] indicates that although stimulation of a D-receptor induces a direct response, similar interaction at an M-receptor leads to an indirect response, involving other, probably cholinergic, receptors. There is also evidence for receptors of two types in the dog bladder;[116] stimulation first produces a twitch response from the nervous receptors, followed by a slower contraction from the muscle receptors. In other tissues, the picture appears to be more complex with the probable existence of receptors which are not specific to 5-HT; Mansour[117] found the effects of 5-HT and amphetamine to be indistinguishable in the liver fluke, and Innes[118] has demonstrated that on cat spleen 5-HT and adrenaline act on the same receptor. The inability of morphine or atropine to inhibit the action of 5-HT on this tissue indicates that the cat spleen has no M-receptors; moreover, the results from adrenaline and D-type antagonists suggest that, although there are receptors similar to those classed as D on the guinea pig ileum, these are not specific for 5-HT on the cat spleen.

Originally, because of the structural and pharmacological similarities between tryptamine and 5-HT,[119] it was assumed that these two indolealkylamines act at the same receptor. However, more recent observations[120] have cast some doubt upon this and two separate receptors for tryptamine and 5-HT have been proposed. An interesting alternative explanation, requiring the existence of only one receptor, has been proposed by Vane:[121] the pharmacological differences between tryptamine and 5-HT, are said to result from the greater permeability of the cell membrane to tryptamine than to the more polar 5-HT. This would result in tryptamine being metabolised by intracellular MAO more rapidly than 5-HT and may account for the

observed pharmacological differences.

The nature of the 5-HT receptor has also been investigated in the molluscan heart,[42] where it is thought that 5-HT acts as a neurotransmitter.[122] This preparation is highly sensitive to 5-HT; low doses cause a positive inotropic effect, while higher concentrations increase muscle tone. The results indicate that tryptamine and phenylethylamine act at the same site as 5-HT and related indolealkylamines, demonstrating that the indole nucleus is not essential for activity. Although tryptamine and alkylated derivatives are less potent than 5-HT, bufotenine and particularly lysergic acid diethylamide (LSD), in contrast to their activities on other peripheral 5-HT receptors, are more active than 5-HT itself. The high activity of LSD on this preparation led Greenberg to suggest that this alkaloid represents the optimum steric arrangement of the indole nucleus and the "ethylamine" side chain for 5-HT-like activity. Hence he described the conformation of 5-HT at the same receptor in terms of this arrangement (see fig. 5). Gyermek,[123] however, questions the

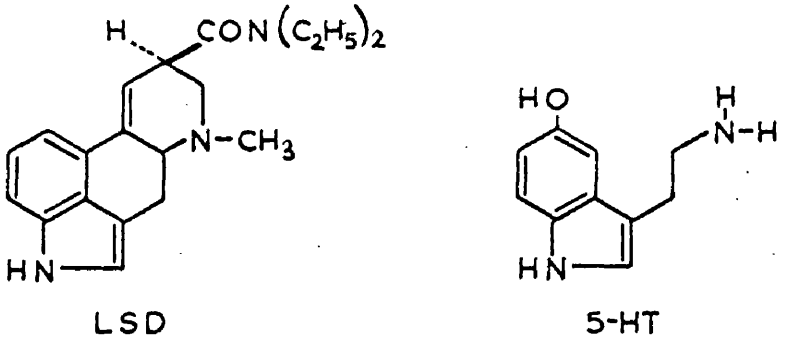


fig. 5

relevance of this optimal conformation of 5-HT for receptor sites other than those of the molluscan heart in which LSD has a stimulant effect. Minor modifications of LSD, such as N- or $C_{(2)}$-substitution, yield analogues with only blocking properties, suggesting that these

modifications affect the intrinsic activity of the molecule rather than its affinity.

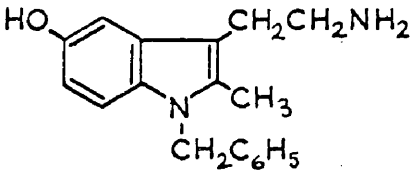
As a consequence of the great interest in 5-HT, a number of related indole derivatives have been synthesised and their pharmacological activity investigated. Although most of these possess antagonistic properties, some have been found to induce 5-HT-like effects. The majority of tests have been carried out on smooth muscle preparations; in general, no correlation has been observed between their chemical structure and their overall pharmacological activity upon these varied tissues, but certain similarities in the responses of rat uterus and rat stomach do make some general comments possible.[82] A 5-hydroxyl group is essential for maximum activity on these preparations; tryptamines possessing a 4-, 6-, or 7-hydroxyl group are progressively less active, while the unsubstituted tryptamine has an activity intermediate between those of 4- and 6-hydroxytryptamines. Offermeier and Ariens[124] attribute this variation in activity to a variation in the affinities of these tryptamines for the receptor rather than to any significant differences in their intrinsic activities. The introduction of other substituents into the indole nucleus, particularly halogens, also decreases activity. Substitution of the ethylamine side chain into the 2-position effectively destroys all activity, as does substitution of a methylamine group in place of the ethylamine side chain of natural tryptamines. Alkyl substitution on the amino group has a variable effect, but similar substitutions on the α-C atom of the side-chain lead to decreased activity, the effect of a methyl group being slight, whereas with larger alkyl groups the effect becomes more pronounced. In general, analogues methylated in the 1-position of the indole nucleus possess similar activities to those of the unsubstituted tryptamines.[125] The effect of such structural variations upon the activities of certain tryptamines and hydroxy-

tryptamines has been discussed recently in relation to the 5-HT receptor by Offermeier and Ariens.[124,126]

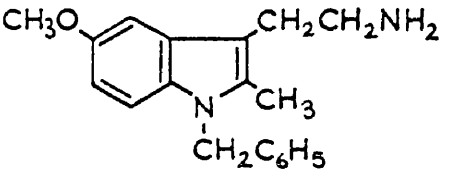
The multitude of 5-HT antagonists which have been prepared and studied pharmacologically have been reviewed by many authors from various aspects; their general cardiovascular properties and their effects upon isolated organs have been discussed by Gaddum.[127] Lysergic acid and its derivatives are the main topic of reviews by Gaddum[128] and Rothlin.[129] A comprehensive review of all antagonists has been published by Jacob,[130] and more recently Gyermek[131] has discussed the peripheral neurotropic antagonists of 5-HT. The compounds regarded as antagonists are those which are thought to prevent the pharmacological effects of 5-HT *in vivo* and *in vitro* without directly interfering with the biosynthesis, the metabolism, or the release of 5-HT.

Many N-alkylated derivatives of 5-HT and tryptamine show reversible and competitive blocking of the spasmogenic action of 5-HT on smooth muscle.[132] Alkylation and arylation at other positions in the molecule, and etherification of the hydroxyl group, often yield compounds capable of antagonising the effects of 5-HT on blood pressure as well as on smooth muscle preparations.[133] Such compounds include the potent 1-benzyl-2-methyl-5-hydroxy-tryptamine (BAS phenol; XVIII), its 5-methoxy derivative (BAS; XIX), the N,N-dimethyl-5-methoxy derivative (BAB; XX) and 1-(p-methoxy-benzyl)-2-methyl-5-hydroxytryptamine (XXI). Recently the corresponding derivatives of tryptophan and 5-HTP have also been prepared and found to possess interesting central effects.[134] Gramine (XXII) and several derivatives such as 2-methyl-5-chlorogramine (XXIII) and 5-benzyloxygramine (XXIV) also exhibit high *in vitro* antagonistic activity[135] but these are generally short acting *in vivo*, probably due to their rapid metabolic inactivation. Certain 4-substituted indole derivatives have been investigated and found to be 5-HT antagonists; these include

4-indolecarboxylic acid diethylamide (XXV) and the N,N-dimethylated indolealkylamines, psilocin (XXVI), benzylpsilocin (XXVII), psilocin benzoate (XXVIII) and psilocybin (XXIX).



XVIII.



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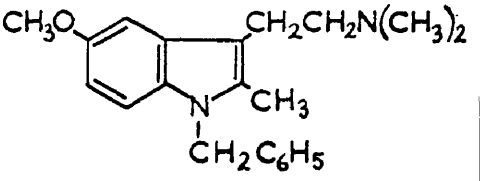

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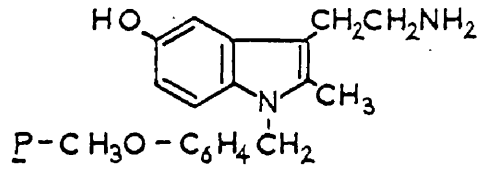
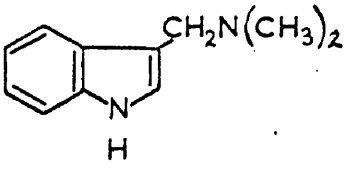


XXI.



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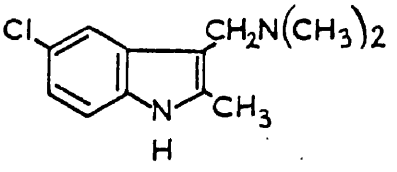

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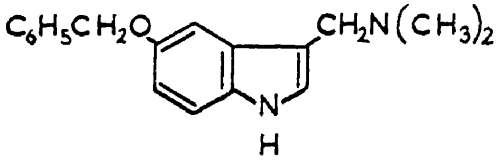
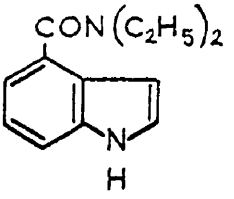
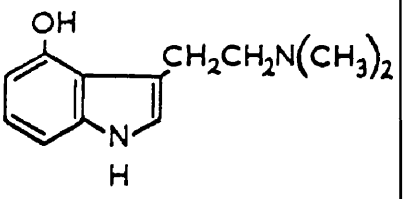


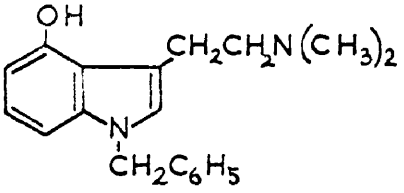
XXIV.



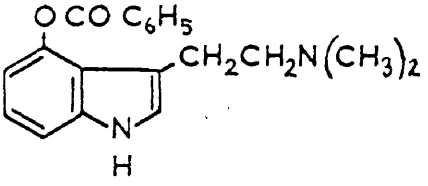
XXV.



XXVI.



XXVII.



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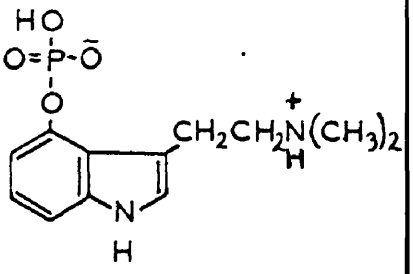
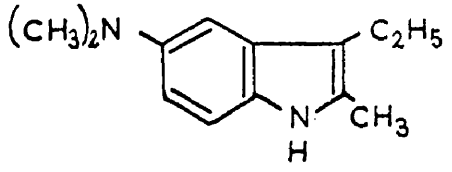
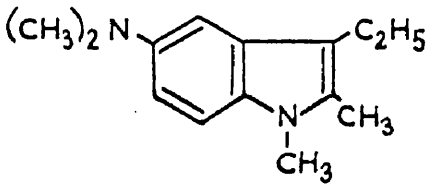


XXIX.

Amino-, alkylated amino-, and nitro-nuclear substituted indoles have also been prepared for their antagonistic properties;[136] the most important of these are the alkylated amino derivatives medmain (XXX) and methylmedmain (XXXI).

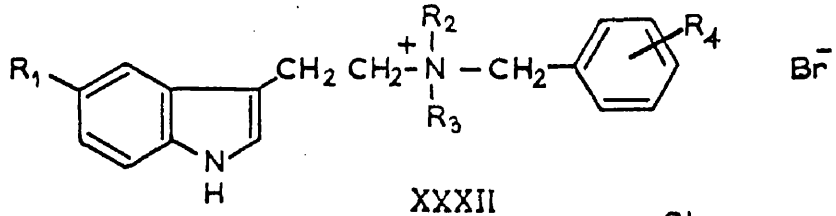


XXX

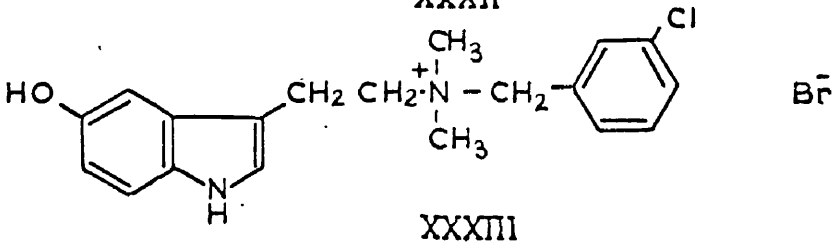


XXXI

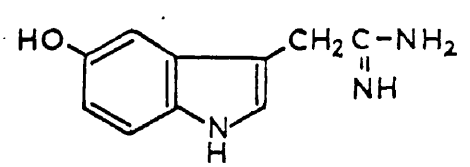
Quaternary ammonium salts of N,N-dialkyltryptamines of general formula XXXII have been prepared and investigated by Gyermek.[137] These compounds, and in particular m-chlorobenzylbufoteninium bromide (XXXIII), are potent neurotropic 5-HT antagonists of peripheral receptors but have low potency on muscular receptors. Indoleacetamidines[138] exhibit similar properties, 5-hydroxyindol-3-ylacetamidine (XXXIV) being particularly potent. A third group of neurotropic blockers are the guanidine derivatives (XXXV);[139] these are less selective than the quaternary ammonium tryptamines or acetamidines and show some 5-HT stimulant properties.



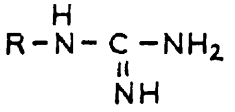
XXXII



XXXIII



XXXIV



XXXV

Tetrahydrocarbazoles, which are analogues of some simple 5-HT antagonists, were investigated by Gaddum and his co-workers[140] and by Shaw and Woolley;[141] certain of these give some protection against the pressor effects of 5-HT.

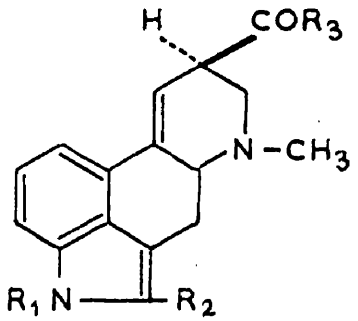
Antihistamines exhibit moderate anti-5-HT potency in isolated organs but this is substantially reduced in vivo. The most potent is cyproheptadine;[142] this blocks the effects of 5-HT on dog blood pressure, spasmogenic effects on rat uterus, and prevents rat paw oedema. There appears to be no correlation between the anti-5-HT activity of antihistamines and their antihistaminic or local anaesthetic activity.

Chlorpromazine[143] blocks the effects of 5-HT upon certain smooth muscle preparations, the capillaries of the rat paw, and in certain instances upon blood pressure of cats under ganglion blockade. The blocking is not specific: chlorpromazine antagonises the bronchoconstrictor action of histamine, acetylcholine and nicotine, and the stimulation of acetylcholine upon the intestine. Other phenothiazines also exhibit anti-5-HT activity but, with a few limited exceptions, this is less than that of chlorpromazine.

Certain sympatholytic drugs (dibenamine, dibenzyline etc.) block, often irreversibly, the action of 5-HT upon the smooth muscle D-receptors of isolated organs. Their effect upon the nervous M-receptors is much less pronounced.[113] Other 5-HT antagonists include certain sympathomimetic amines,[144] morphine-type analgesics,[145] atropine-like drugs[146] and certain antidepressants. Recently certain aminoalkyl substituted benzene derivatives have also been shown to possess strong anti-5-HT properties.[147]

Lysergic acid (XXXVI) and its derivatives form the most

important and potent group of 5-HT antagonists known. Gaddum[148] first noted the high activity of LSD (XXXVII) which prevents, by competitive blocking, the majority of peripheral effects of 5-HT and its related indolealkylamines. Pharmacologically LSD has very pronounced central effects: it induces hallucinations and symptoms similar to those of schizophrenia, and elicits autonomic effects which may be either sympathetic or parasympathetic in nature. Its peripheral effects, often 5-HT-like, include an oxytocic action and a vasoconstrictor action on perfused blood vessels. In vivo, however, the depressive action on the vasomotor centre generally predominates over the vasoconstrictor action and results in an overall depression of blood pressure. On some mollusc hearts LSD is a more potent stimulant than 5-HT itself.



XXXVI	$R_1 = H$;	$R_2 = H$;	$R_3 = OH$
XXXVII	$R_1 = H$;	$R_2 = H$;	$R_3 = N(C_2H_5)_2$
XXXVIII	$R_1 = H$;	$R_2 = H$;	$R_3 = NH\,\underset{CH_3}{CH}\,CH_2OH$
XXXIX	$R_1 = H$;	$R_2 = Br$;	$R_3 = N(C_2H_5)_2$
XL	$R_1 = CH_3$;	$R_2 = H$;	$R_3 = NH\,\underset{C_2H_5}{CH}\,CH_2OH$

The potency of LSD as an hallucinogen and as an antagonist of 5-HT led Woolley and Shaw[149] to suggest that the symptoms of schizophrenia, which are similar to those induced by LSD, might be

caused by a deficiency of 5-HT in the brain brought about by metabolic failure. The structural similarity between 5-HT and LSD appeared to give further support to this concept[150] (see fig. 5).

Later observations, however, tend to contradict the possibility that LSD-induced hallucinations are the result of central 5-HT antagonism. The psychotomimetic potencies of the lysergic acid derivatives appear to be completely unrelated to their ability to antagonise 5-HT peripherally. Ergometrine (XXXVIII), for example, is a most potent 5-HT antagonist but has no psychotomimetic properties and 2-bromolysergic acid diethylamide (BOL; XXXIX) blocks, rather than reinforces, the central effects of LSD.

Evidence that LSD in fact induces slight increases in cerebral 5-HT levels has been reported by Freedman and Giarman.[151] In contrast to those induced by 5-HTP and iproniazid, increases due to LSD could be completely accounted for in particle-bound 5-HT. Although similar elevated levels were observed with other related psychotomimetic drugs, no increases were detected after administration of non-psychotomimetic analogues such as BOL or 1-methyllysergic acid butanolamide (XL). Furthermore the unrelated psychotomimetic drugs, yohimbine, mescaline and certain indoleamines have also been shown to increase brain 5-HT.

Recent evidence indicates that symptoms of schizophrenia may be induced by an altered indole metabolism:[152] the hallucinogenic properties of *N*-alkylated tryptamines are well documented, and Axelrod[153] has demonstrated that *N*-methylation of tryptamine and 5-HT takes place in rabbit lung tissue. Hydroxylation of *N*-alkylated tryptamines[154] has also been proposed as an important step in the biosynthesis of psychoactive molecules. Thus, the possibility of the body synthesising hallucinogenic compounds cannot be excluded.

Enzymatic *O*-methylation[155] of 5-HT and its *N*-acetyl derivative

also takes place in body tissues, the physiological roles of the resulting 5-methoxytryptamines are not clearly understood but melatonin (N-acetyl-5-methoxytryptamine) is thought to participate in the transmission of optical influences to the gonadotrophic-pituitary mechanism. 5-Methoxytryptamine alters the conditioned behaviour of animals and has a high potency on peripheral smooth muscle 5-HT receptors; its effect upon nervous receptors sensitive to 5-HT, however, is negligible. Pharmacologically, melatonin is ineffective on smooth muscle and ganglionic 5-HT receptors, but has a significant action on the central nervous system, influencing the epithalamo-pituitary system. A further derivative of 5-methoxy-tryptamine thought to have physiological significance is the N,N-dimethyl analogue;[156] it exhibits both marked behavioural and peripheral effects, but its precise role in the body is not known.

The general lack of correlation between the central and peripheral potencies of the indolealkylamines demonstrates the difference in structural requirements for central and peripheral activity. For this reason, Gyermek[157] has questioned the use of peripheral receptors, which are influenced by these indolealkyl-amines, as model systems for the hypothetical central nervous system receptors. This makes the assumption, of course, that the lack of correlation in potencies is a direct consequence of the difference in the receptors rather than of differences in the ease of transport of the indolealkylamines to the receptors.

However, due to the high complexity of even the simplest receptor system, the nature of these peripheral receptors has not been completely elucidated. Interpretation of drug action in terms of drug-receptor interactions is complicated by the lack of inform-ation concerning storage sites and inactivating enzyme surfaces, which are certainly present in the whole animal, and may also occur in certain isolated organs. These sites, if present, compete with

the true 5-HT receptor for occupation and, as a consequence, the observed effect induced by a particular drug is the result of a combination of the relative rates of reaction of the drug with the various sites, and the number and location of these sites. The distribution of the drug between these sites is also relevant in determining whether the drug potentiates 5-HT by preferentially reacting with an enzyme inactivating surface, or blocks 5-HT by interacting with the true receptor site. The increases in 5-HT levels which have been reported in certain tissues [151] after the administration of LSD may be explained in terms of this system, LSD preferentially interacting with the inactivating sites for 5-HT leading to a decreased rate of 5-HT metabolism.

Investigations involving 5-HT antagonists are faced with a further complication in that the antagonists are thought to possess a dual mode of action: they may either compete with 5-HT for unoccupied sites, or may attack occupied sites resulting in the replacement and release of 5-HT from these sites. Consequently a complex and variable state may exist with distribution and reditribution of 5-HT occurring between the various sites available.

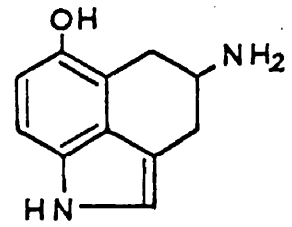
Such complications as outlined above make the study of 5-HT receptors a formidable task and although a variety of methods and approaches have been designed and employed no general conclusions have been drawn regarding their structure. However, the information gained in recent years has provided a general understanding of the complexities associated with receptor study and gives some indications as to the direction that further investigations might take. Analysis of the interactions between specific 5-HT analogues and a simple biologically responsive isolated system would seem to be one approach of particular value in determining the structural requirements and hence the general configuration of certain 5-HT receptors.

DISCUSSION

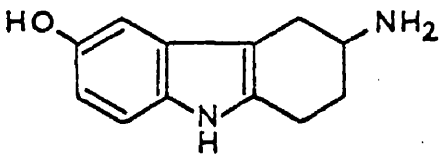
Despite the continued interest shown in 5-HT, little is known concerning its site of action; Greenberg's[42] pictorial representation of the 5-HT receptor in the molluscan heart, as Gyermek[123] has already made clear, cannot be directly accepted as a general structure for 5-HT receptors in other species and other tissues. Even with respect to the molluscan heart, this hypothetical structure was based on the observations of only one compound believed to have the optimum configuration. In an attempt to learn more about the requirements of the 5-HT receptor, the synthesis of a series of compounds structurally related to 5-HT was undertaken.

The intention was to prepare compounds in which the 3-ethylamine side chain of the natural tryptamines was held in various conformations. In this way, the distances between the groups thought to be of particular biological significance (hydroxyl, indole ring nitrogen and amine) would be fixed within fairly small limits and a pharmacological study of such compounds should indicate the most favourable arrangement for optimum biological activity. Using the molecular arrangements associated with maximal activity as model, it should then be possible to describe the configuration of receptor-bound 5-HT and so to draw conclusions concerning the nature of the receptor site under investigation. Studies with various 5-HT-sensitive preparations should also yield information concerning the variation in requirements of receptors of different tissues. Among the most valuable compounds for such an investigation would seem to be the 5-HT analogues XLI-XLVI. Of particular interest would be a study of the action of these compounds on the molluscan heart; if the structure proposed by Greenberg actually has any real significance then the tetrahydrobenz[c,d]-indole (XLI) should provide the best fit and exhibit maximum

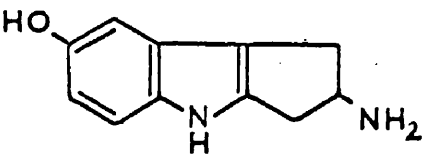
activity, while the remaining compounds, which cannot adopt this conformation, should be considerably less active. This should provide a stringent test for Greenberg's proposals.



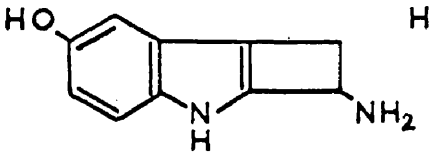
XLI



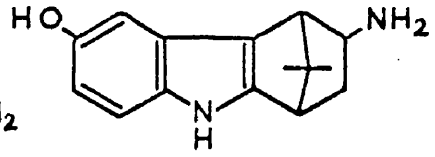
XLII



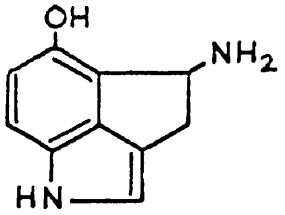
XLIII



XLIV



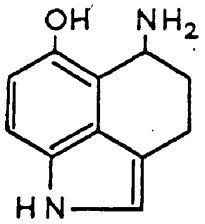
XLV



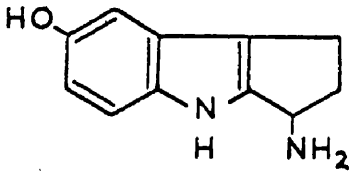
XLVI

Like the natural substrate, 5-HT, the compounds XLI-XLVI possess a 5-hydroxylated indole unit and a primary amino group two carbon atoms removed from the $C_{(3)}$ position of the indole nucleus. The flexible amino-substituted ethyl side-chain of 5-HT is incorporated into a third ring which thereby severely limits the number of conformations the side chain may adopt. Owing to the bridging in the fused bicyclo[2,2,1]heptane derivative (XLV), the unit corresponding to the 5-HT side chain has even greater rigidity. Other compounds of interest but having steric relationships more removed from those thought to be possible in 5-HT are the indole derivatives XLVII-L. The tetrahydrobenz[c,d]indole (L) is in fact a tryptamine analogue, while compounds XLVII-XLIX, although not possessing a unit corresponding to the ethylamine side-chain, still approximate to possible extreme spatial arrangements which the functional groups of tryptamine and 5-HT might adopt at the receptor site. Although extreme conformations of 5-HT are generally to be regarded as unfavourable they should not be completely ignored as it is recognised that drugs may adopt normally unfavourable

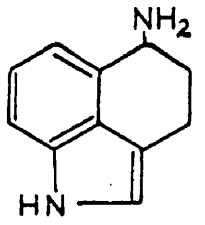
conformations on complexing with the receptor.



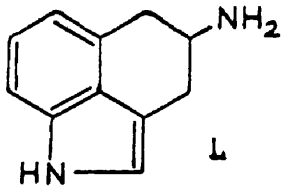
XLVII



XLVIII



XLIX



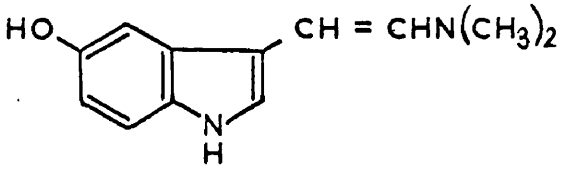
L

The approach of employing "rigid" or "partially rigid" analogues in attempts to elucidate the nature of receptors has been applied to receptors other than those of 5-HT. In particular, several such studies have been made with the acetylcholine receptor; in this connection the work of Belleau and his co-workers[76,158] using 1,3-dioxolane derivatives (see p.21) and of Smissman and LaPidus and their co-workers[159] using substituted decalins may be cited. Suitably substituted piperidine and morpholine derivatives[160] and acetoxytropines[161] have also been prepared as "less flexible" forms of acetylcholine. A further example is provided by the use of suitably substituted cyclopentanes by Belleau and Cooper[162] which enabled them to make deductions concerning the conformation adopted by N-(β-chloroethyl)-2-phenoxyethylamine at the adrenergic receptor.

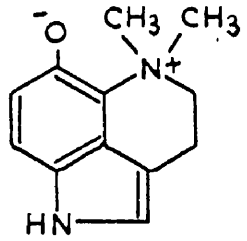
The steroid and diterpenoid skeletons have also been used for the preparation of rigid analogues of proven drugs; bisquaternary ammonium steroids with restricted interonium distances have been synthesised and used in investigations aimed at determining the importance of interonium distance in neuromuscular blocking activity.[163] The physiological activity of certain steroids, such as oestrogens, has also been attributed to the fixed interfunctional

group distance,[164] and in fact a number of diterpenoids bearing two oxygen functions at certain fixed distances also exhibit high oestrogenic activity.[165]

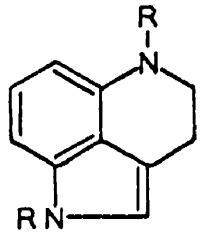
A number of compounds, which may be regarded as less flexible tryptamine and 5-HT analogues, are already known. These, however, generally lack the primary amino function characteristic of tryptamines, the ethylamine side chain being incorporated into a second heterocyclic ring. The closest naturally occurring cyclic analogue of 5-HT is dehydrobufotenine, earlier thought to have the open chain structure (LI)[166] but now known to be the pyrolloquinoline (LII).[167] Revision of the structure of dehydrobufotenine prompted Hester[168] to prepare the corresponding non-hydroxylated tryptamine analogues (LIII; R=H,CH_3). Preliminary pharmacological studies



LI



LII

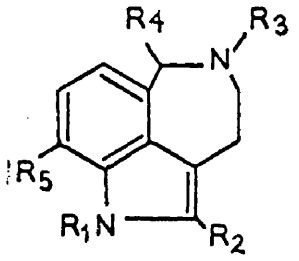


LIII

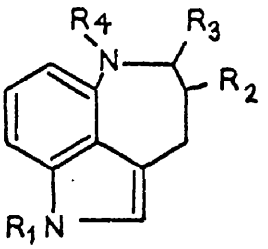
indicated that, like dehydrobufotenine, these compounds possess no pronounced central nervous system effects; the lack of information concerning peripheral activities would suggest that if they do possess such activity it is extremely weak.

Related azepino[5,4,3-c,d]indoles of general structure LIV appear to possess no significant peripheral activity,[169] but stimulation of the central nervous system, at very high doses, has been reported. Recently azepino[4,3,2-c,d]indoles of general structure LV have also been reported[170] in the patent literature, but in a consideration of tryptamine analogues these are of less interest due to the extra methylene group separating the nitrogen

atom from the $C_{(3)}$ position of the indole nucleus. Certain of these compounds were shown to possess tranquillising and anti-inflammatory properties.

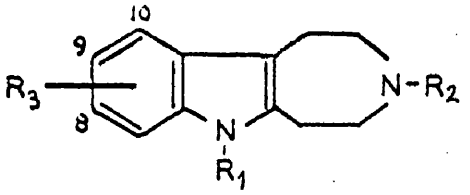


LIV



LV

Other compounds having a different spatial arrangement of the amino nitrogen and which may be considered as rigid tryptamine analogues are the azepino [4,5-b] indoles of general structure LVI. This structure was first described by Harley-Mason and Jackson,[171] and recently the syntheses of the 8-, 9- and 10-methoxy derivatives have been reported in the patent literature;[172] these derivatives have been shown to have antihistaminic activity, but apparently are without anti-5-HT or 5-HT-like properties.



LVI

Pharmacologically, the most important compounds which possess, what has been described as, a rigid tryptamine structure are certain of the indole alkaloids. Invariably, although the unit which corresponds to the ethylamine side chain forms part of a heterocyclic ring and consequently the amino function in these compounds is secondary or tertiary, many do have pharmacological properties which could result from their interfering, in some way, with the normal functioning of 5-HT in the body, as a direct result

of their being structurally similar to the natural substrate. Attention has been drawn to this structural relationship between 5-HT and biologically active indole alkaloids in reviews by Cerletti,[173] dealing with the importance of the indole structure in medicine and by Woolley[174] in his review concerning anti-metabolites.

However, the structural resemblance between 5-HT and pharmacologically active indole alkaloids cannot be the only factor which determines the activities of the latter, as evidenced by the often considerable quantitative changes in pharmacological activity which accompany structural modifications remote from the "tryptamine structure" of the alkaloid. Excellent examples of changes in pharmacological activity with relatively minor structural modifications may be drawn from the investigations carried out on lysergic acid and its derivatives.[175]

Pharmacological studies on the simple tryptamine analogues XLI-L, however, should not be complicated to the same extent as those for the complex alkaloids, and a more direct correlation between steric orientation of the molecule and 5-HT-like activity should be possible. 4-Amino-1,3,4,5-tetrahydrobenz[c,d]indole (L) has already been prepared by Gould and Jacobs[176] and, with a variety of other 4-substituted indoles, has been examined by Uhle and Harris[177] for anti-5-HT activity on the isolated rat uterus. On this preparation the amino compound (L) possessed no significant activity. This observation, together with the findings of Greenberg concerning the steric requirements of the 5-HT receptor of the molluscan heart, would suggest that the receptor of the rat uterus is structurally different from that of the molluscan heart and affords further support for the suggestion that a variety of 5-HT receptors exist in various tissues.

One of the more obvious disadvantages of the use of the compounds XLI-L in attempting to correlate chemical structure with 5-HT-like activity is that they are all substituted α- to the amino function and are either 2- or 4-substituted tryptamines. For this reason it might be preferable to compare their activities with those of the 2,α-dialkyl- and 4,α-dialkyl-5-hydroxytryptamines. The introduction of alkyl substituents into the 2-position of indole derivatives is known to decrease the 5-HT-like activity, probably by affecting the degree of approach of the indole nucleus to the receptor surface.[42] Substituents in the 4-position might also interfere with complex formation. The effect of α-alkyl substitution has been widely examined and it has been shown that while a methyl group has little affect upon peripheral activity, larger groups tend to decrease 5-HT-like activity.[120,178] The α-alkyl group is also known to protect the molecule from rapid metabolic destruction by MAO,[179] and α-methyl- and α-ethyl-tryptamines are in fact reversible MAO inhibitors.[180] α-Methyl- and α-ethyl-5-HT, however, do not possess this inhibitory action. The effect of the non-hydroxylated compounds upon MAO is thought to be responsible for the central effects observed in experimental animals. The inability of the hydroxylated members to induce central effects furnishes further support for this proposal. This hypothesis has now been critically examined by Lessin, Long and Parkes[179] who suggest that a redistribution of 5-HT between free and bound forms may also account for the central effects induced by α-methyl- and α-ethyl-tryptamines. Another possible reason for the central inactivity of α-methyl-5-HT, apart from its inability to inhibit MAO, is that, due to its more polar nature, it might not cross the blood brain barrier. If this is so, and it is known that this is certainly true for 5-HT itself, then attempts to study the planned molecules on the central nervous system could well be complicated by the inability of the hydroxylated members of the series to pass the blood brain barrier. The strategic introduction of a carboxyl

group onto the α-carbon atom of the rigid unit corresponding to the side chain of 5-HT might, however, make the molecule acceptable to the active transport system which has been proposed for the transference of 5-HTP into the brain.

Because of the current interest in LSD, its possible interference with body 5-HT, and its influence upon the structure proposed by Greenberg for the 5-HT receptor, it was decided to commence synthetic work by examining approaches to the structurally related tetrahydrobenz[c,d]indole compounds. Two basic approaches to the syntheses of these derivatives were available: the first using a suitably substituted naphthalene or tetralin derivative onto which is built the pyrrole ring of the indole system; and the second, in which a preformed indole derivative is cyclised to form the third ring of the tricyclic structure. Both of these approaches were developed during attempts to synthesise LSD. The work in this field has been reviewed by Stoll[181] and by Glenn[182] and many pertinent references are included in the paper by Woodward and his co-workers[183] which reports the first total synthesis of lysergic acid. This successful synthesis of lysergic acid employed the second of the synthetic approaches to the tricyclic system.

Various benz[c,d]-indoles, -dihydroindoles and -oxindoles have been prepared by cyclisation of substituted naphthalenes but disubstituted benz[c,d]indoles possessing one substituent in each of the six membered rings do not appear to have been prepared.

Successful syntheses of the required tricyclic system by cyclisation of suitably substituted indole derivatives include those of Uhle[184] using a Dieckmann type condensation with certain β-(4-carboxyindol-3-yl)-propionic acid derivatives; of Plieninger[185] using a similar reaction on diethyl indole-3,4-diacetate, and of Szmuszkovicz[186] using substituted indol-3-yl-succinic anhydrides.

Uhle, however, found that cyclisation of β-(indol-3-yl)-propionic acid derivatives possessing substituents in the side chain led to the formation of naphthalene derivatives instead of the required tetrahydrobenz[c,d]indole structure. Cyclisation of other 3-substituted indole derivatives has also been studied by Plieninger.[187] Direct ring closure of β-(indol-3-yl)-propionic acid into the 4-position has also been attempted, but a low yield of 3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole was isolated resulting from closure into the 2- position.[188] A similar attempt was made using the 5-methoxy derivative but this also resulted in intramolecular acylation to give the corresponding tetrahydrocyclopent[b]indole.[189] It is essential therefore to protect the 2- position from substitution in these reactions if 4-substitution is to be achieved. This was accomplished by Mann and Tetlow[190] using β-(1,2-dimethyl-5-methoxyindol-3-yl)-propionic acid which, on cyclisation, gave the required tetrahydrobenz[c,d]indole. Woodward and his co-workers,[183] in their synthesis of lysergic acid, protected the indole system by hydrogenation of the $C_{(2,3)}$ double bond followed by N-benzoylation.

Synthesis of Derivatives of Benz[c,d]indole

After consideration of the possible routes and the availability of starting materials, the approach reported by Woodward and his co-workers[183] in their synthesis of lysergic acid was selected as the general route to be used in the present work for securing the required tricyclic structure. Thus β-(indol-3-yl)-propionic acid and its 5-methoxy derivative were used as starting materials for the non-hydroxylated and the hydroxylated compounds respectively. Attention was first directed towards the tetrahydrobenz[c,d]-indoles XLIX and L as these, it was considered, would function as model compounds for the corresponding hydroxy analogues XLVII and XLI which would be obtained from the less readily available β-(5-methoxy-indol-3-yl)-propionic acid.

To prevent the propionic acid side chain cyclising onto $C_{(2)}$, the $C_{(2,3)}$ double bond was hydrogenated; the secondary amino group was protected by N-benzoylation. Hydrogenation was effected by a modification of Woodward's method;[183] using an aqueous basic solution of the acid and Raney nickel catalyst the hydrogenation proceded smoothly at room temperature and atmospheric pressure and the required indoline was isolated in good yield. β-(Indol-3-yl)-propionic acid (LVII) of high purity was found to be necessary for this hydrogenation to proceed smoothly; all commercially available starting material was therefore recrystallised from water before use. The reduced acid was not further purified but benzoylated directly using the Schotten-Baumann procedure.

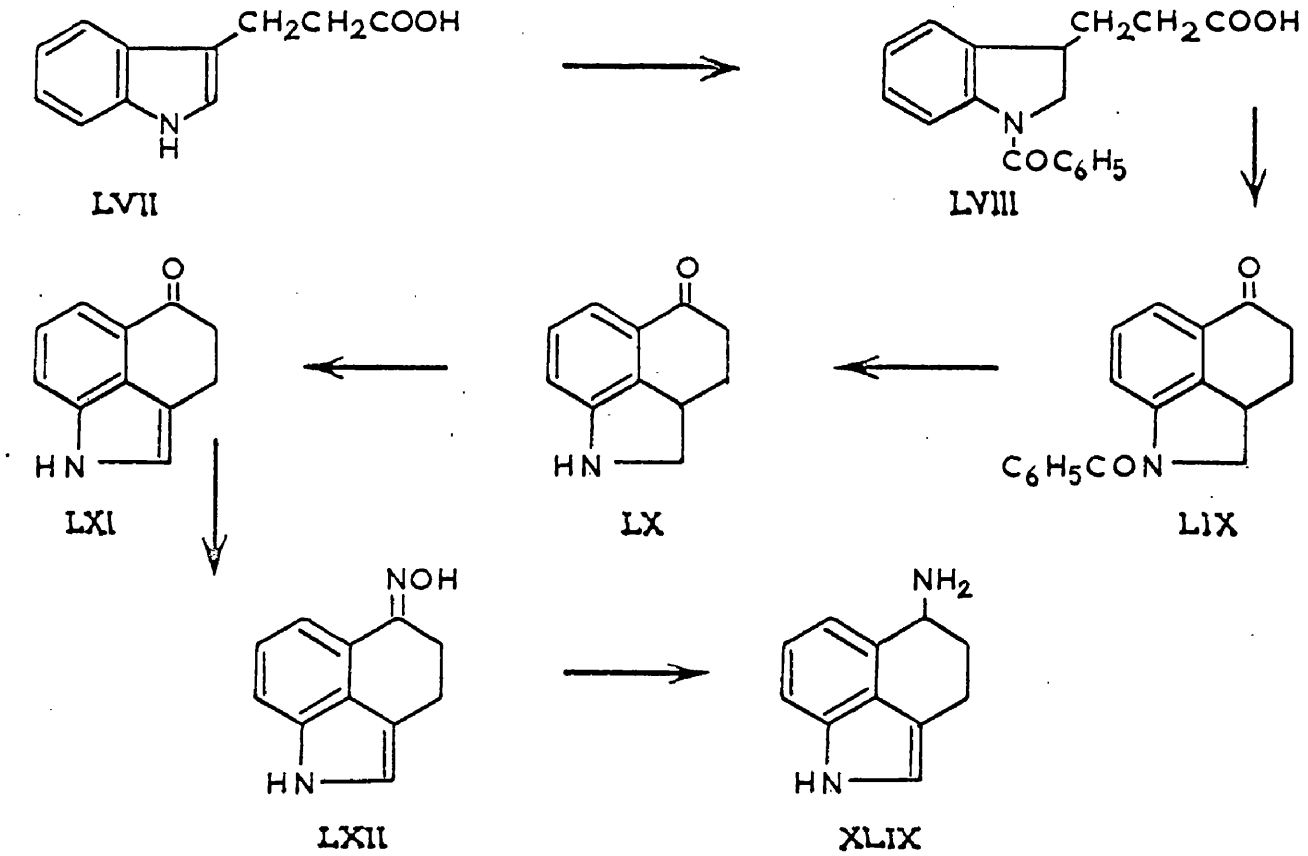


Fig. 6

The N-benzoyl derivative (LVIII) was converted into the acid

chloride and cyclised by an intramolecular Friedel-Crafts acylation using anhydrous aluminium chloride. Slightly higher yields of the cyclic ketone (LIX) were obtained from reactions carried out at 0°. Hydrolysis of the N-benzoyl function in LIX was effected by refluxing in a mixture of glacial acetic acid and hydrochloric acid as described by Woodward and his co-workers;[183] the hexahydrobenz[c,d]-indole (LX) thus obtained was dehydrogenated to the corresponding indole (LXI) using 10% palladium on charcoal in refluxing p-cymene as described by Woodward and his co-workers,[183] or active manganese dioxide in methylene chloride at room temperature according to the method of Jansen, Johnson and Surtees.[191] The oxime (LXII), prepared from this ketone by treatment with hydroxylamine hydrochloride and sodium acetate in aqueous ethanol, was reduced to the required amine (XLIX) using lithium aluminium hydride. The reaction was incomplete when carried out in diethyl ether but in tetrahydrofuran it proceded smoothly and rapidly to yield an unstable basic product. The unstable nature of this free amine appeared to be largely dependent upon the presence of impurities as, on continued recrystallisation, a sample of the pure amine was obtained which, although being rather sensitive to air, was considerably more stable than the original crude reaction product. Its hydrochloride, formed in ether by treatment with hydrogen chloride, was equally unstable. The preparation of other derivatives such as acetate and benzoate also led to appreciable decomposition. Owing to the difficulties associated with the isolation and purification of this amine the yield for the reduction was only 8%.

Although the 4-aminotetrahydrobenz[c,d]indole (L) had been synthesised earlier by Gould and Jacobs[176] via the aminonaphthostyril, an investigation into the possibility of using the tricyclic ketone (LIX) for its preparation was considered worthwhile; if successful, this approach would almost certainly be applicable

to the synthesis of the 6-hydroxy derivative (XLI) from the corresponding 6-methoxytricyclic ketone. The route which appeared to be most convenient and most likely to meet with success is shown in Fig. 7. Although this approach involves many stages, all of the earlier reactions of the sequence have been reported in the literature[183] as giving good yields, and the later stages appeared to be a simple extension of the procedure outlined in the synthesis of the corresponding 5-amino compound (XLIX). The only reservation concerning this route was the possibility of a poor yield in the final conversion of oxime to amine after so many stages.

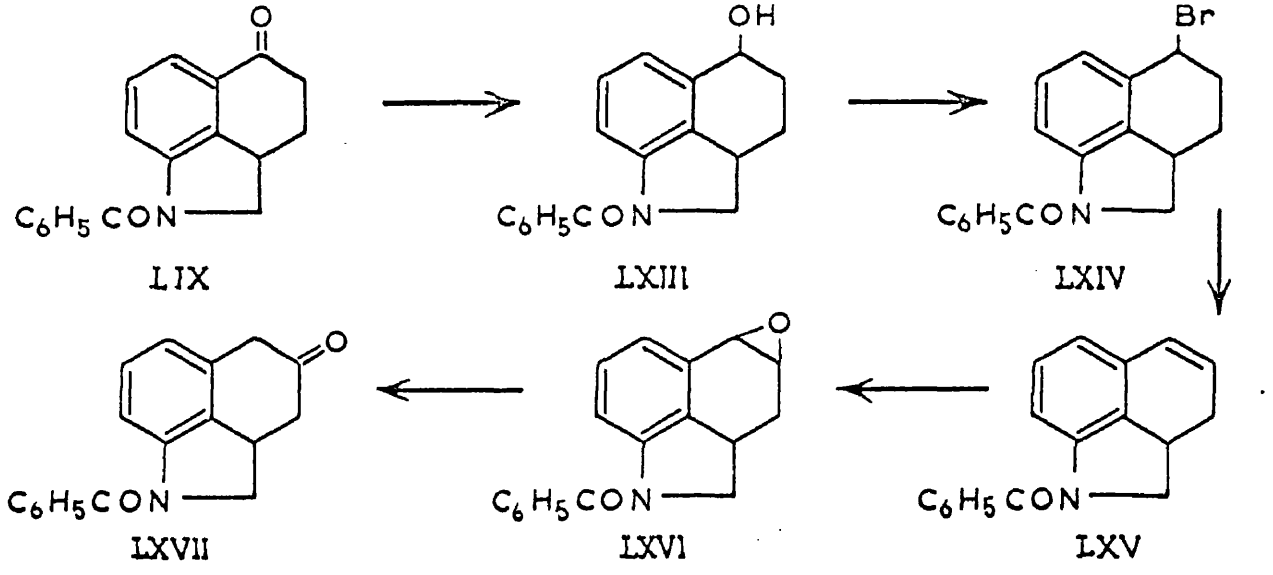


Fig. 7

Conversion of the 5-oxo-hexahydrobenz[c,d]indole (LIX) into the 4-oxo compound (LXVII) was undertaken, however, and reduction of LIX using sodium borohydride furnished the corresponding 5-hydroxy compound (LXIII) which was then converted into the 5-bromo derivative (LXIV) by the action of phosphorous tribromide; subsequent elimination of hydrogen bromide in 2,6-lutidene afforded the tetrahydrobenz[c,d]indole (LXV). On treatment with

perbenzoic acid this unsaturated compound gave the epoxide (LXVI) which on cleavage with magnesium bromide gave the required 4-oxo-material (LXVII) in good yield.

