STUDIES IN THE STEROID GROUP.

THESIS

submitted by

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PREFACE.

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Grateful acknowledgment is made to Dr. O. Rosenheim for his interest in, and helpful criticism of, the work dealing with the acyl migrations; and to the Donaldson Trustees for a Scholarship held during one year.

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C O N T E N T S.

Part I.

Part II.

Part III.

PART I.

EXPERIMENTS IN RELATION TO AN ATTEMPTED

PARTIAL SYNTHESIS OF HYODEOXYCHOLIC ACID FROM CHOLESTEROL.

THE MECHANISM OF ACYL MIGRATION FROM C_3 to C_4

IN THE 3-(aliphatic) MONOESTERS OF 4-HYDROXY-CHOLESTEROL.

INTRODUCTION.

The investigation of the bile acids and the sterols entered a new phase at the beginning of the century, previous work having been primarily concerned with their isolation, analysis and characterisation. Extensive researches on the constitution of these biologically important substances were undertaken, culminating in 1932 with the establishment of the now generally accepted Rosenheim-King formula. The two outstanding workers of this period were Windaus and Wieland, the former[1] iniating his classical investigations on cholesterol in 1903, the latter[2], in the case of the bile acids, in 1912.

The third phase, which is still in progress, is concerned mainly with finer points of structural detail, including stereochemical problems; the re-interpretation of older degradations; and the application of new reactions. There are definite indications that the ultimate goal of complete synthesis may be attained at a not too future date; indeed, many papers have appeared in recent years dealing with the preparation of simple model substances related to the sterols[3]. Already, attempts have been made to synthesise some of the less complex members of the steroid group, in particular, the sex hormones, one notable success being Bachmann's[4] total synthesis of d-equilenin and its three stereoisomers (i.e., l-equilenin and the diastereoisomers

d- and l-isoequilenin), represented by the non-steric
formula (I), below
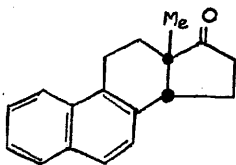
(I)

Among the initial steps in the constitutional
investigation of the bile acids and sterols were the
preparation of the corresponding non-hydroxylated compounds:
STEROLS.   Cholesterol (II)[*] - an unsaturated secondary
alcohol - was converted into its chloride, reduction of
which (with sodium and amyl alcohol) gave an unsaturated
hydrocarbon, cholestene (III)[5].   Catalytic hydrogenation of
the latter yielded the saturated compound cholestane (IV)[6].

The excretory sterol, coprosterol (V) - a saturated
secondary alcohol - was converted into its chloride, sodium
and amyl alcohol reduction of which afforded a hydrocarbon,
coprostane (VI), isomeric with cholestane[6].

The correlation of the fully hydrogenated compounds
was effected by means of an isomer of cholestene (pseudo-
cholestene), obtained[6],[7] by the elimination of hydrogen

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[*]The following degrations, formulae (II) to (X), are
briefly summarised in terms of the modern stereo-structures
on the extending chart between pages 17 and 18.

chloride from cholestene hydrochloride. Catalytic hydrogenation of this substance (VII) in neutral solution, yielded principally coprostane, while cholestane was the exclusive product when an acidic medium was employed[6),8)].

Disregarding modern stereochemical implications, this series of degradations is important in that conclusive proof was obtained as to the identity of the carbon skeletons of cholesterol and coprosterol; furthermore, that their respective parent hydrocarbons, cholestane and coprostane, differ merely in the orientation of one hydrogen atom.

BILE ACIDS.   The bile acids - recognised as saturated polyhydroxy monobasic acids - were transformed into the corresponding hydroxyl-free compounds by dehydration followed by catalytic hydrogenation, or by conversion into the dehydro- (ketonic) acids and treatment according to the method of Clemmensen or Kishner and Wolff[2),9),10)].   In all cases previous to 1923, the product was the saturated acid, cholanic acid (VIII), but in this year a newly discovered acid (from hog bile) named hyodeoxycholic acid (IX) was found to yield an isomer (X)[10)].   To this the name allocholanic acid had been given[*11)].

* In addition to allocholanic acid, two further isomers of cholanic acid have been described within recent years.
   While investigating bufodeoxycholic acid (from toad bile) Okamura (J. Biochem. Japan, 1928, 10, 5; 1929, 11, 103) subjected the corresponding dehydro-acid to Clemmensen reduction and obtained a new acid which he named bufo-cholanic acid. The nature of the (stereo- ?) isomerism in this compound is still obscure.
   The third isomer was secured in 1932 by Wieland and

Here again the important fact was established that most bile acids possess a common carbon framework, yielding on suitable treatment, the parent compound cholanic acid.

Although the constitutions of the bile acids and sterols were treated as separate problems, several workers held the view that there existed a close structural relationship. This followed from their co-occurrence in the animal organism, their similarity in molecular complexity* and finally from the fact that they both gave certain characteristic colour reactions.

The concept that these two series of compounds were closely related structurally has received confirmation only within comparatively recent years, the experimental linking of the two groups being accomplished by interconversions between individual members. The first transitions were

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co-workers (Annalen, 1932, **493**, 272) during an attempt to relate the toad poison, bufotalin, to cholanic acid. Degradation yielded an acid, _isobufocholanic acid_, isomeric with but different from the known cholanic acids. The proposal was put forward that in this case the isomerism was due, perhaps, to epimerism at the C_{17} or C_{20} position.

In 1928 the isolation of an alleged isomer of cholanic acid was reported (Shoda, see Chem. Zentr., 1928, **2**, 679; Kaziro, Z. physiol. Chem., 1929, **185**, 151). This acid was obtained in the usual manner from ursodeoxycholic acid (present in the bile of the bear), but its identity with cholanic acid was established a few years later (Iwasaki, Z. physiol. Chem., 1936, **244**, 181).

* Analyses revealed that cholestane and coprostane, the parent hydrocarbons of the sterol group, have the molecular formula $C_{27}H_{48}$, eight hydrogen atoms short of the corresponding saturated paraffin hydrocarbons, indicative of the presence of a tetracyclic structure. The parent bile acids, cholanic and allocholanic acid, have the molecular formula $C_{24}H_{40}O_2$ again suggesting the presence of four rings.

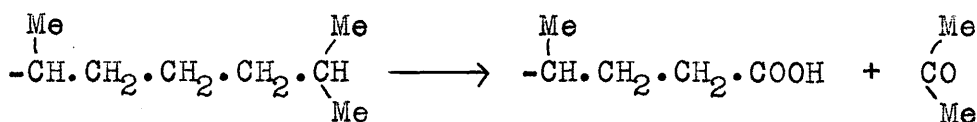carried out in 1919 by Windaus and Neukirchen[11], their
work being based on the following observations:-

Previous investigators having noted the production
of a pleasant smelling substance during the oxidation of
cholesterol and its derivatives, and following a suggestion
by Diels[12] that the odour resembled that of methylhexyl-
ketone, Windaus[13] undertook its isolation.   From the pro-
duct of a large scale chromic acid oxidation of cholesteryl
acetate he isolated, as the semicarbazone, a small amount
of the substance, and identified it as methylisohexylketone.
This fact indicated that the cyclic cholesterol molecule
possessed an aliphatic C_8H_{17} side-chain, the degradation
being formulated:

$$(AcO \cdot C_{19}H_{28}) \cdot \overset{\overset{\displaystyle Me}{|}}{CH} \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{\overset{\displaystyle Me}{\diagup}}{\underset{\underset{\displaystyle Me}{\diagdown}}{CH}} \longrightarrow O = \overset{\overset{\displaystyle Me}{|}}{C} \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{\overset{\displaystyle Me}{\diagup}}{\underset{\underset{\displaystyle Me}{\diagdown}}{CH}}$$
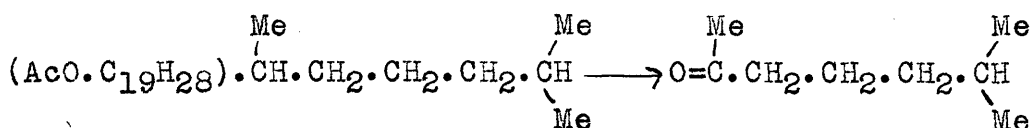
It had also been established that acetone was another
product of these oxidations, and since cholesterol contains
three carbon atoms more than the bile acids, it was suggested
that their carbon skeletons might differ by an isopropyl
group.   Assuming that the isopropyl group was the one present
in the C_8H_{17} side-chain, it appeared that the part of the
cholesterol molecule remaining after fission of acetone would
have the bile acid side-chain, i.e., considering only this
part of the molecule:

$$-\overset{\overset{\displaystyle Me}{|}}{CH} \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{\overset{\displaystyle Me}{\diagup}}{\underset{\underset{\displaystyle Me}{\diagdown}}{CH}} \longrightarrow -\overset{\overset{\displaystyle Me}{|}}{CH} \cdot CH_2 \cdot CH_2 \cdot COOH + \overset{\overset{\displaystyle Me}{\diagup}}{\underset{\underset{\displaystyle Me}{\diagdown}}{CO}}$$

Furthermore, if the bile acids and sterols have a common

nucleus, transitions from one group to the other ought to be possible.

For the purpose of testing these speculations the parent compounds of the sterol group were submitted to chromic acid oxidation. Hydrocarbons were selected in order to avoid the added complication of functional groups (i.e., olefinic linkages and hydroxyl radicals), together with the consequent risk of the cyclic portion of the molecule rupturing at their locations. The chief product obtained from cholestane (IV, extending chart) was ultimately identified as allocholanic acid, while cholanic acid itself was isolated from the coprostane oxidation.

Seven years later, the convergence of the two series was indisputably established[14] by the reconversion of cholanic acid into coprostane as follows. Chromic acid oxidation of the carbinol (XII), obtained from ethyl cholanate (XI) and isopropylmagnesium iodide, yielded a ketone (XIII) (coprostane-24-one), which was reduced to coprostane (XIV) with zinc amalgam.

(XI)

(XII)

(XIV)

(XIII)
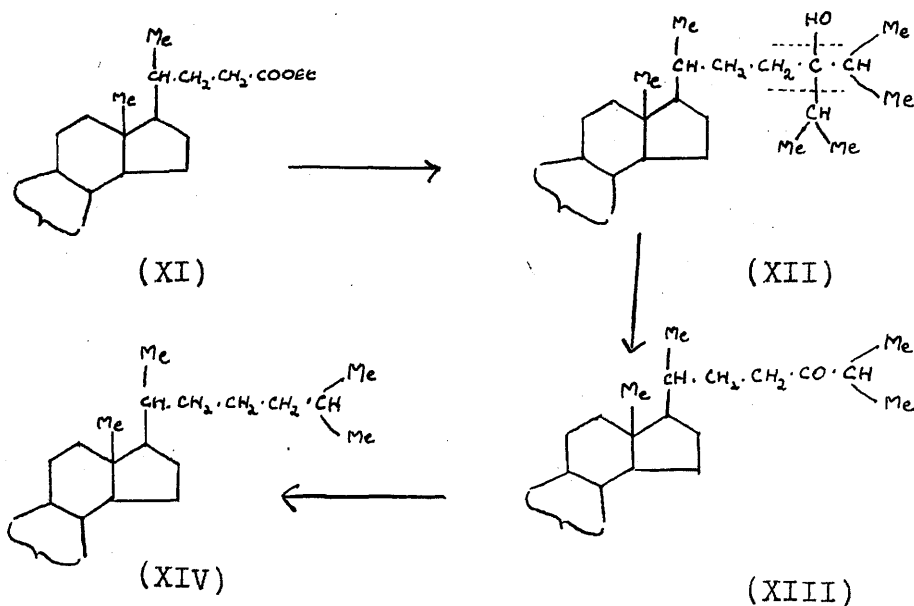
It was thus evident that the parent bile acids exhibit precisely the same isomerism as cholestane and coprostane, and following the establishment of a common carbon framework, the application to one series, of information pertaining to the other, became possible.

While these investigations were being pursued there had been accumulating a large body of data concerning the oxidative disruption of the nucleus.   These degradations were carried out on the natural products themselves (e.g., the sterols, cholesterol and coprosterol; and the bile acids, cholic, deoxycholic, lithocholic and (α-) hyodeoxycholic acid), the hydroxyl groups providing points at which one or more rings could be opened and the products examined.   The collation of these complicated findings represented a difficult

(XV)   (XVI)
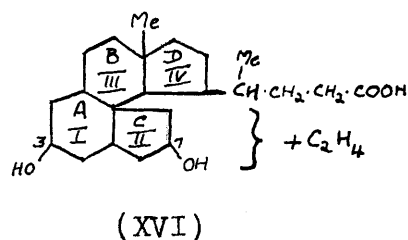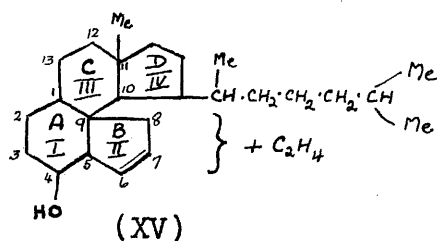
task and was due principally to Wieland and Windaus, who adduced much evidence as to the tetracyclic nature of the steroid molecule, and to the probable size of the rings concerned.   In 1928 they finally advanced the provisional formulae (XV) and (XVI) for cholesterol and deoxycholic acid respectively[*15].

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*   The rings in these structures were formerly differentiated by means of the Roman numerals I-IV; the

These formulations although satisfying the large bulk of experimental facts, had several shortcomings, for example, the impossibility of "fitting in" the remaining two carbon and four hydrogen atoms. They served to stimulate interest in the problem, however, and the task of revision commenced almost immediately.

As a result of measurements on surface films of certain steroids[16] and of an X-ray crystallographic study of ergosterol and some of its irradiation products[17], Bernal inferred that the steroid molecule consisted of an elongated, relatively thin structure; a conclusion which was not in agreement with the calculated dimensions of the Wieland-Windaus formula.

In 1927 Diels[18] had carried out some dehydrogenation experiments with sterols and bile acids, isolating from the resulting mixtures small amounts of three aromatic hydro-carbons. By 1932 one of these had been identified as chrysene (XVII). Rosenheim and King[19] drew attention to this neglected observation, and working on the assumption that the carbon skeleton of chrysene represented the cyclic moiety

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relationship of these rings to those in the modern formula (below) is indicated by the letters A-D, together with the current method of numbering the carbon atoms.
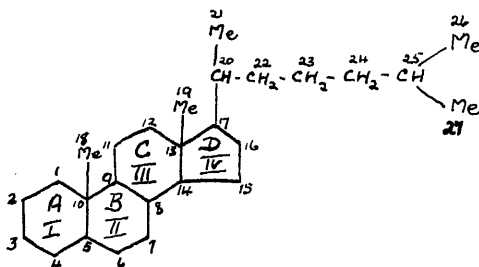
of the steroid molecule, proposed in May 1932 a new
structure, exemplified by formula (XVIII).
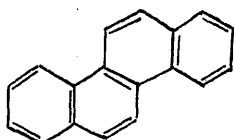
(XVII)   (XVIII)   (XIX)

Deoxycholic Acid

Although this molecule was in conformity with
Bernal's measurements it still did not fit all the known
facts, and three months later both Rosenheim and King[20]
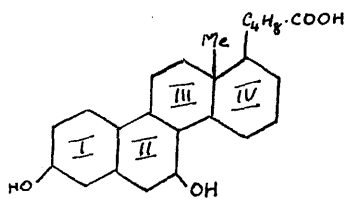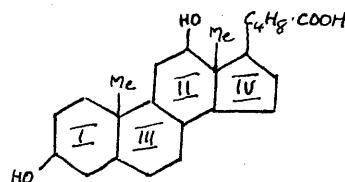and Wieland and Dane[21] modified the formula to that shown
in (XIX) - a structure which has withstood the test of all
subsequent work.

Following the establishment of the perhydro-1,2-
cyclopentenophenanthrene structure of the steroids, study
was directed to their complicated stereochemistry. In this
connection it was found possible to re-apply many of the
older degradations, and the information thus obtained, to-
gether with previous incidental observations on this topic,
were of immediate value. A long series of stereochemical
researches were undertaken, and the sum total of the result-
ing knowledge soon provided a fairly clear picture of the
steroid molecule. It must be stressed, however, that while
the problem has been satisfactorily settled in its essentials,
much work on minor details has still to be completed.

The present position may be summarised briefly as
follows:-

Cholestane and coprostane (XX) have each eight asymmetric carbon atoms (as have also cholanic and allocholanic acid), and of the 256 stereoisomers theoretically possible, only two have been definitely proved to exist in nature.   As with other compounds containing a number of asymmetric centres, one of these has been selected as the point to which all the remaining configurations are referable, and likewise given an arbitrary constellation.   In the case of the steroid molecule, the C_{10}-methyl group has been chosen as the reference point[*22)] and its valency bond is arbitrarily assumed to project upwards from the plane of the molecule (and of the paper) when the structure is represented in the normal manner (XX)[**].

(XX)

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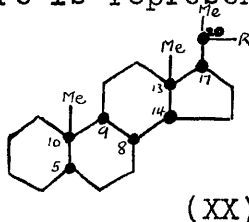
*   Originally, Ruzicka referred all configurations to the C_5-hydrogen, but difficulties were encountered in compounds lacking this reference point.   The assumption had to be made that $\triangle^{4:5}$-steroids were theoretically derived from the coprostane series, and that $\triangle^{5:6}$-compounds were potentially allo- or cholestane structures.

In order to retain the original cis and trans designations of Ruzicka, Miescher and Fischer (J.S.C.I., 1939, 58, 113) have recently suggested the C_9-hydrogen as a convenient point of reference.

**   The attachment to the cyclic portion of the molecule of this and other groups by means of a "solid" valency line, unless otherwise stated, implies that the group lies above the plane of the rings (and of the paper);   the use of a dotted or discontinuous line, as for example, in the case of the C_5-hydrogen atom in formula (XXI), p.12, is to be interpreted on the basis of the attached radical being situated below the plane of the molecule.

Six of the eight asymmetric centres (C_5, C_8, C_9, C_{10}, C_{13} and C_{14}) are conveniently studied in pairs. Thus, carbon atoms 5 and 10 form the junction of rings A and B, and since this part of the molecule has a reduced naphthalene form, cis- and trans-decalin structures are possible. The same consideration applies to rings B and C (C_8 and C_9), and to the hydrindane nature of rings C and D (C_{13} and C_{14}).

As stated on page 3, cholestane and coprostane differ only in the spatial location of one hydrogen atom, and following its identification as that attached to C_5, the two hydrocarbons must exhibit cis-trans isomerism (of the above-mentioned type) with respect to rings A and B. Furthermore, since this is the only difference between the two compounds, and since all other steroids are related to them (all but a few questionable cases), rings B, C and D must have a fixed configuration throughout this class of substances.

It has been established beyond all reasonable doubt, on both physical[23] and chemical grounds[24], that rings A and B in the cholestane series are linked in a trans-decalin manner, while coprostane (and the bile acids) has the corresponding cis-structure.

The evidence for a trans-linking of rings B and C, although somewhat meagre[25], is quite convincing, and for the analogous trans-hydrindane fusion of C and D, almost certain (with reference to the latter, however, see the theoretical

discussion in Part III of this thesis).

While the configuration(s) of C_5 has been compared with the reference group (Me) on C_{10}, that of C_8 has only been related to C_9, and C_{14} to C_{13}. Hence four structures are still possible, for example, in the case of cholestane, formulae (XXI) to (XXIV) below. In order to establish which

(XXI)   (XXII)

(XXIII)   (XXIV)
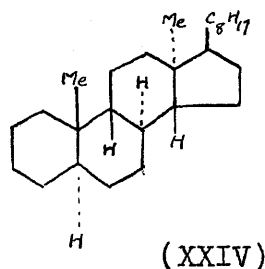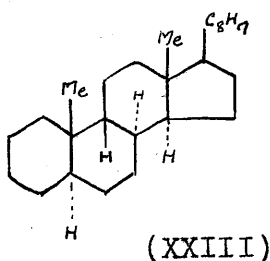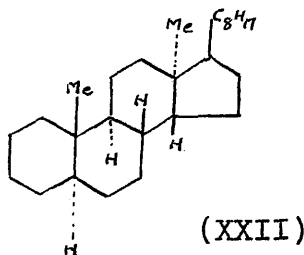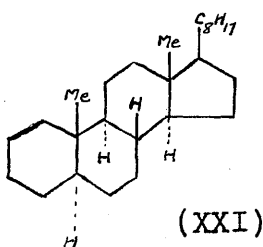
structure belongs to steroids it is necessary to determine the steric relationship between the C_9-H and the C_{10}-Me, and between the C_8-H and the C_{14}-H. Unfortunately, no "chemical" evidence is available to enable the achievement of this correlation, but a strong argument on steric grounds has been advanced suggesting a trans arrangement of the above pairs of groups[26], i.e., that structure (XXI) belongs to steroids.

Giacomello[27] describes the results of an X-ray study of two choleic acids derived from deoxycholic acid.

A Fourier projection of the data thus obtained suggests a similar structure (XXV) for deoxycholic acid.

