The author wishes to express his [illegible] for the guidance and encouragement given during the course of these investigations by Professor [illegible], F.R.S. He also wishes to express his indebtedness to Dr. [illegible] for many invaluable advice and helpful discussion.

A THESIS

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the
requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

ROBERT STEVENSON.

September, 1952.

The author wishes to express his sincere appreciation for the guidance and encouragement given during the course of these investigations by Professor F.S. Spring, F.R.S. He also wishes to acknowledge his indebtedness to Dr. G.T. Newbold for much invaluable advice and helpful discussion.

Historical.

Theoretical.

Experimental.

CONTENTS.

SUMMARY.

The elaboration of the cortisone sidechain, the conversion of ergosterol into 11-oxygenated derivatives, and the degradation of the bile acid sidechain have been investigated.

3β-Hydroxypregna-5:17-diene-21-carboxylic acid and 3β-acetoxypregna-5:17-dien-21-al, both useful intermediates in the synthesis of compounds possessing the characteristic cortisone sidechain, have been prepared by a new route with greater facility. Treatment of dehydroandrosterone acetate with ethoxyethynylmagnesium bromide gave 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol, which on acid rearrangement followed by hydrolysis yielded 3β-hydroxypregna-5:17-dien-21-carboxylic acid, identical with a specimen prepared by a previously established route. 3β-Acetoxy-21-ethoxypregna-5:20-dien-17β-ol and 3β-acetoxy-21-ethoxypregn-5-en-17β-ol were obtained by catalytic hydrogenation of 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol. Acid rearrangement of the former compound, followed by acetylation gave 3β-acetoxypregna-5:17-dien-21-al. Degradation of ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate to dehydroandrosterone acetate semicarbazone proved that the reactions had proceeded without D-ring enlargement.

Ergosterol has been converted by various procedures into 11α-hydroxy and 11-ketosteroids. The action of oxidising agents on ergosteryl-D acetate has been

investigated; treatment with one mol. of performic acid giving 3β-acetoxyergosta-9(11):22-dien-7-one, with two mols. of performic acid giving 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one, with chromic acid giving 3β-acetoxyergosta-8:22-dien-7-one, and with perbenzoic acid giving 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate. 3β:11α-Diacetoxyergosta-8:22-dien-7-one was obtained by mild alkaline treatment, and 7:11-diketoergost-22-en-3β-yl acetate was obtained by strong alkaline treatment, of 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one. Treatment of 5-dihydroergosteryl acetate with bromine gave a tetrabromoergostenyl acetate, treatment of which with sodium iodide gave ergosteryl-D acetate 22:23-dibromide and with zinc gave ergosteryl-D acetate. Oxidation of ergosteryl-D acetate 22:23-dibromide with peracetic or performic acids gave 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one, which yielded 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one on mild alkaline hydrolysis followed by acetylation and 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one on filtration of a benzene solution through alumina. Chromic acid oxidation of the latter compound gave 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate, and hydrogenation of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one in ethanolic potassium hydroxide solution with platinum catalyst gave 3β:11α-dihydroxyergost-22-en-7-one.

In projected degradations of the bile acid sidechain, the oxidation of 3α:12α-diacetoxy-24:24-diphenylchol-23-ene

by selenium dioxide, N-bromosuccinimide, and tertiary-butyl chromate was investigated. The action of the last reagent on cholesteryl acetate to give 7-ketocholesteryl acetate was also studied. Bromination of 3α:12α-diacetoxy-norcholanyl phenyl ketone gave both diastereoisomeric forms of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone. This compound was not successfully dehydrobrominated. Treatment with sodium alkoxides followed by re-acetylation gave two isomeric compounds, $C_{36}H_{50}O_7$, one of which has been identified as 3α:12α:23-triacetoxynorcholanyl phenyl ketone by comparison with a sample prepared by an unambiguous route, and the other is believed to be 3α:12α:24-triacetoxy-23-keto-24-phenylcholane. Two forms of 3α-acetoxy-23-bromo-11-ketonorcholanyl phenyl ketone were also isolated on monobromination of 3α-acetoxy-11-ketonorcholanyl phenyl ketone.

GENERAL INTRODUCTION.

Cortisone (I) (17-hydroxy-11-dehydrocorticosterone or 3:11:20-triketopregn-4-ene-17β:21-diol) is an adrenal cortical hormone, the beneficial effect of which in the treatment of rheumatoid arthritis (1), has stimulated [illegible] investigations on methods of synthesis.

[illegible] on the hormones of the adrenal cortex is [illegible] by the fact that the adrenals are vital organs; in nearly all animals, complete bilateral adrenalectomy leads to death in a few days. The vital function is concerned with the adrenal cortex which appears to operate principally by delivery [illegible] a mixture of substances into the blood, since by injection of suitable cortical extracts, adrenalectomised animals can be kept alive and the numerous insufficiency symptoms prevented or cured.

The first isolation of cortical tissue which would maintain life in the adrenalectomised animal were prepared in 1930 by Hartman and Brownell (2) and by Swingle and Pfiffner (3). Hartman designated the active agent "cortin", a name which became ambiguous when subsequent investigations revealed the presence of at least six individual compounds showing marked activity [illegible] the "amorphous fraction" remaining after [illegible] crystallisable material retains 14-30% of the [illegible]

[illegible] for the quantitative [illegible]

Cortisone (I) (17-hydroxy-11-dehydrocorticosterone or 3:11:20-triketopregn-4-ene-17β:21-diol) is an adrenal cortical hormone, the beneficial effect of which in the treatment of rheumatoid arthritis (1), has stimulated universal investigations on methods of synthesis.

Research on the hormones of the adrenal cortex is based on the fact that the adrenals are vital organs; in nearly all animals, complete bilateral adrenalectomy leads to death in a few days. The vital function is concerned with the adrenal cortex, and appears to operate principally by delivery of a mixture of substances into the blood, since by injection of suitable cortical extracts, adrenalectomised animals can be kept alive and the numerous insufficiency symptoms prevented or cured.

The first extracts of cortical tissue which would maintain life in the adrenalectomised animal were prepared in 1930 by Hartman and Brownell (2) and by Swingle and Pfiffner (3). Hartman designated the active agent "cortin", a name which became ambiguous when subsequent investigations revealed the presence of at least six individual compounds showing marked activity; moreover, the "amorphous fraction" remaining after removal of all crystallisable material retains 14-30% of the activity of the whole extract.

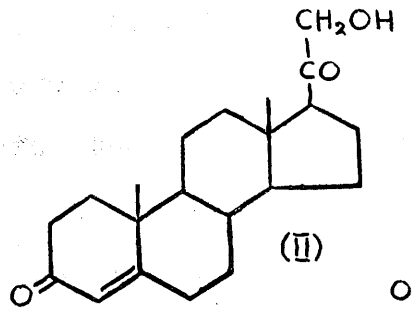
There is no assay method which is generally recognised for the quantitative evaluation of substances possessing

cortical activity; equally, no substance is accepted as a general standard of activity. It becomes increasingly difficult, consequently, to decide whether a substance can be described as an active adrenal cortical hormone, since it may give a positive response in one method of assay and a negative response in another. Many authorities, therefore, restrict themselves to the term "biological activity", always specifying the assay method, rather than "cortical activity". Besides survival tests, other assay methods which depend on the quantitative determination of the degree of a single deficiency symptom, are commonly used. The most important of the symptoms which follow adrenalectomy and which are susceptible to quantitative estimation are:-

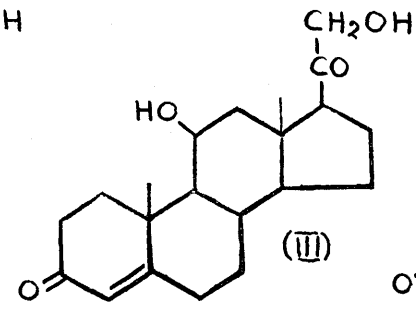
1. Disturbance of the Na^+, Cl^- and water balance (all increased excretion) and K^+ (retention).
2. Increase of the urea content of the blood.
3. Asthenia (inefficiency of muscle).
4. Disturbance of carbohydrate metabolism (decrease in liver glycogen).
5. Reduction in resistance to traumata (cold, mechanical shock, etc.)

Intensive investigation, aimed at the isolation and chemical characterisation of the cortical hormones and initiated in 1934, mainly by E.C. Kendall, J.J. Pfiffner, T. Reichstein and O. Wintersteiner and their collaborators,

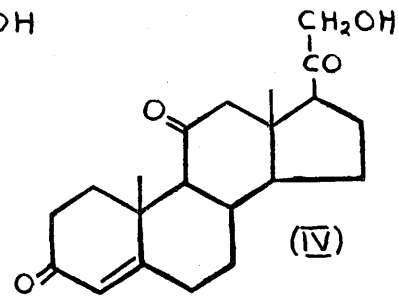
has resulted in the isolation of 28 crystalline substances, six of which (I - VI) are capable of maintaining life in adrenalectomised animals.



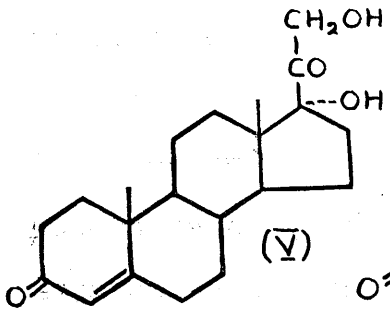
Deoxycorticosterone.



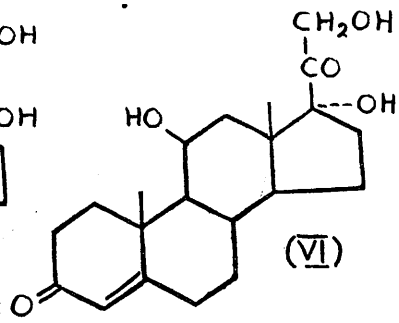
Corticosterone.



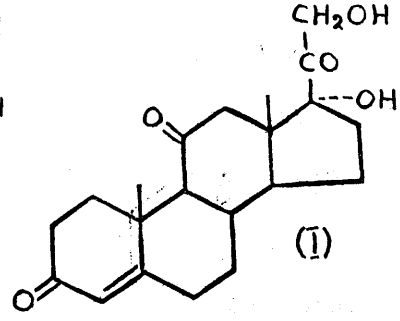
11-Dehydrocorticosterone.



17-Hydroxy-deoxycorticosterone.



17-Hydroxy-corticosterone.



17-Hydroxy-11-dehydro-corticosterone.

Physiological activity was first clearly associated with a crystalline product, when Mason, Myers and Kendall (4,5) established the effectiveness of their Compound E (I, cortisone) in the work performance test of Ingle. The same compound has also been isolated by Wintersteiner and Pfiffner (6) who designated it Compound F, by Reichstein (7) who designated it Compound Fa, and later by Kuizenga and Cartland (8).

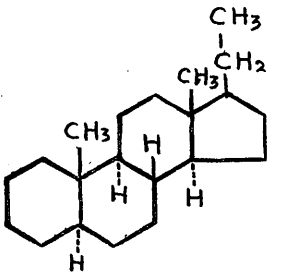
A comprehensive description of the chemical procedures requisite to the isolation of the 28 individual cortical steroids would be superfluous. In recent years, whole glands rather than the dissected cortices were used almost exclusively as starting material, which is also the case for the industrial preparation of the clinical extracts, although it is considered that the steroids originate from the cortex and not from the medulla. First extraction is made with acetone or alcohol, which precipitate protein constituents. In general, advantage is taken of (a) the observation (9, 10) that certain of the highly oxygenated hormones pass from ether or benzene to water on repeated extraction to give a fat-free aqueous concentrate (b) the use of Girard's reagent (7) for the separation of reactive ketones from non-ketonic or inert ketonic material, accomplished in either the formation or hydrolysis of the Girard derivatives and (c) the application of chromatography, extensively employed by Reichstein on the more stable acetates. Ordinary methods of hydrolysis cause decomposition of the sensitive hormones, but hydrolysis of the acetates can be accomplished satisfactorily with aqueous methanol containing potassium carbonate at 20° (11, 12).

The many degradation reactions, transformations, and partial syntheses of these cortical steroids, both active and inactive, which led to the elucidation of the structure

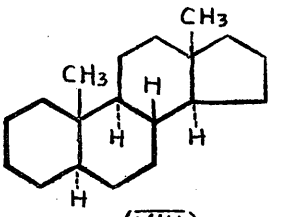
of each individual have been synopsized in excellent reviews by Reichstein and Shoppee (13) and Fieser and Fieser (14, pp. 405 - 473) in which reference to earlier reviews and the original literature are comprehensively listed.

The structural characteristics of the adrenal cortical hormones can be summarised, viz:-

(a) Carbon Ring Skeleton - The parent saturated hydrocarbons are allopregnane (VII) or androstane (VIII).



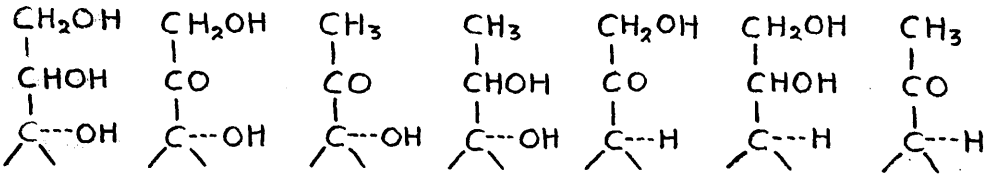
(VII)



(VIII)

(b) The Side Chain

Seven types of two-carbon sidechain at C-17 occur



(i) (ii) (iii) (iv) (v) (vi) (vii)

Cortisone possesses the β-dihydroxyacetone sidechain (ii)

(c) Nuclear Substitution.

The adrenal cortical steroids are either saturated or possess the Δ^4-3-keto unsaturated system. The saturated hormones, with one exception, possess a

3β-hydroxyl group. The other position where substitution can exist is at C-11, either as a ketone or as a hydroxyl group (in which case it is oriented in the β-configuration). Inspection of three-dimensional models makes it apparent that the 11-ketone group and 11β-hydroxyl group are subjected to considerable steric hindrance from the angular methyl groups at C-10 and C-13, a feature which is reflected in their chemical behaviour. The carbonyl group at C-11 is inert to hydroxylamine and phenylhydrazine, does not react under Wolff-Kishner conditions, does not form Girard derivatives and is resistant to catalytic hydrogenation in a neutral medium. Hydrogenation can be accomplished in acetic acid solution, or by means of lithium aluminium hydride to give the 11β-hydroxyl group exclusively (i.e. the molecule is attacked at the unhindered rear, opening the rear bond of the carbonyl group). The 11β-hydroxyl group resists acetylation and has a pronounced susceptibility to dehydration, even by dilute mineral acids.

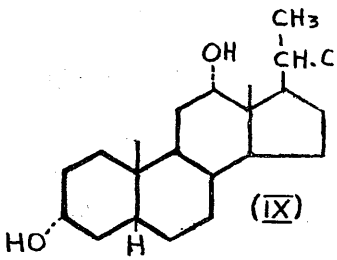
Apart from the development of synthetic methods required for structural elucidation, and in particular with the objective of accomplishing the difficult and important feat of introduction of oxygen functions at C-11 and C-17, it became a matter of considerable importance to obtain the

adrenal cortical hormones in quantities sufficient for clinical evaluation, since only minute amounts could be isolated from beef adrenal glands. An important landmark, in this connection, was the first synthesis of cortisone, starting from deoxycholic acid, achieved by Sarett (15). As a consequence of the availability of cortisone by this route for clinical testing, Hench and Kendall (1) announced the effect of cortisone on rheumatoid arthritis and rheumatic fever; encouraging results have also since been reported on the influence of cortisone on collagen diseases prominent among which are lupus erythematosus, psoriasis, pemphigus and conditions associated with allergy, such as asthma and hay fever. The profound effect of cortisone on rheumatoid arthritis appears to be highly specific (16), no other known compound apart possibly from 17-hydroxycorticosterone (VI) having comparable potency. The prospect, therefore, of preparing simpler analogues of cortisone, which would have a similar effect, is not particularly bright.

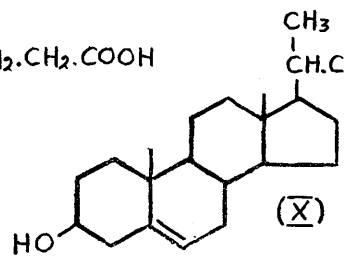
It was apparent by 1949, that in order to provide adequate supplies, the partial synthesis of cortisone from naturally occurring and readily available steroids was the most rational approach. Various starting materials have been considered.

Deoxycholic acid (IX), obtained from ox-bile or from

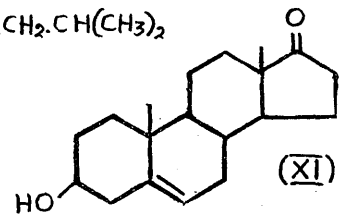
the more abundant bile acid, cholic acid, was the starting material for the first existing method of manufacture. The reactions requisite to the conversion of this acid into cortisone fall into four discrete groups (a) degradation of the bile acid sidechain (reviewed on pp. 137 - 141) (b) the transposition of the oxygen function from C-12 to C-11 (c) the elaboration of the dihydroxyacetone sidechain (reviewed on pp. 11 - 19) and (d) the introduction of the $\alpha\beta$-unsaturated ketone grouping in ring C.



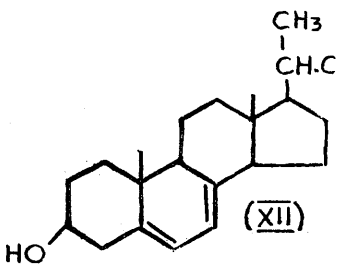
(IX)



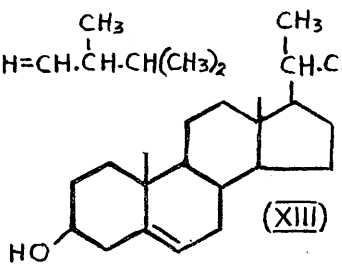
(X)



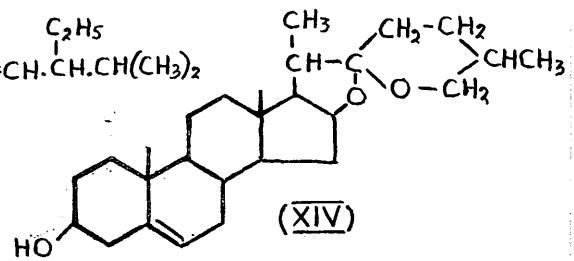
(XI)



(XII)



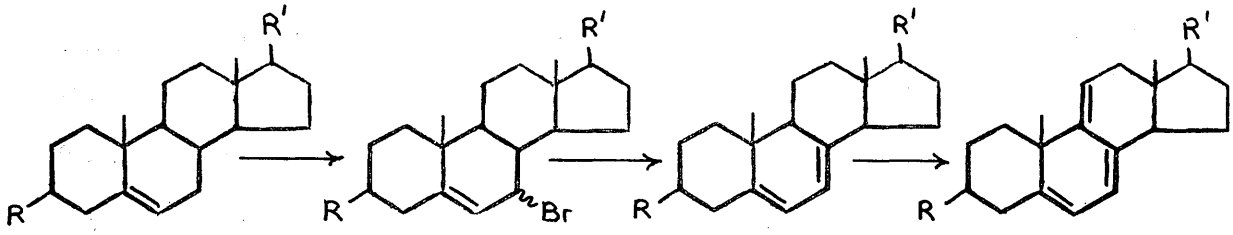
(XIII)



(XIV)

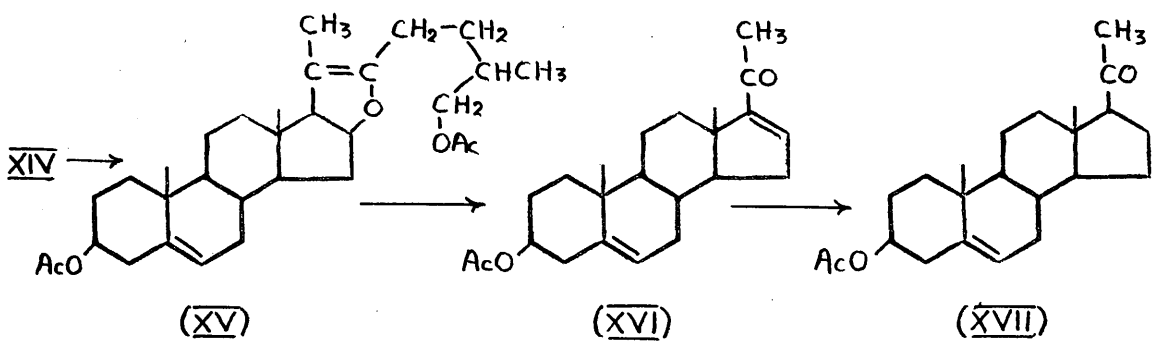
The principal disadvantage in the use of cholesterol (X) is the difficulty of sidechain degradation, the best known oxidation procedures giving the 17-keto steroid, dehydroandrosterone (XI) in only 6-8% yield. The introduction of the 11-oxygen function into this molecule, applicable also to stigmasterol (XIII) and diosgenin (XIV) can be envisaged by the general procedures of allylic

bromination at C-7, dehydrobromination, and mercuric acid oxidation to introduce the $\Delta^{9(11)}$-ethylenic bond, which would serve as the necessary point of attack.



Ergosterol (XII) appears particularly attractive since the sidechain double bond should facilitate degradation (also a feature of stigmasterol), and the $\Delta^{9(11)}$-ethylenic bond can be introduced in one step.

As regards the synthesis of intermediate pregnane derivatives, the most attractive natural materials are the steroidal sapogenins, of which diosgenin (XIV) is an example. Marker and coworkers (17, 18) have shown that treatment with acetic anhydride gives pseudodiosgenin acetate (XV) which an oxidation yields the pregna-5: 16-diene derivative (XVI) which can be converted to pregnenolone acetate (XVII) by hydrogenation.



The subject matter of this dissertation is concerned with various facets of the partial synthesis of cortisone, and has been conveniently subdivided into three sections:-

Section A. The Elaboration of the Cortisone Sidechain - describes a novel route to the characteristic β-dihydroxyacetone grouping, using as a model starting compound a 17-keto steroid, dehydroandrosterone.

Section B. 11-Oxygenated Steroids from Ergosterol - describe methods of introduction of the 11-oxygen function both as 11-keto and 11α-hydroxy groupings, using ergosterol as starting material.

Section C. Degradation of the Bile Acid Sidechain - describes experiments aimed at the synthesis of 20-ketopregnane and 17-ketoandrostane derivatives.

Of the various types of two-carbon sidechains which occur in the natural adrenal cortical steroids, the synthesis of steroids possessing the β-dihydroxyacetone sidechain has proved the most difficult. Many of the methods for the constructions of these sidechains, starting from a 17-ketone or an <u>etio</u> acid, were developed before the cortical steroid sidechains were known to be β-oriented and before additions to the 17-carbonyl group were known to give predominantly products with a 17α-sidechain. Although a synthesis involving such an addition yields chiefly a product of the unnatural series, for example a 17-<u>isoallo</u>pregnane rather than an <u>allopreg</u>nane, it was [illegible]

ELABORATION OF THE CORTISONE SIDECHAIN

[illegible] minor product of the natural series in quantities that sufficed for completion of a particular synthesis. Certain of the <u>isoallo</u>pregnanes, [illegible]

HISTORICAL.

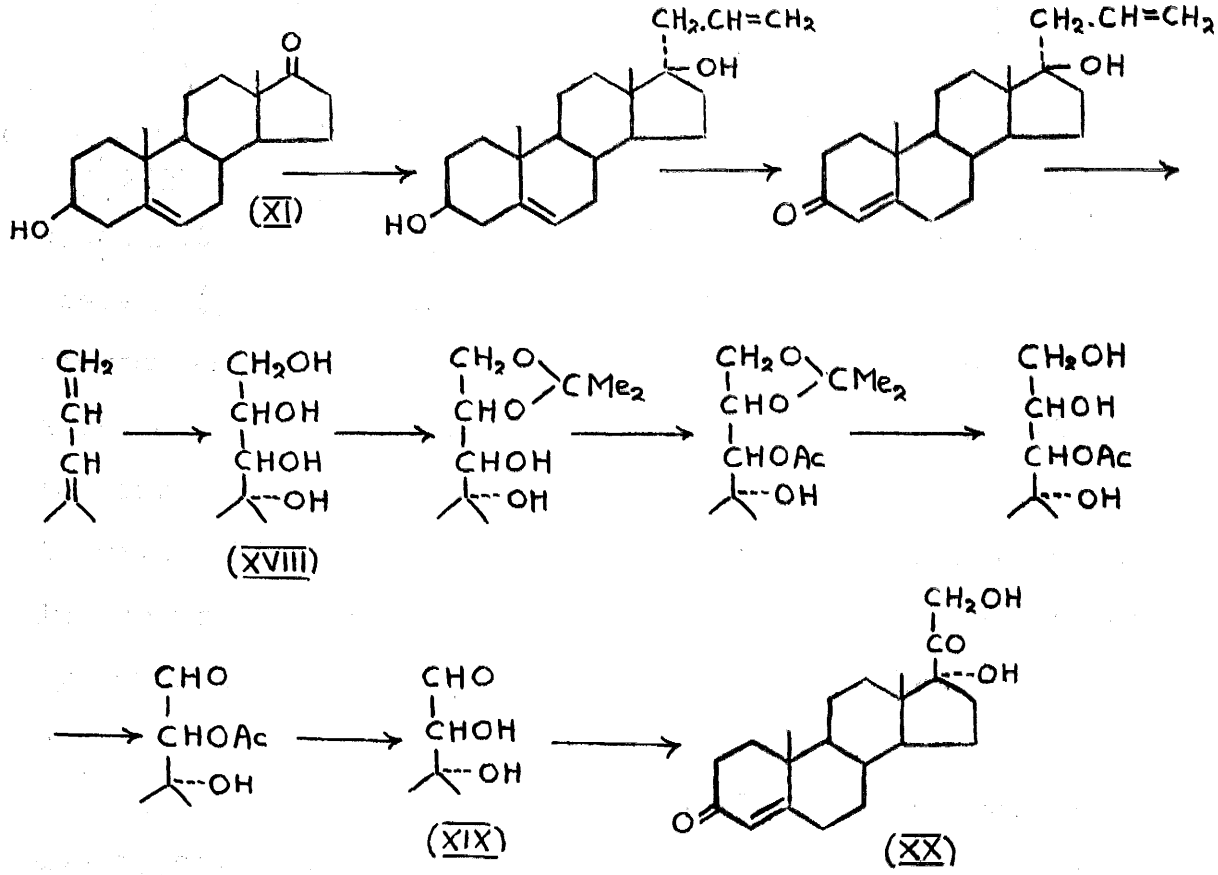
[illegible] are convertible through the corresponding 17-ethylenes into products of the natural series.

The first successful synthesis of the 17:21-dihydroxy-20-ketones with the proper configuration at C-17 was achieved by von Euw and Reichstein (19, 20) in 1940, who utilized the intermediate, w-homopregn-4-en-17α:20:21:22-tetrol-3-one (XVIII), which had previously been obtained by Butenandt and Peters (21) by addition of allylmagnesium [illegible]

Of the various types of two-carbon sidechains which occur in the natural adrenal cortical steroids, the synthesis of steroids possessing the β-dihydroxyacetone sidechain has proved the most difficult. Many of the methods for the constructions of these sidechains, starting from a 17-ketone or an etio acid, were developed before the cortical steroid sidechains were known to be β-oriented and before additions to the 17-carbonyl group were known to give predominantly products with a 17α-sidechain. Although a synthesis involving such an addition yields chiefly a product of the unnatural series, for example a 17-isoallopregnane rather than an allopregnane, it was sometimes possible to isolate the minor product of the natural series in quantities that sufficed for completion of a particular synthesis. Certain of the isoallopregnanes, moreover, are convertible through the corresponding 17-ethylenes into products of the natural series.

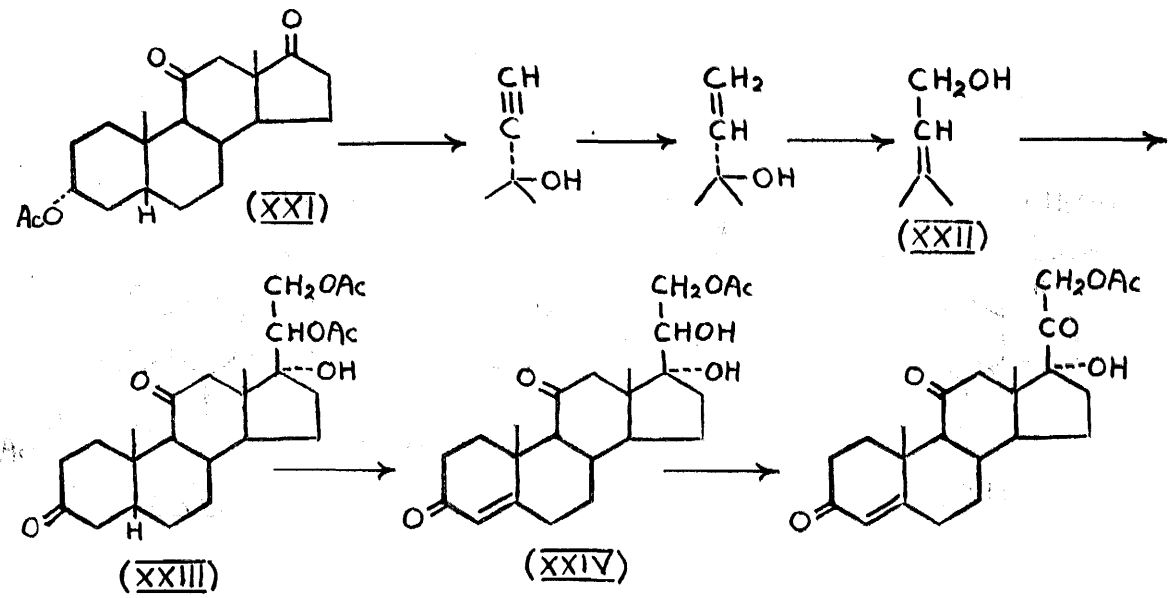
The first successful synthesis of the 17:21-dihydroxy-20-ketones with the proper configuration at C-17 was achieved by von Euw and Reichstein (19, 20) in 1940, who utilised the intermediate, w-homopregn-4-en-17α:20:21:22-tetrol-3-one (XVIII), which had previously been obtained by Butenandt and Peters (21) by addition of allylmagnesium bromide to dehydroandrosterone (XI) followed by Oppenauer oxidation at C-3, dehydration, and hydroxylation to give a mixture of tetrols from which one was isolated in 5%

overall yield. The terminal hydroxyl groups were protected as the acetonide in order to permit selective acetylation at C-20, the glycol group then re-established and cleaved by periodic acid to give the free aldehyde (XIX) which underwent Fischer rearrangement to give the substance, XX, identical with the natural product.



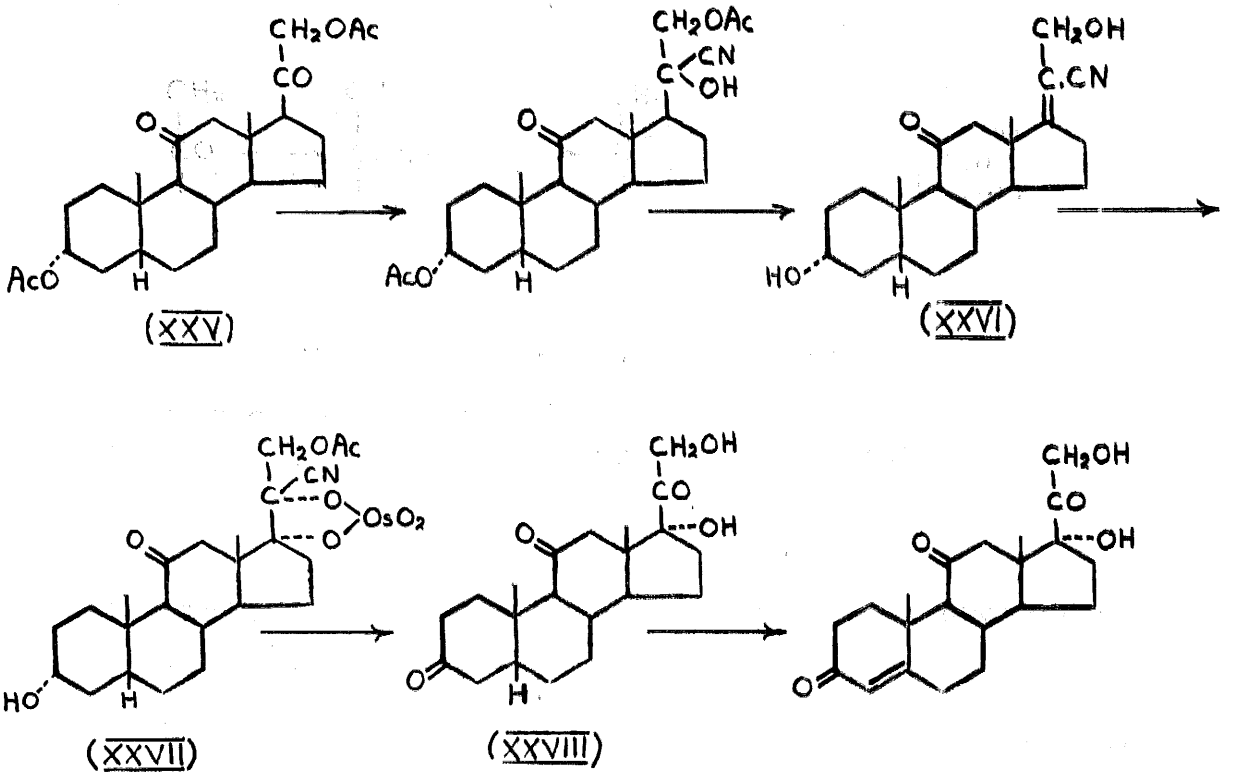
The overall yield from the tetrol is 15% by this route, later improved by the same workers (22) to 30% by direct oxidation of (XVIII) to (XIX) by periodic acid.

Sarett achieved the synthesis of cortisone by two methods. The first method (23) involved the standard



condensation of acetylene with 11:17-diketotestan-3α-yl acetate (XXI), partial hydrogenation and allylic rearrangement to XXII. Protection of the 21-hydroxyl group as the hemisuccinate permitted oxidation at C-3, followed by osmic ester hydroxylation and acetylation to give XXIII. The Δ^4-ethylenic linkage was introduced in the usual way, the diacetate hydrolysed, and converted by partial acetylation to XXIV, and the synthesis completed by cautious oxidation at C-20.

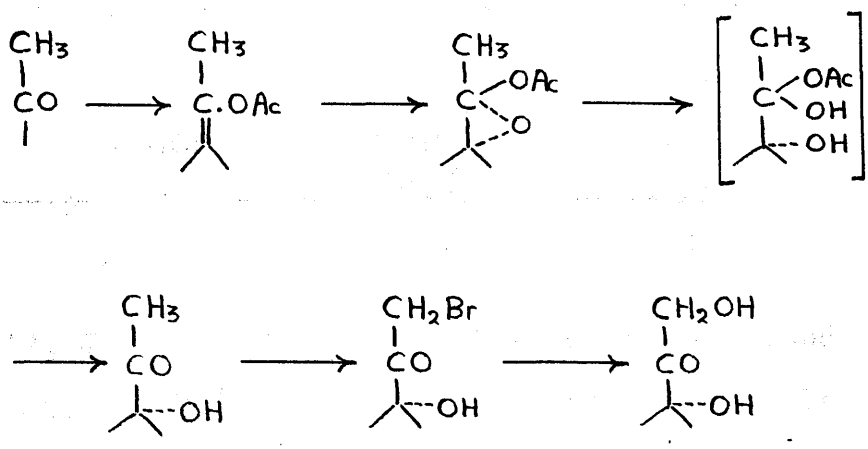
In his second synthesis (24), the starting material was 3α:21-diacetoxy-11:20-diketopregnane (XXV) obtainable with difficulty from deoxycholic acid. Addition of hydrogen cyanide, followed by dehydration and alkaline hydrolysis gave XXVI. Selective acetylation at C-21, followed by osmium tetroxide oxidation gave XXVII, which was conveniently oxidised at C-3, hydrolysed, and the



osmic ester grouping cleaved to give (XXVIII). The synthesis was completed by the Mattox-Kendall procedure (25).

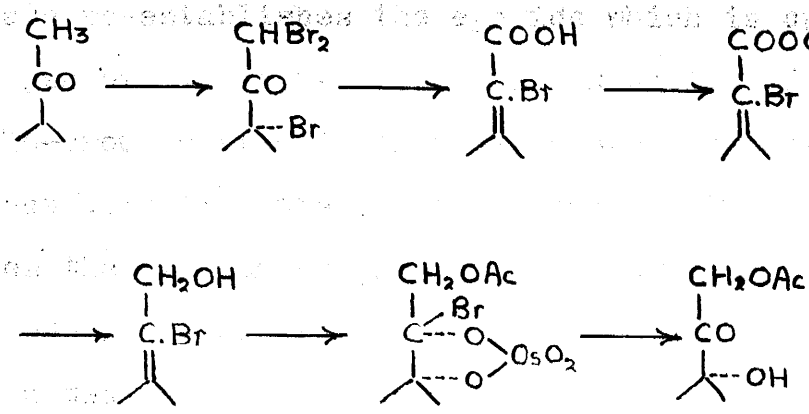
In 1949, Gallagher (26, 27) introduced an elegant synthesis, the main advantage of which is that it does not involve the use of the scarce and expensive osmium tetroxide. Treatment of a 20-ketopregnane derivative with acetic anhydride and p-toluene sulphonic acid gives the enol acetate which, on oxidation with perbenzoic acid, yields the corresponding 17α:20α-epoxide. Alkaline

saponification cleaves the oxide, forming the 17α-hydroxy group. The 21-hydroxyl group is then introduced by controlled bromination and hydrolysis.

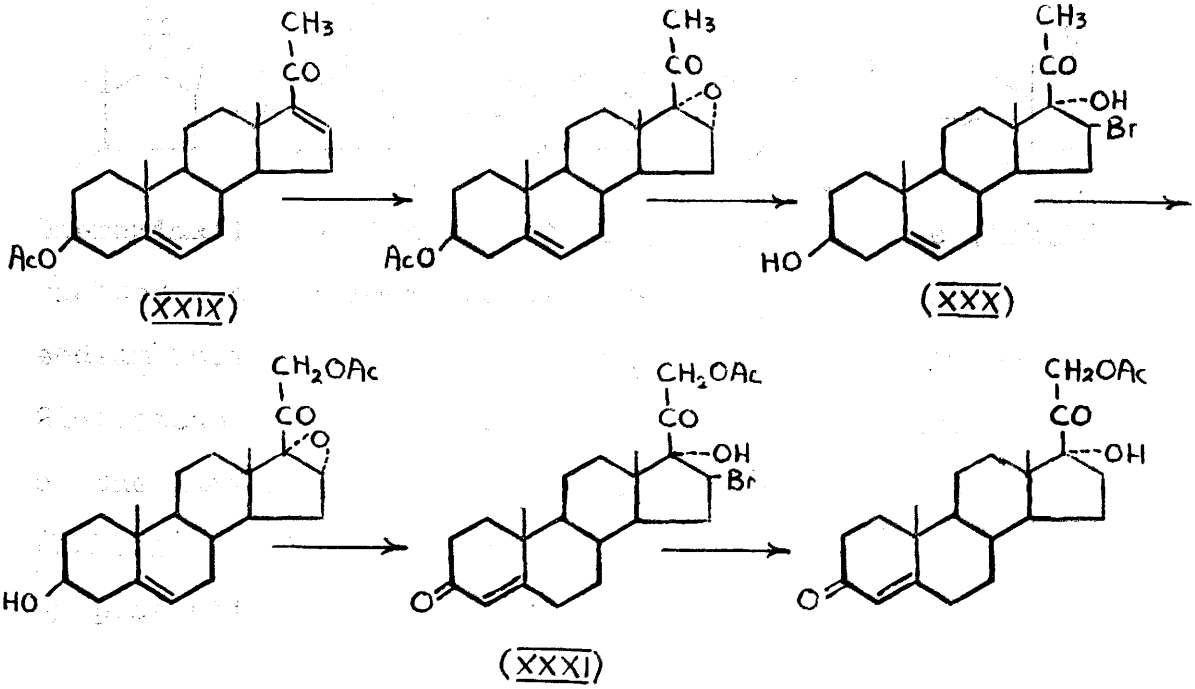


Reichstein's Substances S, L and P and 17 -hydroxyprogesterone have been prepared by this method (26, 27, 28).

The method of Wagner and Moore (29, 30, 31) involves the bromination of a 20-ketopregnane to give the 17;21;21-tribromo derivative which undergoes a modified Favorskii rearrangement with alcoholic potash to the Δ^{17}-20-bromo-21-carboxylic acid. Esterification of the latter compound with diazomethane and reduction with lithium aluminium hydride gives the unsaturated 20-bromo-21-hydroxyl compound, which is then converted by osmium tetroxide oxidation, by a method fundamentally similar to Sarett's to the pregnane derivative possessing the β-dihydroxyacetone sidechain.

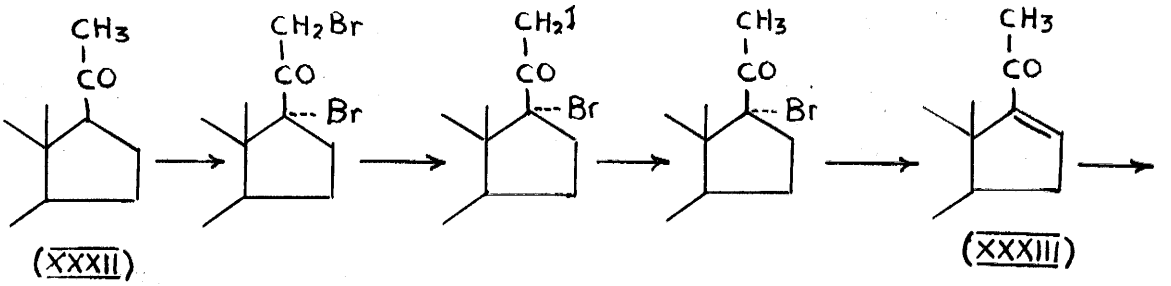


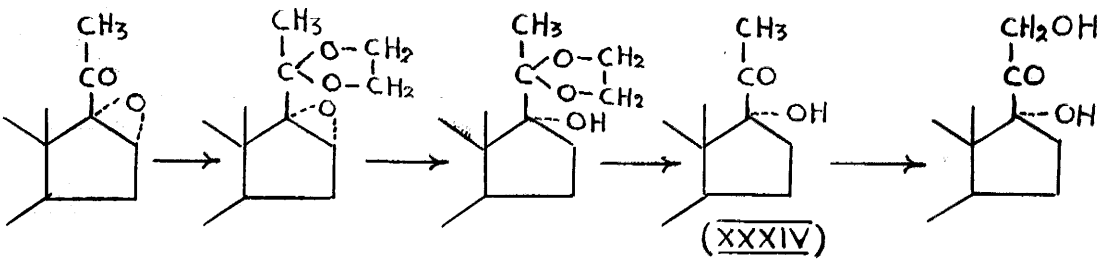
In 1949, Julian (32, 33, 34) introduced a method applicable to Δ^{16}-derivatives (easily obtainable from steroidal sapogenins), an example of which is the synthesis of Reichstein's substance S. Oxidation of XXIX by alkaline hydrogen peroxide gives the 16α: 17α-epoxide, which on bromination in presence of hydrogen bromide yields XXX.



(XXIX) (XXX) (XXXI)

Treatment of the latter with sodium iodide and potassium acetate re-establishes the epoxide which is opened with hydrogen bromide after Oppenauer oxidation to give XXXI. The 16-bromine atom is finally removed by Raney nickel without affecting the 20-keto group. This method raises the problem of providing compounds containing a 16:17-double bond from sources other than the sapogenins, and in two further communications, Julian (35, 36) has achieved this and developed improvements in the sidechain synthesis as shown -

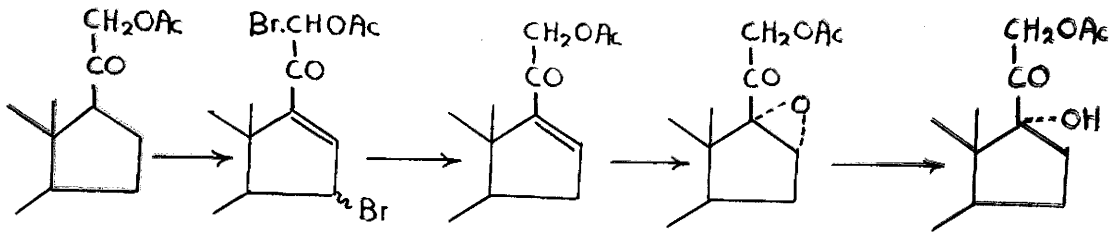




Dibromination of the 20-ketopregnane derivative (XXXII) yielded the 17:21-dibromide, treatment of which with sodium iodide and sodium hydrogen sulphite replaced the 21-bromine atom by hydrogen. Collidine dehydrobromination of the latter gave the necessary Δ^{16}-pregnane derivative (XXXIII). Formation of the 16α:17α-epoxide was followed by protection of the 20-ketone as a cyclic acetal.

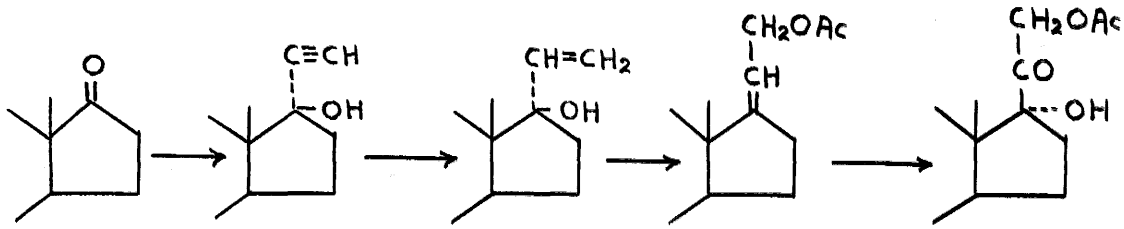
Plattner (37) had previously shown that reductive fission of a 16α:17α-epoxide by lithium aluminium hydride gave the required 17α-hydroxyl group, and using this procedure, followed by regeneration of the 20-ketone, Julian formed the 17α-hydroxy-20-ketone (XXXIV). Bromination and hydrolysis at C-21 (Gallagher's method) completes the synthesis.

A modification of Julian's method has been outlined by Kendall (16). Bromination of the 21-acetoxy ketone (XXXV) obtained from deoxycholic acid gave the dibromide (XXXVI). The bromine atoms were then replaced by treatment with sodium iodide, and the synthesis completed as shown. No experimental details are given.



(XXXV) (XXXVI)

The method of Miescher and Schmidlin (38), finally, produces the cortisone sidechain in one step from a 21-acetoxypregn-17-ene (XXXVII) derivative by oxidation in 48% yield with hydrogen peroxide catalysed by osmium tetroxide.

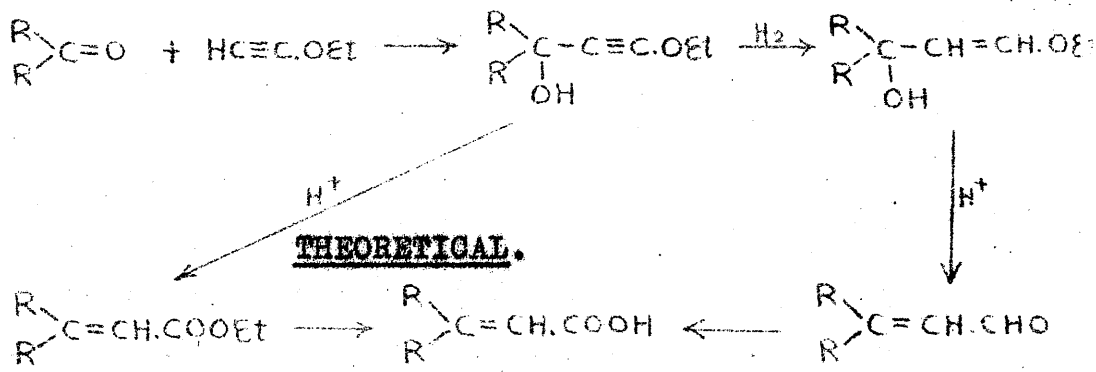


(XXXVII)

The intermediate (XXXVII) is obtainable by the normal condensation of acetylene with a 17-ketosteroid, followed by semihydrogenation and allylic rearrangement.

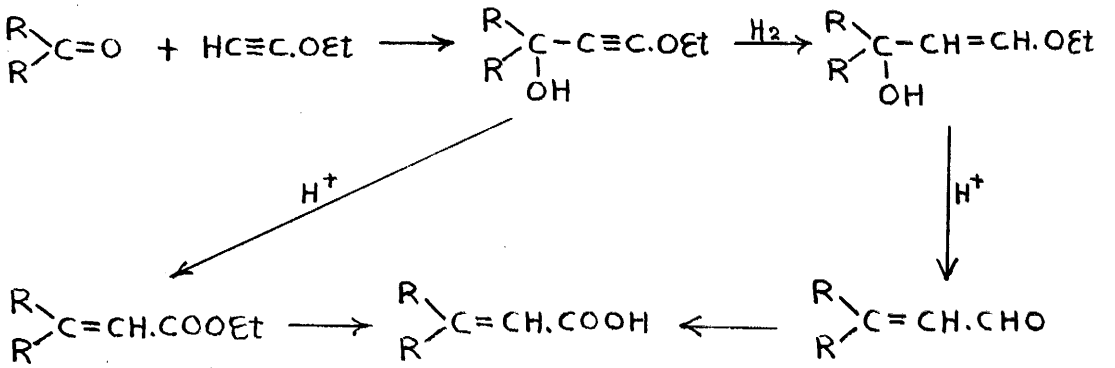
THEORETICAL.

Heilbron, Jones, Julia and Weedon (39) have shown that ethynylethylene, prepared by the method of Jacobs, Cramer and Hanson (40), condenses with ketones (acetone, methyl vinyl ketone and β-ionone) to give ethoxyacetylenic carbinols, which, by semihydrogenation of the triple bond and treatment of the resulting ethoxyvinylcarbinols with dilute mineral acids, are converted into the corresponding αβ-unsaturated aldehydes, as formulated generally below.