Hydrolysis of the amide function in LXVII was not possible employing the conditions described for the 5-oxo compound (LIX): a high melting polymeric solid, resulting from decomposition, was the major product isolated. Other acidic and basic procedures commonly employed for the hydrolysis of amides were also applied but were similarly found to be unsuitable. Hydrolyses attempted on the derived oxime (LXVIII) were also unsatisfactory. Resort was therefore made to the original procedure employed for the 5-oxo compound; the reaction conditions of this hydrolysis were modified, and by excluding oxygen from the reaction mixture and the product during its isolation, the required hexahydrobenz[c,d]indole (LXIX) was obtained in 40% yield. The sensitivity of this compound to

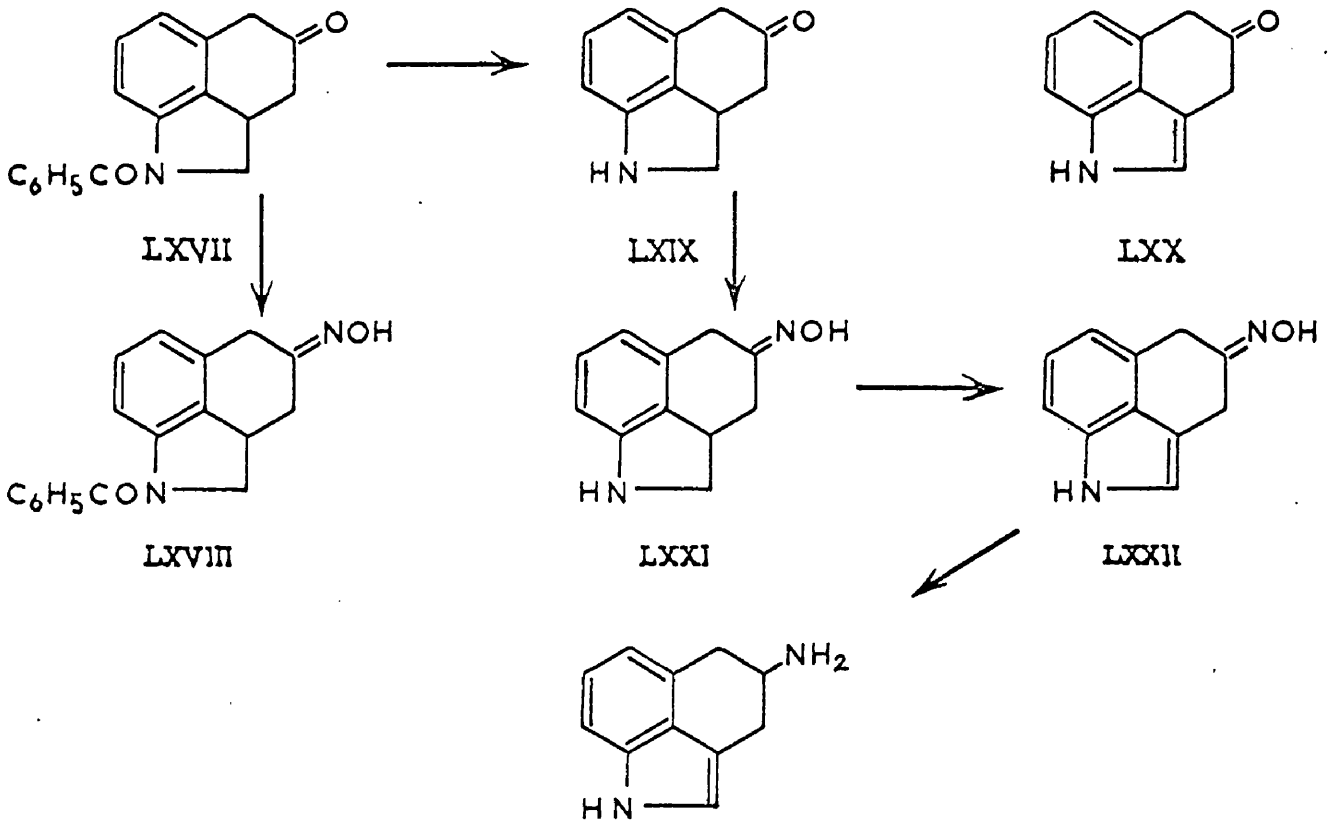
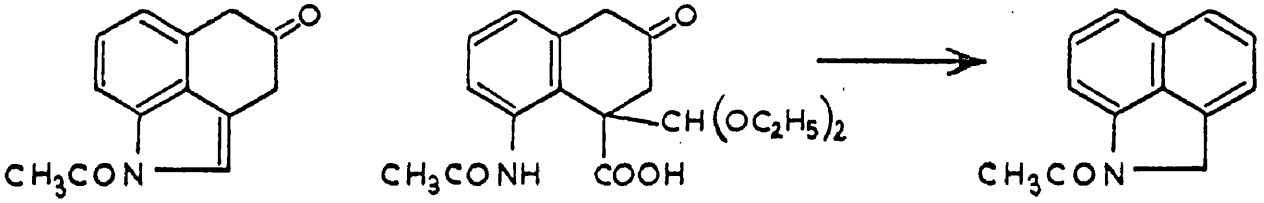


fig. 8

oxygen and its instability in solution prevented its successful recrystallisation. Attempts to dehydrogenate it directly to the corresponding indole (LXX) similarly failed; starting material was recovered, along with decomposition products, after treatment with manganese dioxide and methylene chloride at room temperature, while with identical reagents at elevated temperatures the only chloroform soluble product appeared to lack a carbonyl function and was not indolic. The infrared spectra of the products from attempted palladium-on-carbon induced dehydrogenations indicated that although the keto function was retained no indolic material was formed. The difficulties associated with this dehydrogenation are consistent with the work of Grob and his co-workers[192] who attempted to prepare the N-acetyl derivative (LXXIII) of this compound from the tetralone (LXXIV); this resulted in the formation of an amorphous solid, oxidation products and the naphthalene derivative (LXXV).



LXXIII LXXIV LXXV

fig.9

Conversion of the 4-oxobenz[c,d]indoline (LXIX) into the oxime (LXXI) was achieved by treating the oxo compound with an aqueous ethanolic solution of hydroxylamine hydrochloride and sodium acetate in an atmosphere of nitrogen. The product was stable and easily recrystallised but, due to its hygroscopic nature, some initial difficulties were experienced in its analysis. Attempts to convert this, in an acceptable yield into the corresponding indole by dehydrogenation were, however, unsatisfactory. Although the infrared spectrum was consistent with the required

oximino indole (LXXII) (ν max. 3580, 3480, 3260, 1620, 1600cm.$^{-1}$) only traces of an unstable crystalline solid were isolated, the main product being a high melting amorphous solid. Reduction of this impure product, after removal of amorphous material, in tetrahydrofuran using lithium aluminium hydride gave an equally unstable non-crystalline residue (ν max. 3480, 3300, 1620, 1600cm.$^{-1}$). The reluctance of this amine to crystallise, particularly in the presence of the impurities, has a parallel in the work of Gould and Jacobs[176] who were unable to obtain it in a crystalline form from a pure precursor. Conversion into the hydrochloride by treatment with hydrogen chloride in benzene-ether solution, however, gave a crystalline solid insufficient for full characterisation, but having a melting point close to that recorded in the literature for a specimen prepared from 4-aminonaphthostyril by Gould and Jacobs. The infrared spectrum of the N-acetyl derivative ν max. 3480, 3430, 1660, 1600cm.$^{-1}$ (see fig. 12) also gave a clear indication that the material isolated from the reduction was in fact the required amine (L).

The difficulties encountered in the above approach, their detrimental effect upon the overall yield, and the limited supply of β-(5-methoxyindol-3-yl)-propionic acid, together made an alternative and more efficient route to the 4-amino compound a necessity if it was to be used in the synthesis of the corresponding 6-hydroxy compound (XLI). With the successful synthesis of the required tricyclic system possessing a 5-oxo function completed, an obvious second approach was the introduction of a suitable substituent on the active methylene group adjacent to the keto group. Bromination of 5-oxo-hexahydrobenz[c,d]indole (LIX) using pyridine bromide perbromide gave the 4-bromo derivative (LXXVI) as reported by Woodward and his co-workers.[183] However, attempts to replace this bromine atom by a dimethylamino function to give

the dimethylamino-ketone (LXXVIII) were unsuccessful; under a variety of conditions a product lacking ketonic absorption was obtained. By analogy with the observations of Woodward and his co-workers, working with the same bromo-ketone (LXXVI) and methylamine, this product was assumed to result from aromatisation of the second six membered ring to give the naphthalene derivative (LXXVII).

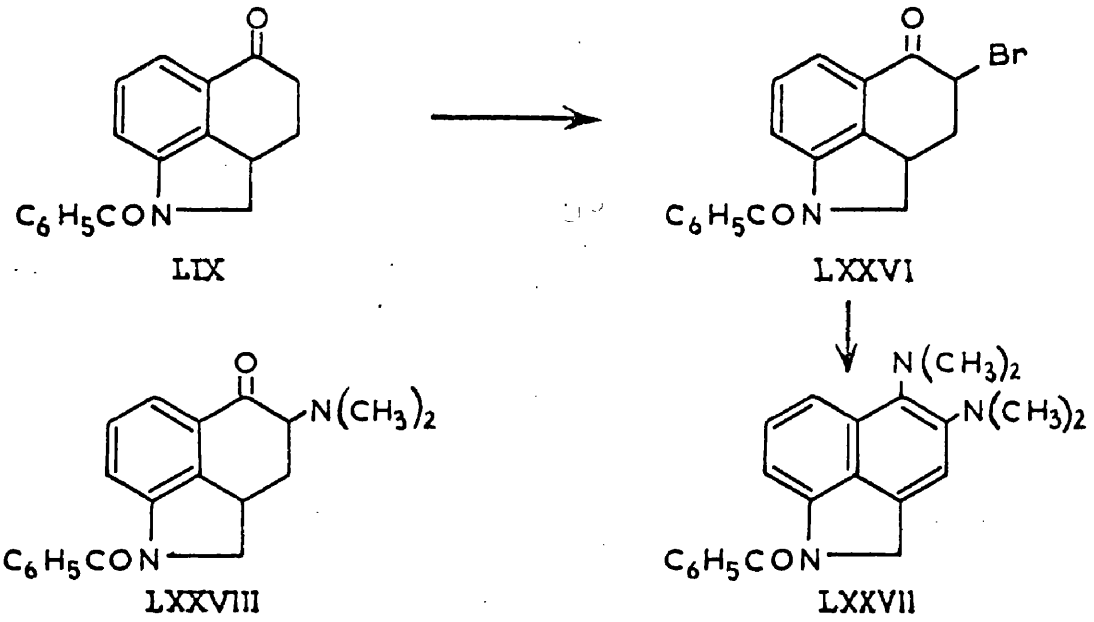


fig. 10

The rearrangement of tetra- and hexahydrobenz[c,d]indoles into the corresponding naphthalene derivatives has hindered many attempted syntheses of lysergic acid. It has been discussed in some detail by Grob and Payot,[193] Woodward and his co-workers,[183] and by Cohen, Heath-Brown and Rees.[194]

Investigations into the possibility of using the bromo-ketone (LXXVI) as an intermediate in the proposed synthesis were then discontinued, largely on account of the apparently formidable task of determining the exact conditions for the replacement of bromine by an amino function. Moreover, conversion of the key amino-ketone (LXXVIII) into the N,N-dimethyl derivative of the

required amine (L) still involved several stages including indolisation. The possibility of introducing other suitable substituents, such as a nitro group, onto the α- position of the keto function was also examined but found to be equally unsatisfactory.

An approach using the known acetamido-ketone (LXXIX) was therefore examined; this compound, which can be converted in two stages into the required amine - namely reduction of the oxo function and hydrolysis of the N-acetyl group - was first prepared by Stoll and his co-workers,[195] and has the apparent advantage that it already possesses the indole system. Although this was considered to be an advantage at the time, subsequent experimental work showed that reactions carried out on the unprotected tetrahydrobenz[c,d]indole system frequently lead to the formation of complicated mixtures, probably arising from oxidation and general decomposition. The oxo-tetrahydrobenz[c,d]indole (LXI) was nitrosylated with amyl nitrite in the presence of potassium t-butoxide, the product reduced with zinc and acetic acid, and the amine thus generated acetylated *in situ* with acetic anhydride to give the required product (LXXIX). The starting material and reagents for this reaction were carefully purified and the reaction conditions described in the literature exactly duplicated. The yield of product, however, was a fraction of that reported and although this reaction was repeated several times and minor modifications made to the procedure, no significant increase in yield could be achieved. The melting point of the product was also significantly lower than that reported but the authenticity of this material was verified by analysis and by the preparation of the p-nitrophenylhydrazone, which had the melting point reported in the literature.[195]

Removal of the keto function was next considered: the Wolff Kishner reaction was unsuitable as, under the strongly basic

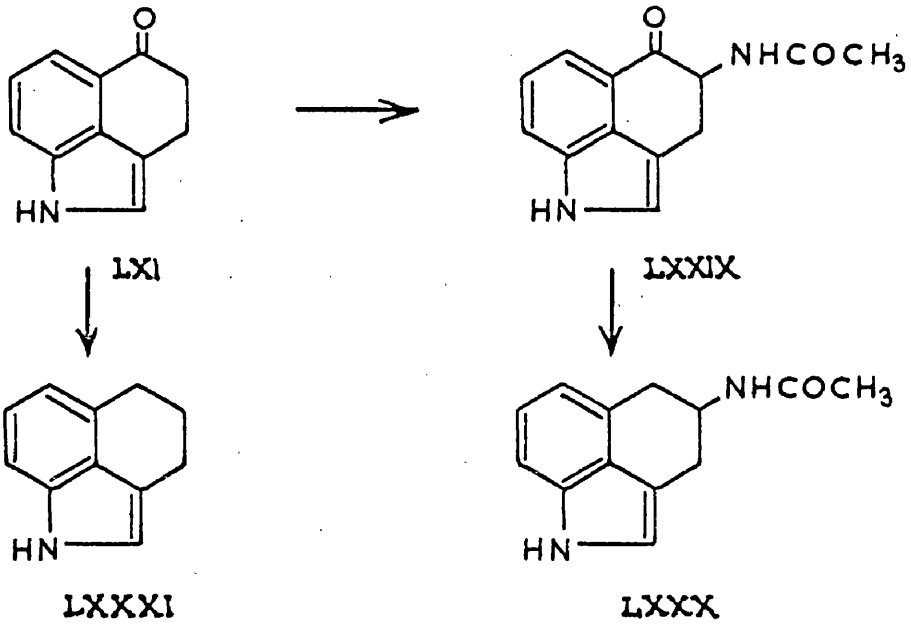
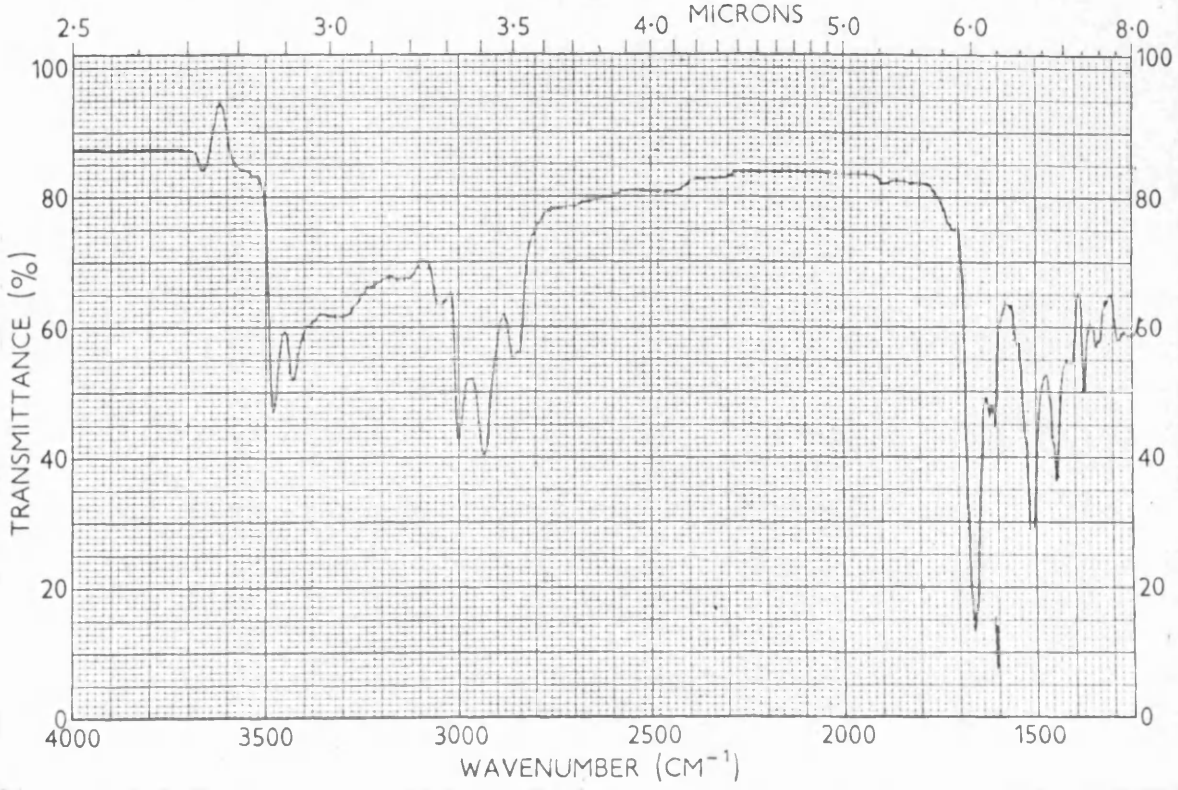


fig. 11

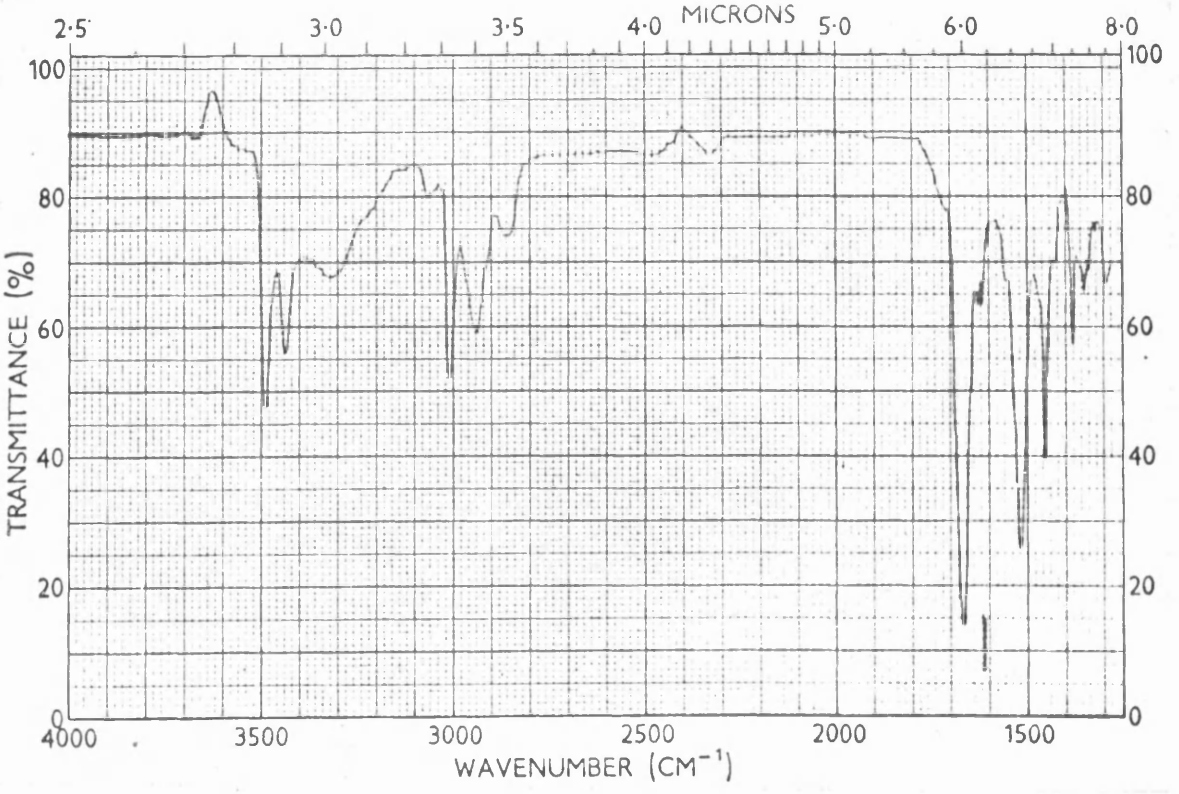
conditions of the reaction, hydrolysis of the N-acetyl function would almost certainly occur with the resultant liberation of the labile free α-amino-ketone. Although reduction of the indole $C_{(2,3)}$ double bond,[196] and elimination of the amide group[197] are both possible side reactions, the Clemmensen reduction was considered a more favourable approach to the acetyl derivative (LXXX) of the required amine. Should hydrolysis of the N-acetyl function occur under these conditions, then the more stable amino-ketone hydrochloride would result (see Woodward and his co-workers,[183] and Stoll, Rutschmann and Petrzilka[195]). In order to establish whether the $C_{(2,3)}$ double bond would suffer reduction under the conditions of the Clemmensen reaction, experiments using 5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (LXI) as a model compound were first undertaken. 1,3,4,5-Tetrahydrobenz[c,d]indole (LXXXI) was obtained in good yield, although there was infrared evidence (ν max. 3380cm.$^{-1}$) for a small amount of $C_{(2,3)}$ double bond reduction. Reduction in the indole ring was confirmed by the further reduction of the tetrahydro product (LXXXI) to the hexahydro derivative by prolonged reaction, and also by the reduction of indole and tetrahydrocarbazole under similar conditions.

The reaction was then carried out on the acetamido-ketone (LXXIX) employing the optimum conditions described. Various unsuccessful attempts were made to purify and crystallise the resulting product. The infrared spectrum of this material retained the absorption maximum at 1660cm.$^{-1}$, which in the starting material was attributed to the N-acetyl carbonyl stretching, but lacked the higher frequency shoulder attributed to the 5-oxo function, which indicated that although the material retained the N-acetyl function the keto group had been removed. Additional evidence came from the fact that, in contrast to the starting material, the product did not form an oxime. A change in absorption frequency of the N-H of the amide function was also observed. In the acetylamino-ketone (LXXIX) this absorption occurred at 3400cm.$^{-1}$ whereas in the product the corresponding absorption was at 3430cm.$^{-1}$ The lower frequency N-H absorption observed in the acetylamino-ketone (LXXIX) could result simply from the presence of the adjacent keto function or possibly from intramolecular hydrogen bonding between the keto group and the hydrogen atom of the amide function. In either case removal of the keto group would be expected to result in a change in the absorption frequency of the N-H group. A comparison of the infrared spectrum of the impure LXXX with that of the acetyl derivative of the impure 4-amino-1,3,4,5-tetrahydrobenz[c,d]indole (L) prepared from the oxime (LXXII) showed that they were very similar (see fig. 12). It was therefore concluded that the Clemmensen reduction had given rise to the acetyl derivative of the required amine (L).

While this work was in progress, Sprenger, Cannon and Koelling[198] reported their failure to convert the aminotetralone hydrochloride (LXXXII) into the aminotetralin (LXXXIII) by a Clemmensen reduction. These workers obtained none of the required product but isolated the rearranged naphthalene derivative (LXXXIV) and the deaminated tetralin (LXXXV). Similar results were obtained



Infrared spectrum of impure LXXX (obtained from 4-acetamido-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (LXXIX) by Clemmensen reaction).



Infrared spectrum of acetyl derivative of L (obtained from 4-oxo-1,3,4,5-tetrahydrobenz[c,d]indole oxime (LXXII) by reduction).

fig. 12

on the simpler non-methoxylated aminotetralone hydrochloride. No mechanism was proposed for the formation of these products but it would appear that in our case the N-acetyl function or the indole nucleus markedly protects the acetylamino-ketone (LXXIX) from similar deamination and rearrangement. Partial hydrolysis of the N-acetyl function, followed by the rearrangements analogous to those described above, however, might be responsible for the difficulties encountered in obtaining the product in a crystalline form.

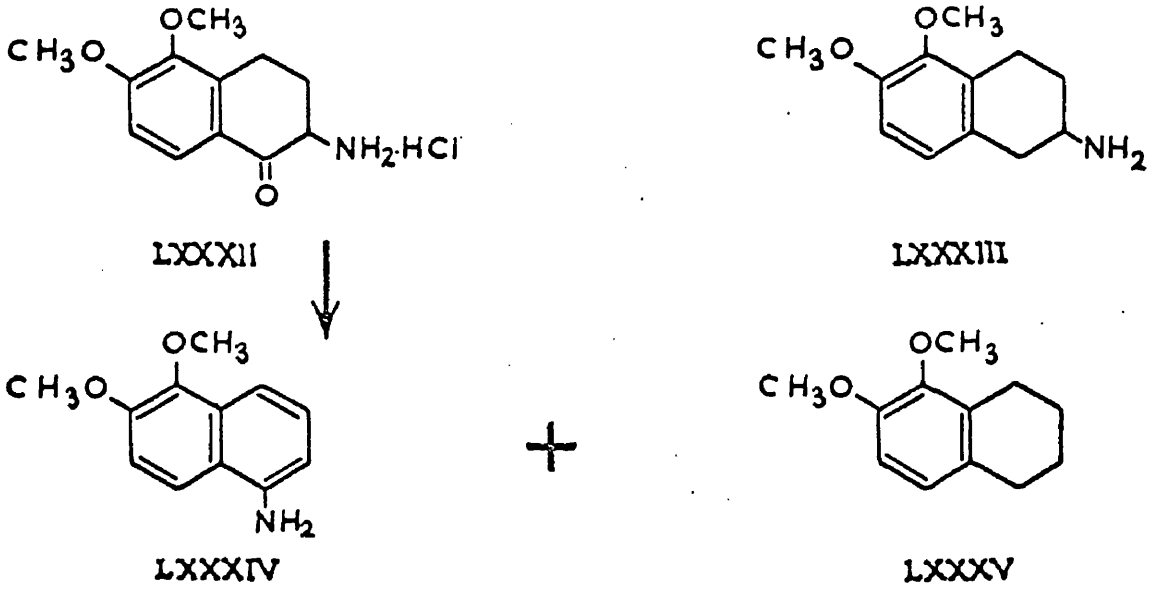


fig. 13

Although insertion of the acetylamino group α to the keto function proceded in low yield, and the Clemmensen reduction gave a non-crystalline product, it was considered that the sequence was capable of application to the synthesis of the 6-hydroxy analogue. The most attractive feature of the route lay in the fact that 4-amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLI) would be only two stages removed from 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX) which would be a useful intermediate for the synthesis of 5-amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLVII). However, as 4-amino-1,3,4,5-tetrahydrobenz[c,d]indole (L) had been prepared earlier by Gould and Jacobs,[176] and the present

investigations as just described had served their purpose in opening up a possible route for the synthesis of the more important 6-hydroxy analogue, further attempts to more fully characterise the 4-amino compound (L) were not undertaken.

The preparation of the corresponding 6-hydroxytetrahydrobenz-[c,d]indoles was initiated by examining the routes available for the synthesis of β-(5-methoxyindol-3-yl)-propionic acid (XCII). 5-Methoxyindole (XCI), prepared according to the methods of Koelsch[199] and of Blaikie and Perkin[200] as shown in fig. 14, was initially chosen as the starting material and its conversion into the required 3-propionic acid examined. The literature methods for the preparation of β-(indol-3-yl)-propionic acid from indole, using acrylic acid[201] and propiolactone[202] were first employed; 5-methoxyindole (XCI) was heated under reflux with acrylic acid in a mixture of acetic acid and acetic anhydride and a satisfactory yield of

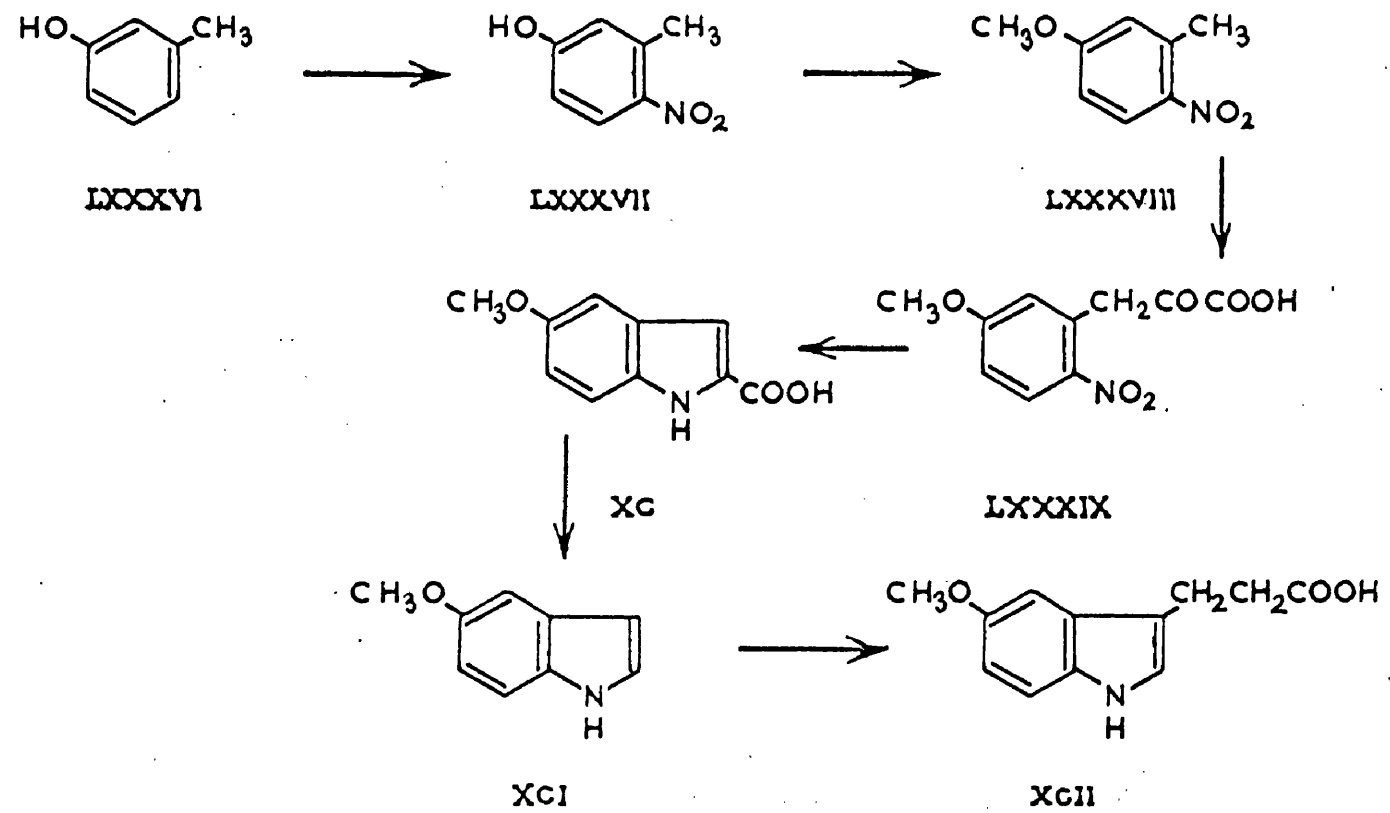


fig. 14

the propionic acid (XCII) was obtained. The method using propiolactone was less satisfactory and, although the infrared spectrum of the product was as expected for the required product, no β-(5-methoxyindol-3-yl)-propionic acid was obtained in a crystalline form. These methods were, however, discarded in favour of the more efficient route initially described by Barret, Perkin and Robinson,[203] and later modified by Renson[189] (see fig. 15):

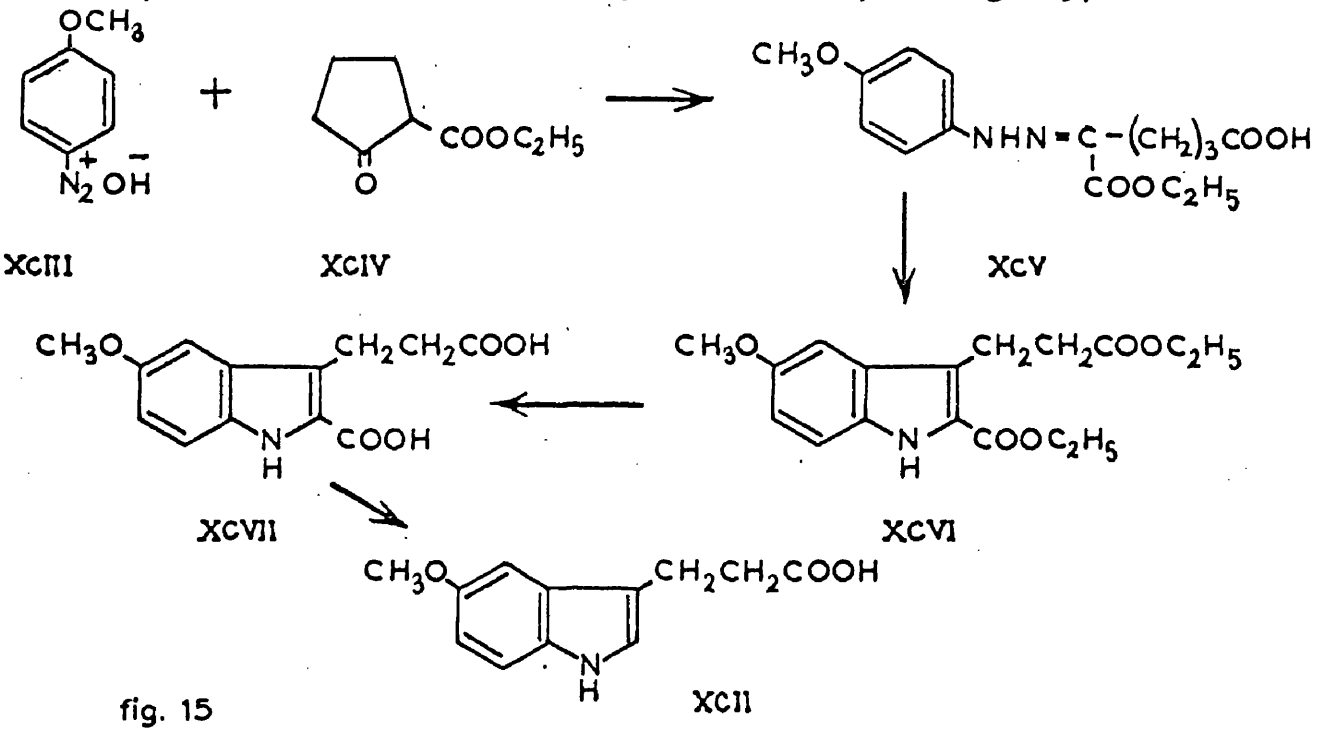


fig. 15

p-methoxyphenyldiazonium hydroxide (XCIII) was condensed with ethyl 2-oxo-cyclopentanecarboxylate (XCIV), prepared from diethyl adipate by a Dieckmann reaction,[204] to give the p-methoxyphenylhydrazone (XCV). Cyclisation of this hydrazone to the indole derivative (XCVI), followed by hydrolysis to the di-acid (XCVII) and subsequent decarboxylation, gave the required β-(5-methoxyindol-3-yl)-propionic acid (XCII). The formation of the phenylhydrazone was carried out at 0° and necessitated the slow addition of the diazo and the ethyl 2-oxo-cyclopentanecarboxylate solutions to a sodium acetate solution at 0°. An efficient method of transference with effective cooling was therefore devised which led to high yields of the p-methoxyphenylhydrazone (XCV).

Shortly after the completion of this stage of the work, what appears to be a simpler synthesis of the methyl ester of β-(5-methoxyindol-3-yl)-propionic acid was reported in the patent literature.[205] p-Methoxyphenylhydrazine is condensed with methyl 3-formylbutyrate in methanol containing sodium acetate, and the product treated with methanol containing hydrogen chloride.

Hydrogenation of β-(5-methoxyindol-3-yl)-propionic acid could not be achieved employing the conditions previously reported for the reduction of the $C_{(2,3)}$ double bond in the corresponding unsubstituted indolepropionic acid; even at elevated temperature and pressure, no reduced material was isolated. Attempts were therefore made to hydrogenate the corresponding methyl ester by the procedure originally described by Blout and Robinson[206] for the conversion of methyl β-(indol-3-yl)-propionate to its indoline derivative. The hydrogenation was carried out in methanol containing a trace of acid with platinum oxide as catalyst. Hydrogen uptake was rapid, even at room temperature and atmospheric pressure, and continued with no apparent break until well in excess of one mole of hydrogen had been absorbed (see fig. 16). Although some

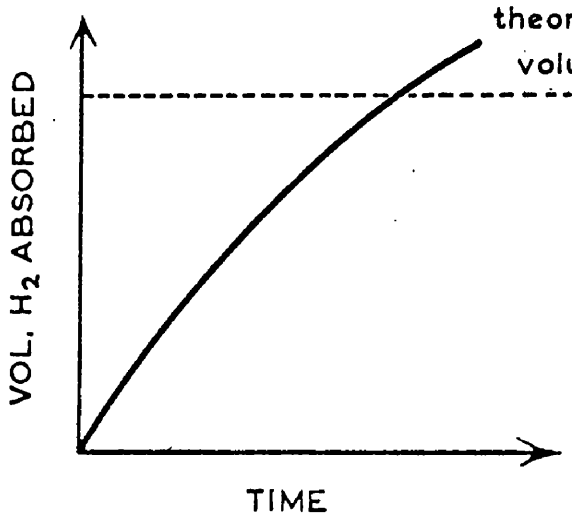


fig. 16

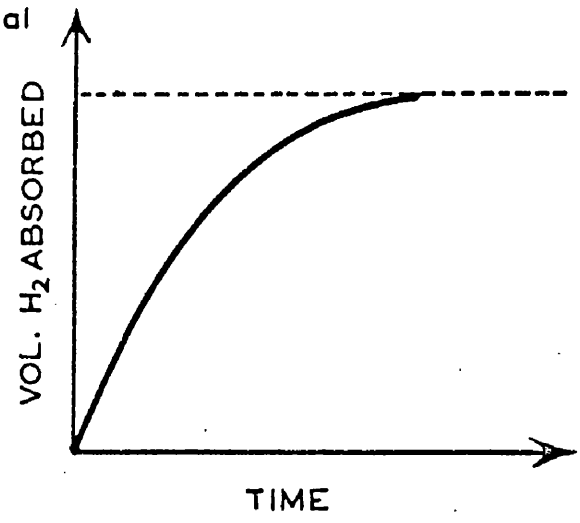


fig. 17

of the required material was isolated in low yield as the N-benzoyl derivative (XCIX), unchanged starting material was also recovered, indicating that reduction was also taking place at other centres. It was apparent that unless a more efficient method of hydrogenation could be found, the syntheses of the two required hydroxy-amino compounds could not be achieved economically by this approach. A detailed study of this hydrogenation was therefore carried out. Catalytic reductions of the free acid, as well as of the derived methyl and ethyl esters were examined, in aqueous basic, and acidic and neutral alcoholic solutions, using Raney nickel, platinum oxide, palladium on carbon, and rhodium on carbon as the catalysts. Raney nickel and palladium on carbon proved insufficiently active, and the hydrogenation proceeded only at a very low rate. Rhodium on carbon, although inducing a rapid hydrogen uptake, brought about unselective hydrogenation. Platinum oxide appeared most efficient for the required reduction and the optimum conditions appeared to be those already described by Blout and Robinson. However, during the course of this work, Smith and Utley[207] reported the smooth reduction of indole, 3-methylindole and various tetrahydrocarbazoles to the corresponding indoline derivatives by hydrogenation at room temperature and atmospheric pressure using platinum oxide catalyst in ethanol and fluoroboric acid. This system was therefore applied to the hydrogenation of β-(5-methoxyindol-3-yl)-propionic acid; although it was rather unsatisfactory for the free acid, the ethyl ester (XCVIII) was smoothly and rapidly reduced. Only one mole of hydrogen was consumed (see fig. 17) and a reproducible high yield of the required indolinepropionic ester was isolated. This was directly hydrolysed in aqueous sodium hydroxide to the free acid which was N-benzoylated *in situ* using the Schotten-Baumann procedure to give β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (XCIX) in a reproducible yield of 62%. It is of interest that after this work was completed,

the preparation of β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid, along with various other derivatives was reported in the patent literature[205] using particularly forcing conditions involving hydrogenation of methyl β-(1-benzoyl-5-methoxyindol-3-yl)-propionate in methanol with Raney nickel as catalyst at 222 atmospheres for 48 hr. It is apparent therefore that the conditions described in the present work represent a much simpler procedure.

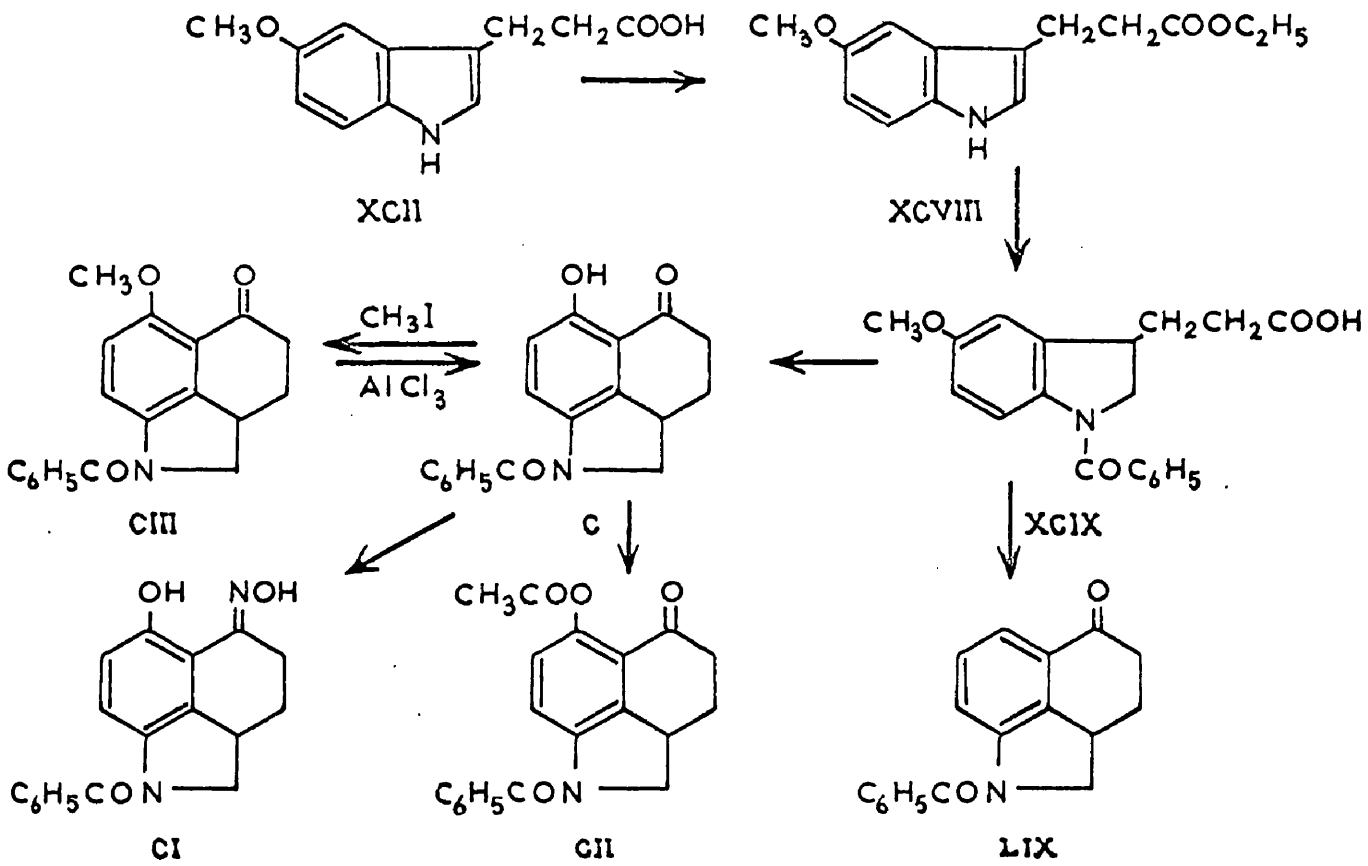


fig. 18

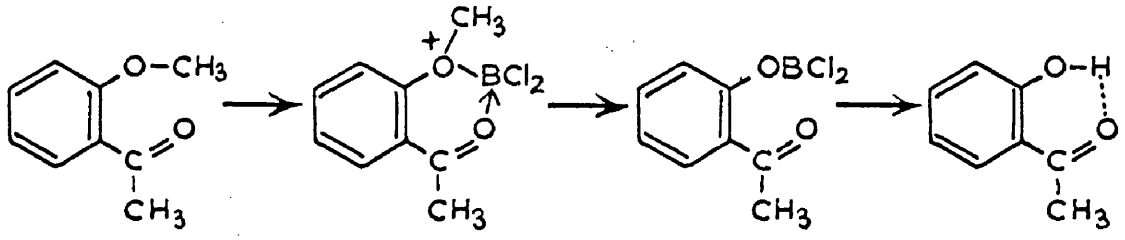
β-(1-Benzoyl-5-methoxyindolin-3-yl)-propionic acid (XCIX) was then converted into its acid chloride by treatment with thionyl chloride, and cyclised by an intramolecular Friedel-Crafts acylation using an analogous procedure to that described above for the cyclisation of β-(1-benzoylindolin-3-yl)-propionic acid (see p. 51). The major product was not the 6-methoxy compound (CIII), but was in

fact 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz [c,d]-indole (C). This was characterised as the oxime (CI), prepared using hydroxylamine hydrochloride and sodium acetate in aqueous ethanolic solution, and as the O-acetyl derivative (CII) which was formed in pyridine by the action of acetyl chloride.