(XXV)
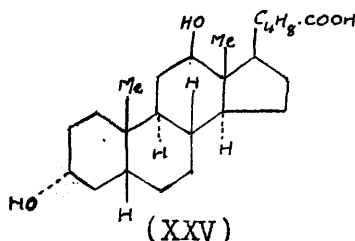
Two centres of asymmetry remain[*], namely, those at C_{17} and C_{20}. The side-chain attached to C_{17} is now supposed to be cis to the C_{19}-Me[27)], and a few authentic cases of inversion at this centre in "synthetic" products have been reported[28)]. Only one reliable instance of stereoisomerism at C_{20} is on record[29)], but the exact configuration obtaining in bile acids and sterols is as yet undetermined.

The presence of nuclear hydroxyl groups at C_2, C_3, C_4, C_5, C_6, C_7, C_{11}, C_{12}, C_{14}, C_{16} and C_{17} in the various classes of naturally occurring steroids and "synthetic" products produce additional centres of asymmetry (with the exception of those at C_5, C_{14} and C_{17}) at each one of which the possibility of epimerism arises.

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[*] There is an additional asymmetric carbon atom at C_{24} in ergosterol and the phytosterols, but nothing is known regarding its configuration. Some cases of isomerism may be attributable to inversion at this centre.
The explanation of the isomerism met with in ergosterol derivatives may also be sought for in geometrical isomerism about the $C_{22}:C_{23}$-double bond - what is the exact spatial location of the groups attached to the $C_{22}:C_{23}$-component in ergosterol, stigmasterol, zymosterol, etc.?

The following description is limited to the stereochemistry of the C_3-hydroxyl group.

Both configurations of the C_3-OH occur in natural products, and it has been established that bile acids and sterols have this group on opposite sides of the plane of the molecule. Ruzicka[30] infers, on the basis of the Auwers-Skita[31] hydrogenation rule as applied to the reduction of cholestane-3-one and coprostane-3-one, that sterols have their C_3-OH cis to the C_{10}-Me, while bile acids have the epi or trans configuration*. This hydrogenation rule - which states that with reference to a group attached to an adjacent atom, hydrogenation in neutral solution yields the trans, and in acid media the cis structure - is ambiguous, however, and may be applied to either the C_5-H or the C_5-C_6 cyclic component giving exactly opposite configurations in each case, e.g.,

$$\xrightarrow[\text{neutral soln.}]{+H_2}$$

Referring to C_5-H   or   Referring to C_5-C_6

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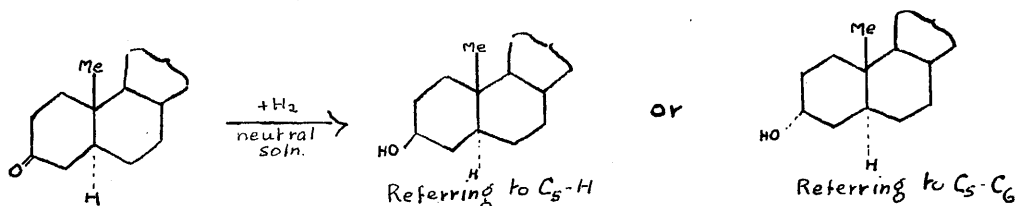
* The following methods of nomenclature are in general use:

	I.	II.
C_3-OH cis to C_{10}-Me	normal or β -configuration.	
C_3-OH trans to C_{10}-Me	epi or λ -configuration.	

I was originally applied to sterols and II to bile acids. It is now common practice to denote configurations of other hydroxyl groups by the Greek letters. For example, anthropodeoxycholic acid has both its C_3- and C_7-hydroxyl groups trans to the C_{10}-Me and is named 3($\propto$), 7($\propto$)-dihydroxy-cholanic acid; ursodeoxycholic acid, which is its C_7-epimer, is 3($\propto$), 7(β)-dihydroxy-cholanic acid.

It now appears that Ruzicka made a fortunate choice when he selected the C_5-H as the reference group for the Auwers-Skita rule, because Lettré[32] has since confirmed his formulations on direct chemical grounds (i.e., by the lactonisation and otherwise of certain acids derived from the rupture of ring B).

While 3(β)-hydroxy-steroids (e.g., sterols) yield sparingly soluble molecular complexes with the saponin, digitonin, no precipitates are formed with their epimers (e.g., epi-sterols, bile acids, etc.). This well-established fact provides a valuable means of separating mixtures of both classes of compounds, and of determining the steric orientation of this group in a new substance.

Other methods of establishing configurations have been suggested:-

The p-toluenesulphonates of saturated sterols form methyl ethers, their epimers, unsaturated compounds, on treatment with boiling methyl alcohol[33]. This method suffers from the defect, however, that it has not been tested out on a sufficiently wide range of compounds.

Ruzicka and co-workers[34] find that the acetates and benzoates of epimeric sterols having rings A and B transfused, are more readily saponified than the corresponding coprostane analogues.

Configurations based on either of the above three methods are subject to the limitation that exceptions are or

may be encountered[22),[33)], but taken in conjunction with other data valuable evidence for a particular orientation can be adduced.

Bergmann[35)] discusses certain cases where an absolute configuration can be ascribed.

Several correlations of stereoisomerism and physical properties have been made. Thus, differences in the specific gravity and molecular refraction of cholestane and coprostane enabled Ruzicka[23)] to confirm the respective trans and cis decalin nature of rings A and B in these hydrocarbons. The most important development in recent years has been attempts to relate stereoisomerism and optical activity, and of the various workers in this field, Callow and Young[36)], and Bernstein and co-workers[37)] have been the most successful, particularly the latter. An application of van 't Hoff's principle of optical superposition enabled the former investigators to formulate a number of empirical rules, such as, for example, the general increase in dextrorotation of about 220° following the formation of a $\triangle^{4:5}$ -ethylenic linkage. Bernstein[37)] has pointed out, however, the general inapplicability of van't Hoff's principle on theoretical grounds, and utilising modern theories of optical activity, he has been able to evolve a method of calculating the optical rotation of (almost ?) any steroid. The excellent agreement between the observed and calculated values of a large number of compounds has led him to propose, in the few cases

where these rotations differ widely, that the substances
in question have not the structures assigned to them.   In
a second paper[38] he extends his system to include esters
such as acetates, benzoates, etc., in fact any derivative,
and he makes the suggestion that this method offers an
alternative means to dehydrogenation for the detection of a
perhydro-1,2-cyclopentenophenanthrene structure.
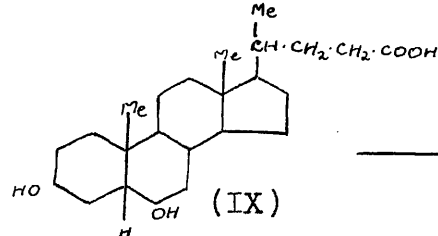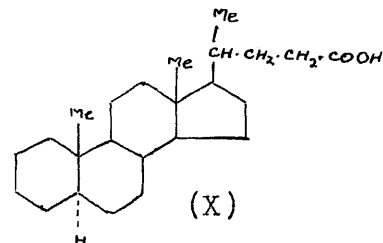
(α-) Hyodeoxycholic acid → Allocholanic acid
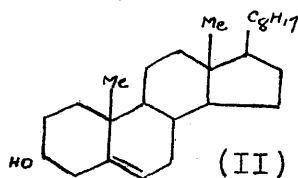
Cholesterol (II) → Cholestene (III) → Cholestane (IV)
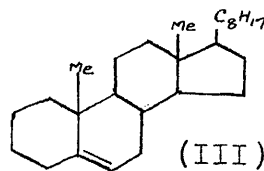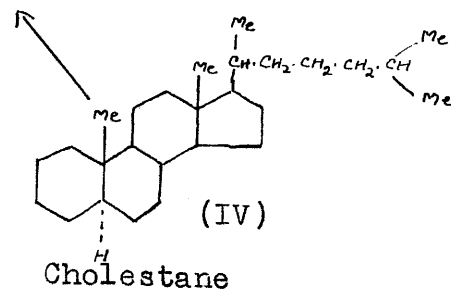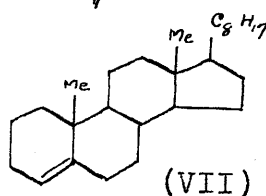
Pseudocholestene (VII)
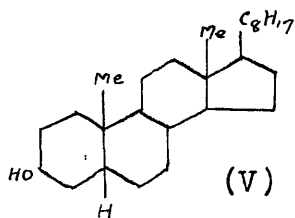
Coprosterol (Coprostanol) (V) → Coprostane (VI)
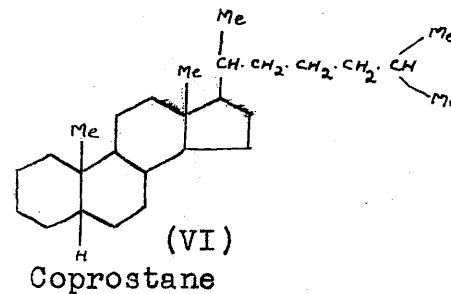
Cholic acid
Lithocholic acid
etc. → Cholanic acid (VIII)
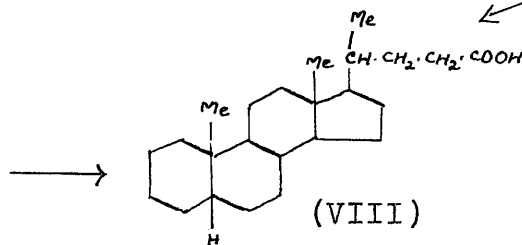
THEORETICAL.

Hyodeoxycholic acid was discovered by Strecker[39] in 1846 during an examination of pig bile. It occurs in the latter conjugated with glycine, and the early studies were mainly directed to the characterisation of this compound, that is, glycohyodeoxycholic acid ("hyocholinic acid"). Although it was recognised as belonging to the bile acid series its chemistry was neglected for some eighty years, when Windaus initiated the attack on its constitution. In 1923 he published a paper[40] describing the preparation (from pig bile) and properties of hyodeoxycholic acid. Some preliminary degradations therein, formed the basis of subsequent work, and three years later he was able to announce that the problem had been settled in its essentials[24].

The modern interpretation of his results proves that hyodeoxycholic acid is a 3,6-dihydroxy-cholanic acid (XXVI). Until 1926, however, it was thought to belong to the allo or cholestane series, since its dehydro- acid yields allocholanic acid on Clemmensen reduction (see p.3). This fact is now simply explained as follows:-

Chromic acid oxidation of hyodeoxycholic acid (XXVI) yields the corresponding diketo- acid (XXVII) ("α"-dehydrohyodeoxycholic acid; 3,6-diketocholanic acid). This compound is unstable, however, and rearranges to 3,6-diketoallocholanic acid (XXVIII) ("β"-dehydrohyodeoxycholic acid) on

treatment with dilute acid or alkali.   It is evident, there-
fore, that a Clemmensen reduction of the "α"-acid (XXVII) must
involve its preliminary allomerisation to the "β"-isomer (XXVIII),
which then reduces normally to allocholanic acid (XXIX).

(XXVI)   (XXVII)

(XXVIII)   (XXIX)
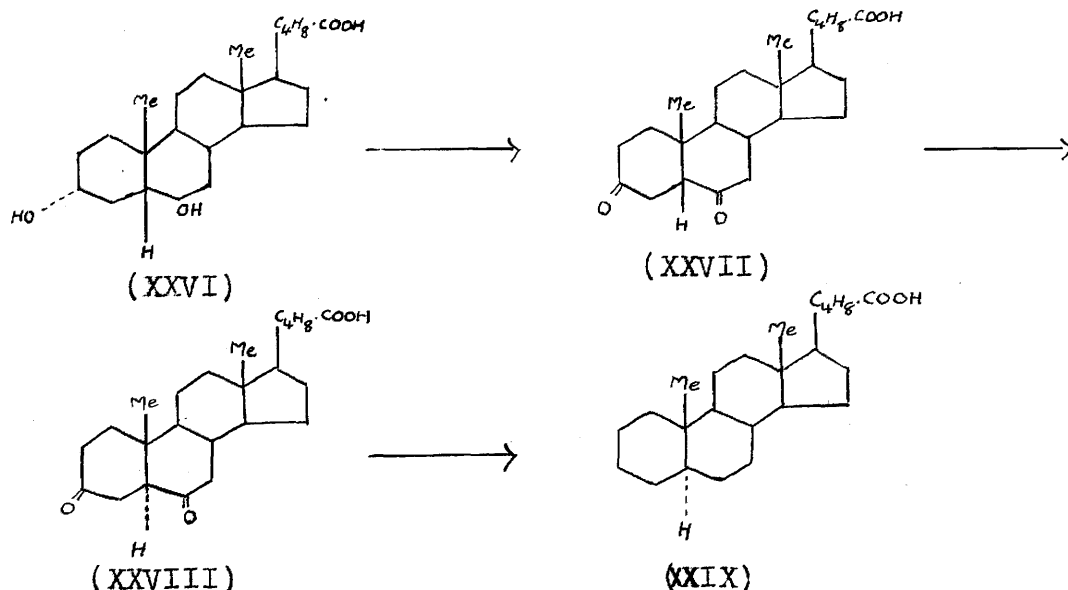
Like the other naturally occurring bile acids, the
C_3-OH in this compound has the customary α-configuration[41],
but no degradation yet effected enables the orientation of
the C_6-OH to be fixed.

While investigating the alleged presence of epimeric
bile acids in nature, Kimura[42] isolated from pig bile a new
acid which, like hyodeoxycholic acid, yielded "α"-dehydro-
hyodeoxycholic acid on oxidation with chromic acid.   The new
acid, named β-hyodeoxycholic acid, must therefore be a
stereoisomer of (α-) hyodeoxycholic acid.   Applying an
analogous reaction sequence, formulae (XXX) to (XXXII), to
that employed by Wieland and co-workers[41] in the case of

the α-isomer, formulae (XXXIII) to (XXXV), Kimura[42] proved
that β-hyodeoxycholic acid had its C_3-OH in the β-con-
figuration.

(XXX)

(XXXIII)

(XXXI)

(XXXIV)

(XXXII)

(XXXV)
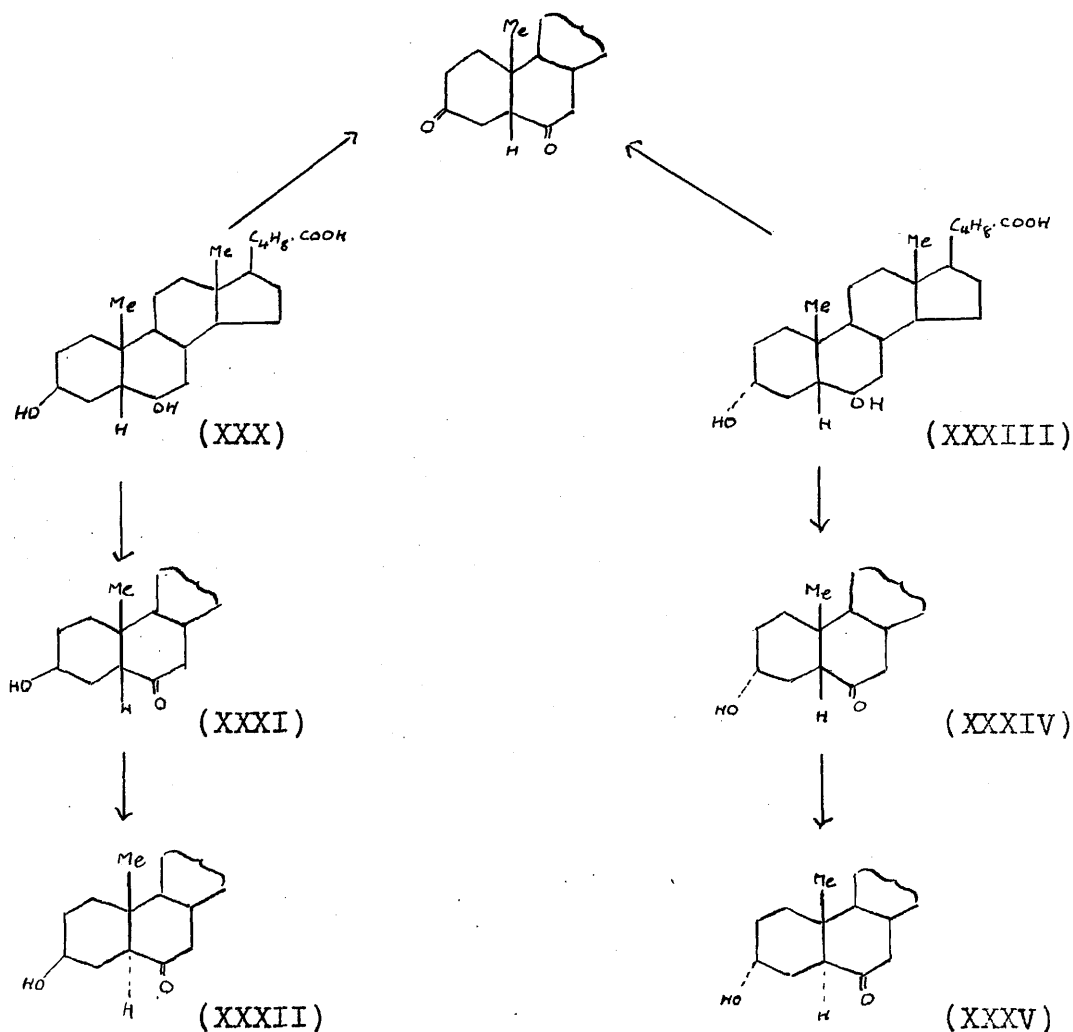
On the basis of the above degradations alone, he
postulates that the two acids are C_3-epimers, but the
possibility of the C_6-OH in the β-acid having a different
configuration cannot be excluded.

The formulae employed in the above paper[42] appear
to imply a specific (β-) configuration for the C_6-OH in both

the α- and β-hyo acids; their confusing nomenclature, how-
ever, offers a possible clue - see formulae (XXXVI) and
(XXXVII).   Why two groups, C_3-OH and C_6-OH, situated on oppos-
ite sides of the ring system in (XXXVI) should have the same
prefix (α) can only be explained as follows.   While the terms
α and β as applied to the C_3-OH in (XXXVI) and (XXXVII) indi-
cate its specific trans and cis relationship, respectively,
to the C_{10}-Me; the term α as applied to the C_6-OH is pre-
sumably to be interpretated as implying an arbitrary (i.e.,
yet unknown) configuration.   The formula for α-hyodeoxy-
cholic acid is taken from a paper by Sugiyama[43], where the
same nomenclature is again employed.
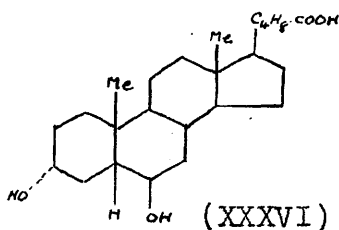
(XXXVI)

"α-3,6-Dihydroxy-cholanic
acid"

(α-Hyodeoxycholic acid)

(XXXVII)
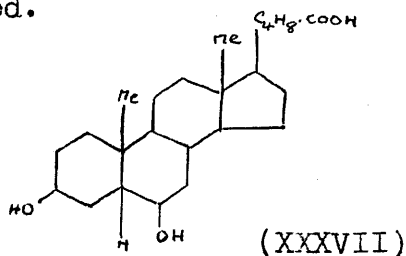
" β-3-α-6-Dihydroxycholanic
acid"

(β-Hyodeoxycholic acid)

Until the configuration of the C_6-OH has been settled
the two acids are preferably named: α-hyodeoxycholic acid,
3(α),6-dihydroxy-cholanic acid; β-hyodeoxycholic acid,
3(β),6-dihydroxy-cholanic acid.

Another point arises from Kimura's paper, namely,
the question whether the new acid is a natural product, or
merely formed by epimerisation during the process of iso-
lation.   The method employed involved the preliminary

removal of α-hyodeoxycholic acid and 3-hydroxy-6-keto-allocholanic acid (also present in pig bile[41]) by salting out with sodium chloride, followed by precipitation of the crude β-glycohyodeoxycholic acid* with hydrochloric acid. The glyco acid was then <u>hydrolysed in the autoclave with aqueous alcoholic potash</u> yielding impure β-hyodeoxycholic acid on acidification.   Since the salting out of the α-acid can hardly be considered to result in its <u>quantitative</u> removal from solution, the subsequent hydrolysis under such drastic conditions might conceivably have led to epimerisation of the residual acid.

Summing up, two problems in connection with the chemistry of α- and β-hyodeoxycholic acid await solution, namely:-

1) the establishment of the configuration of the C_6-OH in both acids, and

2) the question as to whether or not the β-stereoisomer is a naturally occurring bile acid.

The present investigation was undertaken with the object of settling these outstanding problems, the proposed methods of attack being as follows:-

Since there appeared to be no convenient means of determining the configuration of the C_6-OH in α- and β-

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*   This β-glycohyodeoxycholic acid is undoubtedly identical with the "β-hyoglycocholic acid" isolated from pig bile by Jolin (Z. physiol. Chem., 1887, <u>11</u>, 417; 1888, <u>12</u>, 512; 1889, <u>13</u>, 205).

hyodeoxycholic acid (and since a supply of these acids was not available), a synthetic route was considered to be the best approach to the solution of the problem.   In other words, the preparation of both acids by oxidative shortening of the principal side-chain in coprostane-3,6-diols of known stereochemical structure.

Regarding the second problem, two methods of attack are applicable: the direct method, i.e., the attempted isolation of the β-acid from pig bile in such a manner that epimerisation of hydroxyl groups is rendered unlikely; and the indirect method, whereby the α-acid is subjected to the experimental conditions employed in the isolation of the β-stereoisomer, the product being examined for the presence or otherwise of the latter.   A negative result from the indirect procedure would indicate that the β-acid is a natural product; a positive result, on the other hand, would provide evidence to the contrary, final confirmation of which would necessarily have to be obtained by the direct approach.