This method of preparing αβ-unsaturated aldehydes was first noted by Van Dorp and Arens (41, 42, 43) in investigations in the Vitamin A field. Heilbron (39) noted moreover that the ethoxyacetylenic carbinols rearrange readily in the presence of dilute mineral acid to produce αβ-unsaturated ethyl esters, which can be hydrolysed to the αβ-unsaturated acids. This constitutes an alternative route to the well known

Heilbron, Jones, Julia and Weedon (39) have shown that ethoxyacetylene, prepared by the method of Jacobs, Cramer and Hanson (40), condenses with ketones (acetone, methyl vinyl ketone and β-ionone) to give ethoxyacetylenic carbinols, which, by semihydrogenation of the triple bond and treatment of the resulting ethoxyvinylcarbinols with dilute mineral acids, are converted into the corresponding αβ-unsaturated aldehydes, as formulated generally below.

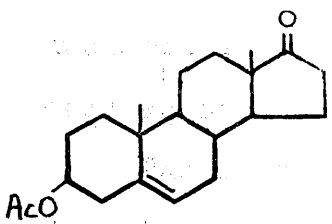


This method of preparing αβ-unsaturated aldehydes was first noted by Van Dorp and Arens (41, 42, 43) in investigations in the Vitamin A field. Heilbron (39) noted moreover that the ethoxyacetylenic carbinols rearrange readily in the presence of dilute mineral acid to produce αβ-unsaturated ethyl esters, which can be hydrolysed to the αβ-unsaturated acids. This constitutes an alternative route to the well known Reformatsky reaction. The conversion of ethoxyacetylenic carbinols into acids and aldehydes has also recently been

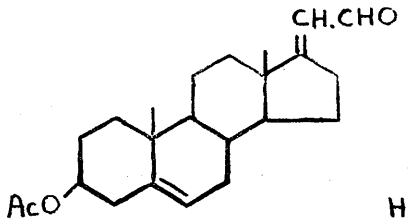
described by Russian workers (44, 45).

Using corresponding conditions, it was decided to investigate the reaction between dehydroandrosterone acetate (XI) and ethoxyethynylmagnesium bromide with the object of developing an efficient route of the $\alpha\beta$-unsaturated aldehyde, 3β-acetoxypregna-5:17-dien-21-al (XXXVIII) and the $\alpha\beta$-unsaturated acid, 3β-hydroxypregna-5:17-diene-21-carboxylic acid (XXXIX).

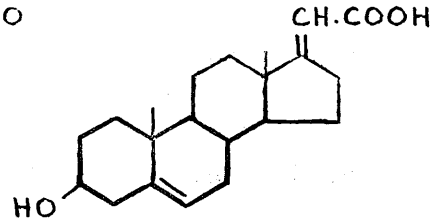
These two pregnane derivatives were considered interesting intermediates in the synthetic elaboration of the various two-carbon oxygenated sidechains of adrenal cortical hormones, since the $\Delta^{17(20)}$-ethylenic link provides a point of attack for the introduction of the 17- and 20- oxygen functions, and the 21-hydroxyl group is easily formed by reduction of either the 21-aldehyde or 21-carboxyl groups.



(XI)



(XXXVIII)



(XXXIX)

NOTE:- Throughout this work, dehydroandrosterone refers to the compound derived from, and possessing the same nuclear configuration as, cholesterol. It is also

referred to in chemical literature as dehydroisoandrosterone, dehydroepiandrosterone, trans-dehydroandrosterone, and t-dehydroandrosterone).

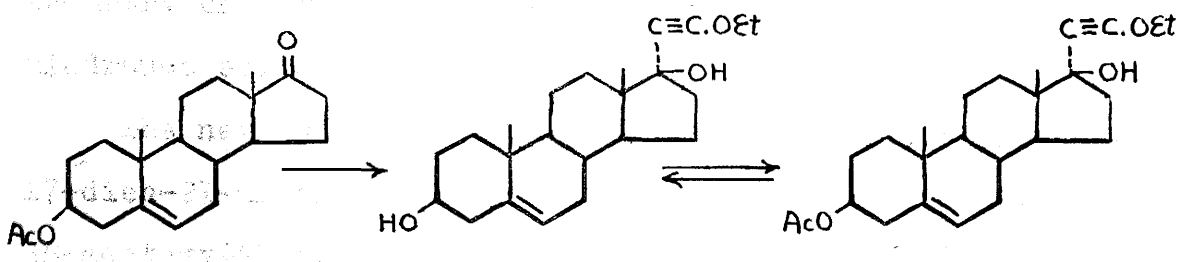
The preparation of ethoxyacetylene was carried out substantially by the method described by Jacobs et al (40)

$$CH_3CHO \longrightarrow Br_2CH.CH(OEt)_2 \longrightarrow Br.CH=CH.OEt \longrightarrow HC\equiv C.OEt$$

The dibromoacetal was prepared by bromination of paraldehyde at -10° as described by Heilbron (39). Newbold's modification (46) whereby the theoretical quantity of bromine is used, and the reaction mixture irradiated by ultra-violet light, was employed to give the required product in satisfactory yield. Treatment with zinc dust gave bromoethoxyethylene and about 20% unchanged dibromoacetal. Atmospheric distillation of bromoethoxyacetylene with finely powdered potassium hydroxide gave ethoxyacetylene, which being a rather unstable liquid, was stored in ether solution at 0° and used as soon as possible.

The ethereal solution of ethoxyacetylene was added dropwise to ethyl magnesium bromide. An immediate reaction occurred on addition of dehydroandrosterone acetate (XI) in benzene (due to its sparing solubility in ether) to produce a complex which rapidly darkened. Working up of the reaction product gave a brown resin from which a small quantity of 21-ethoxypregn-5-en-20-yn-3β:

17β-diol (XL) was crystallised. Chromatography of the crude mother liquor residues separated (XL) and its acetylated derivative, 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (XLI) in approximately equal amounts. These two derivatives were characterised by their interconversion by acetylation and hydrolysis procedures. In view of this partial hydrolysis of the 3β-acetoxy group during the reaction, it was found more expedient in later preparations to re-acetylate the crude product by pyridine-acetic anhydride prior to chromatographic purification. In experiments in which a larger excess of ethoxyethynyl magnesium bromide was employed, no radical alterations in yield were obtained.

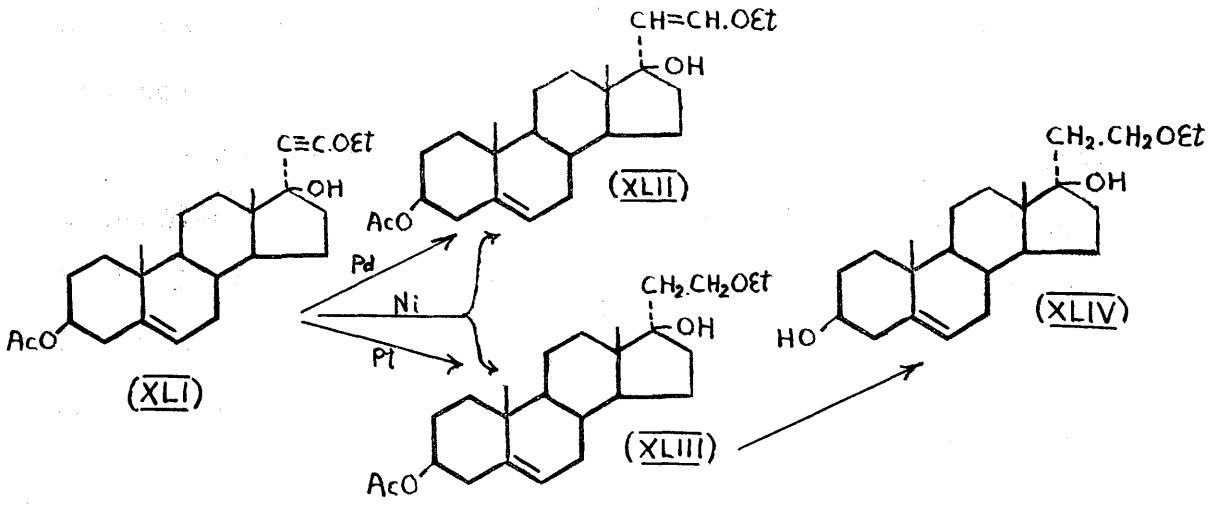


(XL) (XLI)

The configurations assigned to the ethoxyacetylenic and hydroxyl groups are based on analogous reactions involving steric hindrance effects associated with position 17. This aspect has been reviewed by Fieser and Fieser (14 pp. 410-412) and Gallagher and Kritchevsky (47). They have concluded that the attack of the entering group proceeds more readily from the α- or rear face. A survey of the known reactions demonstrates

without exception the validity of this "rule of the rear" which has been inclusively stated:- that when there is a plane of symmetry at C-17, the entering group always attaches to C-17 in the α-configuration. Thus catalytic reduction, lithium aluminium hydride, Grignard reagents, potassium acetylide, osmium tetroxide and perbenzoic acid all result in the attachment of the entering group preponderantly in the α-configuration at C-17. Some rationalisation of this observed phenomenon can be afforded from inspection of Stuart models. The front side of C-17 is the same distance from the angular carbon as the rear side is from C-12, but the vibrating methyl group can dominate more space than the restricted 12-methylene group and so exert a short-range or bond-hindrance effect.

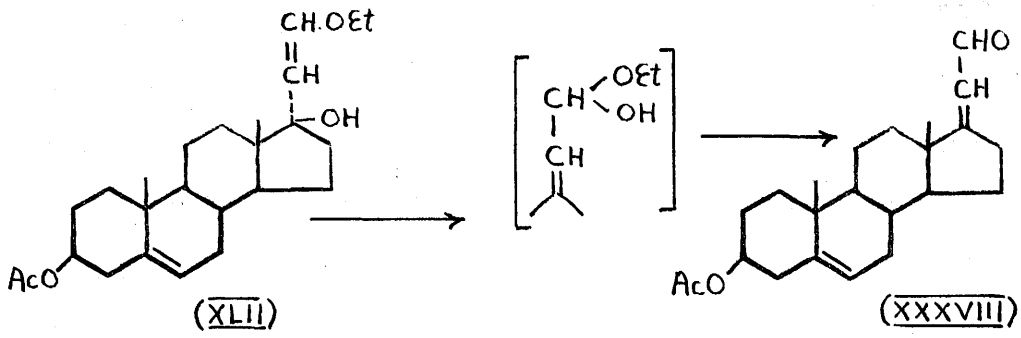
The next stage in the synthesis of 3β-acetoxypregna-5:17-dien-21-al (XXXVIII) consisted of the hydrogenation of 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (XLI) to 3β-acetoxy-21-ethoxypregna-5:20-dien-17β-ol (XLII).



Using ethyl acetate as solvent, and palladised calcium carbonate as catalyst, the required intermediate (XLII) was obtained satisfactorily, when the reaction was stopped on the uptake of one mol. of hydrogen. When Raney nickel was employed as catalyst under similar conditions, however, two products were isolated. One was identified as the expected 3β-acetoxy-21-ethoxypregna-5:20-dien-17β-ol (XLII) and the other compound, $C_{25}H_{40}O_4$ is considered to be 3β-acetoxy-21-ethoxypregn-5-en-17β-ol (XLIII), since, while giving a yellow colour with tetranitromethane in chloroform (indicating unsaturation) no reaction was apparent with Brady's reagent, indicating saturation of the sidechain (compare the reaction with XLII). It was characterised by hydrolysis to the corresponding alcohol, 21-ethoxypregn-5-en-3β:17β-diol (XLIV) which could be sublimed unchanged under reduced pressure. Subsequent experiments showed that XLIII could also be obtained in good yield by using Adam's platinum catalyst in ethyl acetate solution, reduction ceasing after two mols. of hydrogen had been absorbed.

The conversion of 3β-acetoxy-21-ethoxypregna-5:20-dien-17β-ol (XLII) to 3β-acetoxypregna-5:17-dien-21-al (XXXVIII) was simply effected by treatment with hot dilute sulphuric acid, re-acetylation and chromatographic purification. The product exhibited characteristic

light absorption at 2430 Å (ε=17,600) and was characterised by formation of its 2:4-dinitrophenylhydrazone.

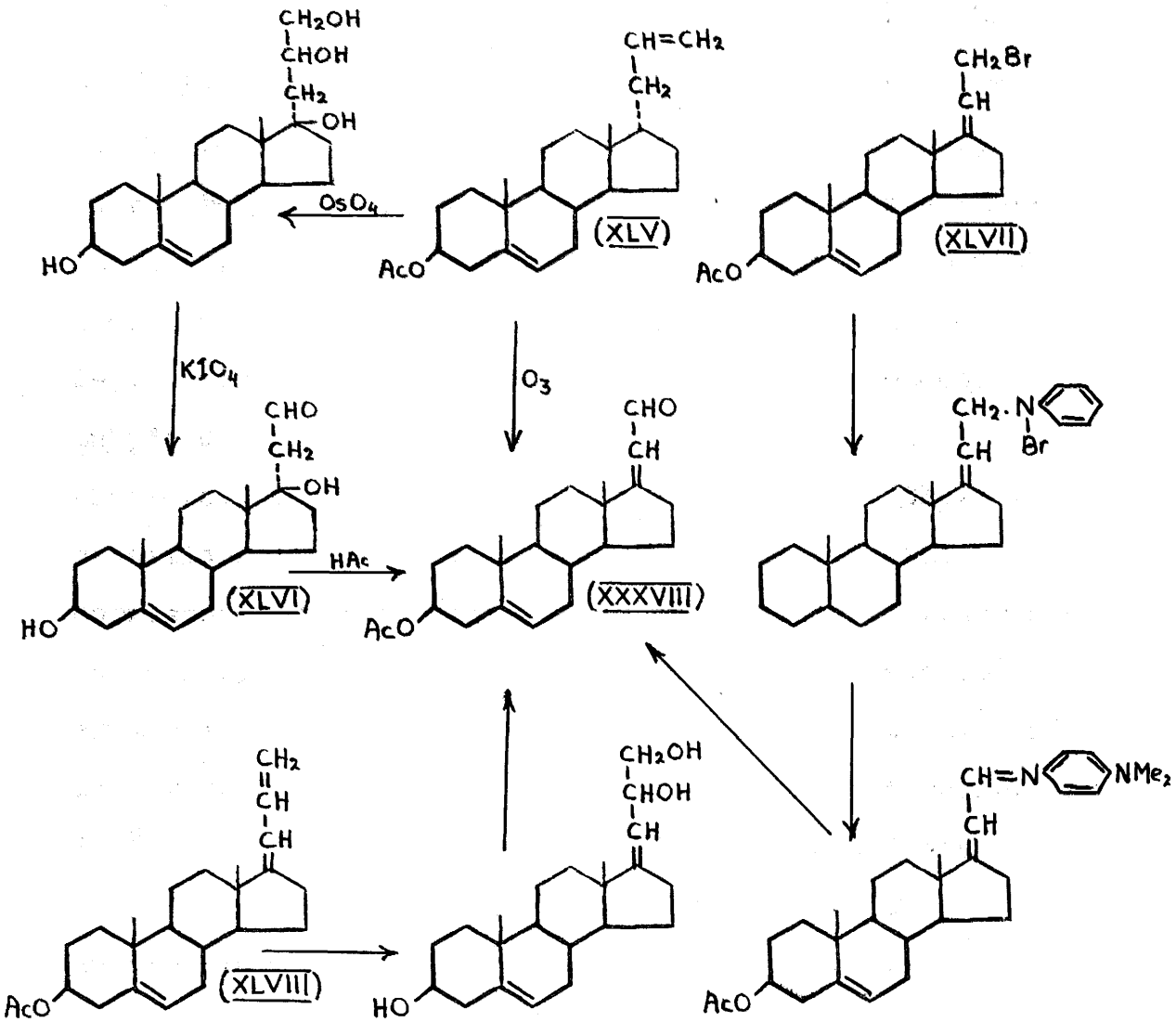


(XLII) (XXXVIII)

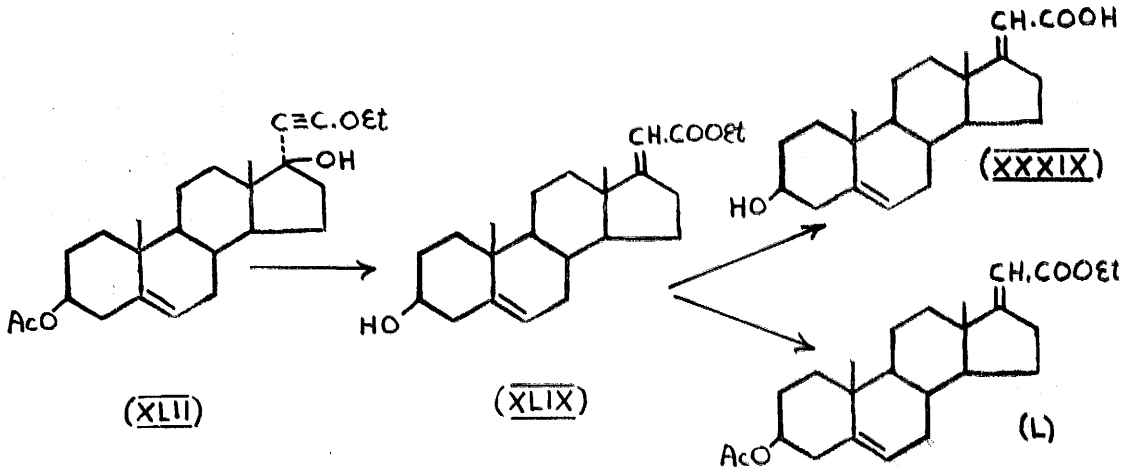
This derivative was also obtained in excellent yield by treatment of a methanolic solution of XLII with Brady's reagent i.e., the acidic reaction conditions being sufficient to effect the mild allylic rearrangement. The unsaturated aldehyde also gave a semicarbazone m.p. 245° in good agreement with that obtained by Miescher, Wettstein and Scholz (48). It was also revealed subsequently that the rearrangement of (XLII) to (XXXVIII) could be effected by simple sublimation under reduced pressure, the product being identical to that obtained by mineral acid rearrangement.

The $\alpha\beta$-unsaturated aldehyde has previously been prepared as outlined below, but these routes are inconvenient in that the starting compounds are accessible only with difficult and the overall yields are low. Miescher, Wettstein and Scholz (48) formed (XXXVIII) by dehydration of 3β-acetoxypregn-5-en-17β-ol-21-al (XLVI) prepared by osmium tetroxide oxidation of 17-allylandrost-5-en-3β:17β-diol (XLV) followed by periodic fission. The

same authors also described the conversion of (XLV) to (XXXVIII) by ozonisation after nuclear protection. Reich (49) applied the Krohnke procedure (50, 51) to the allyl bromide (XLVII) by successive treatment with pyridine, nitrosodimethylaniline and hydrochloric acid to give the required $\alpha\beta$-unsaturated aldehyde. Fuchs and Reichstein (52) obtained (XXXVIII) by periodic fission of the mixture of the glycols obtained from (XLVIII) as shown.



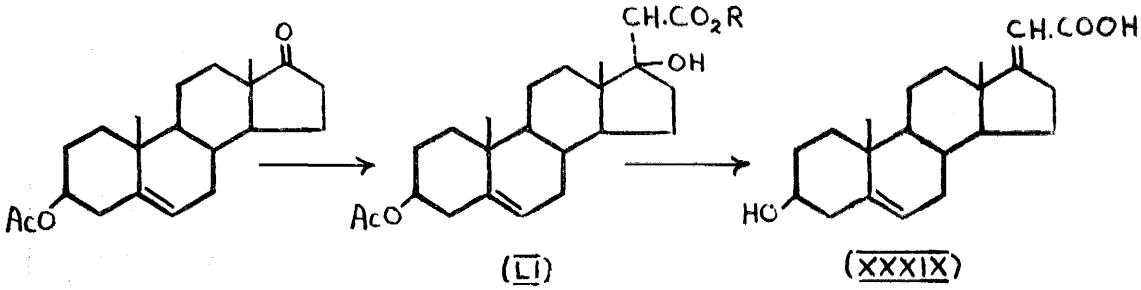
The conversion of 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (XLII) to 3β-hydroxypregna-5:17-diene-21-carboxylic acid (XXXIX) was next investigated. By



(XLII) (XLIX) (XXXIX) (L)

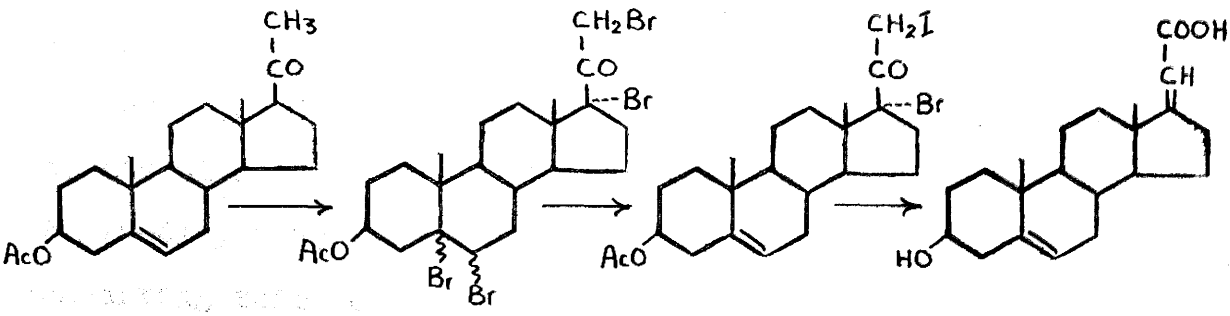
refluxing a methanolic solution of (XLII) with dilute sulphuric acid, and working up in the usual manner, ethyl 3β-hydroxypregna-5:17-diene-21-carboxylate (XLIX) was obtained i.e., sidechain rearrangement occurs with simultaneous hydrolysis of the acetate group at position C-3. It was readily acetylated by pyridine-acetic anhydride to give (L). Both (XLIX) and (L) show characteristic light absorption at 2200Å, and their specific rotations are in good agreement with the corresponding methyl esters, as prepared by Plattner and Schreck (53). It is possible that the acetate (L) exists in dimorphic forms, as two isomers m.p. 111° and 155° were isolated which, on admixture, melted sharply at 111°. The hydrolysis of (XLIX) to (XXXIX) was effected by normal alkaline hydrolysis. The intermediate potassium salt was rather surprisingly soluble in methanol, and insoluble in water.

3β-Hydroxypregna-5:17-diene carboxylic acid (XXXIX) was first obtained by Reichstein, Muller, Meystre and Sutter (54) who subjected dehydroandrosterone acetate to the Reformatsky reaction with zinc and ethyl bromoacetate to give the intermediate (LI) which on hydrolysis and dehydration gave the required α,β-unsaturated acid (XXXIX) This work was repeated in part by Plattner and Schreck (53) who, by using a different dehydration procedure,



(LI) (XXXIX)

obtained the acid (XXXIX) with differing physical constants. Marker and his associates (55, 56) obtained the acid from pregnenolone acetate, by bromination, treatment with potassium iodide and Favorskii rearrangement of the 17:21-dihalogenated-20-ketone. Julian and Karpel (35) have re-investigated this reaction, and shown that it takes the course shown, but do not give experimental details

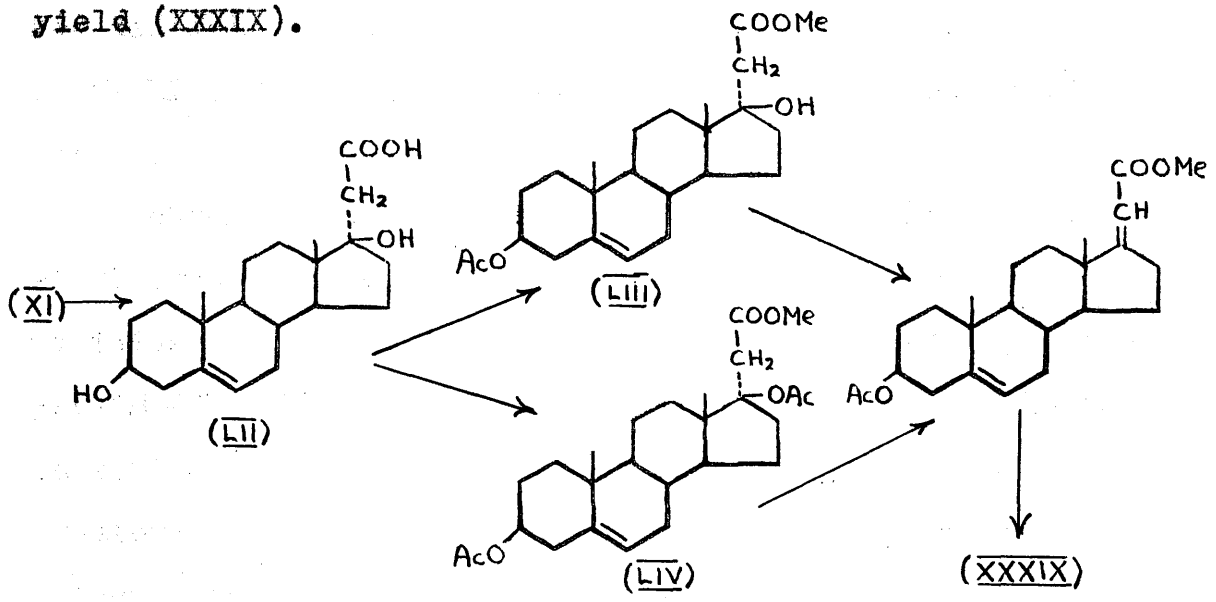


or physical constants for the $\alpha\beta$-unsaturated acid. As prepared by this ethoxyacetylene route, the $\alpha\beta$-unsaturated acid had physical constants differing from those previously recorded in the literature, which in themselves differed markedly (see table). For this reason, it was deemed necessary to prepare an "authentic" specimen of the same acid by a previously described route for direct comparison purposes. Subsequent communications by Heusser, Eichenberger and Plattner (64) and Magrath et al (65) (see Appendix) reported constants in good agreement with those reported in this dissertation.

Authors	Ref.	m.p.	$[\alpha]_D$	Light Absorption.
Reichstein et al	54	217-8°	-	-
Plattner & Schreck	53	249°	-82°	2300 Å log 4.2
Marker et al	55	249°	-	-
Marker & Crooks	56	252-3°	-	-
Magrath et al	65	245°	-	-
Heusser et al	64	243-4°	-82°	2200 Å log 4.10
Experimental		243°	-81°	2180 Å log 4.20

Reichstein's prpcedure (54) consisted of hydrolysing the product obtained by the interaction of dehydroandrosterone acetate with zinc and ethyl bromoacetate to give 3β:17β-dihydroxypregn-5-ene-21-carboxylic acid (LII), converting this to the methyl 3β-acetoxy ester, and

effecting the dehydration with either thionyl chloride or anhydrous copper sulphate, followed by hydrolysis to yield (XXXIX).



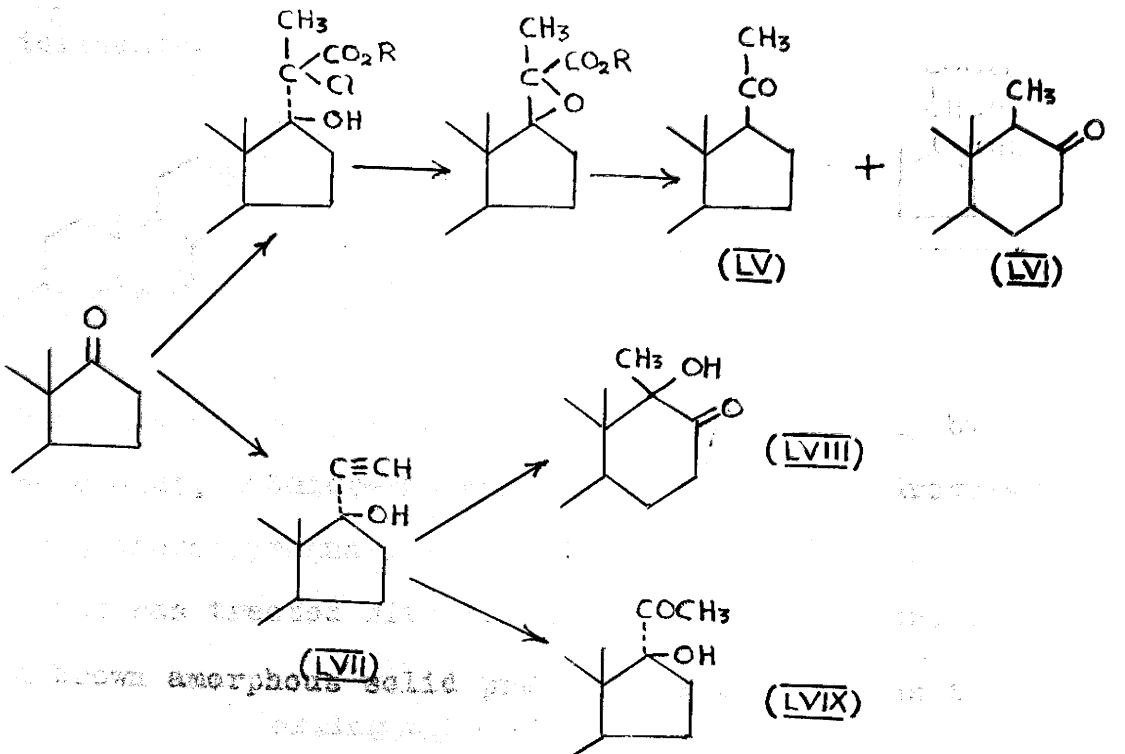
The work was repeated in part by Plattner & Schreck (53) who claimed that the introduction of the 17:20 ethylenic bond proceeded smoothly by vacuum distillation of the methyl 3:17-diacetoxy ester (LIV). They explain the divergence in melting point from Reichstein's product as due to impurity (this is borne out by an indifferent microanalysis of Reichstein's product, and also by the known difficulty of uniformly eliminating the tertiary hydroxyl group of 17β-hydroxyl steroids (cf. Lardon and Reichstein (57); Reich, Sutter and Reichstein (58); and Hardegger and Scholz (59)). An alternative explanation postulated was that the acids were geometrical ("cis-trans") isomers about the $\Delta^{17(20)}$-ethylenic bond. Marker et al (55), whose product agreed in melting-point with Plattner's, suggested that in Reichstein's case, as no degradative

proof of structure is afforded, the product might conceivably be a ring unsaturated (Δ^{16}) isomer. It was decided, therefore, to prepare the authentic specimen of (XXXIX) by Plattner's modification of Reichstein's method.

Dehydroandrosterone acetate reacted with zinc and ethyl bromoacetate under the catalytic influence of pyridine. Alkaline hydrolysis of the product yielded 3β:17β-dihydroxypregn-5-ene-21-carboxylic acid. It was esterified by diazomethane, the reaction proceeding with greater facility when anhydrous methanol was used as solvent, rather than the more customary solvent, ether, in which the reactant was sparingly soluble. Diacetylation by refluxing with acetic anhydride, according to Plattner, yielded a product which did not crystallise readily. Vacuum distillation of this product, to remove the elements of acetic acid and introduce the 17:20-ethylenic bond gave a pale yellow amorphous solid, with light absorption at 2190Å (ε = 6,400). In comparison with the light absorption for ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate, (2200Å, ε = 17,000), this suggests a product in only 30% yield. This behaviour is in agreement with the findings of Magrath et al (65) who also found this dehydration procedure incapable of consistent repetition, but in one instance obtained a 50% yield. The amorphous distillation product was hydrolysed by methanolic potassium hydroxide to give (XXXIX) (m.p. 243°) undepressed with

the specimen prepared by the ethoxyacetylene route, and exhibiting the characteristic light absorption at 2180Å.

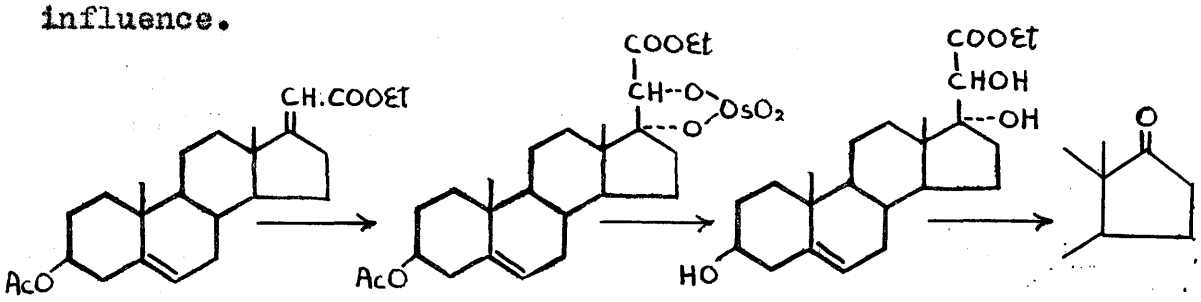
It was felt desirable, to prove by degradation, that condensation of dehydroandrosterone acetate with ethoxyacetylene and the subsequent rearrangement to products possessing $\Delta^{17(20)}$-unsaturation, has proceeded without D-ring enlargement. This phenomenon of D-homosteroid formation (for leading references, vide 14, pp. 377-380) has previously been encountered in different synthetic methods for the introduction of the two-carbon sidechain. The Darzens condensation of dehydroandrosterone acetate with ethyl $\alpha\alpha$-dichloropropionate, followed by alkaline treatment and decarboxylation gave a mixture of the expected product (LV) and the isomeric D-homo derivative (LXI).



Similarly, attempts to hydrate the 17-ethinylcarbinol (LVII) have given both the D-homohydroxyketone (LVIII) and the normal product (LVIX).

The degradation of the compound designated as ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate (L) to dehydroandrosterone acetate would prove unequivocally both the existence of the $\Delta^{17(20)}$-ethylenic linkage and the normal steroid nuclear structure. To achieve this degradation, three oxidative procedures were envisaged:- (a) osmic ester hydroxylation followed by periodate fission (b) chromic acid oxidation (c) ozonisation.

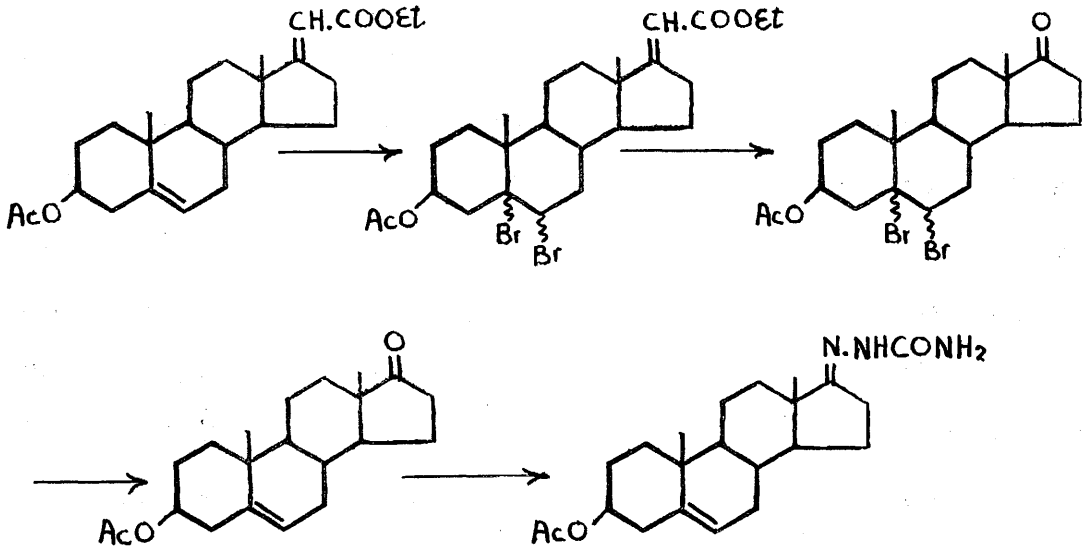
In the cis-hydroxylation of ethylenic groups by osmium tetroxide (60) protection is afforded to a Δ^{5}-ethylenic bond by a 3-acetoxy group. The effect of the 21-carboxylic group on the $\Delta^{17(20)}$ double bond is unknown, but the corresponding alcohol has no protective influence.



The method attempted was that used successfully by Butenandt, Schmidt-Thomé and Paul (61) for hydroxylation of 3-acetoxypregna-5:17-diene. The αβ-unsaturated ester was treated with 1.1 mols. of osmium tetroxide. A brown amorphous solid precipitated, which was treated

with sodium sulphite to decompose the osmic ester. The product, however, was shown to be ethyl 3β-hydroxypregna-5: 17-diene-21-carboxylate, i.e., starting material with hydrolysis of the 3-acetoxy group.

The second method consisted of protection of the Δ^5-ethylenic bond by addition of one mol. of bromine, chromic acid cleavage of the $\Delta^{17,(20)}$ double bond, debromination by zinc, and isolation of the ketone by its semicarbazone.

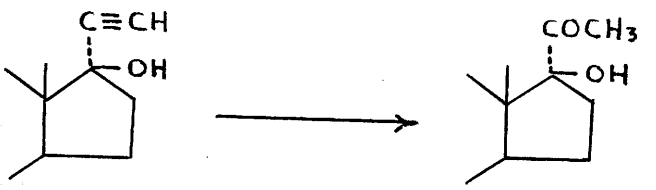


Bromination of (L) in acetic acid proceeded as expected, the Δ^5-ethylenic bond being preferentially attacked, as shown by the brominated product have light absorption consistent with an α,β-unsaturated ester. The brominated product was treated with 5 molar equivalents of chromic anhydride with ice-cooling, then debrominated successfully by zinc dust (as shown by negative Beilstein test). Working up of the reaction product by the usual

procedure yielded, however, only unchanged starting material, the oxidising conditions apparently being too mild to cleave the stable $\Delta^{17(20)}$ ethylenic bond. The stability of the $\Delta^{17(20)}$-20-bromo-21-carboxyl system to oxidation has been noted by Wagner & Moore (29, 30, 31) such a grouping being resistant to chromic acid and potassium permanganate.

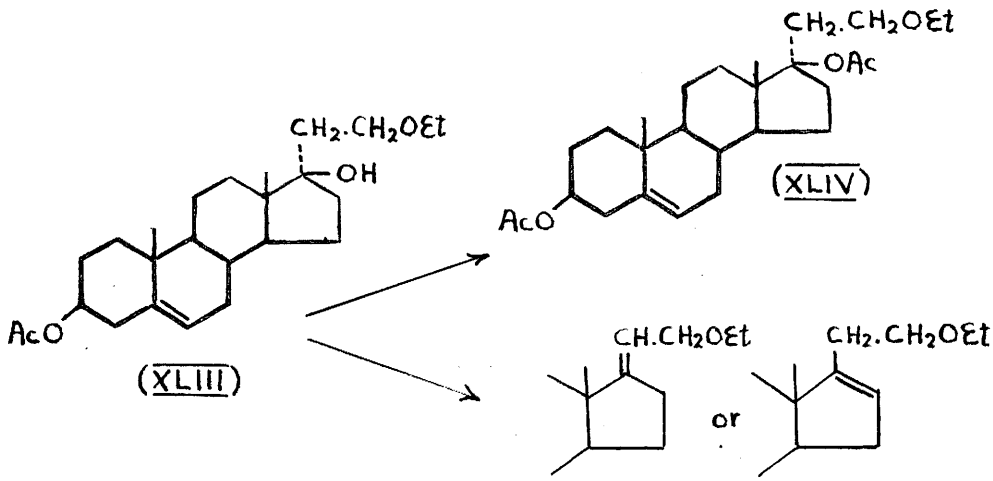
In the final successful attempt, the bromination was conducted in chloroform, the ozonolysis performed at -5^{o} and the debromination by zinc and acetic acid on the steam bath. The reaction product was dissolved in methanol, from which dehydroandrosterone acetate semicarbazone was isolated in the usual manner, possessing the same physical properties an an authentic specimen. Marker, Crooks Shabica and Jones (55) degraded the corresponding acid similarly. This degradation proves, as required, that the synthesis have proceeded without ring enlargement.

The action of mercury acetamide upon 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (XL) was investigated. Goldberg and Aeschbacher (62) showed that the corresponding compound lacking the 21-ethoxy substituent underwent normal hydration on treatment with this reagent to give the methyl ketone:-



In this case, however, a mixture of dehydroandrosterone and its acetate was obtained: i.e., a rather anomalous elimination of the ethoxyacetylene fragment had occurred.

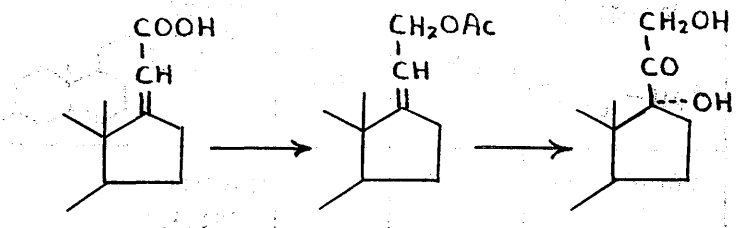
Attempts to dehydrate 3β-acetoxy-21-ethoxypregn-5-en-17β-ol (XLIII) by high vacuum sublimation or treatment with phosphorus oxychloride in pyridine were unsuccessful. Under forcing acetylation conditions, followed by pyrolysis, a low yield of 3β:17β-diacetoxy-21-ethoxypregn-5-ene (XLIV) was isolated.

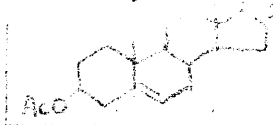
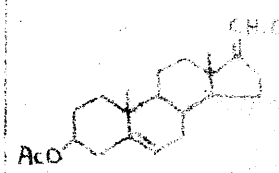
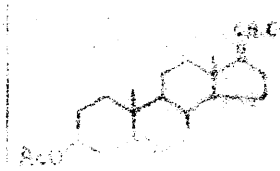


After 3β-acetoxypregna-5:17-dien-21-al and 3β-hydroxypregna-5:17-diene-21-carboxylic acid had been prepared by the routes herein described, and the experiments aimed at the rigorous proof of their constitution were in progress, communications appeared by Heusser, Eichenberger and Plattner (63, 64) and Magrath, Morris, Petrow and Royer (65) describing the preparation of these compounds and some of the intermediates. A discussion of, and comparison with, the methods and physical constants reported by these

workers is included in the Appendix of this section. The condensation of dehydroandrosterone acetate with ethoxyacetylene has also been described in a patent communication by Arens and van Dorp (66).

The reduction of the $\alpha\beta$-unsaturated acid system by lithium aluminium hydride (31, 64) followed by acetylation and oxidation by Miescher and Schmidlin porcedure formally completes the synthesis of the β-dihydroxyacetone sidechain.

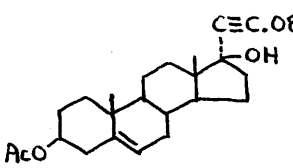
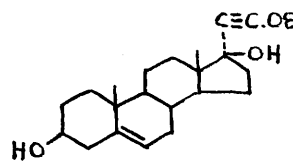
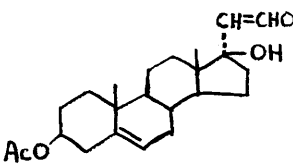
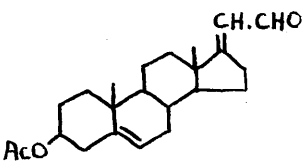
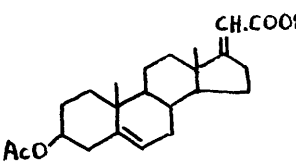
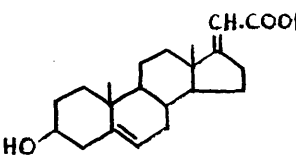
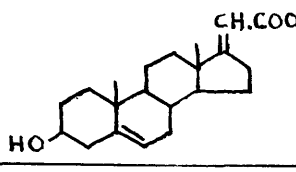


	XLII	$C_{23}H_{34}O_4$	$[\alpha]_D$ -58°	$[\alpha]_D$
	XXXVII	$C_{23}H_{32}O_3$	m.p. 183-185° $[\alpha]_D$ -57° 243mμ (17,600)	m.p. $[\alpha]_D$ 244mμ
	L	$C_{25}H_{36}O_4$	m.p. 111-112° (also m.p. 155°) $[\alpha]_D$ -66° 220mμ	m.p. 115 $[\alpha]_D$ 227mμ $[\alpha]_D$ -70°
	XLIX	$C_{23}H_{34}O_3$	m.p. 181° $[\alpha]_D$ -73°	m.p. 178 $[\alpha]_D$

APPENDIX.

(Comparison of results described in this report with those obtained by Heusser, Eichenberger & Plattner (63, 64) and Magrath, Morris, Petrow and Royer (65).

The physical constant of the compounds prepared in common are tabulated below:-

Structure	No.	Formula	Experimental	(63, 64)	(65)
	XLI	$C_{25}H_{36}O_4$	m.p. 140-141° $[\alpha]_D$ -104°	m.p. 139-140° $[\alpha]_D$ -122°	m.p. 140-141°
	XL	$C_{23}H_{34}O_3$	m.p. 146-149° s. 55-65° $[\alpha]_D$ -114°	m.p. 140-141° s. 74-84° $[\alpha]_D$ -124°	
	XLII	$C_{25}H_{38}O_4$	m.p. 145-147° $[\alpha]_D$ -58°	m.p. 138·5-139·5 $[\alpha]_D$ -56°	
	XXXVIII	$C_{23}H_{32}O_3$	m.p. 183-185° $[\alpha]_D$ -57° 243mμ (17,600)	m.p. 184-185° $[\alpha]_D$ -60° 244mμ (27,000)	
	L	$C_{25}H_{36}O_4$	m.p. 111-112° (also m.p. 155°) $[\alpha]_D$ -66° 220mμ log 4·23	m.p. 115-116° $[\alpha]_D$ -69° 222mμ log 4·40	m.p. 117° $[\alpha]_D$ -70°
	XLIX	$C_{23}H_{34}O_3$	m.p. 181° $[\alpha]_D$ -73° 220mμ log 4·21	m.p. 178-180° $[\alpha]_D$ -78·5° 222mμ log 4·22	
	XXXIX	$C_{21}H_{30}O_3$	m.p. 243° $[\alpha]_D$ -81° 218mμ log 4·20	m.p. 243-244° $[\alpha]_D$ -82° 222mμ log 4·10	m.p. 245°

The most noteworthy experimental differences from (63, 64) are summarised:-

(a) Treatment of dehydroandrosterone acetate (XI) with ethoxyethynyl magnesium bromide gave 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (XL) and the corresponding 3β-hydroxy compound (XL) in comparable yields. Whereas the solvent for the Grignard reaction in this case was benzene, the Swiss workers used ether. In our hands (XI) was insoluble in ether in the concentrations specified.

(b) Semihydrogenation of (XL) to the vinyl compound (XLII) was performed in pyridine with a palladium-calcium carbonate catalyst by the Swiss workers, who claimed that hydrogenation ceased when 1 mole of hydrogen had been absorbed. In this work, the semihydrogenation was performed in ethyl acetate with a palladium-calcium carbonate catalyst and did not cease after absorbing 1 mole of hydrogen. When Raney nickel was used as catalyst, however, a mixture of (XLII) and the sidechain saturated compound (XLIII) was obtained. Using platinum catalyst, moreover, (XLIII) was prepared in good yield, the reaction ceasing when two moles of hydrogen had been absorbed.

(c) In this work, the rearrangement of (XLII) into (XXXVIII) was effected by treatment with hot dilute sulphuric acid followed by re-acetylation.

The Swiss workers performed the rearrangement with hydrochloric acid-dioxan. At room temperature with 0.2N acid, the 3-acetoxy group was preserved; warming with 0.5N acid hydrolysed the 3-acetoxy group. This discrepancy in the intensity of light absorption of the $\alpha\beta$-aldehyde in this report and (63) is noteworthy.

(d) In this work, the 3-acetoxy ethynyl compound (XLI) was converted to the 3-hydroxy $\alpha\beta$-unsaturated ethyl ester (XLIX) by hot dilute methanolic sulphuric acid, which was in turn hydrolysed by ethanolic potassium hydroxide to the $\alpha\beta$-unsaturated acid (XXXIX). The Swiss workers performed the rearrangement by dilute sulphuric acid in dioxan-water, obtaining the 3-acetoxy ethyl ester (L) at room temperature, and the 3-hydroxy ethyl ester (XLIX) on heating at 90°. Magrath et al (65), however, heat with 3N sulphuric acid and yet claim to obtain the 3-acetoxy derivative (L) in contrast to the findings of the Swiss workers and this report.

EXPERIMENTAL.

All m.p's were determined using a standardised N.P.L. thermometer.

Specific rotations were determined in chloroform solution (except where otherwise stated) in a 1 dm. tube at room temperature.

Ultraviolet absorption spectra were measured in ethanol solution (except where otherwise stated) using a Unicam SP. 500 spectrophotometer.

Microanalyses were determined by Dr. A.C. Syme and Mr. Wm. McCorkindale, of the Chemistry Department, Royal Technical College, Glasgow, and Messrs. Weiler and Strauss, Oxford.

The alumina used for chromatographic purposes was that supplied by Savory and Moore, Grade II (except where otherwise stated), standardised according to Brockmann.

Dibromoacetal.

(cf. Heilbron, Jones, Julia & Weedon J. 1949, 1823)
(Newbold. J. 1950, 3347)

To paraldehyde (220 g; equivalent to 5 moles acetaldehyde), cooled to -10°, was added cautiously over 4 hours dry bromine (1600 g; 10 moles) with constant stirring, external cooling to control the vigorous reaction and ultra-violet irradiation. After standing a further 3½ hours, free bromine was still present. Absolute ethanol (2 litres; precooled to -10°) was then added over 1½ hours (cautiously at first due to copious fuming) with continued stirring and cooling. After standing overnight the reaction mixture was poured into water (2 litres) and ice (2 kg.), the lower layer separated, washed with sodium carbonate solution, water, and dried (Na_2SO_4). The aqueous phase, after neutralisation with sodium carbonate, was extracted with ether (4 x 500 c.c.), similarly washed and dried, and the ether evaporated. The two extracts were then combined (720 g.) and distilled under vacuum (water-pump) The fractions arbitrarily collected were,

312 g. b.p. < 83°/30 mm.

271 g. b.p. 83-120°/30 mm.

124 g. b.p. >120°/30 mm.

Each fraction was then fractionated separately, and the total fraction (298 g.) b.p. 85-110°/13 mm. retained.

Newbold (loc. cit) gives b.p. 96-103°/11 mm.

Bromoethoxyethylene.

(cf. Jacobs, Cramer, & Hanson, J.Amer.Chem.Soc., 64, (1942) 223).