Cleavage of the O-alkyl bond, as observed during this cyclisation, is frequently encountered[190,208] during Friedel-Crafts reactions which involve substitution of the acyl group ortho to the alkoxy group. Dealkylation of ethers in general under acid conditions is well documented and requires no further discussion, but the possible facilitation of this reaction by an adjacent oxo function during an aluminium chloride catalysed reaction deserves some comment. Although aluminium chloride is also known to be capable of demethylating[209] methoxyl groups remote from ketonic functions, the conditions necessary for this process are generally more severe than those employed in the cyclisation of the acid chloride of XCIX. It was of interest therefore to determine whether the neighbouring oxo function formed in the cyclisation was critically influencing the demethylation of the 6-methoxy group, or whether the methoxy group was itself sufficiently labile to suffer cleavage before cyclisation. For this purpose both the methoxy-ketone (CIII) (prepared by the action of methyl iodide on the corresponding hydroxy-ketone (C) under the influence of sodium ethoxide) and β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (XCIX) were each separately subjected to the conditions under which the acid chloride of β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (XCIX) had been cyclised. On examination of the respective products, the methoxy-ketone (CIII) was found to have undergone 40% cleavage to the corresponding phenol (C) whereas the methoxyindolinpropionic acid (XCIX) was recovered unchanged. These observations would indicate that the demethylation occurs during or after cyclisation and not prior to cyclisation. The lower yield of hydroxy-ketone (C)

isolated from the model reaction on the methoxy-ketone (CIII) as compared with that isolated from the cyclisation of β -(1-benzoyl-5-methoxyindolin-3-yl)-propionyl chloride could be explained if the process of cyclisation (attack on the indole 4-position by the acylium ion) was, in some way, assisting demethylation of the neighbouring methoxyl group. Thus with this extra driving force being absent in the preformed methoxy-ketone (CIII), a lower rate of demethylation of this compound would be expected. However, a simpler and more feasible explanation for the difference in demethylation yields is that the acid chloride of XCIX is more freely soluble, and also is more reactive towards aluminium chloride than is the methoxy-ketone (CIII).

Possibly related to these demethylation reactions are those recently reported[210] for the structurally similar methoxy-ketones of the type CIV, which on treatment with boron trichloride yield the corresponding phenols. It has been proposed that boron trichloride forms a cyclic complex (CV) involving the keto function, and this assists demethylation to give the hydroxy-ketone (CVII). The similar electronic configuration of boron trichloride and aluminium trichloride and their similar acceptor properties would suggest that a scheme analogous to that shown in fig. 19 might possibly be operative with aluminium chloride. This would account



CIV CV CVI CVII

fig. 19

for the facilitated demethylation observed in the methoxy-ketone (CIII) and similarly in the cyclisation product of β-(1-benzoyl-5-methoxyindolin-3-yl)-propionyl chloride.

The second product, isolated in low yield, from the cyclisation of the acid chloride of β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (XCIX) was the demethoxylated compound 1-benzoyl-5-oxo-1,2,-2a,3,4,5-hexahydrobenz[c,d]indole (LIX), identical in all respects with an authentic sample prepared as described on p. 51. Although cleavage of an O-alkyl bond of ethers is commonly encountered during Friedel-Crafts reactions the formation of the demethoxylated material LIX, resulting from O-aryl cleavage, is difficult to rationalise. Recently W. Schäfer and Leute[211] have reported that acetylation of 1,2,4,5-tetramethoxybenzene results in the formation of 2,4,5-trimethoxyacetophenone but although a methoxy group is lost during this reaction it is by replacement rather than by a simple O-aryl bond cleavage.

The intramolecular hydrogen bonding which is reported[190] to exist in 6-hydroxy-5-oxo-1,2-dimethyl-1,3,4,5-tetrahydrobenz[c,d]-indole was observed in the hydroxy-ketone (C). The infrared spectrum of this material in chloroform possessed no absorption above 3200 $cm.^{-1}$, indicating that the O-H stretching frequency had been significantly lowered. Moreover, the absorption due to the carbonyl stretching mode of the 5-keto group was lowered to 1660 $cm.^{-1}$ as compared with that at 1680 $cm.^{-1}$ observed in the non-hydroxylated material (LIX). Methylation, acetylation or benzylation yielded products, the infrared spectra of which indicated that hydrogen bonding had been destroyed and the keto absorption frequency was increased to 1670 $cm.^{-1}$ Further evidence for the existence of hydrogen bonding was furnished by the difficulties experienced in methylating or benzylating the 6-hydroxyl group. This finding is consistent with those of Mann and Tetlow[190] who obtained only 30% methylation of 6-hydroxy-5-oxo-

1,2-dimethyl-1,3,4,5-tetrahydrobenz[c,d]indole, and of Hunt and Rickard[212] who found 1-acetyl-7-hydroxyindoline considerably more difficult to methylate than the corresponding 5- or 6-hydroxy derivatives; this they attributed to hydrogen bonding between the 7-hydroxyl and the carbonyl of the N-acetyl function.

The hydroxy-ketone (C) was debenzoylated by treatment with a mixture of acetic acid and hydrochloric acid and the resulting indoline derivative (CVIII) dehydrogenated in good yield to the corresponding indole (CIX) by means of manganese dioxide in methylene chloride. The increase in conjugation resulting from dehydrogenation lowered the frequency of the 5-carbonyl stretching absorption still further to 1640cm.$^{-1}$ A similar, though less marked, decrease in frequency was observed on debenzoylation and aromatisation of 1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LIX); the absorption due to the keto function of the starting material appears at 1680cm.$^{-1}$ whereas the corresponding absorption in 5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (LXI) occurs at 1670cm.$^{-1}$ It is to be noted that where the keto function is not in conjugation with the aromatic system, as in 1-benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXVII), absorption occurs at a frequency of 1700cm.$^{-1}$

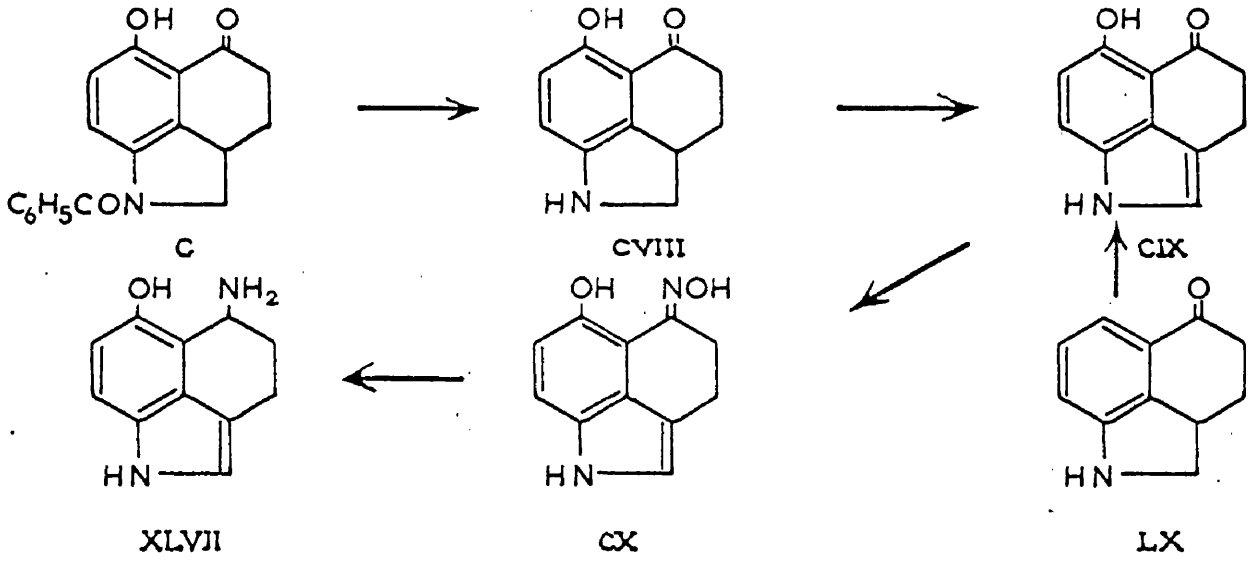


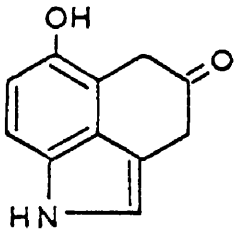
fig. 20

The tricyclic indole (CIX) was converted into the oxime (CX), using hydroxylamine hydrochloride and sodium acetate in aqueous ethanol, and the product was reduced to the required amine (XLVII) with lithium aluminium hydride in tetrahydrofuran. The intermediate compounds C - CX proved stable and no special precautions were required during their preparation. The amine (XLVII), however, was extremely unstable in the presence of oxygen and the reduction and the isolation of the product were therefore carried out under nitrogen. As it was not possible to recrystallise this amine without considerable decomposition, it was converted into the more stable hydrochloride by treatment with hydrogen chloride in a mixture of benzene and ether under nitrogen. Under the acidic conditions of formation this hydrochloride was also unstable, but when pure it was considerably less labile. The precipitated hydrochloride was freed from excess hydrogen chloride by repeated centrifugation and decantation using sodium-dried ether. The hydrochloride was dried in a stream of nitrogen and recrystallised from ethanol to give colourless cubic crystals of 5-amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLVII) hydrochloride. The crystals quickly darkened and decomposed on heating so that no melting point for this material could be recorded. Analyses of the hydrochloride were inconsistent and varied considerably according to the time and temperature of drying, and to the time of analysis. This was attributed to a slow oxidation of the material in the air. Attempts to isolate stable derivatives, such as the O,N-diacetyl and O,N-dibenzoyl compounds, for analysis were unsuccessful.

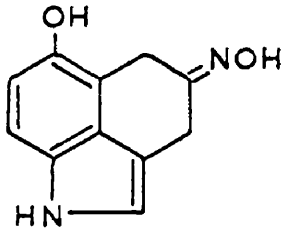
In an attempt to simplify the reaction sequence leading to 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX), the simultaneous hydroxylation and dehydrogenation of the corresponding non-hydroxylated indoline (LX) was examined. This approach to 5-hydroxyindoles has been developed and widely employed by

Teuber[213] who has successfully hydroxylated and indolised dihydroskatole, 2-phenylindoline and a variety of other indolines using Fremy's salt. This method has also been used for the preparation[214] of what was thought to be 12-hydroxylysergic acid from 2,3-dihydrolysergic acid. Although this method proved successful when applied to the indoline (LX), converting it into 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX), the yield was too low to make the method of practical application. The conditions of the reaction were modified within small limits and the reaction times widely varied in an attempt to improve the yield but without success. The protected indoline, 1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole (LIX), was also treated with Fremy's salt but only unchanged starting material was isolated.

A most valuable intermediate for the preparation of 4-amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLI) would be the hydroxy-ketone (CXI); the 4-keto-indoline (LXIX) and the oxime (LXXI)(see p.54) were therefore both treated with Fremys salt in an attempt to prepare this material, or better the oxime (CXII), but without success. Both chemical and spectral evidence indicated that the major product of the reactions was starting material contaminated with various decomposition products. No material possessing absorption in the infrared attributable to an indolic N-H was isolated.



CXI

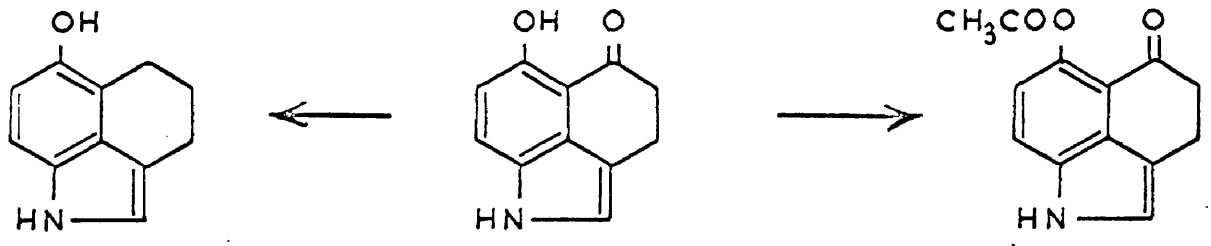


CXII

Because hydroxylation of the indoline (LXIX) or the corresponding oxime (LXXI) was not possible under the conditions employed,

attention was concentrated upon the direct nitrosylation of 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX). The product of nitrosylation, after reduction to the α-amino-ketone hydrochloride, diacetylation, and removal of the 5-oxo function should, in theory, furnish the diacetyl derivative of the required 4-amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLI). Before examining the nitrosylation stage, trial Clemmensen reductions were conducted using 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]-indole (CIX) as a model compound to ensure that the hydrogen bonded 5-oxo function could be preferentially reduced without the simulataneous reduction of the indolic $C_{(2,3)}$ double bond. Conditions similar to those employed for the reduction of the non-hydroxylated material (LXI) gave the required 6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (CXIII)[215] in 50% yield. Thus the intramolecular hydrogen bonding in the starting material did not apparently have any significant effect upon the rate of reduction of the 5-oxo-function.

Attempts to introduce the acetylamino group adjacent to the oxo function of the hydroxy-ketone (CIX) resulted in the formation of a black tarry product from which no pure material could be isolated. Chromatography of the material on silica gel gave a series of fractions, some of which possessed an infrared absorption maximum attributable to the indolic N-H, but none possessed absorption in the 3400 or 1650cm.$^{-1}$ region, characteristic of a secondary amide.



CXIII CIX CXIV

fig. 21

The nitrosylation, reduction and acetylation reactions were repeated on the O-acetyl derivative (CXIV) in an attempt to stabilise the products but with no more success. The only crystalline solid isolated from this reaction sequence was the hydroxy-ketone (CIX) indicating that the acetoxy group was too labile to be used as a protecting group in this reaction. Infrared evidence for the presence of any material possessing an amide function was lacking. The benzyl group, which should be less labile than the acetyl function, and yet easily removed by hydrogenation, was therefore employed as a protecting group. Attempts to benzylate the hydroxy-ketone (CIX) directly to give the corresponding benzyloxy derivative (CXVII) were unsuccessful. Owing to the intramolecular hydrogen bonding between the hydroxyl group and the 5-keto function in CIX, prolonged treatment with benzyl chloride in aqueous ethanolic sodium hydroxide was necessary to effect benzylation. Under these conditions sufficient decomposition would appear to have taken place to prevent the effective isolation of the required benzyloxy product (CXVII). This difficulty, however, was resolved using an alternative route to the benzyloxy-ketone (CXVII) involving the benzylation of the protected 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (C). The rate of benzylation of this material was slow, but the reaction was not accompanied by decomposition. Prolonged and repeated treatment of the hydroxy compound (C) was therefore possible, and an almost complete conversion of hydroxy-ketone (C) into benzyloxy-ketone (CXV) was recorded. The product (CXV) was hydrolysed using aqueous ethanolic sodium hydroxide and the resulting indoline (CXVI), obtained in excellent yield, was dehydrogenated by treatment with active manganese dioxide in methylene chloride. The impure product was separated from insoluble polymeric material and chromatographed on silica gel; elution with chloroform gave the required 6-benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole

(CXVII) in satisfactory yield. A sample of this material was readily debenzylated to the hydroxy-ketone (CIX) by hydrogenation in methanol using palladium-on-carbon catalyst.

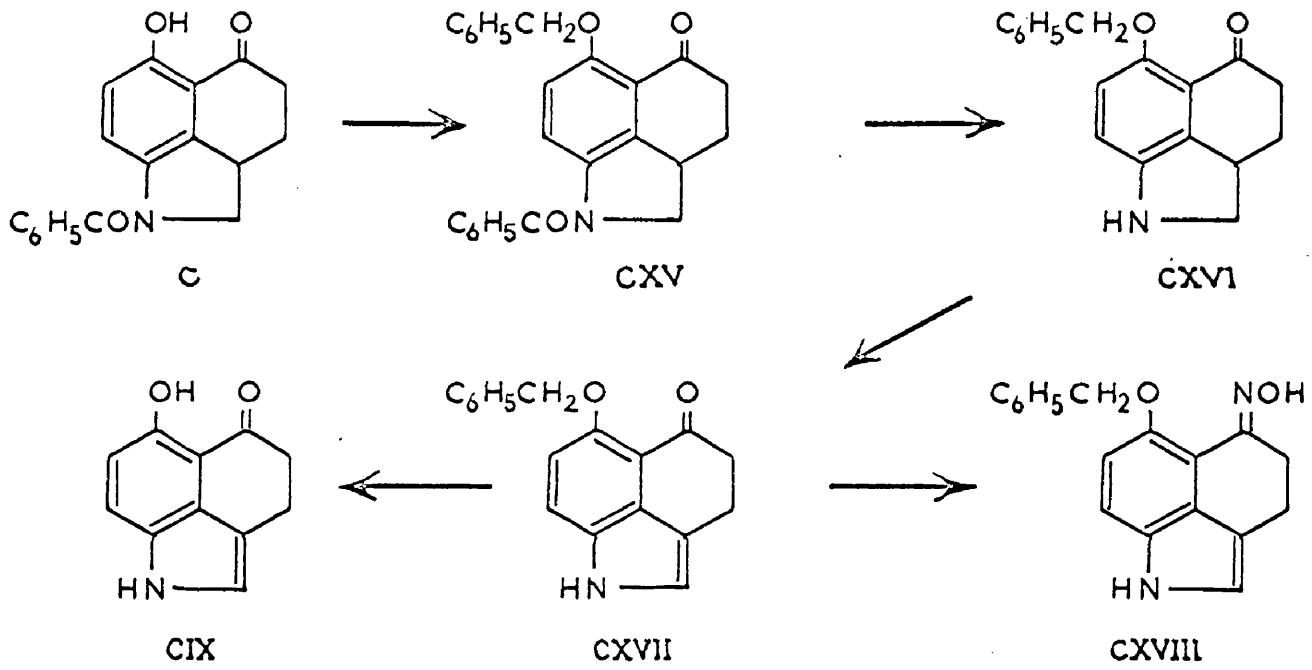
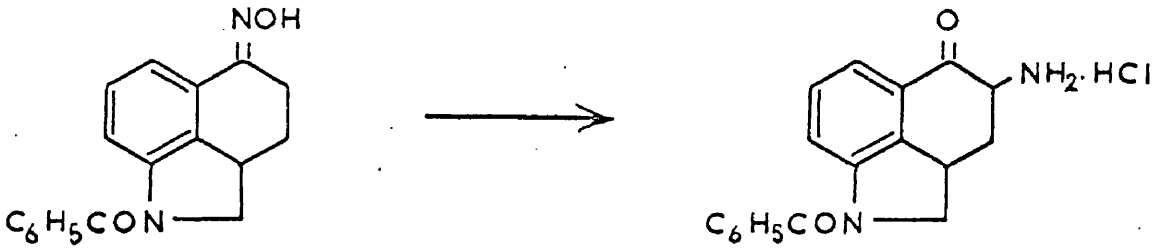


fig. 22

Treatment of 6-benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]-indole with amyl nitrite, and subsequent in situ reduction with zinc and acetic acid, and acetylation gave a crude product which possessed five distinct, strong infrared absorption maxima in the 1650-1800cm.$^{-1}$ region. Thin layer chromatography indicated that this dark oil was a complex mixture, and repeated column chromatography failed to give any satisfactory separation. No material possessing an infrared spectrum consistent with that of the required 4-acetamido-6-benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz-[c,d]indole could be detected. Difficulties were also experienced in attempts to synthesise the required amino-ketone by rearrangement of the p-tosyl derivatives of 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole oxime (CX) and the 6-benzyloxy derivative

(CXVIII). Although this approach was successfully employed by Woodward and his co-workers[183] for the conversion of 1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole oxime (CXIX) into the corresponding amino-ketone (CXX), it was completely unsatisfactory for the unprotected indole derivatives of the present work. The



CXIX CXX

fig. 23

oximes were prepared in the usual manner with hydroxylamine hydrochloride, but attempts to convert these into their p-tosyl derivatives by treatment with p-toluenesulphonyl chloride gave solids which could not be purified by crystallisation and which, from infrared evidence, appeared to contain little, if any, indolic material. Attempts to rearrange the impure tosylate with potassium ethoxide, were unsuccessful; no products possessing a keto function or an indolic N-H group were isolated.

The difficulties which were encountered in the preparation of these tetrahydrobenz[c,d]indole derivatives and which led to the eventual abandonment of attempts to synthesise the 6-hydroxy-4-amino analogue (XLI) illustrate the very labile nature of the unprotected tetrahydrobenz[c,d]indole system under a variety of conditions. Oxidation to deeply coloured and frequently amorphous products and rearrangement to naphthalene derivatives seem to be two particularly favoured processes. The successful synthesis of 4-amino-1,3,4,5-tetrahydrobenz[c,d]indole (L) from the corresponding aminonaphthostyril by Gould and Jacobs,[176] however, indicates

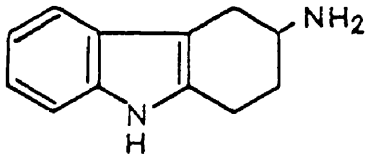
that the intermediate stages in the preparation of 4-amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLI) might be better carried out on a suitably substituted naphthalene derivative which could be reduced to the required tetrahydrobenz[c,d]indole in the final stage of the reaction sequence. A re-examination of the possibility of using a suitably 3,5-disubstituted-8-amino-naphthoic acid would therefore be considered as the next worthwhile step towards the synthesis of this compound. An interesting paper, in this respect, has recently been published by Girardet and Russo[216] in which 5-hydroxynaphthoic acid is converted into 5-hydroxy-8-aminonaphthoic acid; an additional 3-substituent capable of conversion into an amino group would make a very valuable intermediate.

Synthesis of Derivatives of Tetrahydrocarbazole

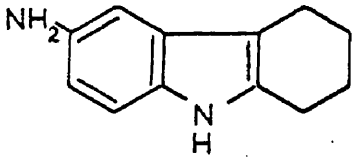
Although a variety of amino- and hydroxy- substituted tetrahydrocarbazoles have been reported in the literature and many tested for pharmacological activity, none possessing a structure directly related to the 5-HT unit appear to have been synthesised. 3-Amino-1,2,3,4-tetrahydrocarbazole (CXXI) which lacks the 6-hydroxyl group and thus is a tryptamine analogue, has been prepared and shown to possess MAO inhibitory action.[217] 6-Aminotetrahydrocarbazole (CXXII) and its derivatives have been synthesised by Woolley and Shaw[218] as antimetabolites of 5-HT. 1-Amino-6-methoxy-1,2,3,4-tetrahydrocarbazole (CXXIII), of interest because of its structural similarity to the pharmacologically active 5-methoxytryptamine has also recently been prepared,[219] but its pharmacological properties do not appear to have been examined.

Preparations of 6-hydroxy-1,2,3,4-tetrahydrocarbazole (CXXIV) include those of Teuber[220] by hydroxylation of the corresponding hexahydrocarbazole; of Beer, Broadhurst and Robertson[221] by de-

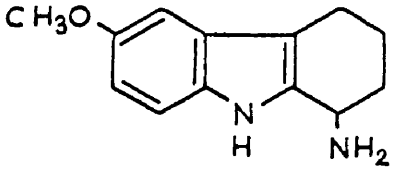
benzylation of the 6-benzyloxy compound; and of Milne and Tomlinson[222] who, using pyridine hydrochloride, demethylated 6-methoxy-1,2,3,4-tetrahydrocarbazole. Little has been reported concerning the pharmacological activity of this substance.



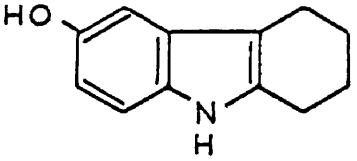
CXXI



CXXII



CXXIII



CXXIV

The most commonly employed procedure for the synthesis of the tetrahydrocarbazole system makes use of the Fischer indole reaction;[223] a suitably substituted phenylhydrazine is condensed with a cyclohexanone derivative and the product cyclised under acid conditions. A suitably para-substituted phenylhydrazine and a cyclohexanone bearing a 4-substituent, which could readily be converted into an amino group, were therefore required if this method was to be applied to the preparation of 3-amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole (XLII). Cyclisation of the mono p-methoxy- or p-benzyloxy-phenylhydrazone of cyclohexan-1,4-dione to give a 3-oxo-1,2,3,4-tetrahydrocarbazole was considered initially as a route to the required 3-amino-tetrahydrocarbazole but, although successful analogous approaches have been made to 1- and 4-oxo-1,2,3,4-tetrahydrocarbazoles[224] through cyclisation of the corresponding monophenylhydrazones of cyclohexan-1,2- and -1,

3-diones, this method could not be successfully applied to the synthesis of the corresponding 3-oxo compound. Unlike cyclohexan-1,2- and -1,3-dione, the 1,4-dione does not form a monophenylhydrazone but on treatment with one equivalent of phenylhydrazine yields a mixture of dihydrazone and unreacted starting material.[225] Cyclisation of the dihydrazone in sulphuric acid has been shown to yield some 3-oxo-tetrahydrocarbazole as a minor product by cleavage of one C=N bond but the method is not of practical application. A substituted cyclohexanone possessing a protected 4-keto group, or a 4-substituent capable of being converted into a keto function, was therefore required. Harley-Mason and Pavri[225] have prepared 3-hydroxy-1,2,3,4-tetrahydrocarbazole by cyclisation of the phenylhydrazone of 4-benzoyloxycyclohexanone followed by hydrolysis of the 3-benzoyl function, but they reported that all attempts to oxidise this hydroxy group to the keto function were unsuccessful. None of the conditions or reagents employed in these attempts, however, were reported, and as there are examples of the oxidation of substituted cyclohexanols to the corresponding cyclohexanones in the literature,[226] it was considered worthwhile to re-examine this oxidation.

4-Acetoxycyclohexanone (CXXV), which has been obtained in good yield by Aldersley, Burkhardt and their co-workers,[227] was selected as the starting material for this present synthesis. Cyclohexan-1,4-diol was diacetylated, selectively hydrolysed to the mono-acetate, and the newly generated hydroxyl group oxidised with chromic acid to give the required acetoxy-ketone (CXXV).

p-Benzyloxyphenylhydrazine hydrochloride (CXXVI) was chosen in preference to p-methoxyphenylhydrazine as the second component of the Fischer reaction as it was apparent from the work of Beer, Broadhurst and Robertson[221] that the 6-hydroxy-1,2,3,4-tetrahydrocarbazole would be more efficiently obtained by debenzylation

than by demethylation. The hydrazine was prepared as the hydrochloride (CXXVI) according to the method of Mentzer, Beaudet and Bory;[228] p-benzyloxynitrobenzene, prepared by the action of benzyl chloride upon p-nitrophenol, was reduced using Raney nickel and hydrazine hydrate to the amine, which was subsequently diazotised and further reduced with stannous chloride to the required phenylhydrazine hydrochloride (CXXVI).

p-Benzyloxyphenylhydrazine hydrochloride (CXXVI), on refluxing with 4-acetoxycyclohexanone (CXXV) in glacial acetic acid containing sodium acetate, gave the required acetoxy-benzyloxy-1,-2,3,4-tetrahydrocarbazole (CXXVII). Hydrolysis of the 3-acetoxy group, using aqueous ethanolic sodium hydroxide gave, in almost quantitative yield, 6-benzyloxy-3-hydroxy-1,2,3,4-tetrahydrocarbazole (CXXVIII). Consistent with the difficulties experienced by

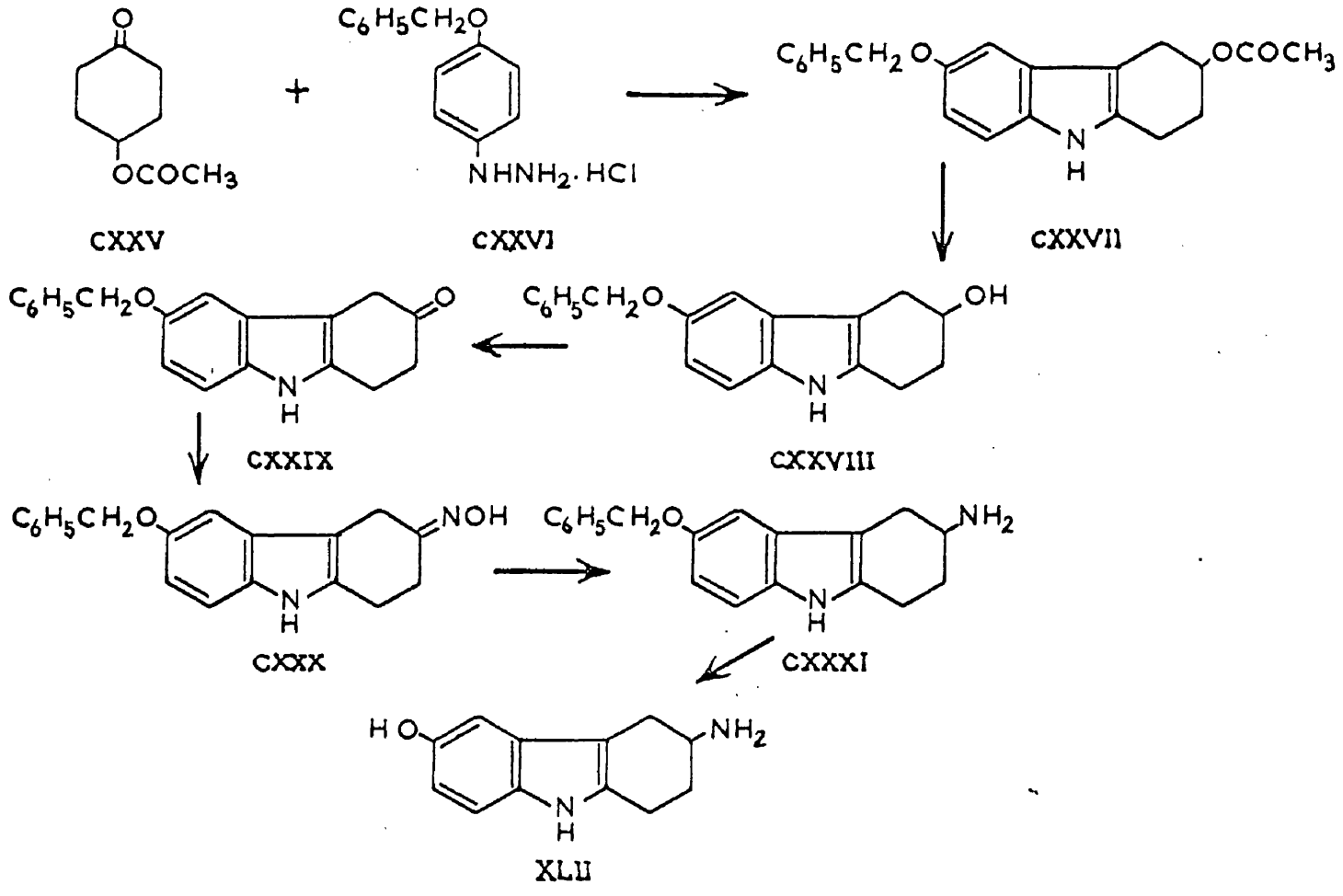


fig. 24

Harley-Mason and Pavri[225] in the oxidation of 3-hydroxy-1,2,3,4-tetrahydrocarbazole, initial investigations into the oxidation of the 6-benzyloxy analogue (CXXVIII) also failed. However, after several unsuccessful attempts the hydroxy compound (CXXVIII) was oxidised smoothly and in a satisfactory yield to the ketone (CXXIX) using aluminium isopropoxide and cyclohexanone in toluene.[226] Very recently, Teuber and his co-workers[229] also failed to oxidise 3-hydroxy-1,2,3,4-tetrahydrocarbazole to the corresponding ketone; under their conditions the hydroxycarbazole and unchanged starting material were isolated.

Treatment of the ketone (CXXIX) with hydroxylamine hydrochloride and sodium acetate in aqueous ethanol gave the oxime (CXXX) which was reduced to the benzyloxy-amine (CXXXI) using lithium aluminium hydride in ether. The N-acetyl derivative, formed by the action of acetic anhydride upon CXXXI, and the hydrochloride, precipitated in ether by treatment of CXXXI with hydrogen chloride, were prepared for the purposes of characterisation. Debenzylation of 3-amino-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (CXXXI) in almost quantitative yield was effected by hydrogenation at atmospheric pressure in methanol using palladium on carbon as catalyst. Difficulties were encountered in the recrystallisation of the 3-amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole (XLII); although it could be recrystallised it darkened quickly on heating in most solvents. It was, however, readily purified by fractional sublimation, and appeared to be stable in an atmosphere of nitrogen.

Synthesis of Derivatives of Cyclopent[b]indole

The route chosen to 3-amino-7-hydroxy-1,2,3,4-tetrahydrocyclopent[b]indole (XLVIII) was that shown in fig. 25 and involved the preparation of two known intermediates, the p-methoxyphenylhydrazone (CXXXIII) and the cyclisation product (CXXXIV). These were prepared

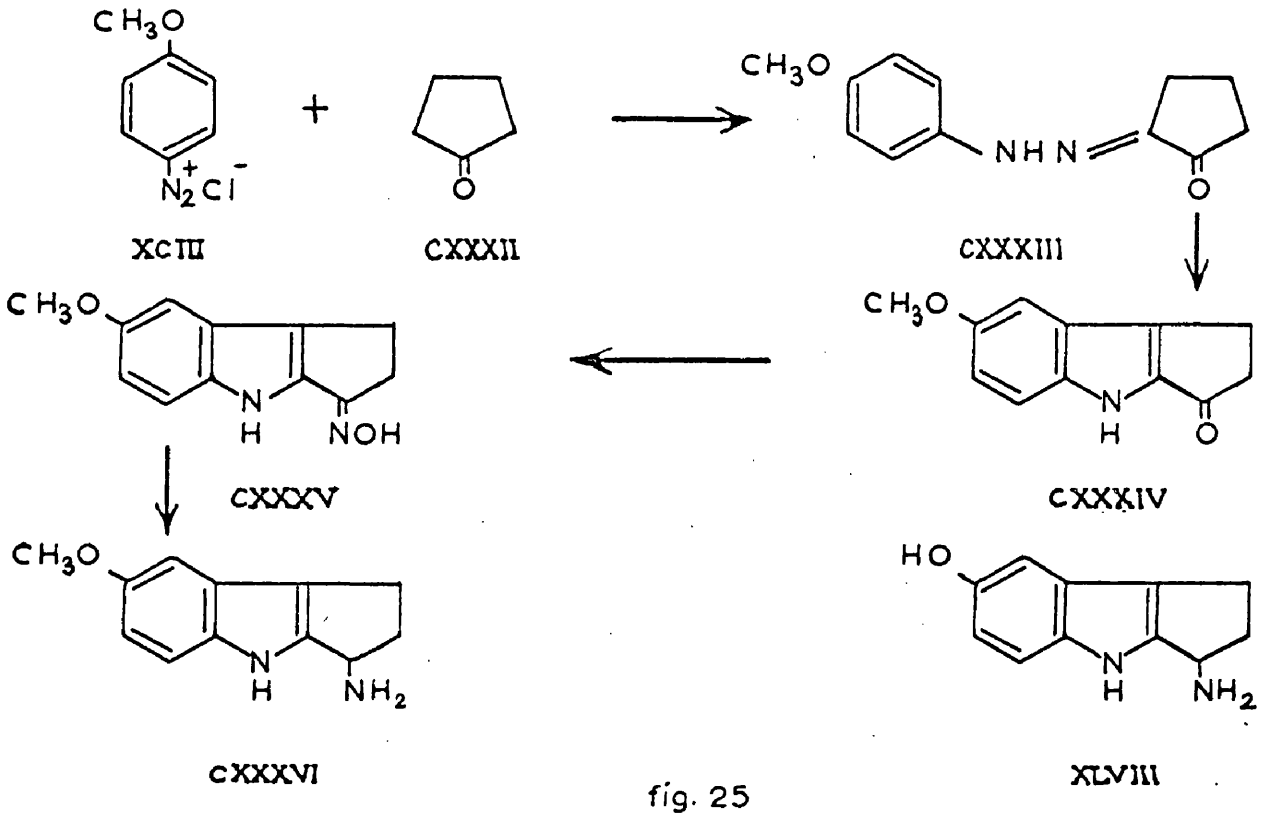


fig. 25

by Renson[189] during his investigation into the mode of cyclisation of β-(5-methoxyindol-3-yl)-propionic acid, by an adaptation of the method described by Elks, Elliot and Hems[230] for the synthesis of 3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole. The mono p-methoxyphenylhydrazone (CXXXIII), prepared by a Japp-Klingermann reaction using p-methoxyphenyldiazonium chloride (XCIII) and cyclopentanone (CXXXII) in the presence of ethyl formate and sodium ethoxide, was cyclised to the cyclopent[b]indole (CXXXIV) in refluxing aqueous sulphuric acid. Although the conditions employed for the preparation of the p-methoxyphenylhydrazone (CXXXIII) and the ketone (CXXXIV) were the same as those described by Renson,[189] and moreover, although the melting points corresponded to those reported by this author, some doubt arose concerning the authenticity of the ketone (CXXXIV) since the infrared data recorded by us for this compound was not consistent with that in the literature. Renson reported equal maxima at 3490 and 1750cm.$^{-1}$ which he attributed to the indole N-H and the 3-keto function respectively, whereas

our specimen of this ketone showed weak absorption at 3480cm.$^{-1}$ attributable to the indole N-H and a considerably stronger absorption at 1675cm.$^{-1}$ ascribable to the carbonyl stretching mode. However, analyses of the p-methoxyphenylhydrazone (CXXXIII), the ketone (CXXXIV), and the products of later stages in the route, indicated that the ketonic material prepared by us did have the required structure.

In agreement with the reports of earlier workers[189,230] investigating this system, the cyclisation of the p-methoxyphenylhydrazone (CXXXIII) occurred in a low and variable yield. This variation in yield from one cyclisation reaction to another on the same material was, almost certainly, due to the difficulties associated with duplicating the reaction conditions of the heterogeneous system. An attempt to improve this situation by the addition of ethanol to render the mixture homogenous was unsuccessful, as were similar attempts using a wide range of acids and solvents. Less acidic solvents, such as glacial acetic acid and dilute mineral acids were ineffective and mainly unchanged starting material was recovered, whereas more forcing conditions employing phosphoric acid and more concentrated mineral acids gave predominently decomposition products. Prolonged reaction also frequently led to decomposition products.

Because of the persistently low yields experienced in the cyclisation of the phenylhydrazone other routes to the cyclopent-[b]indole system, such as intramolecular acylation of β-(5-methoxyindol-3-yl)-propionic acid, and a Dieckmann cyclisation of ethyl β-(2-ethoxycarbonyl-5-methoxyindol-3-yl)-propionate, were considered. However, attempts by other workers to employ these reactions in the non-methoxylated series would indicate that these approaches would be even less efficient than the cyclisation of

the phenylhydrazone (CXXXIII). The latter procedure was therefore retained for the preparation of the keto intermediate (CXXXIV).

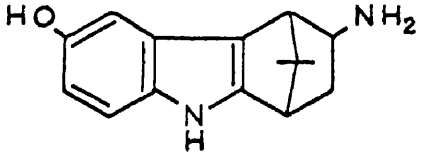
The ketone (CXXXIV) was extremely resistant to oximation; treatment with hydroxylamine hydrochloride in refluxing pyridine for 30hr. was necessary to effect the conversion, and even under these forcing conditions 20% unchanged starting material was recovered from the product. This was recycled and a high yield of the oxime (CXXXV) was eventually obtained. Reduction of the oxime in dry tetrahydrofuran using lithium aluminium hydride gave the stable 3-amino-7-methoxy-1,2,3,4-tetrahydrocyclopent[b]indole (CXXXVI). Attempts were made to demethylate this material to the required hydroxy-amine (XLVIII), using hydrobromic acid,[215,231] aluminium chloride[209] in a variety of solvents, and pyridine hydrochloride[232] but these resulted in insoluble black tars devoid of infrared absorption in the 3470cm.$^{-1}$ region characteristic of the indolic N-H. The most promising results were obtained by treatment of the methoxy-amine (CXXXVI) with boron tribromide in methylene chloride at -80°;[233] a crystalline solid having the chemical properties to be expected of a hydroxy-amine was isolated but the yield was extremely low and could not be increased sufficiently to make characterisation of the product possible.

To overcome the problems associated with removal of the methyl group attempts were made to prepare 3-amino-7-benzyloxy-1,2,3,4-tetrahydrocyclopent[b]indole which, it was thought, would readily undergo debenzylation on hydrogenation. This approach, however, was not of practical application owing to the extremely low yield obtained on cyclisation of the mono p-benzyloxyphenylhydrazone of cyclopentan-1,2-dione. Insufficient of the required 7-benzyloxy-3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole could be prepared to continue with this route.

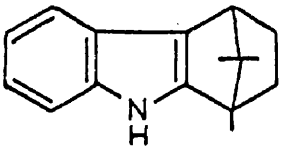
Owing to the difficuties encountered during attempts to prepare the unprotected 3-amino-7-hydroxy-1,2,3,4-tetrahydrocyclopent[b]indole (XLVIII), original plans to synthesise the corresponding 2-amino derivative (XLIII) were abandoned.

Comments Concerning the Synthesis of Other 5-HT Analogues of the Series

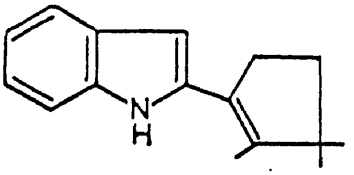
Early unsuccessful attempts to prepare the bicyclo[2,2,1]heptan[b]indole (XLV) were modelled upon the reported[234] cyclisation of the phenylhydrazone of camphor into the camphor-indole (CXXXVII). The Fischer indolisation employed in this synthesis, however, has recently been shown[235] to give the 2-substituted indole (CXXXVIII) and not the claimed camphor-indole. Synthetic work towards the bicyclo[2.2.1]heptan[b]indole was therefore



XLV



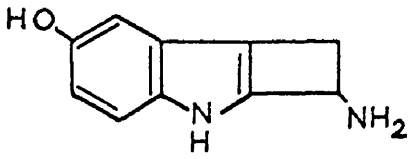
CXXXVII



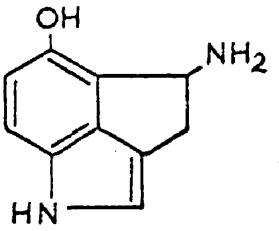
CXXXVIII

discontinued after this observation was published as an entirely new approach to the bridged system would have been required.

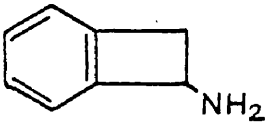
No attempt was made to prepare the cyclobutan-indole (XLIV) or the cyclopent[c,d]indole (XLVI) as it was thought that the syntheses of these sterically strained compounds would be accompanied by even greater synthetic difficulties than those encountered during the syntheses of the benz[c,d]indole derivatives. These compounds were also considered to be of less pharmacological interest than the LSD-like benz[c,d]indoles. An approach which might furnish the cyclobutene derivative (XLIV), however, would



XLIV



XLVI



CXXXIX

be one based upon the successful synthesis of 1-aminobenzocyclobutene (CXXXIX) by Skorcz and Robertson.[236] This synthesis was based on the procedures of Bunnett and Skorcz[237] and of Horner, Kirmse and Muth[238] and employed 2-chlorocyanoethylbenzene as the starting material. Probably the most convenient starting point for the preparation of the cyclobutanindole (XLIV) would be 5-benzyloxy-2-chloro-3-cyanoethylindole.

EXPERIMENTAL

General.- Infrared spectra were measured in chloroform solutions except where otherwise stated with a Perkin Elmer 237 instrument. Melting points are uncorrected and were recorded on a Kofler hot-stage apparatus.

β-(1-Benzoylindolin-3-yl)-propionic Acid (LVIII).- To a solution of β-(indol-3-yl)-propionic acid (25 g.) in N aqueous sodium hydroxide (150 ml.) was added Raney nickel[239] (50 g.) and the mixture shaken in an atmosphere of hydrogen at atmospheric pressure and room temperature until no more hydrogen was absorbed (2,300 ml.). Decolourising charcoal was used to deactivate the nickel, and the filtered solution made acidic with concentrated hydrochloric acid. Unreacted precipitated β-(indol-3-yl)-propionic acid (1.5 g.) was filtered off, and the filtrate made basic with sufficient concentrated aqueous sodium hydroxide to give an approximately 10% sodium hydroxide solution. Benzoyl chloride (22 ml.) was slowly added to the cooled (0°) basic solution and the mixture shaken for 3 hr. at room temperature. Acidification of this solution, cooled to 0°, with concentrated hydrochloric acid gave a mixture of impure product and benzoic acid. After filtration, benzoic acid was removed by repeated washing with hot water, and the remaining solid recrystallised from ethanol to give β-(1-benzoylindolin-3-yl)-propionic acid (24 g., 72%), m.p. 152-153° (lit.,[183] 151-153°).

1-Benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LIX).- β-(1-Benzoylindolin-3-yl)-propionic acid (30 g.) was dissolved in distilled thionyl chloride (50 ml.) and the mixture kept at room temperature for 30 min. and then at 50° for a further 30 min. After distillation of excess thionyl chloride at reduced pressure, a solution of the impure acid chloride in dry carbon disulphide (55 ml.) was added dropwise over 45 min. to a stirred suspension of anhydrous

aluminium chloride (62.5 g.) in carbon disulphide (450 ml.) cooled to 0°. When addition was complete, the reaction mixture was allowed to attain room temperature over 30 min. and then refluxed with continued stirring for a further 1 hr. The complex formed was decomposed by the addition of ice (125 g.), concentrated hydrochloric acid (62.5 ml.) and water (125 ml.) to the stirred and well-cooled mixture. Carbon disulphide was removed at reduced pressure, the organic material extracted into benzene, and the benzene extract, after washing with 2N aqueous sodium hydroxide and water, was dried over anhydrous sodium sulphate. The solvent was distilled at reduced pressure and the residue recrystallised from benzene to give 1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz-[c,d]indole (24 g., 85%), m.p. 146-148° (lit.,[183] 146-147°).