Arranging the four theoretically possible coprostane-3,6-diols as shown in formulae (XXXVIII) and (XXXIX) below, (XXXVIII A) and (XXXVIII B) differ only with respect to the configuration of the C_3-OH, the same applying to (XXXIX A) and (XXXIX B).   Now, if the spatial orientation of the groups attached to the C_6-carbon in α- and β-hyodeoxycholic acid be the same, these acids would be capable of "partial syn-thesis" from the pair of coprostanediols represented by

either (XXXVIII) or (XXXIX).

(XXXVIII)

A.

B.

(XXXIX)

A.

B.

(XL)
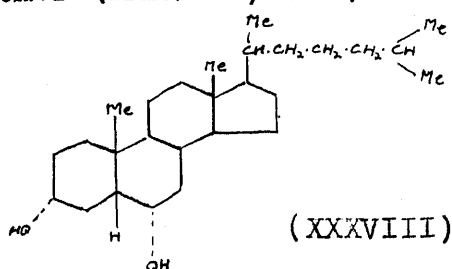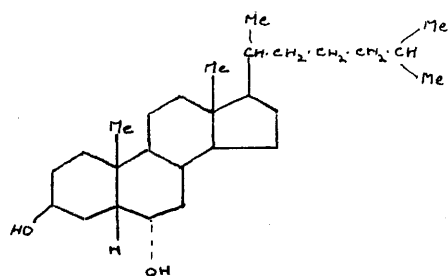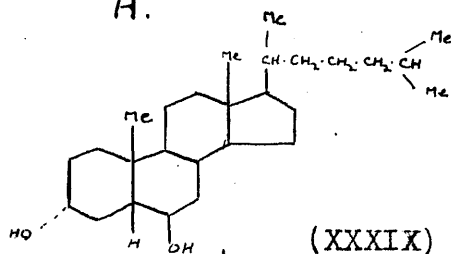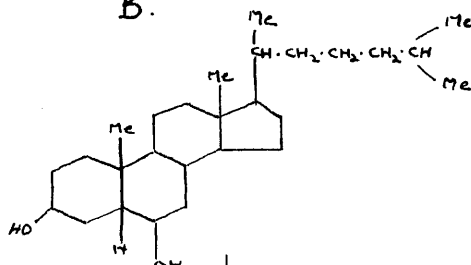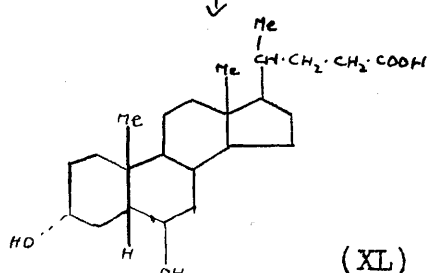
A.
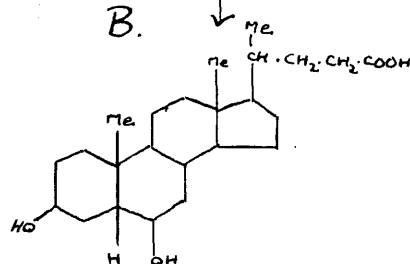
B.

As will be evident from the later discussion (p.26) the preparation of the epimeric coprostanediols (XXXIX) was thought to provide a convenient starting-point.   Thus, in the first instance the problem resolved itself into the preparation of coprostane-3(α),6(β)-diol (XXXIX A) and coprostane-3(β),6(β)-diol (XXXIX B), followed by their degradation to the respective bile acid structures (XLA) and (XL B), and comparison of the physical and chemical properties of the latter with those of the hyo-acids (α- and β- respectively).

In the event of insufficient quantities of the coprostanediols being available for the inevitably wasteful

degradations to the bile acid series, and in the event of the preparation of the necessary quantities being impracticable, there remains an alternative course of action. Namely, the possibility of lengthening the $C_4H_8 \cdot COOH$ side-chain of the hyo- acids to that of cholesterol (by the method employed by Wieland and Jacobi[14] (p.6) in the case of cholanic acid) and making the comparisons in the coprostane series*, i.e.,

It was proposed to effect the above "partial

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syntheses" of α- and β-hyodeoxycholic acid using cholesterol as starting-material, and to employ the reaction sequence illustrated in the formulae, (XLI) to (XLVII), below. The bracketed numbers over the arrows refer to notes giving further information.

Cholesteryl acetate

Triol-I diacetate (XLI)

(XLII)

(XLIII)

(XLIV)

(XLV)

(XLVI)

A.

(XLVII)

A.

B.

B.
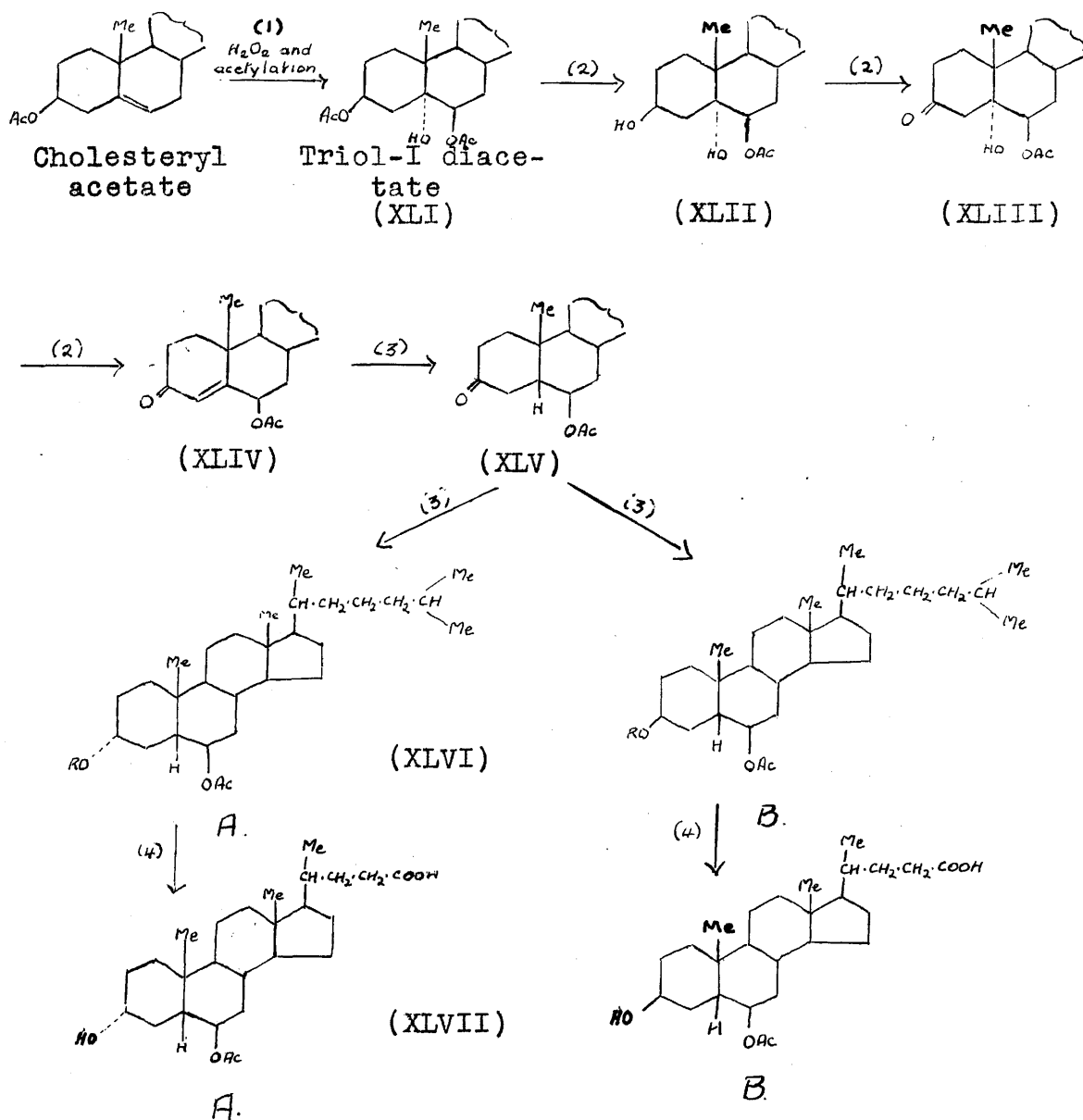
(1)   Spring[44] states that:

" The stereochemical relationship of the two isomeric 3(β), 5,6-trihydroxycholestanes (triols-I and -II) has been examined by Ellis and Petrow[45].   Triol-I, which is obtained by oxidation of cholesterol with hydrogen peroxide[46] or by hydrolysis of α-cholesterol oxide[47], has been shown by Criegee[48] to be a trans-α-glycol (C_5-C_6).   Triol-II is prepared by the oxidation of cholesterol with either potassium permanganate[49] or osmic acid[50] and, as is to be expected from its method of preparation, is a cis-α-glycol[48]. Since oxidation of the isomeric triols yields different 5-hydroxy-3,6-diketocholestanes[49], it follows that the two triols differ only in orientation around C_5.   Ellis and Petrow have now shown that the 3-keto-5,6-diol monoacetate (XLVIII) derived from the 6-monoacetate (XLIX) of triol-I gives a 2-bromo-derivative which with alkali yields a trans-annular oxide (L).   The alternative mechanism whereby the bromine atom is introduced at C_4 to yield a C_4-C_5 oxide is excluded because of the stability of the oxide obtained.   Oxidation of

(XLVIII)   (L)

(XLIX)   (LI)   (LII)

(LIII)   (LIV)
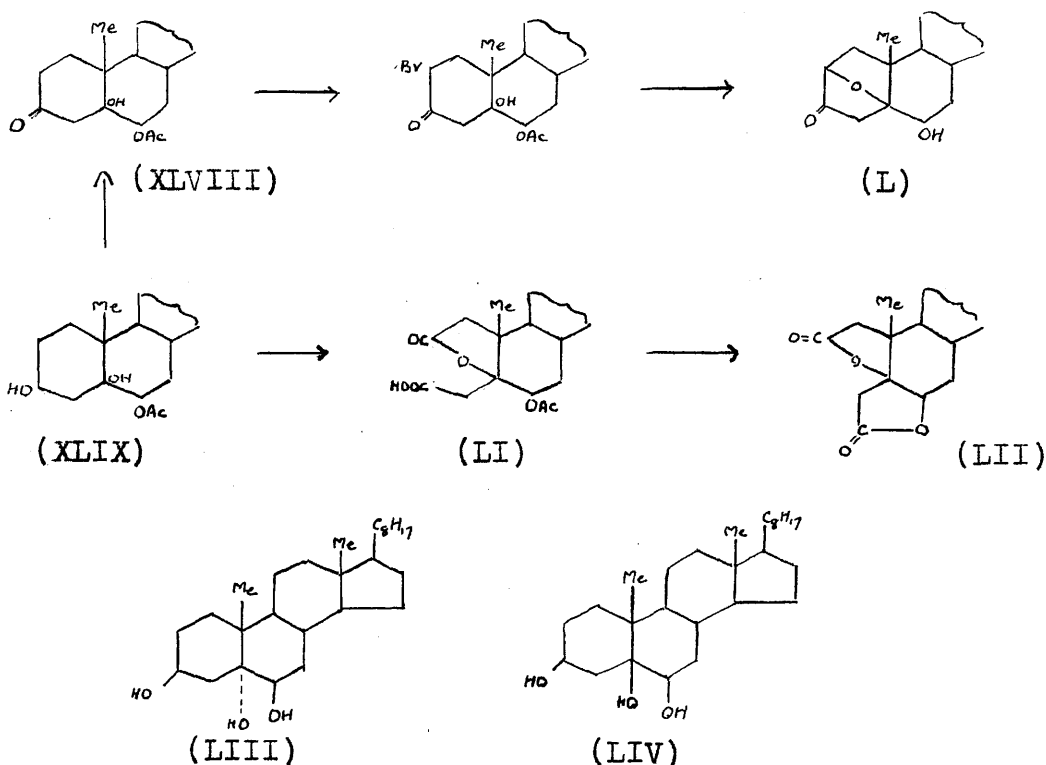
the 6-monoacetate (XLIX) of triol-I gives the lactonic acid (LI), hydrolysis of which gives the dilactone (LII).   Thus bromination of the 3-ketone (XLVIII) gives a 2-bromo-derivative and oxidation leads to $C_2 \| C_3$ rupture, a behaviour known to be characteristic of C_3-keto-steroids in which rings A and B are trans-fused[51].   Ellis and Petrow conclude that this orientation

pertains in the 3-keto-5,6-diol monoacetate and consequently in the parent triol-I, which is therefore represented by (LIII) and triol-II by (LIV).   Unfortunately these decisions are dependent upon the assumption that the course of bromination and oxidation of saturated 3-keto-steroids is not altered by the replacement of the C_5-hydrogen by a hydroxyl group."

"The configurations (LIII) and (LIV) adopted for triols-I and -II, respectively, appear to be independent of the assumption made by Ellis and Petrow.   First, the immediate lactonisation of the (not isolated) $C_2 \| C_3$ dicarboxylic acid to the lactonic acid (LI) and of the (not isolated) hydroxy-lactone-acid to the dilactone (LII) requires that rings A and B in triol-I be <u>trans</u>-fused.   Secondly, the oft-observed fact that cis-hydroxylation of $3(\beta)$-hydroxy-$\triangle^5$-steroids by means of osmic acid is inhibited by acetylation finds an explanation if triol-II is represented by (LIV) (cf. Ehrenstein[52] below).

Ehrenstein[52] has prepared two stereoisomeric 3,5,6-triols (LV) and (LVII) in the pregnane series;   (LV) by treatment of $\triangle^5$-pregnene-20-one-3-ol acetate (LVI, R = Ac) with hydrogen peroxide, and (LVII) by the action of osmic acid on the corresponding free alcohol (LVI, R = H).   These two compounds differ from the cholestanetriols (LIII) and (LIV) in that they yield, on oxidation, the <u>same</u> diketo-compound (LVIII), and hence the configuration of the C_5-OH is

(LV)   (LVI)   (LVII)

(LVIII)
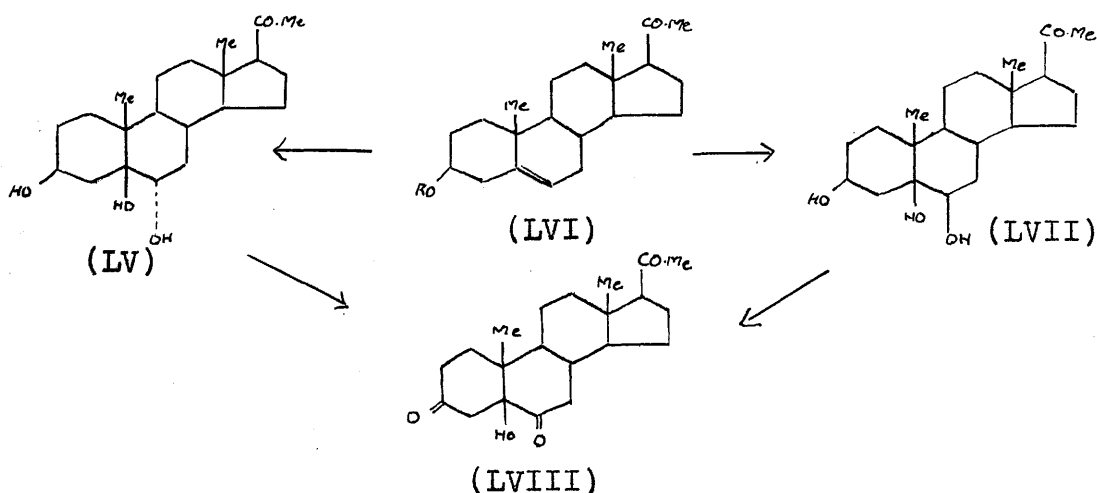
identical in both.   Thus while the cholestanetriols are

stereoisomeric with respect to the configuration of the C_5-OH,

the pregnanetriols differ solely in epimerism at the C_6-OH.

Ehrenstein proposes the above configurational formulae (LV)

and (LVII) on the basis of some observations due to

Butenandt[53] and Westphal[54].   These workers find that when

the C_3-OH (β-) of dehydroisoandrosterone is esterified, no

addition of osmic acid to the C_5:C_6 double bond occurs, and

explain this on steric grounds, i.e., in $\triangle^5$-steroids having

a C_3-OH in the β-position (C_3-OH cis to C_{10}-Me), acylation

should sterically hinder the addition of osmic acid, provided

that the latter leads to a <u>cis</u>-linkage of rings A and B.

In this connection Ehrenstein[55] makes the following

interesting statement:-

"Ellis and Petrow recently investigated the stereo-
chemical configuration of the cholestane-3,5,6-triols.   The
triol obtained by treating cholesterol with osmic acid was
assigned the coprostane configuration, which is in agreement
with the stereochemical considerations of our previous
paper[52]" (outlined above).   "The triol obtained by means of
hydrogen peroxide was assigned the cholestane configuration.
The fact that in our series the procedure with hydrogen per-
oxide apparently furnished the coprostane configuration is
no contradiction.   Probably the hydrogen peroxide treatment
of cholesteryl acetate and of 5-pregnene-20-one-3-ol acetate
respectively furnished in each case a mixture of two "trans"
forms, one possessing the coprostane configuration and the
other the cholestane configuration.   It appears that in the
experiments with cholesterol the cholestane epimer was secured
from this mixture, whereas in our experiments with pregnenonol
the coprostane epimer showed the greater tendency to
crystallise."

(2)   Ellis and Petrow[45] obtained an excellent yield (85%)

of 6(β)-acetoxy-cholestane-3,5-diol (XLII) by treating an

alcoholic solution of 3(β),6(β)-diacetoxy-cholestane-5(α)-ol
(triol-I diacetate) (XLI) with one equivalent of potassium
hydroxide at room temperature.   Chromic acid oxidation of
this monoacetate gave 6(β)-acetoxy-cholestane-5(α)-ol-3-
one (XLIII) which was dehydrated to 6(β)-acetoxy- $\triangle^4$-
cholestene-3-one (XLIV), with either boiling acetic anhydride
or thionyl chloride and pyridine at the boiling point[45].
They characterised this latter material by further oxidation
to the known $\triangle^4$-cholestene-3,6-dione (LXIII), and by its
hydrolysis to the corresponding unsaturated keto-alcohol
(LXI).   The semicarbazone of the latter was found to be
identical with that of Dane (see below).

Dane[56] prepared the same unsaturated keto-alcohol
(LXI) by refluxing cholestenone dibromide (LIX) (obtained
from cholesterol dibromide by oxidation) with sodium acetate
in alcohol and hydrolysing the resulting monobromo-compound
(LX) with methyl alcoholic potash at room temperature.   The

(LIX)   (LX)   (LXI)   (LXII)
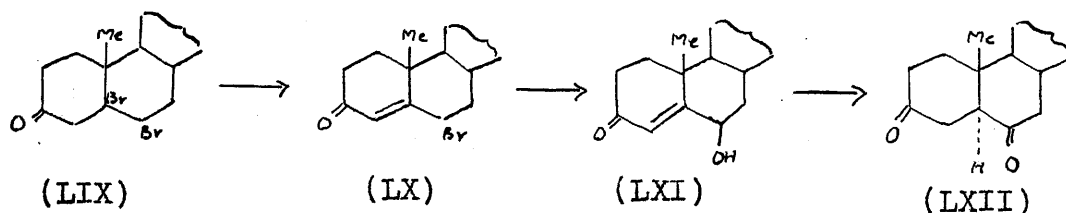
product could only be isolated as the semicarbazone.

Since it does not appear possible to regenerate the
ketone from the semicarbazone (on account of the facile
isomerisation of (LXI) to cholestane-3,6-dione (LXII) by
hot mineral acid or alkali[45]) this method is unsuitable
for preparative purposes.

Chromic acid oxidation of cholesterol in glacial
acetic acid yields in addition to cholestane-3,6-dione-5(α)-
ol (LXIV) (identical with the diketo-alcohol obtainable from
"triol-I") and $\triangle^4$-cholestene-3,6-dione (LXIII), a small
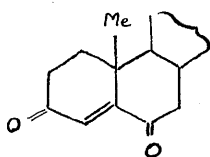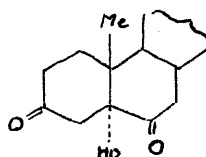
(LXIII)   (LXIV)

amount of a compound which Mauthner and Suida[57] named
"α-oxycholestenol".   This substance has been shown to be a
$\triangle^4$-cholestene-6-ol-3-one and may be identical with (LXI).
Thus:

	m.p.	Acetate m.p.
"α-oxycholestenol" ------------	180°	101-102°
$\triangle^4$-cholestene-6(β)-ol-3-one --	192°	101.5°

(3)   Extending earlier work on reductions in the sterol
group, Ruzicka[30] established the stereochemical relation-
ships outlined below.   These he based on the Auwers-Skita
hydrogenation rule (see p.14).