To a solution of dibromoacetal (298 g.) in ethanol (380 c.c. 95%), preheated to refluxing, was added zinc dust (140 g; activated by treatment with 3N hydrochloric acid, washing with water, ethanol, filtering and drying at 90°) at such a rate as to maintain refluxing without heating (45 minutes). The mixture was refluxed for a further 45 minutes, cooled, filtered from excess zinc, and poured into $\frac{N}{I}$ ammonium chloride solution (1620 c.c.) containing ice. A solid white complex which formed decomposed on addition of concentrated hydrochloric acid (20 c.c.). The lower oily bromoethoxyethylene layer so produced was separated, washed with $\frac{N}{I}$ ammonium chloride solution (100 c.c.), ice water (60 c.c.), and dried ($CaCl_2$). The aqueous phase was extracted with ether (3 x 200 c.c.) washed with ice water and dried ($CaCl_2$). The ether was evaporated, the products combined, and distilled through a small column.

The yield of bromoethoxyethylene b.p. 44-52°/25 mm. was 62 g. Unchanged dibromoacetal (41 g; b.p. 60-70°/25mm) was recovered.

Ethoxyacetylene.

(cf. Jacobs, Cramer & Hanson. J.Amer.Chem.Soc., 64, (1942) 223)

Bromoethoxyethylene (62 g.) mixed with powdered potassium hydroxide (125 g.) in a distillation flask was

heated at atmospheric pressure in an oil bath. A vigorous reaction ensued and at a bath temperature of 90-110°, ethoxyacetylene distilled rapidly and was condensed in a cooled receiver (acetone-carbon dioxide). It was dried (Na_2SO_4), redistilled under vacuum and stored in ether solution at 0°.

The yield of ethoxyacetylene b.p. 26-30°/300 mm. was 7.5 g. Jacobs et al (loc.cit) give b.p. 27.5-28.5°/ 300 mm.

Reaction of Dehydroandrosterone Acetate with Ethoxyacetylene.

(a) Ethyl magnesium bromide, prepared from ethyl bromide (3.8 g.) and magnesium (0.86 g.) in ether (30 c.c.) was treated with stirring at 0° with a solution of ethoxyacetylene (2.5 g.) in ether (10 c.c.) added dropwise. When the addition was complete, the mixture was stirred a further 15 minutes at 0° and allowed to rise to 10° over 15 minutes. A solution of dehydroandrosterone acetate (3.3 g.) in benzene (30 c.c.) was then added over 10 minutes (with formation of a white complex) and the solution heated on the water bath for 1½ hours, after the addition of more benzene (10 c.c.). The reaction mixture became very viscous, and darkened in colour. After standing overnight at room temperature, it was treated with saturated ammonium chloride solution, and the organic layer with added benzene washed several times with saturated ammonium chloride solution, then water and dried (Na_2SO_4). Evaporation of the extract gave a brown resin (4.5 g.) which on dissolving in a minimum

quantity of benzene and adding light petroleum (b.p. 40-60°) yielded crystals contaminated with gum. This product was filtered with the aid of light petroleum (b.p. 40-60°) to give a solid (200 mg.) m.p. 50°, resolidifying and remelting ca. 140°. A further 650 mg. of this material was obtained by treatment of the gummy mother liquor with light petroleum (b.p. 40-60°) and crystallising from benzene - light petroleum (b.p. 40-60°). This latter material (500 mg.) in benzene solution was filtered through a column (2 x 10 cm.) of alumina and the benzene eluant (1000 ml.) collected. Removal of the benzene gave a solid residue (410 mg.) m.p. 45-50°, resolidifying ca. 80°, and remelting at 146-149°. Crystallisation from benzene - light petroleum (b.p. 40-60°) gave 21-ethoxypregn-5-en-20-yn-3β:17β-diol as needles.

m.p. 55-65° and 146-149° with solidification at 80°.

$[\alpha]_D$ -114° (C = 1.9 in chloroform).

Found: C, 76.8 ; H, 9.7.

$C_{23}H_{34}O_3$ requires C, 77.1 ; H, 9.6%

The gummy mother liquors from this substance were evaporated and the residual brown resin (3.9 g.) dissolved in benzene (50 c.c.), and chromatographed on a column (25 x 3 cm.) of alumina.

Eluant.	Volume.	Residue.		
Benzene.	1000 c.c.	Solid	1.30 g.	A.
"	1.400 c.c.	Gum	0.95 g.	B.
"	500 c.c.	Gum	0.50 g.	C.

Fraction A on crystallisation from light petroleum (b.p. 60-80°) gave needles (1.0 g.) m.p. 125-135°; two further crystallisations from the same solvent gave laminae of 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol.

m.p. 140-141°

$[\alpha]_D$ -103° (C = 0.6 in chloroform)

Found: C, 75.4 ; H, 9.1

$C_{25}H_{36}O_4$ requires C, 75.0 ; H, 9.1%

Fraction B on crystallisation from benzene - light petroleum (b.p. 60-80°) gave 21-ethoxypregn-5-en-20-yn-3β: 17β-diol (710 mg.) m.p. 55-65°, solid at 80°, remelting at 145°, while fraction C gave 150 mg. of the same compound m.p. 50-60°, solid at 80°, and remelting at 140°. Both specimens had the same fusion characteristics when individually admixed with the first isolated material.

(b) 3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol is most conveniently prepared by direct acetylation of the crude reaction product. In a typical experiment from dehydroandrosterone acetate (10 g.) and the other reactants in the proportions given above, the benzene solution from decomposition of the reaction mixture with ammonium chloride was evaporated and the residue taken up in dry pyridine (30 c.c.) and acetic anhydride (30 c.c.) and heated on the steam bath for ½ hour. Working up in the usual way gave a residual oil (15.5 g.) which was dissolved in benzene (100 c.c.) and adsorbed in a column (23 x 3 cm.) of alumina. Elution with one litre of benzene gave an

oil (7.2 g.) which rapidly solidified; further elution gave negligible residues. Crystallisation of the solid from benzene - light petroleum (b.p. 60-80°) gave 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (5.7 g.) m.p. 138-140° as small slightly yellow laths.

Hydrolysis of 3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol.

3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (100 mg.) was added to a solution of potassium hydroxide (300 mg.) in water (1 c.c.) and methanol (5 c.c.) and the mixture heated under reflux for 30 minutes. Working up in the usual manner gave 21-ethoxypregn-5-en-20-yn-3β:17β-diol as needles m.p. 55-65°, solid at 80°, remelting at 145-148° from benzene - light petroleum (b.p. 60-80°).

Acetylation of 21-Ethoxypregn-5-en-20-yn-3β:17β-diol.

A solution of 21-ethoxypregn-5-en-20-yn-3β:17β-diol in acetic anhydride (2 c.c.) and pyridine (2 c.c.) was heated on the steam bath for 30 minutes. Working up in the usual manner gave 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol as laths m.p. 138-140° alone or mixed with the preparation previously described.

For analysis purposes, it is conveniently purified by chromatographing on alumina, and eluting with benzene containing ½% ethanol, followed by crystallisation from light petroleum (b.p. 60-80°).

Hydrogenation of 3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol.

(a) 3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (500 mg.) in ethyl acetate (25 c.c.) was added to pre-reduced

palladium-calcium carbonate catalyst (250 mg: 2% Pd) in ethyl acetate (25 c.c.) and shaken in an atmosphere of hydrogen at atmospheric pressure and temperature until 27 c.c. had been absorbed (12 mins; theory for 1 mole 30 c.c.) The catalyst was removed by filtration, the solvent removed under reduced pressure, and the solid residue crystallised from methanol to give 3β-acetoxy-21-ethoxypregna-5;20-dien-17β-ol (380 mg.) as iridescent leaflets.

m.p. 145-147°, with sintering at 140°

$[\alpha]_D$ -58° (C = 2.1 in chloroform)

Found: C, 74.8 ; H, 9.4

$C_{25}H_{38}O_4$ requires C, 74.6 ; H, 9.4%

(b) 3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (600 mg.) in ethyl acetate (20 c.c.) was added to a suspension of Raney nickel catalyst (100 mg.) in ethyl acetate (15 c.c.) and shaken at atmospheric pressure and temperature in hydrogen until 35 c.c. had been absorbed (45 minutes: theory for 1 mole 36 c.c.) After filtration of the catalyst and removal of solvent under reduced pressure, three recrystallisations of the residual solid from light petroleum (b.p. 60-80°) gave 3β-acetoxy-21-ethoxypregn-5-en-17β-ol as rosettes of prismatic needles (100 mg; m.p. 159-160°). It gives a light yellow colour with tetranitromethane in chloroform.

m.p. 159-160°

$[\alpha]_D$ -79° (C = 1.6 in chloroform).

Found: C, 73.9 ; H, 10.1

$C_{25}H_{40}O_4$ requires C, 74.2 ; H, 10.1%

The mother liquors from the crystallisation of this compound were evaporated to dryness under reduced pressure and the residue crystallised four times from methanol to give 3β-acetoxy-21-ethoxypregna-5;20-dien-17β-ol (110 mg.) m.p. 144-146° alone or admixed with preparation (a) above.

(c) 3β-Acetoxy-21-ethoxypregn-5-en-17β-ol (250 mg.) was shaken at atmospheric pressure and temperature with hydrogen in presence of platinum from Adams' catalyst (100 mg.) in ethyl acetate (35 c.c.) until 28 c.c. was absorbed (theory for 2 moles is 30 c.c.) After filtration of the catalyst and removal of the solvent under reduced pressure, the residue was crystallised from light petroleum (b.p. 60-80°) to give 3β-acetoxy-21-ethoxypregn-5-en-17β-ol as needles m.p. 159-160°, undepressed on mixing with preparation from (b) above.

Hydrolysis of 3β-Acetoxy-21-ethoxypregn-5-en-17β-ol.

The compound (78 mg.) in ethanol (8 c.c.) was heated under reflux with potassium hydroxide (500 mg.) in water (2 c.c.) for 1½ hours. Working up in the usual way gave a neutral fraction which was crystallised only with difficulty from aqueous methanol to give 21-ethoxypregn-5 en-3β: 17β-diol as felted needles.

m.p. 139-141° sintering at 80°

$[\alpha]_D$ -72° (C , 0.6).

Found: C, 76.2 ; H, 10.5.

$C_{23}H_{38}O_3$ requires C, 76.2 ; H, 10.6%

It sublimed unchanged under reduced pressure (10^{-4} mm.) at a bath temperature of 150-200°. On drying a sample for analysis, it was noted that heating at 56° under vacuum caused considerable softening. It was dried by standing 10 hours in air and 5 hours in vacuum.

3β-Acetoxypregna-5:17-dien-21-al.

(a) Acid Rearrangement Method.

3β-Acetoxy-21-ethoxypregna-5:20-dien-17β-ol (100 mg.) in methanol (10 c.c.) and sulphuric acid (2.5 c.c: 10% w/w) was refluxed for three hours. The colourless solution was concentrated under reduced pressure, treated with water and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and the ether removed to give a solid which was dissolved in pyridine (2 c.c.) and acetic anhydride (2 c.c.) allowed to stand overnight, then evaporated to small bulk under reduced pressure. The residue was diluted with water, extracted with ether, and the extract washed successively with dilute hydrochloric acid, sodium hydrogen carbonate solution, water and dried (Na_2SO_4). Evaporation of the extract gave a solid residue (68 mg.) sintering ca. 155° and melting at 165-175°. This was dissolved in benzene-light petroleum (b.p. 60-80°) (1:4, 25 c.c.) and adsorbed on alumina (2g:). The column

was washed with the same mixture (10 c.c.) then with benzene - light petroleum (b.p. 60-80°)(1:1, 20 c.c.) and finally with benzene (10 c.c.). The residues on evaporation were respectively 17 mg. 27 mg. and 13 mg. of solids. These were combined and crystallised from aqueous acetone to give 3β-acetoxypregna-5:17-dien-21-al as needles.

m.p. 183-185°

$[\alpha]_D$ -57° (C, 1.5)

Found: C, 77.6 ; H, 9.3

Calc. for $C_{23}H_{32}O_3$ C, 77.5 ; H, 9.1%

Light absorption: maximum at 2430Å (ϵ = 17,600)

(b) Vacuum Sublimation Method.

3β-Acetoxy-21-ethoxypregna-5:20-dien-17β-ol (80 mg.) on heating at bath temperature of 140-180° at 10^{-4} mm. Hg. gave a white sublimate (softens 150-160°, melts at 172°). Three further resublimations under the same conditions gave a solid (30 mg.) m.p. 173°, giving a yellow colour with tetranitromethane in chloroform and a red precipitate with Brady's reagent. Two recrystallisations from ether - light petroleum (b.p. 40-60°) gave 3β-acetoxypregna-5:17-dien-21-al, m.p. 181-184°. The mixed m.p. with an authentic specimen m.p. 181-184° (as prepared in (a) above) was 181-183°.

Found: C, 77.1 ; H, 9.2.

Calc. for $C_{23}H_{32}O_3$ C, 77.5 ; H, 9.1%

Light absorption: maximum at 2430Å (ϵ = 16,700).

3β-Acetoxypregna-5:17-dien-21-al 2:4-dinitrophenylhydrazone.

(a) 3β-Acetoxy-21-ethoxypregna-5:20-dien-17β-ol in methanol gave an immediate red precipitate with Brady's reagent. Crystallisation of the solid from benzene - light petroleum (b.p. 60-80°) gave 3β-acetoxypregna-5:17-dien-21-al 2:4-dinitrophenylhydrazone as small red blades.

m.p. 278° (decomp.)

Found: C, 65.1 ; H, 6.8

$C_{29}H_{36}O_6N_4$ requires C, 64.9 ; H, 6.7%.

Light absorption in chloroform: maxima at 2600 (ε = 16,300) , 3050 (ε = 11,200) and 3900Å (ε = 32,300)

(b) A solution of 3β-acetoxypregna-5:17-dien-21-al in methanol, on treatment with Brady's reagent, gave almost immediately a red precipitate which separated from benzene - light petroleum (b.p. 60-80°) as small red blades m.p. 278° (decomp.) alone or with preparation (a).

3β-Acetoxypregna-5:17-dien-21-al semicarbazone.

(cf: Miescher, Wettstein & Scholz: Helv.Chim.Acta, 22, 894 (1939)).

Prepared from the aldehyde, the semicarbazone separated from aqueous methanol as needles.

m.p. 245° (decomp.) (loc.cit. m.p. 245-246°)

Found: : N 10.4

Calc. for $C_{24}H_{35}O_3N_3$: N 10.2%.

Light absorption: maximum at 2750Å (ε = 34,400).

Ethyl 3β-hydroxypregna-5:17-diene-21-carboxylate.

3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (500 mg.) in methanol (25 c.c.) to which sulphuric acid (5 c.c. 10% $^{w}/_{w}$)

had been added was refluxed for 1 hour, concentrated to half volume under reduced pressure and treated with water. The precipitated solid was taken up in ether (ca. 70 c.c.) and the ethereal solution washed successively with water (50 c.c.), sodium hydroxide solution (3 x 50 c.c.) and water (2 x 50 c.c.). The alkaline extracts on acidification became opalescent, and deposited a trace of solid after several days. The ethereal solution was dried (Na_2SO_4), the ether removed, and the residue crystallised twice from ethanol to give <u>ethyl 3β-hydroxypregna-5:17-diene-21-carboxylate</u> (240 mg.) as prismatic needles.

m.p. 181°.

$[\alpha]_D$ -72° (C, 0,8)

Found: C, 77.0 ; H, 9.2.

$C_{23}H_{34}O_3$ requires C, 77.1 ; H, 9.6%.

Light absorption: maximum at 2200Å (ϵ = 16,500)

<u>Ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate</u>.

Ethyl 3β-hydroxypregna-5:17-diene-21-carboxylate (105 mg.) in dry pyridine (2 c.c.) and acetic anhydride (3 c.c.) was allowed to stand 14 hours at 15°, followed by 5 minutes at 100°. Evaporation under reduced pressure was followed by addition of water and ether extraction: the ethereal extract was washed successively with dilute hydrochloric acid, sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of the ether left a solid residue crystallised twice from ethanol to give

ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate (80 mg.) as needles.

m.p. 111°.

$[\alpha]_D$ -66° (C, 0.7).

Found: C, 74.6 ; H, 9.1.

$C_{25}H_{36}O_4$ requires C, 75.0 ; H, 9.1%.

Light absorption: maximum at 2200Å (ε = 17,000)

The compound also forms needles from methanol, m.p. 155° (Found: C, 74.2 ; H, 8.75%.), exhibiting light absorption maximum at 2200Å (ε = 17,600). A mixture of equal parts of the forms m.p. 155° and 111° melts sharply at 111°.

3β-Hydroxypregna-5:17-diene-21-carboxylic acid.

Ethyl 3β-hydroxypregna-5:17-diene-21-carboxylate (100 mg.) in ethanol (30 c.c.) was treated with a solution of potassium hydroxide (1.0 g.) in water (5 ml.) and the mixture refluxed for four hours. Removal of the solvents under reduced pressure gave a solid, insoluble in water, ether, and chloroform. The solid was dissolved in hot methanol, the solution acidified with sulphuric acid (10% w/w), and the resulting granular precipitate (80 mg.), m.p. 220-230° which separated removed by filtration. Two recrystallisations from aqueous acetone gave 3β-hydroxypregna-5:17-diene-21-carboxylic acid as fine needles. It could be sublimed unchanged at 220°/10^{-4} mm. Hg.

m.p. 243-244°

$[\alpha]_D$ -81° (C, 0.7 in dioxan)

Found: C, 76.2 ; H, 9.1.

Calc. for $C_{21}H_{30}O_3$: C, 76.3 ; H, 9.2%.

Light absorption: maximum at 2180Å (ε = 15,900)

3β;17β-Dihydroxypregn-5-en-21-carboxylic acid.

(cf: Reichstein, Muller, Meystre & Sutter Helv.Chim.Acta. 22, 741 (1939))

To zinc powder (2:33 g.: purified by washing rapidly with hydrochloric acid (2 %), water, ethanol, acetone, ether, warming under vacuum at 100° for 15 minutes and used immediately) was added a solution of dehydroandrosterone acetate (2.0 g.) and ethyl bromoacetate (4 g.) in dry benzene. The addition of a few crystals of iodine did not initiate the reaction. A drop of pyridine, added to catalyse the reactions, caused the solution to turn apple green in colour, but no spontaneous refluxing occurred. The reaction mixture was then heated, with exclusion of moisture, with stirring and refluxing for 5 hours, whilst a greenish-white gummy precipitate was produced. The mixture was then cooled, decomposed with ice and hydrochloric acid, extracted with ether and the extract washed successively with water, dilute hydrochloric acid, NaOH solution and water and dried (Na_2SO_4). The residue obtained on evaporation was dissolved in methanol (33 c.c.) treated with aqueous potassium hydroxide (50%: 5 c.c.) and refluxed for 30 minutes. Water was then added, and the methanol removed under reduced pressure to leave a semi-solid

residue. To remove neutral products, this residue was extracted with ether, and the ether extracts washed with sodium carbonate solution.

The alkaline extracts (ca. 600 c.c.) were acidified with hydrochloric acid to Congo red, and thoroughly extracted with ether. The dried (Na_2SO_4) extract on evaporation gave a crude solid (1.7 g.) m.p. 165-175°. Four recrystallisations from acetone (300 c.c.) gave 3β;17β-dihydroxypregn-5-en-21-carboxylic acid as leaflets.

m.p. 246° (loc. cit. gives m.p. 246-247°).

Methyl 3β:17β-dihydroxypregn-5-en-21-carboxylate.

A solution of diazomethane (Organic Syntheses, Coll. Vol. II. p. 165) in ether was added to a suspension of 3β;17β-dihydroxypregn-5-en-21-carboxylic acid (1.0 g.) in ether. The mixture was allowed to stand for 2 hours, during which the suspension remained. It was then refluxed to remove excess diazomethane, the ether removed by evaporation and the residue dissolved in chloroform, washed with $\frac{N}{1}$ sodium hydroxide solution, water, and dried (Na_2SO_4). Evaporation of the chloroform left a white crystalline solid which was recrystallised from methanol to give methyl 3β:17β-dihydroxypregn-5-en-21-carboxylate m.p. 141-7°. The alkaline extract was acidified with dilute hydrochloric acid to precipitate unchanged acid, which was collected, dried, taken up in absolute methanol, and re-esterified as previously with diazomethane in ether.

Recrystallisation of the combined products gave methyl 3β:17β-dihydroxypregn-5-en-21-carboxylate (600 mg., m.p. 145-148°) (Reichstein, Muller, Meystre & Sutter give m.p. 145-149°).

Methyl 3β:17β-diacetoxypregn-5-en-21-carboxylate.

(cf: Plattner & Schreck, Helv.Chim.Acta, 22, (1939) 1178).

Methyl 3β:17β-dihydroxypregn-5-en-21-carboxylate (600 mg.) was heated under reflux in acetic anhydride (10 c.c.) for 36 hours, during which the solution darkened considerably. After removal of the solvent under reduced pressure, water was added, the mixture extracted with ether and the extract washed with water (3 x 30 c.c.) $\frac{N}{2}$ sodium hydroxide solution (2 x 30 c.c.) and water (2 x 25 c.c.) After drying (Na_2SO_4) removal of the ether gave a pale brown gum, which did not crystallise readily. It was not further purified.

Methyl 3β-acetoxypregna-5:17-diene-21-carboxylate.

Methyl 3β:17β-diacetoxypregn-5-en-21-carboxylate (as prepared above) was heated under reduced pressure (5×10^{-3} mm. Hg.). It melted at bath temperature of 70° and sublimed as a yellow amorphous solid at bath temperature ca. 175°. It was resublimed three times and the sublimate collected over range 110-190°. The yield of the crude product was 271 mg.

Light absorption : maximum at 2190Å (ε = 6,400). In comparison with the light absorption of the corresponding ethyl ester (prepared as previously described) with maximum

at 2200Å (ϵ = 17,000) it is concluded that the specimen of methyl 3β-acetoxypregna-5;17-diene-21-carboxylate, obtained by this method, has a purity of only 40%.

3β-Hydroxypregna-5:17-diene-21-carboxylic acid.

Methyl 3β-acetoxypregna-5:17-diene-21-carboxylate (270 mg; as prepared above) was heated under reflux for 2 hours with methanolic potassium hydroxide solution (10%: 75 c.c.) cooled, diluted to 250 c.c. with water, and extracted with ether to remove neutral constituents (evaporation of this extract gave a negligible residue). The aqueous phase was then acidified to Congo red with 2N hydrochloric acid, and extracted with ether. The ether extract was repeatedly washed with water, dried (Na_2SO_4) and the ether removed to give a pale brown gum which crystallised from acetone (55 mg: m.p. 233-237°). Two more recrystallisations gave 3β-hydroxypregna-5:17-diene-21-carboxylic acid, m.p. 241.5-243° alone or on admixture with a specimen of the acid (m.p. 243°) prepared by the ethoxyacetylene synthesis.

Light absorption: maximum at 2180Å (ϵ = 14,700)

Evaporation of the acetone crystallisation mother liquors left a gum product which could not be crystallised.

Attempted Degradations of Ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate to Dehydroandrosterone (as semicarbazone).

Method 1. By hydroxylation.

To a solution of ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate (600 mg.) in absolute ether (9 c.c.) was added a solution of osmium tetroxide (382 mg. 1.1. mol.) in

absolute ether (20 c.c.). The reaction mixture, which darkened within a few hours, was kept in the dark for three days, after which a dark brown amorphous solid had separated. The ether was removed under reduced pressure to leave a brown oily residue, which was dissolved in ethanol (30 c.c.) and refluxed for 1½ hours with a solution of crystalline sodium sulphite (5 g.) in water (20 c.c.). Ethanol (50 c.c.) was then added, the precipitate allowed to settle, and the supernatant liquor decanted. The precipitate was digested with ethanol (2 x 75 c.c.), the liquor being filtered off on each occasion. On concentration of the combined ethanol extracts under reduced pressure, a white solid separated, and precipitation was completed by addition of water (50 c.c.). The mixture was extracted with ether (4 x 50 c.c.), the extract washed with water (50 c.c.), dried (Na_2SO_4), and the ether removed to leave a white solid residue (370 mg. m.p. 145-165°). Recrystallisation from methanol gave a product m.p. 180°, undepressed on admixture with ethyl 3β-hydroxypregna-5:17-diene-21-carboxylate (m.p. 181°) i.e., starting material with acetate grouping hydrolysed.

Light absorption: maximum at 2200Å (ϵ = 16,000).

Method 2. By Chromic Acid Oxidation.

To a solution of ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate (200 mg.) in glacial acetic acid (8 c.c.), bromine (80 mg: 1 mol.) in glacial acetic acid (6 c.c.) was added dropwise with shaking. There was immediate fading

of colour to pale yellow, and complete decolorisation after 15 minutes. (1 drop of the reaction solution, withdrawn and made up to 40 ml. with spectroscopic ethanol, gave an absorption maximum at 2100-2140Å). Chromic anhydride (167 mg: 5 atoms O) in 83% acetic acid (1 c.c.) was added dropwise with ice-cooling over 15 minutes. The first drop turned green, but successive drops did not appear to be reduced as the solution retained a reddish-brown colouration, which remained after heating at 40° for 10 minutes. The excess chromic acid was reduced by addition of 3 drops of methanol. Zinc dust (99 mg: 3 mols.) was then added, the mixture shaken at room temperature for 2 hours and the mixture diluted with water. A precipitate was produced which coagulated into a sticky mass. The supernatant liquor was removed by decantation, extracted with ether (3 x 30 c.c.) the coagulated precipitate dissolved in this extract, and combined extract successively washed with water (30 c.c.), sodium hydroxide solution (30 c.c. $\frac{N}{1}$), water (30 c.c.), and dried (Na_2SO_4). Removal of the ether under reduced pressure gave a solid in the matrix of a gum. This was dissolved in a minimum of hot ethanol, and after standing overnight, 3 crystalline forms (needles, spheres, and feathers) were apparent in very small quantities.

Attempts to form the known dehydroandrosterone acetate semicarbazone (m.p. 275°) gave only a product m.p. 94-98°, giving a negative Beilstein reaction and light absorption at 2200Å. Recrystallisation from benzene-methanol raised the

m.p. to 107-109°. The remainder of the product after these attempts was then recrystallised from methanol to give unchanged starting material m.p. 108-110°, undepressed on admixture with authentic specimen.

Method 3. By Ozonolysis.

To a solution of ethyl 3β-acetoxypregna-5:17-diene-21-carboxylate (240 mg.) in chloroform (10 c.c.) was added bromine (96 mg.) in chloroform (9.6 c.c.) dropwise with shaking at -5° over half an hour. The bromine colour disappeared instantaneously. Ozone was then bubbled through the chloroform solution at -5° for 25 minutes. The solution turned brown immediately, but went colourless on standing at room temperature for 5 minutes. Removal of the chloroform under reduced pressure below 30° left a clear gum, which was taken up in glacial acetic acid (5 c.c.) and heated on the steam bath with zinc dust (200 mg.) for 1 hour. Water (30 c.c.) was then added, the mixture extracted with ether (2 x 20 c.c.) and the extract washed with saturated sodium hydrogen carbonate solution (2 x 25 c.c. and water (25 c.c.). Removal of the ether left a wet brown oily residue, which was dissolved in methanol (7 ml.) and a solution of semicarbazide hydrochloride (200 mg.) and crystalline sodium acetate (300 mg.) in water (3 drops) added. After refluxing the mixture on the steam bath for 10 minutes, a white crystalline solid (45 mg. m.p. 260-265°) crystallised out. One crystallisation from benzene-methanol gave dehydroandrosterone acetate semicarbazone

m.p. 270-273°, undepressed on admixture with an authentic specimen m.p. 273-276°.

Light absorption: maximum at 2250Å (ε = 13,400).

Oxidation of 3β-acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol with mercury acetamide.

3β-Acetoxy-21-ethoxypregn-5-en-20-yn-17β-ol (500 mg.) in ethanol (10 c.c.) was refluxed for 20 hours with mercury acetamide (1.0 g.). Mercury was removed from the reaction mixture by precipitation with hydrogen sulphide, and the filtrate evaporated to dryness under reduced pressure. The residue was extracted with ether (5 x 10 c.c.) the combined extract washed with water (2 x 15 c.c.) dried (Na_2SO_4), and evaporated to give a gum (400 mg.) which was dissolved in benzene (10 c.c.) and adsorbed on a column (15 x 1.5 cm.) of alumina. The column was washed with benzene (200 c.c.) and the eluate evaporated to give a crystalline solid (170 mg., m.p. 153-158°) which separated from light petroleum (b.p. 40-60°) as fine needles m.p. 167-169°, undepressed by dehydroandrosterone acetate.

$[\alpha]_D$+3° (C, 0.5 in ethanol) Lit. gives $[\alpha]_D$+4° (ethanol)

Found: C, 76.7 ; H, 9.2.

Calculated for $C_{21}H_{30}O_3$: C, 76.3 ; H, 9.15%

Light absorption: maximum at 2910Å (ε = 51).

The semicarbazone formed needles from benzene-methanol m.p. 273-276° undepressed by authentic specimen.

Light absorption: maximum at 2240Å (ε = 13,400)

The dehydroandrosterone acetate was further characterised by hydrolysis. The compound (60 mg.) was allowed to stand

at 20° with methanolic potassium hydroxide (10 c.c., 5%) worked up by means of ether to give dehydroandrosterone as felted needles from light petroleum (b.p. 40-60°) m.p. 139° undepressed by an authentic specimen.

The alumina column was further washed with benzene (100 c.c.) to give negligible residue: elution with ether (200 c.c.) gave a solid (150 mg.) which separated from light petroleum (b.p. 40-60°) as felted needles m.p. 139°, undepressed on admixture with dehydroandrosterone.

$[\alpha]_D$ +9° (C, 0.6 in ethanol) Lit. gives $[\alpha]_D$ +11° (ethanol)

Found: C, 78.7 ; H, 10.1.

Calculated for $C_{19}H_{28}O_2$: C, 79.1 ; H, 9.8%

Light absorption: maximum at 2900Å (ϵ = 62).

3β:17β-Diacetoxy-21-ethoxypregn-5-ene.

3β-Acetoxy-21-ethoxypregn-5-en-17β-ol (100 mg.) was heated for 18 hours at 135° with acetic anhydride (1 ml.) and pyridine (1 ml.). The dark solution was evaporated under reduced pressure, and the residue extracted with ether. The ethereal solution was evaporated and the residue distilled at a bath temperature of 160-170°/10^{-3} mm. to give a viscous yellow brown oil (50 mg.). This was dissolved in benzene (10 c.c.) and adsorbed a column (7 x 1 cm.) of alumina. Elution with benzene -1% ethanol (200 c.c.) gave a crystalline material (45 mg.) which separted from light petroleum (b.p. 40-60°) as needles, and believed to be 3β:17β-diacetoxy-21-ethoxypregn-5-ene.

m.p. 128-130°

Found: C, 72.9 ; H, 9.0.

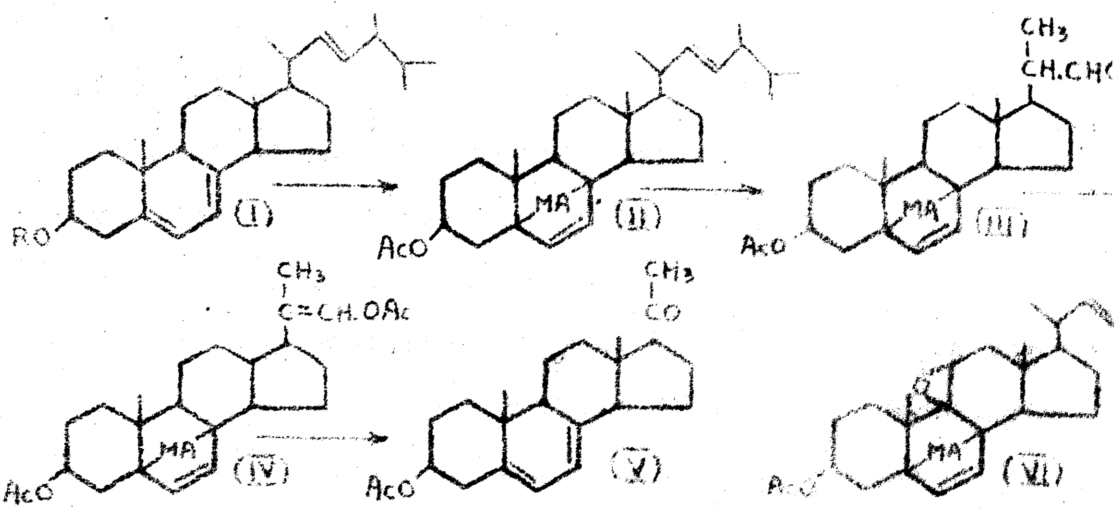
$C_{27}H_{42}O_5$ requires C, 72.6 ; H, 9.4%.

11-OXYGENATED STEROIDS FROM ERGOSTEROL.

HISTORICAL.

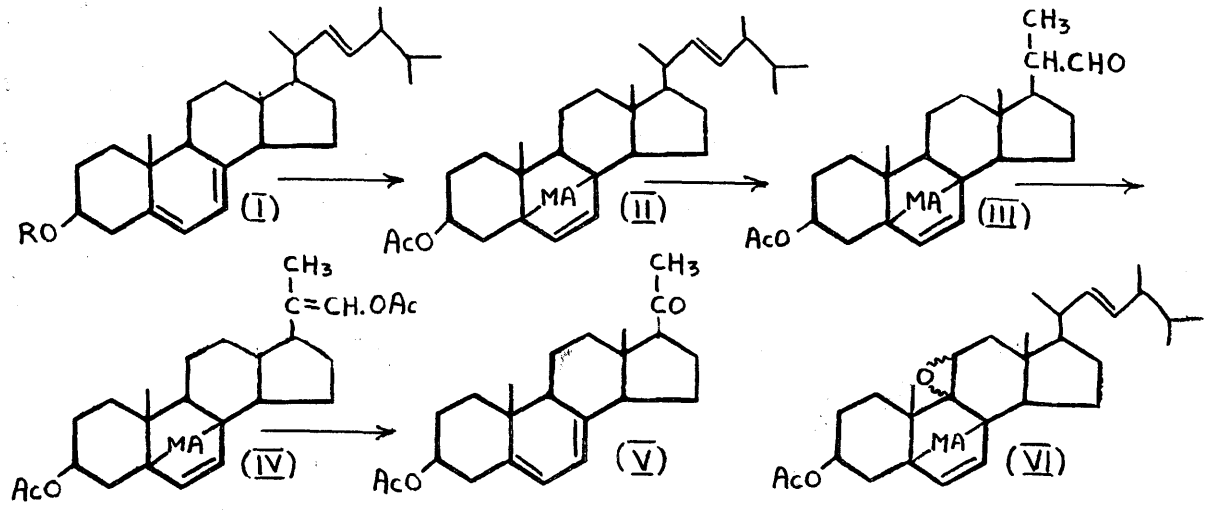
...use of ergosterol (I; R = H) as a starting mate... partial synthesis of adrenal cortical hormones w...

preferential attack on the sidechain double bond. Thus, treatment of the maleic anhydride adduct (II) with ozone



gave an aldehyde (III) which was converted into the enol

The use of ergosterol (I; R = H) as a starting material for the partial synthesis of adrenal cortical hormones was first suggested by Bergmann and Stevens (67) "because of the comparative ease with which it may be converted to derivatives like dehydroergosterol which possess unsaturation at C-11 and which might lend themselves to the introduction of oxygen at this point. In addition, the 22:23-double bond was expected to facilitate removal of the sidechain to permit its replacement by one of the typical sidechains of adrenal cortical hormones". Considerable progress in the latter direction was made by these workers, who showed that protection of the ring B conjugated double bonds of ergosteryl acetate (I; R = Ac) by means of maleic anhydride permitted preferential attack on the sidechain double bond. Thus, treatment of the maleic anhydride adduct (II) with ozone

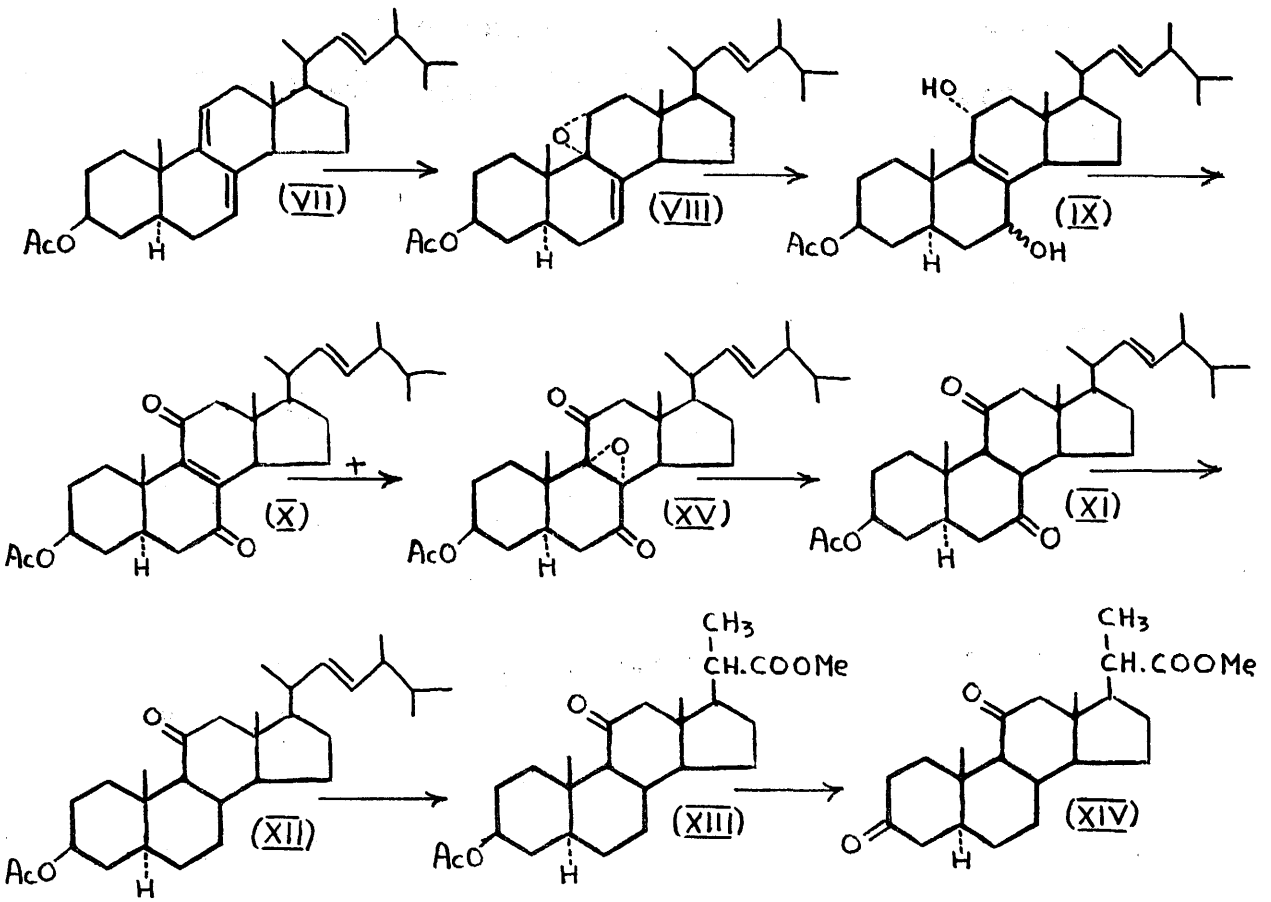


gave an aldehyde (III) which was converted into the enol acetate (IV), ozonolysis of which, followed by pyrolysis, gave 3β-acetoxypregna-5:17-dien-20-one (V). This

procedure has been substantiated by Antonucci, Bernstein, Giancola and Sax (68). The attempts of Bergmann and Stevens to introduce an oxygen function into the 11-position, starting from the maleic anhydride adduct of dehydroergosteryl acetate 22:23-dibromide, were less successful in that, although the epoxide (VI) was obtained, pyrolysis of this was accompanied by aromatisation of ring B.

The successful introduction of 11-oxygen functions into the steroid nucleus starting from ergosterol was first announced (May 1951) by Tishler and coworkers (69). Since that time, a spate of publications has appeared, describing various oxidation procedures and rearrangements directed towards the formation of 11-oxygenated steroids, starting from ergosteryl-D acetate (VII) (obtained by standard methods from ergosterol) or analogous $\Delta^{7,9(11)}$-dienic steroids. Many of these communications are in outline form, no experimental details being given. The experiments described in this dissertation, performed during the same period, have in part been reported in a series of publications (70, 71, 72, 73).

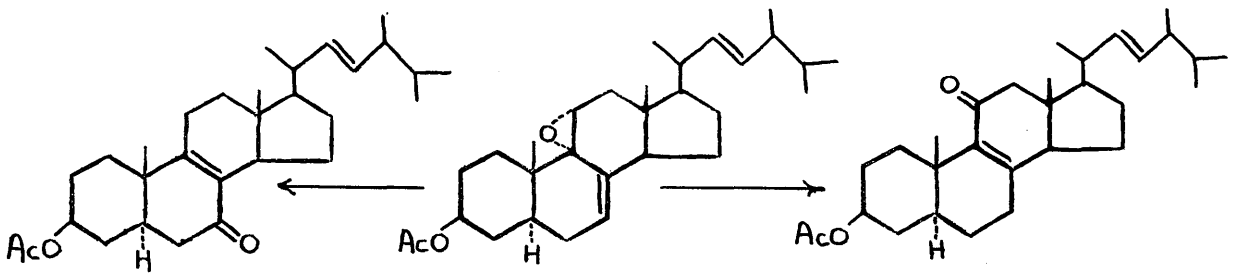
The method described by Tishler (69) involves the oxidation of ergosteryl-D acetate (VII) with perbenzoic acid to give an epoxide (VIII), believed to be a Δ^{7}-9α:11α-epoxide (74) which on hydrolytic rearrangement gives 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (IX). Oxidation of the latter compound with chromic acid gives 7: 11-diketoergosta-8:22-dien-3β-yl acetate (X) converted



to 7:11-diketoergost-22-en-3β-yl acetate (XI) by treatment with zinc and acetic acid. Wolff-Kishner reduction of (XI) gives 11-ketoergost-22-en-3β-yl acetate (XII), converted through (XIII) to the known compound (XIV). These transformations were also applied to the analogous 7:9 (11)-dienes obtained from cholesterol, stigmasterol, and diosgenin.

Jeger and coworkers (75) described with experimental details the same route (VII)→(VIII)→(IX)→(X)→(XI)→(XII). Under their conditions, chromic acid oxidation gave 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (XV) as well as (X). Both the diketone (X) and the epoxydiketone

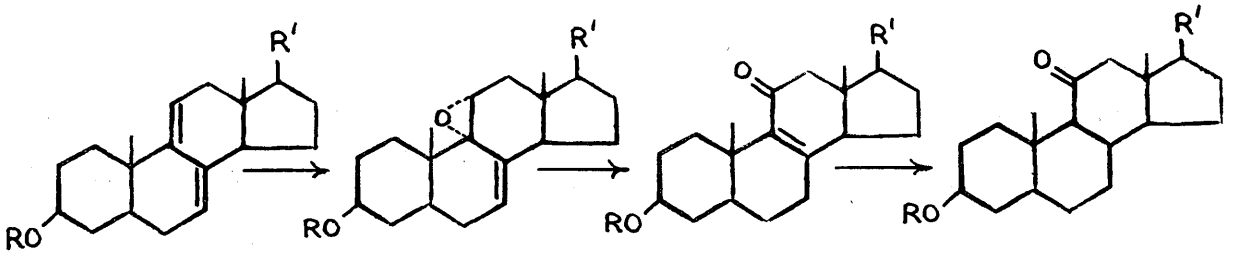
(XV) give the same product (XI) on reduction with zinc and acetic acid. The corresponding compounds were also obtained by analogous reactions starting from methyl



(XVI) (VIII) (XVII)

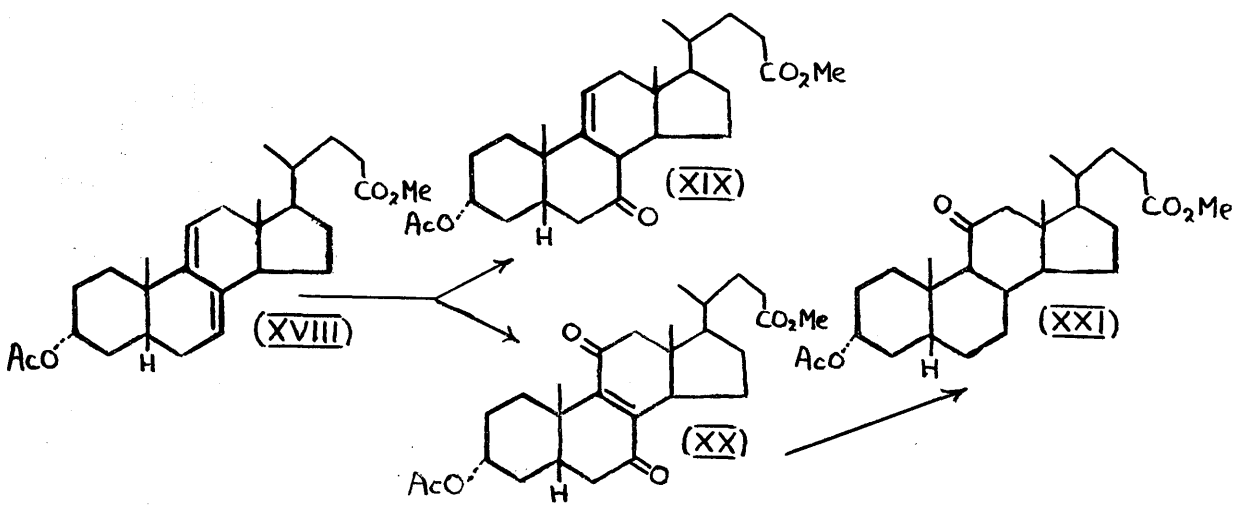
3α-acetoxychola-7:9(11)-dienate, obtained from cholic acid. The same authors report the rearrangement of the epoxide (VIII) to 3β-acetoxyergosta-8:22-dien-7-one (XVI) by treatment with mineral acids, and to 3β-acetoxyergosta-8:22-dien-11-one (XVII) by treatment with boron trifluoride or ferric chloride (76). Similar transformations are also described by Jeger and coworkers (78) in the androstane and cholestane series.

The structure assigned by Jeger to XVII rested essentially on the non-reactivity of the carbonyl group and its non-identity with the Δ^{8}-7-ketone and the $\Delta^{8(14)}$-7-ketone. Irrefutable proof of the structure of this ketone, as well as a new and shorter path to 11-ketoergost-22-en-3β-yl acetate (XII), is provided by the observation of Tishler et al (74) that the Δ^{8}-ethylenic linkage of XVII could be selectively reduced by the action of lithium and liquid ammonia to give XII in high yield. The general availability of this most



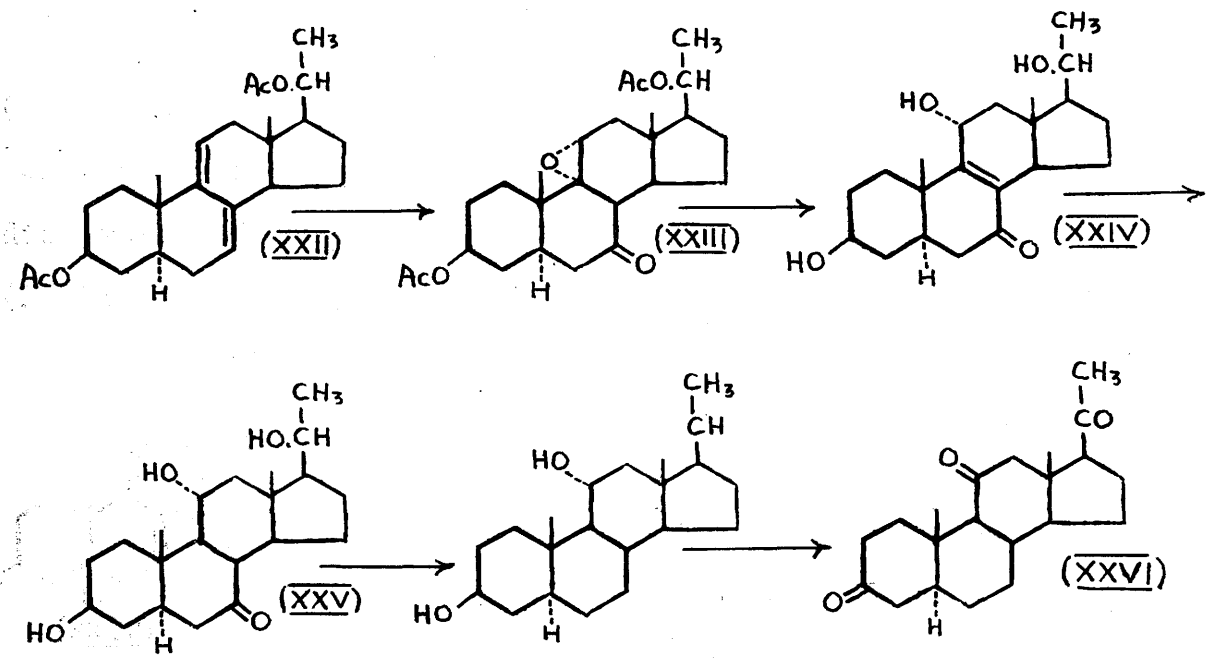
convenient route is demonstrated by its application to 7:9-dienic esters derived from sapogenins (74, 77). Djerassi and coworkers (77) have shown also that reduction of the Δ^{8}-11-ketones by lithium and liquid ammonia can also produce 11α-hydroxy steroids under certain conditions.

Fieser, Herz and Huang (79) oxidised methyl 3α-acetoxychola-7:9(11)-dienate (XVIII) with sodium dichromate to give a mixture of methyl 3α-acetoxy-7-ketochol-9(11)-enate (XIV) and methyl 3α-acetoxy-7:11-diketochol-8-enate (XX). The latter compound was converted, by the established method of zinc and acetic acid reduction followed by Kishner-Wolff reduction, to methyl 3α-acetoxy-11-ketocholanate (XXI). This



oxidative procedure was also applied to the 7:9(11)-diene obtained from cholesterol.