5-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LX).- The benzoyl derivative (LIX) was hydrolysed as reported in the literature[183] using a mixture of glacial acetic acid and concentrated hydrochloric acid to give 5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole in 80% yield, m.p. 124-126° (lit.,[183] 124-126°).

5-Oxo-1,3,4,5-tetrahydrobenz[c,d]indole (LXI).- The indoline derivative (LX) was dehydrogenated using active manganese dioxide[240] according to the method of Jansen, Johnson and Surtees[191] to give 5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole in 48% yield, m.p. 157-161° (lit.,[183] 160-161°).

5-Oxo-1,3,4,5-tetrahydrobenz[c,d]indole Oxime (LXII).- 5-Oxo-1,3,4,5-tetrahydrobenz[c,d]indole (1 g.) in ethanol (30 ml.), was heated under reflux with a solution of hydroxylamine hydrochloride (0.6 g.) and anhydrous sodium acetate (1.2 g.) in water (30 ml.) for 30 min. Much of the ethanol was then removed at reduced pressure and the remaining solution poured onto ice and water (40 g.). The precipitated oxime was filtered off, and additional oxime

obtained by concentration of the filtrate. This material was recrystallised from water to give 5-oxo-1,3,4,5-tetrahydrobenz[c,d]-indole oxime (0.8 g., 72%), m.p. 157-160° (lit.,[193] 167-169°) (Found: C, 70.7; H, 5.6. Calc. for $C_{11}H_{10}N_2O$: C, 70.9; H, 5.4%).

5-Amino-1,3,4,5-tetrahydrobenz[c,d]indole (XLIX).- 5-Oxo-1,-3,4,5-tetrahydrobenz[c,d]indole oxime (1.4 g.) in dry tetrahydrofuran (100 ml.) was refluxed with lithium aluminium hydride (1.4 g.) for 7 hr. Excess lithium aluminium hydride was decomposed by careful addition of water, the tetrahydrofuran removed at reduced pressure, and the residue extracted with ether. The ethereal solution was washed with water and extracted with dilute hydrochloric acid. The acidic solution, after neutralisation with concentrated ammonia, was extracted with ether, and the ethereal solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. Crystallisation of the residue from benzene gave 5-amino-1,3,4,5-tetrahydrobenz[c,d]indole (0.1 g., 8%), m.p. 117-120° (Found: C, 76.4; H, 6.8. $C_{11}H_{12}N_2$ requires C, 76.7; H, 7.0%); νmax. 3495, 3430, 1610, 1585 $cm.^{-1}$

The hydrochloride was formed by treatment of an ethereal solution of the amine with dry hydrogen chloride; it was recrystallised from ethanol to give colourless plates, m.p. 168-170°.

Treatment of a solution of the amine in pyridine with acetic anhydride gave the N-acetyl derivative which was recrystallised from benzene, m.p. 116-118°; νmax. 3495, 3330, 1635, 1585 $cm.^{-1}$

1-Benzoyl-5-hydroxy-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXIII).- The ketone (LIX) was reduced in ethanol using sodium borohydride according to the method of Woodward and his co-workers,[183] but contrary to the observations of these authors a higher yield of product was isolated when no sodium hydroxide was added to the reaction solution. The product was recrystallised from ethyl acetate to give 1-benzoyl-5-hydroxy-1,2,2a,3,4,5-hexa-

hydrobenz[c,d]indole (8.5 g., 84%), m.p. 182-185° (lit.,[183] 182-183°).

1-Benzoyl-1,2,2a,3-tetrahydrobenz[c,d]indole (LXV).- This material was prepared via 1-benzoyl-5-bromo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXIV) from the hydroxy compound (LXIII) as already described by Woodward and his co-workers.[183] The use of five times the volume of benzene in the bromination reaction was the only modification made to the reported procedure. Elimination of hydrogen bromide from the bromo compound in 2,6-lutidine gave the required 1-benzoyl-1,2,2a,3-tetrahydrobenz[c,d]indole in 73% yield (lit.,[183] 32%), m.p. 96-98° (lit.,[183] 95.5-96.5°).

1-Benzoyl-4,5-epoxy-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXVI).- A solution of perbenzoic acid was prepared and standardised against sodium thiosulphate according to the method described by Braun.[241] Oxidation of 1-benzoyl-1,2,2a,3-tetrahydrobenz[c,d]indole, using this solution as described by Woodward and his co-workers,[183] gave 1-benzoyl-4,5-epoxy-1,2,2a,3,4,5-hexahydrobenz[c,d]indole in 83% yield, m.p. 99-106° (lit.,[183] 104-105°).

1-Benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXVII).- The epoxy compound (LXVI) was rearranged using anhydrous magnesium bromide to give 1-benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole in 75% yield, m.p. 147-149° (lit.,[183] 147-149°).

1-Benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole Oxime (LXVIII).- 1-Benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (2 g.) dissolved in ethanol (80 ml.) was heated under reflux with hydroxylamine hydrochloride (1.2 g.) and anhydrous sodium acetate (2.4 g.) in water (20 ml.) for 1 hr. Much of the ethanol was removed at reduced pressure and the remaining mixture poured onto ice and water. The cream-coloured precipitate was filtered off, washed, dried, and recrystallised from ethanol to give 1-benzoyl-4-

oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole oxime (1.5 g., 71%), m.p. 169-172° (Found: C, 73.5; H,5.8. $C_{18}H_{16}N_2O_2$ requires C, 73.95; H, 5.5%); ν max. 3580, 3280, 1640, 1620, 1600 cm.$^{-1}$

Attempted Hydrolysis of 1-Benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole Oxime (LXVIII).- Method A. A solution of the benzoyl derivative (LXVIII) (0.4 g.) in ethanol (15 ml.) and 2N hydrochloric acid (10 ml.) was kept at room temperature for 4 days, neutralised with ammonia, and the product isolated by extraction. The infrared spectrum of the non-crystalline residue thus obtained showed that little hydrolysis of the N-benzoyl function had occurred, but an absorption maxima at 1710 cm.$^{-1}$ suggested that the 4-oxo-function had been generated.

Method B. The benzoyl derivative (LXVIII) (0.6 g.) in ethanol (12 ml.) was heated under reflux with 2N sodium hydroxide (6 ml.), and samples taken at regular intervals. The material was isolated by extraction and the infrared spectra prepared. Hydrolysis of the 4-oximino function appeared to occur at a similar rate to the required N-benzoyl cleavage, and no crystalline material was isolated.

4-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXIX).- 1-Benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (1 g.) dissolved in glacial acetic acid (10 ml.) and concentrated hydrochloric acid (3 ml.) was gently refluxed under nitrogen for 2.5 hr. After removal of the acidic mixture at reduced pressure, the residue was extracted with water, the aqueous solution filtered under nitrogen, and the filtrate made basic with aqueous ammonia and extracted several times with ether. The ethereal solution was washed with water, dried over anhydrous sodium sulphate and the ether removed at reduced pressure to yield 4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.25 g., 40%), m.p. 138°; ν max. 3390, 1710, 1620, 1600 cm.$^{-1}$

This material was very unstable in the air and was therefore

isolated and kept under oxygen-free nitrogen. On exposure to oxygen it decomposed to an amorphous brown solid (m.p. > 350°) which was insoluble in organic solvents. Owing to the difficulties associated with recrystallisation of this product it was used for the next stage without further purification.

Attempted Hydrolysis of 1-Benzoyl-4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXVII).- Earlier unsuccessful attempts to cleave the N-benzoyl function and to isolate the resulting 4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole included: the procedure already outlined for the hydrolysis of the corresponding 5-oxo compound, using glacial acetic acid and hydrochloric acid; methods employing aqueous, ethanolic, and aqueous ethanolic mixtures of hydrochloric acid at room and elevated temperatures; and reactions employing basic conditions, such as aqueous and ethanolic sodium hydroxide. These reactions, from infrared data, either gave unchanged starting material or resulted in a complex mixture of highly coloured decomposition products.

Attempted Dehydrogenation of 4-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXIX).- Method A. 4-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.2 g.) in methylene chloride (6 ml.) was shaken with active manganese dioxide[240] (0.8 g.) at room temperature for 24 hr. The manganese dioxide was filtered off, extracted with methylene chloride, and the combined solution evaporated to dryness. The infrared spectrum of the residue was consistent with impure starting material and indicated that little, if any, dehydrogenation had occurred. There was little absorption at 3495 $cm.^{-1}$ attributable to the indolic N-H. Under more vigorous conditions infrared spectra showed that destruction of the 4-keto function occurred before dehydrogenation of the $C_{(2,3)}$ double bond.
Method B. 4-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.1 g.) in p-cymene (4 ml.) was heated under reflux with 10% palladium on

carbon (0.1 g.) for 1 hr. under nitrogen. The solvent was removed at reduced pressure, the residue extracted with benzene, and the benzene solution filtered free of palladium on carbon and evaporated to dryness. The infrared spectrum of the residue (ν max. 1710, 1690, 1640 $cm.^{-1}$) possessed no absorption at 3495 $cm.^{-1}$ attributable to the indolic N-H. The residue was insoluble in dilute acid.

4-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole Oxime (LXXI).- A solution of 4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.25 g.) in ethanol (35 ml.) was refluxed for 1.5 hr. under nitrogen with hydroxylamine hydrochloride (0.15 g.) and anhydrous sodium acetate (0.3 g.) in water (5 ml.). The ethanol was then removed at reduced pressure, excess water added, and the resulting precipitate filtered off, washed, and dried. Recrystallisation of the precipitate from methanol gave the hygroscopic 4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole oxime (0.17 g., 63%), m.p. 150-153° (Found: C, 69.9; H, 6.4. $C_{11}H_{12}N_2O$ requires C, 70.2; H, 6.4%); ν max. 3580, 3390, 3280, 1620, 1600 $cm.^{-1}$

4-Oxo-1,3,4,5-tetrahydrobenz[c,d]indole Oxime (LXXII).- 4-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole oxime (0.68 g.) in methylene chloride (150 ml.) was shaken with active manganese dioxide[240] (2.8 g.) for 25 hr. The manganese dioxide was filtered off, extracted with methylene chloride, and the combined methylene chloride solution washed with acetic acid then water, and dried over anhydrous sodium sulphate. The solvent was removed at reduced pressure to yield a non-crystalline unstable residue which was kept under nitrogen. The infrared spectrum (νmax. 3580, 3480, 3260, 1620, 1600 $cm.^{-1}$) was consistent with that to be expected for the required indole-oxime, but this could not be obtained in a crystalline form; all attempts to do so resulted in the formation of a black insoluble amorphous solid (m.p. $>$ 350°). The product was not

purified but used directly for the next stage of the synthesis.

Attempted Preparation of 4-Amino-1,3,4,5-tetrahydrobenz[c,d]-indole (L).- Impure 4-oxo-1,3,4,5-tetrahydrobenz[c,d]indole oxime (0.2 g.) in dry tetrahydrofuran (100 ml.) was heated under reflux with lithium aluminium hydride (0.3 g.) for 16 hr. in an atmosphere of nitrogen. After decomposition of the excess lithium aluminium hydride with water the tetrahydrofuran was removed at reduced pressure, the resulting mixture extracted with ether and the ethereal solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at reduced pressure. During extraction of the product the solutions were kept in an atmosphere of nitrogen and the product, when isolated, was stored under nitrogen. The infrared spectrum (ν max. 3480, 3300, 1620, 1600 cm.$^{-1}$) indicated that the residue contained a substantial quantity of the required amino product but this could not be purified. It was therefore dissolved in a mixture of benzene and ether and, by treatment with dry hydrogen chloride, converted into the hydrochloride. The white precipitate was centrifuged and washed several times with ether and dried at reduced pressure. Insufficient material was obtained for complete characterisation, m.p. 200-212° (decomp.) (lit.,[176] m.p. 215-222°).

Acetylation of the impure amine in pyridine using acetic anhydride gave a non-crystalline product which had an infrared spectrum (ν max. 3480, 3430, 1660, 1600 cm.$^{-1}$) indicating that it was the impure N-acetyl derivative of the required amine.

1-Benzoyl-4-bromo-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LXXVI).- This material was prepared by the bromination of 1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole using pyridine hydrobromide perbromide according to the method described by Woodward and his co-workers.[183] Yield 76%, m.p. 181-181.5° (lit.,[183]

180.5-181.5°); ν max. 1690, 1645, 1595 cm.$^{-1}$

Attempted Preparation of 1-Benzoyl-4-dimethylamino-5-oxo-1,2,-2a,3,4,5-hexahydrobenz[c,d]indole (LXXVIII).- 1-Benzoyl-4-bromo-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (2.3 g.) and dimethylamine (0.95 g.) in dry benzene (40 ml.) were heated under reflux in an atmosphere of nitrogen for 20 hr. The mixture was cooled to room temperature, dimethylamine hydrobromide filtered off, and the benzene solution washed with ice-water and extracted with cold 2N hydrochloric acid. The acidic extract was neutralised with cold 2N sodium hydroxide, extracted with ether, and the ethereal solution washed with water, dried using anhydrous sodium sulphate, and evaporated to dryness. All attempts to crystallise the residue were unsuccessful and the infrared spectrum (νmax. 1645, 1595 cm.$^{-1}$) indicated that no 5-oxo function remained.

The reaction was repeated several times varying the reaction temperature over the range 0° to 80° and employing different reaction times (from 2 hr. to 7 days) but none of the required amino-ketone was isolated.

4-Acetamido-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (LXXIX).- 5-Oxo-1,3,4,5-tetrahydrobenz[c,d]indole (1 g.) in a solution of potassium (0.25 g.) in t-butanol (20 ml.) was warmed to 60° for 15 min. and the mixture then stirred at room temperature while amyl nitrite (1.25 ml.) was added over 30 min. After stirring for a further 30 min. at room temperature the mixture was cooled to 5°, and ice-water (20 ml.), glacial acetic acid (14 ml.) and zinc dust (2 g.) added and stirring continued for 2 hr. at 5°.

The solution of amino-ketone thus generated was then treated with acetic anhydride (4 ml.), chloroform (32 ml.) and crystalline sodium acetate (19 g.) and the mixture stirred for 3 hr. at 0°. After separation, the aqueous layer was extracted with chloroform

and the combined chloroform solution washed with aqueous sodium bicarbonate, water, dried over anhydrous sodium sulphate and evaporated to dryness. The oily residue was crystallised in methylene chloride to give 4-acetamido-5-oxo-1,3,4,5-tetrahydrobenz[c,d]-indole (0.17 g., 13%). m.p. 179-184° (lit.,[195] 207-209°) (Found: C, 68.5; H, 5.1. Calc. for $C_{13}H_{12}N_2O_2$: C, 68.4; H, 5.3%); ν max. 3480, 3400, 1665, 1620 cm.$^{-1}$ The p-nitrophenylhydrazone crystallised from ethanol, m.p. 262° (lit.,[195] 263-264°).

1,3,4,5-Tetrahydrobenz[c,d]indole (LXXXI).- A solution of 5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.5 g.) in ethanol (25 ml.) and 6N hydrochloric acid (25 ml.) was heated under reflux with zinc amalgam (1 g.) for 1.5 hr. After filtering free from zinc the solution was reduced in volume at reduced pressure and the residual mixture extracted with chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness. The oily residue was distilled at 15 mm. pressure to give a colourless crystalline solid which was recrystallised from petroleum to give 1,3,4,5-tetrahydrobenz[c,d]indole (0.21 g., 45%), m.p. 57-58° (lit.,[242] 55-56°).

Attempted Clemmensen Reduction of 4-Acetamido-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (LXXIX).- 4-Acetamido-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.2 g.) in ethanol (15 ml.) and 6N hydrochloric acid (15 ml.) was heated under reflux with zinc amalgam (1 g.) for 1.5 hr. The solution was filtered free from zinc, reduced in volume at reduced pressure and the remaining aqueous mixture extracted with chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness to give a non-crystalline residue (0.13 g.) having an infrared spectrum consistent with that to be expected for the N-acetyl derivative of the required 4-amino-1,3,4,5-tetrahydrobenz[c,d]indole. (see p. 60).

Treatment of the impure residue with hydroxylamine hydrochloride and sodium acetate in aqueous ethanol gave a solid which, from infrared evidence, contained no detectable amount of the 5-oximino derivative.

5-Methoxy-2-nitrotoluene (LXXXVIII).- This material was prepared in a 57% yield from m-cresol via 5-hydroxy-2-nitrosotoluene and 5-hydroxy-2-nitrotoluene according to the procedure described by Koelsch,[199] m.p. 55° (lit.,[199] 55°).

5-Methoxyindole (XCI).- Conversion of 5-methoxy-2-nitrotoluene into 5-methoxyindole was achieved in 25% yield employing the method of Blaikie and Perkin.[200] Condensation of the nitrotoluene with ethyl oxalate gave 5-methoxy-2-nitrophenylpyruvic acid which on cyclisation gave 2-carboxy-5-methoxyindole, and on subsequent thermal decarboxylation the required 5-methoxyindole, m.p. 54-55° (lit.,[200] 55°).

Attempted Preparation of β-(5-Methoxyindol-3-yl)-propionic Acid (XCII).- 5-Methoxyindole (1 g.) in tetralin (20 ml.) was heated under reflux with sodium hydroxide (0.3 g.) and propiolactone (0.5 ml.) for 4 hr. Much of the solvent was then removed at reduced pressure, excess water added and the mixture extracted with chloroform. The aqueous basic solution was acidified with 4N hydrochloric acid, the precipitated oil extracted with chloroform and the resulting solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at reduced pressure. The infrared spectrum of the residue was very similar to that of β-(5-methoxyindol-3-yl)-propionic acid but no crystalline material could be isolated. The duration and temperature of the reaction were varied without any significant change in the purity of the product.

β-(5-Methoxyindol-3-yl)-propionic Acid (XCII).- 5-Methoxyindole (1 g.) in a mixture of glacial acetic acid (6 ml.) and acetic anhydride (2 ml.) was heated under reflux with acrylic acid (1.1 g.) for 3 hr. and then allowed to stand at room temperature overnight. The volatile material was removed at reduced pressure, and the oily residue added to 4N sodium hydroxide (25 ml.). Cooling and scratching this mixture converted the impure acid to the crystalline sodium salt which was filtered off, washed with water, and acidified with 2N hydrochloric acid. The resulting solid was recrystallised from water to give β-(5-methoxyindol-3-yl)-propionic acid (0.74 g., 50%), m.p. 136°, not depressed by admixture with an authentic sample. The ethyl ester had m.p. and mixed m.p. 72-73°.

Ethyl 2-Oxo-cyclopentanecarboxylate (XCIV).- This material was prepared in 70% yield by a Dieckmann condensation of diethyl adipate as described by Pinkney,[204] b.p. 81-82°/3 mm., (lit.,[204] 108-111°/15 mm.).

Ethyl Hydrogen α-Ketoadipate p-Methoxyphenylhydrazone (XCV).- This material was prepared in 94% yield from p-methoxyphenyldiazonium chloride and ethyl 2-oxo-cyclopentanecarboxylate in neutral solution at 0°, according to the method described by Renson,[189] and based on the earlier synthesis of Robinson and his co-workers.[203] The p-methoxyphenylhydrazone was not purified but used directly for the next stage.

Ethyl β-(2-Ethoxycarbonyl-5-methoxyindol-3-yl)-propionate (XCVI).- Cyclisation of the phenylhydrazone (XCV) was achieved using a refluxing mixture of ethanol and sulphuric acid as described by Robinson and his co-workers.[203] The product was distilled at reduced pressure to give the required diethyl ester in 58% yield, b.p. 215-220°/5 mm., m.p. 110° (lit.,[203] 110°).

β-(2-Carboxy-5-methoxyindol-3-yl)-propionic Acid (XCVII).- The diethyl ester (XCVI) was hydrolysed with refluxing ethanolic potassium hydroxide as described by Robinson and his co-workers.[203] The required di-acid was obtained, after recrystallisation from water, in 92% yield, m.p. 223-227° (lit.,[203] 225°).

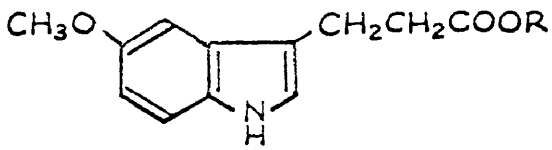
β-(5-Methoxyindol-3-yl)-propionic Acid (XCII).- Decarboxylation of the di-acid (XCVII) was effected thermally in diphenylamine at 250° according to the method of Robinson and his co-workers.[203] After recrystallisation from water, the required product was obtained in 72% yield, m.p. 136° (lit.,[203] 136°).

Ethyl β-(5-Methoxyindol-3-yl)-propionate (XCVIII).- β-(5-Methoxyindol-3-yl)-propionic acid was esterified by treatment with ethanol containing 10% hydrogen chloride. Yield 79%, m.p. 72-72.5°.

Methyl β-(5-Methoxyindol-3-yl)-propionate.- Method A. The ester was prepared in 81% yield by treatment of the acid with methanol containing hydrogen chloride according to the method described by Robinson and his co-workers,[203] m.p. 97-99° (lit.,[203] 100°). Method B. Diazomethane in ether (250 ml.) (prepared from Diazald (3 g.)) was added to a solution of β-(5-methoxyindol-3-yl)-propionic acid (8 g.) in methanol (70 ml.) at 0° and the mixture kept at room temperature for 3 hr. The methanol, ether, and excess diazomethane were then removed at reduced pressure, and the residue recrystallised from methanol to give methyl β-(5-methoxyindol-3-yl)-propionate (8.1 g., 95%), m.p. 99-100°.

Attempted Hydrogenation of β-(5-Methoxyindol-3-yl)-propionic Acid and its Methyl and Ethyl Esters to the Corresponding Indolines.- The reactions listed below were carried out in a general purpose hydrogenation apparatus at room temperature and atmospheric pressure. No attempt was made to purify the resulting indolines but where products were obtained these were benzoylated directly using benzoyl

chloride in aqueous sodium hydroxide.



R	Solvent	Catalyst	Observations
H	Aqueous sodium hydroxide	Raney nickel	
		Platinum oxide	Very slow hydrogen uptake and a considerable quantity of starting material isolated.
		Palladium-on-carbon	
		Rhodium-on-carbon	Rapid hydrogenation, 2 moles of hydrogen taken up. Starting material (50%) isolated. Product (5%).
H	Ethanol	Platinum oxide	Rapid hydrogenation, 1.5 moles of hydrogen taken up. Starting material (40%) isolated. No product.
H	Ethanol and fluoroboric acid	Platinum oxide	Rapid hydrogenation, 1 mole of hydrogen taken up. Product (20%), (isolation difficult).
CH_3	Methanol and hydrochloric acid	Raney nickel Palladium on carbon	Very slow hydrogen uptake and a considerable quantity of starting material isolated.
		Rhodium on carbon	Rapid hydrogenation, 1.7 moles of hydrogen taken up. No product.
		Platinum oxide	Moderate hydrogenation rate, 1.3 moles of hydrogen taken up. Starting material (25%) isolated. Product (20%).

R	Solvent	Catalyst	Observations
C_2H_5	Ethanol and hydrochloric acid	Raney nickel	Very slow hydrogen uptake and a considerable quantity of starting material isolated.
		Palladium-on-carbon	
		Rhodium-on-carbon	Rapid hydrogenation, variable hydrogen uptake. No product.
		Platinum oxide	Moderate hydrogenation rate, 1.3 moles of hydrogen taken up. Starting material (20-40%) isolated. Variable yield of product (17, 25, 37, 50%).

Attempts were also made to hydrogenate the sodium salt of the acid at increased pressures as described for the hydrogenation of β-(indol-3-yl)-propionic acid by Woodward and his co-workers.[183] However, only starting material was isolated.

β-(1-Benzoyl-5-methoxyindolin-3-yl)-propionic Acid (XCIX).- To a suspension of prehydrogenated platinum oxide (1.25 g.) in ethanol (30 ml.) and 40% aqueous fluoroboric acid (30 ml.) was added a solution of ethyl β-(5-methoxyindol-3-yl)-propionate (31 g.) in ethanol (300 ml.) and fluoroboric acid (290 ml.). The total mixture was shaken in an atmosphere of hydrogen at room temperature and atmospheric pressure until the theoretical volume of hydrogen (3100 ml.) had been absorbed. The mixture was then filtered, ethanol removed at reduced pressure and the remaining aqueous solution made basic with dilute ammonia and extracted with chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness to give impure ethyl β-(5-methoxyindolin-3-yl)-propionate (24 g.), having ν max. 3380, 1725, 1595 $cm.^{-1}$

Without further purification the impure ester was hydrolysed under reflux in ethanol (120 ml.) with 10% aqueous sodium hydroxide

(240 ml.) for 5 min. The solution was then cooled to 0°, benzoyl chloride (26 ml.) added, and the mixture shaken at room temperature for 3 hr. The mixture, cooled to 0°, was made acid with concentrated hydrochloric acid and the precipitated mixture of benzoic acid and the required benzoyl material filtered off, washed with hot water (to remove benzoic acid) and the residue recrystallised from ethanol to give β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (25 g., 62%), m.p. 169-170° and 180.5° (lit.,[205] 179.5-181°) (Found: C, 70.1; H, 6.1; N, 4.0. Calc. for $C_{19}H_{19}NO_4$ requires C, 70.15; H, 5.9; N, 4.3%); ν max. 1720, 1640, 1600 $cm.^{-1}$

Neutralisation of the ammoniacal solution, after extraction of the reduced material with chloroform, precipitated unchanged starting material (1.2 g.).

1-Benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole (C).- β-(1-Benzoyl-5-methoxyindolin-3-yl)-propionic acid (13 g.) in freshly distilled thionyl chloride (22 ml.) was kept at room temperature for 30 min. and then warmed gently for a further 30 min. After removal of excess thionyl chloride at reduced pressure, the acid chloride was dissolved in dry carbon disulphide (22 ml.) and added dropwise over 30 min. to a stirred and cooled (0°) suspension of anhydrous aluminium chloride (26 g.) in carbon disulphide (200 ml.). When the addition was complete the mixture was allowed to attain room temperature over 30 min. and then heated under reflux for a further 2 hr. with continued stirring. The reaction mixture was then cooled in ice and the complex decomposed by slow addition of ice (50 g.) and 4N hydrochloric acid (90 ml.). The carbon disulphide was removed at reduced pressure, the remaining aqueous mixture extracted with benzene and the benzene solution washed with water and shaken with 5N sodium hydroxide (20 ml.). The resulting yellow precipitate was filtered off, washed with a little water and dried to give 1-benzoyl-6-hydroxy-1,2,2a,3,4,5-

hexahydrobenz[c,d]indole sodium salt (11.4 g., 90%), m.p.> 350°. On acidification this sodium salt gave a colourless solid which was recrystallised from ethanol to give 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole m.p. 163.5°, (Found: C, 73.2; H, 5.25. $C_{18}H_{15}NO_3$ requires C, 73.7; H, 5.2%); ν max. 1660, 1640, 1600 $cm.^{-1}$

The benzene filtrate, after removal of precipitated sodium salt, was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at reduced pressure. The residue was recrystallised from benzene to give 1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (1 g.), m.p. 145-148° (lit.,[183] 146-147°). The infrared spectrum was identical to that of an authentic sample and no m.p. depression was observed on admixture of the samples.

Action of Aluminium Chloride upon β-(1-Benzoyl-5-methoxyindolin-3-yl)-propionic Acid (XCIX).- To a cooled, stirred suspension of anhydrous aluminium chloride (2 g.) in dry carbon disulphide (20 ml.) was slowly added a suspension of β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (1 g.) in carbon disulphide (10 ml.). When the addition was complete the mixture was allowed to attain room temperature and then heated under reflux for a further 2 hr. with continued stirring. The mixture was then cooled, ice (5 g.) and 4N hydrochloric acid (10 ml.) added, and carbon disulphide removed at reduced pressure. The residual aqueous mixture was extracted with benzene and the benzene solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The residue (0.94 g.) was recrystallised from ethanol to give β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (0.86 g.), m.p. 177-179°, not depressed by admixture with starting material. The infrared spectra of the two samples were identical.

1-Benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole Oxime (CI).- 1-Benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.23 g.) was dissolved in pyridine (5 ml.) containing hydroxylamine hydrochloride (0.2 g.) and the mixture kept at room temperature for 15 hr. It was then poured onto a mixture of ice and water and the precipitate filtered off, washed with water, dried and recrystallised from ethanol to give 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole oxime (0.15 g., 62%), m.p. 265° (decomp.) (Found: C, 70.4; H, 5.2. $C_{18}H_{16}N_2O_3$ requires C, 70.1; H, 5.2%); ν max. 3580, 3260, 1640, 1600 $cm.^{-1}$

6-Acetoxy-1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole (CII).- To a solution of 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,-3,4,5-hexahydrobenz[c,d]indole (0.2 g.) in pyridine (10 ml.) was added acetyl chloride (0.6 ml.), and the mixture kept at room temperature for 30 min. It was then poured onto ice and water, the product extracted with chloroform and the chloroform solution washed with water, 2N hydrochloric acid, water until neutral, and dried over anhydrous sodium sulphate. Evaporation of this solution to dryness gave a residue which on recrystallisation from ethanol furnished the required 6-acetoxy-1-benzoyl-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.13 g., 57%), m.p. 151-153.5° (Found: C, 71.3; H, 5.5. $C_{20}H_{17}NO_4$ requires C, 71.6; H, 5.1%); ν max. 1760, 1670, 1640, 1590, 1255 $cm.^{-1}$

1-Benzoyl-6-methoxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole (CIII).- To a refluxing solution of 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (1 g.) in ethanol (27 ml.) containing sodium (0.1 g.) was slowly added methyl iodide (0.8 ml.) and heating continued for a further 3 hr. After removal of ethanol at reduced pressure, water was added to the residue and the mixture extracted with chloroform. The chloroform solution was shaken with 20% aqueous sodium hydroxide, the unchanged starting material (0.2 g.)

filtered off as the sodium salt, and the remaining solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. Recrystallisation of the residue from ethanol gave 1-benzoyl-6-methoxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.6 g., 71%) (based on starting material consumed), m.p. 154-156°(Found: C, 74.4; H, 5.7. $C_{19}H_{17}NO_3$ requires C, 74.3; H, 5.6%); ν max. 1670, 1635, 1590 $cm.^{-1}$

Action of Aluminium Chloride upon 1-Benzoyl-6-methoxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (CIII).- The procedure and conditions employed in this reaction were similar to those described for the reaction carried out to determine the effect of aluminium chloride upon β-(1-benzoyl-5-methoxyindolin-3-yl)-propionic acid (XCIX): methoxy-ketone (CIII; 0.6 g.) and aluminium chloride (1.2 g.) were employed. After extraction of the reaction mixture with benzene, the benzene solution was shaken with 5N sodium hydroxide (5 ml.) and the yellow solid precipitated was filtered off, washed with a little water and dried to give 1-benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole sodium salt (0.25 g.). The unprotected hydroxy compound (C) was liberated on acidification of the sodium salt in aqueous solution; recrystallisation from ethanol gave 1-benzoyl-6-hydroxy-5-oxo-1,2,-2a,3,4,5-hexahydrobenz[c,d]indole (0.2 g.), m.p. 160-163°, not depressed on admixture with an authentic sample.

The benzene solution was washed with water, dried over anhydrous sodium sulphate, and the solvent removed at reduced pressure. Recrystallisation of the residue (0.4 g.) from methanol afforded 1-benzoyl-6-methoxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole, m.p. 150-154°, not depressed on admixture with an authentic sample. The infrared spectrum was identical with that of the starting material.

6-Hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (CVIII).- 1-Benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole sodium salt (8 g.) was dissolved in glacial acetic acid (80 ml.) and concentrated hydrochloric acid (100 ml.) and the solution heated under reflux for 20 hr. The acid mixture was removed at reduced pressure, the residue extracted with water and the aqueous solution filtered and made basic with concentrated ammonia. The product was extracted three times with chloroform and the chloroform solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at reduced pressure. Recrystallisation of the residue from ethanol gave 6-hydroxy-5-oxo-1,2,2a,-3,4,5-hexahydrobenz[c,d]indole (2.4 g., 50%), m.p. 121-126° (Found: C, 70.1; H, 6.4. $C_{11}H_{11}NO_2$ requires C, 69.8; H, 5.9%); νmax. 3380, 3250, 1650, 1600 cm.$^{-1}$ Further extraction of the ammoniacal solution with chloroform gave, after the usual work up and recrystallisation from benzene, 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz-[c,d]indole (0.16 g.), m.p. 190-194° (Found: C, 70.7; H, 5.4. $C_{11}H_9NO_2$ requires C, 70.6; H, 4.9%); νmax. 3480, 1640, 1605 cm.$^{-1}$

6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX).- 6-Hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (1.7 g.) in methylene chloride (60 ml.) was shaken for 24 hr. at room temperature with active manganese dioxide[240] (6.8 g.). The mixture was then filtered, the manganese dioxide extracted in a Soxhlet extractor using methylene chloride and the combined methylene chloride solution washed with 2N hydrochloric acid and water, and dried over anhydrous sodium sulphate. The solvent was removed at reduced pressure and the residue recrystallised from benzene to give 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (1.2 g., 70%), m.p. 193-195°.

Hydroxylation of 5-Oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (LX) to 6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX).- To a solution of potassium nitrosodisulphonate (2.6 g.) in water (100 ml.) and Sorensen's buffer solution* (50 ml.) was added 5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.5 g.) in acetone (35 ml.). The mixture was shaken at room temperature for 10 min. and then extracted with chloroform. The chloroform solution was washed with water, 2N hydrochloric acid and extracted with aqueous sodium hydroxide. The basic solution was acidified with hydrochloric acid, the product extracted with chloroform and the chloroform solution washed with water and dried over anhydrous sodium sulphate. After removal of the solvent the residue was recrystallised from benzene to give 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.07 g.), m.p. 190-195°, not depressed by admixture with an authentic sample. The infrared spectrum of this material was identical with that of the authentic sample.

The reaction time was varied from 2 min. to 30 min. in an attempt to increase the yield but 10 min. was found to be the optimum time.

6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole Oxime (CX).- A solution of 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (1.2 g.) in ethanol (140 ml.) was heated under reflux with hydroxylamine hydrochloride (0.9 g.) and anhydrous sodium acetate (1.8 g.) in water (30 ml.) for 75 min. Much of the solvent was then removed at reduced pressure, excess ice and water added and the oxime thus precipitated extracted into chloroform. The chloroform

* - The Sorensen's buffer solution[243] (pH 7) was prepared from potassium dihydrogen phosphate (0.175 g.) and disodium hydrogen phosphate (0.362 g.) dissolved in water (50 ml.).

solution was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness at reduced pressure. Recrystallisation of the residue from ethanol gave 6-hydroxy-5-oxo-1,3,4,-5-tetrahydrobenz[c,d]indole oxime (0.95 g., 73%), m.p. 172-176.5° (Found: C, 65.5; H, 4.95. $C_{11}H_{10}N_2O_2$ requires C, 65.3; H, 5.0%); νmax. 3580, 3480, 3250, 1630, 1600 cm.$^{-1}$

5-Amino-6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (XLVII).- 6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole oxime (0.65 g.) in dry tetrahydrofuran (55 ml.) was heated under reflux with lithium aluminium hydride (0.65 g.) for 7.5 hr. Excess lithium aluminium hydride was decomposed by the careful addition of water and much of the tetrahydrofuran removed at reduced pressure and room temperature. The remaining aqueous mixture was extracted with ether and the ethereal solution washed with water, dried over anhydrous sodium sulphate and the ether removed at reduced pressure. During the isolation the solutions were kept cool and in an atmosphere of nitrogen; on exposure to air the yellow solution of amine instantly became black. Attempts to prepare a pure sample of the amine (νmax. 3480, 3380, 1615 cm.$^{-1}$) were unsuccessful.

The hydrochloride was prepared by treatment of a solution of the impure amine in a mixture of anhydrous ether and benzene with dry hydrogen chloride. The resulting precipitate was washed and centrifuged several times with anhydrous ether, dried in a stream of oxygen-free nitrogen and recrystallised from ethanol to give colourless needles of 5-amino-6-hydroxy-1,3,4,5-tetrahydrobenz-[c,d]indole hydrochloride (0.03 g.). No m.p. was recorded as the compound slowly darkened and decomposed on heating. (Found: C, 58.0; H, 5.7. $C_{11}H_{13}N_2OCl$ requires C, 58.8; H, 5.8%).

Attempted Hydroxylation of 4-Oxo-1,2,2a,3,4,5-hexahydrobenz-[c,d]indole (LXIX) to 6-Hydroxy-4-oxo-1,3,4,5-tetrahydrobenz[c,d]-indole (CXI).- A solution of potassium nitrosodisulphonate (1.6 g.) in water (64 ml.) and Sorensen's phosphate buffer solution[243] (32 ml.) was shaken under nitrogen with 4-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (0.32 g.) in acetone (30 ml.) for 10 min. The mixture was then extracted with chloroform and the chloroform solution washed with 2N hydrochloric acid and water, dried over anhydrous sodium sulphate, and evaporated to dryness at reduced pressure. The infrared spectrum of the residue (0.05 g.) possessed no absorption at 1710 or 3480 $cm.^{-1}$ attributable to the 4-oxo function or the indolic N-H respectively.

Neutralisation of the acid washings with sodium bicarbonate and extraction with chloroform gave a dark oil (0.2 g.), the infrared spectrum of which possessed absorption at 1710 $cm.^{-1}$ but not at 3480 $cm.^{-1}$ The oil was insoluble in aqueous sodium hydroxide and could not be obtained in a crystalline state.

6-Hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (CXIII).- 6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.2 g.) in ethanol (40 ml.) and 6N hydrochloric acid (20 ml.) was heated under reflux for 15 min. with amalgamated zinc (0.5 g.). After remaining at room temperature for a further 12 hr., the mixture was filtered, the solution volume reduced and the remaining mixture extracted with chloroform. The chloroform solution was washed with water, dried over anhydrous sodium sulphate and the solvent removed at reduced pressure. The oily residue was distilled at 200°/1 mm. and the colourless crystals obtained were recrystallised from benzene to give 6-hydroxy-1,3,4,5-tetrahydrobenz[c,d]indole (0.09 g., 50%), m.p. 125-126° (lit.,[215] 125-126°).

6-Acetoxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CXIV).- To a solution of 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]-

indole (1 g.) in pyridine (7 ml.) was added acetyl chloride (0.6 ml.) and the mixture kept at room temperature for 2 hr. Addition to ice-water gave an oily solid which was extracted with chloroform and the organic solution washed with water, 2N hydrochloric acid, water, and dried over anhydrous sodium sulphate. Removal of the solvent at reduced pressure gave a residue which was recrystallised from ethanol to give 6-acetoxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]-indole (0.94 g., 91%), m.p. 191-192° (Found: C, 68.4; H, 5.1. $C_{13}H_{11}NO_3$ requires C, 68.1; H, 4.8%); νmax. 3475, 1755, 1670, 1615, 1255 cm.$^{-1}$

1-Benzoyl-6-benzyloxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]-indole (CXV).- 1-Benzoyl-6-hydroxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole sodium salt (6 g.) in N/40 sodium hydroxide (1500 ml.) was heated under reflux with benzyl chloride (4 ml.) in ethanol (200 ml.) for 2 hr. Much of the ethanol was removed at reduced pressure and the residual mixture cooled in ice to precipitate the impure benzyl derivative. After removal of the precipitated material the filtrate was heated under reflux and retreated with benzyl chloride (2 ml.) in ethanol (100 ml.). Heating was continued for 2 hr. when more product was isolated by removal of ethanol and filtration. This process was repeated several times and the combined impure product recrystallised from ethanol to give 1-benzoyl-6-benzyloxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (5.2 g., 67%), m.p. 199-201° (Found: C, 78.8; H, 5.5. $C_{25}H_{21}NO_3$ requires C, 78.3; H, 5.5%); νmax. 1680, 1640, 1595, 1270 cm.$^{-1}$

6-Benzyloxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (CXVI).- 1-Benzoyl-6-benzyloxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (4 g.) in ethanol (600 ml.) was heated under reflux with 5N sodium hydroxide (600 ml.) for 2 hr. Much of the solvent was then removed at reduced pressure, water added, and the mixture

extracted with chloroform. The chloroform solution was washed with 2N sodium hydroxide and water, and dried over anhydrous sodium sulphate. After removal of the chloroform at reduced pressure the residue was recrystallised from ethanol to give 6-benzyloxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (2.8 g., 96%), m.p.132-134° (Found: C, 77.6; H, 6.4. $C_{18}H_{17}NO_2$ requires C, 77.4; H, 6.1%); νmax. 3370, 1670, 1600, 1260 cm.$^{-1}$

Attempted Preparation of 6-Benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CXVII).- 6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.5 g.) in N/4 sodium hydroxide (150 ml.) was heated under reflux with benzyl chloride (0.4 g.) in ethanol (30 ml.) for 2 hr. Much of the ethanol was removed at reduced pressure, the solution extracted with chloroform and the chloroform solution washed with aqueous sodium hydroxide and water, and dried over anhydrous sodium sulphate. Removal of the solvent gave an oily residue which could not be purified by crystallisation. The infrared spectrum (νmax. 3480, 1640, 1605 cm.$^{-1}$) indicated that this product was impure starting material.

More forcing conditions for the benzylation were employed but no pure benzyloxy material could be isolated.

6-Benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CXVII).- 6-Benzyloxy-5-oxo-1,2,2a,3,4,5-hexahydrobenz[c,d]indole (2.5 g.) in methylene chloride (200 ml.) was shaken for 24 hr. at room temperature with active manganese dioxide[240] (10 g.). The mixture was then filtered, the manganese dioxide extracted in a Soxhlet extractor using methylene chloride and the combined methylene chloride solution washed with 2N hydrochloric acid and water, and dried over anhydrous sodium sulphate. The solvent was removed at reduced pressure, the residue shaken with benzene, filtered to remove an insoluble, high melting solid and the benzene - soluble material chromatographed on silica gel.

Elution with chloroform gave a crystalline solid, which was recrystallised from benzene to give 6-benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (1.1 g., 44%), m.p. 152-155.5° (Found: C, 78.1; H, 5.3. $C_{18}H_{15}NO_2$ requires C, 77.9; H, 5.45%); νmax. 3480, 1670, 1600 cm.$^{-1}$

6-Benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole Oxime (CXVIII).- 6-Benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.3 g.) in ethanol (15 ml.) was heated under reflux for 30 min. with hydroxylamine hydrochloride (0.2 g.) and sodium acetate (0.4 g.) in water (7 ml.). Much of the solvent was removed at reduced pressure, excess water added and the solid thus precipitated was filtered off, washed with water and recrystallised from ethanol to give 6-benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole oxime (0.27 g., 85%), m.p. 220° (decomp.) (Found: C, 73.6; H, 5.6. $C_{18}H_{16}N_2O_2$ requires C, 74.0; H, 5.5%); νmax. (Nujol) 3430, 3170, 1600 cm.$^{-1}$

6-Hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (CIX).- 6-Benzyloxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.2 g.) in methanol (12 ml.) was shaken in an atmosphere of hydrogen with palladium on carbon (0.1 g.) until no more hydrogen was absorbed. After removal of the catalyst the filtrate was evaporated to dryness and the residue recrystallised from benzene to give 6-hydroxy-5-oxo-1,3,4,5-tetrahydrobenz[c,d]indole (0.11 g., 81%), m.p. 192-195°.

1,4-Diacetoxycyclohexane.- Cyclohexane-1,4-diol was acetylated with acetic anhydride to give the diacetate in 40% yield, m.p. 101-103° (lit.,[227] 102-103°).

4-Acetoxycyclohexanol.- The diacetate was hydrolysed with ethanolic potassium hydroxide and the product distilled to give

the required monoacetate in 52% yield, b.p. 147-149°/24 mm. (lit.,[227] 136-137°/15 mm.).

4-Acetoxycyclohexanone (CXXV).- Cyclohexane-1,4-diol monoacetate was oxidised to the acetoxy-ketone, using chromic oxide, in 72% yield, b.p. 148°/24 mm. (lit.,[227] 124-126°/14 mm.).

p-Benzyloxyaniline.- p-Benzyloxynitrobenzene was reduced[244] using Raney nickel and hydrazine hydrate to give the required product in 85% yield, m.p. 35-39°.

p-Benzyloxyphenylhydrazine Hydrochloride (CXXVI).- p-Benzyloxyaniline was diazotised and the product reduced with stannous chloride to give the required material in 46% yield, m.p. 180-188° (lit.,[228] 219°).