A catalytic reduction of cholestane-3-one (LXV) in
neutral solution affords dihydrocholesterol (cholestane-
3(β)-ol) (LXIX); which latter is also obtainable by direct
hydrogenation of cholesterol (LXVI), and which returns the
above ketone (LXV) on oxidation with chromic acid. When an
acidic medium is employed, however, the reaction takes a
different course, epidihydrocholesterol (cholestane-3(α)-
ol) (LXX) being the principal product.

Bearing in mind the above-mentioned hydrogenation
rule, coprostane-3-one (LXVIII) behaves entirely analogously:
hydrogenation in neutral solution yielding epicoprosterol
(coprostane-3(α)-ol) (LXXI), and in acid media, coprosterol
(coprostane-3(β)-ol) (LXXII).

(LXV)   (LXVI)   (LXVII)   (LXVIII)

(LXIX)   (LXX)   (LXXI)   (LXXII)
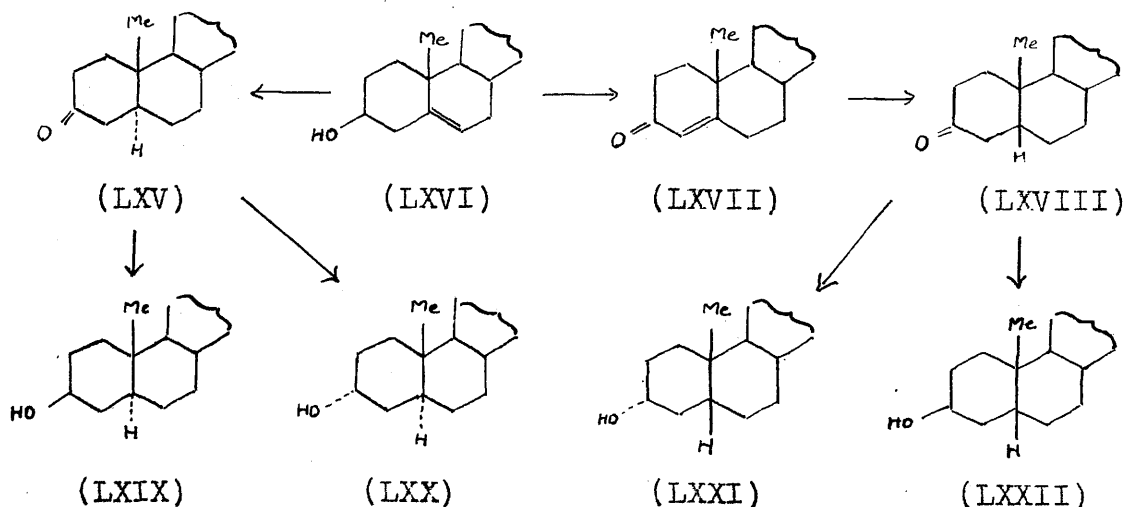
Coprostenone (LXVII) is readily obtainable by oxi-
dation followed by debromination of cholesterol di-
bromide[58], or simply by an Oppenauer oxidation of chol-
esterol itself[59]. Partial hydrogenation (Pd-catalyst) of
this unsaturated ketone (LXVII) yields coprostanone (LXVIII)

(also formed from coprosterol by oxidation).

Since 6(β)-acetoxy-$\triangle^4$-cholestene-3-one (LXXIII) is structurally very similar to coprostenone, it appeared that it would behave analogously on hydrogenation.   Partial reduction, by means of a palladium catalyst giving 6(β)-acetoxy-coprostane-3-one (LXXIV), the further hydrogenation of which (PtO$_2$), in neutral and acid media, should yield 6(β)-acetoxy-coprostane-3(α)-ol (LXXV) and -3(β)-ol (LXXVI), respectively.

(LXXV)

(LXXIII)   (LXXIV)
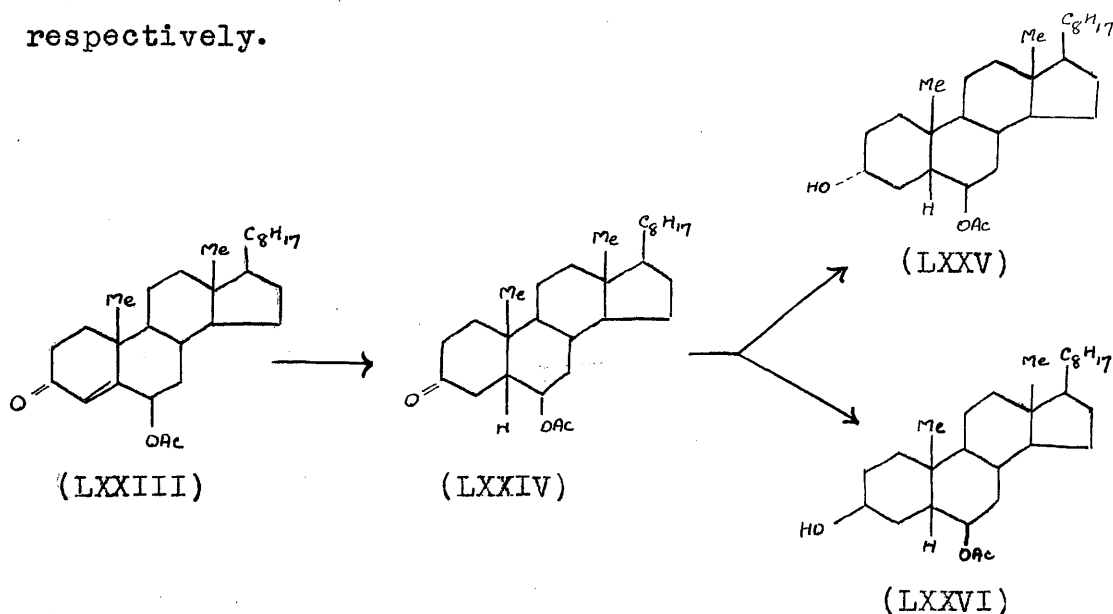
(LXXVI)

Confirmation of these configurations, (LXXV) and (LXXVI), should follow from the oxidation of the corresponding free diols to the same diketone (LXXVII), which ought to rearrange to the known cholestanedione (LXXVIII)[1)60] on treatment with dilute acid or alkali (cf. p.19).   By analogy with

(LXXVII)   (LXXVIII)
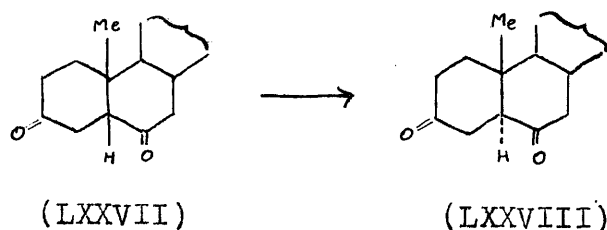
the partial oxidations of α- and β-hyodeoxycholic acid to
the epimeric 3-hydroxy-6-ketocholanic acids, and the allo-
merisation of the latter with acid or alkali (p.20), a
similar series of changes should provide further evidence as
to the cis-linkage of rings A and B in these compounds. The
configurations about C_3 could be settled, prior to the final
stage of the "synthesis", by means of digitonin, and also
from the keto-alcohol (LXXX), obtained from the diol (LXXIX)
corresponding to (LXXVI) above, which ought to be identical
with the 3(β)-hydroxy-cholestane-6-one of Mauthner and Suida[61]

(LXXIX)   (LXXX)
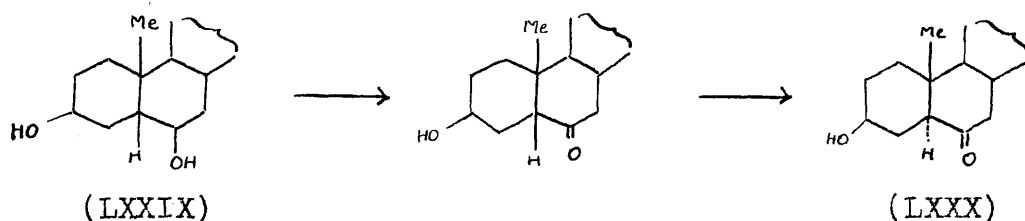
(4)   Hydroxylated C_{17}-C_8H_{17} steroids are degraded to the
corresponding C_{17}-C_4H_8·COOH structures on similar lines to
the partial syntheses of cholanic and allocholanic acids
already discussed (pp.4-6).   Thus chromic acid oxidation of
cholesteryl acetate dibromide (LXXXI) (under well-defined
conditions) followed by debromination and hydrolysis yields
3(β)-hydroxy- $\triangle^5$-cholenic acid (LXXXII) [62].
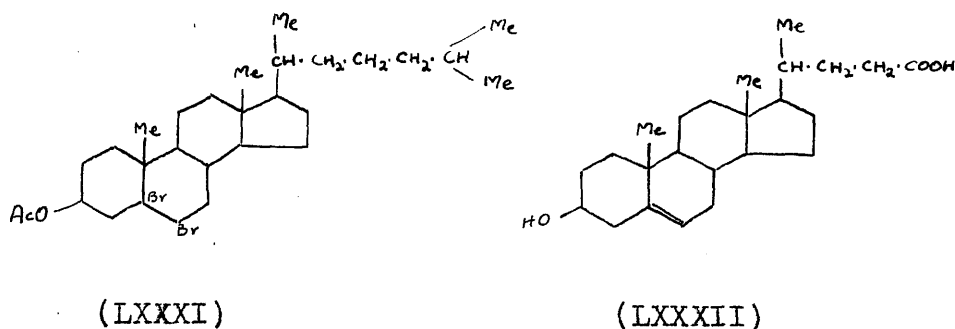
(LXXXI)   (LXXXII)

By an analogous procedure Marker[63][64] obtained "allohyodeoxycholic acid" (3(β),6(β)-dihydroxyallocholanic acid) (LXXXIII) from 3(β),6(β)-diacetoxy-cholestane (LXXXIV). This acid proved to be identical with that of Windaus[24], i.e., the acid obtained by catalytic hydrogenation of "β"-dehydrohyodeoxycholic acid.   Windaus had previously attempted to prepare the above allohyodeoxycholic acid from the stereo-isomeric compound (LXXXV), but oxidative opening of ring B also occurred[64][65].
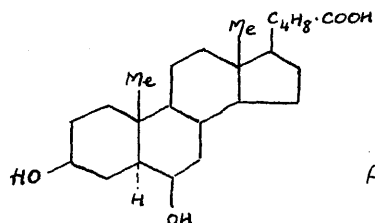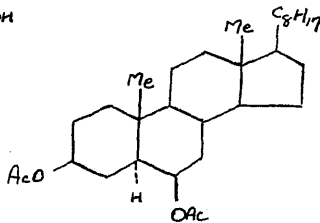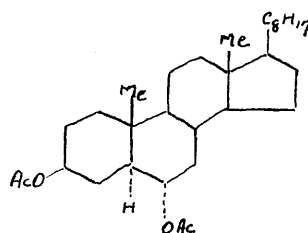
(LXXXIII)   (LXXXIV)   (LXXXV)

Accompanying the above acidic products are neutral ketonic compounds formed by complete removal of the C_8H_{17} side-chain (cf. p.5).   Dehydroisoandrosterone (LXXXVI) and 6(β)-hydroxy-isoandrosterone (LXXXVII) are isolated together with (LXXXII) and (LXXXIII) respectively.
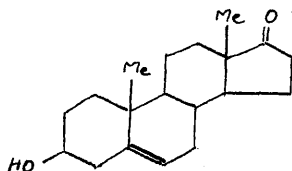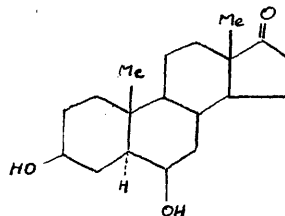
(LXXXVI)   (LXXXVII)

The formulae shown below indicate the proposed structures of various other neutral products which have been

obtained by this type of degradation (usually carried out
under modified conditions)[66].
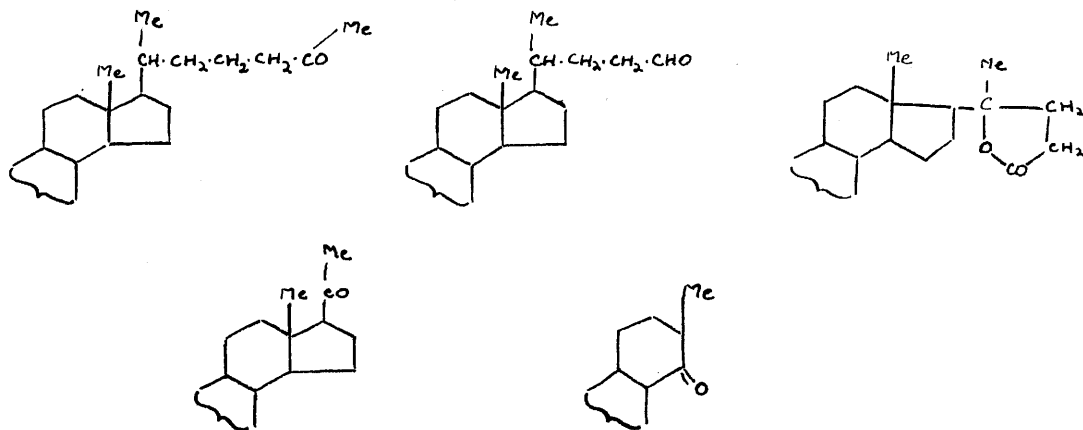
Marker's preparation of "allohyodeoxycholic acid"
provided the analogy for the final stage in the proposed
synthesis, a similar chromic acid oxidation of (XLVI, A)
and (XLVI, B) (after acetylation) being expected to yield
after hydrolysis the respective 3(α),6(β)- and 3(β),6(β)-
dihydroxycholanic acids (XLVII, A) and (XLVII, B).

The formation of the stereoisomeric aetiocholane-
17-one-3,6-diols (LXXXVIII) and (LXXXIX) (from (XLVI,A) and
(XLVI,B) respectively) as by-products in these oxidations
also appeared likely.
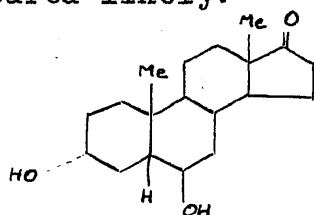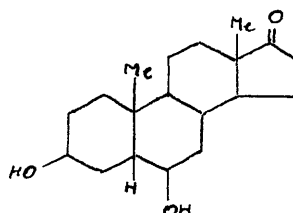
(LXXXVIII)   (LXXXIX)

It would be interesting to test the physiological
properties of these latter compounds.

In 1906 Diels and Abderhalden[67] prepared cholestane-3(β)-ol by reduction of coprostenone with sodium and amyl alcohol. Now, two stereoisomeric cholestane-3,6-diols have been described: one, the 3(β),6(β)- compound (XC), m.p. 194-195°, was obtained[68] by catalytic hydrogenation of $\triangle^{4}$-cholestene-3(β),6(β)-diol*; the other, cholestane-3(β),6(α)-diol (XCI), m.p. 216°, results from a sodium and alcohol reduction of 6-ketocholestanol[69]. The formation of the lower melting diol by sodium and amyl alcohol treatment of 6(β)-acetoxy-coprostenone (XCII) (6(β)-acetoxy- $\triangle^{4}$-cholestene-3-one) should therefore be expected, and the experimental testing of this point was undertaken as a preliminary step before attempting the catalytic hydrogenation of this compound.
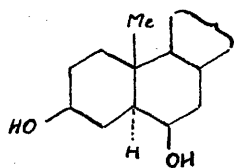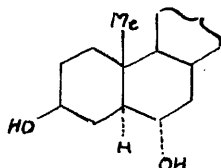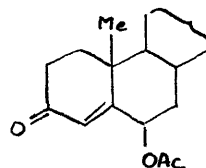
(XC)   (XCI)   (XCII)

A sodium and amyl alcohol reduction of the keto-acetate (XCII) yielded an uncrystallisable oil from which a solid material of m.p. 213-214.5° was isolated after

---

*   The identity of this compound with that of Marker and Krueger (J.A.C.S., 1940, 62, 79) appears to be highly probable, catalytic hydrogenation (PtO$_2$, alcohol) of 6-keto-cholestanol being their method of preparation (see Urushibara, Bull. Chem. Soc. Japan, 1941, 16, 182 and following page).

benzoylation.   Hydrolysis of the latter afforded a compound

of m.p. 217.5-219°.   Since the dibenzoate of cholestane-

3(β),6(α)-diol melts at 211-212°, the identity of the two

substances seemed likely.

In order to prepare an authentic sample of this diol,

and to obviate its laborious preparation by the method of

Windaus[69], a recent finding of Urushibara proved to be of

assistance.   In a paper[64] which admirably summarises the

configurations and inter-relationships of the α- and β-

oxides of cholesterol, the $\triangle^4$-cholestene-3,6-diols and the

cholestane-3,6-diols; he mentions the conversion of

cholesterol α-oxide (XCIII) into cholestane-3(β),6(α)-diol

(XCIV) by the action of sodium and amyl alcohol.   For this

he gave no experimental details, but the procedure seemed

quite straightforward.

(XCIII)   (XCIV)
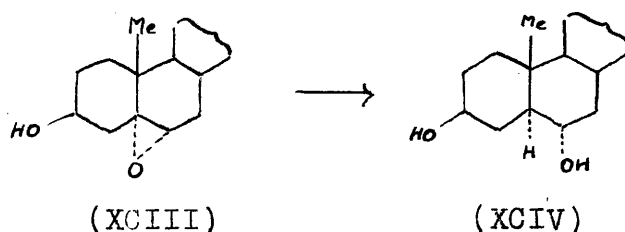
Cholesterol α-oxide was prepared from cholesterol

and monoperphthalic acid in ether solution.   The yield

obtained (87%) compares very favourably with that of Ruzicka

and Bosshard[70] (75%), who employed the less stable per-

benzoic acid.   Sodium and amyl alcohol reduction of the pure

α-oxide gave a small yield of the diol (XCIV), m.p. 218-

219.5^O, the dibenzoate of which melted at 213-214.5^O.

Mixed melting-point determinations of the free alcohol and its dibenzoate, derived from the reduction of 6(β)-acetoxy-coprostenone, with the above specimens of cholestane-3(β),6(α)-diol and its dibenzoate, respectively, proved their identity.   It would appear, therefore, that epimerisation of the C_6-OH in (XC) had occurred during its formation.

When 6(β)-acetoxy-coprostenone was reduced with a palladium catalyst the curious fact was noted that approximately four atoms of hydrogen per molecule of sterol were absorbed.   That the product was saturated and non-ketonic was proved by its failure to hydrogenate further with platinic oxide, and by the non-formation of a semicarbazone.   All attempts to crystallise the hydrogenation gum were unsuccessful, even after benzoylation.

Since the analogous Δ^4-cholestene-3(β),6(β)-diol yields mixtures of cholestane, coprostane, and cholestanol, and cholestane, cholestanol and cholestane-3(β),6(β)-diol, on hydrogenation with palladium and platinum catalysts respectively[68], this reduction probably followed a somewhat similar course.   This had been foreseen, but it had been hoped that the 3-keto group would help to stabilise the molecule.

In view of the reactivity of these structures, the action of sodium and amyl alcohol on Δ^4-cholestene-3,6-diol was investigated.   The product, which was obtained in good yield, melted at 79-80° and was thought to be either impure

$\triangle^5$-cholestene or coprostenone (the latter is very improbable since its reduction by this method has already been discussed on p.37). Mixed melting point determinations proved it to be neither of these. No acetyl derivative resulted from treatment with boiling acetic anhydride, and this fact, together with its failure to yield a 2,4-dinitrophenyl-hydrazone, and its rapid decoloration of bromine water, indicated that it was an unsaturated hydrocarbon. A catalytic hydrogenation of the substance yielded cholestane, and it was observed, moreover, that the amount of hydrogen taken up corresponded to the saturation of the double bond in pseudocholestene ($\triangle^4$-cholestene)[71], which the compound is in all probability.

Since the above catalytic hydrogenation of 6(β)-acetoxy-coprostenone failed to yield the sought coprostanone derivative, and on account of its reduction with platinic oxide as catalyst not being favoured[*], another route to the coprostane-3,6-diols was investigated.

The reduction, by means of sodium and amyl alcohol or catalytically, of the oxide ring in cholesterol β-oxide

---

[*] Ruzicka (Helv. Chim. Acta, 1934, 17, 1407) established that the hydrogenation of coprostenone to the epimeric coprostanols succeeds best when the process is split up into two stages, i.e., hydrogenation with a palladium catalyst to coprostanone, and the further reduction of the latter with a platinum catalyst. Although he indicates that the first step may also be accomplished by means of an old or weak platinum catalyst, this did not appear to offer a solution to the above difficulty. The hydrogenation of 6(β)-hydroxy-coprostenone with a Raney nickel catalyst, however, may be well worth investigating.

(XCV) offered a possible means of preparing coprostane-3(β), 6(β)-diol (XCVI). From the latter the 3(α)-epimer (XCIX) was expected to be obtainable by partial hydrolysis of the diacetate to the 6-monoacetate (XCVII) (see p.45 for analogies), followed by oxidation to the ketone (XCVIII) and catalytic reduction in neutral solution[30].