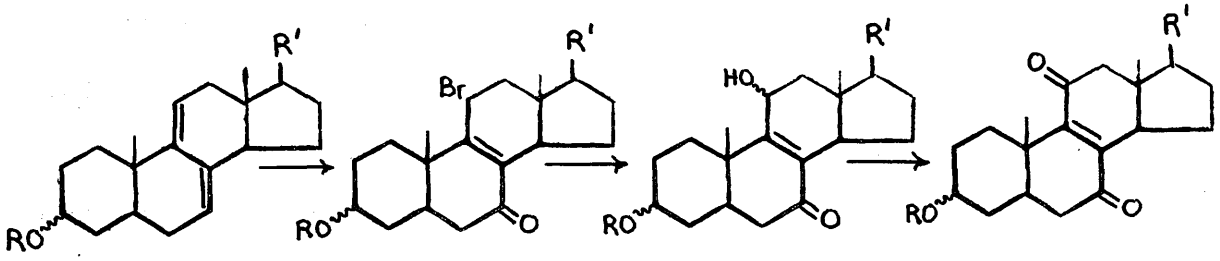
Stork, Romo, Rosenkranz and Djerassi (80) oxidised 3β:20β-diacetoxyallopregna-7:9(11)-diene (XXII) (obtainable from diosgenin or stigmasterol) to give the ketoxide (XXIII), which isomerised on mild alkaline hydrolysis to 3β:11α:20β-trihydroxyallopregn-8-en-7-one (XXIV). Catalytic reduction of the latter gave XXV which on Kishner-Wolff reduction and chromic acid oxidation yielded the known 3:11:20-triketoallopregnane (XXVI). The method was reported to be equally applicable to steroid sapogenins, and experimental details as applied to



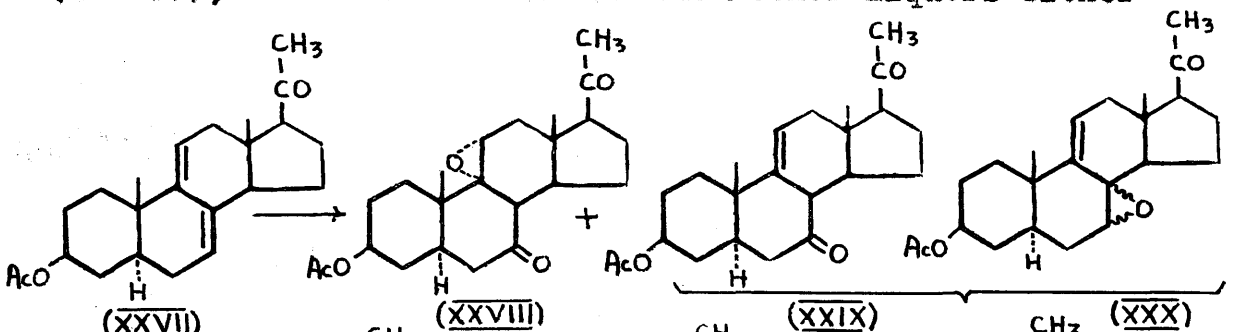
diosgenin, were subsequently published (83).

Fieser and coworkers (81) have indicated that this latter method is inapplicable to the normal series and

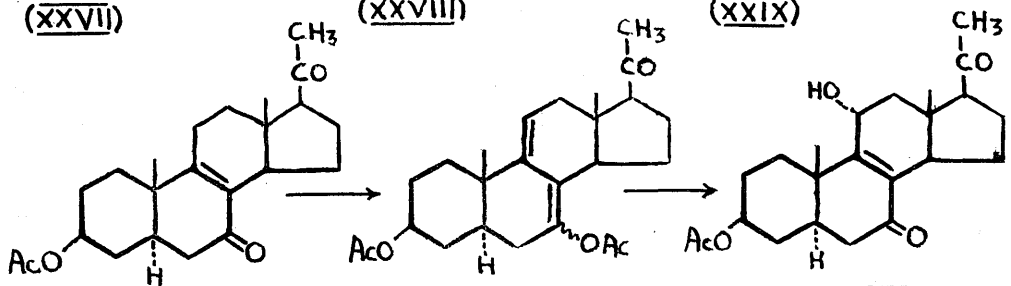
outline a further oxidation procedure which has been applied to ergosteryl-D acetate, cholesta-7:9(11)-dienyl benzoate and methyl 3α-acetoxychola-7:9(11)-dienate. It consists of reaction of the diene with excess N-bromosuccinimide in aqueous t-butanol, followed by addition of silver nitrate and chromic acid oxidation to the enedione compound:-



In a further communication, Djerassi and coworkers (82) extended the examination of the products obtained by performic acid oxidation of 3β-acetoxyallopregna-7:9(11)-dien-20-one (XXVII). Besides the expected ketoxide (XXVIII), there is obtained in the mother liquors either



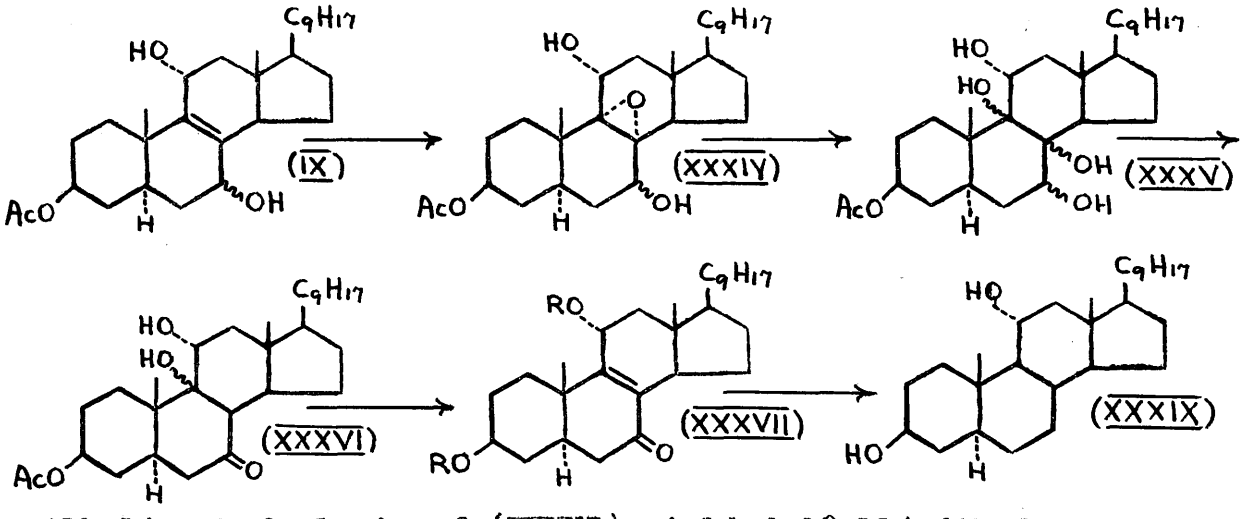
(XXVII) (XXVIII) (XXIX) (XXX)



(XXXI) (XXXII) (XXXIII)

7:20-diketoallopregn-9(11)-en-3β-yl acetate (XXIX) or 7ξ:8ξ-epoxy-20-ketoallopregn-9(11)-en-3β-yl acetate (XXX) or a mixture of both. Saponification of the mother liquors gave 7:20-diketoallopregn-8-en-3β-yl acetate (XXXI) which was preferentially enol acetylated at C-7 by treatment with isopropenyl acetate, to give (XXXII). Treatment of the latter intermediate with monoperphthalic acid gave directly 11α-hydroxy-7:20-diketoallopregn-8-en-3β-yl acetate (XXXIII).

Heusser, Anliker, Eichenberger and Jeger (76) have described variations in their original route (75) to 11-oxygenated ergosterol derivatives. Treatment of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (IX) with monoperphthalic acid gives the corresponding epoxide (XXXIV) which was dehydrated to (XXXVI) by mineral acid, either directly or through the intermediate tetrol (XXXV).

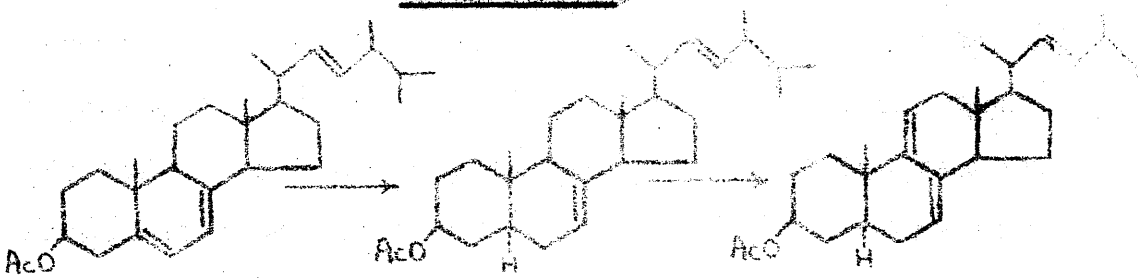


Alkaline hydrolysis of (XXXVI) yielded 3β:11α-dihydroxypregna-8:22-dien-7-one (XXXVII) which was converted to (XXXIX) by selective hydrogenation of Δ^8-ethylenic linkage followed by Wolff-Kishner reduction of the 7-keto group.

or more particularly the conversion of ergosterol to 11-oxygenated steroids. The approach to this end started from ergosteryl-D acetate (ergosta-7:9(11):22: 3β-yl acetate) (VII), the simplest available derivati ergosteryl acetate (I) possessing unsaturation at C-1 a sidechain ethylenic linkage.

A survey of the existing methods of preparation compound revealed that the most efficient method con of the preparation of 5-dihydroergosteryl acetate (XI followed by oxidation with mercuric acetate to give (

THEORETICAL.

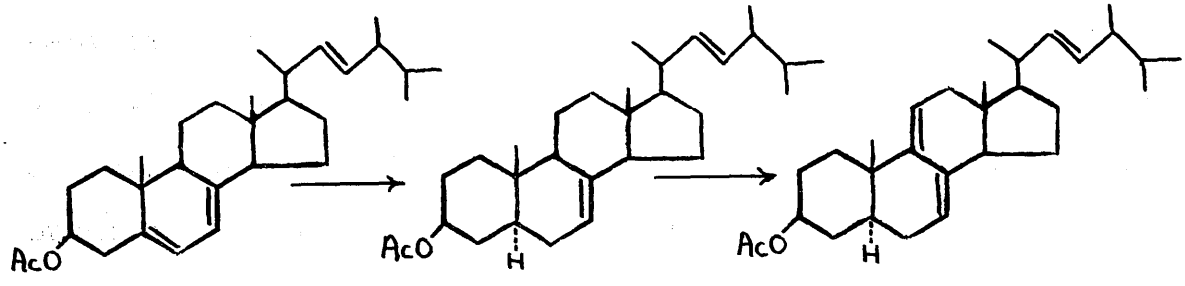


(I) (XI.) (VII)

The preparation of 5-dihydroergosteryl acetate (has been reported by Heilbron and Sexton (34), Wieland Bensnd (35), and Barton and Cox (36) by the partial hydrogenation of ergosteryl esters. The last autho claim that the best method is by hydrogenation of erg

The work described in this section had as its object the development of routes to cortisone from ergosterol, or more particularly the conversion of ergosterol to 11-oxygenated steroids. The approach to this end started from ergosteryl-D acetate (ergosta-7:9(11):22-trien-3β-yl acetate) (VII), the simplest available derivative of ergosteryl acetate (I) possessing unsaturation at C-11 and a sidechain ethylenic linkage.

A survey of the existing methods of preparation of this compound revealed that the most efficient method consisted of the preparation of 5-dihydroergosteryl acetate (XL), followed by oxidation with mercuric acetate to give (VII).



(I) (XL) (VII)

The preparation of 5-dihydroergosteryl acetate (XL) has been reported by Heilbron and Sexton (84), Wieland and Benend (85), and Barton and Cox (86) by the partial hydrogenation of ergosteryl esters. The last authors claim that the best method is by hydrogenation of ergosteryl acetate in chloroform solution using platinum catalyst, obtaining 5-dihydroergosteryl acetate in 30-35% yield after 1½ - 2 hours reaction time. A considerable improvement

is reported here, 5-dihydroergosteryl acetate of high purity being isolated in 90% yield by performing the hydrogenation in benzene solution with Raney nickel catalyst. Using freshly prepared catalyst and Anala R solvent, the hydrogenation could be completed within 15 minutes. Improved procedures have also been recently reported, Panizzon and Kägi (75, p. 2123) obtaining a 90-95% yield with Rupe nickel in ether solution, and Laubach and Brunings (87) obtaining a quantitative yield with Raney nickel in pure dioxan.

The oxidation of 5-dihydroergosteryl acetate to ergosteryl-D acetate by means of mercuric acetate (88, 89) perbenzoic acid (90), and selenium dioxide (91) has been reported, and an analogous reaction, namely the conversion of cholest-7-ene to cholesta-7:9(11)-diene, has been effected by Eck and Hollingsworth (92) by low temperature bromination of the former compound in chloroform solution. Although a modification of the mercuric acetate oxidation procedure, described for the preparation of dehydroergosteryl acetate from ergosteryl acetate (93), was employed in the oxidation of 5-dihydroergosteryl acetate, the method remains far from satisfactory. Although the immediate reaction product isolable in fair yield, the purification of this material to constant optical rotation involved considerable crystallisation losses with consequent lowering the yield of pure product to 30%. Ergosteryl-D acetate was subsequently prepared in reasonable yield by a bromination

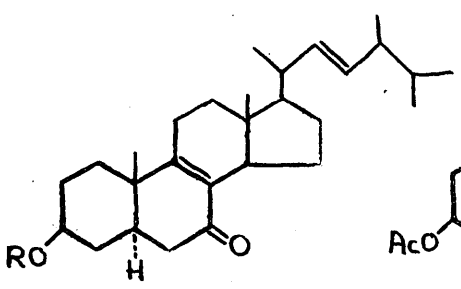
procedure (discussed later) whereby a high purity, as shown by comparison of its melting point, specific rotation and ultra-violet light absorption intensity with previously reported values, was readily obtained.

Oxidant.	Source.	m.p.	$[\alpha]_D$	$\varepsilon^{EtOH}_{2420\text{Å}}$
Bromine	Exptl.	178-180°	+32°	19,000
Mercuric Acetate	Exptl.	176°	+30°	17,000
Mercuric Acetate	(75)	169-170°	+21°	16,000
Mercuric Acetate	(86)	169°	+18°	13,200
Perbenzoic Acid	(90)	171°	+26°	-

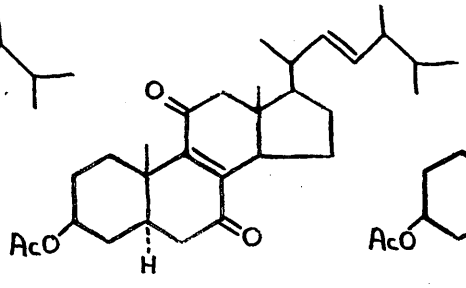
The characteristic ultra-violet light absorption of ergosteryl-D acetate, viz, well defined maximae at 2350 and 2420Å with an inflection at 2510Å, appears to be characteristic of $\Delta^{7,9(11)}$-dienic steroids (94).

Attention was next turned to the oxidation of ergosteryl-D acetate, and experiments involving a number of oxidising agents - chromic acid, performic acid, potassium permanganate and perbenzoic acid - were performed. The action of chromium trioxide in acetic acid was examined under a variety of conditions, in all cases 3β-acetoxyergosta-8:22-dien-7-one (XLI; R = Ac) being isolated in poor yield. This αβ-unsaturated ketone was first obtained by Stavely and Bollenback (95) as a minor product of the oxidation of 5-dihydroergosteryl acetate with chromium trioxide. It was further characterised by conversion to 3β-hydroxyergosta-8:22-dien-7-one (XLI; R = H) by alkaline hydrolysis. Since

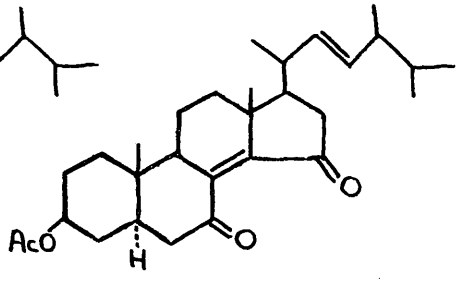
oxidations with chromic acid involving 2 atoms of oxygen yielded unchanged ergosteryl-D acetate as well as the $\alpha\beta$-unsaturated ketone (XLI; R = Ac), the action of a larger excess (3 atoms of oxygen) of chromic acid was investigated. Under these circumstances, a second compound, $C_{30}H_{44-46}O_4$, m.p. 127-128°, $[\alpha]_D$-32°, ε^{2650}_{max} 4,700, was isolated in quantities insufficient to allow a detailed investigation of its structure. It differs from the known 7;11-diketoergosta-8:22-dien-3β-yl acetate (X), and the alternative



(XLI)



(X)

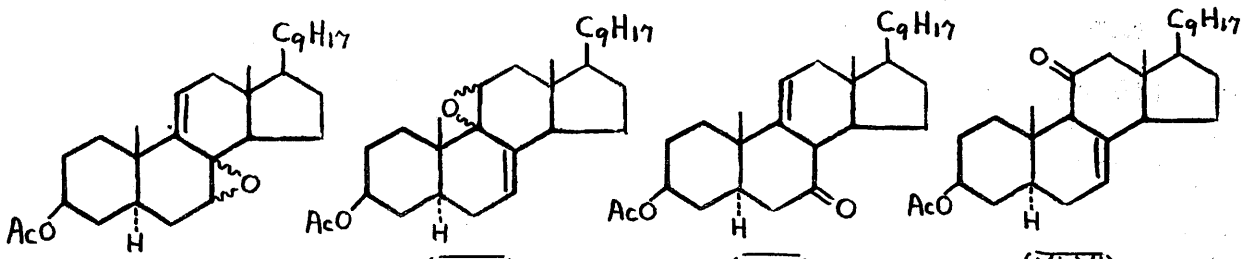


(XLII)

7:15-diketoergosta-8(14)-22-dien-3β-yl acetate (XLII) was considered a possible alternative, since the molecular rotation and light absorption characteristics were in reasonable agreement for those reported (96) for 7:15-diketoergost-8(14)-en-3β-yl acetate ($[\alpha]_D$-24°, ε^{2550}_{max} 5000). The diketodiene (XLII) has since, however, been prepared (97) by two unequivocal routes, and possesses normal light absorption intensity (ε ~10,000) for an unsaturated ketone system. The low intensity of the chromic acid product, considered possibly attributable to the dionene chromophore possessing a <u>cisoid</u> orientation

may be due to the product being a mixture of a normal unsaturated ketone with a compound transparent at 2650Å.

In view of the unpromising yields of the oxidation products described above, the oxidation of ergosteryl-D acetate with hydrogen peroxide in formic acid was next investigated. With one mole of performic acid, a compound, $C_{30}H_{46}O_3$, was isolated in good yield. Since this compound gives only a pale yellow coloration with tetranitromethane, the additional oxygen atom must have added to the nuclear conjugated diene system (in preference to the sidechain double bond), since the diene system of ergosteryl-D acetate, in common with many conjugated dienes gives a dark brown colour wit. tetranitromethane. In support of this decision, it was found that the compound did not exhibit absorption of high intensity above 2200Å. On this basis four possible structures can be envisaged for the compound, oxidation having occurred at either the 9:11- or at the 7:8-ethylenic bonds to give either an epoxide [7:8-epoxyergosta-9(11):22-dien-3β-yl acetate (XLIII) or 9:11-epoxyergosta-7:22-dien-3β-yl acetate (XLIV)] or an unconjugated ketone [7-ketoergosta-9(11):22-dien-3β-yl acetate (XLV) or 11-ketoergosta-7:22-dien-3β-yl acetate (XLVI)] The general instability of the compound, e.g. simple recrystallisation causes the appearance of selective light absorption at 2540Å favours an unconjugated ketone structure rather than an oxide, and its ready conversion (71), either by alkaline or dilute mineral acid hydrolysis, to 3β-hydroxyergosta-8:22-dien-7-one (XLI; R = H) excludes the 11-ketone (XLVI). Further evidence of the presence of the

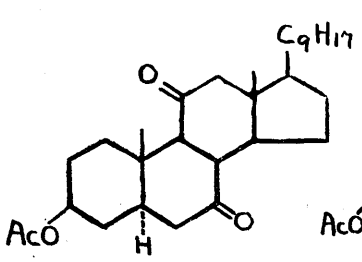


(XLIII) (XLIV) (XLV) (XLVI)

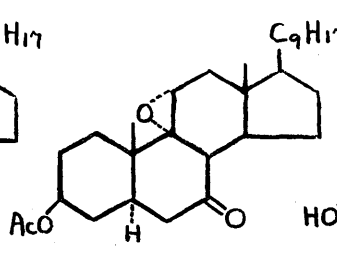
ketone system is apparent on examination of its infra-red spectrum. This shows two well-resolved bands in the carbonyl region at 1740 cm^{-1} (ascribable to the acetate group) and at 1715 cm^{-1} (ascribable to a carbonyl group), proving conclusively that the product obtained by the action of 1 mol. of performic acid on ergosteryl-D acetate is 7-ketoergosta-9(11):22-dien-3β-yl acetate (XLV). Jeger et al (76) and Tishler et al (74) have both recently described preparations of this compound reporting constants (m.p. 176-177°, $[\alpha]_D$-58°) and (m.p. 176-177°, $[\alpha]_D$-43.5°) respectively. It is considered that these preparations give a compound differing from (XLV) described here (m.p. 194-197°, $[\alpha]_D$+20°) in the orientation of the hydrogen atom at C-8, and a discussion of this stereochemical aspect is included later.

Oxidation of ergosteryl-D acetate with two mols. of performic acid gave a compound, $C_{30}H_{46}O_4$, which does not show selective absorption of high intensity above 2200Å. Since the primary product of the performic acid oxidation has been shown to be 7-ketoergosta-9(11):22-dien-3β-yl acetate (XLV), the compound $C_{30}H_{46}O_4$ is either 7:11-diketoergost-22-en-3β-yl acetate (XI) or 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XLVII), if it is assumed, as is later proven by strong alkaline hydrolysis, that the

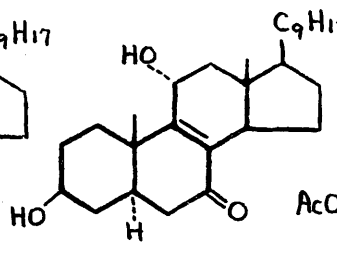
remaining nuclear bond is attacked preferentially to the sidechain double bond. Since the oxidation product differs, however, from the known 7:11-diketone (XI) (69, 75), it is



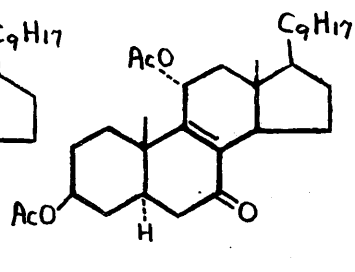
(XI)



(XLVII)



(XLVIII)



(XLIX)

presumably 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XLVII). It has also been prepared directly (71) from 7-ketoergosta-9(11):22-dien-3β-yl acetate (XLV) by addition of one mole of bromine (must protect sidechain rather than 9:11-double bond), followed by oxidation with perbenzoic acid and debromination of the product with zinc dust. The α-configuration is ascribed to the epoxide (XLVII) on the basis of the well-established preferential rear attack by reagents at the 9- and 11- positions (98).

Relatively mild alkaline hydrolysis (2%) of (XLVII) effects its conversion to a compound $C_{28}H_{44}O_3$ shown to be 3β:11α-dihydroxyergosta-8:22-dien-7-one (XLVIII). It shows ultra-violet light absorption normally associated with an αβ-unsaturated ketone, and the infra-red spectrum shows the existence of both the αβ-unsaturated ketone and hydroxyl groups. It readily gives, moreover, 3β:11α-diacetoxyergosta-8:22-dien-7-one (XLIX). Concerning the orientation of the hydroxyl group in XLVIII, the ease of

acetylation at first sight precludes the possibility of the 11β-configuration. Bearing in mind, however, that the effect of the 8:9-ethylenic linkage on the accessibility of an 11β-hydroxyl group was unknown, and might exert an anomalous neighbouring group effect (cf. Heymann and Fieser (99)), it was considered necessary to effect hydrolysis and acetylation in a related compound lacking the 8:9-ethylenic linkage. Correspondingly, it was conclusively shown that the ascribed 11α-configuration is correct, since hydrogenation and alkaline hydrolysis of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (LVII) (which gives (XLIX) on debromination), gave 3β:11α-dihydroxyergost-22-en-3-one (LXIV, R = H).

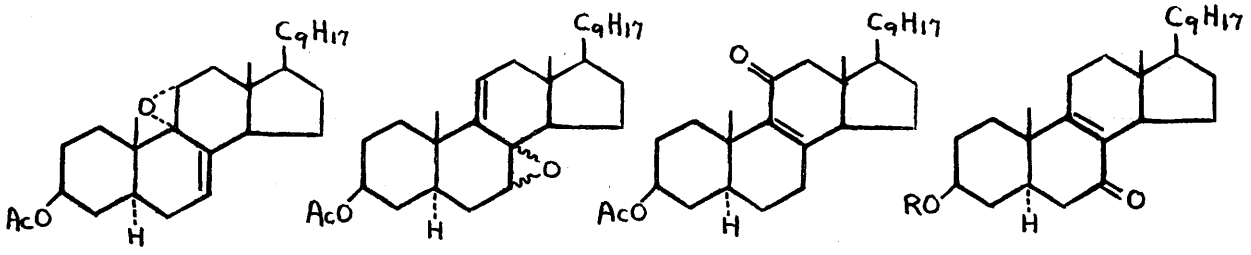
In contrast to treatment with mild alkali, strong alkaline hydrolysis (12%) of 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XLVII) followed by acetylation yielded a reaction mixture from which two components were isolated by chromatography on alumina. One of these proved to be identical with 7:11-diketoergost-22-en-3β-yl acetate (XI), the identity being established by direct comparison with a specimen prepared as described by Heusser et al (75). The mild alkali product, 3β:11α-dihydroxyergosta-8:22-dien-7-one (XLVIII) is assumed to be an intermediate in this isomerisation, and strong alkaline treatment of the diacetate (XLIX) also yields XI (70, 71). Besides providing a route to 11-ketosteroids, this experiment proves that oxidation of ergosteryl-D acetate with performic

acid (2 mols) adds two oxygen functions to the nucleus, and conclusively proves the structure ascribed to XLVIII. A similar strong alkali isomerisation of 6-ketocholest-4-en-3β-yl acetate into 3:6-diketocholestane was observed by Heilbron, Jones and Spring (100) and the conversion of 6β:21-diacetoxypregn-4-en-3:20-dione into 3:6:20-triketoallopregnan-21-ol has been reported by Herzig and Ehrenstein (101).

The second product isolated by chromatography of the acetylated reaction mixture has not been identified. Empirical analysis suggests the formula $C_{32}H_{52}O_5$. It gives a faint yellow colour with tetranitromethane in chloroform (sidechain ethylenic linkage), and does not show high intensity light absorption above 2200Å. The presence of an ethoxyl group in the molecule, taken in conjunction with the non-existence of high intensity absorption, suggests that the $\alpha\beta$-unsaturated ketone chromophore has been destroyed by the base-catalysed addition of ethanol across the activated ethylenic bond, a well-established reaction. The infra-red spectrum shows the presence of acetate and ketone groupings, and the absence of a hydroxyl group.

Parallel with the chromium trioxide and performic acid oxidation experiments described, a study was made of the oxidation of ergosteryl-D acetate with perbenzoic acid, treatment with one mole of this reagent readily yielding a mono-epoxide. Since this epoxide does not exhibit light absorption above 2200Å, it must be either

9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (VIII) or 7ξ:8ξ-epoxyergosta-9(11):22-dien-3β-yl acetate (L). The preparation of this compound was reported almost



(VIII) (L) (XVII) (XLI)

simultaneously by Tishler et al (69), who have since stated their preference for the Δ^7-9α:11α-epoxide structure (74) and by Heusser et al (75) by permonophthalic acid oxidation who also favour structure (VIII). Since both chromic acid and performic acid had attacked the 7:8-preferentially to the 9:11-double bond of ergosteryl-D acetate, it was anticipated that perbenzoic acid would react in an analogous manner; this seemed to be borne out when dilute mineral acid hydrolysis yielded the known 3β-hydroxyergosta-8:22-dien-7-one (XLI; R = H). Since, however, treatment of the epoxide with anhydrous Lewis acids (boron trifluoride or ferric chloride) has been shown to give 3β-acetoxyergosta-8:22-dien-11-one (XVII) in contrast to isomerisation to the 7-ketone in polar media, the assignation of the epoxide group to the 7:8-position on this evidence is no longer tenable. Evidence in favour of the 9α:11α-epoxy structure (VIII) is apparent in the failure of lithium aluminium hydride to effect reductive fission of the oxide. Treatment of the epoxide with this reagent under reflux in ether -

benzene or tetrahydrofuran solution gave ergosterol-D epoxide, characterised by direct comparison with a specimen prepared by mild alkaline hydrolysis of ergosteryl-D acetate epoxide and by re-acetylation to the latter compound. In a review of the reactions of lithium aluminium hydride (102), it was stated that there were no known cases of a failure of lithium aluminium to reduce an epoxide; since this time, however, well authenticated cases have been reported (83, 103, 104) of the failure in the case of steroidal 9α:11α-epoxides, which now appears to be generally characteristic. In view of this last observation and the fact that there are no known transformations which cannot be explained by assumption of the 9α:11α-epoxy structure, the compound obtained by perbenzoic acid oxidation of ergosteryl-D acetate is believed to be 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (VIII). The stability of this compound to alkali is noteworthy in view of the extreme reactivity of 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XLVII) under the same conditions.

The final experiment on the oxidation of ergosteryl-D acetate involved potassium permanganate as the oxidising agent. Under the mild conditions employed, unchanged starting material was recovered in good yield.

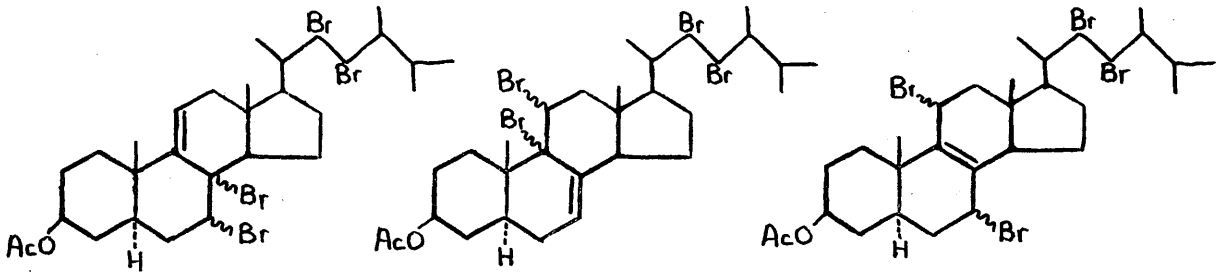
As a consequence of the examination of a possible preparation of ergosteryl-D acetate by bromination of 5-dihydroergosteryl acetate, 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (ergosteryl-D acetate 22:23-dibromide)

became available. It was realised that this compound offered advantages as a starting material in the oxidation investigations of the 7:9(11)-ergostadiene derivatives, since the sidechain ethylenic linkage, adequately protected during oxidations involving excess oxidant and possibly permitting selective nuclear hydrogations, could be easily re-established prior to subsequent sidechain degradation.

Treatment of 5-dihydroergosteryl acetate in ether solution at -60° with bromine gives in about 50% yield a tetrabromoergostenyl acetate which separates directly from the reaction mixture. This compound is only moderately stable in the solid state and in solution in dioxan and benzene, and in most common solvents, e.g. chloroform, alcohol and acetic acid suffers profound decomposition after a short time at room temperature. It is partially debrominated by sodium iodide to 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (ergosteryl-D acetate 22:23-dibromide) (LIV), the structure of which was established by its characteristic 7:9(11)-dienic ultra-violet absorption spectrum and by its facile conversion into ergosteryl-D acetate by debromination with zinc dust.

The structure of the intermediate tetrabromide has not been established with certainty, and the possibilities, 7:8:22:23-tetrabromoergost-9(11)-en-3β-yl acetate (LI), 9:11:22:23-tetrabromoergost-7-en-3β-yl acetate (LII) and 7:11:22:23-tetrabromoergost-8-en-3β-yl acetate (LIII), which could arise either by 1:4 addition of bromine or by allylic

rearrangement of LI or LII, have been considered. The observations that the same tetrabromide can also be obtained



(LI) (LII) (LIII)

by direct bromination of ergosteryl-D acetate, and that sodium iodide removes the nuclear bromine atoms, favour the structures (LI) and (LII), but discrimination between these has not been possible, work in this direction being hampered by extremely labile nature of the compound. Filtration of a benzene solution of the tetrabromide through a column of alumina for example, effects a noteworthy transformation, with adsorption of a dark green colour on the alumina. The isolated product, $C_{30}H_{44}O_2Br_2$, of this treatment (i.e., elimination of two moles of hydrogen bromide) surprisingly does not exhibit high intensity absorption above 2200Å, but on the basis of extremely high intensity absorption at 2100Å, the possibility of aromatisation, either of ring B or C, cannot be excluded.

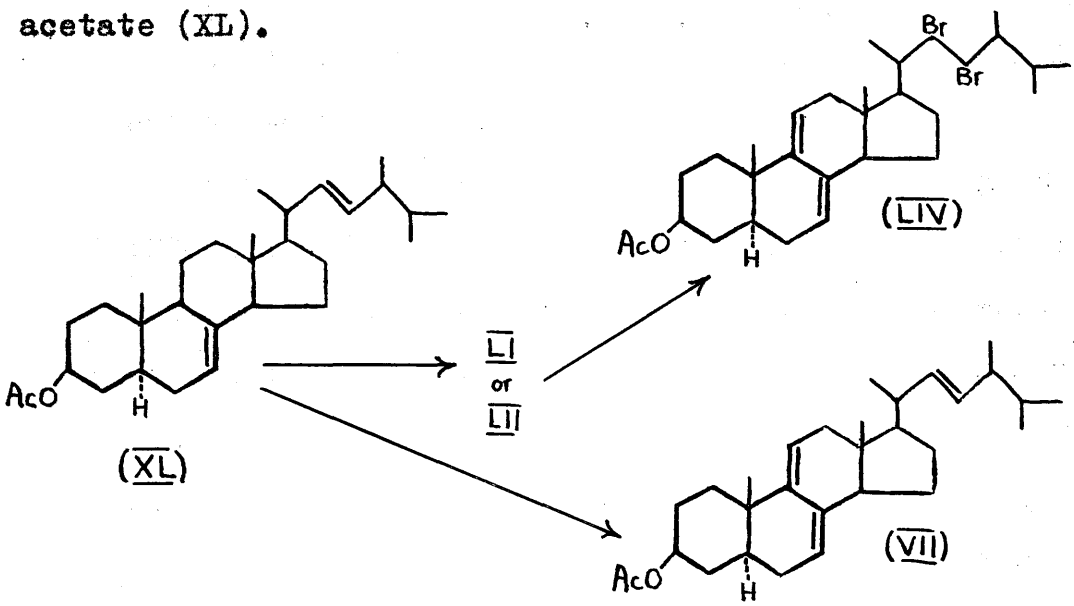
Numerous variations were employed in the reaction conditions of the preparation of tetrabromoergostenyl acetate, the recommended procedure being described in the

experimental section. In general, the ether solvent should be dry, and the temperature should be allowed to rise from -60° to -10° over a period of 2 hours, whereafter the precipitated tetrabromide should be removed from the rapidly decomposing reaction liquor. Attempts to obtain crystalline material from the tetrabromide filtrate proved abortive due to the susceptibility to rearrangement and decomposition in common solvents at room temperature. Treatment of the filtrate with a solution of sodium iodide in ethanol, however, removed excess bromine and caused nuclear debromination, enabling solid material with the characteristic 7:9(11)-diene absorption spectrum (though of diminished intensity) to be isolated. Chromatographic purification of this solid material yielded two fractions which were crystallised to constant melting point, but which are believed, on the basis of their physical constants, to be mixtures each containing ergosteryl-D acetate 22:23-dibromide as one of its constituents; a comparison of the physical constants of possible contaminants suggests the presence of a high laevorotatory impurity.

Compound.	$[\alpha]_D$	%C	%H	Light Absorption ($E^{1\%}_{1\ cm.}$) 2350Å	2420Å
Ergosteryl-D Acetate Dibromide	+32°	60.2	7.75	320	335
Ergosteryl-D Acetate	+30°	82.1	10.6	340	380
5-Dihydroergosteryl Acetate.	-21°	81.8	11.0	0	0
5-Dihydroergosteryl Acetate Dibromide	-5° Calc.	60.0	8.1	0	0
1st Mixture m.p. 165°	+7°	74.9	9.8	290	320
2nd Mixture m.p. 229-231°	-10°	59.9	7.8	210	230

With regard to the first mixture, the high intensity of light absorption and analytical data are in good agreement for a mixture of ergosteryl-D acetate dibromide and ergosteryl-D acetate, but the specific rotation is considerably lower than would be given by such a mixture. The analytical data for the second mixture shows it to be of the dibromide type, $C_{30}H_{46-48}O_2Br_2$, and the light absorption is in agreement for a mixture of the dibromides of ergosteryl-D acetate and 5-dihydroergosteryl acetate. Again, however, the specific rotation is outwith the range given by such a mixture (The specific rotation value for the unknown 5-dihydroergosteryl acetate 22:23-dibromide was calculated from molecular rotation difference theory. Budziarek, Johnson & Spring (private communication) report $[\alpha]_D$-6° for this compound.)

Treatment of solution of tetrabromoergostenyl acetate at room temperature with ethanolic sodium iodide causes immediate liberation of iodine, to give 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (LIV) in almost quantitative yield. This latter compound (LIV) was debrominated in quantitative yield, by heating an ether-ethanol solution with zinc dust, to give ergosteryl-D acetate. Ergosteryl-D acetate was also prepared in less pure form in 70% yield without isolation of the intermediate tetrabromide or dibromide, by direct treatment with zinc dust of the reaction mixture obtained by bromination of 5-dihydroergosteryl acetate (XL).



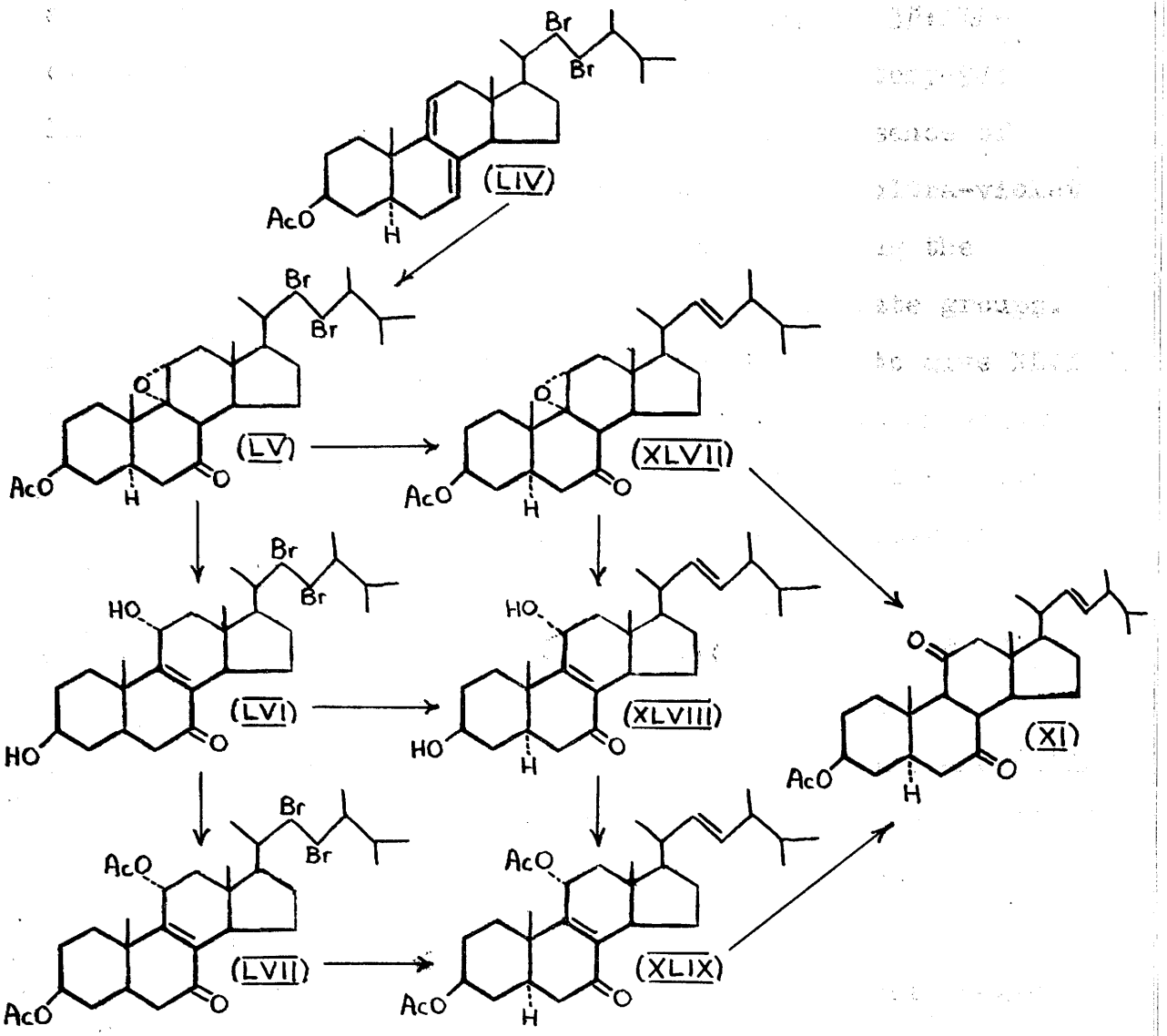
The use of chloroform as the bromination solvent, as described by Eck and Hollingsworth (92) in the corresponding reaction with cholest-7-ene, introduced difficulties, which were mitigated by replacement by ether. Since chloroform has greater solvent powers, the tetrabromide remains in solution, and cannot be purified (as in the use of ether)

by filtration from the more soluble components of the mixture. In working up, moreover, the reaction solution must be maintained at 0°, as at room temperature, decomposition with the typical Tortelli-Jaffé coloration (chloroform, acetic acid, bromine) and rearrangement to mixtures of the ergosterol-B type occurs. In an attempt to obviate these objections, which were considered mainly attributable to the liberation of hydrogen bromide, an experiment was performed in which pyridine was added to the reaction mixture. In this case, the isolated product showed light absorption characteristic of a steroidal 7:9(11)-diene, but the intensity suggested the presence of only 50% ergosteryl-D acetate in a mixture containing laevorotatory contaminants.

Unlike the tetrabromo precursor, ergosteryl-D acetate 22:23-dibromide is extremely stable, and was hydrolysed by alkali to 22:23-dibromoergosta-7:9(11)-dien-3β-ol, characterised by re-acetylation to (LIV). Treatment of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate with hydrogen peroxide in acetic acid gave a compound, $C_{30}H_{46}O_4Br_2$, which was identified as 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (LV), on which the rearrangements and characterisations outlined below have been effected.

The compound (LVII) gave, on treatment with Brady's reagent, a 2:4-dinitrophenylhydrazone, the light absorption of which at 3660Å, is in good agreement for a non-conjugated ketone (105, 106, 107). The identity of the ketone group

was further established by formation, under neutral conditions, of a semicarbazone, and by the characteristic infra-red absorption spectrum. Debromination of



3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (LV) by zinc dust, either in acetic acid or ether-ethanol solution, gave 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (XLVII) identical with the specimen obtained by performic acid oxidation of ergosteryl-D acetate, thus confirming the structure attributed to XLVII on the evidence

previously discussed.

Treatment of the dibromoketoxide (LV) with alkali gave 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (LVI) in an exactly analogous manner to the formation of 3β:11α-dihydroxyergost-8-en-7-one (XLVIII) from 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (XLVII). The presence of the αβ-unsaturated ketone is shown in both the ultra-violet and infra-red spectra, the latter also confirming the presence of hydroxyl groups and absence of acetate groups. LVI was further characterised by debromination to give XLVIII, and by acetylation to 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (LVII). The infra-red spectrum of the latter compound showed the absence of hydroxyl groups (empirical analysis is inconclusive), thus establishing the α-configuration of the 11-hydroxyl group in LVI, and debromination gave the previously prepared 3β:11α-diacetoxyergost-8-en-7-one (XLIX). Both (XLVII) and (XLIX) were converted to 7:11-diketoergost-22-en-3β-yl acetate (XI) by heating under reflux with strong alkali as described previously.

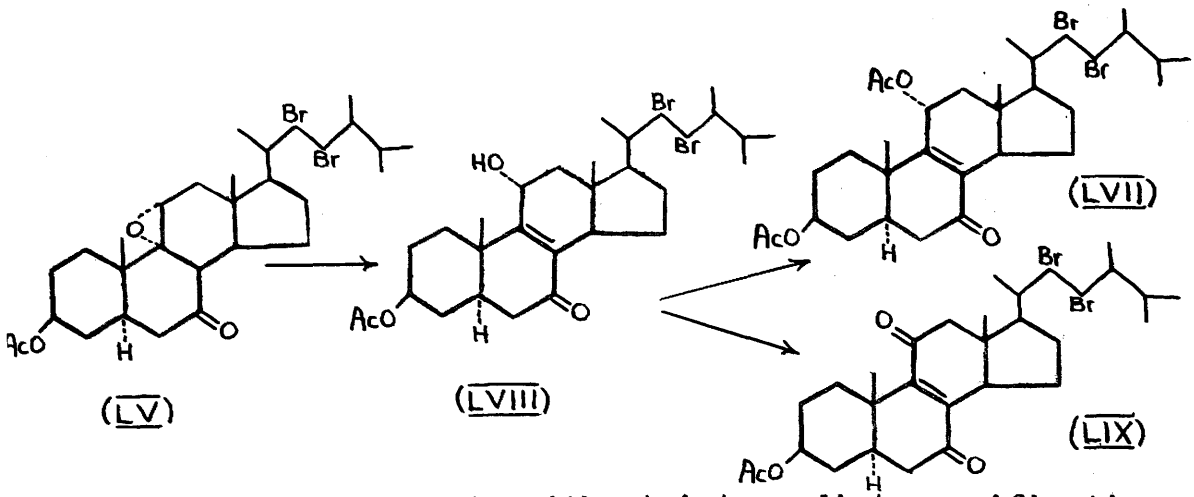
In an attempt to improve the yield of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (LV) obtained by peracetic acid oxidation, attention was next turned to the effect of performic acid on ergosteryl-D acetate 22:23-dibromide (LIV). The reaction mixture yielded an isomer, $C_{30}H_{46}O_4Br_2$, m.p. 220-221° in the crystalline form of needles (from acetone), which differed from the peracetic

acid product, m.p. 234-235° in the crystalline form of plates (from chloroform-methanol), although both possessed the same specific rotation value. In view of its initial non-identification as the known 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (LV), it was considered that this compound might be the isomeric 7ξ:8ξ:9α:11α-diepoxide, but subsequent reactions (a) alkaline hydrolysis to give the known 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (LVI) (b) debromination to give the known 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (XLVII) (c) the formation of a 2:4-dinitrophenylhydrazone identical with that prepared from the known dibromoketoxide, indicated that this isomer was a crystalline variant or dimorphic form of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (LV). As proof of this, the two forms (i) m.p. 220-221°, needles from acetone and (ii) m.p. 234-235°, plates from chloroform-methanol, were interconverted by reversal of the crystallising solvents, and the infra-red spectra were found to be identical within experimental limitation.

A noteworthy feature of the described performic acid oxidations was that they were performed simply by shaking the reactants at room temperature in a one phase solution, made possible by the addition of ethyl acetate.

In an experiment in which the crude performic acid oxidation product was crystallised from a small bulk of solvent, a mixture of crystalline forms was apparent. On chromatographic treatment of this mixture, which did not

exhibit high intensity light absorption, virtually no material was eluted from the column, until methanol was introduced to the eluting solvent. On this behaviour, it was anticipated that the product contained one or more hydroxyl groups, and the possibility that the basicity of the alumina was sufficient to cause the alkaline rearrangement of the 9α:11α-epoxy-7-ketone system to the 11α-hydroxy-8-en-7-one to give 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one (LVIII) suggested itself. The product exhibited light absorption at 2540Å, characteristic of an αβ-unsaturated ketone, and the infra-red spectrum, besides corroborating this, showed absorption maxima in the acetate and hydroxyl regions. Acetylation of this

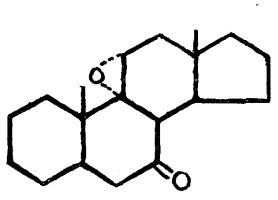


chromatography product, without intermediate purification, gave 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (LVII), and oxidation with chromic acid (1.1 atoms O) gave in good yield 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate (LIX), identified by its ultra-violet light absorption at 2700Å and by direct comparison with an authentic specimen, prepared as described by Budziarek, Johnson and Spring (73).

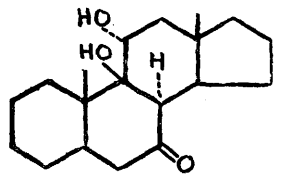
Important features of this procedure are (i) that the chromatographic rearrangement, unlike mild alkali rearrangement, automatically affords selective protection at C-3 and (ii) the oxidation proceeds extremely smoothly without giving a mixture of products as is obtained by oxidations of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate (73).

Chromatography of 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one gave 3β-acetoxy-11α-hydroxyergosta-8:22-dien-7-one in an analogous manner. As in the case of LVIII, the latter compound proved difficult to purify to constant melting-point.