3-Acetoxy-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (CXXVII).- p-Benzyloxyphenylhydrazine hydrochloride (3.25 g.) and 4-acetoxycyclohexanone (2 g.) were heated under reflux with anhydrous sodium acetate (1.1 g.) in acetic acid (40 ml.) for 2 hr. The solution was cooled, poured onto water and the mixture extracted with ether. The ethereal solution was washed with water, aqueous bicarbonate, water, and dried using anhydrous sodium sulphate. The ether was removed at reduced pressure and the residue recrystallised from aqueous ethanol to give 3-acetoxy-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (3.5 g., 84%), m.p. 143-151° (Found: C, 74.9; H, 6.3; N, 4.05. $C_{21}H_{21}NO_3$ requires C, 75.2; H, 6.3; N, 4.2%); νmax. 3475, 1725, 1595, 1250 cm.$^{-1}$

3-Hydroxy-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (CXXVIII).- A solution of 3-acetoxy-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (15 g.) in ethanol (300 ml.) was heated under reflux with sodium hydroxide (3.75 g.) in water (75 ml.) for 30 min. Much of the solvent was removed at reduced pressure, the remaining solution

diluted with water, and the solid thus precipitated filtered off and recrystallised from aqueous ethanol to give 6-benzyloxy-3-hydroxy-1,2,3,4-tetrahydrocarbazole (12.5 g., 95%), m.p. 144-147° (Found: C, 78.1; H, 6.4. $C_{19}H_{19}NO_2$ requires C, 77.8; H, 6.5%); νmax. 3600, 3475, 1625, 1595 cm.$^{-1}$

6-Benzyloxy-3-oxo-1,2,3,4-tetrahydrocarbazole (CXXIX).- To a boiling solution of 6-benzyloxy-3-hydroxy-1,2,3,4-tetrahydrocarbazole (13.5 g.) and cyclohexanone (70 ml.) in toluene (300 ml.) was added aluminium isopropoxide (4 g.) in toluene (60 ml.) such that the rate of addition was equal to the rate of distillation of the toluene. Distillation was then continued until the volume of the solution was reduced to 100 ml. Saturated aqueous sodium potassium tartrate (60 ml.) was added, and the mixture steam distilled to give approximately 60 ml. distillate. The residual mixture was extracted with chloroform and the chloroform solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was recrystallised from ethanol to give 6-benzyloxy-3-oxo-1,2,3,4-tetrahydrocarbazole (8.4 g., 63%), m.p. 161-175° (Found: C, 78.1; H, 6.0. $C_{19}H_{17}NO_2$ requires C, 78.3; H, 5.9%); νmax. 3475, 1710, 1595 cm.$^{-1}$

6-Benzyloxy-3-oxo-1,2,3,4-tetrahydrocarbazole Oxime (CXXX).- 6-Benzyloxy-3-oxo-1,2,3,4-tetrahydrocarbazole (1.9 g.) in ethanol (160 ml.) was heated under reflux with a solution of hydroxylamine hydrochloride (1.3 g.) and anhydrous sodium acetate (2.6 g.) in water (15 ml.) for 1 hr. Much of the ethanol was then removed at reduced pressure and the residual solution poured onto ice- and water. The precipitated solid thus formed was filtered off, washed with water and recrystallised from benzene to give 6-benzyloxy-3-oxo-1,2,3,4-tetrahydrocarbazole oxime (1.5 g., 75%), m.p. 160° (Found: C, 74.8; H, 5.6. $C_{19}H_{18}N_2O$ requires C, 74.5;

H, 5.9%); νmax. 3580, 3475, 3300, 1595 cm.$^{-1}$

3-Amino-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (CXXXI).- 6-Benzyloxy-3-oxo-1,2,3,4-tetrahydrocarbazole oxime (2.25 g.) in sodium-dried ether (250 ml.) was heated under reflux with lithium aluminium hydride (2 g.) for 24 hr. Water was then carefully added to decompose the excess lithium aluminium hydride and the resulting mixture separated. The aqueous layer was extracted with ether and the combined ethereal solution washed with water and extracted with 2N hydrochloric acid. The acid solution was washed with ether, made basic with aqueous ammonia, and extracted with ether. The ethereal solution of amine was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. Recrystallisation of the residue from benzene gave 3-amino-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (1.5 g., 71%), m.p. 131-135° (Found: C, 77.9; H, 6.5. $C_{19}H_{20}N_2O$ requires C, 78.0; H, 6.9%); νmax. 3475, 1625, 1595 cm.$^{-1}$

The hydrochloride was formed by passing dry hydrogen chloride through an ethereal solution of the amine, and was recrystallised from methanol, m.p. 238-240°.

The N-acetate was formed in dry pyridine by treatment of the amine with acetic anhydride, and was recrystallised from benzene, m.p. 157-163°, νmax. 3475, 3440, 1660, 1595 cm.$^{-1}$

3-Amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole (XLII).- To a solution of 3-amino-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (0.65 g.) in methanol (35 ml.) was added 10% palladium-on-carbon (0.3 g.) and the mixture shaken in an atmosphere of hydrogen at room temperature and atmospheric pressure until no more hydrogen was absorbed. The mixture was then filtered free of catalyst and the filtrate evaporated to dryness to give the impure hydroxy-amine. This material was purified by sublimation at 200°/1 mm. to give

3-amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole (0.43 g., 97%), m.p. 250° (decomp.) (Found: C, 71.6; H, 6.7. $C_{12}H_{14}N_2O$ requires C, 71.25; H, 7.0%); νmax. (Nujol) 3370, 3280, 1620, 1595 cm.$^{-1}$

The diacetate was formed in pyridine using acetic anhydride and the product recrystallised from benzene, m.p. 117-121°, νmax. 3475, 3440, 1750, 1655, 1590 cm.$^{-1}$

Cyclopentane-1,2-dione Mono p-Methoxyphenylhydrazone (CXXXIII).- To a solution of sodium (6 g.) in ethanol (85 ml.) at -20° was added a cold (-20°) mixture of cyclopentanone (21 g.) and ethyl formate (18.5 g.). The total mixture was kept at -20° for 30 min. and then overnight at 0°. The precipitate which separated on standing was dissolved in ice and water and the resulting solution neutralised with aqueous acetic acid and slowly added to a stirred solution of p-methoxyphenyldiazonium hydroxide maintained at 0°. The diazo solution was prepared by treatment of p-anisidine (24.5 g.), in 6N hydrochloric acid (95 ml.) at 0°, with sodium nitrite (16 g.) in water (40 ml.) and neutralised with aqueous sodium hydroxide. The red precipitate, formed on addition of the cyclopentanone and ethyl formate solution to p-methoxyphenyldiazonium hydroxide, was collected, washed with water and recrystallised from ethanol to give cyclopentane-1,2-dione mono-p-methoxyphenylhydrazone (26 g., 48%) (based on cyclopentanone), m.p. 189-190° (lit.,[189] 189-190°) (Found: C, 66.2; H, 6.7. Calc. for $C_{12}H_{14}N_2O_2$: C, 66.0; H, 6.5%).

7-Methoxy-3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole (CXXXIV).- Cyclopentane-1,2-dione mono p-methoxyphenylhydrazone (5 g.) was heated under reflux with 10% aqueous sulphuric acid (55 ml.) for 45 min. After cooling, the precipitated black solid was collected and recrystallised from ethanol to give 7-methoxy-3-oxo-1,2,-3,4-tetrahydrocyclopent[b]indole (1.5 g., 33%), m.p. 267-268°

(lit.,[189] 268°) (Found: C, 71.4; H, 5.6. Calc. for $C_{12}H_{11}NO_2$: C, 71.6; H, 5.5%); ν max. 3480, 1675 cm.$^{-1}$ ν max. (Nujol) 3150, 1690, 1650 cm.$^{-1}$ The yield of product in this reaction was variable and 33% represents the maximum yield obtained. Other reaction conditions and reaction times were employed but those described above were found to be optimum.

7-Methoxy-3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole Oxime (CXXXV).- 7-Methoxy-3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole (0.5 g.) was heated under reflux with hydroxylamine hydrochloride (3.7 g.) in pyridine (50 ml.) for 30 hr. The mixture was allowed to cool, poured onto ice and water and the solid precipitated filtered off and recrystallised from ethanol to give 7-methoxy-3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole oxime (3.8 g., 88%) (based on starting material consumed), m.p. 212-214° (Found: C, 66.5; H, 5.4. $C_{12}H_{12}N_2O_2$ requires C, 66.6; H, 5.6%); νmax. 3580, 3470, 3240, 1645 cm.$^{-1}$

The ethanolic mother liquors on concentration gave starting material (0.97 g.).

3-Amino-7-methoxy-1,2,3,4-tetrahydrocyclopent[b]indole (CXXXVI).- 7-Methoxy-3-oxo-1,2,3,4-tetrahydrocyclopent[b]indole oxime (0.5 g.) was refluxed with lithium aluminium hydride (0.5 g.) in dry tetrahydrofuran (40 ml.) for 19 hr. The mixture was cooled, water carefully added to decompose excess lithium aluminium hydride, and the tetrahydrofuran removed at reduced pressure. The residual mixture was extracted with ether and the ethereal solution washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness. Recrystallisation of the residue from benzene gave 3-amino-7-methoxy-1,2,3,4-tetrahydrocyclopent-[b]indole (0.23 g., 49%), m.p. 151-157° (Found: C, 70.9; H, 6.7. $C_{12}H_{14}N_2O$ requires C, 71.25; H, 7.0%); ν max. 3470, 3370, 1625, 1585 cm.$^{-1}$

Attempted Demethylation of 3-Amino-7-methoxy-1,2,3,4-tetrahydrocyclopent[b]indole (CXXXVI).- Method A.[215,231] The methoxy-amine (0.25 g.) was heated under reflux with a mixture of glacial acetic acid (30 ml.) and concentrated hydrobromic acid (10 ml.) for 3 hr. The resulting dark coloured solution was poured onto ice and water, neutralised with sodium bicarbonate, and extracted with chloroform in a continuous extractor. After washing and drying the chloroform solution, it was evaporated to dryness to give a residue (0.07 g.), the infrared spectrum of which possessed no absorption attributable to the indolic N-H in the 3470 $cm.^{-1}$ region.

The composition of the acid mixture and the reaction time were varied but none of the required hydroxy-amine (XLVIII) was isolated.

Method B.[209] The methoxy-amine (0.11 g.) was heated under reflux in benzene (50 ml.) with finely divided anhydrous aluminium chloride (0.3 g.) for 12 hr. The mixture was cooled, water added and the black precipitate (0.08 g.) thus formed filtered off, washed and dried. This material was insoluble in aqueous sodium hydroxide and organic solvents; the infrared spectrum (Nujol) possessed no absorption attributable to the indolic N-H. The filtrate was separated, the aqueous layer neutralised with sodium bicarbonate, washed with benzene and the combined benzene solution washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The oily residue (0.02 g.) was insoluble in aqueous sodium hydroxide and the infrared spectrum indicated that it was impure starting material.

The reaction was repeated using carbon disulphide and nitrobenzene as solvents in place of benzene, and the reaction times were varied, but none of the required product was isolated.

Method C.[232] The methoxy-amine (0.2 g.) was intimately mixed with pyridine hydrochloride (0.6 g.) and heated under nitrogen at 140° for 4 hr. Water was added to the cooled mixture and the black precipitate (0.16 g.) thus formed filtered off, washed and dried. The infrared spectrum and the solubilities of this material were similar to those of the black solid described in method B. Similarly neutralisation and chloroform extraction of the aqueous filtrate gave, after removal of chloroform, a residue (0.04 g.) similar to that thought to be starting material in method B. Shorter reaction times and lower reaction temperatures merely increased the yield of impure starting material isolated from the neutralised aqueous phase.

Method D.[233] To a solution of the methoxy-amine (0.2 g.) in dry methylene chloride (40 ml.) cooled to -80° was added boron tribromide (0.5 ml.), and the mixture kept at -80° for 30 min. The solution was allowed to attain, and remain at, room temperature for a further 3 hr. On careful addition of water a black solid was precipitated with infrared spectrum and solubility characteristics similar to those of the black solids of methods B and C. After removal of this material the aqueous filtrate was washed with ether, neutralised with sodium bicarbonate, and the fine precipitate thus formed taken up into chloroform. This solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness to give a trace of impure yellow solid, m.p. 180-190°. This material was insoluble in water, had low solubility in organic solvents, but was readily soluble in aqueous acid and base. Insufficient of this material was obtained to achieve a complete characterisation.

INTRODUCTION

The realisation, early in this century, that many organic substances were sensitive to light aroused considerable interest and this has led to the development of a novel and now rapidly expanding branch of chemistry. Several excellent reviews[245,246] of photochemistry are available, and more specialised reviews dealing with photobiology[247] and related aspects[248] of photochemistry have recently been published. The introduction of a Journal of Photochemistry and Photobiology, first published in 1962, reflects the interest which has been shown in this field of study.

Since the early studies of Ciamician and Silber, and Paterno using sunlight as the energy source, ultraviolet, visible, and even fluorescent light have also been shown to induce structural modifications in light-sensitive molecules. The availability of a variety of high intensity ultraviolet sources coupled with their convenience in use now make ultraviolet irradiation a practical technique of general application. Mercury arc lamps have been employed widely for this purpose. Light-sensitive molecules possess structural features termed chromophores which absorb incident light of particular frequencies. The energy gain associated with this process raises the molecule to an excited state and generally involves the transition of n or π electrons from the ground state to antibonding π orbitals. Although other electronic transitions are also known to occur on absorption of light, these two fundamental processes, refered to as $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, are generally regarded as being responsible for most of the commonly encountered photochemical reactions. A notable exception is the $n \rightarrow \sigma^*$ transition thought to be involved in the photolysis of halogeno compounds.[249] Dissipation of the excitation energy may occur by a variety of processes: the energy may be wholly or partially converted into thermal energy by

collision, it may be re-emitted as fluorescence or phosphorescence, or finally it may be utilised in bond redistribution associated with photochemical reactions involving structural rearrangement, addition or fragmentation.

These photochemical processes are generally regarded, and diagrammatically represented, as involving radical species, but the precise nature of the intermediates is still in some doubt:[250] the data obtained from stereospecific rearrangements may be accounted for by stabilised radical intermediates or by synchronous processes.

Although light-induced reactions are of fundamental interest and are now known to have some preparative value in organic chemistry, they do also present certain problems; unless adequate protection is provided against light, for example, these photochemical processes lead to the decomposition of light-sensitive substances during storage. Coloured glass bottles and opaque containers such as aluminium or card are often employed for the protection of such substances, and stabilisers which decrease the rate of decomposition have also been added to certain preparations.[251]

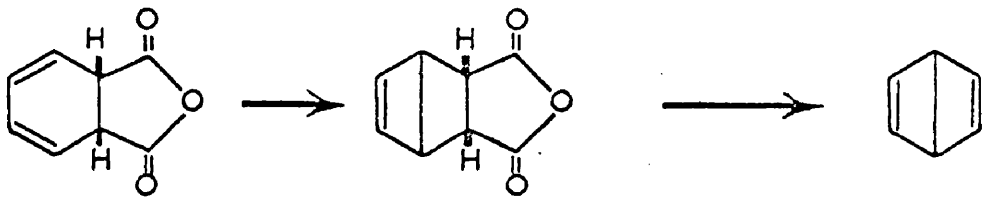
Drugs, often quite complex molecules, frequently possess absorbing chromophores which render them light-sensitive and the storage of these substances is of particular concern since the decomposition products frequently have pharmacological properties markedly different from those of the parent drug. Several reports of changes in pharmacological activity of preparations on exposure to light have been made, but in relatively few instances have these investigations been followed up by structural determinations of the products. In the presence of air, the problems associated with the storage of light-sensitive substances

are further complicated as certain compounds, particularly those possessing unsaturated centres, undergo photochemically-induced oxidation. The well documented deterioration of chlorpromazine, for example, is thought to be the result of photo-oxidation, as is the well known colouration and decomposition of amines on exposure to light and air. The processes involved in photochemical oxidation have been extensively studied and several reviews[252] have appeared in the literature on this subject. It will not, therefore, be discussed further in this brief outline of photochemical processes.

Certain substances, unable to absorb light directly, have been shown to undergo light-induced structural changes in the presence of a sensitiser, a substance capable of absorbing light and transfering the energy to the non-absorbing species. Low concentrations of sensitisers are generally sufficient for photosensitisations; these may be impurities in the non-absorbing material, or, in drug preparations, addititives such as colouring agents. Folic acid, in preparations containing small quantities of riboflavin, is photochemically decomposed with simultaneous reduction of the riboflavin.[253] Riboflavin has a similar sensitising effect[254] in mixtures of the vitamin and ascorbic acid which are also degraded on exposure to sunlight.

Although some molecules, on exposure to light, merely polymerise, others undergo discrete processes which lead to well defined products. These frequently possess highly strained structures which have proved difficult to synthesise by conventional chemical procedures. One such rearrangement is that of cis-1,2-dihydrophthalic anhydride (CXL) which on photolysis[255] yields the bridged structure CXLI and on subsequent treatment

with lead tetraacetate gives bicyclo[2,2,0]hexa-2,5-diene (CXLII), the first synthesis of a "Dewar benzene" structure.



CXL CXLI CXLII

fig. 26

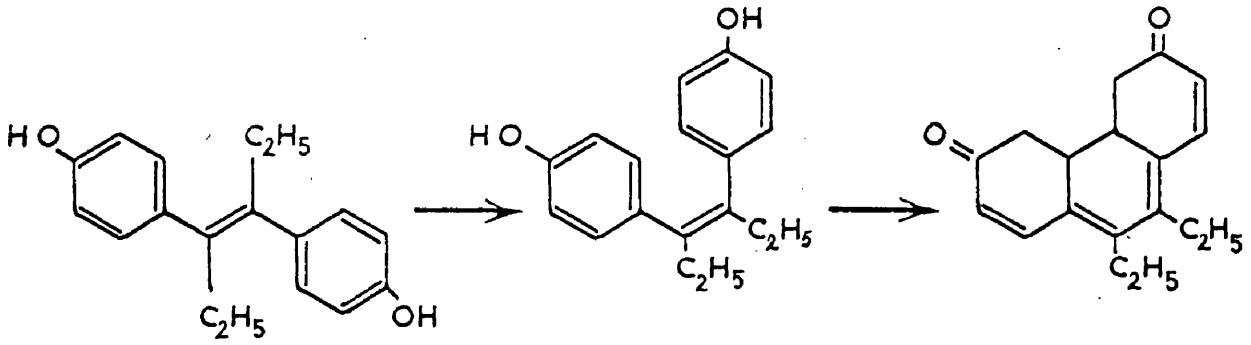
The environment of a molecule has a considerable effect upon the course of a photochemical reaction: in the solid phase, due to the fixed orientation of the molecules in the rigid crystal lattice, interactions are limited to those involving centres which are close together, whereas in solution this limitation no longer exists as the molecules possess a greater degree of freedom and consequently other reactions, excluded in the solid state, frequently predominate. The nature of the solvent employed in solution photolyses often determines which reactions are most favoured. Certain solvents, such as alcohols, may be chemically involved in the photo-induced reaction while others, such as saturated hydrocarbons, being more inert, simply form a dispersion medium for the material under investigation. Solvents which themselves absorb in the ultraviolet, such as acetone and benzene, are known to act as sensitisers. The possibility that some solvents may have an indirect effect upon the reaction by stabilising certain intermediates should not be excluded. The concentration of material, as well as the nature of the solvent, is important in investigations carried out in solution; dilute solutions tend to favour interaction with the solvent or intramolecular rearrangements, whereas in more concentrated solution intermolecular reactions, often resulting in dimerisation, are more commonly encountered.

The absorption of light and the subsequent reactions which

take place, largely depend upon the nature of the absorbing chromophore; it is logical therefore to discuss photochemical transformations in terms of these structures. The photochemical reactions of drug molecules possessing well investigated chromophores such as carbon-carbon double bonds, isolated and conjugated aldehydes and ketones, and aromatic centres are discussed under these headings, while light-sensitive drug molecules which possess other absorbing chromophores are considered in the final section entitled miscellaneous transformations.

Carbon-carbon double bonds.

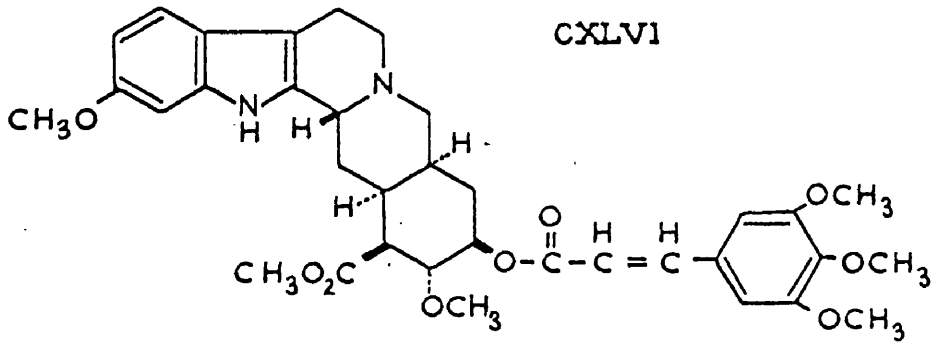
Compounds possessing only isolated carbon-carbon double bonds capable of absorbing light are not, in general, light-sensitive. Suitably substituted unsaturated molecules, however, in which the double bond is conjugated with an additional centre of unsaturation, do absorb ultraviolet light and are subject to photochemical excitation. The most commonly encountered rearrangement of these substances is cis-trans isomerisation about the double bond to give an equilibrium mixture of the two isomers. The less stable isomer frequently predominates. Trans-diethylstilboestrol (CXLIII), on exposure to light,[256] is initially isomerised to the cis isomer (CXLIV), and further irradiation yields the tricyclic diketone (CXLV). This conversion results in considerable loss in



CXLIII CXLIV CXLV

fig. 27

oestrogenic activity, as the trans isomer has several times the activity of the cis form.[257] The Rauwolfia alkaloid, Rescinnamine (CXLVI) undergoes similar isomerisation[258] about the double bond in the $C_{(18)}$ substituent. However, this conversion does not appear to lead to any appreciable change in pharmacological activity of the material, both isomers apparently having similar pharmacological properties.



CXLVI

Another similar conversion of biological importance is that of vitamin A_1, in which the all trans compound is converted into an equilibrium mixture of cis and trans forms,[259] resulting in a significant reduction in vitamin A activity. Vitamin A precursors, α , β and γ -carotene, also undergo similar cis - trans isomerisations: α -carotene, for example, is converted from the all trans form into ten distinct cis - trans isomers on exposure to sunlight.[260]

Cis - trans isomerisation is also known to be involved in the initial processes of vision.[261] Absorption of light by the visual pigment rhodopsin (an addition product of 11-cis-retinal and the protein opsin) induces cis - trans isomerisation of the 11-cis-retinal portion of the molecule. This photochemical reaction is followed by a series of rapid dark reactions and leads to visual excitation. Although certain of these reactions are well understood, the precise process which triggers excitation is still not clear.

The photochemically-induced reactions of both acyclic dienes and heteroannular cyclic dienes have been fully reviewed[262] elsewhere, and as the compounds possessing these chromophores are of little biological importance, these transformations will not be discussed further here.

The transformations of homoannular cyclic dienes, however, are of more significance in a consideration of the photochemically induced changes of drug molecules. Exposure of these substances to light leads to acyclic trienes by ring cleavage, or substituted bicyclo[2,2,0]hexanes by carbon-carbon bridging. In the presence of a sensitiser, homoannular cyclic dienes have also been shown to dimerise. Certain reactions characteristic of the homoannular diene system are exemplified by the well investigated photochemical reactions[263] of ergosterol (CXLVII). On exposure to light ring cleavage of the cyclic diene to the acyclic triene occurs to yield pre-ergocalciferol (CXLVIII); subsequent thermal rearrangement yields vitamin D_2 (CXLIX). The related compounds, tachysterol (CL) and lumisterol (CLI) are reversibly formed from pre-ergocalciferol by further irradiation. Vitamin D_2 is heat and light sensitive; thermal rearrangement yields the stereoisomers pyrocalciferol (CLII) and isopyrocalciferol (CLIII). These in turn are both transformed to the bicyclo[2,2,0]hexene derivatives of general formula CLIV on further irradiation. Ergosterol has also been shown[264] to undergo photochemical dimerisation in alcoholic solution in the presence of the sensitisers eosin or erythrosin to yield bisergostatrienol (CLV). Of the photochemical products of ergosterol, only vitamin D_2 has any significant anti-rachitic activity.

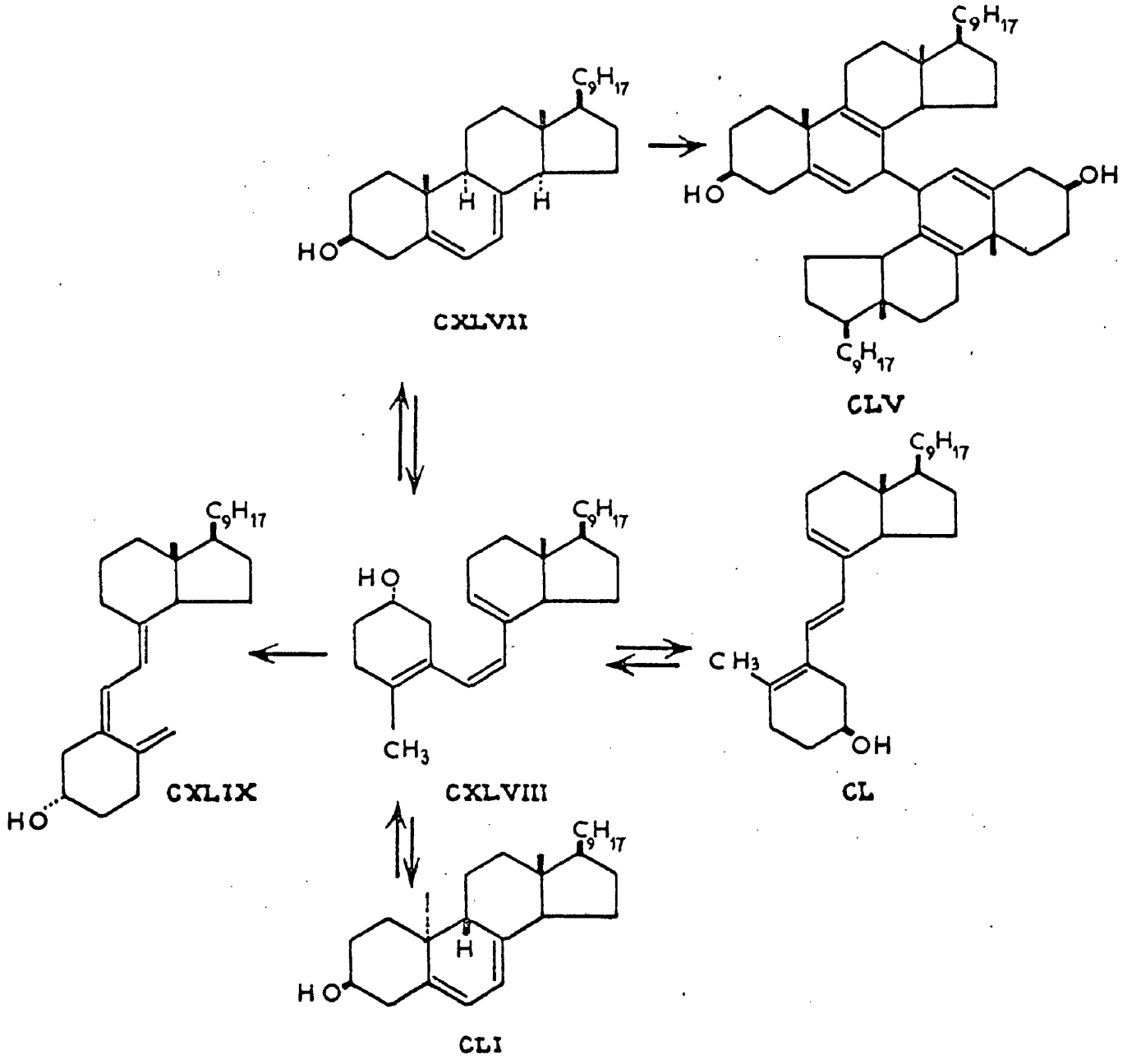


fig. 28

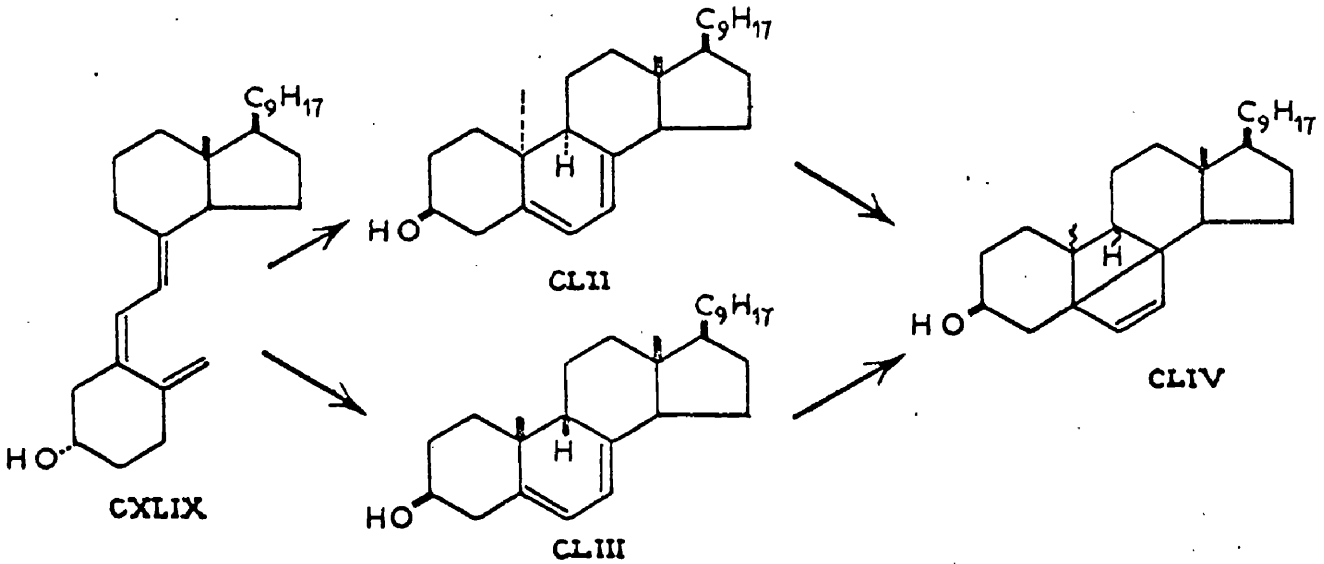
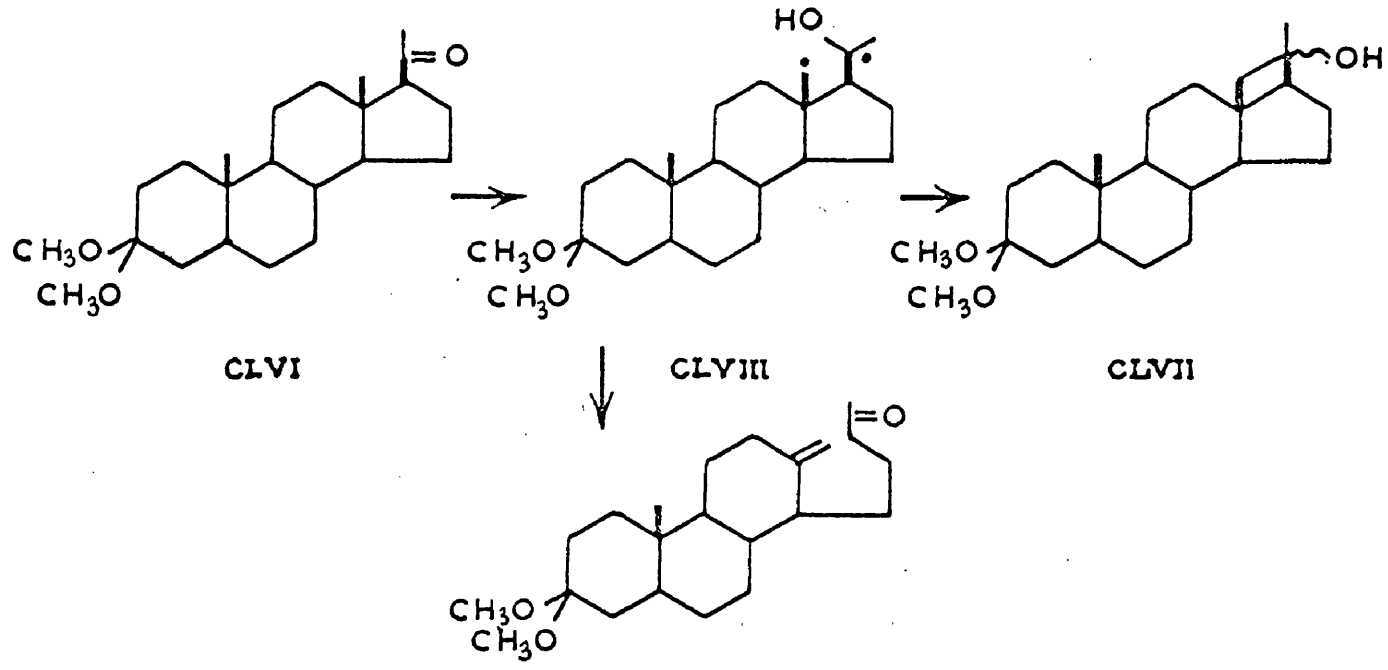


fig. 29

Aldehydes and Ketones

Substances possessing non-conjugated ketones have a weak ultraviolet absorption (ϵ max.$^{10-30}$ at 290mμ), and, therefore, generally undergo photo-induced reactions only slowly. The most commonly observed light-induced reaction of compounds possessing this function involves hydrogen abstraction by the activated carbonyl: this process may be intramolecular if the molecule possesses a γ hydrogen atom and results in the formation of a cyclobutanol derivative, or intermolecular if this is energetically more favourable. Cyclobutanol formation has been observed in 11- and 20-oxo-steroids; in both examples, the excited ketone abstracts a hydrogen atom from the neighbouring angular methyl group; thus 3,3-dimethoxypregnan-20-one (CLVI) is converted[265] into the cyclobutanol derivative (CLVII) probably via the intermediate diradical (CLVIII). An alternative pathway for the decomposition of the diradical (CLVIII) is by D-ring cleavage to yield the ketone (CLIX). Intermolecular hydrogen abstraction generally involves the solvent and results in reduction and pinacol formation.[266]

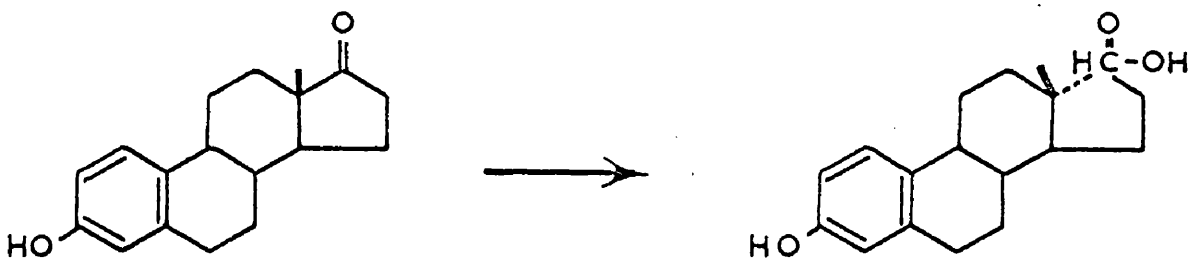


CLVI CLVIII CLVII

CLIX

fig. 30

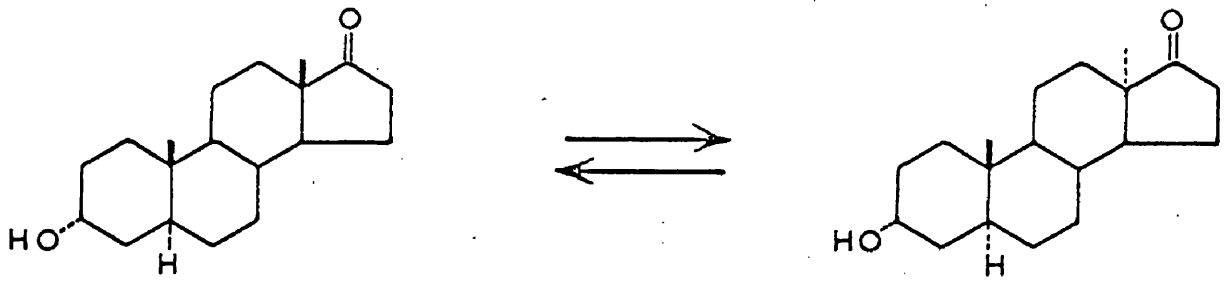
Cleavage of the carbon-carbon bond adjacent to the carbonyl group is another favourable and commonly encountered process in the photolysis of non-conjugated ketones. This process results in fragmentation and decarbonylation if the carbonyl is a terminal function; thus certain 19-oxo-steroids, on exposure to light, lose carbon monoxide and yield the corresponding 19-nor-steroid.[267] Substances in which the carbonyl group is not terminal frequently cleave as described above and react with hydroxylic solvents; the corresponding acids or esters have been isolated. The female sex hormone, oestrone (CLX), for example, on irradiation[268] in aqueous dioxan, yields the acid (CLXI). The intermediate involved in this reaction is thought to be a diradical which on intramolecular hydrogen abstraction rearranges to a ketene; subsequent addition of water yields the acid.



CLX CLXI

fig. 31

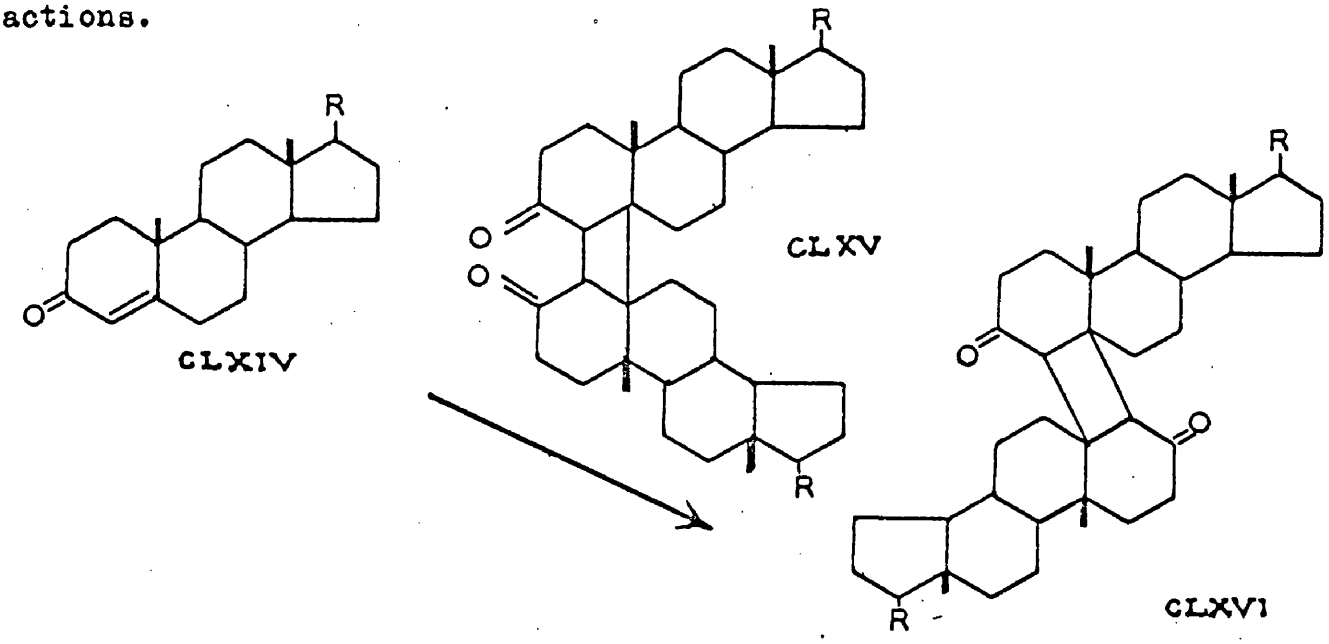
Another pathway available to the diradical, formed on photolysis of the ketone, is recombination to yield the starting material. If the carbon atom adjacent to the ketone is asymmetrically substituted then this recombination process may lead to epimerisation at the asymmetric centre. 17-Oxo-13α- and 17-oxo-13β-steroids are interconvertible by exposure to light; androsterone (CLXII), for example, is epimerised[269] to lumiandrosterone (CLXIII) under the influence of light with accompanying loss of androgenic activity.[269]



CLXII CLXIII

fig. 32

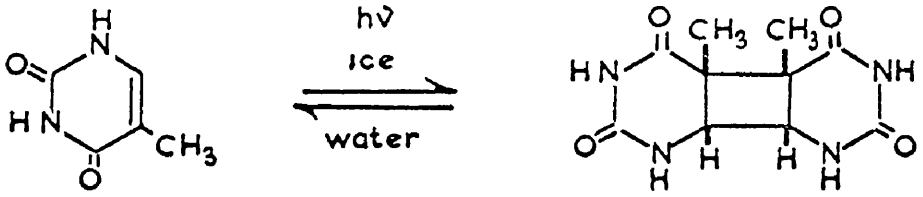
Compounds possessing an α,β -unsaturated ketone, in addition to undergoing the reactions characteristic of carbon-carbon double bonds and non-conjugated ketones, also undergo a variety of other transformations made possible by the conjugation. In the solid state or in concentrated solution, the most favourable process appears to be an intermolecular interaction giving dimeric material possessing a substituted cyclobutane structure. The sex hormones testosterone[270] (CLXIV; R,=OH) and progesterone[271] (CLXIV; R,=$COCH_3$) are dimerised in this way and complete loss of physiological activity has been observed. Of the two possible modes of dimerisation which exist for these compounds, with ketones "head to head" (CLXV) and "head to tail" (CLXVI), only the latter is generally encountered in solution photolyses since this process leads to a product with the least steric and non-bonded interactions.



CLXIV CLXV CLXVI

fig. 33

In the solid phase, however, the configuration of the dimer is determined by the orientation of the molecules in the crystal lattice.[272] Thymine (CLXVII), both in the crystal form and in frozen aqueous solution dimerises[273] in a similar manner on exposure to light to give CLXVIII; this process has been shown to be reversible, the dimer being reconverted into thymine by irradiation in water at room temperature. This dimer has also been

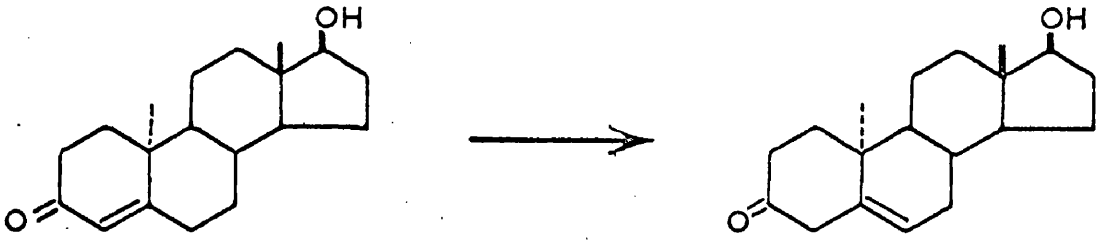


CLXVII CLXVIII

fig. 34

isolated by hydrolysis after irradiation of deoxyribonucleic acid (DNA) with ultraviolet light <u>in vivo</u> and <u>in vitro</u>.[274] It is also present in bacteria which have been irradiated with ultraviolet light.[275]

In more dilute solution, certain 3-oxo-Δ^4 steroids undergo double bond migration; this has been observed[276] in 10α-testosterone (CLXIX), which on irradiation in t-butanol is converted into the corresponding non-conjugated β,γ-unsaturated ketone (CLXX).



CLXIX CLXX

fig. 35

The natural hormone, testosterone (CLXXI), however, does not rearrange in this simple manner but undergoes a series of more

complex transformations[270] which may be represented as shown in fig. 36 to yield the ketonic products CLXXII and CLXXIII.

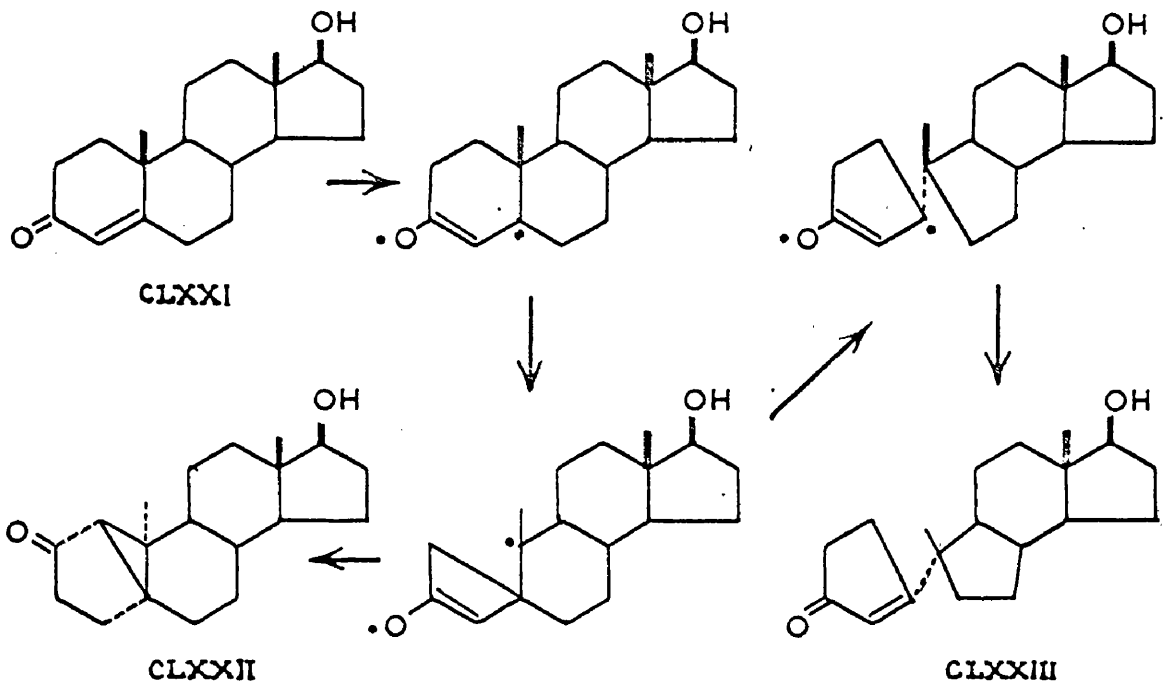
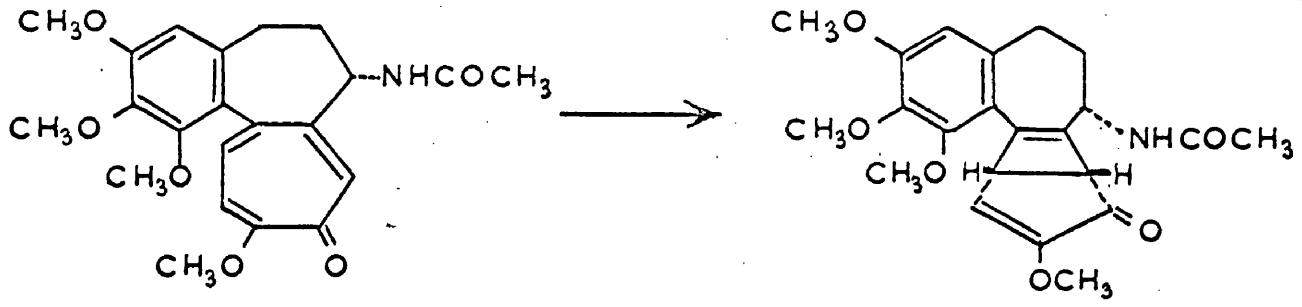


fig. 36

Steroidal α,β -unsaturated ketones also undergo photochemical reduction[270] in many solvents; this may result in the formation of the corresponding saturated ketone or, the formation of a pinacol product. Thus 17β -acetoxytestosterone, on irradiation[270] indiethyl ether yields the pinacol, while in ethanol 17β -acetoxy-5α -androstan-3-one is the major product.