(XCV)   (XCVI)

(XCVII)   (XCVIII)   reduction and hydrolysis   (XCIX)
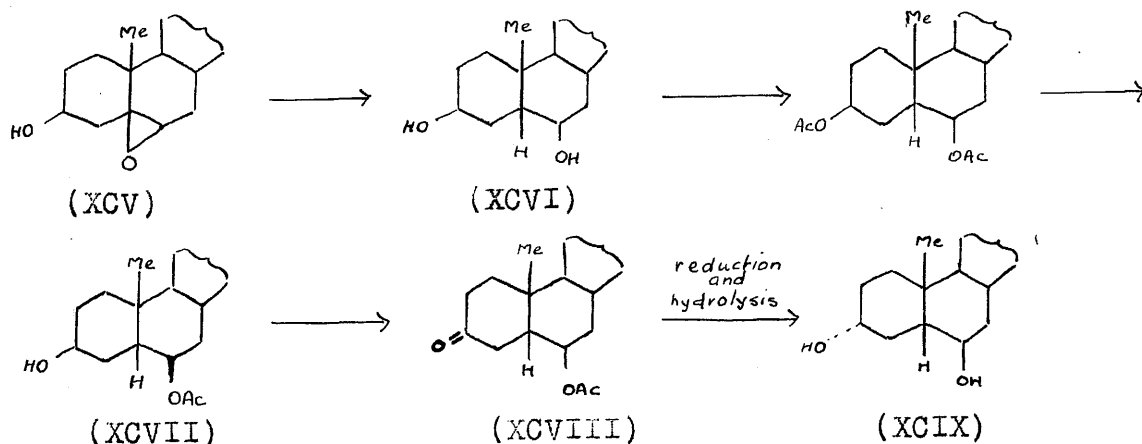
The proposal to attempt hydrogenolysis of cholesterol β-oxide was due to the following considerations:-

Hattori[72] had established the acetolysis of cholesteryl acetate α-oxide (C,R=Ac)* to the 3,6-diacetate (CIV) of "triol-I", also the formation from (C, R=H) of the 6-methyl derivative (CV) by the action of methyl alcohol and concentrated sulphuric acid. Cholesteryl acetate α-oxide and dry hydrogen chloride reacted to give the 6-chloro-compound (CI). Similar treatment of the corresponding β-oxide, however, gave rise to a series of isomeric compounds. Thus, (CIII, R=Ac) with acetic acid and hydrogen chloride

---

* The configurations of the oxide rings, here employed, are those deduced by Urushibara from apparently unambiguous evidence (see reference, p.38). The "solid" valency lines imply the customary cis relationship to the C_{10}-Me; the discontinuous lines, the trans.

yielded (CVII) and (CII) respectively; while the methyl deri-

vative (CVI) was obtained from the free β-oxide (CIII, R=H)[74]*

(C) (CI) (CII) (CIII)

R=Ac R=H R=H R=Ac

(CIV) (CV) (CVI) (CVII)
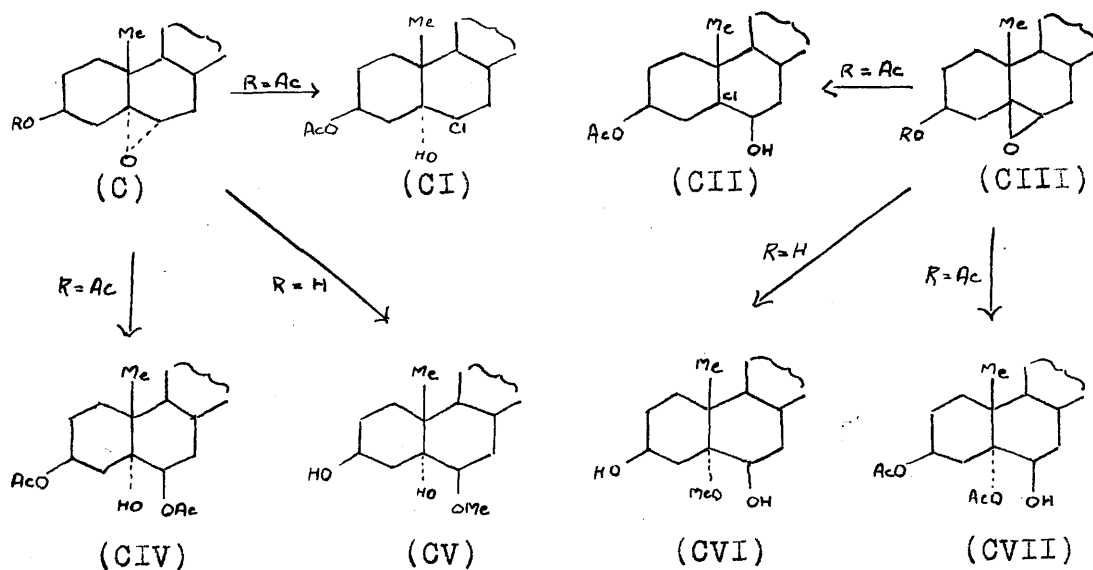
The fission of oxide rings by means of hydrogen

chloride had also been studied by Spring and Swain[73].
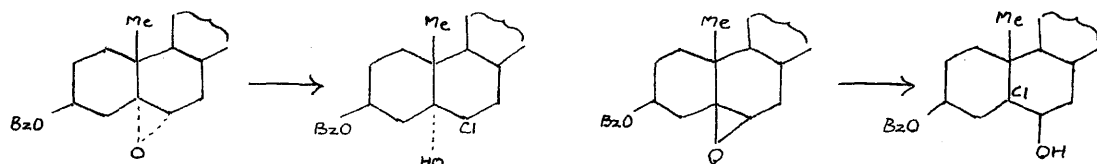
Employing cholesteryl benzoate α- and β-oxides an entirely

analogous series of changes resulted, see formulae above.

---

* Hattori's fission experiments with the β-oxide were carried out with a sample (m.p. 136°) prepared by the action of alcoholic potash on the 5-chloro-derivative of "triol-I". The lower melting point (112-113°) quoted elsewhere in the literature[71] and confirmed in the present investigation (see p. 85) appears to be a peculiar property of the β-oxide as prepared by the action of per-acids on cholesterol and its esters. Hattori advances convincing evidence to support his view that the higher melting specimen is the pure β-oxide, the other being due to association with the α-oxide as a molecular compound. It is curious, nevertheless, that both "forms" yield the same acetate of m.p. 112-113°[74].

This may explain the isolation of cholestane-3(β), 6(α)-diol from sodium and amyl alcohol reductions of both the α- and β-oxides (pp. 38 and 44).

It is evident from the above formulae that the oxide

ring in cholesterol α-oxide tends to split between C_6 and the

oxide oxygen atom; the β-oxide, on the other hand, at the

C_5-O bond.   A hydrogenolysis of the latter may therefore take

place analogously and give rise to a C_6-OH derivative (cf.

footnote, p.44).   (The behaviour of the α-oxide towards

reduction with sodium and amyl alcohol would appear anomalous,

see p.38).   Furthermore, the production of a coprostane

derivative is possible since the molecule already has this

configuration (cf. however, above formulae).

Treatment of cholesteryl benzoate in ether-chloroform

solution with perphthalic acid[*] afforded the α- and β-oxide

benzoates in slightly different yield from those reported by

Spring and Swain[73] in an analogous preparation employing

perbenzoic acid (see below).   This difference, however, is

to be expected.

	α-isomer.	β-isomer.	Total yield.
Perphthalic acid	53%	25%	78%
Perbenzoic acid	46%	38%	84%

A sodium and amyl alcohol reduction of cholesterol

β-oxide, obtained by saponification of the above benzoate,

yielded an oil from which a small amount of crystalline

[*]   After the completion of this work Chakravorty and
Levin (J.A.C.S., 1942, 64, 2317) reported yields of 50-70% of
the α-oxide benzoate by perphthalic acid treatment of chol-
esteryl benzoate, no β-isomer being detected.   The different
conditions employed by them (i.e., oxidation in boiling ether
solution) may account for this discrepancy (cf. also, their
preparation of the α-oxide from cholesterol and perphthalic
acid).

material was isolated.   This proved to be cholestane-3(β),
6(α)-diol (see footnote, p.42).   Benzoylation of the residual
oil afforded larger amounts of the benzoyl derivative of the
same compound, no other substance being isolated from the
mother liquors.   Inversion at C_5 and C_6 had obviously occurred
during this reduction, due probably to the action of sodium
amylate (cf. analogous reduction of 6(β)-acetoxy-coprostenone,
p.39).

An attempted catalytic hydrogenation of the β-oxide
benzoate in ether solution by means of platinic oxide failed.
Since no hexahydrobenzoate was formed the catalyst did not
appear to be highly active, nevertheless, it was effective in
other hydrogenations.   This oxide ring would therefore
appear to be somewhat resistant to catalytic hydrogenation[*].

Owing to an impending appointment by Imperial Chemical
Industries Ltd., Explosives Group, further work on this topic
had to be abandoned.   The above preliminary investigation
indicates however, that "partial synthesis" of the hyodeoxy-
cholic acids probably requires a different approach.

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[*]   Stavely (J.A.C.S., 1942, 64, 2723) has since effected
hydrogenation of cholesterol α-oxide in acetic acid solution
by means of a slow catalytic reduction with palladium black.
The product proved to be a mixture of a cholestane-3,5-diol
monoacetate, cholestane-3-ol acetate and the 3,6-diacetate of
"triol-I."   The principal component was the 3,5-diol, and the
cholestanol is considered to result from this by the elimina-
tion of the C_5-OH.   "Triol-I" diacetate is probably formed by
direct acetolysis of the α-oxide prior to hydrogenation.   The
use of acetic acid as solvent was intentionally avoided in the
present work on account of the possible acetolysis of the β-
oxide benzoate.   Acetic acid may, however, play a part in
Stavely's experiment, and thus account for the failure to
reduce the β-oxide in ether solution.
    Press and Reichstein (Helv.Chim.Acta, 1942, 25, 878)
have reduced the β-oxide of 3(α)-acetoxy-Δ''-cholenic acid

Concurrently with the above attempts to reduce 6-acetoxy- $\triangle^4$ -cholestene-3-one to the (C_3-) epimeric coprostane-3,6-diols the exploration of shorter or more convenient routes to this ketone was undertaken.

Partial hydrolyses of 3,6-diacetoxy-compounds to the corresponding 6-monoacetates are described in the literature. Thus, the 3,6-diacetates of cholestane-3(β), 6(β)-diol[75] (CVIII) and cholestane-3(β),5(α),6(β)-triol[45] (CX) (see p.29), on treatment in alcoholic solution with one equivalent of potassium hydroxide, afford excellent yields of the respective 6-monoacetyl compounds (CIX) and (CXI). While 3(β),6(α)-diacetoxy-pregnane-20-one-5(β)-ol (CXII) can be similarly converted into 6(α)-acetoxy-pregnane-20-one-3(β),-5(β)-diol (CXIII), the C_5- epimeric diacetate (CXIV) does not yield a monoacetate but merely a mixture of unchanged material and fully hydrolysed triol[52)55].

(CVIII)   (CIX)   (CX)   (CXI)

(CXII)   (CXIII)   (CXIV)
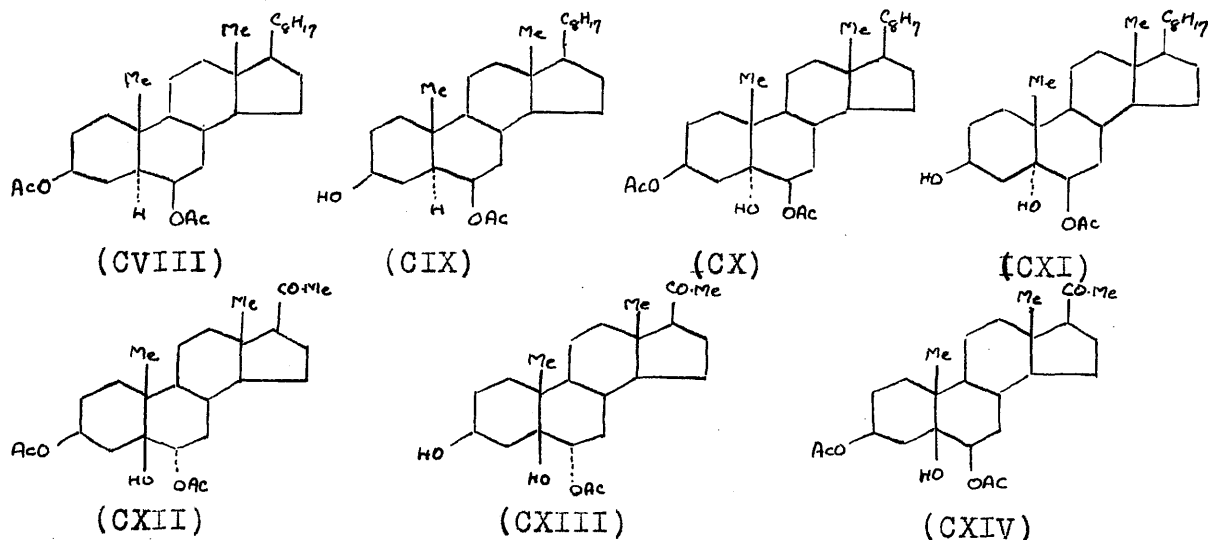
(methyl ester) with Raney nickel and hydrogen under pressure at 100° obtaining a mixture of 3(α),12(β)-dihydroxy-cholanic acid (deoxycholic acid) and 3(α)-hydroxy-cholanic acid (lithocholic acid). Cholesterol β-oxide may yield coprostane-3,6-diol under these conditions.

It appeared, therefore, that it might be possible to effect a partial deacylation of $3(\beta),6(\beta)$-diacetoxy- $\triangle^4$ - cholestene* (CXV) (readily obtainable in 25% yield by selenium dioxide oxidation of cholesteryl acetate, (see pp.47-49) to $6(\beta)$-acetoxy- $\triangle^4$ -cholestene-$3(\beta)$-ol (CXVI), chromic acid oxidation of the dibromide of which should yield the required keto-acetate (CXVII) on debromination with either zinc and acetic acid, or sodium iodide[58].

(CXV)   (CXVI)   (CXVII)
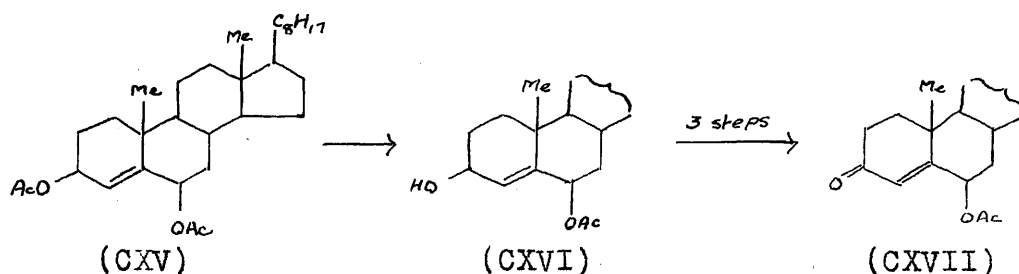
An attempted partial hydrolysis, at room temperature, of the diacetate (CXV), in ether-alcohol solution containing one equivalent of potassium hydroxide, yielded a mixture of unchanged material and the fully hydrolysed diol, no evidence being obtained of the presence of a mono-acylated product.   A second hydrolysis, carried out in more dilute solution and employing the procedure of Ellis and Petrow[45], likewise failed to give the required monoester.

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*   The configurations assigned to the acetoxyl groups in this compound follow from its formation by a Darzen dehydration of cholestane-$3(\beta),5(\alpha),6(\beta)$-triol 3,6-diacetate (Petrow, Rosenheim and Starling, J.C.S., 1938, 677; Urushibara, Bull. Chem. Soc. Japan, 1941, 16, 182), the stereochemical nature of which has already been discussed on page 27.

Thus, while the order of reactivity of the acyl groups in the 3,6-diacetates of the cholestanediol (CVIII), the cholestanetriol (CX) and the pregnanonetriol (CXII) is 3 > 6; that of the same radicals in the pregnanonetriol (CXIV) and in $\triangle^4$-cholestene-3,6-diol (CXV) must be represented as 3 = 6, no empirical rule being apparent.

In order to overcome, in this compound, the equality in the rates of hydrolysis of the 3- and 6-acetyl groups it was proposed to employ a mixed ester, the acyl group in the 3-position of which being so chosen as to exhibit the greater sensitivity towards alkali. For this purpose the 3-carbomethoxylate-6-acetate (CXVIII) of the diol was selected, its preferential hydrolysis to the 6-acetate (CXIX) appearing to be feasible. Some earlier work provided a possible means of preparing this diester:

(CXVIII)   (CXIX)
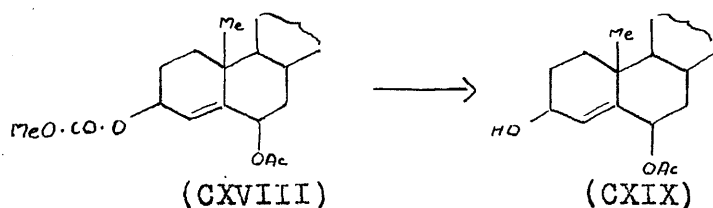
In 1937 Rosenheim and Starling[68], while engaged in a study of the action of selenium dioxide on steroids, submitted cholesterol (CXX) in benzene-acetic acid solution to the action of this oxidant. The sole product of the reaction, an unsaturated diol of melting point 176-177°, proved to be $\triangle^5$-cholestene-3,4-diol (CXXI), mainly on the basis of

its oxidation with lead tetra-acetate to an unsaturated
dialdehyde (CXXII) which on treatment with hydrogen peroxide
yielded Diels' acid (CXXIII).   When cholesteryl esters were
employed (in boiling acetic acid solution), however, the re-
action was found to take a different course.   The principal
crystalline product from a selenium dioxide oxidation of

(CXX)   (CXXI)   (CXXII)   (CXXIII)
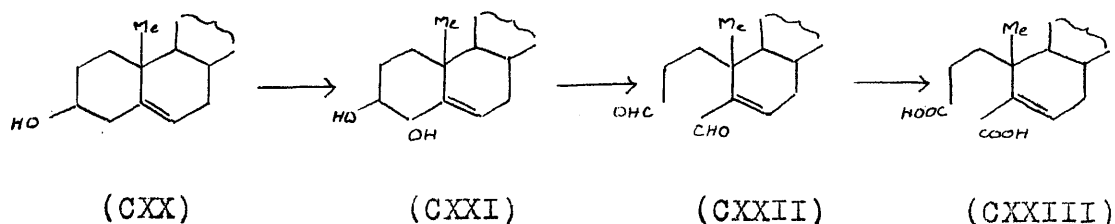
cholesteryl acetate was the diacetate of an isomeric compound,
together with a small amount of a monoacetate of the pre-
viously obtained diol (CXXI).   The new diol (m.p. 257-258°),
like its isomer, readily lost water on treatment with dilute
mineral acid giving coprostenone (CXXV), and in view of this
and other facts Rosenheim and Starling proposed that the two
substances were stereoisomers.   Furthermore, since the
higher melting diol reacted much more slowly with lead
tetra-acetate than the lower melting one, the two compounds
were assigned the respective trans- and cis-α-glycol con-
figurations (CXXIV) and (CXXVI).

(CXXIV)   (CXXV)   (CXXVI)
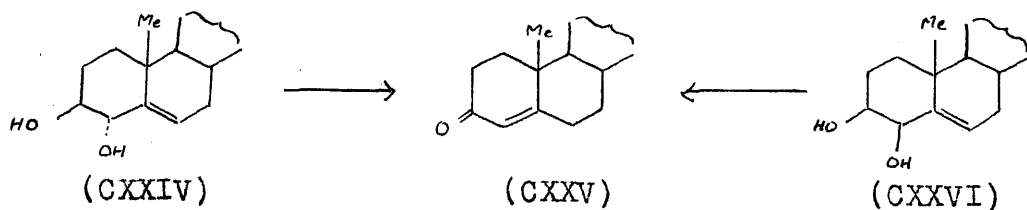
Diol, m.p.257-258°   Diol, m.p.176-177°.