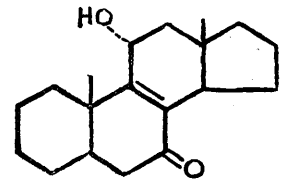
The ease of rearrangement of the ketoxide system (a) to the hydroxyenone system (C) by chromatography or hydrolytic treatment permits speculation on the stereochemistry at C-8, C-9 and C-11. If it is assumed, as is reasonable, that the mechanism consists of hydrolytic



(A)



(B)

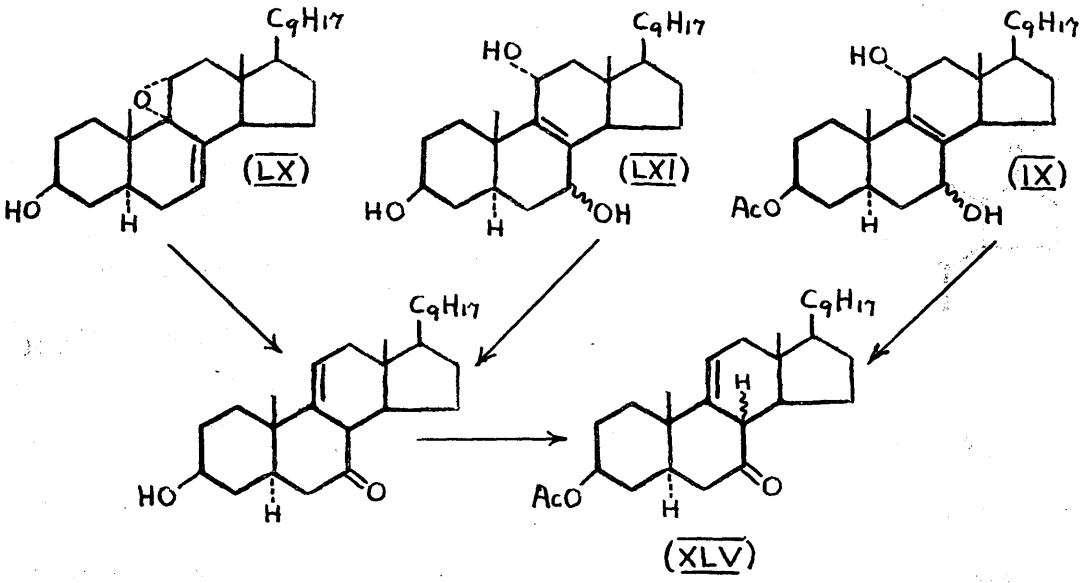


(C)

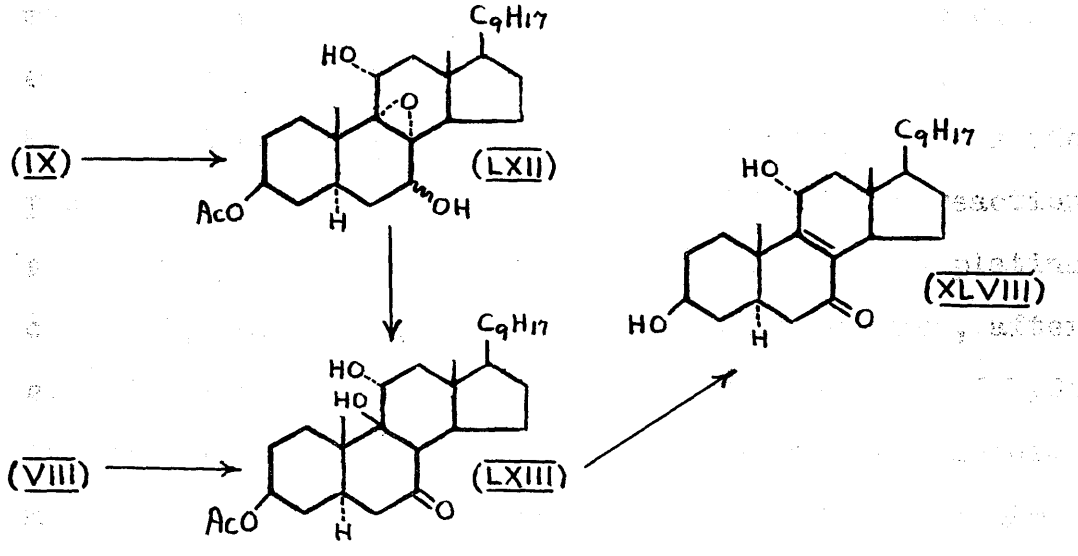
fission of the oxide to give a system (B) which dehydrates to the product (C), and it has been proved that the 11-hydroxyl group has the α-configuration, then the normal <u>trans</u> fission of an epoxide necessitates the 9-hydroxyl group being β-orientated (In the cleavage of oxides, a

Walden inversion occurs at the carbon atom at which the carbon-oxygen bond is ruptured. cf. (14) p. 221 and (108)). Since ease of dehydration is associated with trans-elimination, the hydrogen atom at C-8 must be α-oriented i.e., have the unnatural configuration. Moreover, as 7-ketoergosta-9(11):22-dien-3β-yl acetate (XLV) has been related to 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (XLVII) by bromination, perbenzoic acid oxidation and debromination, the α-configuration at C-8 can also be assumed in XLV.

The isomeric unconjugated ketone, 7-ketoergosta-9(11):22-dien-3β-yl acetate was obtained by Tishler et al (74), both by acidic rearrangement of 9α:11α-epoxyergosta-7:22-dien-3β-ol (LX) and of 3β:7ξ:11α-trihydroxyergosta-8:22-diene LXI under critical control, followed by acetylation, and by Jeger et al (76) by a dehydration of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (IX) under the influence of hydrogen peroxide in acetic acid. The triol monoacetate (IX) has also been converted (76) through the intermediate epoxide (LXII) to 9ξ:11α-dihydroxyergost-22-en-3β-yl acetate (LXIII) identical with that prepared (71) by a series of reactions in which ergosteryl-D acetate monoxide is treated with 1 mol of bromine, then with an excess of perbenzoic acid, and debrominated with zinc dust. Since this latter preparation involved the opening of 9α:11α-epoxide, the 9-hydroxyl group was ascribed the β-configuration. The known difficulty of dehydrating this compound (LXII) by alkaline hydrolysis (Reflux for 2½ days with 2.5% or 8 hours

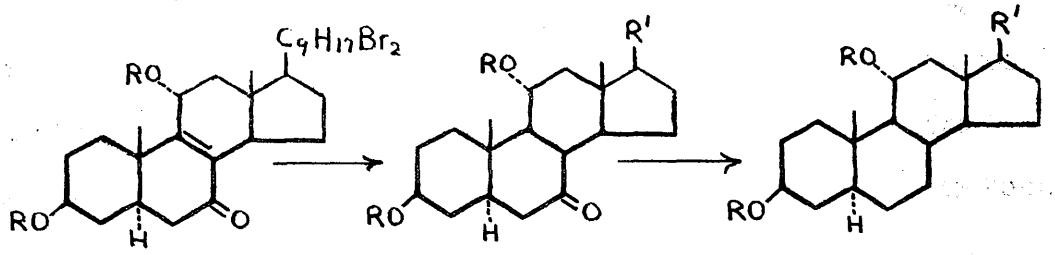


with 5% alkali) to 3β:11α-dihydroxyergosta-8:22-dien-7-one (XLVIII) and its stability to alumina suggest that cis-dehydration is being enforced, and hence the hydrogen atom at C-8 must have the natural or β-configuration.



The final problem investigated was the reduction of the 8:9-ethylenic linkage in the 11-hydroxy-8-en-7-one system, in order to permit the facile removal of the 7-keto group by Wolff-Kishner reduction, thus obviating

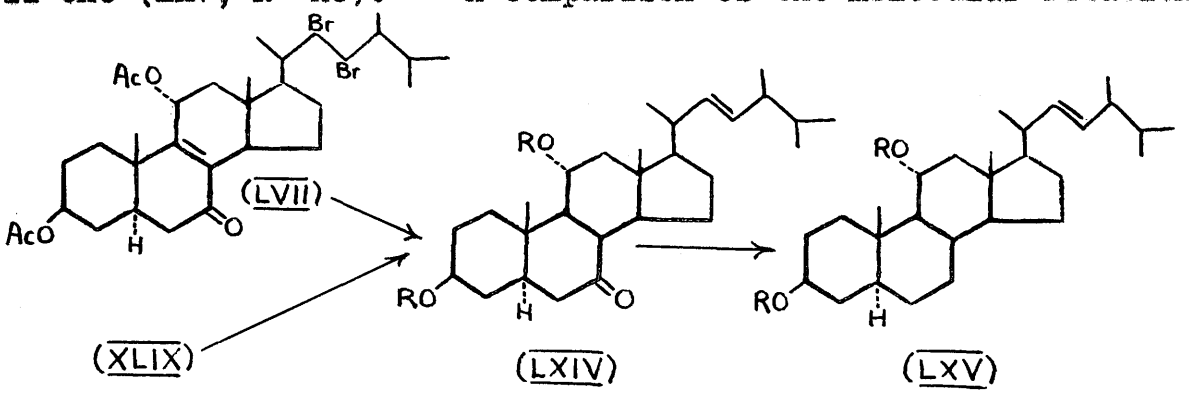
the difficulty of performing this reaction on an $\alpha\beta$-unsaturated ketone.



The compound chosen for this examination was 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one, since due to the known difficulty of hydrogenating an 8:9-double bond (109) it was considered that an unprotected 22:23-double bond would be preferentially reduced. The first method attempted consisted of performing the hydrogenation in ethanol solution with palladium black catalyst; this method was unsuccessful in that after the uptake of 1 mole of hydrogen, the $\alpha\beta$-ketone moiety still existed. Hydrogenation to completion gave a mixture, which suffered profound decomposition on concentration of the reaction solution. A second attempt, using dioxan and platinum catalyst, resulted in the isolation of a mixture, after stopping the reaction on the uptake of one mole of hydrogen. Acetylation and chromatography of this mixture yielded two main fractions one of which was identified as 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (i.e. acetylated starting material). The second fraction was a mixture of products exhibiting 7:9(11)-diene steroidal light absorption of diminished intensity (i.e. hydrogenolysis had occurred);

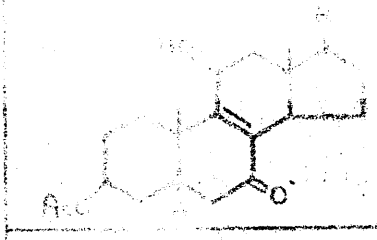
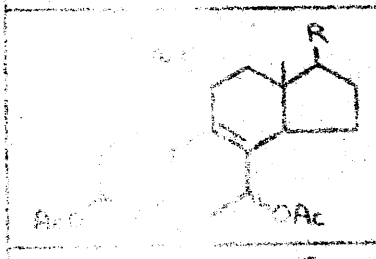
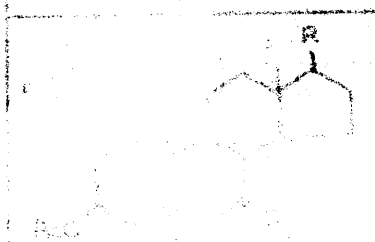
the ultra-violet spectrum, with absorption at 2100Å, also indicated that partial debromination had occurred.

The required selective hydrogenation was finally successfully accomplished by performing the reaction in ethanolic potassium hydroxide. This procedure, for reducing the ethylenic linkage of an $\alpha\beta$-unsaturated ketone, has been previously utilised by Chemerda, Chamberlin, Wilson and Tishler (110) (cf. also Oliveto, Gerold and Hershberg (110)) and by this route 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one was converted to 3β:11α-dihydroxyergost-22-en-7-one (LXIV; R = H), the platinum catalyst also causing debromination. The same compound (LXIV, R = H) was also prepared similarly by Jeger et al, by selective reduction of 3β:11α-diacetoxyergosta-8:22-dien-7-one (XLIX), i.e. without protection of the sidechain ethylenic linkage. The latter workers also effected the necessary Kishner-Wolff reduction to 3β:11α-diacetoxyergost-22-one (LXV; R = Ac). A comparison of the molecular rotations

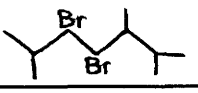
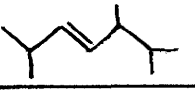
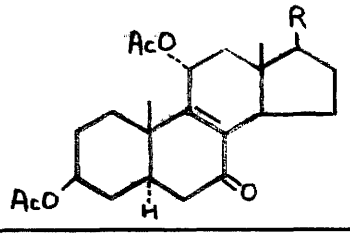
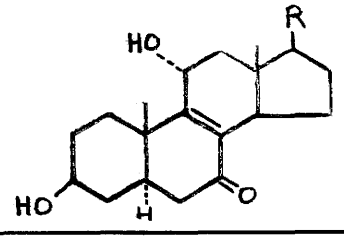
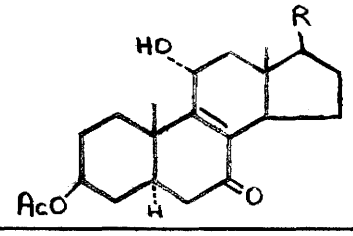
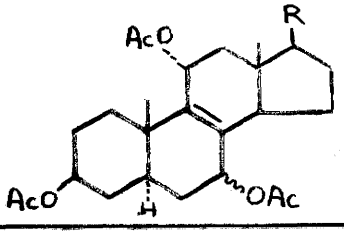
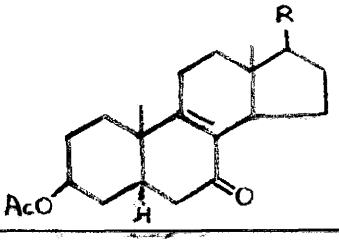


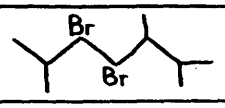
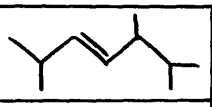
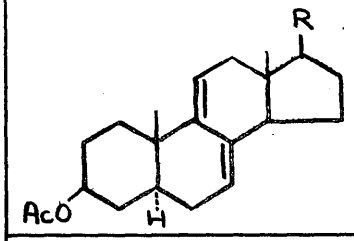
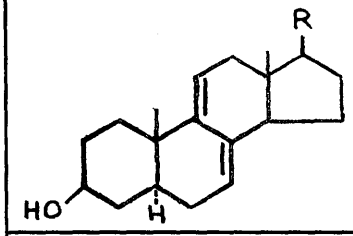
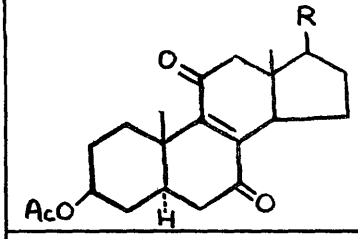
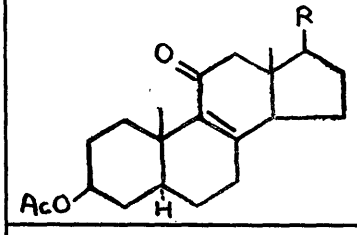
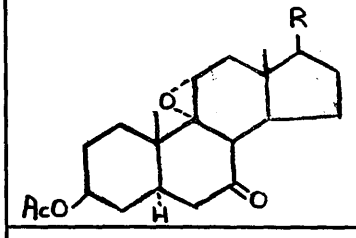
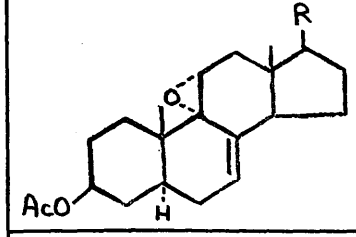
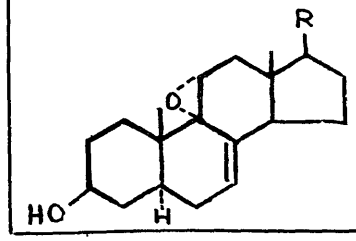
of the various ergosterol derivatives described in the experimental section and in the appropriate publications (70, 71,72,73,75,69) with their corresponding 22:23-dibromides

permits some generalisations. Examination of the 12 pairs of compounds listed shows that in all cases, sidechain bromination results in an increased dextrotation, the values varying between 20 and 100 units, with a mean value of 62 units. With such a variance of nuclear substituents, it is virtually certain that, in some cases, the individual groups interfere with one another's rotation contribution - i.e., exert vicinal action. In this connection, it should be noted that the largest divergences from the mean value exist in those compounds possessing either an 11-keto group or a 9α:11α-epoxy group. If the examination be restricted to the compounds lacking these functions, the values are found to vary between 47 and 76, with a mean value of 59 units.

			-50°
	631 -82°	471 -142°	-60°
	$C_{34}H_{52}O_6Br_2$ +77° 717 +550°	$C_{34}H_{52}O_6$ +88° 557 +490°	-60°
	$C_{30}H_{46}O_3Br_2$ -90°	$C_{30}H_{46}O_3$ -51° 455 -255°	-76°

Comparison of molecular rotations of ergosterol derivatives with their corresponding 22:23-dibromides.

Formula $[\alpha]_D$ Mol. Wt. $[M]_D$			Δ
	$C_{32}H_{48}O_5Br_2$ +18° 673 +121°	$C_{32}H_{48}O_5$ +12° 513 +62°	−59°
	$C_{28}H_{44}O_3Br_2$ +4° 588 +24°	$C_{28}H_{44}O_3$ −6° 429 −26°	−50°
	$C_{30}H_{46}O_4Br_2$ −13° 631 −82°	$C_{30}H_{46}O_4$ −30° 471 −142°	−60°
	$C_{34}H_{52}O_6Br_2$ +77° 717 +550°	$C_{34}H_{52}O_6$ +88° 557 +490°	−60°
	$C_{30}H_{46}O_3Br_2$ −29° 615 −179°	$C_{30}H_{46}O_3$ −56° 455 −255°	−76°

			△
	$C_{30}H_{46}O_2Br_2$ +32° 599 +192°	$C_{30}H_{46}O_2$ +30° 439 +132°	−60°
	$C_{28}H_{44}OBr_2 . CH_3OH$ +26° 589 +152°	$C_{28}H_{44}O$ +30° 397 +119°	−33°
	$C_{30}H_{44}O_4Br_2$ +29° 628 +182°	$C_{30}H_{44}O_4$ +24°, +18·5° 469 +113°, +87°	−69° −95°
	$C_{30}H_{46}O_3Br_2$ +98° 615 +600°	$C_{30}H_{46}O_3$ +110° 455 +500°	−100°
	$C_{30}H_{46}O_4Br_2$ −47° 631 −297°	$C_{30}H_{46}O_4$ −83° 471 −392°	−95°
	$C_{30}H_{46}O_3Br_2$ −25° 615 −154°	$C_{30}H_{46}O_3$ −38° 455 −173°	−19°
	$C_{28}H_{44}O_2Br_2 . CH_3OH$ −27° 604 −163°	$C_{28}H_{44}O_2 . CH_3OH$ −41° 445 −183°	−20°

EXPERIMENTAL.

All m.p's were determined using a standardised N.P.L. thermometer.

Specific rotations were determined in chloroform solution (except where otherwise stated) in a 1 dm. tube at room temperature.

Ultra-violet absorption spectra were measured in ethanol solution (except where otherwise stated) using a Unicam SP.500 spectrophotometer.

Infra-red absorption spectra were determined by Dr. I.A. Brownlie, and micro-analyses by Dr. A.C. Syme and Mr. Wm. McCorkindale, to whom grateful acknowledgements are due.

The alumina used for chromatographic purposes was that supplied by Savory and Moore, Grade II (except where stated), standardised according to Brockmann.

Ergosteryl Acetate.

Acetic anhydride (300 c.c.) was added to a solution of ergosterol (300 g.) in warm pyridine (1800 c.c.; freshly distilled), the air in the flask displaced by nitrogen, and the mixture maintained in the dark at room temperature for 18 hours. The product, which had crystallised, was removed by filtration, washed with hot water, and partially dried at 50°. Crystallisation from chloroform methanol gave ergosteryl acetate as large lustrous leafs, m.p. 173-175°.

$[\alpha]_D$ -93° (C, 1.8)

5-Dihydroergosteryl Acetate.

Raney nickel sludge (Org. Synth., 29, 25) (W6; 15-20 c.c.), washed twice by decantation with benzene (2 x 50 c.c.) was added to a solution of ergosteryl acetate (35 g.) in benzene (300 c.c.; Analar), and the mixture shaken at 17° under slight positive pressure in an atmosphere of hydrogen until 2140 c.c. had been absorbed (13-20 minutes). A blank experiment indicated that the solvent absorbed 150 c.c. The filtered reaction solutions from five such experiments were combined, the solvent removed under reduced pressure to yield a crystalline residue, m.p. 172-174°, which gave a yellow colour with tetranitromethane in chloroform. Crystallisation from chloroform - methanol gave 5-dihydroergosteryl acetate (93 g.) as lustrous plates, m.p. 178-181°, $[\alpha]_D$ -19.5° (C, 2.0). A further quantity (69 g.), m.p. 177-179°, $[\alpha]_D$ -18° (C, 1.8) was obtained

from the mother liquor. Recrystallisation of the product from chloroform - methanol raised the m.p. to 180-182°.

$[\alpha]_D$-20.5° (C, 2.1)

Found: C, 81.4 ; H, 11.0.

Calc. for $C_{30}H_{48}O_2$: C, 81.8 ; H, 11.0%.

No high intensity light absorption above 2200Å.

Ergosteryl-D Acetate.

A warm solution of mercuric acetate (95.6 g., 1.5 mols.) in stabilised glacial acetic acid (1300 c.c.) was added to a solution of 5-dihydroergosteryl acetate (44 g.) in dry chloroform (550 c.c.) and the mixture shaken for 2½ hours. After standing for 12 hours, the mixture was shaken for a further 2½ hours, then the precipitated mercurous acetate (61 g.) removed by filtration. The filtrate, after standing 20 hours, deposited a further quantity (6 g.) of mercurous acetate which was removed; it was then concentrated under reduced pressure below 50° to a volume of 440 c.c., and the resulting precipitate (31 g.) removed by filtration, and washed with acetic acid and cold methanol. The product was dissolved in chloroform, filtered, and crystallised by addition of methanol to give ergosteryl-D acetate (18 g.) as blades, m.p. 168-172°, $[\alpha]_D$+19° (C, 1.0). Recrystallisation of the product from chloroform - methanol to constant specific rotation raised the m.p. to 176°.

$[\alpha]_D$+30° (C, 1.9)

Found: C, 81.8 ; H, 10.5.

Calc. for $C_{30}H_{46}O_2$: C, 82.1 ; H, 10.6%.

Light absorption: maxima at 2350 (ε = 15,500) and 2420Å (ε = 17,000) and an inflection at 2510Å (ε = 12,500).

It gives a dark brown colour with tetranitromethane in chloroform. The crystallisation mother liquors can be taken to dryness, coloured impurities removed by filtration of a benzene - light petroleum solution through alumina, and the filtered solution evaporated to give a colourless residue (constants of order m.p. 160-166°, $[\alpha]_D$ +4°). This material is suitable for similar re-treatment with mercuric acetate to yield more ergosteryl-D acetate.

Ergosterol-D.

Hydrolysis of ergosteryl-D acetate with ethanolic potassium hydroxide in the usual way gave ergosterol-D as felted needles, m.p. 165-167° from chloroform - methanol

$[\alpha]_D$+30° (C, 1.4).

Light absorption: maxima at 2350 (ε = 15,300) and 2420Å (ε = 17,000) and an inflection at 2510Å (ε =12,000)

Oxidation of Ergosteryl-D Acetate with Chromic Acid.

(a) Ergosteryl-D acetate (2.19 g.) in stabilised acetic acid (200 c.c.) was treated dropwise during 45 minutes at 95° with stirring with a solution of chromium trioxide in 95% acetic acid (20 c.c., 1.044N, 2 atoms O equiv.) After

stirring for a further hour at 95°, the reaction solution was concentrated under reduced pressure, diluted with water, and extracted with ether. The ethereal extract was washed successively with water, potassium hydroxide solution (5%) and water and dried (Na_2SO_4). Removal of the ether gave a yellow semi-crystalline solid which on trituration with warm methanol (100 c.c.) yielded a pale yellow solid (800 mg.) m.p. 173-176°, which gave a brown colour with tetranitromethane in chloroform. One crystallisation from chloroform - methanol gave unchanged ergosteryl-D acetate, m.p. 175-176°, alone or mixed with an authentic specimen. Concentration of the methanolic mother liquor yielded solid (310 mg.), m.p. 152-162° which gave a yellow colour with tetranitromethane in chloroform. Recrystallisation of this solid from methanol (10 c.c.) gave plates (110 mg.) m.p. 168-170°, a solution of which in light petroleum (b.p. 40-60°) was chromatographed on a column (12 x 1.5 cm.) of alumina.

	Eluant.	Volume (c.c)	Residue Wt.	
1.	Light petrol (b.p. 40-60°)	140	Nil.	-
2.	Light petrol (b.p. 40-60°)-benzene (4:1).	100	Nil.	-
3.	Light petrol (b.p. 40-60°)-benzene (2:1).	50	Solid.	15 mg.
4.	Light petrol (b.p. 40-60°)-benzene (2:1)	50	Wax	50 mg.
5.	Light petrol (b.p. 40-60°)-benzene (1:1)	50		

	Eluant.	Volume (c.c)	Residue	Wt.
6.	Light petrol (b.p. 40-60°)-benzene (1:1)	50	Solid	Trace.
7.	Light petrol (b.p. 40-60°)-benzene (1:2)	170	Solid	40 mg.
8.	Benzene.	100	Gum	Negligible
9.	Benzene.	100	Nil.	-
10.	Benzene-Methanol (99:1)	100	Nil.	-
11.	Methanol.	100	Gum.	Trace.

Fraction 3 was ergosteryl-D acetate, m.p. 165-170° giving a dark brown colour with tetranitromethane in chloroform. Combined fractions 4 and 5, after one crystallisation from methanol, gave 3β-acetoxyergosta-8:22-dien-7-one as plates, m.p. 203°, raised to 208-211° on three recrystallisations from methanol.

$[\alpha]_D$ -56° (C, 1.2)

Found: C, 79.4 ; H, 10.5.

Calc. for $C_{30}H_{40}O_3$: C, 79.2 ; H, 10.2%.

Light absorption: maximum at 2520Å (ε = 10,100)

Stavely and Bollenback (loc. cit.) give m.p. 206-208° $[\alpha]_D$ -53°. The acid fraction, obtained by potassium hydroxide washing of the ether extract, yielded a dark brown resin (180 mg.).

(b) A solution of ergosteryl-D acetate (2.19 g.) in warm stabilised acetic acid (220 c.c.) was rapidly chilled with vigorous stirring. A solution of chromium trioxide in acetic acid (20 c.c.; 1.044 N, 2 atoms O equiv.) was added dropwise over 4 hours at 15° to the stirred suspension.

After the addition of more chromium trioxide in acetic acid (10 c.c.; 1.044 N) over 20 minutes, the resulting solution was kept at room temperature for 60 hours. Methanol (2 c.c.) was added, the acetic acid removed under reduced pressure, diluted with water, and extracted with ether. The neutral fraction, isolated as in the above experiment, was a light brown resin which solidified on trituration with methanol. This solid (610 mg.), m.p. 154-166° gave a pale yellow colour with tetranitromethane in chloroform and showed light absorption at 2520 and 2640Å; it was dissolved in light petroleum (b.p. 40-60°)-benzene (10 c.c., 4:1) and chromatographed on a column (10 x 2 cm.) of alumina. Washing with the same solvent mixture (500 c.c.) was followed by elution with light petroleum (b.p. 40-60°)-benzene (500 c.c.; 2:1) to give a yellow solid (205 mg.). Slow evaporation of a methanol solution of this solid gave a mixture of long yellow needles and short colourless needles which were separated mechanically. Sublimation of the yellow needles (8.2 mg.) in high vacuum gave a compound (5.1 mg.), m.p. 125-127°.

$[\alpha]_D$ -32° (C, 0.9).

Found: C, 76.3 ; H, 10.1.

$C_{30}H_{44}O_4$ requires : C, 76.9 ; H, 9.5%.

Light absorption: maximum at 2650Å (ε = 4,700).

Crystallisation of the short needles yielded 3β-acetoxyergosta-8:22-dien-7-one. Elution of the column with light petroleum (b.p. 40-60°)-benzene (700 c.c.; 2:1) yielded a solid (360 mg.) m.p. 190-192°, which on crystallisation from

methanol gave 3β-acetoxyergosta-8:22-dien-7-one.

3β-Hydroxyergosta-8:22-dien-7-one.

3β-Acetoxyergosta-8:22-dien-7-one (200 mg.) was heated under reflux for 1 hour with aqueous methanolic potassium hydroxide (25 c.c.; 2%). Isolation of the product in the usual way by means of ether gave 3β-hydroxyergosta-8:22-dien-7-one (80 mg.) as plates, m.p. 175-177° from methanol.

$[\alpha]_D$ -42° (C, 0.8).

Found: C, 78.0 ; H, 11.1

$C_{28}H_{44}O_2.CH_3OH$ requires : C, 78.3 ; H, 10.9%

Light absorption: maximum at 2520Å (ε = 11,100).

Attempted Oxidation of Ergosteryl-D Acetate with Potassium Permanganate.

A solution of potassium permanganate (0.332 g.; 1.05 atoms O) in aqueous acetic acid (90%; 27.7 c.c.) was added dropwise with shaking to a solution of ergosteryl-D acetate (2.19 g.) in chloroform (25 c.c.) and glacial acetic acid (40 c.c.) during which the reaction mixture turned dark brown. After standing at room temperature for 30 minutes, the mixture was diluted with water and extracted with chloroform. After washing with water, the extract was concentrated under reduced pressure to leave a brown semi-crystalline residue which was taken up in ether, washed with saturated sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of the ether left an orange solid (2.14 g.), which on one crystallisation from light petroleum

(b.p. 40-60°) gave ergosteryl-D acetate as small glistening leaflets, m.p. 173-175°, undepressed on mixture with an authentic specimen, m.p. 176°.

Oxidation of Ergosteryl-D Acetate with Performic Acid.

(a) One mol. (With R. Budziarek.)

A mixture of ergosteryl-D acetate (2.2 g.) in benzene (20 c.c.), formic acid (20 c.c.; 90%) and hydrogen peroxide (0.65 c.c.; 30%) was stirred at room temperature for 20 hours. Evaporation of the reaction mixture under reduced pressure below 50°, and crystallisation of the residue from methanol gave 3β-acetoxyergosta-9(11):22-dien-7-one (930 mg.) as needles, m.p. 194-197°.

$[\alpha]_D$ +20° (C, 0.5).

Found: C, 78.8 ; H, 10.2.

$C_{30}H_{46}O_3$ requires: C, 79.2 ; H, 10.2%

It gives a pale yellow colour with tetranitromethane in chloroform. It does not show high intensity light absorption above 2200Å (repeated recrystallisation from methanol caused the appearance of light absorption at 2540Å) Infra-red light absorption: exhibits maxima at 1740 cm^{-1} (attributable to the acetoxy group) and at 1715 cm^{-1} (attributable to the nonconjugated ketone group).

(b) Two mols.

A mixture of ergosteryl-D acetate (2.2. g.) in benzene (20 c.c.), formic acid (20 c.c.; 90%) and hydrogen peroxide (1.2 c.c.; 30%) was stirred at room temperature for 20 hours. Evaporation of the reaction mixture under reduced pressure

below 50°, and crystallisation of the residue from methanol gave 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (360 mg.) as needles, which form slowly from a gel, m.p. 220-223°.

$[\alpha]_D$ -79° (C, 0.8).

Found: C, 76.2 ; H, 9.8.

$C_{30}H_{40}O_4$ requires: C, 76.55 ; H, 9.85%

The compound gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit high intensity above 2200Å.

Oxidation of Ergosteryl-D Acetate with Perbenzoic Acid.

A solution of perbenzoic acid (1.36 mols.) in chloroform (110 c.c.) was added with stirring over 4 hours to a solution of ergosteryl-D acetate (9.0 g.) in chloroform (35 c.c.) maintained at 5°. After standing 12 hours at 0° the reaction mixture was evaporated under reduced pressure at room temperature. The solid residue was dissolved in a minimum volume of boiling acetone, and on cooling deposited 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (ergosteryl-D acetate epoxide) as hexagonal plates (3.9 g.) m.p. 205-207°, which after two recrystallisations from acetone had m.p. 211-213°.

$[\alpha]_D$ -38° (C, 2.2).

Found: C, 79.5 ; H, 10.3

Calc. for $C_{30}H_{46}O_3$: C, 79.2 ; H, 10.2%.

It gives a yellow colour with tetranitromethane in chloroform and does not show selective absorption of high

intensity above 2200Å.

Chamberlin et al. (loc. cit.) report m.p. 202-205°, $[\alpha]_D$-35° and Heusser et al. (loc. cit.) report m.p. 205-207°, $[\alpha]_D$-29.5° of this compound.

9α:11α-Epoxyergosta-7:22-dien-3β-ol (Ergosterol-D epoxide)

A solution of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (400 mg.) in aqueous methanolic potassium hydroxide solution (60 c.c.: 2%) was heated under reflux for 2 hours. The solid (330 mg.) which separated on cooling was washed with water and twice crystallised from methanol to give 9α:11α-epoxyergosta-7:22-dien-3β-ol as flat needles, m.p. 187-189°.

$[\alpha]_D$-41° (C, 1.3).

Found: C, 78.5 ; H, 10.8.

$C_{28}H_{44}O_2.CH_3OH$ requires: C, 78.3 ; H, 10.9%

It gives a yellow colour with tetranitromethane and does not show high intensity light absorption above 2200Å.

9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate.

A solution of 9α:11α-epoxyergosta-7:22-dien-3β-ol (105 mg.) in pyridine (1 c.c.) and acetic anhydride (2 c.c.) was heated on the steam bath for 1 hour. The solid obtained by dilution with water was crystallised twice from acetone to give 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate as plates, m.p. 210-212°, undepressed by an authentic specimen.

$[\alpha]_D$-35° (C, 1.0)

Found: C, 79.3 ; H, 10.3.

Calc. for $C_{30}H_{46}O_3$: C, 79.2 ; H, 10.2%.

Action of Lithium Aluminium Hydride on 9α:11α-Epoxyergosta-7:22-dien-3β-yl Acetate.

(a) In Ether-Benzene.

A suspension of lithium aluminium hydride (500 mg.) in ether (70 c.c.) was added dropwise to a solution of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (500 mg.) in benzene (18 c.c.) and ether (40 c.c.), the mixture heated under reflux for 2 hours, then stirred at room temperature for 1½ hours. After the cautious addition of water with external cooling, dilute sulphuric acid (10%) was added, and the layers separated. The ether extract was washed successively with water, saturated hydrogen carbonate solution and water. Evaporation of the solvent left a white solid m.p. 150-183°, (which did not exhibit high intensity light absorption above 2200Å), which was taken up in pyridine (5 c.c.) and acetic anhydride (7 c.c.) and warmed on the steam bath for 1 hour. On cooling, plates separated, were removed by filtration and washed with a little cold methanol to give 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate, m.p. 209-212°, undepressed on mixing with an authentic specimen, m.p. 210-213°.

(b) In Tetrahydrofuran.

A solution of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (500 mg.) in absolute tetrahydrofuran (15 c.c.) was added dropwise over 15 minutes to a vigorously stirred refluxing solution of lithium aluminium hydride (1.0 g.) in tetrahydrofuran (100 c.c.). The mixture was heated under reflux for 3 hours, kept at room temperature overnight, then decomposed, and the product isolated by means of ether as

described in (a). Removal of the ether gave a solid residue which, after two recrystallisations from methanol gave 9α:11α-epoxyergosta-7:22-dien-3β-ol, m.p. 182-185°, undepressed by authentic specimen, m.p. 187-189°.

Action of Mineral Acids on 9α:11α-Epoxyergosta-7:22-dien-3β-yl Acetate.

(a) 9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate (150 mg.) was refluxed in aqueous methanolic hydrogen chloride (10 c.c.; 0.7%) for 2 hours. The solution was concentrated, and the solid (85 mg.), which separated on cooling, crystallised three times from methanol to give 3β-hydroxyergosta-8:22-dien-7-one as plates, m.p. 173-175°, undepressed with the specimen prepared by alkaline hydrolysis of the acetate.

$[\alpha]_D$ -45° (C, 0.5)

Found: C, 78.5 ; H, 11.1

Calc. for $C_{28}H_{44}O_2.CH_3OH$: C, 78.3 ; H, 10.9%

(b) 9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate (100 mg.) was refluxed in aqueous sulphuric acid (1 c.c.; 10%) and methanol (12 c.c.) for 1½ hours. Working up as in (a) above, gave 3β-hydroxyergosta-8:22-dien-7-one as plates, m.p. 168-173° on one crystallisation from methanol. Acetylation of the alcohol using pyridine and acetic anhydride gave 3β-acetoxyergosta-8:22-dien-7-one which separated from methanol as plates, m.p. 208-210°, $[\alpha]_D$ -55° (C, 1.1).

Action of Mild Alkali on 3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one.

A solution of 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one

(185 mg.) in aqueous methanolic potassium hydroxide (50 c.c.; 2%) was heated under reflux for 1½ hours. The mixture was cooled, diluted with water, extracted with ether and the ether extract washed successively with water, dilute hydrochloric acid and water and dried (Na_2SO_4). Removal of the ether left a white solid, m.p. 207-209°, which was crystallised three times from acetone to give 3β:11α-dihydroxyergosta-8:22-dien-7-one as needles, m.p.215°.

$[\alpha]_D$ -6°, -6° (C, 1.5, 1.2).

Found: C, 78.75 ; H, 10.6

$C_{28}H_{44}O_3$ requires: C, 78.45 ; H, 10.35%.

Light absorption: maximum at 2540Å (ε = 8,100)

The compound gives a faint yellow colour with tetranitromethane in chloroform.

3β:11α-Diacetoxyergosta-8:22-dien-7-one.

Acetylation of 3β:11α-dihydroxyergosta-8:22-dien-7-one by pyridine - acetic anhydride gave 3β:11α-diacetoxyergosta-8:22-dien-7-one as needles, m.p. 175-177°, from methanol.

$[\alpha]_D$ +13° (C, 0.5)

Found: C, 74.8 ; H, 9.7.

$C_{32}H_{48}O_5$ requires: C, 75.0 ; H, 9.4%

Light absorption: maximum at 2520Å (ε = 10,400)

The compound gives a faint yellow colour with tetranitromethane in chloroform.

9ξ:11ξ:22:23-Tetrabromoergost-7-en-3β-yl Acetate.

Dry bromine (5.1 c.c.; 4.4 mols) in glacial acetic acid (50 c.c.) was added rapidly to a solution of

5-dihydroergosteryl acetate (10 g.) in dry anaesthetic ether (1000 c.c.) at 0°. The mixture was cooled immediately to -60°, and allowed to regain room temperature during 2 hours with occasional shaking. The solid (8.3 - 9.0g.) which separated was then immediately removed by filtration, washed with cold ether, and dried at room temperature. Two crystallisations of the amorphous solid from benzene - light petroleum (b.p. 60-80°) gave 9ξ:11ξ:22:23-tetrabromoergost-7-en-3β-yl acetate as felted needles, m.p. 128° (decomp.).

$[\alpha]_D$ +233°, +225° (C, 0.2, 0.2 in dioxan).

$[\alpha]_D$ +238° (C, 1.8 in benzene).

Found: C, 47.7 ; H, 6.4 ; Br, 42.5.

$C_{30}H_{46}O_2Br_4$ requires: C, 47.5 ; H, 6.1 ; Br, 42.2%

The solid decomposes on prolonged standing, and solutions in chloroform, acetone and acetic acid decompose with evolution of hydrogen bromide and considerable decomposition. The ethereal - acetic acid mother liquors, obtained by filtration, decomposed within a few hours at room temperature.

22:23-Dibromoergosta-7:9(11)-dien-3β-yl Acetate.

A solution of sodium iodide (25 g.) in ethanol (500 c.c) was added in one portion to a solution of 9ξ:11ξ:22:23-tetrabromoergost-7-en-3β-yl acetate (9.0 g.) in warm benzene (500 c.c.), causing immediate liberation of iodine. After standing for 20 hours at room temperature, the solution was diluted with water (500 c.c.), the benzene layer separated,

and the aqueous phase extracted with benzene (300 c.c.). The combined benzene solutions were washed with sodium hydroxide solution (2 x 200 c.c.; 1%), then with water and dried (Na_2SO_4). Removal of the benzene gave an orange solid, which was dissolved in a minimum volume of chloroform, precipitated by addition of methanol, and removed by filtration. A solution of this solid in benzene (100 c.c.) was percolated through a column (15 x 2.5 cm.) of alumina, followed by elution with benzene (200 c.c.). Removal of the benzene gave a white solid (6.5 g.), which on crystallation from chloroform - methanol gave 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate as prismatic needles, m.p. 233-234°.

$[\alpha]_D$ +32° (C, 1.4).

Found: C, 60.0 ; H, 7.8 ; Br, 27.4.

$C_{30}H_{46}O_2Br_2$ requires: C, 60.2 ; H, 7.75 ; Br, 26.65%.

Light absorption: maxima at 2350 (ε = 19,000) and 2420Å (ε = 21,000) with an inflection at 2500Å (ε = 13,000)

The compound gives a dark brown colour with tetranitromethane in chloroform.

Ergosteryl-D Acetate.

(a) From Ergosteryl-D Acetate Dibromide.

A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (270 mg.) in ether (35 c.c.) and ethanol (25 c.c.) was heated under reflux for 3 hours in the presence of zinc dust (1.7 g.). After removal of the zinc by filtration, the solution was concentrated, diluted with water, and

extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave plates (180 mg.), m.p. 176-178°. One crystallisation from chloroform - methanol gave ergosteryl-D acetate as elongated plates, m.p. 178-180°, $[\alpha]_D$+32° (C, 2.1).

(b) From 5-Dihydroergosteryl Acetate.

A solution of 5-dihydroergosteryl acetate (2.5 g.) in ether (250 c.c.) at 0° was treated with bromine (1.25 c.c.) in glacial acetic acid (5 c.c.). The mixture was immediately cooled to -60°, and the temperature thereafter allowed to rise to -5° over 1½ hours. Benzene (100 c.c.) and zinc dust (7 g.) were then added, the mixture stirred at 0° for 1 hour and kept overnight at 0°. After filtration, ethanol (250 c.c.) and zinc dust (25 g.) were added to the filtrate, the mixture heated under reflux for 3 hours, and the product (2.3 g. m.p. 160-167°) isolated as in (a) above. Purification of this product by filtration of its solution in benzene through a short column of alumina, followed by crystallisation from chloroform - methanol yielded ergosteryl-D acetate as plates (1.5 - 1.8 g.), m.p. 173-4°, $[\alpha]_D$+28° (C, 2.1).

Found: C, 81.8 ; H, 10.6.

Calc. for $C_{30}H_{46}O_2$: C, 82.1 ; H, 10.6%.

Light absorption: maxima at 2360 (ε = 14,500) and 2420Å (ε = 16,3000) with an inflection at 2500Å (ε = 10,900).

Bromination of 5-Dihydroergosteryl Acetate in Chloroform.

A solution of 5-dihydroergosteryl acetate (5 g.) in

chloroform (150 c.c.) at -75° was treated with bromine (3.82 g.; 2.1 mols) in chloroform (50 c.c.) then almost immediately with pyridine (20 c.c.). After 1 hour, during which the temperature was allowed to rise to 0°, the solvent was removed under reduced pressure below room temperature. The residue was extracted with ether - benzene, the extract washed with water, and debrominated by refluxing the mixture for 3 hours, after the addition of ethanol (50 c.c.) and zinc dust (20 g.). Working up in the usual way gave a non-homogeneous product, which after repeated recrystallisation from chloroform - methanol, had m.p. 172°, $[\alpha]_D$ -7° (C, 2.0).

Light absorption: maxima at 2360 ($E^{1\%}_{1cm}$ 185) and 2420Å ($E^{1\%}_{1cm}$ 198) and an inflection at 2500Å ($E^{1\%}_{1cm}$ 138).

Examination of Mother Liquors from the preparation of 9ξ:11ξ:22:23-Tetrabromoergost-7-en-3β-yl Acetate.

Preliminary experiments to obtain crystalline material from the ethereal filtrate, after removal of the precipitated tetrabromide, proved abortive due to the rapid decomposition at room temperature, and susceptibility to rearrangement in presence of common solvents. Attempts were made, consequently, to stabilise the products by treatment with debrominating agents, before attempting to isolate crystalline material.

Treatment with sodium iodide.

The ether filtrate (ca. 3000 c.c.) from bromination of 5-dihydroergosteryl acetate (30 g.), after removal of

the tetrabromide (17.3 g.) was treated with an excess of ethanolic sodium iodide solution. After standing overnight, water was added, the layers separated, and the aqueous layer extracted with benzene. The combined ether and benzene extracts were washed with water, sodium hydroxide solution, water and dried (Na_2SO_4). Removal of the solvents under reduced pressure caused some decomposition; the residual dark gum was dissolved in warm pyridine, and acetic anhydride added, causing immediate separation of crystalline material. After standing overnight, the solid (13.0 g., m.p. 155-190°) was collected, recrystallised from chloroform - methanol to give two crops of irregular prisms (8.0 g. m.p. 195-200°, sintering ca. 160°) and (2.0 g. m.p. 155-165°), which were recombined (since no homogeneous product was observed after repeated recrystallisation), dissolved in a mixture of benzene (30 c.c.) and light petroleum (b.p. 40-60°, 120 c.c.) and chromatographed on a column (23 x 3.5 cm) of alumina.

Fraction.	Vol(c.c)	Eluant.	Product.	Wt.(g)	m.p.
1.	250	Petrol-Benzene(4:1)	White Solid	Trace.	
2.	100	"	"	2.58	m.p. 157-160°.
3.	300	"	"	3.15	Sinters 158° m.198°
4.	300	" (1:1)	"	1.12	Sinters 175° m.215°
5.	500	" (1:1)	"	1.93	Sinters 195° m.215°
6.	450	Benzene	Gum	0.11	-
7.	250	Chloroform methanol(1:1)	Gum	0.88	-

Fraction 2 gave a red colour with tetranitromethane in chloroform. One recrystallisation from chloroform - methanol gave irregular plates, m.p. 158-160°. Two recrystallisations from the same colvent raised the m.p. to 163-4°, $[\alpha]_D \pm 0°$ (C, 0.7) (Found: C, 73.6 ; H, 9.7 ; Br 9.8%). Two further crystallisations gave plates m.p. 165°, $[\alpha]_D +7°$ (C, 1.0) (Found: C, 74.9 ; H, 9.8 ; Br, 8.6%)

Calc. for $C_{30}H_{46}O_2Br_2$: C, 60.2 ; H, 7.75 ; Br, 26.65%

" " $C_{30}H_{48}O_2$: C, 81.8 ; H, 11.0%

Light absorption: maxima at 2350 ($E^{1\%}_{1cm}$ 291) and 2420Å ($E^{1\%}_{1cm}$ 323) and an inflection at 2500Å ($E^{1\%}_{1cm}$ 217)

Debromination of this mixture (230 mg.) by refluxing an ether - ethanol solution with zinc dust gave, after working up in the usual way, elongated leaflets (140 mg. m.p. 171-4°, $[\alpha]_D +14°$). Repeated recrystallisation from chloroform - methanol gave ergosteryl-D acetate, m.p. 172-174°, $[\alpha]_D +28°$ (C, 1.1) (Found: C, 82.0 ; H, 10.6%).

Light absorption: maxima at 2350 (ε = 13,200) and 2420Å (ε = 17,000) and an inflexion at 2500Å (ε = 11,200)

Fractions 4 and 5 were combined, and thrice recrystallised from chloroform - methanol to give beautiful long needles, m.p. 229-231°.

$[\alpha]_D -8°$, $-10°$ (C, 0.9, 1.8).

Found: C, 59.9 ; H, 7.8

Calc. for $C_{30}H_{46}O_2Br_2$: C, 60.2 ; H, 7.75%

Calc. for $C_{30}H_{48}O_2Br_2$: C, 60.0 ; H, 8.1%

Light absorption: maxima at 2350 (ε = 12,700) and 2420Å (ε = 13,900) and inflection at 2500Å (ε = 9,200)

A mixed m.p. with ergosteryl-D acetate dibromide (m.p. 234-5°, $[\alpha]_D$+32°) was 233-235°.

Debromination of the compound (220 mg.) as above gave elongated plates (140 mg. m.p. 172-175°, $[\alpha]_D$+4°) which on repeated recrystallisation from chloroform - methanol (with deliberate loss) gave ergosteryl-D acetate, m.p. 175-176° $[\alpha]_D$+27° (C, 2.1).

Chromatography of 9ξ:11ξ:22:23-Tetrabromoergost-7-en-3β-yl Acetate.

The tetrabromide (1 g.), dissolved in benzene (60 c.c.) without heating, giving a pale yellow solution, was chromatographed on a column (11.5 x 2.5 cm.) of alumina. A dark green band was immediately formed on the column.

Fraction.	Eluant.	Vol(c.c)	Product.	Wt. (mg.)
1.	Benzene	100	Clear Gum	180
2.	Benzene	100	Froth Gum	450
3.	Benzene	400	Yellow Gum	50
4.	Ether	300	Dark Gum	100
5.	Methanol	200	Dark Gum	

Crystallisation of fraction 2 from chloroform - methanol gave stout needles (160 mg.), m.p. 127-129°. This product gives a strong yellow colour with tetranitromethane in chloroform, but does not liberate iodine from ethanolic potassium iodide solution. Recrystallisation from ethanol yielded a compound, m.p. 134-136°.

$[\alpha]_D$ -3° (C, 1.1)

Found: C, 60.4 ; H, 7.5

$C_{30}H_{44}O_2Br_2$ requires : C, 60.4 ; H, 7.4

$C_{30}H_{46}O_2Br_2$ requires : C, 60.2 ; H, 7.75%

Light absorption: maximum at 2080Å (ε = 39,800) and an inflection at 2200Å (ε = 15,300).

3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one.

A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (4.5 g.) in carbon tetrachloride (100 c.c.) and stabilised glacial acetic acid (600 c.c.) was treated with hydrogen peroxide solution (8 c.c.; 30%) added in one portion, and heated to 95° with stirring over 25 minutes. The mixture was maintained at this temperature for 3 hours. The solution was evaporated under reduced pressure, the partially crystalline residue treated with methanol (30 c.c.), allowed to stand at room temperature overnight, and the solid removed by filtration and washed with cold methanol. Recrystallisation of the product (1.64 g., m.p. 219-224°) from chloroform - methanol gave 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one as plates, m.p. 235°.

$[\alpha]_D$ -45°, -47° (C, 2.3, 1.7)

Found C, 56.9 ; H, 7.4

$C_{30}H_{46}O_4Br_2$ requires: C, 57.1 ; H, 7.35%

The compound does not give a coloration with tetranitromethane in chloroform and shows no high intensity light absorption above 2200Å. Infra-red light absorption: maxima at 1733 cm^{-1} and 1257 cm^{-1} (acetate group) and 1725 cm^{-1} (nonconjugated ketone).

3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one 2:4-dinitrophenylhydrazone.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one in dioxan was treated with Brady's reagent. The precipitate was removed by filtration, recrystallised three times from chloroform - ethanol to give 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one 2:4-dinitrophenylhydrazone as small yellow felted needles, m.p. 217-219° (decomp.)

Found: C, 53.0 ; H, 5.95

$C_{36}H_{50}O_7N_4Br_2$ requires: C, 53.3 ; H, 6.2%

Light absorption in chloroform : maxima at 2460 (ε = 13,000) and 3660Å (ε = 23,600).