The alkaloid colchicine (CLXXIV), used in treatment of acute gout, possesses a conjugated trienone system and this, on irradiation, undergoes carbon-carbon cross bridging to yield β -lumicolchicine (CLXXV);[277] its stereoisomer δ -lumicolchicine,[278] and a product described as α-lumicolchicine,[279] now shown to be the dimer of β-lumicolchicine. This type of cross bridging is also commonly encountered in the photochemistry of seven-membered cyclic conjugated dienones and of highly substituted six-membered cyclic conjugated dienones.



CLXXIV CLXXV

fig. 37

Of particular interest in photochemical studies is the cross-conjugated dienone chromophore (1,4-dien-3-one) which is found in certain naturally occurring and biologically important molecules. This system has attracted considerable interest and large sections of reviews have been devoted to a consideration of the photochemically-induced transformations which occur in molecules possessing this chromophore. α-Santonin (CLXXVI) is one such compound which has been thoroughly investigated. This sesquiterpene, known[280] to be light-sensitive since the beginning of the century, has been widely employed as an anthelmintic. Investigation has shown that the changes which occur on exposure to light are extremely sensitive to the solvent employed: in aqueous acetic acid[281] isophotosantonic lactone (CLXXVII) and photosantonic acid (CLXXVIII) are the major products of ultraviolet irradiation whereas in ethanol,[282] lumisantonin (CLXXIX), postulated to be a non-isolatable intermediate in the conversion of santonin into photosantonic acid in acetic acid, is formed. The mechanism by which lumisantonin is converted into photosantonic acid in acetic acid has provoked considerable discussion[283] but recent investigations support the view that yet another intermediate, of structure CLXXX, is involved.[284] This material has been isolated from the irradia-

tion product of lumisantonin in anhydrous diethyl ether. In basic

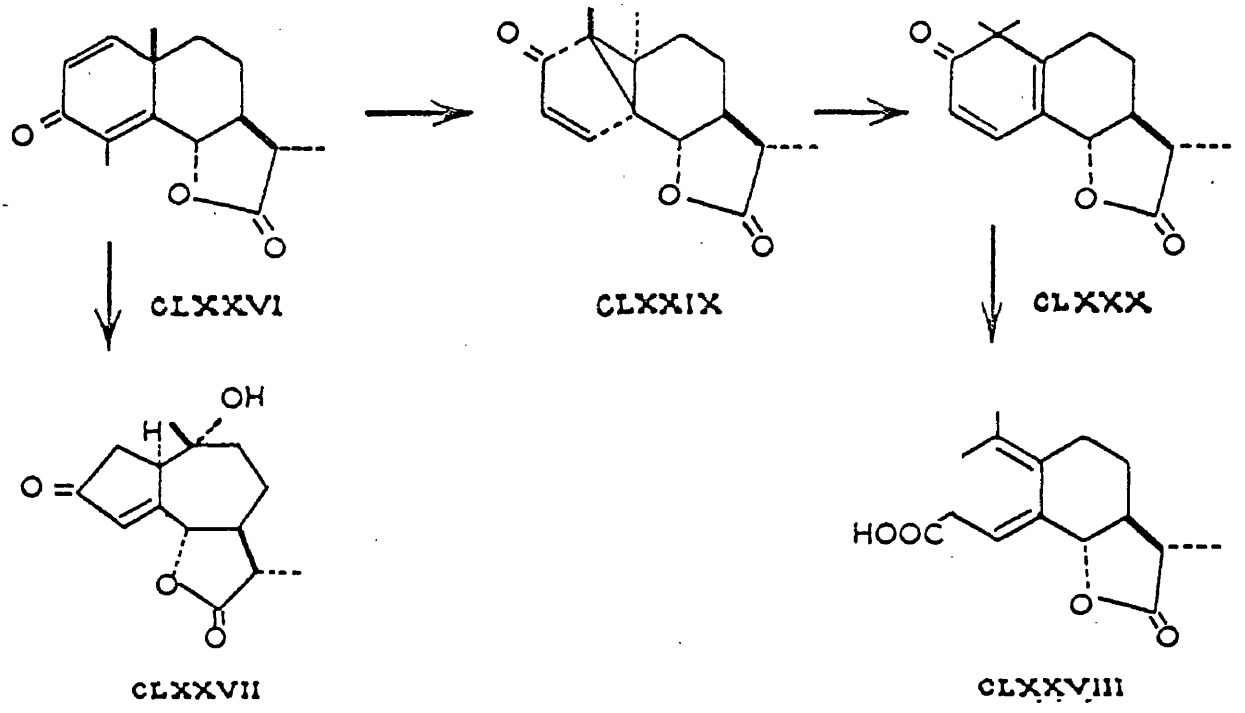
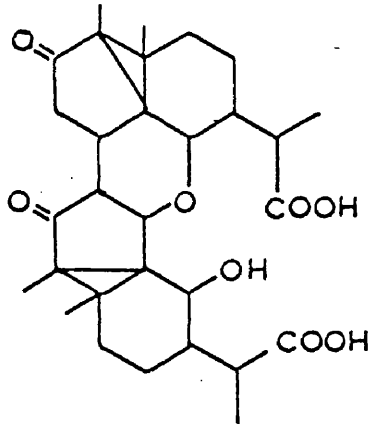


fig. 38

solution[285] the dimeric material, photosantoninic acid (CLXXXI), is the major product. A variety of processes are also known to occur in the solid state, but the structures of the products have not been completely elucidated. Pharmacological testing of



CLXXXI

several of these products has shown that decomposition of α-santonin is paralleled by a decrease in the anthelmintic activity[286] of the preparation. Certain of the products, however, have been shown[287] to possess anti-inflammatory properties.

Similar processesses to those involved in the photo-rearrangement of α-santonin could account for the known decomposition of opthalmic preparations of prednisolone and methyl prednisolone on exposure to light.[288] In ethanolic solution, these cross-conjugated dienone steroids are even decomposed by fluorescent light. The anti-inflammatory agent prednisone also possesses this chromophore, and its acetate (CLXXXII), on exposure to ultraviolet light undergoes rearrangements[289] which are largely dependent upon the nature of the solvent. In acetic acid, a rearrangement similar to that which occurs in the conversion of santonin into isophotosantonic lactone takes place, but in ethanol B-ring cleavage at $C_{(9,10)}$ occurs to give the proposed diradical (CLXXXIII) which on rearrangement gives the unsaturated ketone (CLXXXIV). In dioxan, aromatisation occurs in ring A to yield a phenol with probable structure CLXXXV.

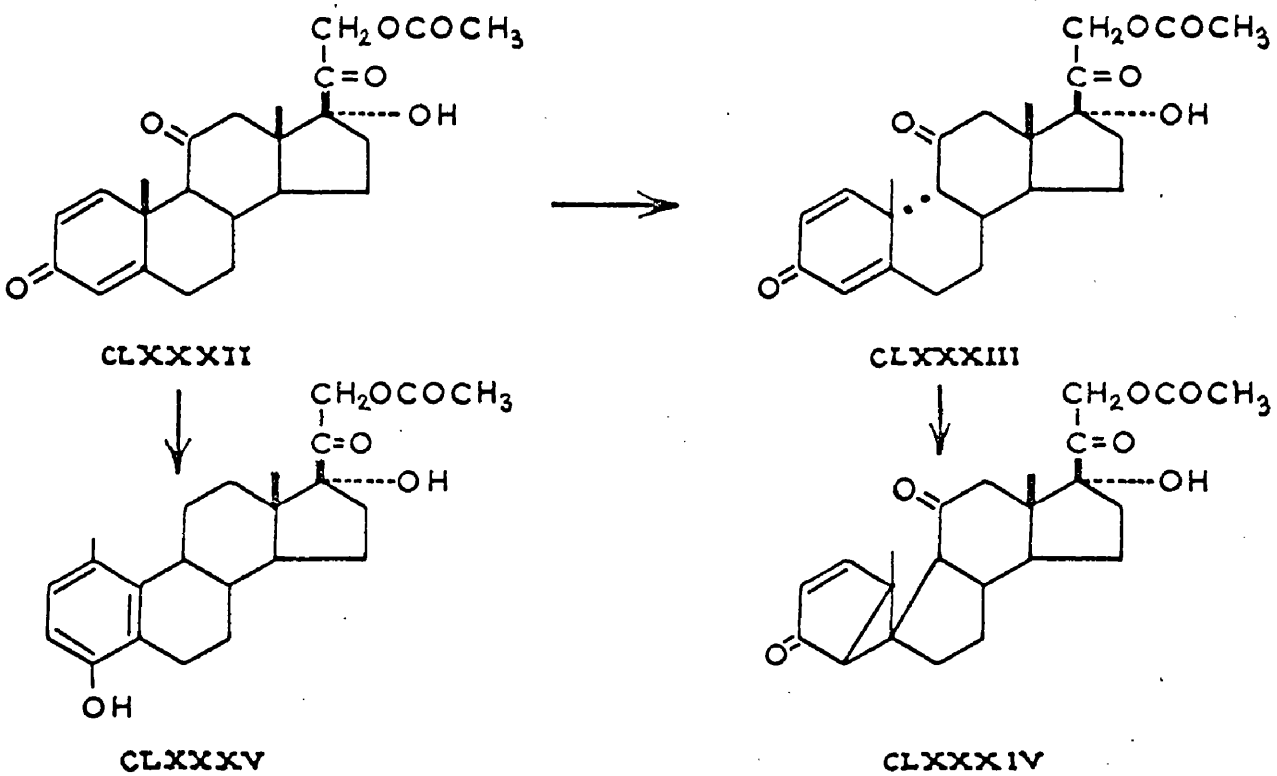
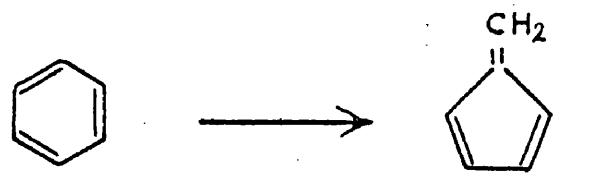


fig. 39

Aromatic Compounds

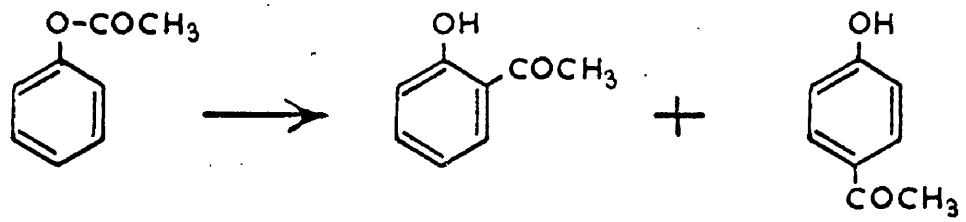
The benzene ring, although capable of absorbing ultraviolet light, is not readily transformed in this way: prolonged irradiation of benzene (CLXXXVI), for example, is necessary for its conversion,[290] in very low yield into fulvene (CLXXXVII). The energy absorbed by



CLXXXVI CLXXXVII

fig. 40

the aromatic nucleus may, however, be transmitted to a suitable substituent of the ring, and in this way functions which are not generally regarded as light-sensitive may undergo photochemically-induced rearrangement. In these instances, the aromatic nucleus is effectively acting as an "internal sensitiser". Phenyl acetate (CLXXXVIII), for example, undergoes a "photochemically-induced Fries rearrangement"[291] on exposure to ultraviolet light to yield a mixture of ortho (CLXXXIX) and para (CXC) hydroxyacetophenone. This process has been employed in a synthesis of the antifungal griseofulvin.[292]

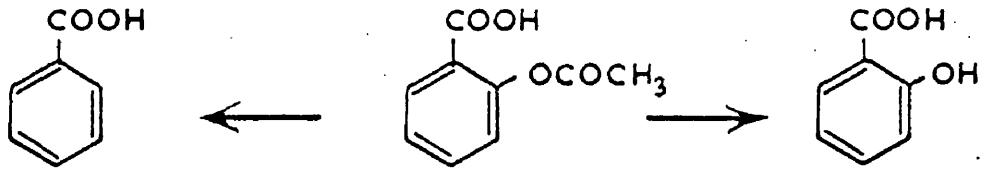


CLXXXVIII CLXXXIX CXC

fig. 41

Acetylsalicylic acid (asprin; CXCI) is also known to be light-sensitive; recent investigations[293] have shown that, in

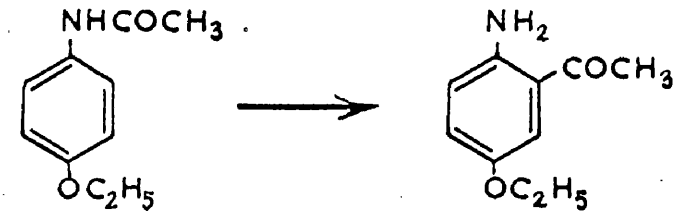
ethanol, hydrolysis to salicylic acid (CXCII) occurs rather than the Fries rearrangement. In the presence of a trace of acid, complete elimination of the acetoxy group has been observed and high yields of benzoic acid (CXCIII) have been isolated. The close proximity of the acetoxy and the carbonyl functions and the intramolecular hydrogen bonding known to exist in acetylsalicylic acid would almost certainly be responsible for this novel reaction.



CXCIII CXCI CXCII

fig. 42

Phenolic ethers, such as the fungicide phenoxyacetic acid, are isomerised by a process analogous to the photochemical Fries rearrangement; p-hydroxyphenylacetic acid and the lactone of the ortho isomer are the major products of the light-induced rearrangement[294] of phenoxyacetic acid. Anilides are similarly rearranged to the corresponding ortho and para substituted anilines. We know that phenacetin (CXCIV), for example, yields 2-amino-5-ethoxyacetophenone (CXCV) as the major product on irradiation in ethanol.[295]



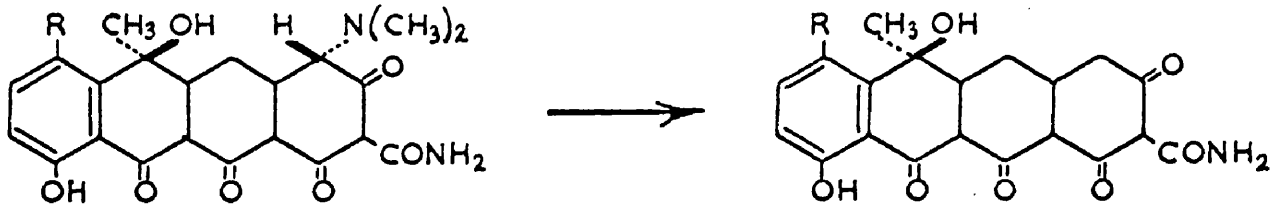
CXCIV CXCV

fig. 43

Polynuclear aromatic hydrocarbons are not easily transformed by ultraviolet light, but dimerisation is one process which has been observed:[296] anthracene, and the carcinogenic hydrocarbons benz[a]anthracene, 4-methylbenz[a]anthracene and 5-methylbenz[a]-anthracene have all been shown to undergo photochemical dimerisation.

Miscellaneous Transformations

Aliphatic and aromatic amines are frequently sensitive to light; aqueous solutions of primary aliphatic amines, after irradiation, have been shown to contain the alcohol corresponding to the amine and ammonia.[297] Secondary and tertiary amines yield a complex mixture of products; diethylamine, for example, in the absence of oxygen yields N,N-diethylbutane-2,3-diamine, 1,3-diethyl-2,4,5-trimethylimidazolidine, tetraethylhydrazine, and N-but-2-enylidene-ethylamine.[298] Tetracycline (CXCVI; R=H) and chlorotetracycline (CXCVI; R=Cl), in methanol, have recently been reported[299] to undergo photochemically-induced deamination to give products of general structure CXCVII; the tertiary amino group was detected as dimethylamine. The light sensitivity of the cate-

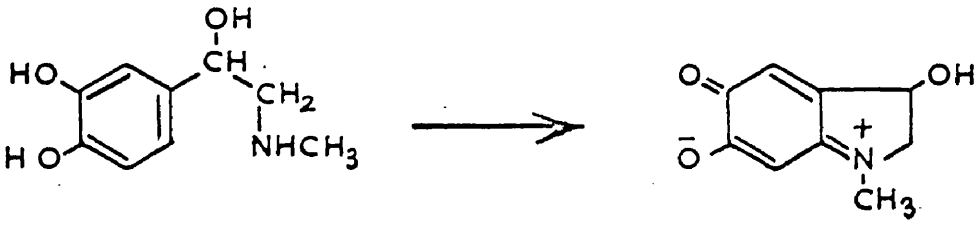


CXCVI CXCVII

fig. 44

cholamine adrenaline is well documented;[300] solutions, on exposure to air and light, have reduced hypertensive properties. In neutral solution, adrenaline (CXCVIII) undergoes an intramolecular oxidative ring closure to yield adrenochrome (CXCIX) which itself

is further polymerised to melanin[301] on exposure to ultraviolet light.



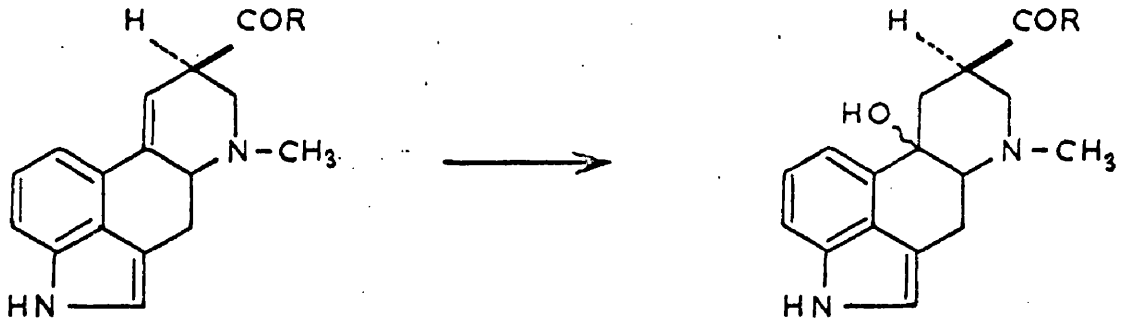
CXCVIII CXCIX

fig. 45

Generally, light-sensitive pharmacologically active molecules on exposure to light yield products with reduced activity. However, phenylephrine is an interesting example for which the converse is true;[302] increased pressor activity has been reported when solutions of this amine are allowed to stand in the presence of oxygen and sunlight. This phenomenon is now attributed to the light-induced hydroxylation of phenylephrine which converts it into the more active adrenaline. A change in the pharmacological properties of chlorpromazine on irradiation has also been observed; irradiation in the absence of oxygen yields products which are reported[303] to possess a significant anti-tumor activity.

Photochemically-induced addition of water to certain unsaturated drug molecules has also been observed, and this frequently leads to products with a considerably reduced pharmacological activity. The naturally occurring alkaloid, ergotamine (CC; R=peptide residue), employed in the treatment of migraine, undergoes such an addition of water to the $C_{(9,10)}$ double bond when exposed to ultraviolet light in the presence of mild acid.[304] The product is a mixture of the two isomeric 10-hydroxy derivatives of general structure CCI (fig. 46). The photo-product, lumiergotamine, has a small fraction of the activity of the parent ergotamine. A similar process has been described for ergometrine,

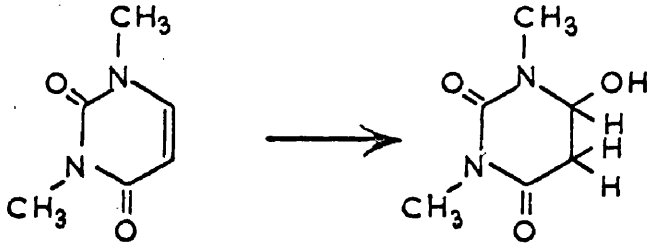
lumi-ergometrine having one hundredth of the anti-5-HT activity of ergometrine on the isolated rat uterus. 1,3-Dimethyluracil



CC CCI

fig. 46

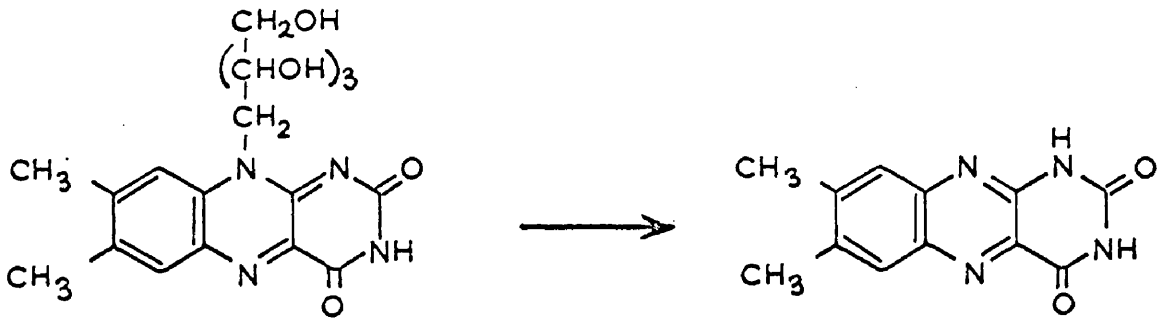
(CCII) also undergoes photochemically-induced addition of water to the $C_{(5,6)}$ double bond to yield preferentially the 6-hydroxy derivative (CCIII).[305]



CCII CCIII

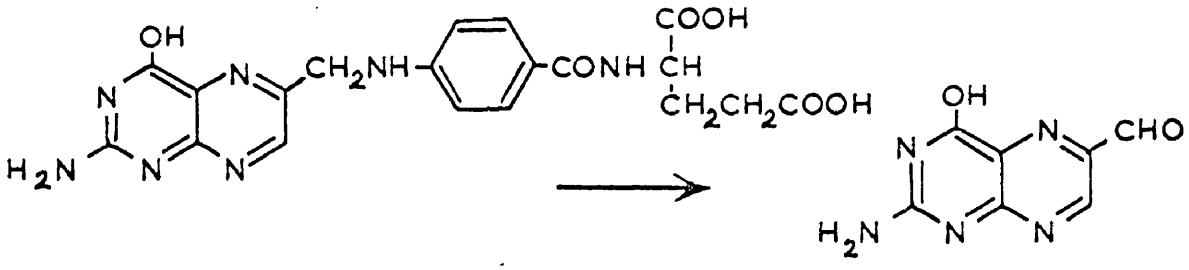
fig. 47

Photochemically-induced fragmentation is another process by which drug molecules may be structurally modified or degraded on exposure to light. Such transformations are inevitably accompanied by change in the pharmacological activities of these preparations. Riboflavin (CCIV) in neutral solution, for example, is cleaved[306] to the tricyclic product CCV by loss of the sugar residue on exposure to ultraviolet light. Similarly, the initial step in the photo-decomposition of folic acid (CCVI) involves the cleavage and loss of the p-aminobenzoylglutamic acid side chain to yield the pteridine (CCVII).[307]



CCIV CCV

fig. 48



CCVI CCVII

fig. 49

Other drug molecules of particular importance which are photochemically decomposed include cyanocobalamin (vitamin B_{12}),[308] used in the treatment of pernicious anaemia, and the widely employed antibiotic cephalosporin C.[309] The beneficial action of cyanocobalamin is reduced by half and the activity of cephalosporin C completely destroyed on exposure to ultraviolet light. The nature of these photo-products has not as yet been determined.

DISCUSSION

The present investigation was concerned with a study of the effect of ultraviolet light on certain hormonal and related steroids. A medium pressure mercury arc light source and a quartz photochemical reactor were employed. The irradiations were carried out in dilute (less than 4 mg/ml) solution, to minimise intermolecular reactions between steroid molecules, and in an atmosphere of nitrogen to prevent photochemical oxidation. In general the photochemical reactions were followed by infrared spectroscopy.

Cortisone acetate (21-Acetoxy-17-hydroxypregn-4-ene-3,11,20-trione)

The effect of ultraviolet light upon the physiologically and pharmacologically important steroid cortisone was first investigated. Like other corticosteroids, cortisone is synthesised from cholesterol in the adrenal cortex and its presence influences carbohydrate, protein, fat and purine metabolism. It also affects electrolyte and water balance, and the functional capacities of the cardiovascular system, the nervous system, the kidneys, skeletal muscle and various other organs and tissues. Corticosteroids also lead to the development in the organism of resistance to noxious stimuli and to environmental changes. Therapeutically, they are employed in substitution therapy when their secretion from the adrenal cortex is insufficient for normal body functioning, and in the treatment of adaptive diseases such as rheumatoid arthritis, asthma and rheumatic fever. Cortisone is frequently employed in the treatment of these diseases in the form of the 17-acetyl derivative.

The effects of X-rays upon methanolic solutions[310] of cortisone acetate (CCVIII) and aqueous suspensions[311] of cortisone have already been investigated by Coleby and Weiss and their coworkers. Unchanged cortisone acetate and a little cortisone were the only materials isolated from the study in methanol, whereas

in water, cortisone is reported to undergo dehydroxylation at $C_{(17)}$ and $C_{(21)}$ and reduction of the $C_{(4,5)}$ double bond. Compounds formed by the addition of water to the $C_{(4,5)}$ double bond are also thought to be products of the irradiation.

The effect of ultraviolet light on a number of steroids, structurally related to cortisone acetate, has been investigated, and many of the resulting photo-products isolated and characterised. A study of the photolysis of cortisone acetate therefore seemed to serve two purposes: firstly, it should indicate the changes which might be expected to occur during the storage of this drug in sunlight, and secondly, from a purely chemical viewpoint, it would be one logical extension to the photochemical work already carried out in this field. Barton and Taylor[289] have studied photolysis of 5α-dihydrocortisone acetate (21-acetoxy-17-hydroxy-5α-pregnane-3,11,20-trione) under a variety of conditions but only unchanged starting material and a little tar was isolated. More recently, however, photochemical reactions involving the 3,- 11-, and 20-keto functions of certain related 3-, 11-, and 20-keto steroids have been shown to take place on prolonged irradiation.

In the present investigation irradiation of cortisone acetate in ethanol gave an oily product, the infrared spectrum of which possessed only weak absorption at 1668 $cm.^{-1}$ indicating that the α,β-unsaturated 3-keto function was destroyed on exposure to ultraviolet light. Exploratory irradiations were carried out employing various reaction times and these, after removal of the solvent and quantitative chromatography of the residue on alumina, indicated that at least three distinct photo-products were formed (see fig. 50). On neutral alumina (grade 3) the first fraction was eluted with a 4:1 benzene-chloroform mixture, the second in a 1:1 mixture of the same solvents, and the final fraction was eluted

with chloroform.

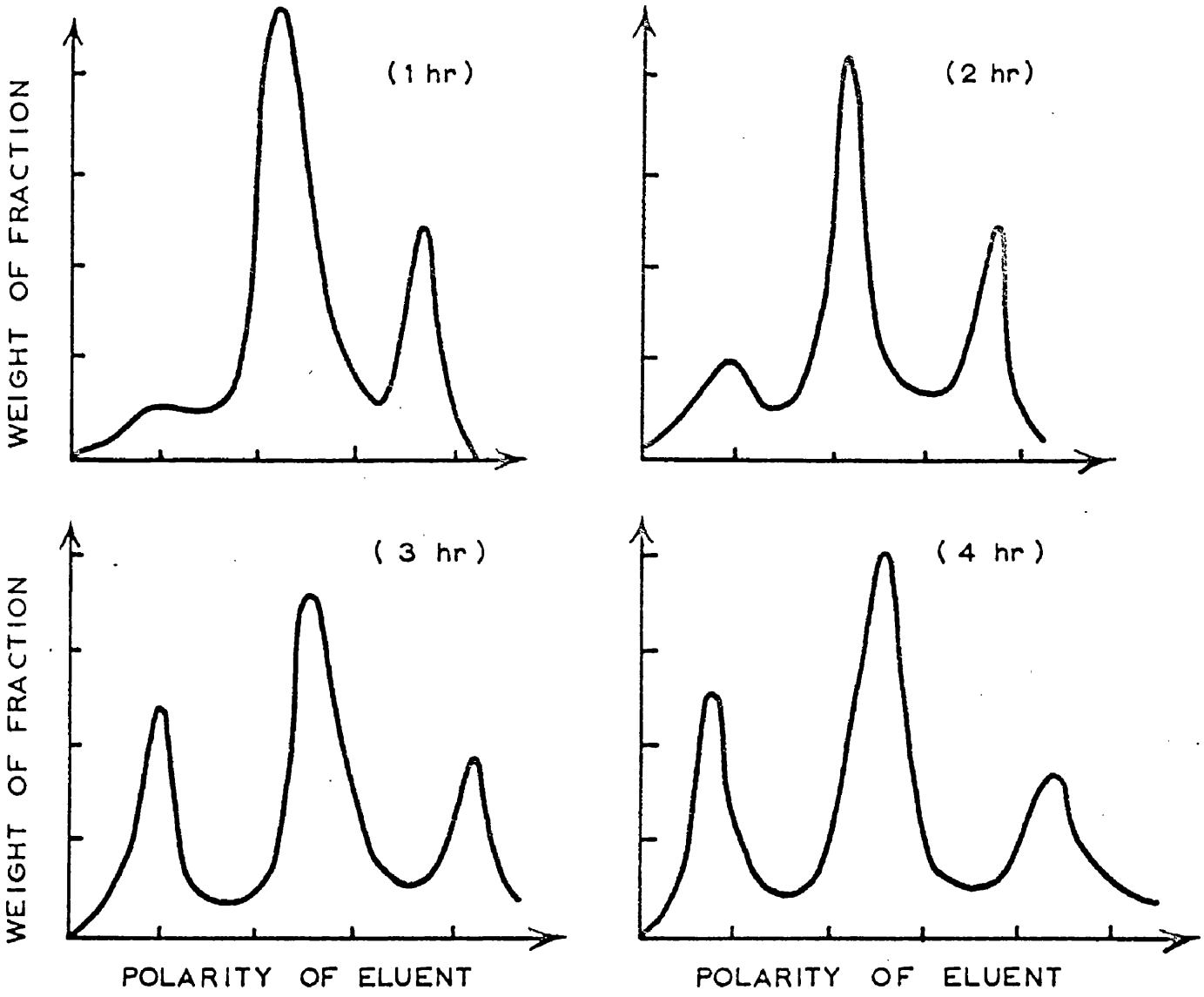


fig. 50

Although thin layer chromatography (T.L.C.) indicated that the first fraction was predominently one component, it could not be induced to crystallise, even after repeated chromatography. Attempts to prepare crystalline derivatives, or derivatives which could be obtained crystalline after further chromatography, were without success.

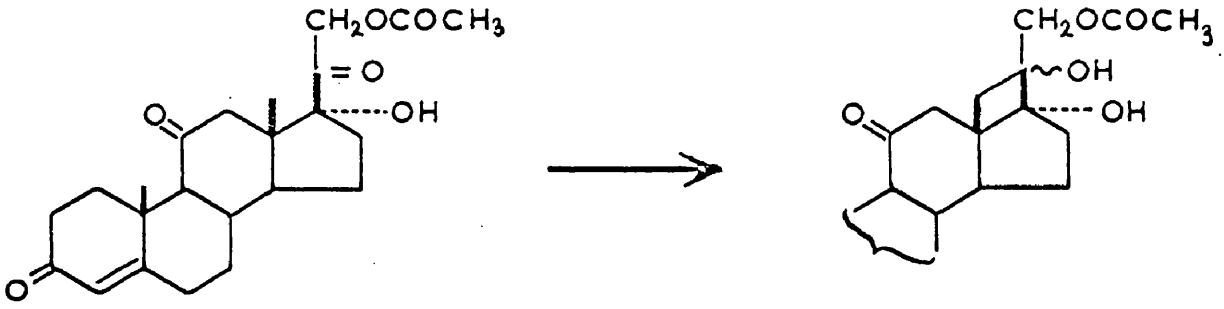
The 1650-1750 $cm.^{-1}$ region of the infrared spectrum of cortisone acetate has been fully interpreted[312] and the maxima at

1748, 1728, 1705, 1668 cm.$^{-1}$ attributed to the acetate carbonyl, and the 20-keto, 11-keto and the 3-keto functions respectively. The infrared spectrum of the first fraction ($\nu_{max.}$ 1740, 1705, 1235 cm.$^{-1}$) indicated that this material, in addition to lacking the unsaturated 3-keto function, also lacked the 20-keto group. Although absorption attributable to the acetoxy carbonyl was present, this was shifted to the lower frequency of 1740 cm.$^{-1}$ Absorption due to the 11-keto group appeared to remain unchanged.

Loss of the unsaturated 3-keto function could occur by a number of photochemical processes as outlined in the introduction (see p.130): direct reduction to the corresponding saturated ketone or reduction and formation of a pinacol[270] at $C_{(3)}$, addition of a molecule of ethanol to the $C_{(4,5)}$ double bond, or migration of the double bond out of conjugation with the keto group[276] to give the corresponding β,γ-unsaturated ketone, are among the simpler processes open to the excited molecule. Other reactions, involving rearrangement in the A ring to give cyclopropane and cyclopentane derivatives have also been reported[270] for α,β-unsaturated 3-keto steroids (see p.132). Dimerisation about the $C_{(4,5)}$ double bond to give cyclobutane derivatives has also been widely reported[270,271] for α,β-unsaturated ketones but this process is generally favoured in a highly concentrated solution, or in the solid state. It is not therefore likely to be an important process in the photolysis of cortisone acetate at the low concentrations employed (< 4 mg/ml).

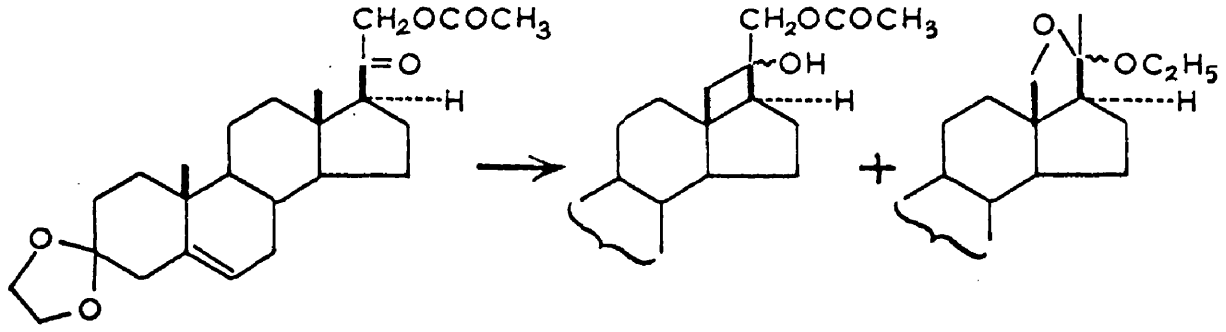
Loss of absorption at 1728 cm.$^{-1}$, attributable to loss of the 20-keto group, can be most easily accounted for by hydrogen abstraction by the excited carbonyl; this process is commonly encountered,[265] and abstraction from the neighbouring $C_{(19)}$ angular methyl group to give a cyclobutanol is a well documented

process. Such a transformation (see fig.51), by introducing a hydroxyl group capable of hydrogen bonding with the neighbouring carbonyl of the acetate, could well account for the observed shift in the frequency of the 21-acetoxy carbonyl absorption. In addition



CCVIII fig. 51 CCIX

to cyclobutanol formation, the irradiation of certain 20-keto-21-acetoxy-substituted pregnanes is also known to yield acetal derivatives; thus the acetoxyketone (CCX), on irradiation,[313] yields the cyclobutanol (CCXI) and the acetal (CCXII). No product having the properties of an acetal was isolated from the irradiation of cortisone acetate.

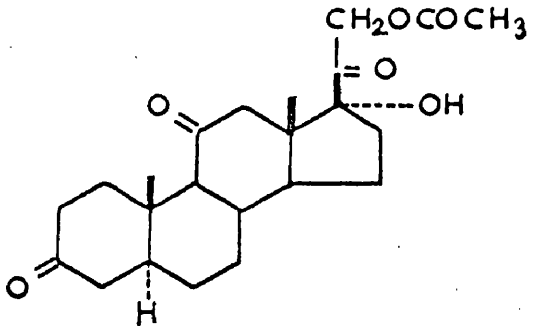


CCX CCXI CCXII

fig. 52

The second fraction, obtained from the chromatography of the total photo-product, was a colourless crystalline solid with infrared maxima at 1748, 1728, 1705 cm.$^{-1}$ and a significantly reduced maximum at 1668 cm.$^{-1}$ The relative intensity of absorption at 1705 cm.$^{-1}$ was increased over that found in the spectrum of cortisone acetate. Although the infrared spectrum and the melting

point remained unchanged, even after rechromatography and repeated recrystallisation from various solvents, this crystalline solid was shown, by ultraviolet absorption measurements, to be a mixture of a product lacking an α,β-unsaturated keto function and cortisone acetate. Attempts to separate this mixture were unsuccessful. It was therefore further irradiated in ethanol and the product isolated as a crystalline solid after concentration of the ethanolic solution. The resulting product lacked absorption at 1668 cm.$^{-1}$ and was shown to be 5α-dihydrocortisone acetate by analysis, mass spectrometry, and by comparison with an authentic sample prepared by catalytic hydrogenation of cortisone acetate according to the procedure of Oliveto, Gerold and Hershberg.[314] The 3-oxime[315] and the 3-2',4'-dinitrophenylhydrazone[316] were also identical with the same derivatives of the authentic sample. 5α-Dihydrocortisone acetate lacks



CCXIII

the important pharmacological properties of cortisone acetate and in fact is the major metabolic product of this corticosteroid.

The third fraction, eluted from the column with chloroform, was a light-coloured oil which could not be obtained in a crystalline form. The infrared spectrum of this material was similar to that of the second fraction in that it retained absorption maxima at 1748, 1728, 1705 cm.$^{-1}$ and lacked absorption at 1668 cm.$^{-1}$, but differed from the spectrum of 5α-dihydrocortisone acetate in that there was no significant change in the relative intensities of the carbonyl maxima from that observed in the spectrum of cortisone

acetate. In particular the intensity of absorption in the 1705 $cm.^{-1}$ region was unchanged, suggesting that the loss of α,β unsaturated keto function was not accompanied by formation of the corresponding saturated 3-keto group.

Under the conditions employed for the irradiation, loss of the keto function at $C_{(3)}$ is most likely to occur by a reduction process. The formation of a pinacol, as described above, is one possibility but attempts to cleave this material, using periodic acid,[317] under a variety of conditions furnished no spectroscopic evidence that the cleavage of a diol had occurred. It may be concluded therefore, with reasonable certainty, that this fraction did not contain any appreciable quantities of a pinacol product. Reduction of the 3-ketone to the corresponding 3-hydroxy compound is another possibility; an analogous reduction has been described by Quinkert and his co-workers[268] in which 5α-cholestan-3-one was photochemically transformed into a mixture containing 5α-cholestan-3α-ol and 5α-cholestan-3β-ol.

The infrared spectrum of the third fraction, showing increased absorption in the 3400 $cm.^{-1}$ region, and the infrared spectrum of the product obtained after treatment of this material with acetic acid and acetic anhydride, together supported the view that a product having an additional hydroxyl group was formed during the irradiation of cortisone acetate. These observations were consistent with the photochemical formation of 21-acetoxy-3,17-dihydroxy-5α-pregnane-11,20-dione, but, without a pure sample, the identity of this product could not be established.

The total photo-product of cortisone acetate in ethanol was also chromatographed on silica gel. Apparently the same separation was achieved on this absorbent as was obtained on the alumina: the only pure crystalline material isolated was 5α-dihydrocortisone

acetate.

Observations, similar to those discussed above, were also made when cortisone acetate was irradiated in methanol rather than in ethanol. A lower yield of the dihydrocortisone, however, was isolated. The irradiations were also repeated using a pyrex filter, which filtered out light with wavelength < 300 mμ; apart from decreasing the rate of disappearance of the unsaturated keto function the filter appeared to have no significant effect upon the nature of the products.

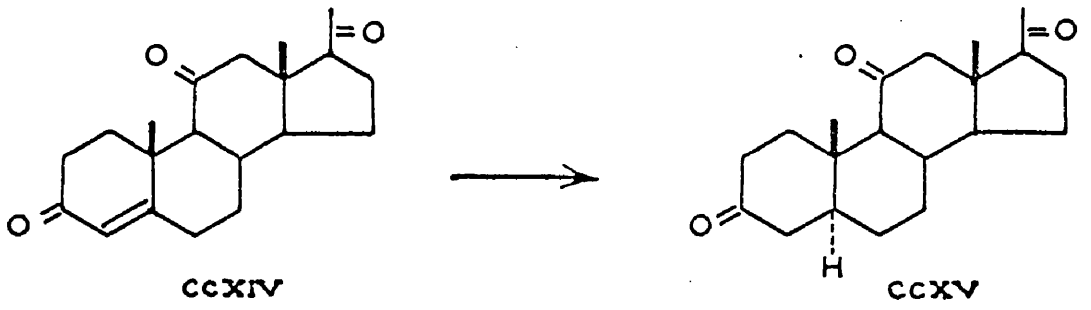
In benzene, cortisone acetate underwent photochemical transformation only very slowly, and the sole product, isolated after several days irradiation, was an amorphous, high melting, white solid. This material was insoluble in all common solvents and could not be vapourised for mass spectrometric measurement. It was assumed to be polymeric.

11-Ketoprogesterone (Pregn-4-ene-3,11,20-trione)

In an attempt to learn more about the nature of the photoproducts of cotisone acetate, the irradiation of simpler molecules which are closely related to cortisone was undertaken. The first steroid studied was 11-ketoprogesterone (CCXIV); this substance lacks only the 17- and 21-hydroxy functions of cortisone but is pharmacologically very much less important. It does, however, possess a significant diabetogenic potency.[318]

Photolysis of 11-ketoprogesterone occurred rapidly in ethanol with loss of the α,β-unsaturated keto function at $C_{(3)}$. The irradiation was therefore continued until the infrared spectrum of a sample completely lacked absorption at 1660 $cm.^{-1}$; the resulting product, isolated by removal of ethanol at reduced pressure and room temperature, was chromatographed on alumina (grade 3). Elution

with a mixture of benzene and petroleum gave, as the major product, 5 α-pregnane-3,11,20-trione. This material was characterised by comparison with an authentic sample,[319] and by comparison of the derived 3,20-dioximes.[320]



CCXIV CCXV

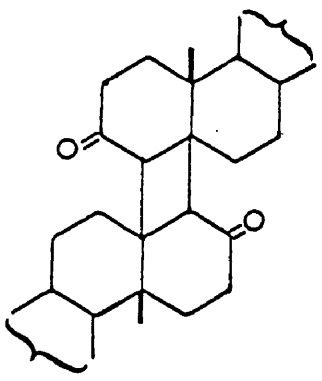
fig. 53

Although 5α-pregnane-3,11,20-trione no longer possesses the α,β-unsaturated keto function, generally thought to be important for the biological activity of these compounds, it has recently been shown[321] to possess considerable depressor effects upon the central nervous system and is claimed to be suitable as a hypnotic, a sedative and an anaesthetic agent.

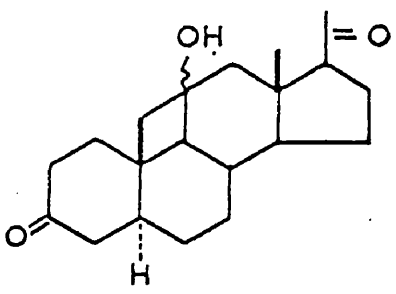
In addition to the major product, 5α-pregnane-3,11,20-trione, three other products were isolated by further elution of the column with more polar solvents. The extremely low yields of these additional photo-products, however, prevented their complete characterisation. Elution with benzene containing a trace of chloroform gave an oil which, in the presence of a little ethanol, deposited a mixture of fine needles with melting point 345°, and colourless cubes which did not melt below 365°. The extremely low yields of these products, their high melting points, and the ease with which they crystallised from a low concentration, together indicate that they are probably dimeric or possibly polymeric. Analogous dimerisations have been reported[270,271] for testosterone and progesterone; the general structure of the dimeric products is now believed to contain a cyclobutane unit as shown in CCXVI. Such "head to tail"

structure could well account for one of our high melting products, while the other product, if dimeric, might have the "head to head" arrangement. A more likely structure for the second high melting product, however, would be a pinacol[270] formed at $C_{(3)}$ as discussed earlier (see p.132).

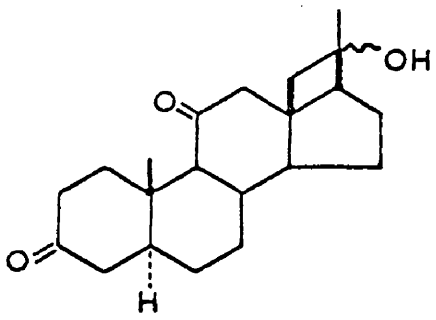
The third minor product was eluted, in 2% yield, from the column in an equal mixture of benzene and chloroform. Recrystallisation from ethyl acetate gave a well-defined crystalline solid, the analytical figures for which were consistent with a dihydro-11-ketoprogesterone. The melting point, however, ruled out the possibility of it being 5β-pregnane-3,11,20-trione[322] and failure to obtain an α,β-unsaturated ketone by treatment with base suggested that the product was not a β,γ-unsaturated ketone formed by double bond migration. The infrared spectrum indicated that the material possessed a hydroxyl group; as the product was unlikely to be a pinacol (it could not be cleaved by treatment with periodic acid[317]) the hydroxyl group probably resulted from cyclobutanol formation (see p.128). Both 11- and 20-keto functions in similar steroid molecules, when photochemically excited, are known[265] to be capable of abstracting a hydrogen atom from the neighbouring $C_{(18)}$ or $C_{(19)}$ angular methyl group; 11-ketoprogesterone, therefore, possessing both these keto functions, may form two such products, CCXVII and CCXVIII. However, due to the very limited quantity of material available cyclobutanol formation could not be proven.