Butenandt and Hausmann[76] had been independently studying the same oxidation, and a few months later published a paper in which they described the action of selenium dioxide on cholesteryl acetate in acetic anhydride solution. Employing this medium, equal amounts (25% yields of each) of the diacetates of the above diols were obtained. They pointed out that while the constitution of the lower melting diol had been firmly established by Rosenheim and Starling, that of the alleged stereoisomer rested on a less secure experimental basis. Additional work carried out by them (illustrated in the scheme below) proved conclusively that this latter substance was not a stereoisomer of the 176-177° melting compound, but that it was the isomeric $\triangle^4$-cholestene-3,6-diol, and they suggested its formation from the 3,4-diol by allylic rearrangement. Finally, since the diols were

(Reference compound)

(Reference compound)
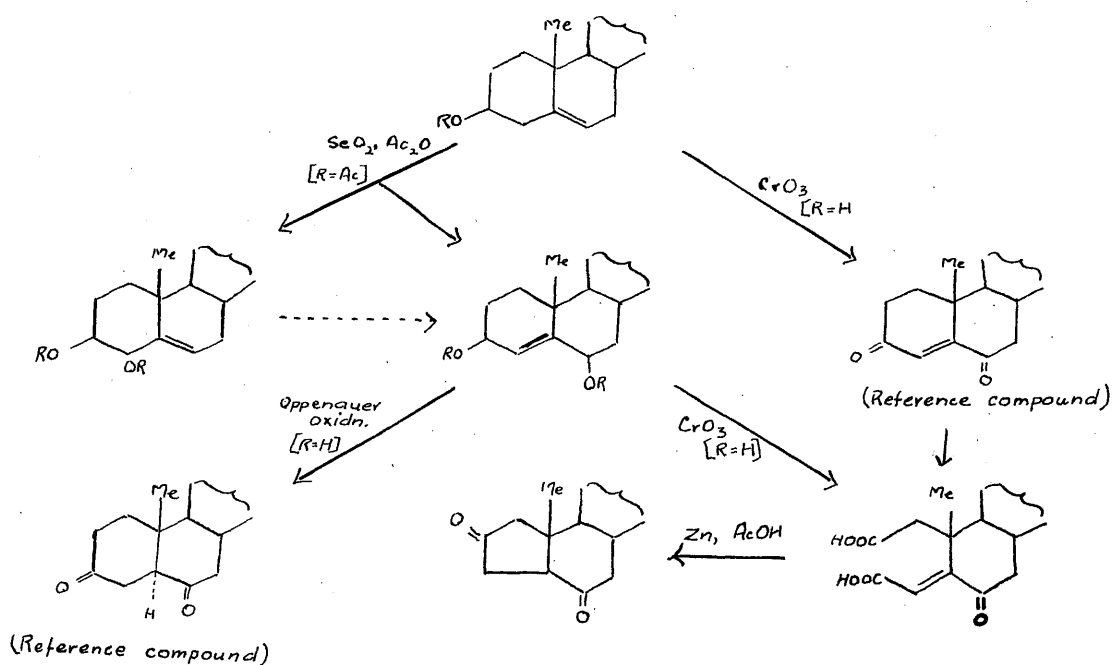
no longer to be regarded as stereoisomers the evidence for
the first obtained compound being a cis-glycol had now to
a large extent broken down[*].

The oxidation of cholesteryl benzoate (in acetic
acid solution) with selenium dioxide was also studied by
Rosenheim and Starling[68], and in this case approximately
equal amounts of the 3-benzoate (CXXVII) of the cis-3,4-diol
and the 3-benzoate-6-acetate (CXXVIII) of the 3,6-diol were
formed.
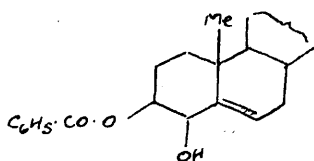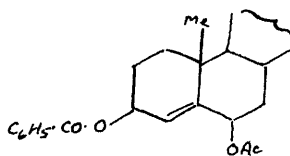
(CXXVII)   (CXXVIII)

Consideration of the above facts indicated that a
selenium dioxide oxidation of carbomethoxy-cholesterol in
either acetic anhydride or acetic acid solution ought to
yield (in addition to the 3-carbomethoxylate-4-acetate
(CXXIX, R = Ac) or a mixture of this with the 3-carbo-
methoxylate (CXXIX, R = H), respectively, of the 3,4-diol)
the required 3-carbomethoxylate-6-acetate (CXXX), and experi-
ments were undertaken with a view to testing this analogy.

The carbomethoxy- and carbethoxy- (see p.61) deri-
vatives of cholesterol used in this investigation were
prepared according to the method of Robberecht[77], i.e., by

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* In more recent papers, however, this compound is
still referred to as a cis-diol and in view of the evidence
obtained in the present research (p.70), the name cis- $\triangle^5$ -
cholestene-3,4-diol is retained, see also footnote on p.52

pyridine treatment of an ice-cold solution of cholesterol
in a mixture of anhydrous benzene and the appropriate
chloroformic ester.

(CXXIX)   (CXXX)
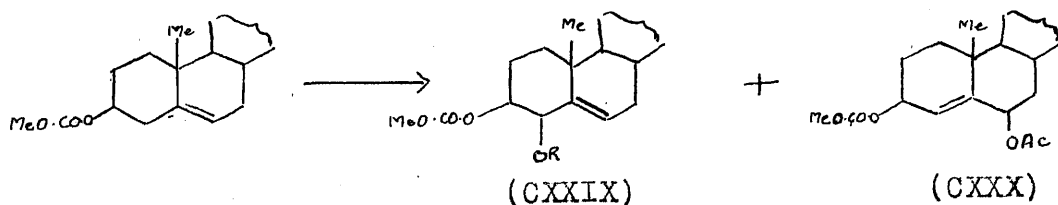
(R = Ac and/or H)

An acetic anhydride solution of carbomethoxy-
cholesterol was oxidised with selenium dioxide and the pro-
duct was worked up exactly as described by Butenandt and
Hausmann[76] in the case of cholesteryl acetate.   From the
resulting dark-brown gum only one crystalline substance
(provisionally named compound A) was isolated by solvent
treatment.   Hydrolysis of this compound, m.p. 160.5-161°,
yielded cis-$\triangle^{5}$-cholestene-3,4-diol, identified by mixed
melting point with a specimen prepared from cholesterol
according to the directions of Rosenheim and Starling[68].
Further attempts to isolate from the gum the expected mixed
ester of the unsaturated 3,6-diol failed, but its presence
was assumed since saponification of the non-crystallisable
residues yielded a small amount of the sparingly soluble
3,6-diol.

Since this method failed to yield the required com-
pound the oxidative procedure of Rosenheim and Starling
(i.e. the employment of acetic acid instead of acetic
anhydride as solvent) was applied to the ester, resulting

in the isolation from the reaction mixture of two crystalline
products.   One, m.p. 160.5-161°, was identical with that
obtained from the previous oxidation; whilst the other,
m.p. 173-173.5° (compound B), was thought to be the required
material, but the curious fact was established that it also
yielded the cis-3,4-diol on hydrolysis.   Again, it was
possible to isolate the expected mixed ester only in the
form of the free diol.   Unlike the similar oxidations of
the acetate and benzoate of cholesterol, the carbomethoxy-
late yielded two derivatives of cis- $\triangle^{5}$ -cholestene-3,4-
diol, and the further examination of this anomaly was
obviously of interest.

The failure of compound A (i.e., the common product
from both oxidations) to acetylate indicated that both
hydroxyl-groups were acylated, and it was considered probable
that the substance was 3-carbomethoxy-4-acetoxy-cholesterol[*].
Its analysis provided support for this view and it was de-
cided to settle the matter by synthetic means.

A cooled solution of the cis-α-glycol in a mixture
of anhydrous benzene, ether and excess methyl chloroformate
was treated with pyridine and the product isolated as
described in the "Experimental", p.91.   This substance,

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[*]   The mixed esters of the cis-3,4-diol are more con-
veniently described by this nomenclature.   Thus, the "tri-
vial" and systematic names, 4-hydroxy-cholesterol and cis-$\triangle^{5}$-
cholestene-3,4-diol, respectively, are to be regarded as
synonymous.

m.p. 157.5-159.5°, gave analytical figures consistent with those of a monocarbomethoxy-derivative and on acetylation it was converted into a material of melting point 160.5-161°, identical with compound A. The monocarbomethoxy-compound was further characterised by benzoylation and analysis of the resulting carbomethoxylate-benzoate.

Compound A is, therefore, a carbomethoxy-acetyl-derivative of 4-hydroxy-cholesterol, probably the 3-carbomethoxylate-4-acetate. This latter follows from its formation from carbomethoxy-cholesterol and from the observation that previous mono-acylations of the diol have been proved to occur at the 3-position[68][78] (Note 1, p.74).

In a recent Note in Nature[79], Rosenheim stated that "the remarkable fact has --- been established that an acyl migration from C_3 to C_4 occurs when the aliphatic-3-monoesters" of the cis-3,4-diol "are warmed to 90° in acetic acid solution." Now, Petrow and Starling[78] had prepared 3-acetyl-4-hydroxy-cholesterol by treatment (at room temperature) of a pyridine solution of the diol with one equivalent of acetic anhydride, and since compound B yielded this diol on hydrolysis and analysed as its mono-acetyl-derivative, it was thought to be the hitherto un-described 4-monoacetate. This opinion was invalidated, however, when the substance was found to be incapable of further acylation (with either acetic anhydride or benzoyl chloride) and another explanation had to be sought.

For this rearrangement and other allied topics
Rosenheim[79] proposed two alternative reaction mechanisms.
Quoting, "this novel reaction, which has no analogy in the
steroid series, becomes intelligible on the assumption of
an intramolecular rearrangement and the intermediate forma-
tion of the labile pentacyclic compound" (CXXI).   "Rupture
of the bridge-linkage followed by the allylic change usual
in these compounds then leads to" (CXXXII).   He states that
"an alternative mechanism of acyl migration, involving the
formation of ortho-carbonic esters as intermediates, will
be discussed in the detailed account of this work".   (This
paper has not yet been published).
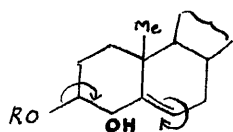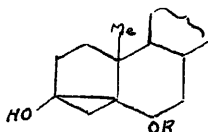
(CXXXI)   (CXXXII)

The latter mechanism is presumably to be interpreted
as shown in the formulae below:

(CXXXIII)   (CXXXIV)
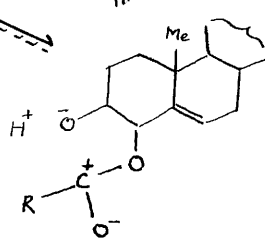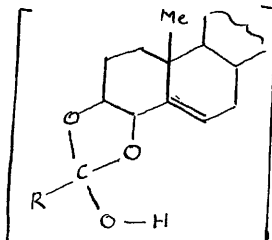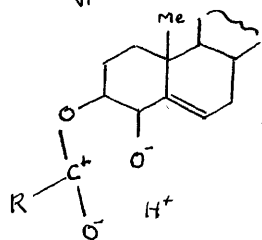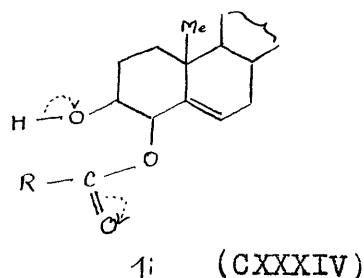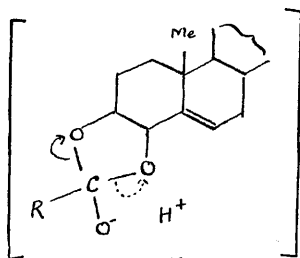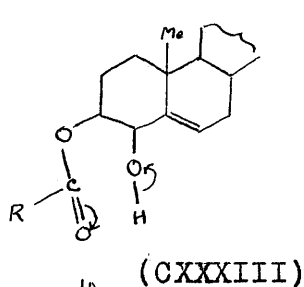
Whether or not the above changes are to be represented as a series of equilibria remains to be seen (see, however, p.65), but in any event, it is necessary to postulate an incipient ionisation (influenced by the acetic acid medium) of the hydrogen atom attached to the C_4-oxygen atom in (CXXXIII) --- for the reverse changes (i.e. equilibrium) to occur the C_3-hydroxyl in (CXXXIV) must likewise be assumed to ionise.

The first stage in the selenium dioxide oxidation of an ester of cholesterol is undoubtedly the formation of the 3-monoester (CXXXVII) of the 3,4-diol.   While the latter is in the "nascent" or "activated" state (CXXXV) a portion of it may be assumed to suffer allylic rearrangement, and since this change involves an anionotropic shift, the presence of the large excess of acetate ions (i.e., the acetic acid medium) over the hydroxyl ions thus formed must inevitably result.in the formation of a di-ester (CXXXVI) of the 3,6-diol.   This explains why the selenium dioxide acetic acid oxidation of cholesteryl benzoate yields the 3-benzoate-

(CXXXV)

(CXXXVI)

(CXXXVII)
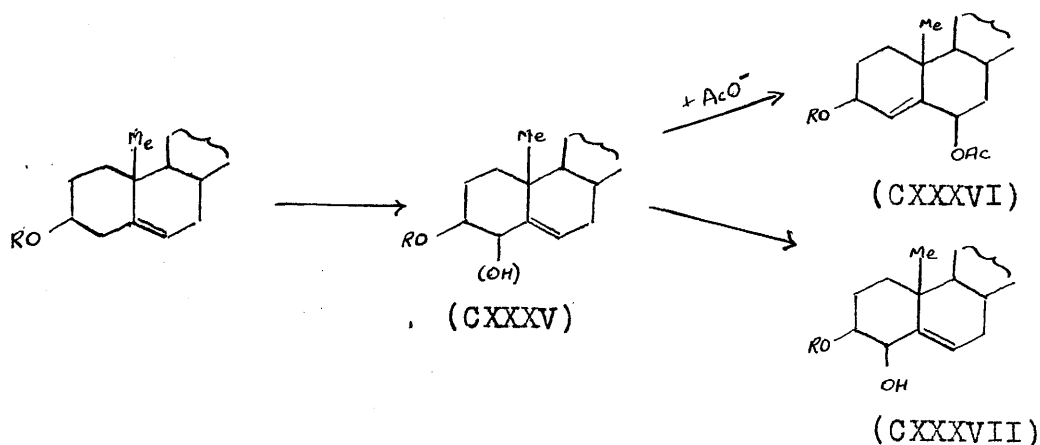
6-acetate of the 3,6-diol and the unacetylated 3-monobenzoate
of the 3,4-isomer.   The medium employed now appears to in-
fluence the ultimate course of the reaction.   When hot (105-
110°) acetic anhydride is used as the solvent the complete
acetylation of the remaining 3-monoester (CXXXVII) is to be
expected.   With acetic acid as solvent, however, this ester
may react simultaneously in two manners:- a) part becoming
acetylated as before (this is highly probable with boiling
acetic acid as solvent, but unlikely when a mixture of this
and benzene is employed), and b) the remainder, provided it
is an aliphatic-3-monoester (Note 2, p.74), tending to re-
arrange according to either the "intra-annular" or the
"ortho-ester" mechanism.

The above formulations provide an explanation for
the facts so far known (with the exception of the non-forma-
tion of the 3,6-diol, or a derivative thereof, from chol-
esterol itself (see p.67), i.e.,

1) the formation of the non-acylated 3,4-diol in the oxida-
tion of cholesterol in acetic acid-benzene solution;

2) the formation of the diacetates of the isomeric diols in
the selenium dioxide-acetic anhydride oxidation of chol-
esteryl acetate;

3) the formation of the 3- and (very probably) the 4-mono-
acetates (the latter by rearrangement) of the cis-diol,
together with the diacetates of the 3,4- and 3,6-diols, in
the selenium dioxide-acetic acid oxidation of the same acetate;

4) the formation of the 3-benzoate of 4-hydroxy-cholesterol*
and the 3-benzoate-6-acetate of the isomeric γ-diol in the
selenium dioxide-acetic acid oxidation of cholesteryl
benzoate, and, finally

5) the formation of the 3-carbomethoxylate-4-acetate of the
α-glycol and the 3-carbomethoxylate-6-acetate of the iso-
meric compound (isolated only as the free diol) in the
selenium dioxide-acetic anhydride oxidation of carbomethoxy-
cholesterol.

In view of this a theoretical study was made of the
selenium dioxide-acetic acid oxidation of carbomethoxy-
cholesterol in the hope of finding a clue to the nature of
the anomalous compound B.

As stated previously, the first step leads presumably
to the formation of 3-carbomethoxy-4-hydroxy-cholesterol
(CXXXIX), which in its "nascent" condition (CXXXVIII) is
assumed to be partially converted, by allylic rearrangement,
to the 3-carbomethoxylate-6-acetate of the 3,6-diol (this was
only isolated as the free diol). The remaining 3-monoester
(CXXXIX) may now suffer two simultaneous changes, a) a portion
becoming acetylated by the boiling acetic acid, and giving
rise to compound A (CXL) (which was isolated); and b) the
remainder tending to undergo rearrangement.

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*   The fact that this compound is not partially
acetylated may be explained on the grounds of the steric
effect produced by the greater bulk of the benzoyl radical.

Up to this point it is not possible to formulate any likely side-reaction leading to the formation of a second derivative (compound B) of the 3,4-diol, and its production must, therefore, occur subsequently.

(CXXXVIII)

(CXXXIX)

(CXL)

(CXLI)

(CXLII)

(CXLIII)
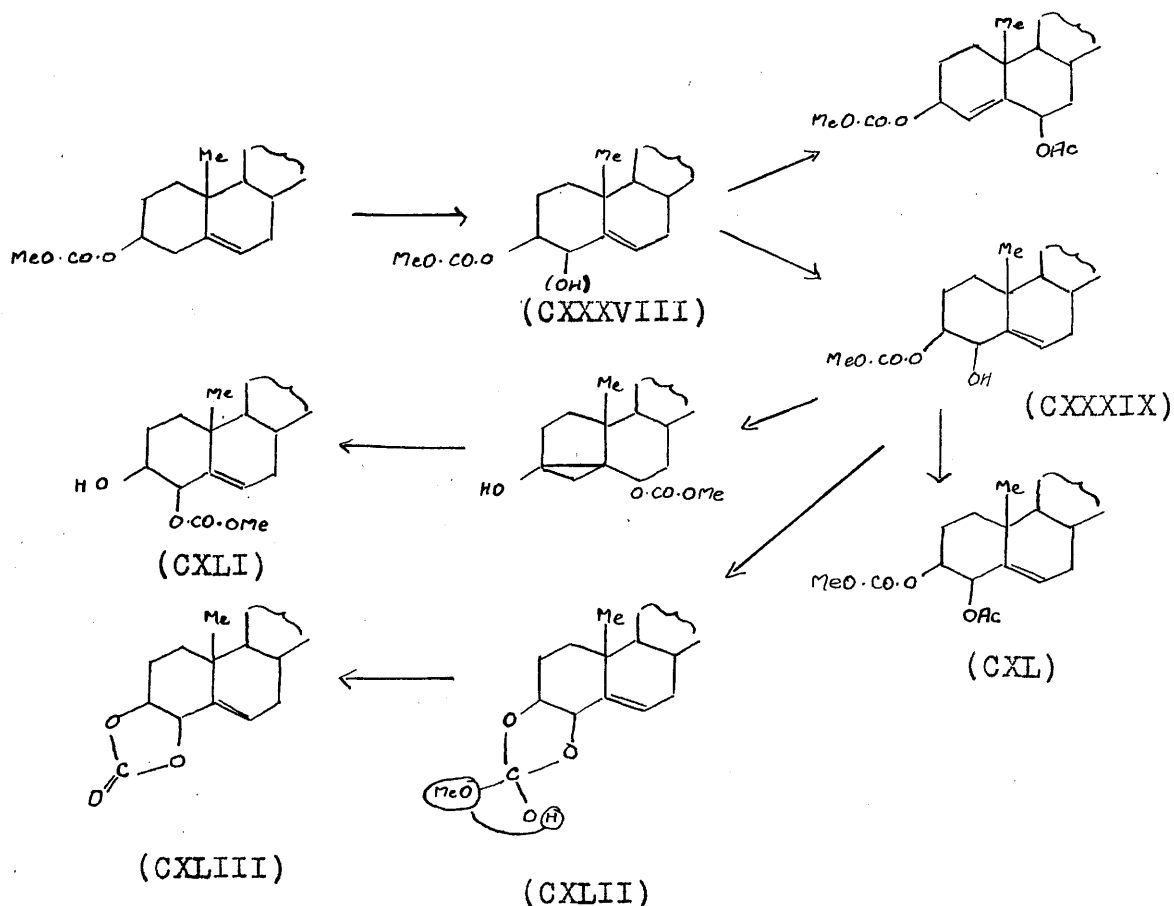
The "intra-annular" mechanism for the rearrangement of 3-carbomethoxy-4-hydroxy-cholesterol, formulae (CXXXIX) to (CXLI), similarly failed to yield a substance other than the 4-carbomethoxylate (CXLI), and since compound B cannot be further acylated this possibility must be ruled out. When the "ortho-ester" mechanism was applied, however, it was observed that a tri-ester of orthocarbonic acid (CXLII) resulted as an intermediate; a structure which might

conceivably lose methyl alcohol under the experimental con-
ditions of the oxidation (i.e., boiling acetic acid).   The
product from the fission of methyl alcohol is the intra-
molecular $\propto$-glycol carbonate (CXLIII), and this compound
was immediately recognised as having the observed properties
of compound B, i.e., both hydroxyl groups are esterified and
its percentage carbon and hydrogen content agrees with the
figures obtained experimentally.

The "synthetic" preparation of the glycol carbonate
(CXLIII) by the action of phosgene on the free diol offered
a means of testing the above hypothesis.

Some experiments carried out in the sex hormone
group by Dirscherl[80] provided a working basis.   In order
to obtain the chloroformic ester (CXLV) he found that it
was necessary to heat, in a sealed tube for one hour at 70°,
a benzene solution of oestrone (CXLIV) and ten times the
theoretical amount of phosgene in pyridine.   When phosgene
was passed into an alkaline solution of the follicular

(CXLIV)   (CXLV)
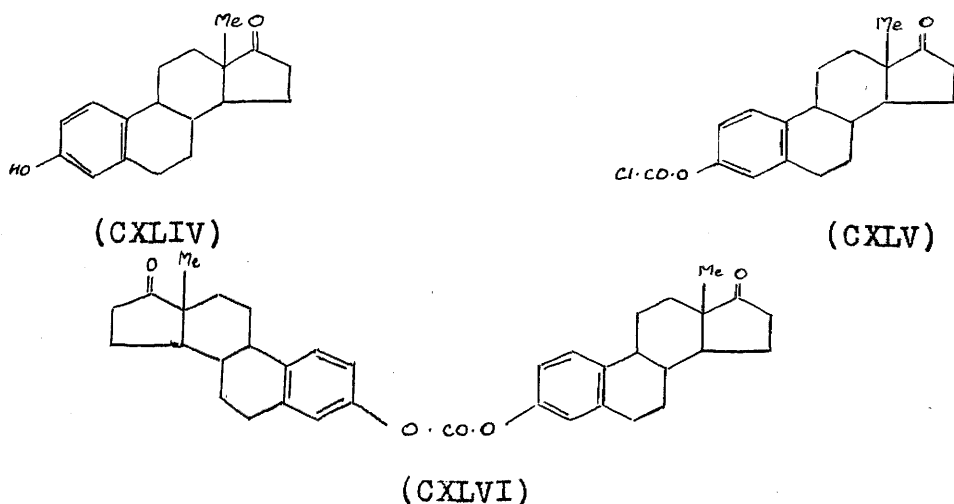
(CXLVI)

hormone, however, the bimolecular carbonate (CXLVI) was produced.