3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one semicarbazone.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one in ethanol - dioxan was treated with a solution of semicarbazide hydrochloride and sodium acetate in water. The precipitate was removed by filtration, after standing overnight, washed with water, and the product (m.p. 233°) recrystallised twice from methanol - chloroform to give 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one semicarbazone as prisms, m.p. 236-237°

Found: C, 54.2 ; H, 7.3

$C_{31}H_{49}O_4N_3Br_2$ requires: C, 54.1 ; H, 7.2%.

Light absorption: maximum at 2280Å (ε = 14,600)

3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one.

(a) A stirred solution of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (1.35 g. m.p. 218-221°) in stabilised glacial acetic acid (150 c.c.) was treated at 95° over 2 hours with zinc dust (11 g.) added portionwise. After a further 2 hours at 95°, the reaction mixture was filtered, concentrated under reduced pressure until solid separated, and diluted with water. The mixture was extracted with ether (3 x 50 c.c.), the extract washed successively with sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of the ether gave a solid residue (0.90 g.; m.p. 208-213°). The product was twice crystallised from methanol, from which 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one was obtained as needles (which formed slowly from an initial gel), m.p. 223-224°, undepressed when mixed with a specimen prepared by performic acid oxidation of ergosteryl-D acetate.

$[\alpha]_D$-79° (C, 0.8)

Found: C, 76.5 ; H, 9.95

Calc. for $C_{30}H_{46}O_4$: C, 76.55 ; H, 9.85%

The compound does not show high intensity light absorption above 2200Å and gives a faint yellow coloration with tetranitromethane in chloroform.

(b) A solution of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (386 mg., m.p. 229-233°) in ether (80 c.c.) and ethanol (80 c.c.) was heated under reflux for 3 hours with zinc dust (6.5 g.) The solution was then

filtered, diluted with water, and extracted with ether. The ether extract was washed with water, evaporated, and the residue crystallised once from methanol to give 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one, m.p. 218-221° as clusters of needles (forming from an initial gel).

Treatment of 3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one with 12% Alkali.

To a solution of 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (600 mg.; m.p. 208-213°) in ethanol (65 c.c.) was added a solution of potassium hydroxide (10.0 g.) in water (15 c.c.), and the mixture heated under reflux for 16 hours. It was then diluted with water, extracted with ether, the ether extract repeatedly washed with water, and the ether removed to leave a yellow gum. The dried product was treated with pyridine (10 c.c.) and acetic anhydride (10 c.c) on the steam bath for 1 hour, and the acetylated product, obtained in the usual way by means of ether, isolated as an orange-brown gum. This was dissolved in benzene (40 c.c.) and adsorbed on a column (10 x 2 cm.) of alumina.

Fraction.	Eluant.	Vol(c.c)	Product.	Wt. (mg.)
1.	Benzene	40	Gum	5
2.	"	40	Yellow Gum	180
3.	"	50	Pale Yellow Solid.	120
4.	"	110	Gum-solid	15
5.	"	280	Solid	55
6.	"	200	Solid	Trace.
7.	Benzene-Ether	300	Gum	Trace.

Fraction.	Eluant.	Vol(c.c)	Product	Wt.(mg.)
8.	Ether	200	Dark Gum	-
9.	Methanol	100	Dark Gum	-

Fraction 3 was crystallised from methanol as long fine needles, m.p. 173-5°. Recrystallisation from the same solvent gave a compound, m.p. 176-177°.

$[\alpha]_D$ -15° (C, 0.7)

Found C, 74.4 ; H, 10.0 ; $-OC_2H_5$ 9.5

$C_{32}H_{52}O_5$ requires: C, 74.4 ; H, 10.1 ; $-OC_2H_5$ 8.7%

The compound does not show high intensity light absorption above 2200Å, and gives a faint yellow coloration with tetranitromethane in chloforom.

Infra-red light absorption: maxima at 1733 cm^{-1} and 1245 cm^{-1} (acatate group), 1710 cm^{-1} (ketone group), 973 cm^{-1} (sidechain ethylenic group) and at 1030 cm^{-1} (ethoxyl group?).

Fraction 2, on crystallisation from methanol gave the same product, m.p. 174-176° undepressed by the specimen obtained from fraction 3.

Fraction 5, on crystallisation from methanol, gave 7:11-diketoergost-22-en-3β-yl acetate as clusters of needles, m.p. 196-198°.

$[\alpha]_D$ -28° (C, 0.9)

Found: C, 76.5 ; H, 10.1

Calc. for $C_{30}H_{46}O_4$: C, 76.55 ; 9.85%.

It does not show high intensity light absorption above 2200Å

A mixture with a specimen (m.p. 197-199°, $[\alpha]_D$ -31°) prepared as described by Heusser et al (loc. cit.) who report

m.p. 195-196° $[\alpha]_D$-27° had m.p. 196-199°. Chamberlin et al (loc. cit) give m.p. 197-200°, $[\alpha]_D$-30° for the same compound.

22:23-Dibromo-3β:11α-dihydroxyergost-8-en-7-one.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (400 mg., m.p. 212-224°) in methanolic potassium hydroxide solution (3%; 40 c.c.) was heated under reflux for 70 minutes. The solution was cooled, diluted with water, and extracted with ether. The extract was washed successively with water, dilute hydrochloric acid and water, and concentrated until a solid (300 mg; m.p. 219-221°) separated. Recrystallisation of this solid from methanol gave 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one as prismatic needles, m.p. 227-229°.

$[\alpha]_D$+4°, +4° (C, 0.9, 2.0)

Found: C, 57.0 ; H, 7.65.

$C_{28}H_{44}O_3Br_2$ requires: C, 57.1 ; H, 7.5%

Light absorption: maximum at 2520Å (ε = 8,300)

Infra-red light absorption: maxima at 3495 cm^{-1} (hydroxyl group) and 1665cm^{-1} (αβ-unsaturated ketone).

3β:11α-Diacetoxy-22:23-dibromoergost-8-en-7-one.

A solution of 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (130 mg.) in pyridine (2 c.c.) and acetic anhydride (3 c.c.) was heated at 100° for 1½ hours. The mixture was then treated with water, extracted with ether,

and the product isolated in the usual manner to give 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (130 mg; m.p. 156°) as needles from chloroform - methanol. After recrystallisation from the same solvents, it had m.p. 161-163° (air dried) or m.p. 201-202° (prolonged drying at 100° in vacuo).

$[\alpha]_D$ +18°, +16° (C, 0.6, 1.6)

Found: C, 57.2 ; H, 7.4

$C_{32}H_{48}O_5Br_2$ requires: C, 57.1 ; H, 7.2%

Light absorption: maximum at 2510Å (ε = 10,000)

Infra-red light absorption: maxima at 1738cm^{-1} and 1243cm^{-1} (acetate group) and 1690cm^{-1} (αβ-unsaturated ketone).

Debromination of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one.

A solution of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (124 mg.) in ether (15 c.c.) and ethanol (15 c.c.) was heated under reflux for 3 hours with zinc dust. The zinc was removed, the filtrate concentrated, diluted with water and extracted with ether. After washing with water, the ether extract was concentrated, diluted with methanol, and the crystalline product (60 mg., m.p. 160-164°) collected. Two recrystallisations from aqueous methanol gave 3β:11α-diacetoxyergost-8:22-dien-7-one as needles, m.p. 172-174°, $[\alpha]_D$ +11° (C, 1.0).

Found: C, 75.4 ; H, 9.6.

Calc. for $C_{32}H_{48}O_5$: C, 75.0 ; H, 9.4%.

Spring et al (70, 71) give m.p. 175-177°, $[\alpha]_D$+13° and m.p. 173-174°, $[\alpha]_D$+11°, and Heusser et al (76) give m.p. 184-185°, $[\alpha]_D$+12° for this compound.

Debromination of 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one.

A solution of 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (160 mg.) in methanol (50 c.c.) was heated under reflux for 3 hours. Working up in the usual way gave 3β:11α-dihydroxyergosta-8:22-dien-7-one as needles from acetone, m.p. 214-215°, undepressed when mixed with a specimen prepared by hydrolysis of 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one.

Oxidation of Ergosteryl-D Acetate Dibromide with Performic Acid.

(a) A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (2.32 g.) in warm ethyl acetate (100 c.c.) was treated with formic acid (40 c.c.; 98-100%) and hydrogen peroxide solution (1.73 c.c.; 30%). The suspension was shaken at room temperature for 44 hours whereafter solution was complete. Water (300 c.c.) was added, the layers separated, and the aqueous phase extracted with ethyl acetate (3 x 100 c.c.). The combined ethyl acetate extracts were washed successively with water, saturated sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of the solvent under reduced pressure left a solid residue, which gave irregular crystals (650 mg., m.p. 214-217°) on crystallisation from ethanol (120 c.c.). Three

recrystallisations from acetone gave 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one as long well-defined needles, m.p. 220-221°.

$[\alpha]_D$ -48° (C, 1.0)

Found: C, 57.2 ; H, 7.5

Calc. for $C_{30}H_{46}O_4Br_2$: C, 57.1 ; H, 7.35%

It does not exhibit high intensity light absorption above 2100Å, and does not give a colour with tetranitromethane in chloroform.

(b) A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (5.0 g.) in ethyl acetate (200 c.c.) was treated with formic acid (80 c.c; 98-100%) and hydrogen peroxide solution (3.75 c.c; 30%). The solid product was isolated as in (a), and crystallised twice from acetone and once from ethanol to give a mixture (625 mg.; m.p. 217-219°) of prisms and needles. This mixture was taken up in benzene (30 c.c.) and chromatographed on a column (12 x 2 cm.) of alumina. Elution with benzene (400 c.c.) gave no residue. Elution with benzene - ether (400 c.c.; 3:1) gave a solid (55 mg.), and benzene - ether (400 c.c.; 1:2) gave a solid (5 mg.). After elution with ether (250 c.c.) gave no residue, ether - methanol (100 c.c.; 1:1) gave a solid (530 mg.; m.p. 199-201°). Crystallisation of the latter from aqueous methanol gave crude 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one (contaminated with amorphous material), m.p. 202-203°. Recrystallisation from benzene - light petroleum petroleum (b.p. 60-80°) gave

long needles, separated mechanically from the amorphous impurity, which on one more recrystallisation from the same solvents had m.p. 206-207°, undepressed on mixing with a specimen prepared by chromatography of pure 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (m.p. 220°) Alkaline hydrolysis of the solid residues obtained from the original acetone - ethanol mother liquors yielded by fractional crystallisation, 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one, m.p. 232°, $[\alpha]_D$+2°. (Found C, 57.4 ; H, 7.6).

3β-Acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one.

A solution of 3β-acetoxy-22;23-dibromo-9α:11α-epoxyergostan-7-one (needles, m.p. 220°) (152 mg.) in benzene (20 c.c.) was chromatographed on a column (12 x 2 cm) of alumina to give the following fractions:-

1.	400 c.c.	Benzene.	Nil.	
2.	300 c.c.	Benzene-Ether (1:1)	Solid	20 mg.
3.	200 c.c.	" " (1:1)	Solid	Trace.
4.	400 c.c.	Ether	Solid	Trace.
5.	150 c.c.	Ether-Methanol(1:1)	Solid	110 mg.

Three recrystallisations of the ether - methanol fraction gave 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one m.p. 206-207°.

$[\alpha]_D$-13° (C, 1.5)

Found: C, 56.9 ; H, 7.6

$C_{30}H_{46}O_4Br_2$ requires: C, 57.1 ; H, 7.35%.

Light absorption: maximum at 2520Å (ε = 7,800)

Infra-red light absorption: maxima at 1732 cm^{-1} (acetate) 1669 cm^{-1} ($\alpha\beta$-ketone) and 3700 cm^{-1} (hydroxyl).

Acetylation of the compound (90 mg., m.p. 199-201°) by pyridine - acetic anhydride gave a solid (75 mg., m.p. 153°), recrystallised from chloroform - methanol to give 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one as prismatic needles, m.p. 162-163° (undepressed by authentic specimen, m.p. 161-163°) and 202-203° (after drying). A second crop of smaller needles, m.p. 197-200° (with softening at 160°) was obtained from the crystallisation mother liquors.

3β-Acetoxy-11α-hydroxyergosta-8:22-dien-7-one.

A solution of 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (240 mg.) in benzene (40 c.c.) was chromatographed on a column (12 x 2 cm.) of alumina to give the following fractions:-

1.	400 c.c.	Benzene	Nil
2.	400 c.c.	Benzene-Ether (1:1)	Solid 44 mg.
3.	400 c.c.	Ether	Nil.
4.	100 c.c.	Ether-Methanol (3:1)	Solid 180 mg.

Two recrystallisations from aqueous methanol and two from methanol gave 3β-acetoxy-11α-hydroxyergosta-8:22-dien-7-one as felted needles m.p. 185-188° (unsharp, sintering at 160°)

$[\alpha]_D$ -30°, -30° (C, 0.7, 0.6)

Found: C, 76.3 ; H, 10.1

$C_{30}H_{46}O_4$ requires: C, 76.55 ; H, 9.85%

Light absorption: maximum at 2540Å (ε = 7,200)

22:23-Dibromo-7:11-diketoergost-8-en-3β-yl Acetate.

Chromium trioxide (21 mg; 1.1 atoms O) in stabilised acetic acid (0.63 c.c.) was added in one portion to a solution of 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one (177 mg.; m.p. 199-201°) in stabilised acetic acid (25 c.c.) followed by sulphuric acid (2N; 2 drops). The mixture was shaken for 5 minutes, whereafter it had turned green, and a fine precipitate began to appear. After standing at room temperature overnight, the solution was filtered, the precipitate washed with acetic acid and water to leave a solid (20 mg., m.p. 265°). The combined washings and filtrate were diluted with water, extracted with ether, and the ether extract washed successively with water, saturated sodium hydrogen carbonate solution, water and dried (Na_2SO_4). Removal of the ether gave a yellowish-white solid (140 mg.) insoluble in methanol. Two recrystallisations from chloroform - methanol gave 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate as plates, m.p. 249-250°, undepressed by an authentic specimen, m.p. 248-250°.

$[\alpha]_D$ +29° (C, 1.0).

Light absorption: maximum at 2700Å (ε = 8,100).

Characterisation of Performic Acid Product (needles, m.p. 220-221°) as 3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one.

(a) Alkaline hydrolysis in the usual way gave 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one, as felted needles, $[\alpha]_D$ +5° (C, 1.8), m.p. 229-230°, undepressed by a specimen

m.p. 227° (obtained by hydrolysis of the dibromoketoxide, plates, m.p. 234°).

Re-acetylation of the diol (28 mg.) by pyridine - acetic anhydride gave 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one as needles (27 mg.), m.p. 155-157°. After drying in vacuo for 2 hours, it had m.p. 197-198° with softening at 157°.

(b) Debromination by zinc dust in ether - ethanol solution in the usual way gave 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one, as needles formed from a gel, from methanol, m.p. 221°, $[\alpha]_D$-82° (C, 1.5).

(c) Treatment of a solution of the compound in dioxan with Brady's reagent gave a 2:4-dinitrophenylhydrazone, m.p. 219-221° after 3 recrystallisations from chloroform - methanol. A mixed m.p. with a sample m.p. 217-219° (obtained from the dibromoketoxide, plates, m.p. 234°) was 218-220°. (Found: C, 53.5 ; H, 6.6%).

Light absorption in chloroform: maxima at 2460 (ε=13,000) 3660Å (ε = 23,400).

(d) The compound (needles from acetone, m.p. 221°) was recrystallised once from chloroform - methanol to give 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one as plates, m.p. 234-235°, $[\alpha]_D$-47° (C, 1.6).

3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (m.p. 234°, plates from chloroform - methanol) was recrystallised from acetone as well-defined needles, m.p. 215-220°. Two recrystallisations from acetone raised the

m.p. to 220-221°, $[\alpha]_D$-48° (C, 0.9).

Hydrogenation of 22:23-Dibromo-3β:11α-dihydroxyergost-8-en-7-one.

(a) Palladium black-ethanol.

22:23-Dibromo-3β:11α-dihydroxyergost-8-en-7-one (315 mg.) in ethanol (130 c.c.) was shaken in an atmosphere of hydrogen with palladium black prereduced catalyst (200 mg.) After taking up 1 mol. of hydrogen, a light absorption determination on the solution showed the continued existence of the αβ-unsaturated ketone system. The solution was then shaken for 12 hours, the catalyst removed, and the solvent removed under reduced pressure. At high concentration, profound decomposition occurred leaving a very dark residue, which could not be crystallised, and only amorphous material was isolated on chromatography.

(b) Platinum-Dioxan.

22:23-Dibromo-3β:11α-dihydroxyergost-8-en-7-one (367 mg.) in dioxan (30 c.c.) was shaken in an atmosphere of hydrogen at room temperature with prereduced platinum oxide catalyst (140 mg.) until 15 c.c. of hydrogen had been absorbed (85 minutes; theo. for 1 mole is 14.7 c.c.). The catalyst was then removed, the solution slightly concentrated, diluted with water, and the product (m.p. 210-212°, yellow colour with tetranitromethane) removed by filtration. After two recrystallisations from acetone, the product had m.p. 219-220°, and light absorption maxima at 2060 ($E^{1\%}_{1cm}$ 41) and 2520Å ($E^{1\%}_{1cm}$ 123).

Acetylation of this product by pyridine - acetic anhydride in the usual way gave a solid (304 mg. m.p. 142-187°) from methanol, which was dissolved in light petroleum (b.p. 40-60°) and chromatographed on a column of alumina. After elution with light petroleum (b.p. 40-60°; 200 c.c.) and light petroleum-benzene (1:1, 200 c.c.), benzene (130 c.c.) gave a solid (32 mg.) which gave needles, m.p. 217-218°, $[\alpha]_D$+7° (C, 1.1) from chloroform - methanol. Light absorption exhibits maxima at 2100 ($E^{1\%}_{1cm}$ 78), 2350 ($E^{1\%}_{1cm}$ 89), 2420 ($E^{1\%}_{1cm}$ 95) and 2500Å ($E^{1\%}_{1cm}$ 69). The product is presumably a mixture of ergosteryl-D acetate (dibromide) and a transparent laevorotatory compound, possibly 3β:11α-diacetoxyergost-22-en-7-one ($[\alpha]_D$-68°). Elution with benzene - ether (2:1) gave unchanged 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (130 mg.) m.p. 155-159 .

3β:11α-Dihydroxyergost-22-en-7-one. (With R. Budziarek)

A solution of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (250 mg.) in ethanolic potassium hydroxide solution (100 c.c.; 0.1N) was shaken with hydrogen for 2 hours at room temperature with pre-reduced platinum catalyst (50 mg.). The solution was filtered, concentrated to a volume of 30 c.c., diluted with water, and extracted with ether. Removal of the ether left a crystalline residue, recrystallisation of which from aqueous acetone gave 3β:11α-dihydroxyergost-22-en-7-one as long fine needles, m.p. 206-207°.

$[\alpha]_D$ -76° (C, 0.8)

Found: C, 74.8 ; H, 10.9

Calc. for $C_{28}H_{46}O_3H_2O$: C, 74.95 ; H 11.0%

It does not exhibit high intensity light absorption above 2200Å, and gives a faint yellow coloration with tetranitromethane in chloroform.

3β:11α-Diacetoxyergost-22-en-7-one. (With R. Budziarek)

Acetylation of 3β:11α-dihydroxyergost-22-en-7-one by pyridine and acetic anhydride gave, in the usual way, 3β:11α-diacetoxyergost-22-en-7-one as plates from methanol, m.p. 141-142°.

$[\alpha]_D$ -68° (C, 0.6).

Found: C, 74.8 ; H, 10.0

Calc. for $C_{32}H_{50}O_5$: C, 74.7 ; H, 9.8%

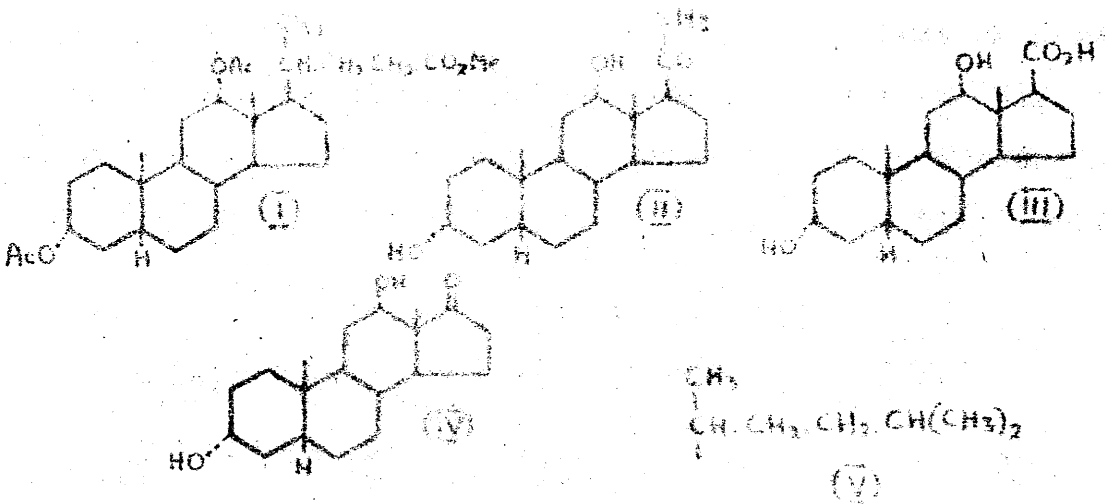
It does not exhibit high intensity light absorption above 2200Å, and gives a faint yellow coloration with tetranitromethane in chloroform.

DEGRADATION OF THE BILE ACID SIDECHAIN

HISTORICAL.

[illegible] bile acids represent one of the most abundantly [illegible] starting materials for the synthetic preparation of hormones, considerable effort has been devoted to the [illegible] of the [illegible] sidechain.

[illegible] oxide, may [illegible] after [illegible] of [illegible] and [illegible] desoxycholate ([illegible] 112), yields only [illegible] IV. No [illegible] by the extension,

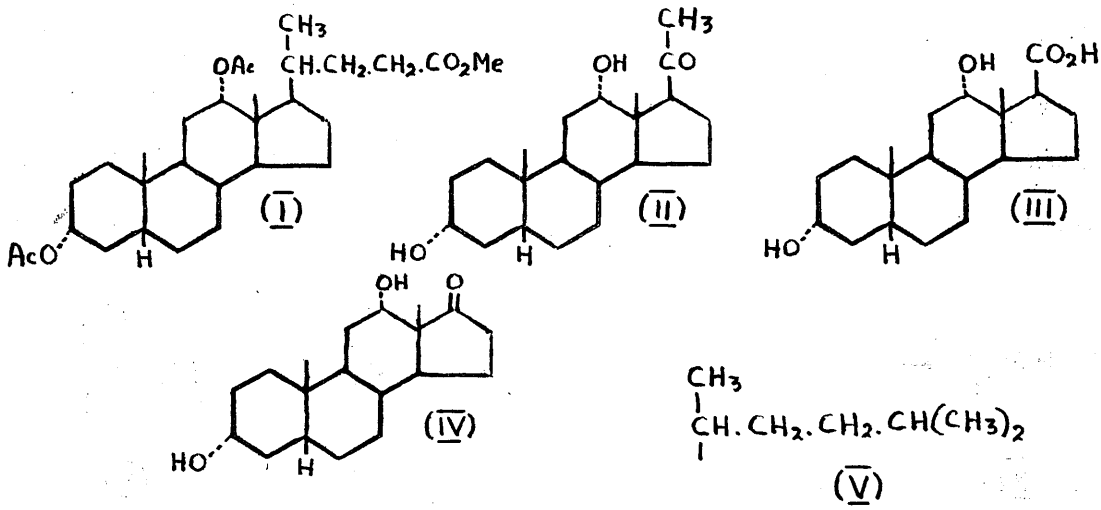


prior to oxidation, of the bile acid [illegible] to the [illegible] aliphatic norsterol sidechain (V).

[illegible] classical Barbier-Wieland degradation (114, 115)

Since bile acids represent one of the most abundantly available starting materials for the synthetic preparation of hormones, considerable effort has been devoted to the development of improved methods of degradation of the characteristic (γ-substituted n-valeric acid) sidechain.

Unlike cholesterol, which, as acetate dibromide, may be oxidised directly with chromic acid to give (after debromination and suponification) moderate amounts of pregnenolone, 3β-hydroxyetiochol-5-enic acid and dehydroandrosterone, the diacetate of methyl deoxycholate (I), oxidised under similar conditions (111, 112), yields only traces of the diacetates of II, III, and IV. No worthwhile improvement is effected (113) by the extension,



prior to oxidation, of the bile acid sidechain to the saturated aliphatic norsterol sidechain (V).

The classical Barbier-Wieland degradation (114, 115) which involves the successive oxidation of the carbinols obtained by the Grignard reaction on the esters and lower homologues, as shown below, affords the desired products

in 2-5% yield. This route has been applied in several

(i) $CH_3.CH(CH_2.CH_2.CO_2Me) \longrightarrow CH_3.CH(CH_2.CH_2.C(OH).Me_2) \longrightarrow CH_3.CH(CH_2.CO_2H)$ Norcholanic Acid

(ii) $\longrightarrow$ Ester $\longrightarrow CH_3.CH(CH_2.C(OH).Ph_2) \longrightarrow CH_3.CH(COOH)$ Bisnorcholanic Acid

(iii) $\longrightarrow$ Ester $\longrightarrow CH_3.CH(OH).CH(Ph_2) \longrightarrow CH(COOH)$ Etiocholanic Acid

instances, and improved in several details. Hoehn and Mason (116,117) studied the various steps, and improved the yields by alterations in experimental conditions. The greatest loss in this synthesis occurs in the oxidation of the ternordiphenylethylene (V) to the etio acid (IX) which proceeds in only 15% yield. The latter authors described a longer, but preferable route through the ketone (VI), the benzal derivative (VII) and the glyoxal (VIII), increasing the yield at this stage to 40%. Kendall and his associates

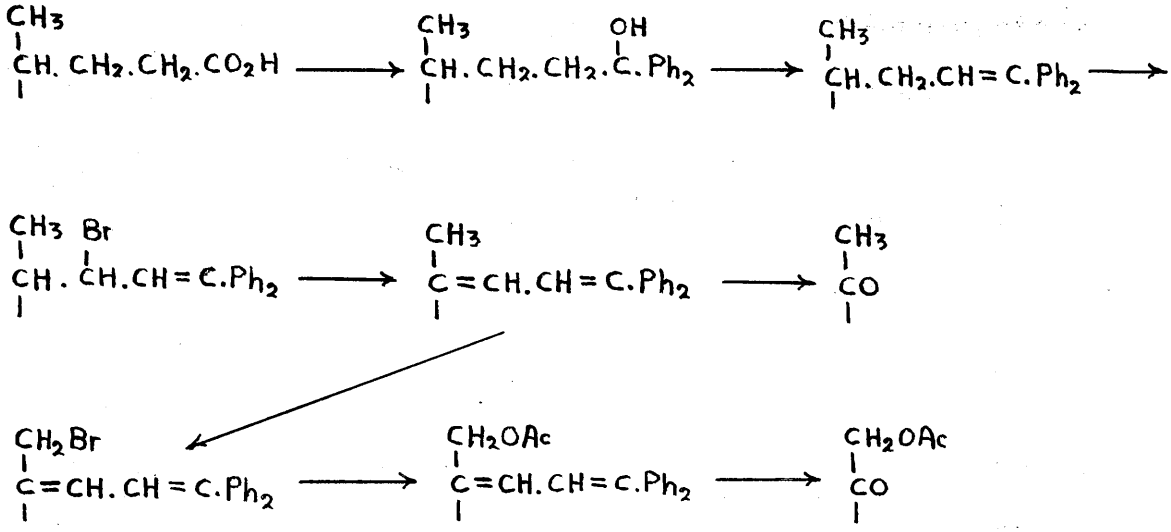
$CH_3.C(=C.Ph_2) \longrightarrow CH_3.CO \longrightarrow CH(=CHPh).CO \longrightarrow CHO.CO \longrightarrow COOH$

(V) (VI) (VII) (VIII) (IX)

(118) have also described improvements in yield by varying experimental conditions.

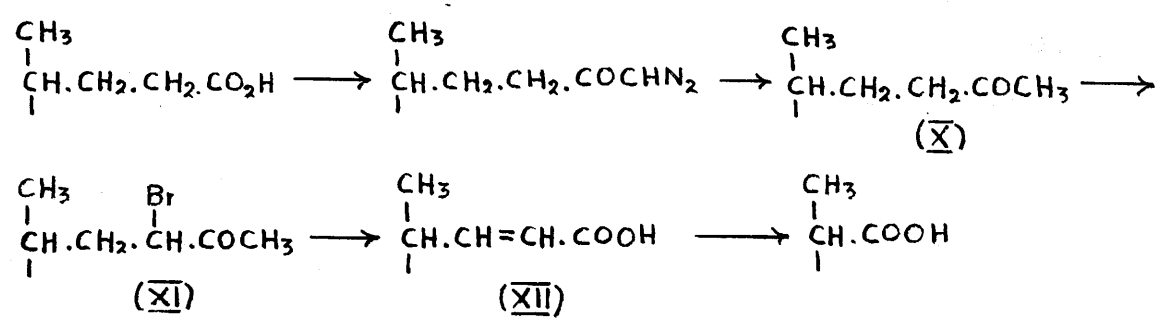
For the degradation of the bile acid sidechain to a 20-methyl ketone, this method is now superseded by the Meystre-Miescher method in which the initial diphenylethylene is converted by Ziegler bromination and dehydrohalogenation

into a diphenyldiene that on oxidation affords the 20-methyl ketone. Meystre and Wettstein (125, 128) also showed that



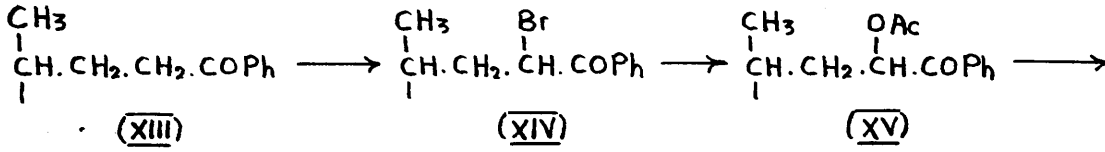
allylic bromination at C-21 occurs on the diphenyldiene, and the 21-bromine atom can be replaced by hydroxy, acyloxy or alkoxy groupings, thus giving the ketol sidechain directly. The degradative procedure has been applied to deoxycholic acid (118, 119, 129), to cholic acid (120), to cholanic acid (121), to 3β-hydroxyallocholanic acid and lithocholic acid (122). It can also be applied in the presence of nuclear ketone groups (127) or in the presence of nuclear unsaturation (123, 124, 126), e.g., the synthesis of progesterone from 3β-hydroxychol-5-enic acid.

The method of Hollander and Gallagher (130) was applied to cholanic acid and is outlined below. The yields in the

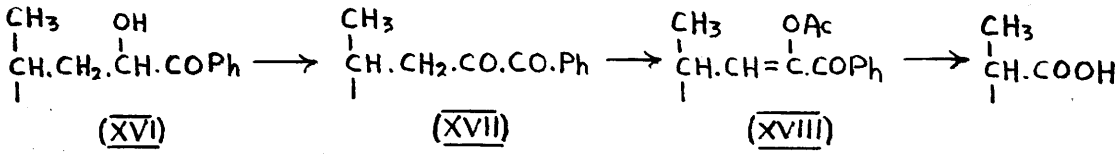


steps leading to the methyl ketone (X) i.e., formation of the acid chloride, treatment with diazomethane and zinc and hydrochloric acid, were satisfactory. The α-bromoketone (XI) proved difficult to purify, however, (possibly due to a 23-isomeric mixture or presence of a 25-bromo derivative) and dehydrohalogenation gave only an oily product (XII), which on oxidation afforded only minute amounts of isomeric bisnorcholanic acids.

Another method, reported by Jacobsen (131) in a preliminary note, appears more promising. Starting from the phenyl ketone (XIII) of cholic acid, obtained by the action



(XIII) (XIV) (XV)



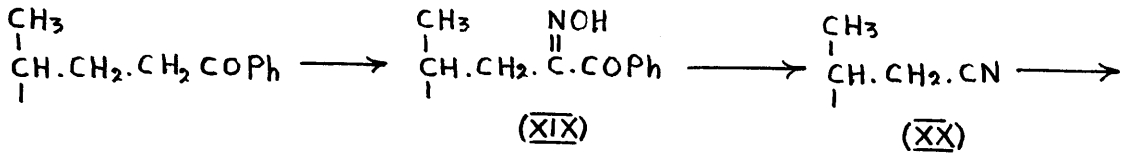
(XVI) (XVII) (XVIII)

of diphenylcadmium on the acid chloride, and proceeding through the α-bromo (XIV) and α-acetoxy derivatives (XV), the α-hydroxyketone (XVI) was oxidised by copper sulphate to the α-diketone (XVII). The derived enol acetate (XVIII) was then oxidised to the bisnor acid.

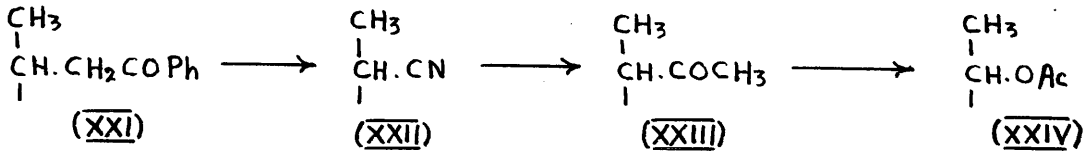
The route investigated by Brink, Clark and Wallis (132) involved the reaction of bromine with the silver salts of bile acids to form compounds in which the carboxyl group is replaced by a bromine atom. In this way, 23-bromocholane was prepared from cholanic acid in 25% yield, but could not

be further degraded by dehydrohalogenation with collidine, piperidine, silver nitrate or sodium ethoxide.

The most recently described method, due to Ercoli and de Ruggieri (133) also utilised the phenyl ketone, and proceeded by the isonitroso derivative (XIX). Fission of (XIX) with thionyl chloride gave benzoic acid and the nitrile (XX), which was directly treated with phenylmagnesium bromide to give the bisnorcholanyl phenyl ketone (XXI). Similar stepwise treatment gave the nitrile



(XIX) (XX)

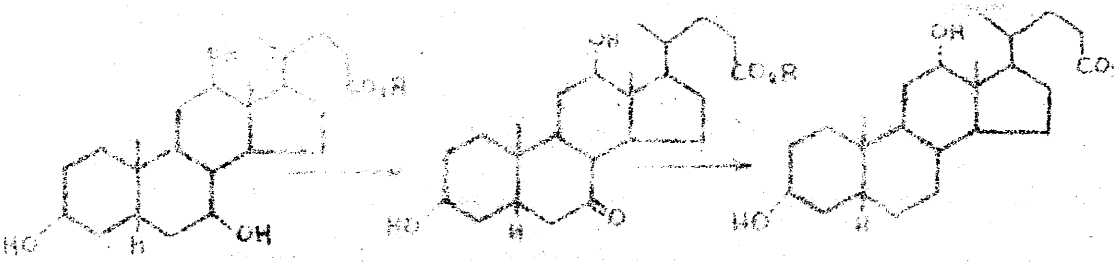


(XXI) (XXII) (XXIII) (XXIV)

(XXII), which was converted to the methyl ketone (XXIII) and treated with perbenzoic acid, inserting the oxygen atom as required to give the 20-acetoxy derivative (XXIV).

[illegible] deoxycholic acid is an important starting [illegible] for the partial synthesis of sex hormones, and [illegible] obtainable from ox bile is approximately about one eighth [illegible] isolated in the same process, [illegible] to realise efficient methods of [illegible] latter to the former. The present [illegible] the fact that the hydroxyl groups of [illegible] are oxidised preferentially in the order 7α-12 [illegible] the selective oxidation of the 7-position [illegible] by Wolff-Kishner reduction.

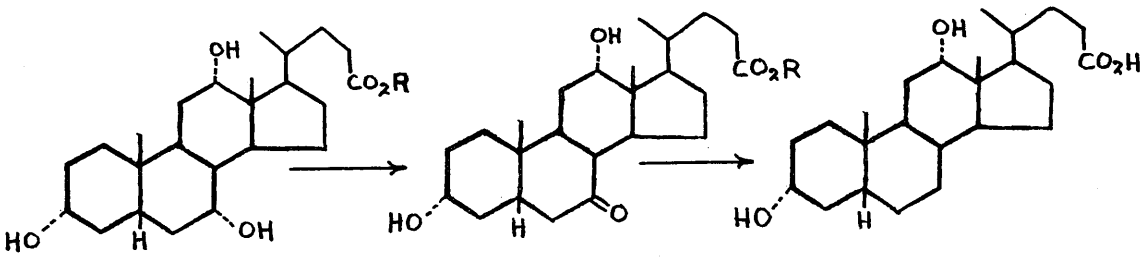
THEORETICAL.



(XXV) (XXVI) (XXVII)

[illegible] and Dane (134) showed that 7α:12α-dihydroxycholanic acid could be converted into 12α-hydroxy-7-ketocholanic acid, and Iwasaki (135) effected the oxidation of 3α:7α-dihydroxycholanic acid to 3α-hydroxy-7-ketocholanic acid. Shortly afterwards, Kaziro and Shimada (136) demonstrated selective oxidation of deoxycholic acid to 3α-[illegible] ketocholanic acid, thus establishing the order of [illegible] susceptibility as C-7>C-12>C-3 and correctly [illegible] the cholic acid should be convertible to 3α:12α-dihydroxy-7-[illegible]

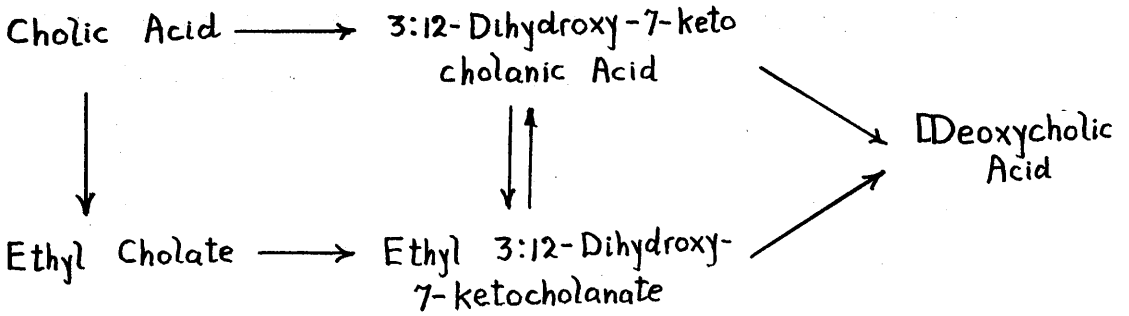
Since deoxycholic acid is an important starting material for the partial synthesis of sex hormones, and the amount obtainable from ox bile is approximately about one eighth as great as the cholic acid isolated in the same process, much research has been done to realise efficient methods of converting the latter to the former. The present best procedures, making use of the fact that the hydroxyl groups of cholic acid are oxidised preferentially in the order 7α-12α-3α, all involve the selective oxidation at the 7-position followed by Wolff-Kishner reduction.



Wieland and Dane (134) showed that 7α:12α-dihydroxycholanic acid could be converted into 12α-hydroxy-7-ketocholanic acid, and Iwasaki (135) effected the oxidation of 3α:7α-dihydroxycholanic acid to 3α-hydroxy-7-ketocholanic acid. Shortly afterwards, Kaziro and Shimada (136) demonstrated the selective oxidation of deoxycholic acid to 3α-hydroxy-12-ketocholanic acid, thus establishing the order of attack susceptibility as C-7>C-12>C-3 and correctly inferred that cholic acid should be convertible to 3α:12α-dihydroxy-7-ketocholanic acid. This actual conversion was first reported by Haslewood(137, 138) by the action of potassium chromate

in acetic acid buffered with sodium acetate, followed by Wolff-Kishner reduction in 40% overall yield. Many other oxidation procedures have since been described. Gallagher and Long (139) oxidised ethyl cholate with chromium trioxide in aqueous acetic acid to give the 7-keto derivative in 40% yield. Two procedures for the large-scale oxidation of cholic acid are described by Hoehn and his associates (140, 141), in one case involving oxidation of the free acid by bromine at -5° (40% yield), and the other consisting of chromic acid oxidation of the ethyl ester (33% yield). It is interesting to note that the microbiological oxidation of cholic acid (142) by the bacillus Alcaligenes faecalis follows the same path as chemical oxidation, giving 3α:12α-dihydroxy-7-ketocholanic acid, then 3α-hydroxy-7:12-diketo-cholanic acid, and finally 3:7:12-triketocholanic acid. In regard to the reduction of the 7-keto grouping, the yield (24%) in the normal Wolff-Kishner reduction (139) is improved to 57% by the modification of Huang-Minlon (143).

In the initial stages of this investigation of possible degradations of the bile acid sidechain, deoxycholic acid was not available, and preliminary small-scale experiments were performed as outlined below as a guide to the ease of its accessibility from cholic acid. Cholic acid (XXV; R = H) and its ethyl ester (XXV ; R = C_2H_5) prepared by the method of Cortese (144), were oxidised respectively by the methods of Haslewood (137) and Hoehn (140) and the



7-keto derivatives (XXVI; R = H) and(XXVI; R = C_2H_5) characterised by their interconversion by hydrolysis and esterification. These latter intermediates were then individually subjected to identical Wolff-Kishner reductions, and deoxycholic acid isolated as its acetic acid and ether complex. From these experiments, it was apparent that the method of Haslewood, using the free acid, was preferable, giving higher yields (about 25% overall) and not requiring isolation of the intermediate 7-keto derivative (XXVI, R = H). Recently, Fieser and Rajagopalan (145) have improved the conversion yield to 54% by applying the Haslewood conditions to methyl 3-carbethoxycholate. The same authors also claim that deoxycholic acid is prepared with great efficiency (68% yield) and ease by conducting the oxidation of cholic acid with N-bromosuccinimide in aqueous sodium hydrogen carbonate solution, followed by Huang-Minlon reduction. The alcholic groups at positions 3 and 12 remain unattacked by excess reagent, making the method conveniently applicable to the preparation of deoxycholic acid from the total crude acids of saponified bile.

Most of the deoxycholic acid used in these investigations was obtained conveniently, however, from crude commercial sodium deoxycholate by mineral acid precipitation from aqueous solution, filtration, extraction of fats by hot benzene, and purification by recrystallisation from aqueous acetone.

In the first projected degradation of the sidechain, the required starting compound was 3α:12α-diacetoxy-24:24-diphenylchol-23-ene (XXIX), obtainable through 3α:12α:24-trihydroxy-24:24-diphenylcholane (XXVIII) (not isolated) by the action of excess phenylmagnesium bromide on methyl deoxycholate (XXVII; R = CH_3). The diphenylethylene (XXIX) was prepared by modification of described methods (146, 116), and isolated by chromatographic purification. The action of three oxidising agents, namely selenium dioxide, N-bromosuccinimide, and tertiary-butyl chromate on XXIX was investigated. The use of selenium dioxide as an oxidising agent has been adequately reviewed (147, 148). It can oxidise methyl or methylene groups adjacent to carbonyl groups, as in (a), or ethylenic (b) or acetylenic groups (c). A different reaction (d) whereby oxygen does not enter the final product, but where dehydrogenation occurs, can also happen.

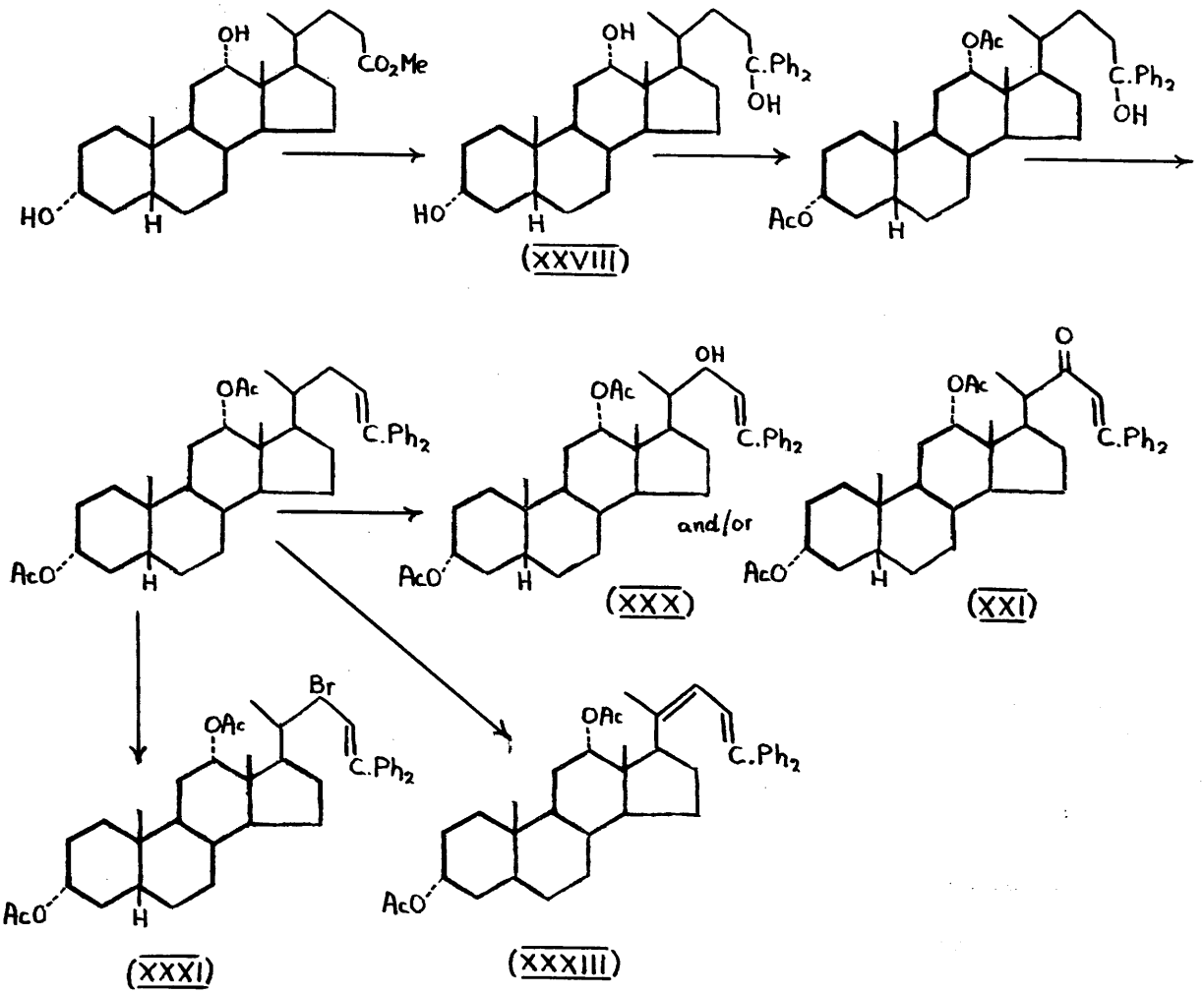
(a) $R.CO.CH_2.R' \xrightarrow{SeO_2} R.CO.CO.R' + Se + H_2O$

(b) $2\ R.CH_2.CH{=}CH.R' \xrightarrow{SeO_2} 2\ R.CH(OH).CH{=}CH.R' + Se$

(c) $2\ R.CH_2.C{\equiv}C.R' \xrightarrow{SeO_2} 2\ R.CH(OH).C{\equiv}C.R' + Se$

(d) $2\ R.CH_2.CH_2.R' \xrightarrow{SeO_2} 2\ R.CH{=}CHR' + Se + H_2O$

In view of these observations, products of this type (XXX), (XXXI), (XXXII) and (XXXIII) were anticipated.



Several attempted oxidations, performed by heating a mixture of selenium dioxide and 3α:12α-diacetoxy-24:24-diphenylchol-23-ene in anhydrous acetic acid under reflux, proved fruitless, in that only unchanged starting material was recovered after removal of the precipitated colloidal selenium by filtration through alumina and repeated recrystallisation.

The allylic bromination of 3α:12α-diacetoxy-24:24-

diphenylchol-23-ene (XXIX) by N-bromosuccinimide, and subsequent dehydrobromination of 3α:12α-diacetoxy-22-bromo-24:24-diphenylchol-23-ene (XXXII) to 3α:12α-diacetoxy-24:24-diphenylchola-20:23-diene (XXXIII) has been reported by **Miescher** and coworkers (119, 120). In the first communication, dehydrobromination was effected by dimethylaniline, and the mixture re-acetylated, and XXXIII was reported as existing in dimorphic forms m.p. 140-142° and 184° with $[\alpha]_D$+197°. In the second improved procedure, bromination was accompanied by incandescent illumination, and the dehydrohalogenation performed by simple refluxing. Under these conditions, the product showed a double melting point, m.p. 144-146° and 179-180° with $[\alpha]_D$+184°. Employing the improved procedure, but increasing the dehydrobromination refluxing time to 7 hours, 3α:12α-diacetoxy-24:24-diphenylchola-20:23-diene (XXXIII) was obtained by direct crystallisation as feathered needles, m.p. 142-144° and $[\alpha]_D$+212°. The yield was increased by treating the mother liquors with dimethylaniline and re-acetylating. The ultra-violet light absorption was comparable in location and intensity to the product described (119, 120).

The introduction of tertiary-butyl chromate as an oxidising agent is due to Oppenauer and Oberrauch (149). The principal feature claimed for this reagent is that it acts much more selectively than the classical chromium trioxide-acetic acid mixture. For example, primary alcohols are oxidised to the corresponding aldehydes in almost theoretical

yield, with only small amounts of the carboxylic acids as secondary products. Another striking feature is its ability to oxidise methylene groups activated by adjacent ethylenic centres to carbonyl groups (i.e. allylic oxidation) with a selectivity of the same order as that of selenium dioxide. It is claimed that cholesteryl acetate is oxidised by this novel reagent to 7-ketocholesteryl acetate in 92% yield, as against the 28% yield obtained by normal chromic acid oxidation (150). Despite the statement by these authors that allylic oxidation does not proceed with diphenylethylenes (no experimental details and no actual compounds are given), the action of tertiary-butyl chromate on 3α:12α-diacetoxy-24:24-diphenylchol-23-ene was considered worthy of investigation. Since, however, many experimental details in the use of this reagent were incomplete, discrepancies exist between the theoretical and experimental sections and the general description is capable of divergent interpretations, model experiments were performed initially on the oxidation of cholesteryl acetate as a guide to optimum experimental conditions. Subsequent application of these conditions to 3α:12α-diacetoxy-24:24-diphenylchol-23-ene resulted in considerable decomposition, with the isolation of an acid fraction believed to be impure 3α:12α-diacetoxynorcholanic acid, and a neutral fraction from which only traces of unidentified crystalline material could be recovered.