CCXVI



CCXVII

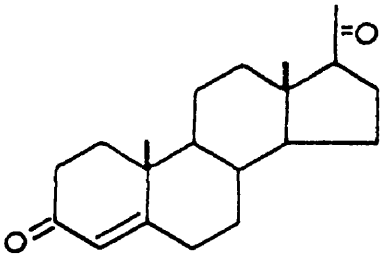


CCXVIII

Progesterone (pregn-4-ene-3,20-dione)

The female sex hormone, progesterone, is a still simpler steroid molecule which retains certain of the functional groups of cortisone acetate; it was therefore considered worthy of photochemical investigation. In the body it is secreted by the corpus luteum and functions to prepare the oestrogen sensitised uterus to receive the fertilised ovum and to maintain pregnancy to its full term. It finds clinical application in substitution therapy, where insufficient of the hormone is produced by the body, and in inhibition therapy when endogenous production of certain other hormones is too high for normal body functioning.

Like 11-ketoprogesterone, progesterone (CCXIX) lacks the 17-hydroxy and 21-acetoxy groups of cortisone acetate, and in addition lacks the 11-keto function. Thus only the α,β-unsaturated keto and the 20-keto functions remain for photochemical excitation.



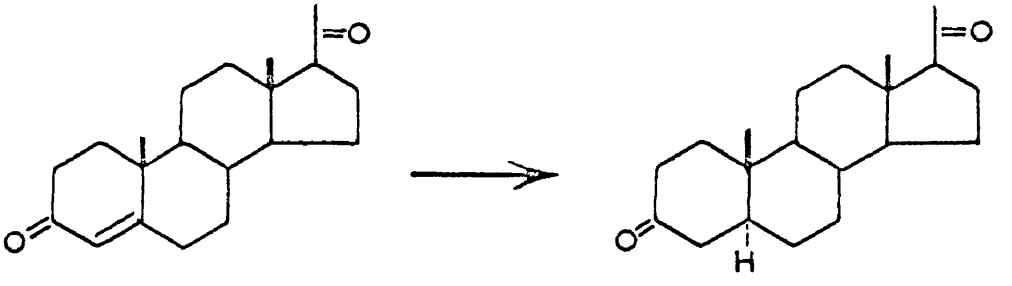
CCXIX

Coleby, Keller and Weiss[310] have already studied the effect of X-rays upon methanolic solutions of progesterone and have isolated in low yield 5α-pregnane-3,6,20-trione. The effect of air and sunlight upon progesterone has been investigated by Ritter and his co-workers:[323] in hydrocarbon and alcoholic solvents little change was reported, but in halogenated solvents such as carbon tetrachloride, there was substantial evidence that progesterone was converted into a mixture of substituted Δ^4-3-keto steroids, including 6-keto-progesterone, 4-chloroprogesterone and 4-hydroxyprogesterone. These

products were separated by chromatography and characterised by infrared spectra, chromatographic retention times and other physical properties. Although similar observations were made when solutions were irradiated with light from a Xenon lamp, differences were observed when a mercury lamp was employed as source.

An earlier study of the photochemistry of progesterone was made by Butenandt and his co-workers:[271,324] in benzene and hexane solution, progesterone is photochemically converted into the dimeric cyclobutane derivative (CLXVI; R=$COCH_3$).

In the present investigation, progesterone was irradiated in ethanol in an atmosphere of nitrogen until a sample no longer possessed infrared absorption in the 1660 $cm.^{-1}$ region, indicating that the α,β-unsaturated 3-keto function had been completely destroyed. The oily product was then chromatographed on neutral alumina (grade 3) whereupon elution with a mixture of benzene and petroleum gave a white crystalline solid which melted over a 100° temperature range. Although T.L.C. indicated that this solid consisted of three components, of which two were present only in trace amounts, the material could not be readily purified by recrystallisation. Fractional sublimation, however, was more effective and pure 5α-dihydroprogesterone (5α-pregnane-3,20-dione) (CCXX) was obtained in 30% yield. This material was characterised by analysis, infrared spectra, and finally by comparison with an authentic sample.



CCXIX CCXX

fig. 54

Further elution, with a mixture of benzene and chloroform, resulted in an oily fraction which, on standing in the presence of a little ethanol, deposited fine needle-like crystals. These were formed in an extremely low yield, in the order of 0.1%, and melted sharply at 332-335°, suggesting that this product was probably dimeric. However, it was not the same product as that described by Butenandt and Wolff[271] from the irradiation of progesterone in benzene. The infrared spectrum, prepared from a potassium chloride micro-disc, possessed absorption due to the 20-keto function and due to a hydroxyl group. Attempts to obtain a mass spectrum, in order to determine the molecular weight, were unsuccessful as the material could not be vapourised.

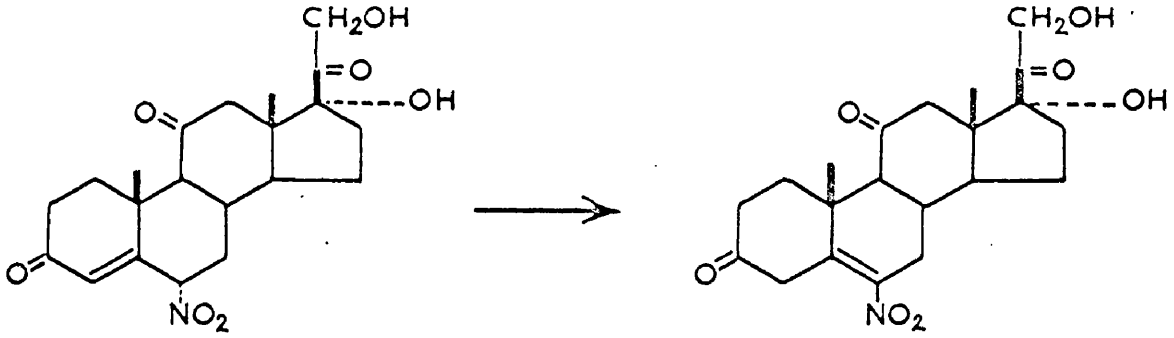
What appeared to be the major product from this irradiation was eluted from the column with chloroform and accounted for 40% of the starting material. Although a variety of attempts, including repeated chromatography, T.L.C., and sublimation, were made to obtain a pure crystalline sample, these were all unsatisfactory. The infrared spectrum (νmax. 3590, 3410, 1695 cm.$^{-1}$) indicated that this material retained the 20-keto function and possessed an additional hydroxyl group but appeared to have lost the 3-keto function. Confirmation for the presence of a hydroxyl group was furnished by the infrared spectrum of the oil obtained after treatment of the impure material with acetyl chloride and pyridine. The spectrum lacked the absorption attributed to the hydroxyl group (νmax. 3590, 3410 cm.$^{-1}$) and possessed additional absorption at 1730 and 1250 cm.$^{-1}$ due to the O-acetyl function. Owing to the difficulties associated with obtaining a crystalline sample of this product it could not be identified, although it is likely to be a 3-hydroxy-5α-pregnan-20-one.

6-Nitro Steroids

The current interest in the photochemistry of nitro compounds[325,326] and the recent preparation of 6-nitro derivatives of such substances as cortisone, progesterone and 17α-acetoxyprogesterone (17-acetoxy-6α-nitro-pregn-4-ene-3,20-dione) by Ringold and his co-workers[327,328] made a study of the photochemistry of certain of these compounds an interesting extension to the work already discussed. Of the 6-nitro-steroids prepared, it would appear that only 17α-acetoxy-6α-nitro-progesterone has any significant pharmacological activity.[328] This material, in the Clauberg assay, has 3-4 times the activity of 17α-acetoxyprogesterone which in turn is significantly more active than progesterone itself. The effect of 6-substitution upon the activity of certain biologically important steroids has recently been discussed by Ringold.[329]

17α-Acetoxy-6α-nitroprogesterone, prepared by nitration of 17α-acetoxyprogesterone according to the method of Ringold and his co-workers,[328] was irradiated in ethanol in an atmosphere of nitrogen. The resulting reaction was followed by infrared; the absorption at 1675 $cm.^{-1}$, due to the α,β-unsaturated 3-keto function, and at 1555 $cm.^{-1}$, due to the 6-nitro function, rapidly decreased. Additional maxima appeared at 1710 $cm.^{-1}$, probably resulting from saturation of the unsaturated 3-keto function, and a weak absorption at 1525 $cm.^{-1}$ attributable to the appearance of an α,β-unsaturated nitro group. Similar infrared observations were made when 6α-nitrocortisone (17,21-dihydroxy-6α-nitropregn-4-ene-3,11,20-trione) was irradiated in ethanol. Although repeated attempts were made to isolate crystalline products from these photolyses using various chromatographic methods, these were unsuccessful. T.L.C. showed the total products of irradiation of both 6α-nitrocortisone and 17α-acetoxy-6α-nitroprogesterone to be highly complex mixtures.

α,β -Unsaturated nitro functions are known to be capable of photochemical excitation, but isolated nitro groups are generally regarded as being more stable to light. A possible explanation, therefore, for the rapid photo-decomposition of 6α-nitrocortisone and 17α-acetoxy-6α-nitroprogesterone is that, on irradiation, the $C_{(4,5)}$ double bond migrates into conjugation with the 6-nitro function, giving the photochemically more labile α,β-unsaturated nitro system. Migration of the double bond in certain 3-keto Δ^4 steroids to the $C_{(5,6)}$ position has already been established as a definite photochemical process; the light-induced transformation[276] of 10α-testosterone (CLXIX), for example, results in the isomeric Δ^5 product (CLXX) (see p.131). Taking this reaction as a model,



CCXXI CCXXII

fig. 55

6α-nitrocortisone (CCXXI) would give the α,β -unsaturated nitro compound (CCXXII).

To determine the possible light-induced transformations which might result after such a double bond migration, a simple α,β -unsaturated 6-nitro steroid, lacking any additional chromophores, was required for photochemical investigation. The most readily available material for such a study was 6-nitrocholesteryl acetate (3β -acetoxy-6-nitrocholest-5-ene) (CCXXIII), and this was prepared according to the method of Anagnostopoulos and Fieser[329a] by nitration of cholesteryl acetate. The photochemistry of this compound

was studied in ethanol solution in an atmosphere of nitrogen.

Shortly after the completion of this investigation two preliminary accounts of the photochemistry of 6-nitrocholesteryl acetate were published; Pinhey and Rizzardo[330] investigated the photolysis in hexane and aqueous dioxan, while Chapman and his co-workers[331] employed acetone as the solvent.

In the present investigation[332] 6-nitrocholesteryl acetate was irradiated in ethanol (concentration 3 mg/ml) until the infrared spectrum of a sample lacked absorption at 1510 $cm.^{-1}$, characteristic of the unsaturated nitro group. The oily product, when dissolved in a little petroleum, gave a white crystalline solid as the major product. Infrared evidence (νmax. 3580, 3290, 1685, 1598 $cm.^{-1}$) and conversion of this material into cholest-4-ene-3,6-dione (CCXXIV) on treatment with pyruvic acid together indicated that the product was a mono-oxime of cholest-4-ene-3,6-dione. Early attempts to characterise the product as the 6-oxime, which was initially thought to be the more likely product as it would not necessitate a nitrogen migration during the photolysis, were consistently unsuccessful. However, treatment of cholest-4-ene-3,6-dione with hydroxylamine hydrochloride and sodium acetate gave a mono-oxime identical to that obtained from the irradiation, indicating[333] that the photochemical product was in fact the 3-oxime (CCXXV). This was confirmed by the unambiguous synthesis of cholest-4-ene-3,6-dione 3-oxime from the known 6-ethoxycholesta-4,6-dien-3-one (CCXXVI)[334] which was converted into the oxime (CCXXVII) and selectively hydrolysed to the required product in aqueous acetic acid.

Although the same oxime was isolated from irradiations carried out in acetone,[331] the major product in this solvent was a mixture of the 6α- and 6β-nitro derivatives of 3β-acetoxycholest-4-ene. In hexane and dioxan,[330] 3β-acetoxy-6β-nitrocholest-4-ene was the major product

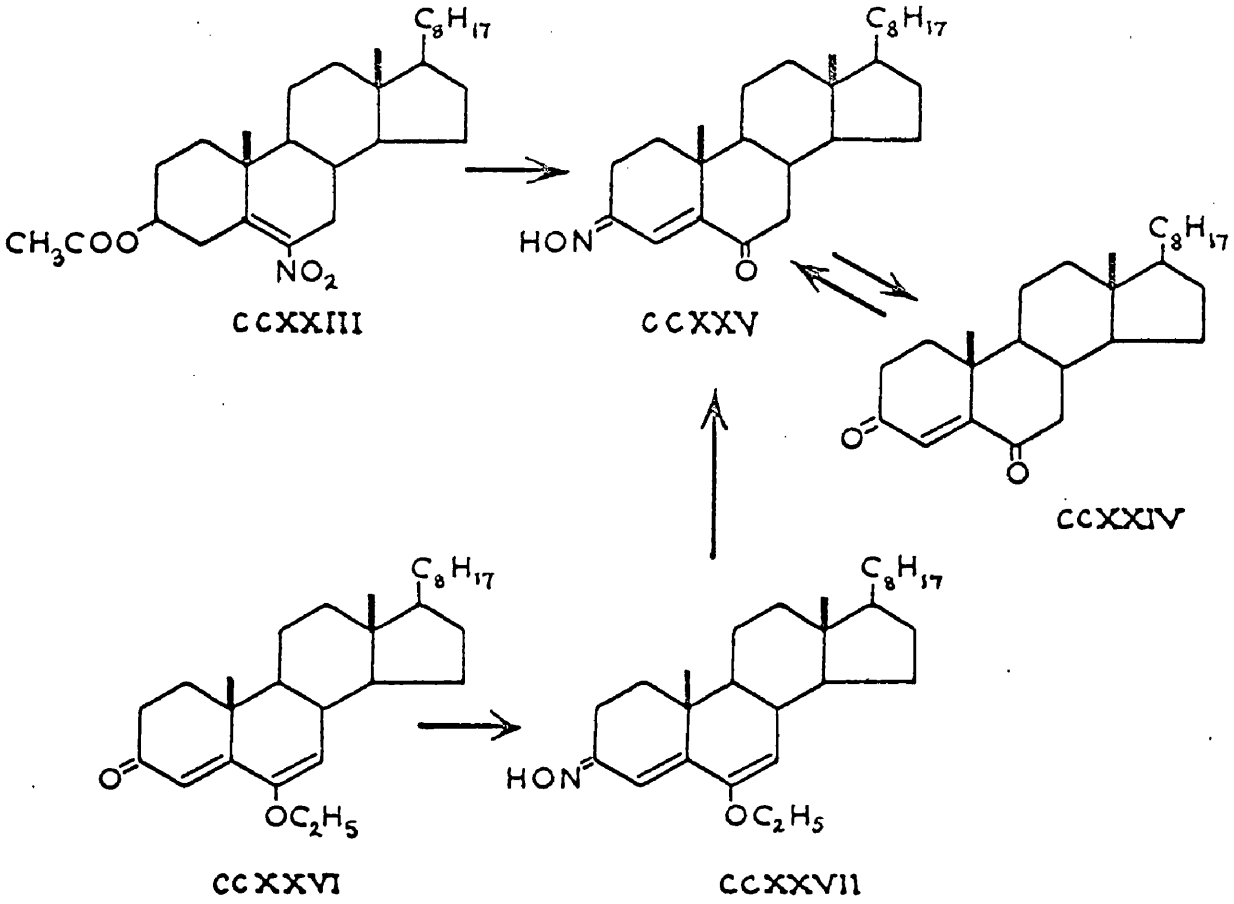


fig. 56

and no cholest-4-ene-3,6-dione 3-oxime was isolated. Chapman[331] suggested that the formation of the oxime during irradiations carried out in acetone could well be due to the ability of acetone to act as a sensitiser. However, isolation of the oxime in a higher yield, from irradiations employing ethanol as solvent now makes this proposal questionable. Our yield of oxime was not reduced by the use of a pyrex filter, indicating that it is the nature of the solvent rather than the light of wavelength less than 300mμ which is thedetermining factor in the formation of cholest-4-ene-3,6-dione 3-oxime.

The residue remaining after removal of oxime was chromatographed on silioa gel and an additional two photo-products isolated. The first of these was eluted in 7% yield with a benzene and chloroform

mixture and was identical to a sample of 3β-acetoxycholest-4-en-6-one prepared according to the method of Heilbron, Jones and Spring.[335] The second, isolated in 2.5% yield by elution with petroleum and benzene, proved to be 6-nitrocholesta-3,5-diene (CCXXVIII). It was characterised by infrared (ν max. 1625, 1500 cm.$^{-1}$), analysis, and by comparison with an authentic sample prepared from 3β-chloro-6-nitrocholest-5-ene[336] by elimination in quinoline. The method of elimination was based upon the procedure employed by Mauthner and Suida[337] for the conversion of cholesteryl chloride into cholesta-3,-5-diene. The same nitrodiene has been isolated previously from the photolysis[330] of 6-nitrocholesteryl acetate in hexane and aqueous dioxan where it was postulated to be an intermediate in the formation of the oxime (CCXXV). Later work[331] demonstrated that this nitrodiene (CCXXVIII) is photochemically transformed into the oxime in acetone and we found the same conversion takes place in good yield in ethanol.

A possible explanation for the formation of the oxime from the nitrodiene is shown in fig. 57: the nitrodiene (CCXXVIII), formed by elimination of the 3-acetoxy group, undergoes rearrangement of the nitro function to the nitrite (CCXXIX) which then cleaves giving a NO radical which in turn attacks the steroid at the reactive centre at $C_{(3)}$. Rearrangement of the resulting product provides the oxime (CCXXV).

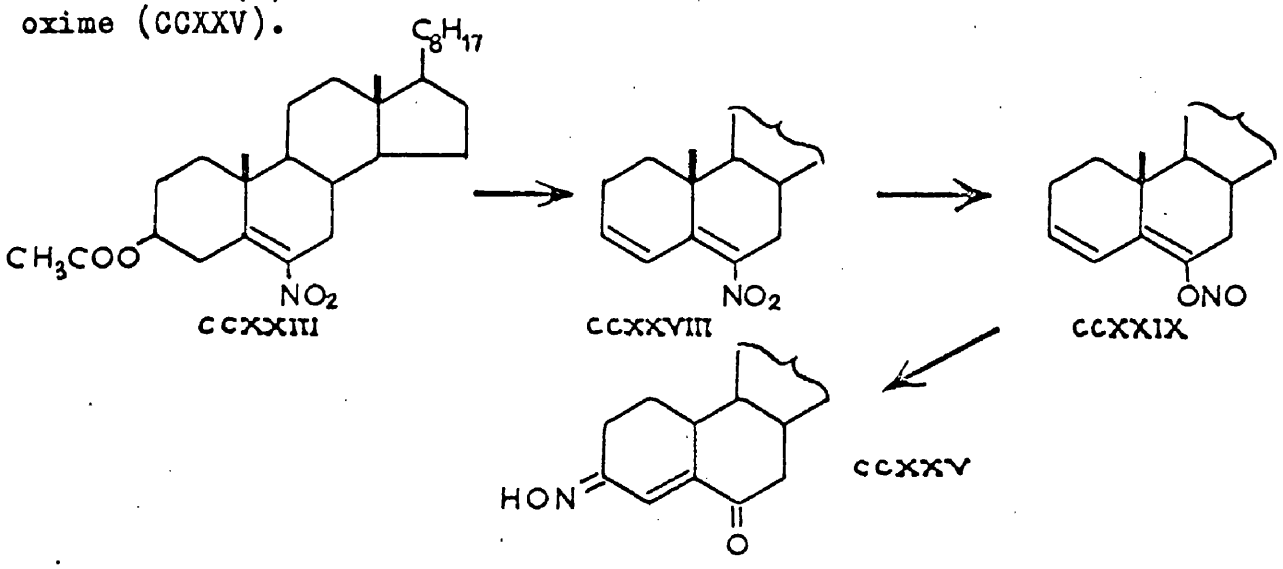
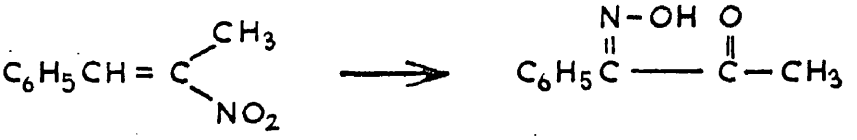
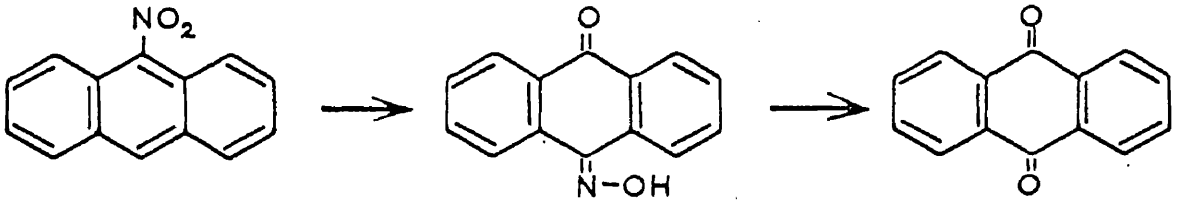


fig. 57

Conversion of an α,β -unsaturated nitro function into the corresponding nitrite, followed by cleavage and attack by the NO radical is consistent with the mechanism proposed by Chapman and his co-workers[326] for the photochemical conversion of nitrostyrenes and nitroanthracenes into the oximino and keto products shown in fig. 58. Photolytic cleavage of a nitrite and recapture of the



CCXXX CCXXXI



CCXXXII CCXXXIII CCXXXIV

fig. 58

resulting NO radical by the parent molecule has also been established as the mechanism of the Barton reaction in which nitrites are converted into hydroxy-oximes as illustrated in fig. 59. Many excellent papers and reviews[338] concerning the Barton reaction have been

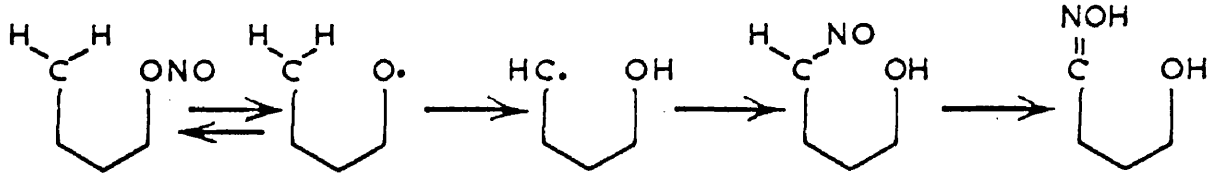
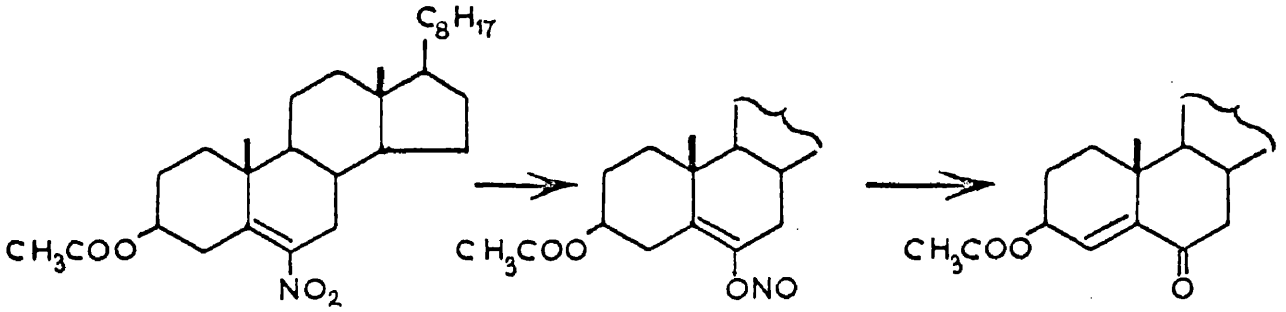


fig. 59

published. Rearrangement of the nitro function of 6-nitrocholesteryl acetate to the corresponding nitrite without elimination of the 3-acetoxy function is almost certainly the first step in the formation of 3β -acetoxycholest-4-en—6-one (CCXXXV). Cleavage of this nitrite could be expected to result in attack by the NO radical at $C_{(5)}$ but such a transformation was not observed and must therefore be assumed to be unfavourable. The alternative, more favourable process, resulting in the formation of 3β -acetoxycholest-4-en—6-one (CCXXXV) would appear to involve cleavage of the nitrite (CCXXXVI) accompanied by loss of hydrogen at $C_{(4)}$.



CCXXXIII CCXXXVI CCXXXV

fig.60

In all the irradiations of 6-nitrocholesteryl acetate in ethanol, no product arising from double bond migration from $C_{(5)}$ to $C_{(4)}$ was obtained, although such products accounted for the majority of the starting material employed in the irradiations carried out in other solvents.

6-Nitrocholesteryl acetate was also irradiated in cyclohexane and the product chromatographed on silica gel. Elution with a mixture of petroleum and benzene gave a non-crystalline solid which, from infrared data (ν max. 1725, 1640, 1550 $cm.^{-1}$), was thought to be a mixture of 6α-nitro and 6β -nitro derivatives of 3β -acetoxycholest-4-ene as reported in the photolysis of 6-nitrocholesteryl acetate in acetone.[331] All attempts to separate these isomers were unsuccessful.

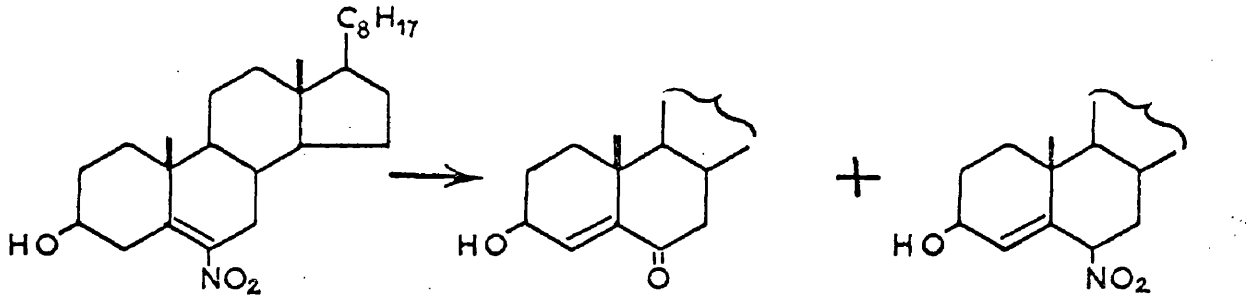
Elution with chloroform gave, as the major product, 3β -acetoxycholest-4-en-6-one (CCXXXV). This material was more efficiently separated on neutral alumina (grade 4) and yields in the order of 40% were obtained by elution with petroleum. Thus the photolysis of 6-nitrocholesteryl acetate in cyclohexane constitutes a useful simple synthesis of 3β -acetoxycholest-4-en-6-one which is usually prepared[335] from 6-nitrocholesteryl acetate by chemical methods. The much increased yield of this product compared with that reported[330] (2-3%) for the irradiation in hexane is most likely a consequence of the use of light of a shorter wavelength: a quartz photochemical reactor was used in our work, whereas a pyrex filter was employed for the irradiation reported using hexane as the solvent.

The influence of the substituent at $C_{(3)}$ upon the conversion of 3-substituted 6-nitrocholest-5-enes into cholest-4-ene-3,6-dione 3-oxime was also studied. The oxime (CCXXV) was obtained by the photolysis of 3β -chloro-6-nitrocholest-5-ene and the trifluoroacetate of 6-nitrocholesterol in ethanol in yields of the same order as that obtained by the irradiation of 6-nitrocholesteryl acetate in ethanol.

In contrast to these observations, the oxime (CCXXV) was not formed during the irradiation of 6-nitrocholesterol (CCXXXVII) in ethanol. The failure of this substance to yield the oxime is thought to be a direct result of the greater difficulty of elimination of the 3β -hydroxyl group as compared with the acetoxy, trifluoroacetoxy and chloro substituents. Without prior formation of the nitrodiene (CCXXVIII) the formation of the oxime is excluded, and other photochemical processes predominate.

The major product from the irradiation of 6-nitrocholesterol (CCXXXVII) in ethanol was 3β -hydroxycholest-4-en-6-one (CCXXXVIII)

which was obtained in 37% yield after chromatography of the impure product on silica gel. A second crystalline solid, melting over



CCXXXVII CCXXXVIII CCXXXIX

fig. 61

a large range, was eluted with a mixture of benzene and chloroform. After repeated recrystallisations from methanol a solid was isolated which was identical to a sample of 6β-nitrocholest-4-en-3β-ol (CCXXXIX) prepared according to the method described by Jones and his co-workers.[339] The nature of the other constituent contaminating this crystalline material was not determined but, from infrared evidence, it could well be the 6α-nitro isomer.

From a consideration of our results in ethanol and cyclohexane, and from those reported by Pinhey[330] in hexane and aqueous dioxan, and those of Chapman[331] in acetone, it would appear that certain distinct pathways are available to the photochemically excited molecule. Firstly it may undergo elimination of the 3-substituent to give the nitrodiene (CCXXVIII), followed by conversion into the 3-oxime (CCXXV). This process is favoured in ethanol and acetone and when the 3-substituent is easily eliminated. Secondly the 6-nitro function may rearrange to the nitrite, undergo cleavage, and give the 3-substituted cholest-4-en-6-one. This process is favoured in the absence of oxime formation. The third pathway open to the excited molecule is by double bond migration to give the corresponding Δ^4-steroid. Rearrangement of this type is known to occur readily in the photolysis[276] of certain α,β-unsaturated ketones

to give the corresponding β,γ-unsaturated compounds. The possibility that 3β-acetoxy-6-nitrocholest-4-ene might be an intermediate in the formation of the nitrodiene (CCXXVIII) and thus the oxime was considered, but irradiation of the 3β-acetoxy-6-nitrocholest-4-ene in ethanol indicated that this was not so. Infrared data from samples of the irradiation solution showed the nitrosteroid decomposed on exposure to ultraviolet light but no oxime was isolated from the product.

The extent to which any of these processes predominate over the other two appears to depend largely on the solvent employed in the irradiation, the $C_{(3)}$ substituent, and the wavelength of the light employed.

EXPERIMENTAL

Irradiation Procedure.- In general the irradiations were carried out in solution at a concentration of 4 mg./ml. or less in an atmosphere of nitrogen. A quartz Hanovia photochemical reactor fitted with a medium pressure mercury arc was employed. Adequate cooling was provided to maintain the solution at room temperature. The reactions were followed by infrared spectra of samples taken at regular intervals, and the product isolated by removal of the solvent at reduced pressure.

Irradiation of Cortisone Acetate (21-Acetoxy-17-hydroxy-pregn-4-ene-3,11,20-trione (CCVIII) in Ethanol.- (a) Cortisone acetate (2 g.) in ethanol (600 ml.) was irradiated for 30 min. and the product chromatographed quantitatively on neutral alumina (grade 3). This was repeated employing irradiation times of 1, 2, 3 and 4 hr. Three main fractions were obtained from these irradiations: the first (νmax. 1740, 1705, 1235 cm.$^{-1}$) was eluted with a 4:1 mixture of benzene and chloroform; the second (ν max. 1748, 1728, 1705, 1668, 1235 cm.$^{-1}$), was eluted with a 1:1 mixture of benzene and chloroform; and the third (νmax. 1748, 1728, 1705, 1235 cm.$^{-1}$), was eluted with chloroform.

(b) Cortisone acetate (2 g.) in ethanol (600 ml.) was irradiated until the infrared spectrum of a sample no longer contained a maximum at 1668 cm.$^{-1}$ due to the unsaturated 3-keto function. The oil, remaining after removal of the ethanol, was then chromatographed on neutral alumina (grade 3); elution with a 1:1 mixture of benzene and chloroform gave a crystalline solid which was repeatedly recrystallised from ethanol to give a product (0.5 g.), m.p. 220-224° (Found: C, 65.6; H, 7.6%); ν max. 1748, 1728, 1705, 1668, 1615, 1235 cm.$^{-1}$

The crystalline product (0.5 g.) was dissolved in ethanol (300 ml.) and re-irradiated until the infrared spectrum of a

sample of the solution completely lacked absorption in the 1665-1670 cm.$^{-1}$ region. The ethanolic solution was then reduced to a small volume at reduced pressure, allowed to crystallise, and the product (0.35 g.) recrystallised from ethanol, m.p. 215-222° (Found: C, 63.7; H, 8.6%); νmax. 1748, 1728, 1705, 1235 cm.$^{-1}$ Molecular weight was shown to be 404 by mass spectrograph. Recrystallisation of this material from a mixture of acetone and benzene gave a product (0.27 g.) shown by comparison with an authentic sample, to be 21-acetoxy-17-hydroxy-5α-pregnane-3,11,20-trione, m.p. 225-229° (lit.,[314] 225-227°) (Found: C, 68.2; H, 7.6. Calc. for $C_{23}H_{32}O_6$: C, 68.3; H, 8.0%); ν max. 1748, 1728, 1705, 1235 cm.$^{-1}$

The 3-2',4'-dinitrophenylhydrazone had m.p. 207-212° (lit.,[316] 208-211°) and the 3-oxime had m.p. 276° (lit.,[315] 276-279°).

21-Acetoxy-17-hydroxy-5α-pregnane-3,11,20-trione.- Cortisone acetate was hydrogenated in ethyl acetate according to the method of Oliveto, Gerold and Hershberg[314] using palladium on carbon as catalyst. The product was recrystallised from acetone to give 21-acetoxy-17-hydroxy-5α-pregnane-3,11,20-trione in 65% yield, m.p. 225-229° (lit.,[314] 225-227°).

Irradiation of 11-Ketoprogesterone (Pregn-4-ene-3,11,20-trione) (CCXIV) in Ethanol.- 11-Ketoprogesterone (1 g.) in ethanol (600 ml.) was irradiated until the infrared spectrum of a sample no longer possessed absorption at 1660 cm.$^{-1}$ due to the unsaturated 3-keto function. The product was chromatographed on neutral alumina (grade 3). Elution with a 3:1 mixture of benzene and petroleum gave as the major product colourless plates which were recrystallised from ethanol to give 5α-pregnane-3,11,20-trione (0.44 g., 43%), m.p. 212-215° (lit.,[319] 217-221°). This material was identical with an authentic sample of 5α-pregnane-3,11,20-trione. The 3,20-

dioxime had m.p. 242-246° (lit.,[320] 244-246°).

A 10:1 mixture of benzene and chloroform eluted an oily fraction (0.01 g.) from the column, which on addition of ethanol, deposited a very small quantity (0.001 g.) of a mixture of cubic crystals, m.p. > 360° and needle-like crystals, m.p. 345°. The yields of these two products were too low to allow characterisation. Further elution with a 1:1 mixture of benzene and chloroform gave a fraction which, in the presence of ethanol, gave colourless needles (0.02 g.). These were recrystallised from ethanol, m.p. 224-225° (Found: C, 76.3; H, 9.3. Calc. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.15%); ν max. 3310, 1705, 1695 cm.$^{-1}$

Irradiation of Progesterone (pregn-4-ene-3,20-dione) (CCXIX) in Ethanol.- Progesterone (2 g.) in ethanol (600 ml.) was irradiated until the infrared spectrum of a sample no longer possessed absorption at 1663 cm.$^{-1}$ due to the unsaturated 3-keto function. The product was chromatographed on neutral alumina (grade 3). Elution with a 3:1 mixture of benzene and petroleum gave a product, m.p. 80-180°, which could not be readily purified by recrystallisation. T.L.C. on alumina showed it to contain at least three components and fractional sublimation gave 5α-pregnane-3,20-dione (0.2 g., 10%), m.p. 198-199° (Found: C, 79.5; H, 10.4. Calc. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2%); ν max. 1708, 1695 cm.$^{-1}$

A very small quantity (approximately 0.001 g.) of fine needle-like crystals, m.p. 332-335°, were obtained from a fraction eluted with a 3:1 mixture of benzene and chloroform after the addition of ethanol. Elution of the column with chloroform gave an oily fraction (0.4 g.); νmax. 3590, 3410, 1695 cm.$^{-1}$ This material could not be obtained in a crystalline form by addition of various solvents, by chromatography on neutral alumina or silica gel, or by treatment with hydroxylamine hydrochloride and sodium acetate. Treatment with acetyl chloride and pyridine gave a non-crystalline

product with νmax. 1730, 1695, 1250 cm.$^{-1}$

Irradiation of Progesterone (CCXIX) in Benzene.- Progesterone (1 g.) in benzene (50 ml.) was irradiated in a quartz flask for 15 hr. and the precipitated crystalline dimer (0.3 g., 33%) collected. This material did not melt but underwent slow decomposition at temperatures greater than 340° (lit.,[271] m.p. 340°).

6α-Nitrocortisone (17,21-Dihydroxy-6α-nitropregn-4-ene-3,11,20-trione (CCXXI).- This material was prepared in an overall yield of 9% according to the method of Ringold and his co-workers.[328] Cortisone acetate was converted into the enol acetate, nitrated, and the resulting 6β-nitrocortisone 17,21-diacetate isomerised and hydrolysed to the required 6α-nitrocortisone by treatment with potassium hydroxide in methanol, m.p. 227-230° (lit.,[328] 230-232°).

17α-Acetoxy-6α-nitroprogesterone (17-Acetoxy-6α-nitropregn-4-ene-3,20-dione).- 17α-Acetoxyprogesterone was converted into the 6β-nitro derivative by nitration of the derived enol acetate as described by Bowers, Ibanez and Ringold.[328] This material was converted into the 6α-isomer by treatment with potassium hydroxide in methanol. The overall yield was 36%, m.p. 203-205° (lit.,[328] 203-205°).

Irradiation of 6α-Nitrocortisone (CCXXI) in Ethanol.- 6α-Nitrocortisone (1 g.) in ethanol (600 ml.) was irradiated for 30 min.; after this time the infrared spectrum of the product (νmax. 1745, 1725, 1705 cm.$^{-1}$) possessed no absorption at 1665 cm.$^{-1}$ due to the unsaturated keto function, or at 1555 cm.$^{-1}$ due to the 6-nitro group. Chromatography of this material on silica gel and alumina gave no significant separation of the products, and no crystalline substances were isolated.

Irradiation of 17α-Acetoxy-6α-nitroprogesterone in Ethanol.- 17α-Acetoxy-6α-nitroprogesterone (1 g.) in ethanol (600 ml.) was irrad-

iated and chromatographed as described for 6α-nitrocortisone. The photolysis was equally rapid and the product, isolated after 25 min., lacked absorption at 1675 and 1555 cm.$^{-1}$ The oily product had νmax. 1725, 1710, 1250 cm.$^{-1}$ No crystalline material was isolated.

6-Nitrocholesteryl Acetate (3β-Acetoxy-6-nitrocholest-5-ene) (CCXXIII).- Cholesteryl acetate was nitrated with fuming nitric acid using the conditions described by Anagnostopoulos and Fieser.[329a] 6-Nitrocholesteryl acetate was obtained in 81% yield, m.p. 102-104° (lit.,[329a] 103-104°).

Irradiation of 6-Nitrocholesteryl Acetate (CCXXIII) in Ethanol.- 6-Nitrocholesteryl acetate (2 g.) in ethanol (600 ml.) was irradiated until the infrared spectrum of a sample no longer contained a maximum at 1510 cm.$^{-1}$ due to the unsaturated nitro group. The crude product was dissolved in petroleum from which a white crystalline precipitate was deposited. This was recrystallised from ethanol to give cholest-4-ene-3,6-dione 3-oxime (0.67 g., 38%), m.p. 221-222°; λmax 286 mμ (ϵ1.7 × 10^4); ν max. 3580, 3290, 1685, 1600 cm.$^{-1}$ The infrared spectrum was identical to that of an authentic sample of cholest-4-ene-3,6-dione 3-oxime; no depression in m.p. was observed on admixture of the two samples. (Found: C, 78.1; H, 10.5; N, 3.5. $C_{27}H_{43}NO_2$ requires: C, 78.4; H, 10.5; N, 3.4%).

The petroleum residues were chromatographed on silica gel. Elution with a 1:1 mixture of petroleum and benzene gave 6-nitrocholesta-3,5-diene (0.046 g., 2.5%) after recrystallisation from ethanol, m.p. 72-73.5° (lit.,[330] 72-73°); ν max.1500, 1625 cm.$^{-1}$ (Found: C, 78.2; H, 10.2. $C_{27}H_{43}NO_2$ requires: C, 78.4; H, 10.5%). Elution with a 4:1 mixture of benzene and chloroform gave 3β-acetoxycholest-4-en-6one (0.130 g., 6.9%) after crystallisation from ethanol, m.p. 109°, identical with authentic material (lit.,[335] 110°).

Cholest-4-ene-3,6-dione 3-Oxime (CCXXV).- (a) A solution of 6-ethoxycholesta-4,6-dien-3-one[334] (1.3 g.) in ethanol (50 ml.) was heated under reflux for 1 hr. with hydroxylamine hydrochloride (0.25 g.) and sodium acetate (0.30 g.) in water (5 ml.). After removal of ethanol at reduced pressure, water was added and the precipitate filtered off, washed, and dried. Without further purification, the 6-ethoxy-3-oximinocholesta-4,6-diene thus obtained was heated under reflux for 5 min. in 85% acetic acid (10 ml.). The crystalline precipitate was filtered off, washed with water, and recrystallised from ethanol to give cholest-4-ene-3,6-dione 3-oxime (0.72 g., 57%), m.p. 221-222°.
(b) Cholest-4-ene-3,6-dione (2 g.) in ethanol (40 ml.) was heated under reflux for 45 min. with hydroxylamine hydrochloride (0.36 g.) and anhydrous sodium acetate (0.70 g.) in water (10 ml.). After removal of ethanol at reduced pressure, water was added and the precipitate filtered off, washed with water, and recrystallised from ethanol to give cholest-4-ene-3,6-dione 3-oxime (0.90 g., 44%), m.p. 221-222°.

Cholest-4-ene-3,6-dione (CCXXIV).- Cholest-4-ene-3,6-dione 3-oxime (0.15 g.) was heated under reflux for 9 hr. in glacial acetic acid (8 ml.) with sodium acetate (0.06 g.) and pyruvic acid (0.25 ml.) in water (2 ml.). The crystalline precipitate was filtered off and recrystallised from methanol to give cholest-4-ene-3,6-dione (0.11 g., 79%), m.p. 122-125°. This material was identical with an authentic sample (lit.,[340] 124-125°).

6-Nitrocholesta-3,5-diene (CCXXVIII).- A solution of 3β-chloro-6-nitrocholest-5-ene[336] (0.30 g.) in quinoline (10 ml.) was heated under reflux for 15 min., poured into 4N hydrochloric acid, and the product extracted with chloroform. The chloroform extract was washed with dilute hydrochloric acid, water, and then dried. The solvent was removed at reduced pressure, and the oily residue

chromatographed on silica gel. Elution with a 4:1 mixture of benzene and petroleum gave 6-nitrocholesta-3,5-diene (0.072 g., 27%) which was recrystallised from ethanol, m.p. 72-73°.

3β-Acetoxycholest-4-en-6-one (CCXXXV).- 5α-Cholestan-3-ol-6-one, prepared by treatment of 6-nitrocholesteryl acetate with zinc dust and acetic acid, was converted into the 3-acetoxy derivative and subsequently brominated to give 3β-acetoxy-5α-bromocholestan-6-one. On elimination of hydrogen bromide in pyridine the bromo compound gave the required 3β-acetoxycholest-4-en-6-one in an overall yield of 10%, m.p. 110° (lit.,[335] 110°).

Irradiation of 6-Nitrocholesta-3,5-diene (CCXXVIII) in Ethanol.- 6-Nitrocholesta-3,5-diene (0.17 g.) was irradiated in ethanol (400 ml.) until the infrared spectrum no longer contained a maximum at 1500 $cm.^{-1}$ due to the unsaturated nitro group. The crude product was dissolved in petroleum from which crystalline cholest-4-ene-3,6-dione 3-oxime (0.08 g., 47%) separated. After recrystallisation from ethanol, the oxime had m.p. 221-222°.

Irradiation of 6-Nitrocholesteryl Acetate (CCXXIII) in Cyclohexane.- (a) 6-Nitrocholesteryl acetate (1 g.) in cyclohexane (600 ml.) was irradiated for 40 min. when there was no evidence in the infrared spectrum of any unsaturated nitro group. The product was chromatographed on neutral alumina (grade 4). Crystalline material (0.41 g., 44%) with νmax. 1735, 1690, 1635 $cm.^{-1}$ was eluted from the column with petroleum and had m.p. 109° after recrystallisation from methanol; this material was identical with authentic 3β-acetoxycholest-4-en-6-one (lit.,[335] 110°).

Other compounds containing saturated and unsaturated nitro groups (νmax. 1550, 1510 $cm.^{-1}$) were shown to be present in the residues.

(b) 6-Nitrocholesteryl acetate (2.3 g.) was again irradiated in

cyclohexane (950 ml.), and the crude product chromatographed on silica gel (45 g.). A non-crystalline fraction (0.45 g.) was eluted with a 4:1 mixture of benzene and petroleum, and had ν max. at 1725, 1640, 1550 cm.$^{-1}$ This material is believed to be a mixture of the 6α-nitro and 6β-nitro derivatives of 3β-acetoxycholest-4-ene.

3β-Acetoxycholest-4-en-6-one with m.p. 110° from methanol (0.50 g., 24%) was eluted from this column with chloroform.