Since the latter procedure did not appear to be applicable to the non-phenolic 3,4-diol the method of esterification in non-aqueous solvent was employed. An attempted acylation of 4-hydroxy-cholesterol with one equivalent of phosgene failed, resulting in the starting-material being recovered unchanged. When eight equivalents of the acid chloride were used, however, a homogeneous material was isolated and a mixed melting point determination proved its identity with compound B. No evidence of the formation of a bimolecular product corresponding to (CXLVI) was observed.

The intramolecular carbonate (CXLIII) represents a new structure in the field of sterols, an analogy to this being the recent preparation of the acetone compound (CXLVII) of androstene-3,16,17-triol[81]. An attempt to prepare a similar derivative of the cis-diol (CXLVIII) yielded an uncrystallisable gum which was not further investigated.
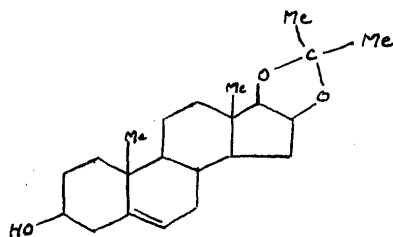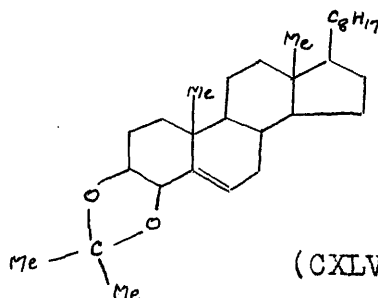
(CXLVII)   (CXLVIII)

The hydrogen chloride employed as condensing agent probably caused partial dehydration to coprostenone (cf. p.48).

The establishment of the proposed structure of compound B rendered highly probable the orthocarbonate mechanism of acyl migration and it was decided to divert attention to this topic.

Assuming this mechanism to be correct it ought to be possible to prepare the glycol carbonate, a) by an analogous selenium dioxide-acetic acid oxidation of carbethoxy-cholesterol --- the intermediate (CL) losing ethyl alcohol, and b) by hot acetic acid treatment of 3-carbomethoxy- (CLI) and 3-carbethoxy-4-hydroxy-cholesterol (CXLIX) (the first hypothetical intermediates in the oxidation of carbomethoxy- and carbethoxy-cholesterol, respectively).

(CXLIX)

(CL)

(CLI)
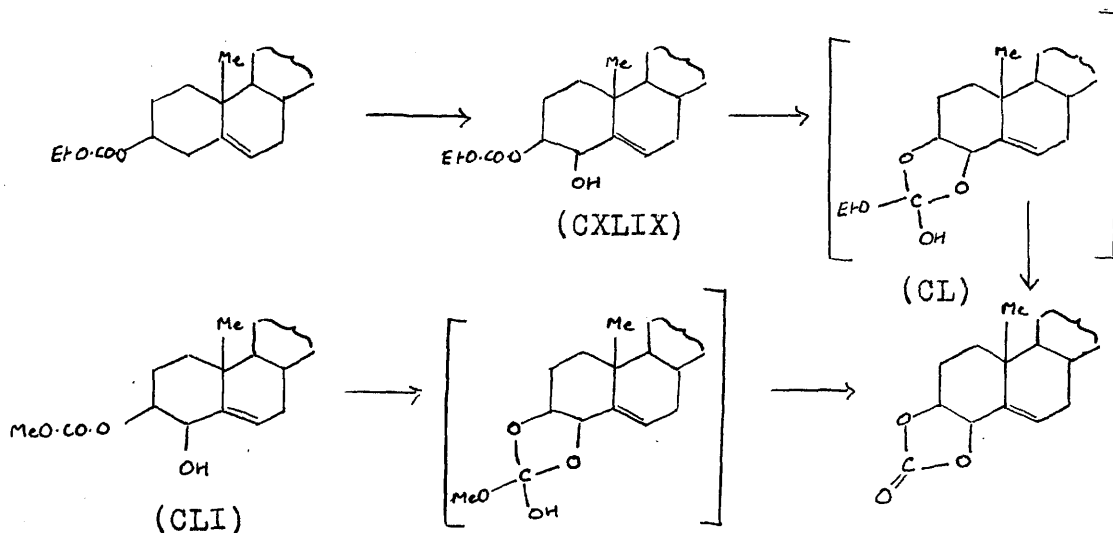
Selenium dioxide oxidations of carbethoxy-cholesterol were also of interest in connection with the original purpose of this investigation, and it was hoped that in this case the desired mixed ester (here, the 3-carbethoxylate-6-acetate of the 3,6-diol) might be capable of isolation.

Carbethoxy-cholesterol in acetic anhydride solution was oxidised under the same conditions as for the carbo-

methoxy- derivative and the product was isolated in the usual manner.   Treatment of this material, the customary gum, with selective solvents yielded two pure compounds: one, compound C, melted at 163-163.5°;   the other, compound D, melted at 121-122.5°.

The higher melting substance yielded 4-hydroxy-cholesterol on hydrolysis and proved to be fully acylated, i.e., incapable of acetylation with <u>boiling</u> acetic anhydride. An analysis of the material supported the contention that it was the carbethoxy analogue of compound A (i.e., 3-carbeth-oxy-4-acetoxy-cholesterol) and its synthetic preparation was undertaken on exactly similar lines.   4-Hydroxy-cholesterol yielded a monocarbethoxy-derivative which was characterised by the preparation of its acetate and benzoate.   The identity of the synthetic 3-carbethoxylate-4-acetate and compound C was established by mixed melting point.

Hydrolysis of compound D, on the other hand, yielded $\triangle^4$-cholestene-3,6-diol and it analysed as a carbethoxylate-acetate of the latter.   This substance is undoubtedly the sought 3-carbethoxylate-6-acetate (CLII)

(CLII)
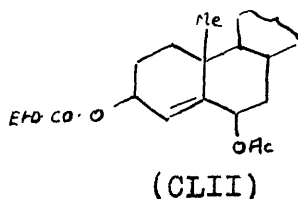
A selenium dioxide-acetic acid oxidation of

carbethoxy-cholesterol was therefore to be expected to yield the following three substances:- 3-carbethoxy-6-acetoxy-$\triangle^4$-cholestene-3-ol (compound D), and the 3-carbethoxylate-4-acetate (compound C) and carbonate (compound B) of cis- $\triangle^5$-cholestene-3,4-diol. Trial experiments with samples of the three compounds showed that compounds B and D were soluble in relatively small amounts of cold acetone, while it had already been established that C was crystallisable from this medium. Finally, that compound D was soluble in cold ligroin, compound B being sparingly soluble under these conditions.

The **solid** material obtained from such an oxidation was crystallised successively from acetone, ligroin and absolute alcohol, small amounts of the three compounds being isolated and identified by mixed melting point with the previously prepared materials.

Before attempting to prepare the glycol carbonate from the 3-carbomethoxy- and 3-carbethoxy derivatives of 4-hydroxy-cholesterol and in order to acquire more information on the conditions required (see p.53), the rearrangement of the 3-monoacetate to 4-acetoxy-cholesterol was studied.

The 3-acetate (m.p. 189.5-190.5°) was prepared by treatment at room temperature of a pyridine solution of 4-hydroxy-cholesterol with one equivalent of acetic anhydride[78]. A saturated solution of the monoacetate in acetic acid at 90°

was maintained at this temperature for one hour and allowed
to crystallise.   Purification of the crude material thus
deposited yielded the 4-monoacetate (m.p. 161-163°) which
was identified by benzoylation to the 3-benzoate-4-acetate
(m.p. 165.5-166.5°) of Rosenheim and Starling[68] (which
they obtained by acetylation of the 3-benzoate from, a) a
selenium dioxide oxidation of cholesteryl benzoate, and
b) the diol in pyridine with one molecular proportion of
benzoyl chloride).   The isomeric 3-acetate-4-benzoate
(m.p. 128-129°) was obtained by similar treatment of the
higher melting monoacetate[82].

Returning to the two mechanisms of acyl migration
proposed by Rosenheim (p.54) it is evident that the "intra-
annular" mechanism involves anionotropic changes; the
"orthocarbonate" mechanism, on the other hand, prototropic
changes.   In other words, the former is intermolecular and
the latter intramolecular with respect to the acetyl group.
Now, the rearrangement of the 3-monoacetate in acetic acid
does not allow of any differentiation between these mechan-
isms; but, if it be carried out, for example, in propionic
acid, then the 4-propionate would result (intermolecularly)
from the former and the 4-acetate (intramolecularly) from
the latter.   The following statement of Rosenheim (see
p.53) "The remarkable fact has --- been established that an
acyl migration from C_3 to C_4 occurs when the aliphatic
3-monoesters are warmed to 90° in acetic acid solution"

would, therefore, appear to support the "orthocarbonate" mechanism.   It was decided, however, to confirm this on the above lines.

A hot propionic acid solution of the 3-acetate was heated on the water-bath for one hour, a higher temperature being employed to compensate for the weaker acidic strength of this acid.   In this case, owing to the relatively large amount of unchanged 3-acetate present, it was not possible to isolate the rearrangement product as such, but a facile separation was effected after benzoylation;   trial experiments with the isomeric acetate-benzoates having shown the 3-acetate-4-benzoate to possess the greater solubility in methyl alcohol.   The more insoluble component was isolated and proved to be identical with the 3-benzoate-4-acetate, showing that the rearrangement product was the 4-acetate and not 4-propionyloxy-cholesterol, i.e., that the intramolecular mechanism was responsible for this change.

In both of the above rearrangements the reaction did not proceed to completion, a mixture of the 3- and 4-acetates being formed.   Employing acetic acid as the medium, and prolonging the heating to six hours a mixture was again produced.   These facts would seem to indicate the establishment of an equilibrium between the two isomers under the above conditions.

In 1939 Marker and Rohrmann[83] submitted cholesteryl acetate, in benzene-acetic acid solution, to the action of selenium dioxide, isolating from the resulting gum two

crystalline products. Crystallisation of one from methyl alcohol gave a pure substance melting at 163-165°, and of the other from ether-methyl alcohol, a compound of melting point 189-191°. Both yielded 4-hydroxy-cholesterol and its diacetate on hydrolysis and acetylation respectively, and both analysed as monoacetates of the diol. They stated that a mixture of the two compounds melted over an intermediate range of 163-184°, and in view of this they proposed that the two substances were polymorphic forms of 3-acetyl-4-hydroxy-cholesterol.

The close agreement in melting point between these substances, m.p's. 189-191° and 163-165°, and the 3- and 4-monoacetates of the 3,4-diol (m.p's. 189.5-190.5° and 161-163°), respectively, indicated that they might be isomers and not polymorphs, thus necessitating a reinvestigation of the above oxidation. Unfortunately, when this was repeated only the lower melting substance was isolated. A direct comparison between this (and its benzoate) and the 4-acetate (and the 3-benzoate-4-acetate) showed them to be identical, hence disproving Marker and Rohrmann's contention that the lower melting compound was the 3-acetate. The other product not being available, attempts were made to prepare a higher melting polymorph of the 4-acetate by crystallisation from ether-methyl alcohol, i.e., the solvent employed by the above workers for the purification of the 189-191° melting substance; and to prepare similarly a lower melting

modification of the 3-acetate by recrystallisation from methyl alcohol; but in both cases the compounds were recovered unchanged. It would appear, therefore, that the products obtained by Marker and Rohrmann were the 3- and 4-acetates, an error having been made in the mixed melting point determination. A re-examination of the latter revealed that while a mixture of the 3- and 4-acetates in the ratio 1:1 gave a melting point of approximately 140-147°, one in the ratio 3:1 melted over an intermediate range (165-179°), thus offering a rational explanation for the above observation.

A further point emerges from Marker and Rohrmann's oxidation of cholesteryl acetate, namely, the fact that no derivative of $\triangle^4$-cholestene-3,6-diol was isolated. The same negative result was obtained on repeating this reaction but unfortunately no specific search was made for this substance. The previously observed absence of a similar compound in the analogous oxidation of cholesterol itself[68] (see p.47), however, supports this view, and it would seem that the allylic change does not occur at temperatures below that of boiling acetic acid. Hence, for this isomerisation to occur, the 3,4-diol molecule would appear to become sufficiently activated only at temperatures in the region of 118°.

The proposed transformation of 3-carbomethoxy-4-hydroxy-cholesterol into the carbonate was studied under somewhat similar conditions as existed during the oxidation

of carbomethoxy-cholesterol, i.e., an approximately 95%
acetic acid solution of the compound was boiled for two
hours.   A longer reaction period was employed to facilitate
a more complete conversion.   As expected, a high yield of
the carbonate was obtained.   Similar treatment of the
carbethoxy analogue likewise yielded the same result, and
it was further established that boiling propionic acid could
be substituted for acetic acid in this reaction.   An attempt
was made to effect a rearrangement, in acetic acid at 90°,
of 3-carbomethoxy-4-hydroxy-cholesterol to the 4-carbo-
methoxy isomer, but the intermediate orthoester again lost
(methyl) alcohol, in part, giving a mixture of the carbonate
and unchanged starting material.

The correlation of the new esters of 4-hydroxy-
cholesterol with those already described in the literature
was successfully accomplished by the synthesis of the
3-carbomethoxy-4-acetyl- and 3-carbethoxy-4-acetyl-deriva-
tives from the 4-monoacetate.   Namely, by treatment of its
pyridine solution with methyl and ethyl chloroformate,
respectively.   The failure of the isomeric 3-acetate to
carbomethoxylate under more vigorous conditions would seem
to indicate steric hindrance in the 4-position when the
3-hydroxyl group is acylated.   This is supported by the pre-
viously observed non-formation of di-carbomethoxy- and di-
carbethoxy-derivatives of the diol, when the latter is
treated with a large excess of the appropriate chloroformic

ester in pyridine solution (p.52).

Finally, in order to complete this work an attempt was made to reverse the change from the monoester structure to that of the carbonate. The idea was based on the observation of Tschitschibabin[84)] that neutral carbonic esters (CLIII) react with a Grignard reagent to form, presumably, the adduct (CLIV), which on hydrolysis yields esters of the type shown in formula (CLV). The highest yields were
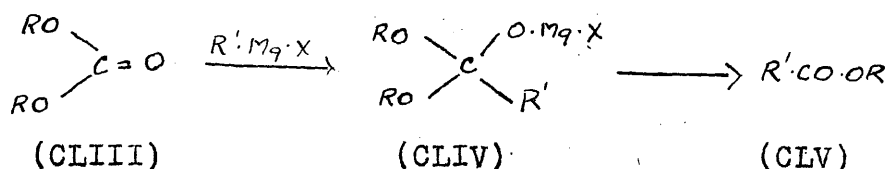
obtained when the first part of the reaction was carried out in an atmosphere of hydrogen.

Methylmagnesium iodide and the steroidal carbonate (CLVI) should, accordingly, form the intermediate (CLVII), hydrolysis of which should yield either the 3- or 4-monoacetate (e.g., CLVIII), or a mixture of both, depending on
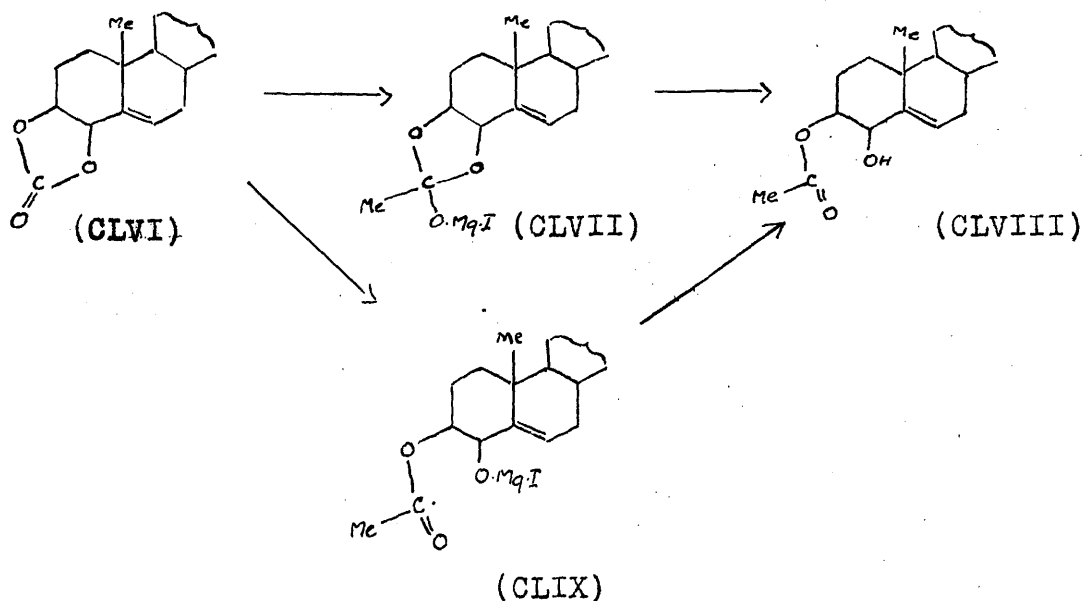
the relative stability of the CO-O bonds in the orthoester
(CLVII).

(Note. The addition of the Grignard compound to one of the
CO-O bonds in (CLVI), instead of to the carbonyl group, leads
to the same ultimate result, e.g., (CLVI)$\longrightarrow$(CLIX)$\longrightarrow$(CLVIII)).

When the reaction was carried out, however, no mono-
acetate was isolated from the hydrolysate. The sole product
of the reaction was a low melting compound which proved to be
identical with that obtained from the sodium and amyl alcohol
reduction of $\triangle^4$-cholestene-3,6-diol, i.e., presumably,
pseudocholestene. The formation of this substance can only
be accounted for on the basis of a reducing action of the
Grignard reagent accompanied by migration of the olefinic
linkage.

In connection with the possible <u>cis</u>-glycol nature of
Rosenheim and Starling's 4-hydroxy-cholesterol, support for
this is provided by the relatively facile formation of the
carbonate by rearrangement of 3-carbomethoxy- and 3-carbethoxy-
cholesterol, and by phosgene treatment of the free diol.

Although 3-carbomethoxy-6-acetoxy- and 3-carbethoxy-
6-acetoxy- $\triangle^4$-cholestene are formed by selenium dioxide
oxidation of the appropriate cholesteryl esters, the low
yields obtained, together with the difficulty in separating
them from the accompanying gum, renders impracticable their
preparation by this method. The small amount of the

3-carbethoxy-6-acetoxy derivative actually isolated and remaining after determining its nature was insufficient to test its partial hydrolysis to the 6-acetyl compound.

SUMMARY OF WORK ON ACYL MIGRATIONS.

Selenium dioxide oxidations of carbomethoxy- and carbethoxy-cholesterol (CLX, R = Me & Et, see extending chart between pp.73 and 74) in acetic anhydride solution yield the respective 3-carbonate-4-acetates (CLXI, R = Me & Et) of 4-hydroxy-cholesterol, together with the analogous 3-carbonate-6-acetates (CLXII, R = Me & Et) of the isomeric 3,6-diol. When acetic acid is substituted for its anhydride in these reactions there is formed, in addition, the intra-molecular carbonate (CLXIII) of the 3,4-diol. The constitution of the latter follows from its formation by phosgene treatment of the free diol (CLXIX); that of the mixed esters (CLXI, R = Me & Et) from their preparation by carbomethoxy-lation and carbethoxylation of the same compound (CLXIX), followed by acetylation of the resulting 3-monoesters (CLXV, R = Me & Et). The latter compounds are assigned this structure because previous monoacylations have been observed to take place at the 3-position. The acyl migration which occurs when 3-acetyl-4-hydroxycholesterol (CLXVIII) is warmed to 90° in acetic acid provides a means of correlating these esters (CLXI, R = Me & Et) with those already known, since they are formed on treatment of the resulting 4-acetate (CLXIV) with the appropriate chloroformic ester.

The observation that the 3-monoacetate also yields 4-acetoxy-cholesterol on rearrangement in propionic acid

demonstrates the intramolecular nature of the change. This

fact is held to support an orthoester mechanism of acyl

migration involving the formation, in this case, of an inter-

mediate orthoacetic ester (CLXVII). Proof of this mechanism

follows from the formation of the glycol carbonate (CLXIII)

by treatment of the 3-carbomethoxy- and 3-carbethoxy-

derivatives (CLXV, R = Me & Et) with hot acetic acid. That

is, these compounds, which are undoubtedly intermediates in

the selenium dioxide oxidation of the corresponding esters

of cholesterol, rearrange to the triester forms (CLXVI,

R = Me & Et) of orthocarbonic acid which then lose methyl

and ethyl alcohol, respectively, becoming "fixed" in this

form as the carbonate structure (CLXIII).