Tertiary-butyl chromate can be prepared according to the equation

$$2\ (CH_3)_3.C.OH + CrO_3 \longrightarrow \begin{matrix}(CH_3)_3CO\\(CH_3)_3.CO\end{matrix}\!\!>CrO_2 + H_2O$$

by adding chromium trioxide to an excess of tertiary-butanol. This mixture is then dissolved in a non-polar solvent (light petroleum, benzene, or carbon tetrachloride) and can be concentrated to 30% strength. Such a solution is referred to as Solution A by Oppenauer. The oxidising power can be increased by complete removal of the excess butanol (by vacuum concentration or aqueous extraction) to give Solution B. A substantial increase in oxidising power can be achieved by addition of anhydrous organic acids, e.g., 5-25% acetic acid, to give Solution C. In the theoretical description, the introduction of a small amount of acetic anhydride is recommended.

In the first experiment performed in the oxidation of cholesteryl acetate (XXXIV), the experimental conditions employed were those described by Oppenauer (i.e., Solution C in absence of acetic anhydride) in which a 92% yield of 7-ketocholesteryl acetate was claimed. The basis on which this yield is calculated is not clear, since the product, for which the only physical constant give is a low melting point, and a low mixed melting point, is impure and there is an obvious typographical error in the weight given either of the starting material or the product. In my hands, this method gave a product in excellent yield which was an equimolecular mixed crystal of cholesteryl acetate and

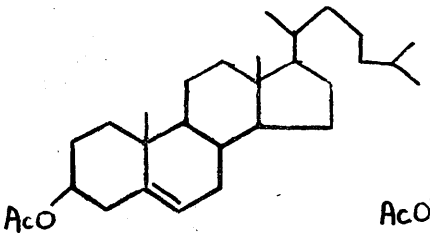
7-ketocholesteryl acetate (XXXV), as shown by analytical, light absorption and specific rotation data as summarised in the table below:-

	m.p.	$[\alpha]_D$	Light Absorption	%C	%H
Oppenauers Product.	147-152°	-	-	-	-
This Report	128-130°	-75°	234 (6,700)	79.6 Found 79.8	10.7 Found 11.0
Cholesteryl Acetate	114-115°	-47°	234 (0)	81.3 Calc	11.3 Calc.
7-Ketocholesteryl Acetate.	158°	-103°	235 (12,000)	78.5 Calc	10.85 Calc.
Equimolecular Mixture.		-75°	234.5 (6,000)	79.5 Calc	10.85 Calc

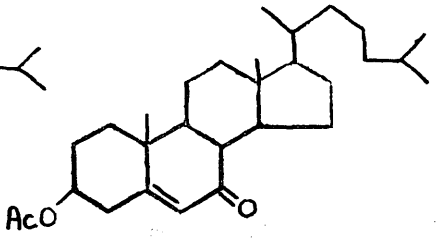
Since in this experiment, the ratio of atoms of oxygen to molecules of cholesteryl acetate was 4, a second experiment using the ratio of 16, i.e., increasing the excess of oxidising agent, was performed in an attempt to complete the oxidation. Under these conditions, also, a mixture of (XXXIV) and (XXXV) was obtained with a slightly higher percentage of the 7-keto component. Pure 7-ketocholesteryl acetate was finally obtained in 55% yield using an oxidising ratio of 8 in the presence of acetic anhydride. It is reasonable to assume that the effect of the anhydride is to react with the water produced in the reaction, thus maintaining anhydrous conditions, necessary to complete the oxidation. The oxidising reagent was also satisfactorily prepared by using chromyl chloride instead of chromium trioxide, and prepared in this way gave 7-ketocholesteryl

acetate in 60% yield.

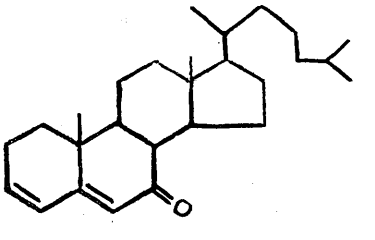
When the equimolecular mixed crystal of cholesteryl acetate and 7-ketocholesteryl acetate was chromatographed on acid washed alumina in an effort to produce separation, the products isolated were cholesteryl acetate and cholesta-3:5-dien-7-one (XXXVI) (oxycholesterylene), which has apparently arisen by removal of the elements of acetic acid from 7-keto-cholesteryl acetate. This transformation has been



XXXIV



XXXV

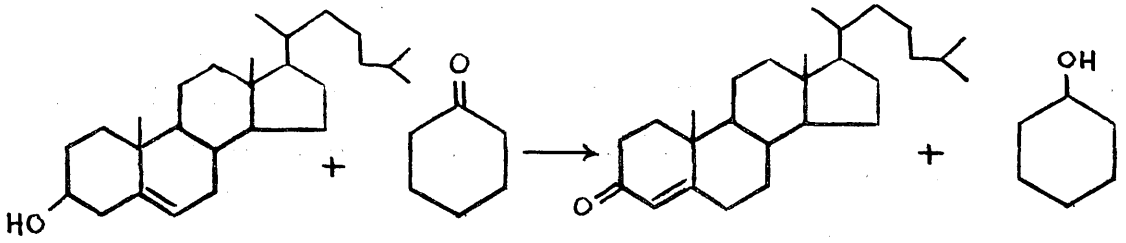


XXXVI

previously effected by treatment of XXXV with alcoholic alkali (151) or alcoholic hydrochloric acid (152). Although the change on alumina has not been reported, Bergstrom and Wintersteiner (153) found that when cholesterol was aerated in colloidal solution, 7-ketocholesterol and cholesta-3:5-dien-7-one were isolated. Since, however, the dienone (XXXVI) was isolated by acetylation of the reaction mixture followed by chromatography, it is conceivable that it has been produced from the intermediate (XXXV), and not as a direct oxidative aeration product.

Cholestenone was chosen as a reference compound to investigate whether tertiary-butyl chromate exerted its known allylic oxidising properties on an $\alpha\beta$-unsaturated ketone to Produce, in this case, the known 3:6-diketocholest-

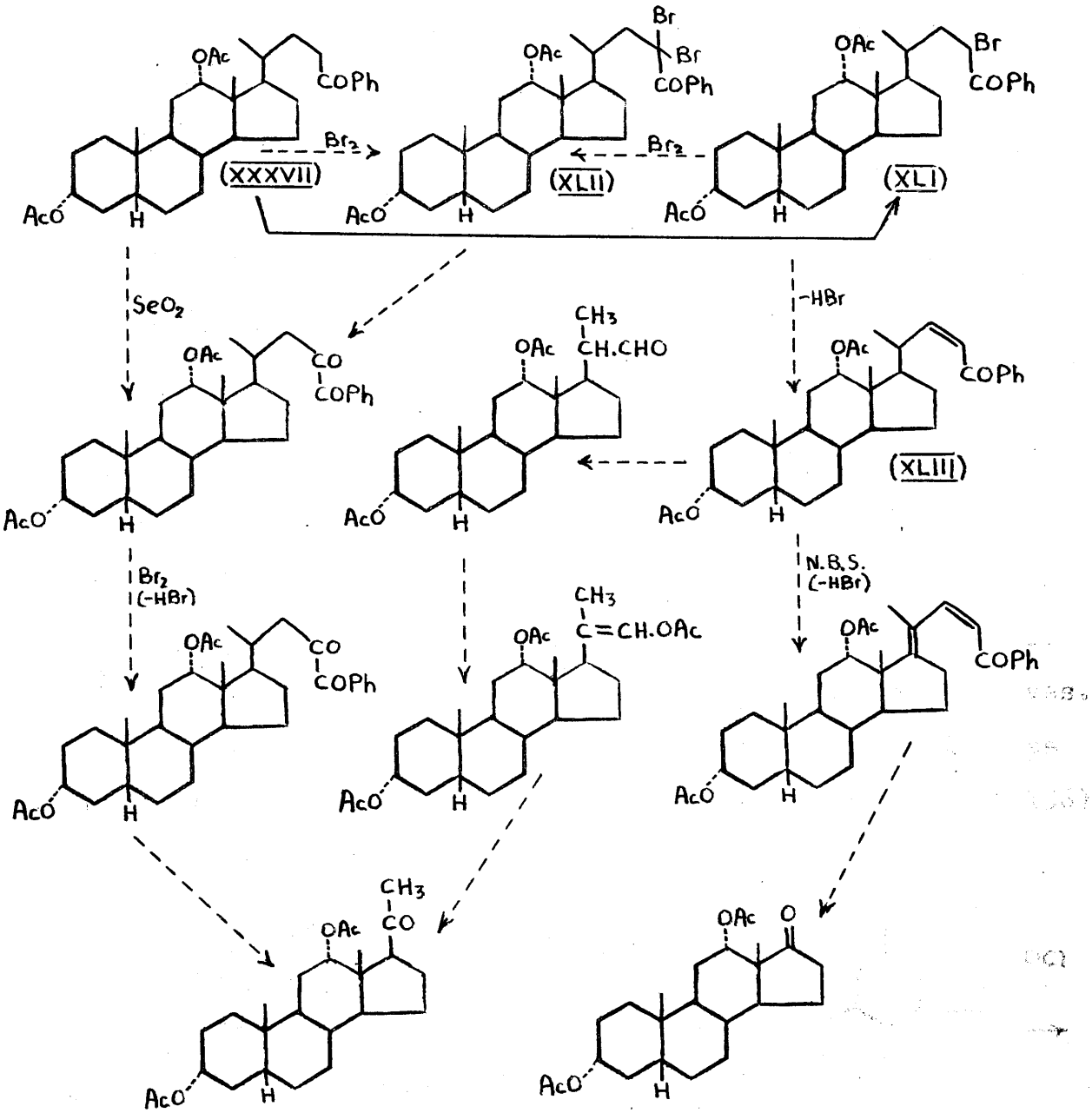
4-ene. Cholestenone was prepared by two routes, the first consisting of the customary Oppenauer oxidation of cholesterol by aluminium tertiary-butoxide (154). The second method employed makes use of the novel hydrogen exchange reaction with cyclohexanone in the presence of Raney nickel catalyst (155). As regards ease of preparation,



yield and quality of product, this method did not offer any advantages over the older method. Treatment of cholestenone with tertiary-butyl chromate gave a 20% yield of an amorphous acid fraction, and examination of the neutral fraction revealed only unchanged starting material. On this evidence, it appears feasible that oxidation may be carried out at other centres of attack in a molecule without prior protection of the unsaturated ketonic system.

Since attempts to improve the degradation of the bile acid sidechain from the intermediate diphenylethylene derivative were unsuccessful, attention was turned to possible degradative routes from the phenyl ketone obtained from deoxycholic acid. It was considered that this derivative would be free from the disadvantages attending the degradation of the methyl ketone (cf.Hollander & Gallagher's method in Historical Section). The degradative routes

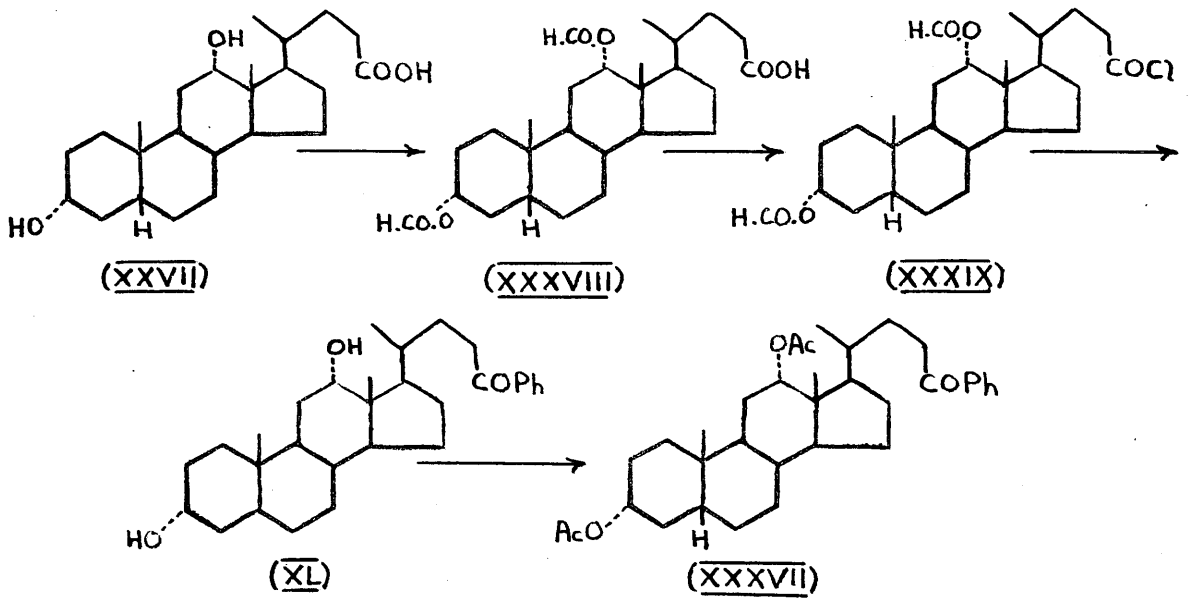
envisaged, leading to 17- and 20- ketones, and outlined below are self-explanatory.



Alkyl and aryl norcholanyl ketones have previously been prepared from bile acids, and their use as starting materials for a convenient degradation of the bile acid sidechain considered. Hollander and Gallagher (130) prepared norcholanyl methyl ketone by the action of diazomethane on

cholanic acid chloride followed by treatment with zinc dust. Bromination of this methyl ketone produced difficulties in the purification of the product due "to traces of the 25-bromoketone or by the presence of the two possible epimeric 23-bromoketones". Jacobsen (131) has reported, without experimental details, the preparation of 3α:7α:12α-triacetoxynorcholanyl phenyl ketone by the action of diphenylcadmium on cholic acid chloride, and also the formation of the corresponding 23-bromo and 23-acetoxy derivatives. The general applicability of this procedure, involving the interaction of acid chlorides with the appropriate cadmium or zinc aryl or alkyl, has been demonstrated by Hoehn and Moffett (156) and Cole and Julian (157) who describe in detail the preparation of a number of phenyl and methyl ketones from various bile acid derivatives.

3α:12α-Diacetoxynorcholanyl phenyl ketone (XXXVII) was prepared essentially by the method of Hoehn and Moffett (156), by the route shown:-



(XXVII) (XXXVIII) (XXXIX)

(XL) (XXXVII)

Formylation of deoxycholic acid (XXVII) by the method of Cortese and Baumann (158, 159) gave 3α:12α-diformoxycholanic acid (XXXVIII). Treatment of the latter compound with thionyl chloride gave the corresponding acid chloride (XXXIX) which was treated without purification, with diphenylcadmium, prepared as described by Gilman and Nelson (160). Alkaline hydrolysis of the reaction mixture gave 3α:12α-dihydroxynorcholanyl phenyl ketone (XL) which on re-acetylation gives 3α:12α-diacetoxynorcholanyl phenyl ketone (XXXVII), characterised by formation of its 2:4-dinitrophenylhydrazone. Both (XL) and (XXXVII) show characteristic ultra-violet light absorption at 2420Å, attributable to the phenyl ketone chromophore.

The bromination of 3α:12α-diacetoxynorcholanyl phenyl ketone was next investigated. By monobromination in acetic acid at room temperature, 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (XLI) was obtained in good yield, and shown to exist in two easily interconvertible forms. On crystallisation from methanol and drying in air, the bromoketone had m.p. 110° $[\alpha]_D$ +93° and the analysis was consistent for 1 molecule of bromoketone containing ½ molecule of methanol. Simple heating in vacuum sufficed to cause its conversion to a form m.p. 167° and $[\alpha]_D$ +95°. The change is presumably due simply to solvation, as the higher-melting form reverts to the lower-melting form on recrystallisation from methanol. This behaviour has also been noted by Cole, Julian, Magnani and Meyer (161) who obtained 3α:12α-diacetoxy-23-bromonorcholanyl

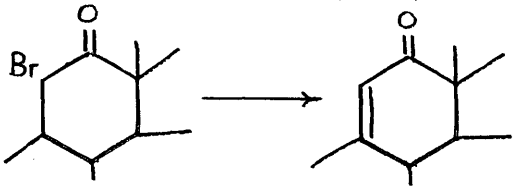
phenyl ketone similarly, describing "the labile form m.p. 106-108°, $[\alpha]_D$+91°" by crystallisation from acetone, and "the stable form, m.p. 175°, $[\alpha]_D$+105°" from boiling ethanol. When, however, the monobromination was performed in more concentrated solution at 30-40°, instead of at room temperature, in addition to the main product, a second well-defined epimeric bromoketone was isolated in about 10% yield. This form is considered to be the diastereoisomer due to the creation of a new centre of asymmetry at C-23.

Two unsuccessful attempts were made to prepare 3α:12α-diacetoxy-23:23-dibromonorcholanyl phenyl ketone (XLII). In the first, in which one mol. of bromine was added to 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone in acetic acid, no uniform homogeneous product could be isolated. In the second, two mols. of bromine were added to 3α:12α-diacetoxynorcholanylphenyl ketone in acetic acid. In this case, the monobrominated phenyl ketone was isolated in good yield. The parent phenyl ketone was also recovered unchanged on treatment with N-bromosuccimide. Attempted oxidation with selenium dioxide gave a crude product from which only starting material was recovered.

Attention was next turned to methods of dehydrobrominating 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone to produce 3α:12α-diacetoxynorchol-22-enyl phenyl ketone (XLIII), and the normal procedure of treatment with tertiary bases was first examined. Heating under reflux with collidine caused considerable decomposition, and isolation only of a brown amorphous product, which gave

dark brown gum products after chromatography. Similar treatment using pyridine also gave only dark amorphous products.

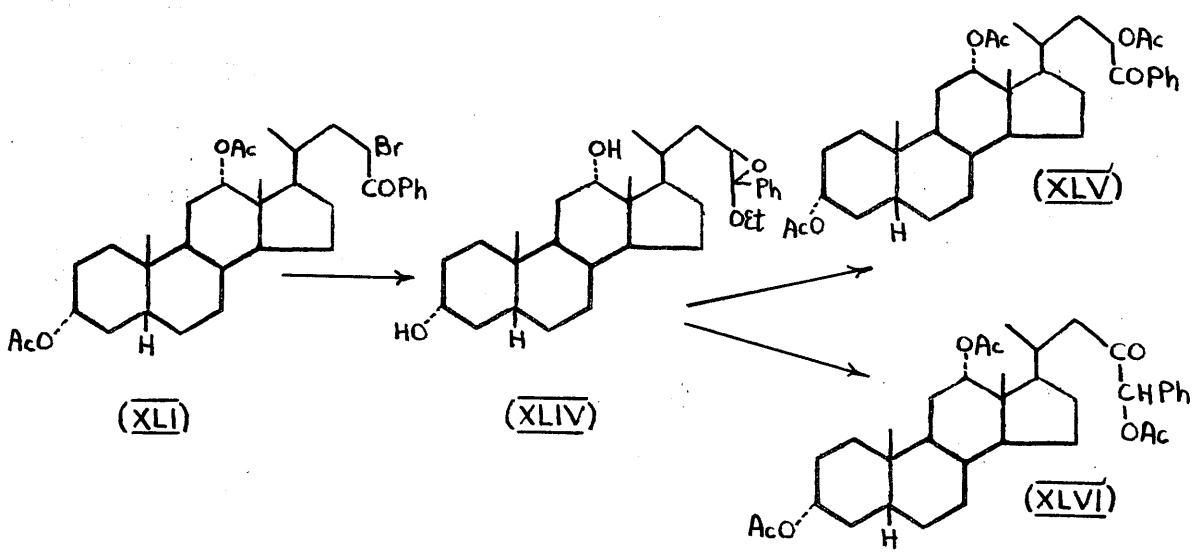
Sodium ethoxide has been used as a dehydrohalogenating agent on steroid compounds by Chakravorti and Wallis (162) in their studies on 3α-acetoxy-11-bromo-12-ketocholanic acid. Hicks, Berg and Wallis (163), appreciating that



αβ-unsaturated ketones are often unstable in alkali, modified the conditions and obtained the dehydrobrominated product in 65% yield. It was decided, therefore, to use these experimental conditions. In a preliminary experiment, a solution of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone in absolute ethanol was heated under reflux for 30 minutes with sodium ethoxide, then the solvent removed. Examination of the aqueous extract revealed the presence of bromide ions, and the crude product, after washing, gave a negative Beilstein reaction, indicating removal of the 23-bromine atom. A product, isolated from a mixture in poor yield, proved difficult to purify, and inconsistent analyses were obtained, due probably to the existence of the hydroxyl groups (from hydrolysis of acetoxy groups) causing solvation. The light absorption data, however, (discussed later), was consistent with the replacement of the bromine atom by either a hydroxyl or ethoxy group.

In view of this experiment, it was considered

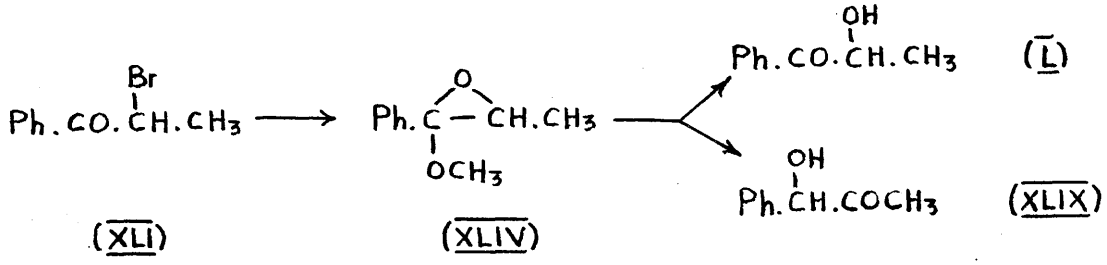
advantageous in subsequent experiments to re-acetylate the reaction mixture before examining the products. Under these conditions, two isomeric compounds, $C_{36}H_{50}O_7$, were isolated by fractional crystallisation and chromatography. The structure of one of these compounds, m.p. 173°, $[\alpha]_D$+90° with light absorption at 2450Å, has been established as 3α:12α:23-triacetoxynorcholanyl phenyl ketone (XLV) by comparison with a specimen prepared by an unambiguous route. The other compound, m.p. 215°, $[\alpha]_D$+146°, possessing no high intensity light absorption above 2200Å is believed to be 3α:12α:24-triacetoxy-23-keto-24-phenylcholane (XLVI). It is apparent from the light absorption data that the phenyl radicle and ketone group are no longer in conjugation, which can be explained by the formation of XLVI (as also can XLV) from the postulated intermediate 23:24-epoxy-3α:12α-dihydroxy-24-ethoxy-24-phenylcholane (XLIV).



In order to prove conclusively that the ethoxy radicle was absent in the end-products (XLV) and (XLVI), the

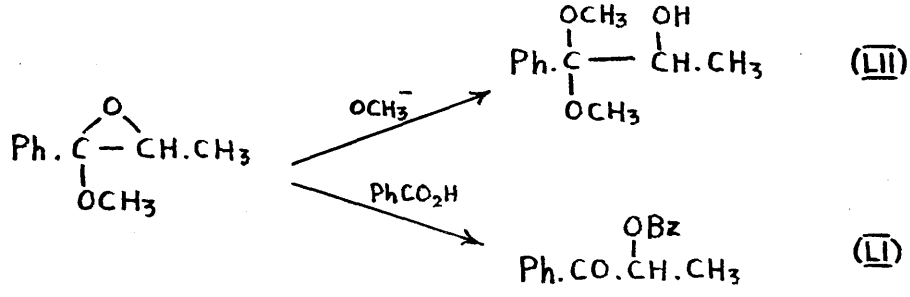
bromoketone (XLI) was treated similarly with sodium methoxide, and the reaction mixture re-acetylated. The same two compounds were isolated, showing that the alkoxy radicle is absent in the products.

A precedent for the formation of XLV and XLVI from the phenylbromoketone (XLI), through an intermediate postulated as XLIV is apparent in the work of Temnikova and Kropacheva (164). These authors showed that treatment of α-bromopropiophenone (XLVII) with sodium methoxide gave 1:2-epoxy-1-methoxy-1-phenylpropane (XLVIII) which on hydrolysis with 5% sulphuric acid gave phenylacetylcarbinol (XLIX) and methylbenzoyl carbinol (L).



(XLI) (XLIV) (L) (XLIX)

The epoxyether formation was confirmed by Stevens, Malik and Pratt (165) who showed, moreover, that cleavage of the epoxide with benzoic acid gave α-hydroxypropiophenone benzoate (LI), and with warm methanol gave α-hydroxypropiophenone dimethyl acetal (LII).



(LII) (LI)

The mechanism for the formation of α-hydroxyketals from

certain α-haloketones with alcoholic sodium alkoxides via epoxyether intermediates has been postulated by Ward (166) and by Kohler and Addinall (167). Whereas the formation of α-acetoxy phenyl ketone (XLV) is easily explained by these analogies, the method of formation of the acetoxybenzyl ketone (XLVI) is less clear, although it is feasible that it has arisen by the hydrochloric acid washing of the sodium alkoxide product.

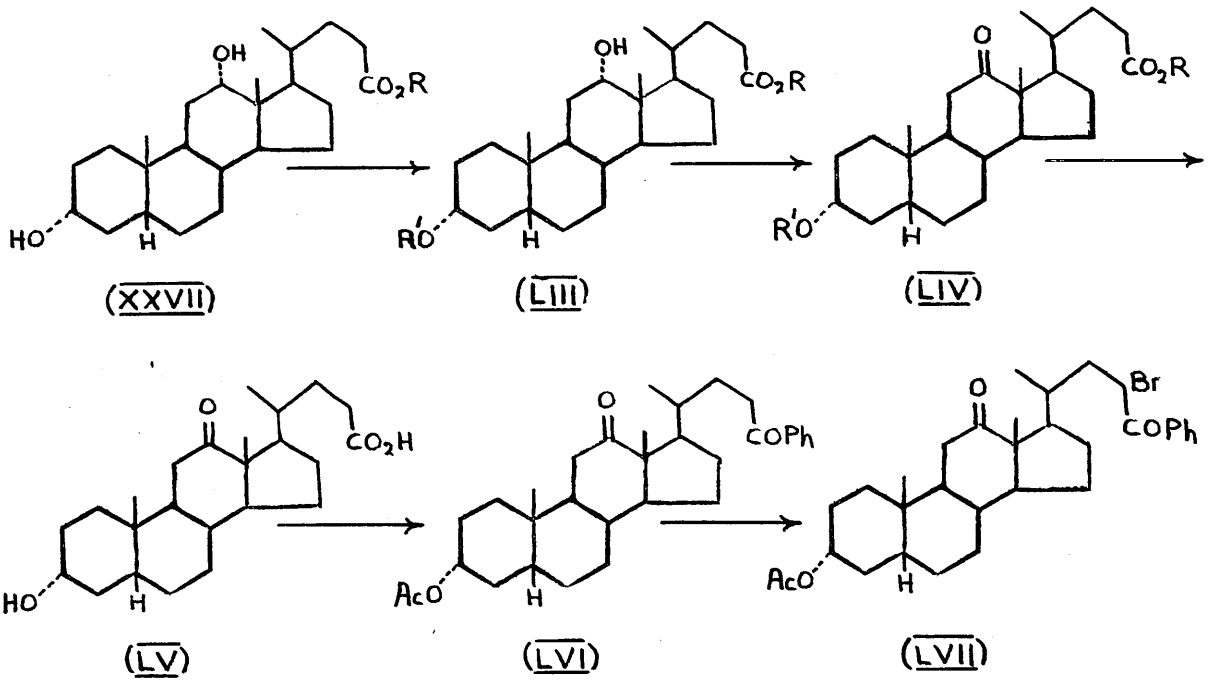
In order to establish unequivocally the structure of 3α:12α;23-triacetoxynorcholanyl phenyl ketone, an authentic specimen was prepared by replacement of the bromine atom in 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone with the acetoxy group. This was achieved by heating a solution of the bromoketone in ethanol under reflux with fused potassium acetate. When the bromoketone was heated at 100° with potassium acetate in glacial acetic acid, it was recovered unchanged.

When 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone was treated with strong alkali (Marker-Lawson conditions), the neutral fraction, obtained in only 40% yield, was an amorphous solid. Crystallisation from methanol gave, in 5% yield, a product m.p. 179-182°, $[\alpha]_D$ +36°, believed to be 3α:12α:23-trihydroxynorcholanyl phenyl ketone. It appears that the α-hydroxyketone is unstable in presence of strong alkali. An appreciable amorphous acid fraction was also isolated. Due to the nature of the products, and the poor yield of crystalline material, this reaction was not further investigated.

The light absorption characteristics of 3α:12α-diacetoxynorcholanyl phenyl ketone (XXXVII) and its 23-bromo (XLI) and 23-acetoxy (XLV) derivatives are of some interest. The parent phenyl ketone exhibits maximum light intensity absorption at 2420Å, in good agreement with that reported for acetophenone, 2410Å, by Campbell, Linden, Godshalk and Young (168). The introduction of α-substituents causes a marked increase in the wavelength of maximum absorption, the bathochromic shift amounting to 3mμ in the case of the α-acetoxy derivative, and 14mμ in the case of the α-bromo derivative. In comparison with acetophenone, α-bromoacetophenone exhibits maximum absorption at 2480Å, (169), i.e., a bathochromic shift of 7mμ. This behaviour has previously been noted in the steroid nucleus (170). Campbell et al (168) have drawn attention to the fact that the spectrum of a phenyl ketone (acetophenone) is similar to, and the intensity the same order as, an aromatic ring in conjugation with an ethylenic linkage, and Djerassi et al (171) have shown, in a study of the effect of bromine substitution on the absorption spectra of αβ-unsaturated ketones, that in the presence of a phenyl group in conjugation with either the double bond or carbonyl group, the bathochromic shift to the introduction of a bromine atom is 8-14mμ.

The investigation on bromination of norcholanyl phenyl ketones has been extended by application to the corresponding 3α-acetoxy-12-ketonorcholanyl phenyl ketone (LVI). The general procedure for the preparation of the necessary

intermediate, 3α-hydroxy-12-ketocholanic acid (LV) consists of protection of the 3α-hydroxy group of deoxycholic acid (or its ester), followed by chromic acid oxidation and alkaline hydrolysis. The 3-hydroxyl group has been



protected by formation of the hemisuccinate (172, 173), acetate (174, 175, 176, 177) and benzoate (178). Using the method of Kendall (178) methyl 3α-benzoxy-12α-hydroxycholanate (LIII; R = Me, R^{1} = B_z) was prepared, and oxidised by chromic acid in a two phase system to give methyl 3α-benzoxy-12-ketocholanate (LIV; R = Me, R^{1} = B_z) in high yield. The latter compound was not purified, but hydrolysed directly to give the desired acid (LV). Although this procedure gave the keto acid in good yield, the method of Kaziro and Shimada (136) (cf. also Bergstrom and Haslewood (179)), whereby deoxycholic acid is oxidised directly under controlled conditions, proved preferable, a comparable

yield being obtained with more ease and rapidity.

3α-Hydroxy-12-ketocholanic acid was converted to 3α-acetoxy-12-ketonorcholanyl phenyl ketone (LVI) by the diphenylcadmium route outlined previously for the corresponding 3α:12α-diacetoxy series, and the constants of the intermediates proved to be in good agreement with those reported by Hoehn and Moffett (156) 3α-Hydroxy-, 3α-formoxy-, and 3α-acetoxy-12-ketonorcholanyl phenyl ketone all show light absorption maximum intensity at 2410Å (attributable to the phenyl ketone system) and a low intensity inflection at 2700-2800Å (attributable to the 12-keto grouping).

By treatment of 3α-acetoxy-12-ketonorcholanyl phenyl ketone with one mol. of bromine in glacial acetic acid at 30-35°, 3α-acetoxy-23-bromo-11-ketonorcholanyl phenyl ketone (LVII) was isolated in two distinct forms, considered to be diastereoisomers due to the creation of an asymmetric centre at C-23. Both forms exhibited identical light absorption at 2550Å (ϵ = 10,900), the bathochromic shift of 14 mμ showing conclusively that bromine had entered, as expected, at C-23 in preference to C-11.

EXPERIMENTAL.

All m.p's were determined using a standardised N.P.L. thermometer.

Specific rotations were determined in chloroform solution (except where otherwise stated) in a 1 dm. tube at room temperature.

Ultraviolet absorption spectra were measured in ethanol solution (except where otherwise stated) using a Unicam SP. 500 spectrophotometer.

Microanalyses were determined by Dr. A.C. Syme and Mr. Wm. McCorkindale, of the Chemistry Department, Royal Technical College, Glasgow, and Messrs. Weiler and Strauss, Oxford.

The alumina used for chromatographic purposes was that supplied by Savory and Moore, Grade II (except where otherwise stated), standardised according to Brockmann.

Ethyl Cholate.

(cf. Cortese J.Amer.Chem.Soc., 59 (1937), 2532)

To ethanol (95%; 250 c.c), previously cooled to 0°, was added fuming sulphuric acid (20-30% SO_3; 10 c.c.) and cholic acid (25 g.). After shaking for 5 hours, the insoluble impurities were removed by decantation, the mixture kept at 0° for 24 hours, then poured slowly with stirring into a solution of sodium hydroxide (20 g.) in water (2 litres). Ethyl acetate (50 c.c.) was stirred in to neutralise excess alkali, the product removed by filtration after standing at 0° for 10 hours, and dried in vacuo at room temperature. One crystallisation from light petroleum (b.p. 40-60°)-ethyl acetate (4:1) yielded ethyl cholate (25.6 g.) , m.p. 153-4°. It was not purified further.

Cortese (loc.cit) gives m.p. 162-163° for the pure ester.

Ethyl 3α:12α-dihydroxy-7-ketocholanate.

Method 1. (cf. Hoehn & Linsk J.Amer.Chem.Soc. 67 (1945), 312)

To a solution of ethyl cholate (22 g.) in acetic acid (125 c.c.; 70%) cooled to -5°, was added chromic acid solution (22 c.c.; N) with stirring over 1½ hours. After stirring for a further 30 minutes, the reaction mixture was poured into water and extracted with benzene (4 x 25 c.c.). The extract was washed successively with water, dilute hydrochloric acid and water, then the solvent removed under reduced pressure. Crystallisation of the residue from methanol yielded ethyl 3α:12α-dihydroxy-7-ketocholanate (3.3g)

m.p. 153-156°. Less pure product was obtained in subsequent crops.

Fieser and Rajagopalan (145) give m.p. 158-159°

Method 2. (cf. Haslewood Biochem.J. 37 (1943), 109)

To a solution of cholic acid (10 g.) in warm glacial acetic acid (100 c.c.) was added crystalline sodium acetate (20 g.). The solution was then cooled to 20°, and treated with a solution of potassium chromate (6.34 g.) in water (20 c.c.), added with agitation until solution was complete. After 24 hours, the solution was diluted with water (700 c.c.). Sodium chloride (100 g.) was added, the mixture allowed to stand for a further 24 hours, then filtered. The filter was washed with sodium hydroxide solution (100 c.c.; N) and water (200 c.c.), these washings added to the filtrate, and the mixture boiled for 10 minutes to coagulate the chromium salts. Sodium chloride (50 g.) and sulphuric acid (200 c.c; N) where then added with shaking. After standing 16 hours, the precipitate was collected, washed with cold water, partially dried and taken up in ethanol. The ethanol solution was filtered and the solvent removed under reduced pressure to leave crude 3α:12α-dihydroxy-7-ketocholanic acid as a yellow gum (7.1 g.). The gum (1 g.) was heated under reflux with ethanol (4 c.c.) and concentrated sulphuric acid (1 c.c.), diluted with water and extracted with ether. The ether extract was washed successively with water, sodium carbonate solution and water, the ether evaporated and the residue crystallised and recrystallised from methanol to give ethyl 3α;12α-dihydroxy-7-ketocholanate

(500 mg.) m.p. 157°, undepressed by a specimen prepared by Method 1.

Haslewood (loc. cit) gives m.p. 155-157°.

3α:12α-Dihydroxy-7-ketocholanic acid.

Ethyl 3α:12α-dihydroxy-7-ketocholanate (300 mg.) was added to a solution of sodium (70 mg.) in ethanol (2.5 ml.) and water (1 drop), and the mixture heated under reflux for 30 minutes. After cooling, water (200 c.c.) was added, the mixture heated to boiling and acidified with sulphuric acid (5 c.c.; N). The precipitate (230 mg.) was collected after 2 days, and after drying at 65-70° had m.p. 132-148°. (The m.p. is largely dependent on the degree of hydration which varies with the drying conditions). Crystallisation from ethyl acetate gave 3α:12α-dihydroxy-7-ketocholanic acid as needles, m.p. 195°.

Hoehn and Linsk (7) give m.p. 197-199° for material crystallised from ethyl acetate.

Deoxycholic Acid.

(a) Kishner-Wolff reduction of ethyl 3α:12α-dihydroxy-7-ketocholanate.

Ethyl 3α:12α-dihydroxy-7-ketocholanate (3.0 g.), added to a mixture of sodium (1.2 g.) in ethanol (15 c.c.) and hydrazine hydrate (7 c.c.; 85%) was heated at 180-210° for 3 hours in a stainless steel tube. After cooling, the product was extracted with water (500 c.c.), acidified with dilute sulphuric acid, and the suspended precipitate gently warmed to disperse gels. After standing overnight, the

precipitate was collected, washed with water and dissolved in cold ethanol. The solution was filtered and the solvent removed under reduced pressure to leave a yellow gum which crystallised on addition of ether (10 c.c.). Filtration gave the deoxycholic acid - ether complex (700 mg.), m.p. 149-153°.

(b) Kishner-Wolff reduction of 3α:12α-dihydroxy-7-ketocholanic acid.

A solution of 3α:12α-dihydroxy-7-ketocholanic acid (6.0 g.; gum as prepared by Haslewood method) in ethanol (15 c.c.) treated exactly as in (a) gave the deoxycholic acid - ether complex (2.30 g.) m.p. 147-152°.

Recrystallisation from acetic acid gave the deoxycholic acid - acetic acid complex as needles m.p. 137°.

(c) Sodium Deoxycholate.

Commercial sodium deoxycholate (200 g, Allen and Hanbury) was dissolved in warm water (2 litres), cooled, and the dark brown liquor acidified by dropwise addition with vigorous stirring of a mixture of concentrated hydrochloric acid (50 c.c.) and water (200 c.c.). After standing overnight, the precipitated acid was filtered, partially dried in vacuum over potassium hydroxide, and extracted by suspending it in refluxing benzene. Dryness was ensured by distilling off the benzene - water azeotrope, then the acid was removed by filtration. (evaporation of the filtrate yielded a residue (9 g.) of benzene soluble material which was not examined). The acid was recrystallised

from acetone - water (4:1) to yield deoxycholic acid (130 g.) m.p. 168°.

Methyl Deoxycholate.

Concentrated hydrochloric acid (8 c.c.) was added to a solution of deoxycholic acid (130 g.) in methanol (500 c.c.) After standing overnight at 0°, the mixture was neutralised by addition of sodium hydrogen carbonate, concentrated under reduced pressure, and the product allowed to crystallise. Filtration gave methyl deoxycholate (101 g.) m.p. 65-78°.

The m.p. of this ester (solvated with methanol) is not a satisfactory criterion of purity.

3α:12α:24-Trihydroxy-24:24-diphenylcholane.

cf. Organic Syntheses 24, 41.

A solution of methyl deoxycholate (100 g.: 0.25 M) in dry benzene (1 litre) was added with stirring over 1 hour to a solution of phenyl magnesium bromide (made from magnesium (96 g.; 4M) and bromobenzene (628 g.; 4M) in ether (1100 c.c.) and the mixture heated under reflux for 3 hours. The ether was then distilled off, benzene (1 litre) added and the mixture heated under reflux for a further 20 hours after which it was cooled and added cautiously with vigorous stirring to crushed ice (3 kg.) containing concentrated hydrochloric acid (1 litre). The benzene layer was separated, washed successively with aqueous hydrochloric acid (5N, 2 x 1 litre), sodium carbonate solution (5%, 2 x 1 litre) and water (2 x 1 litre).

Evaporation of the solvent left a viscous syrup which was taken up in methanol (500 c.c.) and hydrolysed (to remove unchanged ester) by refluxing for 1 hour with a solution of potassium hydroxide (15 g.) in water (50 c.c.). The reaction mixture was then concentrated under reduced pressure (crystals of diphenyl were observed in the distillate), the residue extracted with ether, and the ether extract washed with water. Removal of the ether gave a syrup, which was dried by dissolving in benzene (500 c.c.) and removing the solvent by distillation. The desired product, obtained as a viscous syrup, was not further purified.

3α:12α-Diacetoxy-24:24-diphenylchol-23-ene.

3α:12α:24-Trihydroxy-24:24-diphenylcholane (gum as obtained above) was taken up in dry pyridine (200 c.c.) and acetic anhydride (400 c.c.). After heating on the water bath for 20 hours, the cooled reaction mixture was poured on to ice to produce an oil which solidified on trituration. The solid was removed by filtration, taken up in ether (500 c.c.), washed successively with water (500 c.c.), dilute hydrochloric acid (5N; 1 litre), sodium carbonate solution (10%; 600 c.c.) and water (1 litre) and dried (Na_2SO_4). Removal of the ether gave 3α:12α-diacetoxy-24;24-diphenylcholan-24-ol as a viscous syrup which was dissolved in glacial acetic acid (200 c.c.). The solution was distilled for 6 hours from a 4 foot helix packed column employing a high reflux ratio. The remaining solvent was then removed under reduced pressure and the residual viscous

oil poured into water to give a product which solidified on trituration. This solid was dissolved in ether (200 c.c.) washed successively with water, saturated hydrogen carbonate solution and water, the ether removed and the residue dried by benzene co-distillation. A benzene solution was chromatographed on a column (26 x 2.7 cm.) of alumina, and the column developed with the same solvent. Evaporation of the eluate (1 litre) yielded a pale yellow gum which gave 3α:12α-diacetoxy-24:24-diphenylchol-23-ene (36.5 g.) m.p. 145° as needles from n-propyl ether - light petroleum (b.p. 40-60°). Recrystallisation from ethanol raised the m.p. to 159°.

$[\alpha]_D$ +89.4° (C, 2.0)

Light absorption: maximum at 2520Å (ε = 15,400)

Found: C, 80.8 ; H, 9.0

Calc. for $C_{40}H_{52}O_4$: C, 80.5 ; H, 8.8%

Hoehn & Mason (J.Amer.Chem.Soc. 60 (1938), 1493) give m.p. 160°, $[\alpha]_D$ +118° (in alcohol).

Organic Syntheses 24, 41 gives m.p. 159-160°.

Experiments on the Oxidation of 3α:12α-Diacetoxy-24:24-diphenylchol-23-ene.

(a) Using selenium dioxide.

Anhydrous selenium dioxide (5.0 g.) was added to a solution of 3α:12α-diacetoxy-24:24-diphenylchol-23-ene (5 g.) in glacial acetic acid (200 c.c.) and the mixture refluxed for 30 hours, during which the solution darkened considerably and an odour of hydrogen selenide was perceptible. The solvent was then removed under reduced pressure, the residue taken up in ether, filtered, washed successively

with water, saturated sodium hydrogen carbonate solution, water, refiltered and dried (Na_2SO_4). Removal of the ether gave a dark red rum, which was dissolved in benzene (100 c.c.), filtered through a column (8 x 2 cm.) of alumina, and eluted with the same solvent (1100 c.c.). Removal of the benzene left a dark brown gum (4.5 g.) still contaminated with selenium, which, after repeated recrystallisation from ethanol, yielded unchanged starting material, m.p. 159-161°.

(b) Using N-bromosuccinimide.

cf. Meystre, Frey, Wettstein & Miescher Helv.Chim.Acta 27, (1944), 1815.

Meystre, Neher, Ehmann & Miescher Helv.Chim.Acta 28, (1945), 1252.

N-Bromosuccinimide (6 g.) was added to a solution of 3α:12α-diacetoxy-24:24-diphenylchol-23-ene (20 g.) in carbon tetrachloride (200 c.c.) and the mixture heated under reflux, with irradiation from two 100 watt lamps, for 20 minutes. The succinimide was then removed by filtration, the filtrate heated under reflux for 7 hours (during which hydrogen bromide was evolved), then taken to dryness under reduced pressure. The gum residue was crystallised from acetone, and recrystallised thrice from acetone - methanol to give 3α:12α-diacetoxy-24:24-diphenylchola-20:23-diene (10.0 g.) feathered needles, m.p. 137-139°. The acetone crystallisation mother liquors were evaporated to dryness and the residue heated under reflux for 1 hour with dimethylaniline (10 c.c.), diluted with water and extracted with ether. The ether extract was washed with dilute

hydrochloric acid and water, dried (Na_2SO_4), evaporated to dryness and acetylated by heating with pyridine (10 c.c.) and acetic anhydride (10 c.c.) for 1 hour on the water bath. Working up in the usual way gave, on crystallisation from aqueous acetone, a further yield (2.5 g.) of the same product, m.p. 135-140°. Recrystallisation for analysis raised the m.p. to 142-144°.

$[\alpha]_D$+212° (C, 0.6).

Found: C, 80.5 ; H, 8.7

Calc. for $C_{40}H_{50}O_4$: C, 80.5 ; H, 8.5%

Light absorption: maximum at 3050Å (ε = 26,000)

Meystre et al (118) give m.p. 140-142° and 184°, $[\alpha]_D$+197° for this compound.

(c) Using tertiary-Butyl Chromate.

Glacial acetic acid (2 c.c.) and acetic anhydride (1 c.c.) was added to a solution of 3α:12α-diacetoxy-24:24-diphenylchol-23-ene (1 g.; 1.7 mM) in carbon tetrachloride (5 c.c.). A solution of tertiary-butyl chromate (≡ 8.84 mM CrO_3) in carbon tetrachloride (10 c.c.) was added dropwise over 30 minutes with stirring, then stirred for 2 hours at room temperature, 2 hours at 50°, and the reaction mixture allowed to stand overnight. The resulting sludge was then shaken for 30 minutes with a saturated aqueous solution of oxalic acid, the organic phase separated, and the aqueous phase extracted several times with carbon tetrachloride. The combined extracts were washed with water, $\frac{N}{2}$ sodium hydroxide solution (produced a yellow aqueous layer after precipitate

and emulsion formation) and water.

The alkali soluble fraction was acidified with hydrochloric acid and extracted with ether. Removal of the ether from the dried extract left a yellow gum (200 mg.) which gave a solid m.p. 174-184° from ether - light petroleum. A recrystallisation from the same solvent raised the m.p. to 184-190°.

The carbon tetrachloride extract was dried (Na_2SO_4), and the solvent removed under reduced pressure to leave an oily gum which could not be crystallised. Half of this product was taken up in methanol, and treated with Brady's reagent to give an orange precipitate (12 mg.). Two crystallisations from chloroform - methanol gave an orange dinitrophenylhydrazone, m.p. 233°.

Light absorption: maxima at 2460Å ($E^{1\%}_{1cm}$ 560) and 3900Å ($E^{1\%}_{1cm}$ 710).

The remainder of the product was dissolved in benzene and chromatographed on benzene. Elution with benzene (350 ml.) gave a colourless oil, which deposited crystals (40 mg.) after standing several weeks. Filtration yielded a solid m.p. 133-137°.

Light absorption: maximum at 2460-2500Å ($E^{1\%}_{1cm}$ 22).

Subsequent development of the column yielded only amorphous and gum products.

Preparation of tertiary-Butyl Chromate.

(a) From Chromium Trioxide.

Oppenauer & Oberrauch Anales asoc.quim.argentina 37 (1949), 246.

Chromium trioxide (30 g.) was dissolved in tertiary-butanol (86 c.c.) with external cooling. The solution was then diluted with carbon tetrachloride (400 c.c.), anhydrous sodium sulphate added, and the mixture stirred vigorously whilst the less dense aqueous layer disappeared, after which it was allowed to stand overnight. The solution was evaporated under reduced pressure, after removal of the sodium sulphate, to a volume of 160 c.c., then made up to 200 c.c. with more carbon tetrachloride.

(b) From Chromyl Chloride.

Chromyl chloride (15.5 g.) was added slowly with shaking and cooling to tert-butanol (37.0 g.), allowed to stand 15 minutes, then diluted with carbon tetrachloride (250 c.c.). The mixture was then stirred vigorously with anhydrous sodium sulphate, and allowed to stand overnight. After removal of the sodium sulphate, the solution was concentrated under reduced pressure to a volume of 100 c.c.

Action of Tertiary-Butyl Chromate on Cholesteryl Acetate.

(1) Using 4 atoms of oxygen per mole of cholesteryl acetate

Tertiary-butyl chromate solution (50 c.c.; 4 atoms O equivalent; prepared as in (a) above), to which glacial acetic acid (10 c.c.) had been added, was added dropwise with vigorous stirring over 2 hours to a solution of cholesteryl acetate (10 g.) in carbon tetrachloride (6 c.c.). The dark reaction mixture was allowed to stand at room temperature for 48 hours after which a heavy gel had separated. The mixture was then stirred with a saturated aqueous solution of oxalic

acid until evolution of carbon dioxide ceased (2 hours). The carbon tetrachloride layer was then separated, and the aqueous layer twice extracted with the same solvent. The combined carbon tetrachloride extracts (100 c.c.) were washed successively with water, sodium carbonate solution, water and dried (Na_2SO_4). Acidification of the alkaline extract yielded a negligible acid fraction. Removal of the carbon tetrachloride under reduced pressure left a white crystalline solid (9.7 g.), m.p. 107-121°. Recrystallisations from methanol yielded a mixed crystal of cholesteryl acetate and 7-ketocholesteryl acetate (1:1) as fine needles, m.p. 128-130°. A solution in chloroform gave a yellow colour with tetranitromethane.

$[\alpha]_D$ -75° (C, 1.0)

Calc. for 1:1 mixture $[\alpha]_D$ -75°

Light absorption: maximum at 2340Å (ϵ = 6,800)

Calc. for 1:1 mixture : ϵ = 6,100

Found: C, 79.6, 79.8 ; H, 10.7, 11.0

Calc. for $C_{29}H_{48}O_2 \cdot C_{29}H_{46}O_3$: C, 79.5 ; H, 10.85%

(2) Using 16 atoms of oxygen per mole of cholesteryl acetate.

Cholesteryl acetate (5.0 g.) in carbon tetrachloride (8 c.c.) was treated with tertiary-butyl chromate solution (100 c.c.) and acetic acid (16 c.c.) and the reaction mixture worked up as in(1). After repeated recrystallisations from methanol, the mixed product of cholesteryl acetate and 7-ketocholesteryl acetate had m.p. 136-138°.