6-Nitrocholesteryl Trifluoroacetate (6-Nitro-3β-trifluoroacetoxycholest-5-ene).- 6-Nitrocholesterol (2 g.) was dissolved in trifluoroacetic anhydride (10 ml.) and the solution heated gently under reflux for 90 min. On addition of this solution to water, a white precipitate of 6-nitrocholesteryl trifluoroacetate was obtained (1.72 g., 70%) and recrystallised from ethanol, m.p. 117-118.5°; ν max. 1780, 1515, 1155 cm.$^{-1}$ (Found: C, 66.0; H, 8.6. $C_{29}H_{44}F_3NO_4$ requires: C, 66.0; H, 8.4%)

Irradiation of 6-Nitrocholesteryl Trifluoroacetate in Ethanol.- 6-Nitrocholesteryl trifluoroacetate (1 g.) was irradiated in ethanol (400 ml.) as described for 6-nitrocholesteryl acetate. Cholest-4-ene-3,6-dione 3-oxime (0.31 g., 40%), m.p. 220-222° was obtained by crystallisation from petroleum.

Irradiation of 3β-Chloro-6-nitrocholest-5-ene.- 3β-Chloro-6-nitrocholest-5-ene (0.65 g.) was irradiated in ethanol (600 ml.) for 45 min., and the oily product chromatographed on silica gel. Elution with a mixture of 3:1 chloroform and benzene gave cholest-4-ene-3,6-dione 3-oxime (0.13 g., 21%) which was recrystallised from ethanol, m.p. 221-222°.

Irradiation of 6-Nitrocholesterol (CCXXXVII) in Ethanol.- 6-Nitrocholesterol (2 g.) in ethanol (650 ml.) was irradiated as described for 6-nitrocholesteryl acetate. The crude product was chromato-

graphed on silica gel (40 g.). Crystalline material with $\nu_{max.}$ 3590, 1545 $cm.^{-1}$ in the infrared spectrum was eluted from the column with a 1:1 benzene and chloroform mixture. White needles having m.p. 152-153° were separated by repeated recrystallisation using methanol, and proved to be 6β-nitrocholest-4-en-3β-ol (lit.,[339] 151-152°). There was no m.p. depression on admixture with an authentic sample, and the infrared spectra of the two samples were identical.

Elution of the silica gel column with 4:1 chloroform and methanol mixture gave 3β-hydroxycholest-4-en-6-one (0.47 g., 37%) which had m.p. 151° after recrystallisation from methanol. This was identified by comparison with an authentic sample (lit.,[335] m.p. 151°).

REFERENCES

1. C. Singer and E.A. Underwood, "A Short History of Medicine", Clarendon Press, Oxford, 2nd. edn., 1962.

2. J.F. Danielli and E.N. Harvey, J. Cell. Comp. Physiol., 1935, 5, 483.

3. H.L. Booij, Acta Physiol. et Pharmacol. Neerl., 1954, 3, 536.

4. C.A.M. Hogben, D.J. Tocco, B.B. Brodie and L.S. Schanker, J. Pharmacol., 1959, 125, 275.

   R.P. Walton, J. Amer. Med. Assoc., 1944, 124, 138

5. N.J. Harper, "Progress in Drug Research", Birkhäuser Verlag, Basel, 1962, Vol. IV, p. 221.

6. L.S. Schanker, "Advances in Drug Research", Academic Press, London, 1964, Vol. I, p. 73.

7. W. Wilbrandt and T. Rosenberg, Pharmacol. Rev., 1961, 13, 109.

8. T.H. Wilson and B.R. Landau, Amer. J. Physiol., 1960, 198, 99.

9. L.S. Schanker and J.J. Jeffrey, Nature, 1961, 190, 727.

   L.S. Schanker and J.J. Jeffrey, Biochem. Pharmacol., 1962, 11, 961.

10. E.J. Ariens, "Molecular Pharmacology", Academic Press, New york, 1964, Vol. I, p. 39.

11. A.L. Hodgkin and R.D. Keynes, J. Physiol., 1955, 128, 28.

12. S. Bennet, J. Biophys. Biochem. Cytol., 1956, supl. 2, 99.

13. D.P. Rall and C.G. Zubrod, Ann. Rev. Pharmacol., 1962, 2, 109.

    L.J. Roth and C.F. Barlow, Science, 1961, 134, 22.

    L.S. Schanker, Pharmacol. Rev., 1962, 14, 501.

14. M.A. Chirigos, P, Greengard and S. Udenfriend, J. Biol. Chem., 1960, 235, 2075.

    G. Guroff, W. King and S. Udenfriend, J. Biol. Chem., 1961, 236, 1773.

15. L.D. Prockop and L.S. Schanker, Life Sci., 1962, 1, 141.

    J.R. Pappenheimer, S.R. Heisey and E.F. Jordon, Amer. J. Physiol., 1961, 200, 1.

16. J. Tréfouël, Mme. J. Tréfouël, F. Nitti and D. Bovet, Compt. rend. Soc. Biol., 1935, 120, 756.

D.K. Myers, S.P. Simons and E.G.L. Simons, Biochem. J., 1953, 55, i.

17. M. Florkin and E.H. Stotz, "Comprehensive Biochemistry", Elsevier, New York, 2nd edn., 1965, Vol. XIII.

18. M.B. Slaytor and S.E. Wright, J. Med. Pharm. Chem., 1962, 5, 483.

19. R.T. Williams, "Detoxication Mechanisms", Chapman and Hall, London, 1959, p. 374.

20. J.F. Mead, M.M. Rapport and J.B. Koepfli, J. Amer. Chem. Soc., 1946, 68, 2704.

21. A.H. Beckett and A.F. Casy, J. Pharm. Pharmacol., 1955, 7, 433.

22. M. Martin-Smith and S.T. Reid, J. Med. Pharm. Chem., 1959, 1, 507.

23. A.J. Clark, "The Mode of Action of Drugs on Cells", Edward Arnold, London, 1933.

24. W. Moore, J. Agric. Res., 1917, 10, 365.

25. J.J.H. Holt, Lancet, 1916 (1), 1136.

26. H. Meyer, Arch. exp. Path. Pharmak., 1901, 46, 338.

E. Overton, Arch. ges. Physiol., 1902, 92, 115.

27. J. Traube, Pflüg. Arch. ges. Physiol., 1904, 105, 541.

28. L. Pauling, Science, 1961, 134, 15.

29. S.L. Miller, Proc. Nat. Acad. Sci. U.S.A., 1961, 47, 1515.

30. J.H. Lawrence, W.F. Loomis, C.A. Tobias and F.H. Turpin, J. Physiol., 1946, 105, 197.

R.M. Featherstone and C.A. Muehlbaecher, Pharmacol. Rev., 1963, 15, 97.

31. J. Ferguson, Proc. Roy. Soc., 1939, B127, 387.

32. F. Brink and J.M. Posternak, J. Cell. Comp. Physiol., 1948, 32, 211.

33. L.J. Mullins, Chem. Rev., 1954, 54, 289.

34. K.W. Miller, W.D.M. Paton and E.B. Smith, Nature, 1965, 206, 574.

35. F.W. Schueler, "Chemobiodynamics and Drug Design", McGraw-Hill, New York, 1960, p.140.

36. D.W. Woolley and B.W. Gommi, Arch. Intern. Pharmacodyn., 1966, 159, 8.

C. Chagas, E. Penna-Franca, K. Nishie and E. Gracia, Arch. Biochem. Biophys., 1958, 75, 251.

C. Chagas, Ann. N.Y. Acad. Sci., 1959, 81, 345.

S. Ehrenpreis, Science, 1959, 129, 1613.

S. Ehrenpreis, Biochim. Biophys. Acta, 1960, 44, 561.

37. P.G. Waser, "Curare and Curare-like Agents", Elsevier, Amsterdam, 1959, p.219.

38. P.G. Waser, Pharmacol. Rev., 1961, 13, 465.

A.H. Beckett, N.J. Harper and J.W. Clitherow, J. Pharm. Pharmacol., 1963, 15, 362.

J.P. Long and A.M. Lands, J. Pharmacol. Exptl. Therap., 1957, 120, 46.

B. Belleau and J. Puranen, J. Med. Chem., 1963, 6, 325.

39. A.H. Beckett and A.F. Casy, J. Pharm. Pharmacol., 1954, 6, 986.

A.H. Beckett, A.F. Casy, N.J. Harper and P.M. Phillips, J. Pharm. Pharmacol., 1956, 8, 860.

40. B. Belleau, "Proceedings of the First International Pharmacological Meeting", Pergamon Press, Oxford, 1961, Vol. VII, p. 75.

B.M. Bloom and I.M Goldman, "Advances in Drug Research", Academic Press, London, 1966, Vol. III, p. 121.

41. E.C. Jorgensen, P.A. Lehmann, C. Greenberg and N. Zencker, J. Biol. Chem., 1962, 237, 3832.

42. M.J. Greenberg, Brit. J. Pharmacol., 1960, 15, 375.

43. L.H. Sarett, A.A. Patchett and S.L. Steelman, "Progress in Drug Research", Birkhauser Verlag, Basel, 1963, Vol. V, p. 11.

44. L. Weiss, "The Structure and Function of the Membranes and Surfaces of Cells", University Press Cambridge, 1936, p. 32.

45. M. Nickerson, Pharmacol. Rev., 1957, 9, 246.

46. B. Holmstedt, "Handbook of Experimental Pharmacology", Springer-Verlag, Berlin, 1963, Vol. XV, p. 428.

47. J.A. Montgomery, "Progress in Drug Research", Birkhauser Verlag, Basel, 1965, Vol. VIII, p. 431.

48. C. Tanford, J. Amer. Chem. Soc., 1962, 84, 4240.

49. U. Wesphal, "Mechanism of Action of Steroid Hormones", Pergamon Press, Oxford, 1961, p. 73.

50. E.W. Gill, Progr. Med. Chem., 1965, 4, 72.

51. Reference 50, p. 74.

52. I.C.L. Bruce, C.H. Giles and S.K. Jain, J. Chem. Soc., 1958, 1610.
C.H. Giles and R.B. McKay, J. Biol. Chem., 1962, 237, 3388

53. P. Erlich and J. Morgenroth, Klin. Wochschr., 1910, 682.

54. J.N. Langley, Proc. Roy. Soc., 1906, B78, 170.

55. E. Lucas, J. Physiol., 1907, 36, 113.

56. E. Fischer, Ber., 1894, 27, 2985.

57. J.H. Gaddum, J. Physiol., 1937, 89, 7P.
J.H. Gaddum, Pharmacol. Rev., 1957, 9, 211.

58. E.J. Ariens, Arch. Intern. Pharmacodyn., 1954, 99, 32.
E.J. Ariens and A.M. Simonis, ibid., 1954, 99, 175.
E.J. Ariens and W.M. de Groot, ibid., 1954, 99, 193.
E.J. Ariens, A.M. Simonis and W.M. de Groot, ibid., 1955, 100, 298.

59. R.P. Stephenson, Brit. J. Pharmacol., 1956, 11, 379.

60. M. Nickerson, Nature, 1956, 178, 697.

61. E.J. Ariens, J.M. van Rossum and A.M. Simonis, Pharmacol. Rev., 1957, 9, 218.

62. E.J. Ariens and A.M. Simonis, J. Pharm. Pharmacol., 1964, 16, 137.

63. R.F. Furchgott, At the Symposium on "The Interaction of Drugs with Receptors", Chelsea College of Science and Technology, April, 1965, see reference 64.

64. D. Mackay, J. Pharm. Pharmacol., 1966, 18, 201.

D. Mackay, "Advances in Drug Research", Academic Press, London, 1966, Vol. III, p. 1.

65. A.S.V. Burgen, J. Pharm. Pharmacol., 1966, 18, 137.

66. W.D.M. Paton, Proc. Roy. Soc., 1961, B154, 21.

67. R. Croxatto and F. Huidobro, Arch. Intern. Pharmacodyn., 1956, 106, 207.

68. R.F. Furchgott, Ann. Rev. Pharmacol., 1964, 4, 21.

69. W.D.M. Paton and H.P. Rang, "Advances in Drug Research", Academic Press, London, 1966, Vol. III, p. 57.

70. Reference 50, p. 80.

71. B. Belleau, J. Med. Chem., 1964, 7, 776.

72. B. Belleau, "Advances in Drug Research", Academic Press, London, 1965, Vol. II, p. 89.

73. S. Grisolia, Physiol. Rev., 1964, 44, 657.

74. D.E. Koshland, "The Enzymes", Academic Press, New York, 1959, Vol. I, p. 305.

75. D.J. Triggle, "The Autonomic Nervous System", Academic Press, London, 1965.

76. B. Belleau and G. Lacasse, J. Med. Chem., 1964, 7, 768.

77. G. Werner and A.S. Kuperman, "Handbook of Experimental Pharmacology", Springer-Verlag, Berlin, 1963, Vol. XV, p. 570.

78. J.C. Eccles, "Physiology of Nerve Cells", The John Hopkins Press, Baltimore, 1957.

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

H.H. Dale, Pharmacol. Rev., 1954, 6, 7.

B. Katz, Proc. Roy. Soc., 1962, B155, 455.

L. Brown, Proc. Roy. Soc. B, 1965, 162, 1.

79. V. Erspamer, R.C. Sci. Farmitalia, 1954, 1, 1.

80. K.E. Hamlin and F.E. Fischer, J. Amer. Chem. Soc., 1951, 73, 5007.

81. M.E. Speeter, R.V. Heinzelmann and D.I. Weisblat, J. Amer. Chem. Soc., 1951, 73, 5514.

B. Asero, V. Colò, V. Erspamer and A. Vercellone, Annalen, 1952, 576, 69.

M.E. Speeter and W.C. Anthony, J. Amer. Chem. Soc., 1954, 76, 6208.

E.H.P. Young, J. Chem. Soc., 1958, 3493.

A.S.F. Ash and W.R. Wragg, J. Chem. Soc., 1958, 3887.

W.E. Noland and R.A. Hovden, J. Org. Chem., 1959, 24, 894.

G. Bernini, Ann. Chim. Rome, 1953, 43, 559; Chem. Abs., 1955, 49, 2413.

J. Harley-Mason and A.H. Jackson, J. Chem. Soc., 1954, 1165.

R.A. Abramovitch and D. Shapiro, J. Chem. Soc., 1956, 4589.

C.D. Nenitzescu and D. Raileanu, Chem. Ber., 1958, 91, 1141.

S. Pietra, Il Farmaco (Pavia) Ed. Sci., 1958, 13, 75; Chem. Abs., 1958, 52, 13704.

82. V. Erspamer, "Progress in Drug Research", Birkenhauser Verlag, Basel, 1961, Vol. III, p.151.

83. S. Garattini and L. Valzelli, "Serotonin", Elsevier, Amsterdam, 1965.

84. O. Eichler and A. Farah, "Handbook of Experimental Pharmacology", Springer-Verlag, Berlin, 1966, Vol. XIX.

85. V. Erspamer, Pharmacol. Rev., 1954, 6, 425.

86. M. Rand and G. Reid, Nature, 1951, 168, 385.

87. E.P. Benditt, R.L. Wong, M. Arase and E. Roeper, Proc. Soc. Exp. Biol. Med., 1955, 90, 303.

88. A.H. Amin, T.B.B. Crawford and J.H. Gaddum, J. Physiol., 1954, 126, 596.

89. B.M. Twarog and I.H. Page, Amer. J. Physiol., 1953, 175, 157.

90. S. Udenfriend and H. Weissbach, Proc. Soc. Exp. Biol. Med., 1958, 97, 748.

91. T.N. Tozer, N.H. Neff and B.B. Brodie, J. Pharmacol. Exptl. Therap., 1966, 153, 177.

92. D.W. Woolley and E.N. Shaw, Brit. Med. J., 1954, 122.

93. J.H. Gaddum, "20th International Physiological Congress", Bruxelles, 1956, Abs. Rev., 442

94. S. Udenfriend, D.F. Bogdanski and H. Weissbach, Fed. Proc., 1956, 15, 493.

95. Reference 83, p. 205.

96. B.B. Brodie, "5-Hydroxytryptamine", Pergamon Press, London, 1958, p. 64.

P.A. Shore, D. Bogdanski and B.B. Brodie, J. Pharmacol. Exptl. Therap., 1957, 119, 183.

E. Costa, G.L. Gessa, C. Hirsch, R. Kuntzman and B.B. Brodie, Ann. N.Y. Acad. Sci., 1962, 96, 118.

97. A.S. Marazzi and E.R. Hart, Science, 1955, 121, 365.

A.S. Marazzi, Ann. N.Y. Acad. Sci., 1957, 66, 496.

98. A. Iggo and M. Vogt, J. Physiol., 1960, 150, 114.

99. J. Crossland, J. Pharm. Pharmacol., 1960, 12, 1.

100. I.A. Michaelson and V.P. Whittaker, Biochem. Pharmacol., 1963, 12, 203.

L.M. Zieher and E. De Robertis, ibid., 1963, 12, 596.

E.W. Maynert, R. Levi and A.J.D. De Lorenzo, J. Pharmacol. Exptl. Therap., 1964, 144, 385.

101. HH. Gerschenfeld and E. Stefani, J. Physiol., 1966, 185, 684.

102. Reference 82, p. 292.

103. S. Undenfriend, H. Weissbach and D.F. Bogdanski, J. Biol. Chem., 1957, 224, 803.

104. E.A. Zeller, Ann. N.Y. Acad. Sci., 1959, 80, 551.

105. H. Green and J.L. Sawyer, "Progress in Brain Research", Elsevier, Amsterdam, 1964, Vol. VIII, p. 150.

106. B.B. Brodie, A. Pletscher and P.A. Shore, Science, 1955, 122, 968.

A. Pletscher, P.A. Shore and B.B. Brodie, J. Pharmacol. Exptl. Therap., 1956, 116, 46.

107. A. Carlsson, E. Rosengren, A. Bertler and J. Nilsson, "Psychotropic Drugs", Elsevier, Amsterdam, 1957, p. 363

108. B. Gözsy and L. Kato, Science, 1957, 125, 934.

D.A. Rowley and E.P. Benditt, J. Exp. Med., 1956, 103, 399.

109. D.H. LeMessurier, C.J. Schwartz and R.F. Whelan, Brit. J. Pharmacol., 1959, 14, 246.

110. H. Herxheimer, J. Physiol., 1955, 128, 435.

111. T.R. Hendrix, M. Atkinson, J.A. Clifton and E.J. Ingelfinger, Amer. J. Med., 1957, 23, 886.

E. Bülbring and A. Crema, Brit. J. Pharmacol., 1958, 13, 444.

112. E. Bülbring and R.C.Y. Lin, J. Physiol., 1958, 140, 381.

D.J. Boullin, Brit. J. Pharmacol., 1964, 23, 14.

113. M. Rocha e Silva, J.R. Valle and Z.P. Picarelli, Brit. J. Pharmacol., 1953, 8, 378.

114. J.H. Gaddum and Z.P. Picarelli, Brit. J. Pharmacol., 1957, 12, 323.

115. J. Harry, Brit. J. Pharmacol., 1963, 20, 399.

M. Day and J.R. Vane, Brit. J. Pharmacol., 1963, 20, 150.

116. L. Gyermek, Arch. Intern. Pharmacodyn., 1962, 137, 137.

117. T.E. Mansour, Brit. J. Pharmacol., 1957, 12, 406.

118. I.R. Innes, Brit. J. Pharmacol., 1962, 19, 427.

119. J.H. Gaddum, J. Physiol., 1953, 119, 363.

I.H. Page, J. Pharmacol. Exptl. Therap., 1952, 105, 58.

V. Erspamer, Nature, 1952, 170, 281.

120. D.W. Woolley and E.N. Shaw, J. Pharmacol. Exptl. Therap., 1957, 121, 13.

R.B. Barlow and I. Khan, Brit. J. Pharmacol., 1959, 14, 99, 265.

121. J.R. Vane, Brit. J. Pharmacol., 1959, 14, 87.

122. J.H. Welsh, Ann. N.Y. Acad. Sci., 1957, 66, 618.

123. L. Gyermek, "Handbook of Experimental Pharmacology", Springer-Verlag, Berlin, 1966, Vol. XIX, p.476.

124. J. Offermeier and E.J. Ariens, Arch. Intern. Pharmacodyn., 1966, 164, 216.

125. R.G. Taborsky, P. Delvigs, I.H. Page and N. Crawford, J. Med. Chem., 1965, 8, 460.

126. J. Offermeier and E.J. Ariens, Arch. Intern. Pharmacodyn., 1966, 164, 192.

127. J.H. Gaddum, "Ciba Symposium on Hypertension", Churchill Ltd., London, 1954, p. 75.

J.H. Gaddum, "5-Hydroxytryptamine", Pergamon Press, New York, 1957, p. 195.

128. J.H. Gaddum, Ann. N.Y. Acad. Sci., 1957, 66, 643.

129. E. Rothlin, Ann. N.Y. Acad. Sci., 1957, 66, 668.

E. Rothlin, J. Pharm. Pharmacol., 1957, 9, 569.

130. J. Jacob, Actualités Pharmacol., 1960, 13, 131.

131. L. Gyermek, Proc. I.U.P.S. XXII Int. Congr., Leidon, 1962, Excerpta Med., Amsterdam, 1962, I/1, 28.

132. G. Bertaccini and P. Zamboni, Arch. Intern, Pharmacodyn., 1961, 133, 138.

E.N. Shaw and D.W. Woolley, Proc. Soc. Exp. Biol. Med., 1956, 93, 217.

133. D.W. Woolley and E.N. Shaw, Fed. Proc., 1955, 14, 307.

E.N. Shaw and D.W. Woolley, J. Pharmacol. Exptl. Therap., 1956, 116, 164.

134. F. Leonard and W. Tschannen, J. Med. Chem., 1965, 8, 287.

135. V. Erspamer, Science, 1955, 121, 369.

136. D.W. Woolley and E.N. Shaw, J. Pharmacol. Exptl. Therap., 1953, 108, 87.

D.W. Woolley and E.N. Shaw, ibid., 1954, 111, 43.

137. L. Gyermek, J. Med. Chem., 1964, 7, 280.

138. L. Gyermek, Nature, 1961, 192, 465.

139. L. Gyermek, Arch. Intern. Pharmacodyn., 1964, 150, 570.

140. J.H. Gaddum, K.A. Hameed, D.E. Hathway and F.F. Stephens, Quart. J. Exp. Physiol., 1955, 40, 49.

141. E.N. Shaw and D.W. Woolley, J. Amer. Chem. Soc., 1957, 79, 3561.

142. J.R. Parrat and G.B. West, Brit. J. Pharmacol., 1958, 13, 65.

143. L. Gyermek, I. Lázár and A.Z. Csák, Arch. Intern. Pharmacodyn., 1956, 107, 62.

144. R. Jaques, H.J. Bein and R. Meier, Helv. Physiol. Pharmacol. Acta, 1956, 14, 269.

145. M. Medakovic, Arch. Intern. Pharmacodyn., 1958, 114, 201.

146. R. Meier, J. Tripod and E. Wirz, Arch. Intern. Pharmacodyn., 1957, 109, 55.

147. J. Krapcho and C.F. Turk, J. Med. Chem., 1966, 9, 809.
J. Krapcho, E.R. Spitzmiller, C.F. Turk and J. Fried, J. Med. Chem., 1964, 7, 376.

148. J.H. Gaddum, J. Physiol., 1953, 121, 15P.

149. D.W. Woolley and E.N. Shaw, Ann. N.Y. Acad. Sci., 1957, 66, 649.

150. J.M. Robson and R.S. Stacey, "Recent Advances in Pharmacology", Churchill, London, 1962, p. 122.

151. D.X. Freedman and N.J. Giarman, Ann. N.Y. Acad. Sci., 1962, 96, 98.

152. H. Sprince, Ann. N.Y. Acad. Sci., 1962, 96, 399.

153. J. Axelrod, J. Pharmacol. Exptl. Therap., 1962, 138, 28.

154. S. Szara and E. Hearst, Ann. N.Y. Acad. Sci., 1962, 96, 134.
S. Szara and J. Axelrod, Experientia, 1959, 15, 216.

155. R.J. Wurtman, J. Axelrod and L.S. Phillips, Science, 1963, 142, 1071.

156. R.K. Gessner and I.H. Page, Amer. J. Physiol., 1962, 203, 167.

157. Reference 123, p. 480.

158. B. Belleau and J. Puranen, J. Med. Chem., 1963, 6, 325.
D.J. Triggle and B. Belleau, Canad. J. Chem., 1962, 40, 1201.

159. E.E. Smissman, W.L. Nelson, J.B. LaPidus and J.L. Day, J. Med. Chem., 1966, 9, 458.

160. F.W. Scheuler, J. Amer. Pharm. Assoc., 1956, 45, 197.

161. S. Archer, A.M. Lands and T.R. Lewis, J. Med. Pharm. Chem., 1962, 5, 423.

162. B. Belleau and P. Cooper, J. Med. Chem., 1963, 6, 579.

163. M. Alauddin, B. Caddy, J.J. Lewis, M. Martin-Smith and M.F. Sugrue, J. Pharm. Pharmacol., 1965, 17, 59.
R.S. Bigs, M. Davis and R. Wiew, Experientia, 1964, 20, 119.
S.A.P. 63/940, 63/941/1963.

164. F.W. Schueler, Science, 1946, 103, 221.
H.H. Keasling, J. Amer. Pharm. Assoc., 1950, 39, 87.

165. L.F. Fieser and W.P. Campbell, J. Amer. Chem. Soc., 1939, 61, 2528.
C.W. Brandt and D.J. Ross, Nature, 1948, 161, 892.

166. H. Wieland and T. Wieland, Annalen, 1937, 528, 234.

167. F. Marki, A.V. Robertson and B. Witkop, J. Amer. Chem. Soc., 1961, 83, 3341.

168. J.B. Hester, J. Org. Chem., 1964, 29, 1158.

169. M. von Strandtmann, M.P. Cohen and J. Shavel, J. Med. Chem., 1963, 6, 719.
M. von Strandtmann, M.P. Cohen and J. Shavel, ibid., 1965, 8, 200.

170. D.P. 6,509,222/1966; Chem. Abs. 1966, 64, 19565.
U.S.P. 3,249,623; Chem. Abs.,1966, 64, 19620.

171. J. Harley-Mason and A.H. Jackson, J. Chem. Soc., 1955, 374.

172. D.P. 6, 515, 701/1966.

173. A. Cerletti, "Progress in Drug Research", Birkenhauser Verlag, Basel, 1960, Vol. II, 240.

174. D.W. Woolley, "Progress in Drug Research", Birkenhauser Verlag, Basel, 1960, Vol. II, 613.

175. R.H.F. Manske, "The Alkaloids, Chemistry and Physiology", Academic Press, London, 1965, Vol. VIII.

176. R.G. Gould and W.A. Jacobs, J. Biol. Chem., 1939, 130, 407.

177. L.S. Harris and F.C. Uhle, J. Pharmacol. Exptl. Therap., 1960, 128, 358.

178. J.R. Vane, Brit. J. Pharmacol., 1957, 12, 344.

179. A. Lessin, R.F. Long and M.W. Parkes, Brit. J. Pharmacol., 1965, 24, 49.

180. Reference 84, p. 600.

181. A. Stoll, Chem. Rev., 1950, 47, 197.

182. A.L. Glenn, Quart. Rev., 1954, 8, 192.

183. E.C. Kornfeld, E.J. Fornefeld, G.B. Kline, M.J. Mann, D.E. Morrison, R.G. Jones and R.B. Woodward, J. Amer. Chem. Soc., 1956, 78, 3087.

184. F.C. Uhle, J. Amer. Chem. Soc., 1949, 71, 761.

F.C. Uhle and S.H. Robinson, ibid,, 1955, 77, 3544.

F.C. Uhle and L.S. Haris, ibid., 1957, 79, 102.

F.C. Uhle, C.M. McEwan, H. Schroter, Ching Yuan and B.W. Baker, ibid., 1960, 82, 1200.

185. H. Plieninger and W. Muller, Chem. Ber., 1960, 93, 2029.

186. J. Szmuszkovicz, J. Org. Chem., 1964, 29, 843.

187. H. Plieninger and C.E. Castro, Chem. Ber., 1954, 87, 1760.

H. Plieninger and T. Suehiro, ibid., 1955, 88, 550.
H. Plieninger, M. Schach, N. Wittenau and B. Kiefer, ibid., 1958, 91, 2095.

188. K.F. Jennings, J. Chem. Soc., 1957, 497.

189. M. Renson, Bull. Soc. chim. belges, 1959, 68, 258.

190. F.G. Mann and A.J. Tetlow, J. Chem. Soc., 1957, 3352.

191. A.B.A. Jansen, J.M. Johnson and J.R. Surtees, J. Chem. Soc., 1964, 5573.

192. C.A. Grob, W. Meier and E. Renk, Helv. Chim. Acta, 1961, 44, 1525.

193. C.A. Grob and P. Payot, Helv. Chim. Acta, 1953, 36, 839.

194. A. Cohen, B. Heath-Brown and A.H. Rees, Chem. and Ind., 1953, 1179.

195. A. Stoll, J. Rutschmann and Th. Petrzilka, Helv. Chim. Acta, 1950, 33, 2257.

196. E.L. Martin, Org. Reactions, 1942, 1, 155.

197. J. Braun and K. Weissbach, Chem. Ber., 1929, 62, 2416.

198. W.K. Sprenger, J.G. Cannon and H.F. Koelling, J. Org. Chem., 1966, 31, 2402.

199. C.F. Koelsch, J. Amer. Chem. Soc., 1944, 66, 2019.

200. G. Blaikie and W.H. Perkin, J. Chem. Soc., 1924, 296.

201. H.E. Johnson and D.G. Crosby, J. Org. Chem., 1960, 25, 569.

202. H.E. Fritz, J. Org. Chem., 1963, 28, 1384.

203. H.S.B. Barrett, W.H. Perkin and R. Robinson, J. Chem. Soc., 1929, 2942.

204. P.S. Pinkney, Org. Synth., 1943, 2, 116.

205. D.P. 6, 405, 591/1964.

206. B.K. Blout and R. Robinson, J. Chem. Soc., 1931, 3158.

207. A. Smith and J.H.P. Utley, Chem. Commun., 1965, 427.

208. F. Kunckell, Chem. Ber., 1901, 34, 124.

209. B. Asero, V. Colò, V. Erspamer and A. Vercellone, Annalen, 1952, 576, 69.

210. F.M. Dean, J. Goodchild, L.E. Houghton, J.A. Martin, R.B. Morton,

B. Paton, A.W. Price and N. Somvichien, Tetrahedron Letters, 1966, 4153.

211. W. Schäfer and R. Leute, Tetrahedron Letters, 1965, 1843.

212. R.R. Hunt and R.L. Rickard, J. Chem. Soc. (C), 1966, 344.

213. H.J. Teuber and G. Staiger, Chem. Ber., 1956, 89, 489.

214. P.A. Stadler, A.J. Frey, F. Troxler and A. Hofmann, Helv. Chim. Acta, 1964, 47, 756.

215. J.A. Moore and M. Rahm, J. Org. Chem., 1961, 26, 1109.

216. A. Girardet and N. LoRusso, Helv. Chim. Acta, 1966, 49, 471.

217. H. Dressler and M.E. Baum, J. Org. Chem., 1961, 26, 102.

218. D.W. Woolley and E. Shaw, J. Biol. Chem., 1953, 203, 69.
E. Shaw and D.W. Woolley, J. Amer. Chem. Soc., 1953, 75, 1877.
E. Shaw and D.W. Woolley, ibid., 1957, 79, 3561.

219. V.I. Shvedov, L.B. Altukhova, E.K. Komissarova and A.N. Grinev, Khim. Geterotsikh Soedin., Akad. Nauk. Latv. S.S.S.R., 1965, 3, 365; Chem. Abs., 1965, 63, 14800.

220. H.J. Teuber and G. Staiger, Chem. Ber., 1954, 87, 1251.

221. R.J.S. Beer, T. Broadhurst and A. Robertson, J. Chem. Soc., 1952, 4946.

222. A.H. Milne and M.L. Tomlinson, J. Chem. Soc., 1952, 2789.

223. C.U. Rogers and B.B. Corson, Org. Synth., 1963, 4, 884.

224. B. Robinson, Chem. Rev., 1963, 63, 373.

225. J. Harley-Mason and E.H. Pavri, J. Chem. Soc., 1963, 2504.

226. C. Djerassi, Org. Reactions, 1951, 6, 207.

227. J.B. Aldersley, G.N. Burkhardt, A.E. Gilham and N.C. Hindley, J. Chem. Soc., 1940, 10.

228. C. Mentzer, C. Beaudet and M. Bory, Bull. Soc. Chim. France, 1953, 20, 421.

229. H.J. Teuber, D. Cornelius and U. Wolcke, Annalen, 1966, 696, 116.

230. J. Elks, D.F. Elliot and B.A. Hems, J. Chem. Soc., 1944, 624.

231. M. Julia and M. Gaston-Breton, Bull. Soc. Chim. France, 1966, 1335.

232. V. Prey, Ber., 1941, 74B, 1219.
V. Prey, ibid., 1942, 75B, 350.

233. J.F.W. McOmie and M.L. Watts, Chem. and Ind., 1963, 1658.

234. F.Sparatore, Gazzetta, 1958, 88, 755.

235. D. Beck, K. Schenker, F. Stuber and R. Zurcher, Tetrahedron Letters, 1965, 2285.

236. J.A. Skorcz and J.E. Robertson, J. Med. Chem., 1965, 8, 255.

237. J.F. Bunnett and J.A. Skorcz, J. Org. Chem., 1962, 27, 3836.

238. L. Horner, W. Kirmse and K. Muth, Chem. Ber., 1958, 91, 430.

239. A.I. Vogel, "Practical Organic Chemistry", Longmans, London, 1956, 3rd Edn., p. 870.

240. J. Attenburrow, A.F.B. Cameron, J.H. Chapmam, R.M. Evans, B.A. Hems, A.B.A. Jansen and T. Walker, J. Chem. Soc., 1952, 1094.

241. G. Braun, Org. Synth., 1941, 2, 431.

242. F.C. Uhle, C.G. Vernick and G.L. Schmir, J. Amer. Chem. Soc., 1955, 72, 3334.

243. "Documenta Geigy Scientific Tables", J.R. Gergy, Basle, 1959, 5th edn., p.105.

244. D. Balcom and A. Furst, J. Amer. Chem. Soc., 1953, 75, 4334.

245. W.A. Noyes, G.S. Hammond and J.Pitts, "Advances in Photochemistry", Interscience, 1963, Vols. I-IV.

A. Schonberg, "Preparative Organische Photochemie", Springer-Verlag, Berlin, 1958.

P. de Mayo, "Advances in Organic Chemistry", Interscience, New York, 1960, Vol. II, p. 367.

246. P. de Mayo and S.T. Reid, Quart. Rev., 1961, 15, 393.

247. H.R. Seliger and W.D. McElroy, "Light: Physical and Biological Action", Academic Press, New York, 1965.

J.B. Thomas, "Primary Photoprocesses in Biology", North-Holland Publishing Co., Amsterdam.

248. K. Schaffner, "Progress in the Chemistry of Organic Natural Products", Springer-Verlag, New York, 1964, Vol. XXII, p. 1.

S.T. Reid, "Progress in Drug Research"

249. J.R. Majer and J.P. Simons, "Advances in Photochemistry", Interscience, New York, 1964, Vol. II, p. 137.

250. O.L. Chapman, J.B. Sieja and W.J. Welstead, J. Amer. Chem. Soc., 1966, 88, 161.

R.C. Cookson, Pure and Appl. Chem., 1964, 9, 575.

251. J.W. Conine and D.A. Zuck, J. Pharm. Sci., 1963, 52, 63.

252. W. Bergmann and M.J. McLean, Chem. Rev., 1941, 28, 367.

L. Bateman, Quart. Rev., 1954, 8, 147.

G.O. Schenck, Angew. Chem., 1957, 69, 579.

253. S. Scheindlin, A. Lee and I. Griffith, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1952, 41, 420.

254. M. Guillot, Pharm Acta Helv., 1959, 34, 373.

255. E.E. van Tamelen and S.P. Pappas, J. Amer. Chem. Soc., 1963, 85, 3297.

256. D. Banes, J. Assoc. Offic. Agric. Chemists, 1961, 44, 323.

257. E. Walton and G. Brownlee, Nature, 1943, 151, 305.

258. The Joint Committee of the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry on Methods of Assay of Crude Drugs, J. Pharm. Pharmacol., 1959, 11, 211.

259. L. Zechmeister, Experientia, 1954, 10, 1.

260. L. Zechmeister and A. Polgár, J. Amer. Chem. Soc., 1944, 66, 137.

261. G. Wald, 'Light and Life", Johns Hopkins, Baltimore, 1961, p. 724.

H.J.A. Dartnall, "The Eye", Academic Press, New York, 1962, Vol. II.

262. R. Srinivasan, "Advances in Photochemistry", Interscience, New York, 1966, Vol. IV, p. 113.

W.G. Dauben and W. Wipke, Pure and Appl. Chem., 1964, 9, 539.

263. L.F. Fieser and M. Fieser, "Steroids", Reinhold Publishing Corp., New York, 1959, p. 90.

264. H.H. Inhoffen, Naturwiss., 1937, 25, 125.

J.L. Owades, Experientia, 1950, 6, 258.

265. N.C. Yang and D.H. Yang, Tetrahedron Letters, 1960, (4), 10.

266. G. Ciamician and P. Silber, Ber., 1900, 33, 2911.

267. M. Akhtar, Tetrahedron Letters, 1965, 4727.

268. G. Quinkert, B. Wegemund, F. Homberg and G. Cimbollek, Chem. Ber., 1964, 97, 958.

269. A. Butenandt and L. Poschmann, Ber., 1944, 77, 394.

270. B. Nann, D. Gravel, R. Schorta, H. Wehrli, K. Schaffner and O. Jeger, Helv. Chim. Acta, 1963, 46, 2473.

271. A. Butenandt and A. Wolff, Ber., 1939, 72, 1121.

272. M.D. Cohen, Pure and Appl. Chem., 1964, 9, 567.

273. G.M. Blackburn and R.J.H. Davies, J. Chem. Soc. (C), 1966, 2239.

274. A. Wacker, H. Dellweg and D. Weinblum, Naturwiss., 1960, 47, 477.

R. Beukers, J. Ijlstra and W. Berends, Rec. Trav. chim., 1960, 79, 101.

275. R.B. Setlow, R.A. Swenson and W.L. Carrier, Science, 1963, 142, 1464.

276. H. Wehrli, R. Wenger, K. Schaffner and O. Jeger, Helv. Chim. Acta, 1963, 46, 678.

277. O.L. Chapman, H.G. Smith and R.W. King, J. Amer. Chem. Soc., 1963, 85, 803.

278. P.D. Gardner, R.L. Brandon and G.R. Haynes, J. Amer. Chem. Soc., 1957, 79. 6334.

279. O.L. Chapman, H.G. Smith and R.W. King, J. Amer. Chem. Soc., 1963, 85, 806.

280. J.L. Simonsen and D.H.R. Barton, "The Terpenes", Cambridge University Press, Cambridge, 1952, Vol. III, p. 292.

281. D.H.R. Barton, P. de Mayo and M. Shafiq, J. Chem. Soc., 1957, 929.

E.E. van Tamelin, S.H. Levin, G. Brenner, J. Wolinsky and P. Aldrich, J. Amer. Chem. Soc., 1958, 80, 501.

D.H.R. Barton, P. de Mayo and M. Shafiq, J. Chem. Soc., 1958 3314.

282. D.H.R. Barton, P. de Mayo and M. Shafiq, J. Chem. Soc., 1958, 140.

D. Arigoni, H. Bosshard, H. Bruderer, G. Büchi, O. Jeger and L.J. Krebaum, Helv. Chim. Acta, 1957, 40, 1732.

283. Reference 246, p. 410.

284. O.L. Chapman and L.F. Englert, J. Amer. Chem. Soc., 1963, 85, 3028.

285. E. Schott, D. Arigoni and O. Jeger, Helv. Chim. Acta, 1963, 46, 307.

286. P. Trendelenburg, "Handbook of Experimental Pharmacology", Springer-Verlag, Berlin, 1920, Vol. II, p. 404.

287. H. Yamasaki, K. Kondo, T. Uda, T. Yamamoto and K. Endo, J. Chem. Soc. Japan, 1960, 56, 1249; Chem. Abs., 1962, 56, 5366.

288. W.E. Hamlin, T. Chulski, R.H. Johnson and J.G. Wagner, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1960, 49, 253.

289. D.H.R. Barton and W.C. Taylor, J. Chem. Soc., 1958, 2500.

290. H.J.F. Angus, J.M. Blair and D. Bryce-Smith, J. Chem. Soc., 1960, 2003.

291. J.C. Anderson and C.B. Reese, J. Chem. Soc., 1963, 1781.

292. C.H. Kuo, R.D. Hoffsommer, H.L. Slates, D. Taub and N.L. Wendler, Chem. and Ind., 1960, 1627.

293. S.T. Reid and G. Subramanian, Chem. Commun., 1966, 245.

294. D.P. Kelly and J.T. Pinhey, Tetrahedron Letters, 1964, 3427.

295. Personal communication from G. Subramanian.

296. A. Schönberg and A. Mustafa, J. Chem. Soc., 1949, 1039.
A. Schonberg, A. Mustafa and M.Z. Barakat, Nature, 1947, 160, 401.

297. B. Pouyet, Bull. Soc. Chim. France, 1965, 90.

298. L.T. Allan and G.A. Swan, J. Chem. Soc., 1965, 4822.

299. J.J. Hlavka and P. Bitha, Tetrahedron Letters, 1966, 3843.

300. J. Chatonnet and J. Vial, Compt. rend. Soc. Biol., 1945, 139, 112.

301. E. Walaas, Photochem. Photobiol., 1963, 2, 9.

302. F.P. Luduena, A.L. Snyder and A.M. Lands, J. Pharm. Pharmacol., 1963, 15, 538.

303. G. Carraz, D. Gagnaire, A. Dardas, A. Rassat and H. Beriel, Therapie, 1963, 81, 171; Chem. Abs., 1965, 63, 2258.

304. H. Hellberg, Acta Chem. Scand., 1957, 11, 219.
A. Stoll and W. Schlientz, Helv. Chim. Acta, 1955, 38, 585.

305. S.Y. Wang, M. Apicella and B.R. Stone, J. Amer. Chem. Soc., 1956, 78, 4180.
S.Y. Wang, J. Amer. Chem. Soc., 1958, 80, 6196.

306. P. Karrer, H. Salomon, K. Schöpp, E. Schlittler and H. Fritzsche, Helv. Chim. Acta, 1934, 17, 1010.

307. O.H. Lowry, O.A. Bessey and E.J. Crawford, J. Biol. Chem., 1949, 180, 389.

308. J.L. DeMerre and C. Wilson, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 1956, 45, 129.

309. A.L. Demain, Nature, 1966, 210, 426.

310. B. Coleby, M. Keller and J. Weiss, J. Chem. Soc., 1954, 66.

311. R. Allinson, B. Coleby and J. Weiss, Nature, 1955, 175, 720.

312. R.N. Jones, P. Humphries, F. Herling and K. Dobriner, J. Amer. Chem. Soc., 1952, 74, 2820.

313. H. Wehrli, M. Cereghetti, K. Schaffner and O. Jeger, Helv. Chim. Acta, 1960, 43, 367.

314. E.P. Oliveto, C. Gerold and E.B. Hershberg, J. Amer. Chem. Soc., 1952, 14, 2248.

315. S.G. Brooks, R.M. Evans, G.F.H. Green, J.S. Hunt, A.G. Long, B. Mooney and J.L. Wyman, J. Chem. Soc., 1958, 4614.

316. J. Elks and J.F. Oughton, J. Chem. Soc., 1962, 4729.

317. E.L. Jackson, Org. Reactions, 194 , 2, 341.

318. B.J. Magerlein and R.H. Levin, J. Amer. Chem. Soc., 1953, 75, 3654.

319. W. Nagata, C. Tamm and T. Reichstein, Helv. Chim. Acta, 1959, 42, 1399.

320. R. Huttenrauch, Arch. Pharm., 1961, 294, 366.

321. D.P. 91,070/1959; Chem. Abs., 1960, 54, 12504.

322. O. Mancera, H.J. Ringold, C. Djerassi, G. Rosenkranz and F. Sondheimer, J. Amer. Chem. Soc., 1953, 75, 1286.

323. F.J. Ritter, Nature, 1964, 202, 694.

324. A. Butenandt, L.K. Poschmann, G. Failer, U. Schiedt and E. Biekert, Annalen, 1952, 575, 123.

325. A. Mustafa, "Advances in Photochemistry", Interscience, New York, 1964, Vol. II, p. 63.

326. O.L. Chapman, A.A. Griswold, E. Hoganson, G. Lenz and J. Reasoner, Pure and Appl. Chem., 1964, 9, 585.

327. A. Bowers, M.B. Sánchez and H.J. Ringold, J. Amer. Chem. Soc., 1959, 81, 3702.

328. A. Bowers, L.C. Ibanez and H.J. Ringold, J. Amer. Chem. Soc., 1959, 81, 3707.

329. H.J. Ringold, "Mechanism of Action of Steroid Hormones", Pergamon Press, London, 1961, p. 222.

329a. C.E. Anagnostopoulos and L.F. Fieser, J. Amer. Chem. Soc., 1954, 76, 532.

330. J.T. Pinhey and E. Rizzardo, Chem. Commun., 1965, 362.

331. O.L. Chapman, P.G. Cleveland and E.D. Hoganson, Chem. Commun., 1966, 101.

332. G.E.A. Coombes, J.M. Grady and S.T. Reid, Tetrahedron, 1967, 23, 1341.

333. J. Décombe, R. Jacquemain and J. Rabinovitch, Bull. Soc. chim., France, 1948, 447.

334. W.C.J. Ross, J. Chem. Soc., 1946, 737.

335. I.M. Heilbron, E.R.H. Jones and F.S. Spring, J. Chem. Soc., 1937, 801.

336. K. Preis and B. Raymann, Ber., 1879, 12, 224.

337. J. Mauthner and W. Suida, Monatsh, 1903, 24, 648.

338. M. Akhatar, "Advances in Photochemistry", Interscience, New York, 1964, Vol. II, p. 263.

A.L. Nussbaum and C.H. Robinson, Tetrahedron, 1961, 17, 35.

339. W.A. Harrison, Sir E.R.H. Jones, G.D. Meakins and P.A. Wilkinson, J. Chem. Soc., 1964, 3210.

340. L.F. Fieser, J. Amer. Chem. Soc., 1953, 75, 4386.