$[\alpha]_D$-82° (C, 1.0)

Light absorption: maximum at 2350Å (ϵ = 7,800)

Found: C, 80.5 ; H, 11.2

Calc. for $C_{29}H_{48}O_2$: C, 81.25 ; H, 11.3

Calc. for $C_{29}H_{46}O_3$: C, 78.7 ; H, 10.8%.

(3) Using 8 atoms of oxygen per mole of cholesteryl acetate in presence of acetic anhydride.

To a solution of cholesteryl acetate (4.0 g.) in carbon tetrachloride (10 c.c.) was added tertiary-butyl chromate solution (75 c.c.) to which glacial acetic acid (8 c.c.) and acetic anhydride (4 c.c.) had been added. The reaction conditions and isolation procedure adopted in (1) and (2) were followed to yield 7-ketocholesteryl acetate (2.22 g.) m.p. 156-158° (undepressed on mixture with an authentic specimen) as needles from methanol.

$[\alpha]_D$-105° (C, 0.8) Literature value $[\alpha]_D$-103°

Light absorption: maximum at 2340Å (ϵ = 12,300)

(4) Using conditions of (3) and tertiary-butyl chromate prepared as in (b).

Cholesteryl acetate (4.0 g.) in carbon tetrachloride (10 c.c.) was treated as described above with tertiary-butyl chromate solution (50 c.c. prepared as in (b)) to which glacial acetic acid (10 c.c.) and acetic anhydride (3 c.c.) had been added. In this case, the yield of 7-ketocholesteryl acetate, m.p. 155-157° was 2.43 g. (60%). An appreciable quantity of a brown amorphous resin was isolated from the acid fraction.

Chromatography of Mixed Crystal, m.p. 128-130°, of Cholesteryl Acetate and 7-Ketocholesteryl Acetate.

The alumina used (Savory and Moore) was washed with warm aqueous acetic acid (50%), then with distilled water until the washings were neutral. It was re-activated by heating at 200° for 48 hours. The column used was 20 cm. x 1.8 cm. dia. The mixed crystal (1.0 g.) was dissolved in benzene, and developed a pale yellow fluorescence on the column. Fractions were collected in 50 c.c. quantities.

Fraction.	Eluent.	Product.	Wt.(mg)	m.p.
1.	Benzene	White Wax	15	-
2.	"	" "	540	-
3.	"	White Solid	110	106-109°
4.	"	" "	110	106-109°
5.	"	" "	10	-
6.	"	Clear Gum	70	108-110° (crystallised on adding MeOH)
7-9.	"	Traces	-	-
10.	Benzene-Ethanol (1%)	Nil.	-	-
11.	" " (2%)	Nil.	-	-
12.	" " (5%)	Nil.	-	-

Fractions 1 and 2 were combined, and exhibited a light absorption maximum at 2780Å (ϵ = 3000) i.e., indicative of cholesteryl acetate with 15% cholesta-3:5-dien-7-one as impurity. One crystallisation from ethyl acetate gave

cholesteryl acetate m.p. 111-113°, undepressed on mixing with an authentic specimen.

Fractions 3, 4, 5 and 6 were combined and recrystallised from aqueous ethanol to give cholesta-3:5-dien-7-one, m.p. 110°.

$[\alpha]_D$ -297° (C, 0.8)

Light Absorption: maximum at 2770Å (ϵ = 21,600)

Bergstrom and Wintersteiner (loc. cit) give m.p. 109-111°

$[\alpha]_D$ -279° and light absorption at 2800Å (ϵ = 23,000)

Cholestenone.

(a) Oppenauer oxidation of cholesterol.

cf. Organic Syntheses 21, 18.

The only variation in the described procedure consisted in crystalling the gum product by cooling it to -70°, adding acetone (25 c.c.) and allowing the mixture to attain room temperature with trituration. By this method, cholesterol (40 g.) yielded cholestenone (27.5 g.) m.p. 78-80°.

(b) By hydrogen exchange with cyclohexanone.

cf. Kleiderer & Kornfeld J.Org.Chem., 13 (1944) 455.

A mixture of toluene (450 c.c.), cyclohexanone (130 c.c.) Raney nickel (ca. 30 g.; Org.Synth. 21, 15) and cholesterol (15 g.) was heated under reflux for 24 hours. The catalyst was then removed under reduced pressure to leave a brown gum. The residue was taken up in ether and the solution re-filtered. As the gum residue obtained on removal of the ether could not be satisfactorily crystallised, it was dissolved in benzene, and filtered through a column of alumina with washing by

the same solvent. Removal of the benzene left a pale yellow gum, which crystallised from acetone - methanol giving cholestenone (5.7 g.), m.p. 176°.

Action of tertiary-Butyl Chromate on Cholestenone.

Cholestenone (1.90 g.) was treated with a mixture of tertiary-butyl chromate solution (40 c.c.; prepared as in method (a) previously described) to which acetic acid (5 c.c) and acetic anhydride (2 c.c.) had been added, in the usual manner. Evaporation of the solvent from the dried carbon tetrachloride extracts gave a gum (1.35 g.) which was dissolved in hot methanol. On cooling, an oil which separated solidified on standing. The solid (0.9 g. m.p. 64-78°) gave cholestenone m.p. 78-79° on one crystallisation from methanol.

Acidification of the alkaline extract with hydrochloric acid produced a precipitate which was taken up in ether, washed with water, and dried (Na_2SO_4). Removal of the ether gave a solid amorphous residue (300 mg.).

Light absorption: maximum at 2360Å ($E^{1\%}_{1cm}$ 112).

3α:12α-Diformoxycholanic Acid.

cf. Hoehn & Moffett J.Amer.Chem.Soc. 67 (1945), 740

A solution of deoxycholic acid (50 g.) in formic acid (100 c.c.; 90%) was heated at 70-80° for 5 hours. On cooling, the product crystallised and was filtered. One recrystallisation from formic acid gave 3 :12 - diformoxycholanic acid (47 g.), m.p. 194-5°.

Hoehn & Moffett (loc. cit) give m.p. 195-6°

3α:12α-Diformoxycholanic Acid Chloride.

Thionyl chloride (90 c.c.; distilled from quinoline and raw linseed oil) was added to 3α:12α-diformoxycholanic acid (46 g.), and the mixture allowed to stand at room temperature. The acid dissolved with evolution of sulphur dioxide and hydrogen chloride. After 1 hour, dry ether - benzene (1000 c.c.; 1:1) was added, then the solvents removed under reduced pressure. This was repeated twice more to leave the desired product as a gum which was used without further purification.

Diphenylcadmium.

cf. Gilman & Nelson Rec.trav.chim. 55, (1936) 518.

Anhydrous cadmium chloride (52.8 g.) was added in portions with cooling and stirring to a solution of phenyl magnesium bromide, prepared in the usual manner from magnesium (11.70 g.), bromobenzene (53.10 c.c.) and ether (300 c.c.). A dark sludge was formed and a granular precipitate was produced. The mixture was stirred for 30 minutes, and allowed to stand at room temperature for 1½ hours before use.

3α:12α-Dihydroxynorcholanyl Phenyl Ketone.

cf. Hoehn & Moffett J.Amer.Chem.Soc. 67 (1945), 740.

The diphenylcadmium suspension (as prepared above) was added portionwise over 30 minutes with vigorous stirring to a refluxing solution of 3α:12α-diformoxycholanic acid chloride (as prepared above) in dry benzene (600 c.c.).

After heating under reflux for a further 10 minutes, the white solid complex which had separated was decomposed by addition of dilute hydrochloric acid. The layers were separated, and the aqueous layer extracted once with ether. The organic layer and ether extract were combined, washed with dilute hydrochloric acid and water, then steam distilled to remove the solvents and diphenyl. After decanting the water, the glass product was hydrolysed by heating under reflux for 4 hours with methanolic sodium hydroxide (1000 c.c.; 2%). The solvent was then removed under reduced pressure to a volume of 500 c.c. the product allowed to crystallise, and removed by filtration. It was digested with ether (ca. 100 ml.) to remove the colour, cooled and filtered (29 g) One crystallisation from methanol gave pure 3α:12α-dihydroxynorcholanyl phenyl ketone (25 g.).

m.p. 202-204°

$[\alpha]_D$ +45° (C, 0.8 in dioxan).

Light absorption: maximum at 2420Å (ϵ = 12,900)

Hoehn and Moffett give m.p. 203-205° $[\alpha]_D$ +47.5°.

3α:12α-Diacetoxynorcholanyl Phenyl Ketone.

A solution of 3α:12α-dihydroxynorcholanyl phenyl ketone (9.1 g.) in glacial acetic acid (25 c.c.) and acetic anhydride (35 c.c.) was heated under reflux for 1½ hours, then the solvents removed under reduced pressure. The residual brown crystalline solid was recrystallised from acetone to give 3α:12α-diacetoxynorcholanyl phenyl ketone (8.34 g.) m.p. 135-137°.

$[\alpha]_D$ +91° (C, 1.0 in dioxan)

Light Absorption: maximum at 2420Å (ε = 13,500)

Hoehn and Moffett (loc. cit) give m.p. 136-137° $[\alpha]_D$ +92.5° Treatment of the ketone (100 mg.) with Brady's reagent gave a product m.p. 110-120°. It was purified by chromatographing a benzene solution on a column (9 x 2 cm.) of alumina. Elution with benzene (150 c.c) removed a green-yellow band which gave a negligible residue. Elution with more benzene (250 c.c.) removed an orange band which left an orange gum residue. Crystallisation from chloroform - methanol gave 3α:12α-diacetoxynorcholanyl phenyl ketone 2:4-dinitrophenylhydrazone as orange plates m.p. 117-122°. Three further recrystallisations did not alter the melting-point.

Found: C, 67.6 ; H, 7.2 ; N, 8.1

$C_{40}H_{52}O_8N_4$ req. : C, 67.0 ; H, 7.3 ; N, 7.8%

Light absorption: maximum at 3750Å (ε = 28,600)

Action of Selenium Dioxide on 3α:12α-Diacetoxynorcholanyl Phenyl Ketone.

A mixture of 3α:12α-diacetoxynorcholanyl phenyl ketone (536 mg.) selenium dioxide (1.70 g.) and acetic anhydride (6 c.c.) was heated under reflux for 4 hours. Precipitated selenium (730 mg.) was removed by filtration, and the filtrate poured into water to give a dark red oil, which was extracted with ether. After washing with water and drying (Na_2SO_4), the ether was removed to give a dark red oil which could not be crystallised. An aqueous methanolic solution

was treated with active charcoal to remove the remaining selenium; crystallisation from acetone, after this treatment, yielded unchanged starting material (230 mg.)

Action of N-Bromosuccinimide on 3α:12α-Diacetoxynorcholanyl Phenyl Ketone.

A mixture of 3α:12α-diacetoxynorcholanyl phenyl ketone (536 mg.), N-bromosuccinimide (196 mg.) and carbon tetrachloride (15 c.c.) was heated under reflux in a quartz flask with ultra-violet irradiation. After 50 minutes, the solution was cooled, filtered, and the solvent removed under reduced pressure to leave a dark red oil. This was taken up in benzene and chromatographed on alumina (grade III). Elution with benzene gave a brown gum, which crystallised on slow evaporation of an ether - light petroleum (b.p. 40-60°) solution. One recrystallisation from methanol gave a solid (440 mg.), m.p. 133-135°, undepressed on mixing with starting material. Elution of the column with benzene containing ethanol (1%) and chloroform - ethanol gave only small quantities of amorphous materials.

Bromination of 3α:12α-Diacetoxynorcholanyl Phenyl Ketone.

(a) A solution of bromine (596 mg.) in glacial acetic acid (17.42 c.c.) was added dropwise at room temperature to a solution of 3α:12α-diacetoxynorcholanyl phenyl ketone (2.0 g.) in glacial acetic acid (50 c.c.) to which aqueous hydrobromic acid (48%; 2 drops) had been added. The bromine colour faded almost instantaneously and there was

free evolution of hydrogen bromide. The reaction solution was allowed to stand for 1½ hours (during which it darkened), then poured into water, and the granular precipitate extracted with ether. The extract was washed successively with water, saturated hydrogen carbonate solution and water, and the ether removed to leave a pale orange solid; washing with cold methanol removed the coloured impurity. Recrystallisation from methanol gave 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone -I (1.8 g.) as clusters of needles. On air drying, the bromoketone had m.p. 110°.

$[\alpha]_D$ +93° (C, 0.8)

Found: C, 65.4 ; H, 7.8

Calc. for $C_{34}H_{47}O_5Br.\frac{1}{2}CH_3OH$: C, 65.6 ; H, 7.8%

After drying in vacuo at 100°, the bromoketone had m.p. 167°.

$[\alpha]_D$ +95° (C, 1.0)

Found: C, 66.3 ; H, 7.7 ; Br, 13.0

Calc. for $C_{34}H_{47}O_5Br$: C, 66.0 ; H, 7.7 ; Br, 12.8%

Light absorption: maximum at 2560Å (ε = 11,200)

On using a very slow rate of heating in determining the melting point of the air-dried specimen, it softened at 108-110°, and resolidified before melting finally at 167°. On recrystallisation from methanol, the form m.p. 167° reverted to the form m.p. 110°. The phenyl ketone should be pure before bromination; bromination of less pure specimens proceeded only with difficulty and with considerable decomposition, and considerable losses were encountered in purification of product. In the presence of potassium acetate, bromination did not occur.

(b) A solution of 3α:12α-diacetoxynorcholanyl phenyl ketone (8.0 g.) in glacial acetic acid (60 c.c.) to which aqueous hydrobromic acid (48%; 2 drops) had been added was treated dropwise at 30-40° with bromine (1.05 moles) in acetic acid (56 mg. bromine per c.c.). The bromine colour faded, then redarkened rapidly. The reaction mixture was worked up as in (a). On taking the solid product up in methanol, a fraction appeared sparingly soluble, and was removed by filtration. On cooling the methanol solution, 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone-I (6.72 g.) m.p. 108-110° and 167° was obtained. The filtered solid, dissolved in boiling methanol (100 c.c.), gave on cooling 3α:12α-diacetoxy-23-bromo-norcholanyl phenyl ketone-II (810 mg.) as prisms, m.p. 207-210°. Recrystallisation raised the m.p. to 208-210°.

$[\alpha]_D$ +91° (C, 0.4)

Found: C, 66.0 ; H, 7.7.

$C_{34}H_{47}O_5Br$. requires C, 66.3 ; H, 7.7%

Light absorption: maximum at 2550Å (10,200)

Action of Tertiary Bases on 3α:12α-Diacetoxy-23-bromonorcholanyl Phenyl Ketone.

(a) A solution of 3α:12α-diacetoxy-23-bromocholanyl phenyl ketone (200 mg.) in collidine (11 c.c.) was heated under reflux. Removal of the collidine under reduced pressure left a dark brown amorphous residue which was taken up in ether-benzene, and washed successively with water, dilute hydrochloric acid and water. After drying, the solvents

were removed, the residue dissolved in benzene and chromatographed on alumina. No crystalline material could be isolated, one typical dark brown gum fraction having a light absorption maximum at 2360Å ($E^{1\%}_{1cm}$ 107).

(b) A solution of the bromoketone (500 mg.) in pyridine (5 c.c.) was heated under reflux for 5 hours. Working up as in (a) yielded only gum products.

Action of Sodium Ethoxide on 3α:12α-Diacetoxy-23-bromonorcholanyl Phenyl Ketone.

A solution of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (1.0 g.) in absolute ethanol (20 c.c.) was added to a refluxing solution of sodium ethoxide (from 1.2 g. sodium) in absolute ethanol (20 c.c.). The mixture turned yellow immediately. It was heated under reflux for 30 minutes, after which the solvent was removed under reduced pressure. The residue was extracted with ether, washed successively with water, dilute hydrochloric acid, water and dried (Na_2SO_4). Removal of the ether left an amorphous froth solid (600 mg.); digestion of this solid (420 mg.) in light petroleum (50 c.c.; b.p. 80-100°) left a residue of molten gum, removed by decantation. A solid (150 mg. melting unsharply at approximately 80°) separated from the soluble fraction. After three recrystallisations from methanol, it had m.p. 187° with softening at 89-94°. It gave a negative Beilstein test.

Found: C, 79.2, 77.3 ; H, 9.8, 9.1

$C_{30}H_{42}O_3$ requires: C, 79.95 ; H, 9.4

$C_{32}H_{48}O_4$ " C, 77.4 ; H, 9.7

$C_{30}H_{44}O_4$ " C, 76.9 ; H, 9.5%.

Light absorption: maximum at 2450Å ($E^{1\%}_{1cm}$ 147).

Action of Sodium Ethoxide on 3α:12α-Diacetoxy-23-bromonorcholanyl Phenyl Ketone, followed by re-acetylation.

A solution of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (2.0 g.) in absolute ethanol (40 c.c.) was added to a refluxing solution of sodium ethoxide (from 2.4 g. sodium) in absolute ethanol (40 c.c.). The solution darkened immediately. It was heated under reflux for 30 minutes, after which the solvent was removed under reduced pressure, water added, and the residue extracted with ether. The ether extract was washed successively with water, dilute hydrochloric acid and water, the ether removed, and the residue dried by benzene codistillation. Glacial acetic acid (20 c.c.) and acetic anhydride (30 c.c.) were added, the solution heated at 100° for 1½ hours, water added, the mixture extracted with ether, and the acetylated product, in the form of a gum, isolated in the usual way. It was taken up in methanol, and a few drops of water added. After prolonged standing, prisms (150 mg.), m.p. 177-200° separated, exhibiting light absorption at 2450Å ($E^{1\%}_{1cm}$ 44). Recrystallisation from methanol gave 3α:12α:24-triacetoxy-23-keto-24-phenylchlolane as felted rosettes. m.p.215°.

$[\alpha]_D$ +146° (C, 0.4).

Found: C, 72.7 ; H, 8.5.

$C_{36}H_{50}O_7$ requires: C, 72.7 ; H, 8.5%

It exhibited no high intensity light absorption above 2200Å.

The aqueous methanolic mother liquor, after removal of the prisms, was concentrated, and the residue, dried by benzene codistillation, taken up in benzene (25 c.c.), chromatographed on a column (7 x 1½ cm.) of alumina (Grade III). The first benzene eluate (50 c.c.) gave a yellow-brown gum, which yielded yellow gummy crystals (600 mg.) from methanol. From the mother liquor on standing three days, felted needles (215 mg.), m.p. 130° separated. Recrystallisation from methanol gave 3α:12α:23-triacetoxynorcholanyl phenyl ketone as leaflets, m.p. 131° (on air drying) and m.p. 173° (on drying in vacuo at 100°)

$[\alpha]_D$ of form m.p. 131° = +81° (C, 0.5)

$[\alpha]_D$ of form m.p. 173° = +88° (C, 0.4)

Found: C, 72.2, 73.2 ; H, 8.4, 8.6.

$C_{36}H_{50}O_7$ requires: C, 72.7 ; H, 8.5%

Light absorption: maximum at 2450Å (ϵ = 13,300)

The gummy crystals (600 mg.) were rechromatographed. The first benzene eluate (50 c.c.) gave a further 350 mg. of 3α:12α:24-triacetoxy-23-keto-24-phenylcholane.

Action of Sodium Methoxide on 3α:12α-Diacetoxy-23-bromonorcholanyl Phenyl Ketone, followed by re-acetylation.

A solution of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (3.0 g.) in absolute methanol (70 c.c.) was added to a refluxing solution of sodium methoxide (from 3.6 g.

sodium) in absolute methanol (60 c.c.). The mixture was treated, and the product re-acetylated as in the corresponding sodium ethoxide experiment. The re-acetylated gum product yielded a solid (910 mg.) m.p. 149-165°, from aqueous methanol. Recrystallisation from methanol gave 3α:12α:23-triacetoxynorcholanyl phenyl ketone (470 mg.) as leaflets, m.p. 173°, undepressed on mixing with a authentic specimen.

$[\alpha]_D$ +90° (C, 0.6)

Light absorption: maximum at 2450Å (ϵ = 11,600)

The aqueous methanolic mother liquors were concentrated, dried, and chromatographed in benzene solution. Elution with the same solvent (600 c.c.) yielded a crude solid (610 mg.), which after two recrystallisations from methanol gave 3α:12α:24-triacetoxy-23-keto-24-phenylcholane as felted rosettes, m.p. 211-213° undepressed on mixing with a specimen, m.p. 215° obtained by sodium ethoxide method.

Action of Potassium Acetate on 3α:12α-Diacetoxy-23-bromo-norcholanyl Phenyl Ketone.

(a) In Acetic Acid.

A mixture of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (500 mg.) and fused potassium acetate (500 mg.) in glacial acetic acid (6 c.c.) was heated at 100° for 3 hours, and under reflux for 15 minutes. After removal of the solvent under reduced pressure, the residual solid was taken up in ether, washed with water, dried (Na_2SO_4), and the ether removed. One recrystallisation of the residue

from methanol gave unchanged starting material (400 mg.) m.p. 105° and 165°. After drying in vacuo, it had m.p. 165-167°.

(b) In Ethanol.

A mixture of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (500 mg.) and fused potassium acetate (500 mg.) in absolute ethanol (25 c.c.) was heated under reflux for 3 hours. After removal of the solvent, the residual solid was taken up in ether, washed with water, dried (Na_2SO_4), and the ether removed to leave a white froth solid (420 mg.). Crystallisation from methanol gave 3α:12α:23-triacetoxynorcholanyl phenyl ketone (170 mg.), m.p. 155-165°. Recrystallisation from the same solvent raised the m.p. to 173°, alone, or mixed with specimens prepared by the sodium methoxide or sodium ethoxide methods described above.

$[\alpha]_D$ +92° (C, 0.9)

Found: C, 72.4 ; H, 8.5

$C_{36}H_{50}O_7$ requires: C, 72.7 ; H, 8.5%

Light absorption: maximum at 2450Å (ε = 11,200)

Attempted Formation of 3α:12α-Diacetoxy-23:23-dibromonorcholanyl Phenyl Ketone.

(a) By addition of one mole of bromine to the bromonorcholanyl phenyl ketone.

A solution of bromine (52 mg.; 1 mole) in glacial acetic acid (1.52 c.c.) was added at room temperature to a solution of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (200 mg.) in acetic acid to which aqueous hydrobromic

acid (2 drops; 48%) had been added. There was no fading of colour, and hydrogen bromide was evolved only on heating the solution. The mixture , after standing for three days with occasional heating, had darkened considerably. Working up in the usual way by means of ether gave a dark amorphous solid. A methanol solution of this solid deposited a gummy solid m.p. 100-170° after standing several days. After treatment with activated charcoal, a solid m.p. 80-120° was isolated. Repeated recrystallisation from methanol caused variation and divergence of the melting point range.

(b) By addition of two moles of bromine to the parent norcholanyl phenyl ketone.

A solution of bromine (320 mg.; 2 moles) in glacial acetic acid (15.1 c.c.) was added dropwise at room temperature to a solution of 3α:12α-diacetoxynorcholanyl phenyl ketone (536 mg.) in acetic acid (15 c.c.) to which aqueous hydrobromic acid (48%; 2 drops) had been added. The bromine decolouration ceased after the uptake of one mole. After standing for 5 days in the dark at room temperature, the product was isolated in the usual way to give 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (440 mg.) as short needles m.p. 106-108° and 167°.

Action of Strong Alkali (Marker-Lawson conditions) on 3α:12α-Diacetoxy-23-bromonorcholanyl Phenyl Ketone.

A solution of 3α:12α-diacetoxy-23-bromonorcholanyl phenyl ketone (1 g.) in ethanolic potassium hydroxide (20%; 100 c.c.) was heated under reflux for 1 hour, cooled, diluted with water and extracted with ether. The extract,

after washing with water, dilute hydrochloric acid and water, was dried (Na_2SO_4) and evaporated to leave a yellow amorphous solid (400 mg.). This solid crystallised from methanol solution as clusters of needles m.p. 80-90°, resolidifying and remelting at 170°. Repeated recrystallisation from methanol gave 3α:12α:23-trihydroxynorcholanyl phenyl ketone (45 mg.) m.p. 179-182°.

$[\alpha]_D$ +36° (C, 1.3)

Found: C, 74.8 ; H, 9.9

$C_{30}H_{44}O_4CH_3OH$ req. C, 74.4 ; H, 9.7%

Light absorption: maximum at 2440 Å (ε = 9,400)

Methyl 3α-Benzoxy-12-ketocholanate.

cf. McKenzie, Mattox, Engel & Kendall J.Biol.Chem. 173 (1948), 271.

Methyl 3α-benzoxy-12α-hydroxycholanate (46 g, m.p. 73-76°, with methanol of crystallisation; prepared by method of McKenzie, McGuckin and Kendall, J.Biol.Chem. 162 (1946), 555) was dissolved in chlorobenzene (50 c.c.), and the solvent removed under reduced pressure. This procedure was repeated, then the dried residue dissolved in chlorobenzene (170 c.c.) and glacial acetic acid (42 c.c.). A solution of chromium trioxide (8.5 g.) in water (8.5 c.c.) was then added with vigorous stirring. After 1 hour, concentrated sulphuric acid (5 c.c.) was added, and the mixture stirred for a further hour. The mixture was first washed with water (500 c.c.) containing hydrochloric acid (5 c.c.; to minimise emulsion formation), then with water and dried (Na_2SO_4)

The chlorobenzene solution was then concentrated under reduced pressure, methanol added, and the product (44 g. m.p. 88-94°) allowed to settle, first as a syrup which solidified on standing. One recrystallisation from methanol yielded methyl 3α-benzoxy-12-ketocholanate, m.p. 95°.

McKenzie et al (loc. cit) give m.p. 127-128°. Hoehn & Mason (J.Amer.Chem.Soc. 62 (1940), 569) give m.p. 94-95° and note the difficulty of crystallisation.

3α-Hydroxy-12-ketocholanic Acid.

(a) By hydrolysis of methyl benzoxy ester.

Methyl 3α-benzoxy-12-ketocholanate (40 g.) was added to a solution of sodium hydroxide (30 g.) in water (40 c.c.) and methanol (360 c.c.), the mixture heated under reflux for 1 hour, concentrated under reduced pressure and poured on to ice-hydrochloric acid with stirring to give a syrup which solidified on standing. The gummy solid was dissolved in benzene, the solution dried by benzene azeotropic distillation, and the product collected after concentration. After one treatment with activated charcoal in benzene, 3α-hydroxy-12-ketocholanic acid (24 g.) was obtained as white needles from benzene m.p. 155-158°, $[\alpha]_D$ +85° (C, 0.6). Further crops of less pure material were obtained.

(b) By direct oxidation of deoxycholic acid.

cf. Kaziro & Shimada Z.physiol.Chem. 249 (1937), 220.
Bergstrom & Haslewood J. 1939, 540.

A solution of deoxycholic acid (10 g.) in glacial

acetic acid (700 c.c.) and water (50 c.c.) was treated at 0° with a solution of chromium trioxide (2.5 g.) in water (25 c.c.) and acetic acid (25 c.c.) added with stirring over 1 hour. The solution was maintained at 0° for 16 hours diluted with water (1500 c.c.) and the product filtered, and washed with water. The wet precipitate was taken up in benzene, the solution dried by distillation, and concentrated to give 3α-hydroxy-12-ketocholanic acid (7.0g) as needles m.p. 155-158°. After one recrystallisation from the same solvent, it had m.p. 159-161°, $[\alpha]_D$+85° (C, 0.6). If heated rapidly, it melted at 116°, resolidified, and remelted at 160°. Kaziro & Shimada (loc. cit) give $[\alpha]_D$+110° Hoehn & Mason (J.Amer.Chem.Soc., 65 (1943), 550) give $[\alpha]_D$+87° (in dioxan).

3α-Formoxy-12-ketocholanic Acid.

A solution of 3α-hydroxy-12-ketocholanic acid (25 g.) in formic acid (90%; 50 c.c.) was heated at 70-80° for 5 hours, cooled to 0° and removed by filtration. Recrystallisation from formic acid gave 3α-formoxy-12-ketocholanic acid as prismatic needles, m.p. 204-206°, $[\alpha]_D$+107°, +103° (C, 2.0, 1.0 in dioxan). Hoehn & Moffett (loc. cit) give m.p. 207-208°, $[\alpha]_D$+117.5°

3α-Hydroxy-12-ketonorcholanyl Phenyl Ketone.

3α-Formoxy-12-ketocholanic acid (33.5 g.) was

dissolved in thionyl chloride (60 c.c.; distilled from linseed oil), and allowed to stand at room temperature for 1 hour. Benzene-ether (1:1; 100 c.c.) was added, then removed, and this repeated twice more to give 3α-formoxy-12-ketocholanic acid chloride as a pale brown solid. A solution of this solid in dry benzene (300 c.c.) was heated to reflux with vigorous stirring, and treated with diphenylcadmium (prepared from magnesium (7.80 g.), bromobenzene (35-40 c.c.) anhydrous ether (200 c.c.) and anhydrous cadmium chloride (35.2 g.)) added over 30 minutes. The mixture was heated under reflux with stirring for a further 10 minutes, then cooled, and the complex decomposed by addition of dilute hydrochloric acid. The layers were separated, the aqueous layer extracted with ether, and the combined organic layers washed with dilute hydrochloric acid and water, then steam distilled to leave a crystalline solid suspended in water. This product was removed by filtration and heated under reflux in a solution of methanolic potassium hydroxide (3%; 900 c.c.) for 2 hours. The solution was then concentrated until a solid began to separate, water added to complete precipitation, and the product (m.p. 176°) collected. One crystallisation from methanol gave 3α-hydroxy-12-ketonorcholanyl phenyl ketone (21 g.) as fine needles.

m.p. 177-179°

$[\alpha]_D$ +81° (C, 1.4 in dioxan)

Light absorption: maximum at 2410Å (ε = 12,700)
with plateau at 2700-2800Å (ε = 1,000)

Hoehn & Moffett (loc. cit) give m.p. 176-178°, $[\alpha]_D$+77.5°.

3α-Acetoxy-12-ketonorcholanyl Phenyl Ketone.

A solution of 3α-hydroxy-12-ketonorcholanyl phenyl ketone (20 g.) in acetic acid (40 c.c.) and acetic anhydride (60 c.c.) was heated under reflux for 1½ hours. On cooling, 3α-acetoxy-12-ketonorcholanyl phenyl ketone (20.5 g.) separated, was collected and dried in vacuo over sodium hydroxide.

m.p. 197°.

$[\alpha]_D$+91° (C, 1.7)

Light absorption: maximum at 2410Å (ε=12,000) with plateau at 2700-2800Å (ε=500)

Hoehn and Moffett (loc. cit) give m.p. 197°, $[\alpha]_D$+85°.

Monobromination of 3α-Acetoxy-12-ketonorcholanyl Phenyl Ketone.

A solution of 3α-acetoxy-12-ketonorcholanyl phenyl ketone (1.0 g.) in glacial acetic acid (50 c.c.) to which hydrobromic acid (48%; 2 drops) had been added was treated at 30-35° with a solution of bromine (341 mg.) in acetic acid (9.23 c.c.), added dropwise over 5 minutes. After standing at room temperature for 30 minutes, the reaction mixture was poured into cold water (200 c.c.) to give a white flocculent precipitate, which was extracted with ether. After washing successively with water, saturated sodium hydrogen carbonate solution and water, the dried (Na_2SO_4) extract was evaporated under reduced pressure to give a white amorphous residue (1.15 g.). On treating with

hot methanol, it was noted that a fraction was sparingly soluble, and was removed by filtration. On cooling the hot methanol filtrate, 3α-acetoxy-23-bromo-11-ketonorcholanyl phenyl ketone-I, m.p. 155-160°, separated. Repeated recrystallisation from the same solvent raised the m.p. to 169-171°.

$[\alpha]_D$ +114° (C, 1.0)

Found: C, 67.5 ; H, 7.75.

$C_{32}H_{43}O_4Br$ requires: C, 67.2 ; H, 7.6%

Light absorption: maximum at 2550Å (ϵ = 10,900)

The insoluble fraction (70 mg.) from the mixture (350 mg.) was crystallised from a large quantity of methanol to give 3α-acetoxy-23-bromo-11-ketonorcholanyl phenyl ketone-II, (55 mg.) as short fine needles, m.p. 191-193°. After two recrystallisations from methanol, the m.p. was raised to 193°.

$[\alpha]_D$ +102° (C, 1.0)

Found: C, 67.3 ; H, 7.75

$C_{32}H_{43}O_4Br$ requires: C, 67.2 ; H, 7.6%.

Light absorption: maximum at 2550Å (ϵ = 10,900)

LITERATURE CITED.

1. Mason, Kendall, Slocumb & Polley. — Proc. Staff Meet. Mayo Clin 24 (1949), 181.
2. Hartman & Brownell. — Science 72 (1930), 76.
3. Swingle & Pfiffner. — Science 72 (1930), 75.
4. Mason, Myers & Kendall. — J. Biol. Chem. 114 (1936),
5. Mason, Myers & Kendall. — J. Biol. Chem. 116 (1936),
6. Wintersteiner & Pfiffner. — J. Biol. Chem. 116 (1936),
7. Reichstein. — Helv. Chim. Acta 19 (1936),
8. Kuizenga & Cartland. — Endocrinology 24 (1939),
9. Kendall. — Cold Spring Harbour Sympo Quant. Biol. 5 (1937), 299
10. Pfiffner — J. Biol. Chem. 106 (1934),
11. Reichstein & von Euw. — Helv. Chim. Acta. 21 (1938),
12. von Euw & Reichstein. — Helv. Chim. Acta. 25 (1942)
13. Reichstein & Shoppee. — Vitamins & Hormones (Acad. Press) Vol. I., 345.
14. Fieser & Fieser. — Natural Products Related Phenanthrene (Reinhold) 3rd. Edn.
15. Sarett. — J. Biol. Chem. 162 (1946),
16. Kendall. — Chem. Eng. News. 28 (1950),
17. Marker, Wagner, Ulshafer, Wittbecker, Goldsmith & Ruof. — J. Amer. Chem. Soc. 69 (194 2172.
18. Marker. — J. Amer. Chem. Soc. 69 (1947
19. von Euw & Reichstein. — Helv. Chim. Acta. 23 (1940)
20. Reichstein & von Euw. — Helv. Chim. Acta. 23 (1940),
21. Butenandt & Peters. — Ber., 71 (1938), 2688.

1. Hench, Kendall, Slocumb & Polley. Proc.Staff Meet.Mayo Clin. 24 (1949), 181.
2. Hartman & Brownell. Science 72 (1930), 76.
3. Swingle & Pfiffner. Science 72 (1930), 75.
4. Mason, Myers & Kendall. J.Biol.Chem. 114 (1936), 613.
5. Mason, Myers & Kendall. J.Biol.Chem. 116 (1936), 267.
6. Wintersteiner & Pfiffner. J.Biol.Chem. 116 (1936), 291.
7. Reichstein. Helv.Chim.Acta. 19 (1936), 1107.
8. Kuizenga & Cartland. Endocrinology. 24 (1939), 526.
9. Kendall. Cold Spring Harbour Symposia Quant Biol. 5 (1937), 299.
10. Pfiffner & Vars. J.Biol.Chem. 106 (1934), 645.
11. Reichstein & von Euw. Helv.Chim.Acta. 21 (1938), 1181.
12. von Euw & Reichstein. Helv.Chim.Acta. 25 (1942), 988.
13. Reichstein & Shoppee. Vitamins & Hormones (Academic Press) Vol.I., 345.
14. Fieser & Fieser. Natural Products Related to Phenanthrene (Rheinhold), 3rd. Edn.
15. Sarett. J.Biol.Chem. 162 (1946), 601.
16. Kendall. Chem.Eng.News. 28 (1950), 2074.
17. Marker, Wagner, Ulshafer, Wittbecker, Goldsmith & Ruof. J.Amer.Chem.Soc. 69 (1947), 2172.
18. Marker. J.Amer.Chem.Soc. 69 (1947), 2395.
19. von Euw & Reichstein. Helv.Chim.Acta. 23, (1940), 1258.
20. Reichstein & von Euw. Helv.Chim.Acta. 23 (1940), 1114.
21. Butenandt & Peters. Ber., 71 (1938), 2688.
22. von Euw & Reichstein. Helv.Chim.Acta. 24 (1941), 1140.
23. Sarett. J.Biol.Chem. 162 (1946), 601.

24. Sarett. J.Amer.Chem.Soc. 70 (1948), 1454.

25. Mattox & Kendall. J.Amer.Chem.Soc. 70 (1948), 882.

26. Koechlin, Garmaise, Kritchevsky & Gallagher. J.Amer.Chem.Soc. 71 (1949), 3262.

27. Kritchevsky & Gallagher. J.Biol.Chem. 179 (1949), 507.

28. Rosenkranz, Pataki, Kaufmann, Berlin & Djerassi. J.Amer.Chem.Soc. 72 (1950), 4081.

29. Wagner & Moore. J.Amer.Chem.Soc. 71 (1949), 4160.

30. Wagner & Moore. J.Amer.Chem.Soc. 72 (1950), 3655.

31. Wagner & Moore. J.Amer.Chem.Soc. 72 (1950), 5301.

32. Julian, Meyer & Ryden. J.Amer.Chem.Soc. 71 (1949), 756.

33. Julian, Meyer, Karpel & Ryden. J.Amer.Chem.Soc. 71 (1949), 3574.

34. Julian, Meyer, Karpel & Waller. J.Amer.Chem.Soc. 72 (1950), 5145.

35. Julian & Karpel. J.Amer.Chem.Soc. 72 (1950), 362.

36. Julian, Meyer & Ryden. J.Amer.Chem.Soc. 72 (1950), 367.

37. Plattner, Heusser & Fuerer. Helv.Chim.Acta. 31 (1948), 2210.

38. Miescher & Schmidlin. Helv.Chim.Acta. 33 (1950), 1840.

39. Heilbron, Jones, Julia & Weedon. J. 1949, 1823.

40. Jacobs, Cramer & Hanson. J.Amer.Chem.Soc. 64 (1942), 223.

41. Arens & van Dorp. Nature. 160 (1947), 189.

42. Arens & van Dorp. Rec.trav.Chim. 68 (1949), 604.

43. Graham, van Dorp & Arens. Rec.trav.Chim. 68 (1949), 609.

44. Schtschukina & Rubtsov. J.Gen.Chem.Russia. 18 (1948), 1645.

45. Preobrazhenskii & Rubtsov. J.Gen.Chem.Russia. 18 (1948), 1719.

46. Newbold. J. 1950, 3346.

47.	Gallagher & Kritchevsky.	J.Amer.Chem.Soc. 72 (1950), 882.
48.	Miescher, Wettstein & Scholz.	Helv.Chim.Acta. 22 (1939), 894.
49.	Reich.	Helv.Chim.Acta. 23 (1940), 219.
50.	Krohnke & Broner.	Ber. 69 (1936), 2006.
51.	Krohnke.	Ber. 71 (1938), 2583.
52.	Fuchs & Reichstein.	Helv.Chim.Acta. 24 (1941), 804.
53.	Plattner & Schreck.	Helv.Chim.Acta. 22 (1939), 1178.
54.	Reichstein, Muller, Meystre & Sutter.	Helv.Chim.Acta. 22 (1939), 741.
55.	Marker, Crooks, Jones & Shabica.	J.Amer.Chem.Soc. 64 (1942), 1276.
56.	Marker & Crooks.	U.S.Pat.2,359,773 C.A. 39, 2627.
57.	Lardon & Reichstein.	Helv.Chim.Acta. 24 (1941), 1127.
58.	Reich, Sutter & Reichstein.	Helv.Chim.Acta. 23 (1940), 170.
59.	Hardegger & Scholz.	Helv.Chim.Acta. 28 (1945), 1355.
60.	Criegee.	Ann. 522 (1936), 75.
61.	Butenandt, Schmidt-Thomé & Paul.	Ber. 72 (1939), 1112.
62.	Goldberg & Aeschbacher.	Helv.Chim.Acta. 22 (1939), 1185.
63.	Heusser, Eichenberger & Plattner.	Helv.Chim.Acta. 33 (1950), 370.
64.	Heusser, Eichenberger & Plattner.	Helv.Chim.Acta. 33 (1950), 1088.
65.	Magrath, Morris, Petrow & Royer.	J. 1950, 2393.
66.	Arens & van Dorp.	Dutch Pat. 63,015/1949.
67.	Bergmann & Stevens.	J.Org.Chem. 13 (1948), 10.
68.	Antonucci, Bernstein, Giancola & Sax.	J.Org.Chem. 16 (1951), 1356.

69. Chamberlin, Ruyle, Erickson, Chemerda, Aliminosa, Erickson, Sita & Tishler. — J.Amer.Chem.Soc. 73 (1951) 2396.

70. Anderson, Budziarek, Newbold, Stevenson & Spring. — Chem. & Ind. 1951, 1035.

71. Budziarek, Newbold, Stevenson & Spring. — J. 1952, 2892.

72. Anderson, Stevenson & Spring. — J. 1952, 2901.

73. Budziarek, Johnson & Spring. — J. 1952.

74. Schoenewaldt, Turnbull, Chamberlin, Reinhold, Erickson, Ruyle, Chemerda, & Tishler. — J.Amer.Chem.Soc. 74 (1952), 2696.

75. Heusser, Eichenberger, Kurath, Dällenbach & Jeger. — Helv.Chim.Acta. 34 (1951), 2106.

76. Heusser, Anliker, Eichenberger & Jeger. — Helv.Chim.Acta. 35 (1952), 936.

77. Sondheimer, Yashin, Rosenkranz & Djerassi. — J.Amer.Chem.Soc. 74 (1952), 2696.

78. Heusser, Heusler, Eichenberger, Honnegger & Jeger. — Helv.Chim.Acta. 35 (1952), 295.

79. Fieser, Herz & Huang. — J.Amer.Chem.Soc. 73 (1951), 2397.

80. Stork, Romo, Rosenkranz & Djerassi. — J.Amer.Chem.Soc. 73 (1951), 3546.

81. Fieser, Babcock, Herz, Huang & Schneider. — J.Amer.Chem.Soc. 73 (1951), 4052.

82. Djerassi, Mancera, Stork & Rosenkranz. — J.Amer.Chem.Soc. 73 (1951), 4496.

83. Djerassi, Batres, Velasco & Rosenkranz. — J.Amer.Chem.Soc. 74 (1952), 1712.

84. Heilbron & Sexton. — J. 1929, 921.

85. Wieland & Benend. — Ann. 554 (1943), 1.

86. Barton & Cox. — J. 1948, 1354.

87. Laubach & Brunings. J.Amer.Chem.Soc. 74 (1952) 705

88. Windaus & Auhagen. Ann. 472 (1929), 185.

89. Heilbron, Johnstone & Spring. J. 1929, 185.

90. Windaus & Luttringhaus. Ann. 481 (1930), 119.

91. Callow & Rosenheim. J. 1933, 387.

92. Eck & Hollingsworth. J.Amer.Chem.Soc. 64 (1942), 140.

93. Bergmann & Stevens. J.Org.Chem. 13 (1948), 10.

94. Antonucci, Bernstein, Giancola & Sax. J.Org.Chem. 16 (1951), 1891.

95. Stavely & Bollenback. J.Amer.Chem.Soc. 65 (1943) 1290.

96. Stavely & Bollenback. J.Amer.Chem.Soc. 65 (1943) 1285.

97. Dr. D.H.R. Barton. Private communication to Professor F.S. Spring.

98. Fieser. Experientia 6 (1950), 312.

99. Heymann & Fieser. J.Amer.Chem.Soc. 73 (1951) 5252.

100. Heilbron, Jones & Spring. J. 1937, 801.

101. Herzig & Ehrenstein. J.Org.Chem. 16 (1951), 1050.

102. Brown. Organic Reactions, 6,469.

103. Djerassi, Martinez & Rosenkranz. J.Org.Chem. 16 (1951), 1278.

104. Fieser & Rajagopalan. J.Amer.Chem.Soc. 73 (1951) 118.

105. Braude & Jones. J. 1945, 498.

106. Roberts & Green. J.Amer.Chem.Soc. 68 (1946) 214.

107. Djerassi & Ryan. J.Amer.Chem.Soc. 71 (1949) 1000.

108. Furst & Plattner. Abstracts S.409 XII International Congress for Pure & Applied Chemistry.

109. Barton & Cox. J. 1949, 214.

110.	Chemerda, Chamberlin, Wilson & Tishler.	J.Amer.Chem.Soc. 73 (1951) 4052.
	Oliveto, Gerold & Hershberg.	J.Amer.Chem.Soc. 74 (1952) 2248.
111.	Reich.	Helv.Chim.Acta. 28 (1945) 892.
112.	Reich & Reichstein.	Helv.Chim.Acta. 26 (1943) 2102.
113.	Heard, Lawrence & Wasson.	Unpublished observation.
114.	Barbier & Locquin.	Compt.rend. 156 (1913) 1443.
115.	Wieland, Schlichting & Jacobi.	Z.physiol.Chem. 161 (1926) 80.
116.	Mason & Hoehn.	J.Amer.Chem.Soc. 60 (1938) 1493
117.	Mason & Hoehn.	J.Amer.Chem.Soc. 60 (1938) 2824
118.	McKenzie, Mattox, Engel & Kendall.	J.Biol.Chem. 173 (1948) 271.
119.	Meystre, Frey, Wettstein & Miescher.	Helv.Chim.Acta 27 (1944) 1815.
120.	Meystre, Ehmann, Neher & Miescher.	Helv.Chim.Acta 28 (1945) 1252.
121.	Meystre, & Miescher.	Helv.Chim.Acta 28 (1945) 1497.
122.	Meystre & Miescher.	Helv.Chim.Acta 29 (1946) 33.
123.	Meystre, Frey, Neher, Wettstein & Miescher.	Helv.Chim.Acta 29 (1946) 627.
124.	Meystre, Wettstein & Miescher.	Helv.Chim.Acta 30 (1947) 1022.
125.	Meystre & Wettstein.	Helv.Chim.Acta. 30 (1947) 1037.
126.	Meystre & Wettstein.	Helv.Chim.Acta. 30 (1947) 1256.
127.	Meystre & Wettstein.	Helv.Chim.Acta. 30 (1947) 1262.
128.	Meystre & Wettstein.	Experientia 3 (1947), 185.
129.	Ettlinger & Fieser.	J.Biol.Chem. 164 (1946) 451.
130.	Hollander & Gallagher.	J.Biol.Chem. 162 (1946) 549.
131.	Jacobsen.	J.Amer.Chem.Soc. 66 (1944) 662.

132.	Brink, Clark & Wallis.	J.Biol.Chem. 162 (1946), 695.
133.	Ercoli & de Ruggieri.	Il Farmaco 6 (1951), 547.
134.	Wieland & Dane.	Z.physiol.Chem. 210 (1932) 268.
135.	Iwasaki.	Z.physiol.Chem. 244 (1936) 181.
136.	Kaziro & Shimada.	Z.physiol.Chem. 249 (1937) 220.
137.	Haslewood.	Biochem. J. 37 (1943) 109.
138.	Haslewood.	Biochem. J. 38 (1944) 108.
139.	Gallagher & Long.	J.Biol.Chem. 147 (1943) 131.
140.	Hoehn & Linsk.	J.Amer.Chem.Soc. 67 (1945) 312.
141.	Schneider & Hoehn.	J.Amer.Chem.Soc. 65 (1943) 485.
142.	Hoehn, Schmidt & Hughes.	J.Biol.Chem. 152 (1944), 59.
143.	Huang-Minlon.	J.Amer.Chem.Soc. 68 (1946) 2487.
144.	Cortese.	J.Amer.Chem.Soc. 59 (1937) 2532.
145.	Fieser & Rajagopalan.	J.Amer.Chem.Soc. 71 (1949) 3935.
146.		Organic Syntheses 24, 41.
147.	Waitkins & Clark.	Chem.Reviews 36 (1945), 235.
148.	Rabjohn.	Organic Reactions 5, 331.
149.	Oppenauer & Oberrauch.	Anales asoc.quim.argentina 37 (1949), 246.
150.	Windaus, Lettré & Schenk.	Ann. 528 (1935), 98.
151.	Mauthner.	Monat. 17 (1896), 496.
152.	Stavely.	J.Org.Chem. I (1932), 567.
153.	Bergstrom & Wintersteiner.	J.Biol.Chem. 141 (1941) 597.
154.		Organic Syntheses 21, 18.
155.	Kleiderer & Kornfeld.	J.Org.Chem. 13 (1944), 455.
156.	Hoehn & Moffett.	J.Amer.Chem.Soc. 67 (1945), 740.
157.	Cole & Julian.	J.Amer.Chem.Soc. 67 (1945), 1369.

158. Cortese & Baumann. J.Amer.Chem.Soc. 57 (1935) 1393.

159. Cortese & Baumann. J.Biol.Chem. 113 (1936) 779.

160. Gilman & Nelson. Rec.trav.chim. 55 (1936), 518.

161. Cole, Julian, Magnani, & Meyer. J.Amer.Chem.Soc. 67 (1945), 1728.

162. Chakravorti & Wallis. J.Amer.Chem.Soc. 62 (1940), 318.

163. Hicks, Berg & Wallis. J.Biol.Chem. 162 (1946), 633.

164. Temnikova & Kropacheva. Zhur.Obshchei Khim 19 (1949) 1917.

165. Stevens, Malik & Pratt. J.Amer.Chem.Soc. 72 (1950) 4758.

166. Ward. J. 1929, 1544.

167. Kohler & Addinall. J.Amer.Chem.Soc. 52 (1930) 3728.

168. Campbell, Linden, Godshalk, & Young. J.Amer.Chem.Soc. 69 (1947) 882.

169. R. Stevenson. Private observation.

170. Barr, Heilbron, Jones & Spring. J. 1938, 334.

171. Nussbaum, Mancera, Daniels, Rosenkranz & Djerassi. J.Amer.Chem.Soc. 73 (1951) 3263.

172. Schwenk, Riegel, Moffett & Stahl. J.Amer.Chem.Soc. 65 (1943) 549.

173. Hicks, Berg & Wallis. J.Biol.Chem 162 (1946) 633.

174. Wieland, Dane & Scholz. Z.physiol.Chem. 211 (1932) 261.

175. Reichstein & Sorkin. Helv.Chim.Acta. 25 (1942) 797.

176. Gallagher & Long. J.Biol.Chem. 162 (1946) 521.

177. Marker & Lawson. J.Amer.Chem.Soc. 60 (1938) 1334.

178. McKenzie, Mattox, Engel & Kendall. J.Biol.Chem. 173 (1948), 271.

179. Bergstrom & Haslewood. J. 1939, 540.