C_8H_{17}

Me

Me

$RO \cdot CO \cdot O$

(CLX)

Me

$RO \cdot CO \cdot O$

OAc

(CLXI)

Me

$RO \cdot CO \cdot O$

OAc

(CLXII)

Me

O

OC O

(CLXIII)

Me

HO

OAc

(CLXIV)

Me

$RO \cdot CO \cdot O$

OH

(CLXV)

Me

O O

RO C OH

(CLXVI)

Me

O O

Me C OH

(CLXVII)

Me

AcO

OH

(CLXVIII)

Me

HO

OH

(CLXIX)
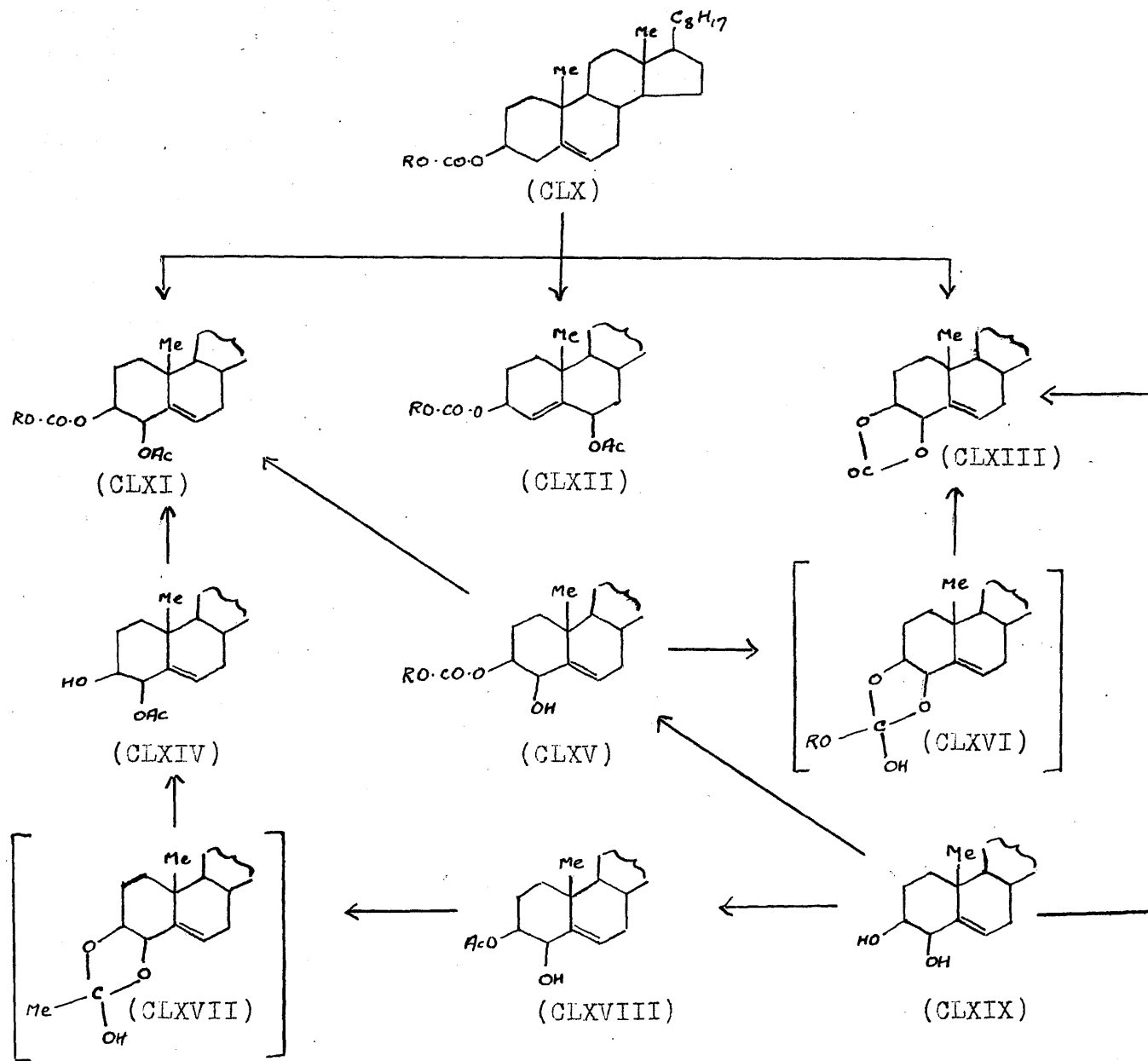
NOTE 1.

The position of the ester group(s) in the various derivatives of the 3,4-diol has now been established (Rosenheim, Nature, 1941, 147, 776; cf., Spring and Swain, J.C.S., 1941, 83), the original assumptions regarding their location having proved to be correct. (For alternative methods of preparing the different monoesters, see, Rosenheim, loc.cit.).

The 3,4- and 3,6-diols are also obtainable by treatment of cholesteryl acetate dibromide (Petrow, J.C.S., 1937, 1077) and cholesterol dibromide (Rosenheim and Starling, J.C.S., 1937, 377), respectively, with alkali acetate. Debromination of cholesteryl acetate dibromide with silver nitrate in pyridine likewise yields the cis-diol on saponification (Petrow, loc. cit.).

Heilbron and co-workers (J.C.S., 1937, 801) have prepared another $\triangle^4$-cholestene-3,6-diol (CLXXIII) by Ponndorff reduction of 3(β)-acetoxy- $\triangle^4$-cholestene-6-one (CLXXII). The latter obtained from 3(β)-acetoxy-cholestane-6-one (CLXX) by way of the monobromide (CLXXI). Unless an inversion has

(CLXX)   (CLXXI)   (CLXXII)   (CLXXIII)
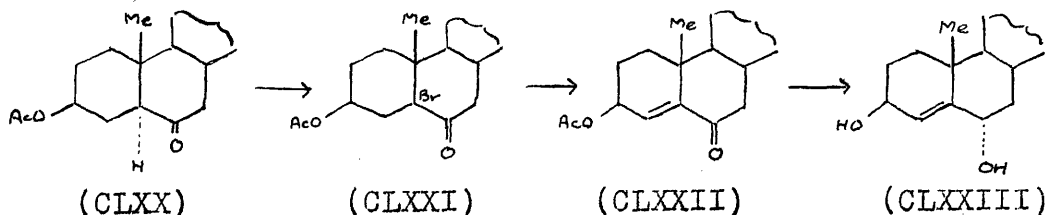
occurred at the C_3-position (cf. however, Heilbron et al., J.C.S., 1940, 1390) this diol must differ from that of Rosenheim and Starling (loc. cit.) solely in the orientation of the C_6-hydroxyl group.

NOTE 2.

The aromatic-3-monoesters of the 3,4-diol do not appear to rearrange in acetic acid at 90° (see p.53). The "reluctance" of the aroyl group to migrate under these conditions may bear some relation to the following analogy in the field of aromatic compounds:-

A Friedel-Crafts' condensation of 2-methyl-naphthalene (CLXXIV) and an aroyl anhydride at 0° results in the formation of the 1-aroyl-2-methyl-naphthalene (CLXXV) (Fieser and Peters, J.A.C.S., 1932, 54, 3742). On heating this product to 150° with AlCl₃-NaCl an isomerisation occurs, the aroyl group migrating to the 6-position (CLXXVI).

When, however, acyl chlorides or anhydrides are
substituted for the aroyl anhydride in the above condensa-
tion at 0°, the 2-methyl-6-acyl-naphthalene (CLXXVIII)
corresponding to (CLXXVI) is obtained.   In this case the
intermediate formation of the 1-acyl derivative (CLXXVII)
is assumed to take place, but the apparently greater
mobility of acyl groups results in the intermediate rearrange-
ment to the 6-acyl compound (see Haworth and Sheldrick, J.C.S.,
1934, 864).

(CLXXV)   (CLXXVI)

(CLXXIV)

(CLXXVII)   (CLXXVIII)
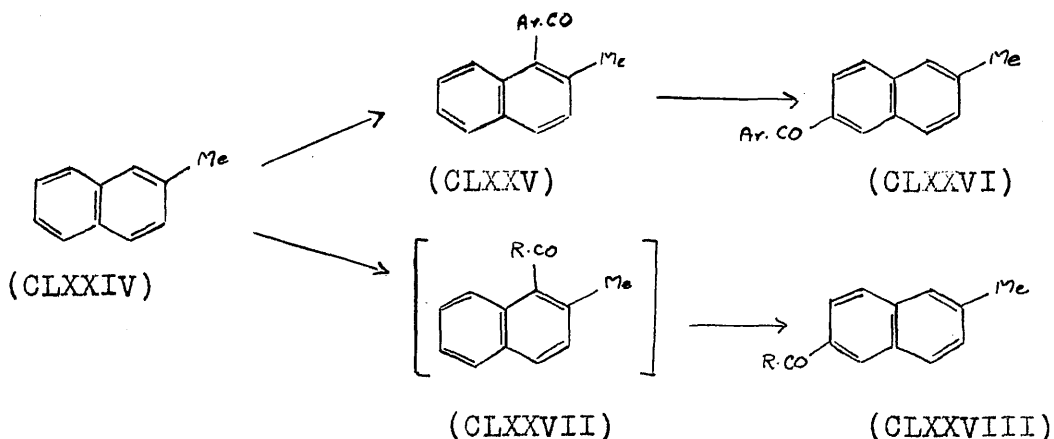
Similarly, when 2-methoxy-naphthalene, acetyl
chloride and aluminium chloride are allowed to react in
nitrobenzene solution, a 70% yield of the 6-acetyl compound
(CLXXIX) is obtained (Haworth and Sheldrick, loc. cit.).
When the condensation is carried out in benzene or carbon
disulphide, however, 1-acetyl-2-methoxy-naphthalene (CLXXX)
results (see Haworth and Sheldrick, loc. cit.)

(CLXXIX)   (CLXXX)
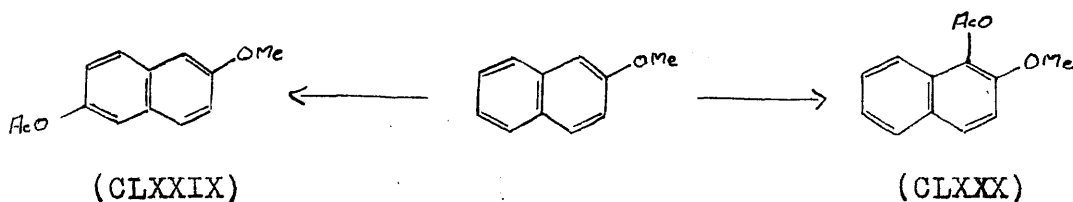
Unfortunately, no condensation of 2-methoxy-naphthalene
with an aroyl chloride appears to have been attempted, but
the formation of the 1-aroyl derivative would seem likely.

<u>ADDENDUM.</u>

Shortly after compilation of this part of the Thesis
Dr. O. Rosenheim very kindly forwarded to me a draft of the
paper promised in Nature, 1941, <u>147</u>, 776 (see p.54).

A slight overlap has occurred, and the following
extract is of interest in connection with the above work
since it provides amplification for some of the points
discussed:-

"An acyl migration from C_3 to C_4 occurs when the
3-monoacetate of the cis-diol (CLXXXII) is warmed to 90°
in acetic acid solution, yielding the 4-monoacetate (CLXXXIII).
The reaction takes place slowly even at 37° and is reversible,
the equilibrium apparently being shifted in favour of the
less soluble 4-monoester.   At temperatures higher than 90°
and rapidly at 118° in boiling acetic acid, the two mono-
esters undergo an allylic rearrangement, the acetyl group

(CLXXXI)

(CLXXXII)   (CLXXXIII)   (CLXXXIV)
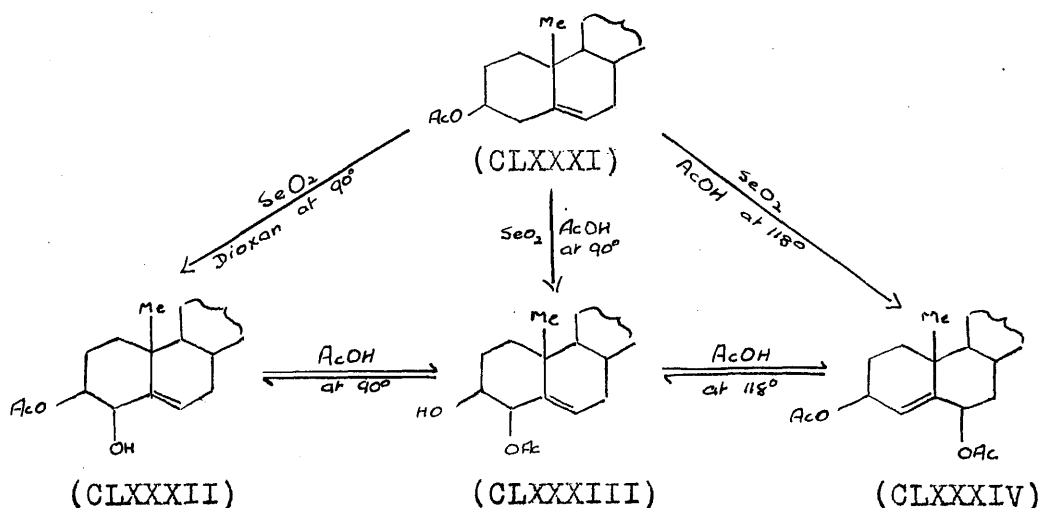
migrating to C_6, and yield the esters of $\triangle^4$ -cholestene-
3,6-diol (CLXXIV).   The latter diol is the main product
when cholesteryl acetate (CLXXXI) is oxidised with selenium
dioxide in boiling acetic acid (Rosenheim and Starling,
J.C.S., 1937, 377; Butenandt and Hausmann, Ber., 1937, <u>70</u>,
1154; Petrow, Rosenheim and Starling, J.C.S., 1938, 677)."

Mention is also made of the formation of the cyclic sulphite (CLXXXV) and the bis-sulphite (CLXXXVI) by thionyl chloride treatment of the 3,4-diol and its 4-monoacetate, respectively.

(CLXXXV) (CLXXXVI)
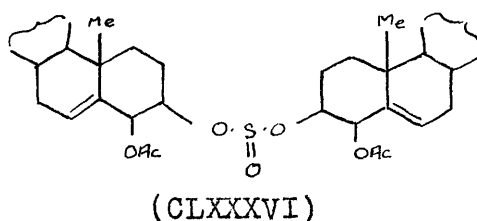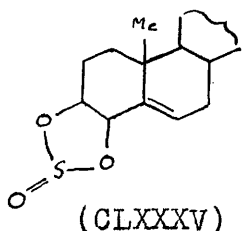
The sulphite is the sulphur analogue of the carbonate studied in the present work, and to a lesser degree, the bis-sulphite and the bimolecular carbonate (CXLVI) formulated on p.59. It would, no doubt, be possible to prepare an analogous derivative of 4-acetoxy-cholesterol on similar lines, i.e., by phosgene treatment of the 4-monoacetate.

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Having read the sections of Part I dealing with the acyl migrations, Dr. Rosenheim drew attention to the following points:-

p.45. He confirms the failure to effect partial hydrolysis of 3,6-diacetoxy- $\triangle^4$ -cholestene by the usual methods, but has successfully obtained the 6-monoacetate "by a method of catalytic hydrolysis which has so far not been applied to steroids".

pp.50, 60 & 70. The fact that the acetone compound of $\triangle^5$ -cholestene-3,4-diol has already been prepared (Rosenheim and Starling, J.C.S., 1937, 380) has been regrettably overlooked. Needless to say, the existence of this compound proves the cis-glycol nature of the diol.

pp. 65 & 102.   Dr. Rosenheim has studied the rearrangement
of the 3-monoacetate in formic and propionic acids
as well as in acetic acid.   It is interesting to
note that the product (and this only) forms molecular
compounds with these acids, the acid of crystallisa-
tion only being lost at 120° in vacuo.   Complete
dissociation occurs, however, when these compounds
are crystallised from 85% alcohol.   In the case of
the rearrangement in propionic acid he did not have
to separate the mixture via the benzoates, the
propionic acid compound being found to separate out
from the reaction solution (benzene-propionic acid)
on cooling.   The low melting point (ca. 140°) of
some of the rearrangement fractions encountered in
the present investigation must, therefore be
attributed to this, and not, as assumed, to admixture
of the isomeric acetates.

pp. 66 & 103.   By a slight modification of Marker and
Rohrmann's procedure both polymorphic forms have been
obtained and identified as the 3- and 4-monoacetates.

p. 77.   For a similar sterol endo-sulphite see,
Jacobs and Elderfield, J. Biol. Chem., 1932, 97, 727,
and Tschesche, Z. physiol. Chem., 1934, 229, 224.
               See also the bis-cholesteryl sulphite of
Daugenbaugh and Allison, J.A.C.S., 1939, 61, 3665.

EXPERIMENTAL.

All melting points are corrected.   Specimens for analysis were dried in the steam-oven and finally in vacuo over sulphuric acid.   The palladium catalyst used was that of Heilbron et al. (J.C.S., 1929, 929), and the platinic oxide was prepared from ammonium chloroplatinate according to the directions of Bruce (J.A.C.S., 1936, 58, 687).

Reduction of 6(β)-acetoxy-$\triangle^{4}$-cholestene-3-one with sodium and amyl alcohol.

6(β)-acetoxy-$\triangle^{4}$-cholestene-3-one was prepared from "triol-I" according to the method of Ellis and Petrow (see p.29).

Sodium (5 g.), in fairly small fragments, was added during the course of about 40 minutes to a boiling solution of the ketone (1 g., m.p. 100-101.5^{o}) in (iso-) amyl alcohol (40 c.c.).   The mixture was refluxed for 3 hours, a further quantity of the alcohol (20 c.c.) being added towards the end of this period in order to destroy the residual sodium. After decomposing the cooled reaction mixture with water (over- night) the yellow layer was separated and the aqueous portion extracted twice with ether.   Addition of the ethereal ex- tracts to the amyl alcohol solution rendered the latter more mobile, whereupon the alkali was washed out with water.   The ether was distilled off on the water-bath;   the amyl alcohol under diminished pressure, remaining traces being removed by steam-distillation.   The product, isolated by means of ether, was a pale yellow, viscous oil which could not be crystallised.

Benzoylation.   The oil was taken up in dry pyridine (10 c.c.) and benzoyl chloride (4 c.c.) added dropwise at 0°.   After

standing for 48 hours at room temperature the mixture was poured into water and extracted twice with ether. The combined extracts, having been previously washed with dilute hydrochloric acid, sodium carbonate solution, and water, and dried over sodium sulphate, were distilled and the residue, an oil, triturated with a small volume of acetone yielding crystals of m.p. 200-207.5^o. One recrystallisation from acetone-chloroform followed by two from ethyl acetate gave elongated leaflets (0.12 g.), m.p. 213-214.5^o. A mixed melting point with authentic cholestane-3(β),6(α)-diol dibenzoate, m.p. 213-214.5^o, (see below) showed no depression.

Saponification of the dibenzoate. The above dibenzoate (50 mg.) was refluxed for one hour with methyl alcoholic potash (10 c.c., 5%) when the hydrolysate was poured into water and ether-extracted. After washing with water the dried (sodium sulphate) etherial solution was distilled and the residue crystallised from acetone. The product was further purified by repeated recrystallisation from dilute alcohol yielding leaflets of m.p. 217.5-219°, undepressed by an authentic specimen of cholestane-3(β),6(α)-diol, m.p. 218-219.5^o (see below).

Cholesterol α-oxide.

An ether solution of perphthalic acid (Böhme, Ber., 1937, 70, 379; Baeyer and Villiger, ibid., 1901, 34, 763), equivalent to 0.166 g. of active oxygen (2 atoms), was added dropwise with stirring to cholesterol (2 g.; 1 mol.) in

ether (50 c.c.) cooled to 0°.　After standing for 3 days at room temperature the liquor was de-acidified with sodium carbonate solution, washed with water, and dried over sodium sulphate.　The residue obtained on removal of the solvent was purified by two crystallisations from ethyl acetate giving narrow nacreous plates (1.8 g.) of m.p. 142-143°.

Cholestane-3(β),6(α)-diol.

Cholesterol α-oxide (1 g.) was treated with sodium (5 g.) and boiling amyl alcohol (40 c.c.) over a period of 4 hours.　The product (isolated as described in the analogous reduction of 6-acetoxy-$\triangle^4$-cholestene-3-one) was a pale yellow, viscous oil, which was dissolved in a small amount of boiling acetone.　On cooling, shining microscopic prisms were deposited and filtered off, m.p.208-213°.　After repeated recrystallisation from dilute alcohol, leaflets of the pure diol (0.11 g.), m.p. 218-219.5°, were obtained.·

Cholestane-3(β),6(α)-diol dibenzoate.

Benzoyl chloride (0.5 c.c.) was added to an ice-cold solution of the above diol (50 mg.) in dry pyridine (2 c.c.) and the mixture was allowed to stand at room temperature for 48 hours.　After decomposing the excess acid chloride with water, the solid was filtered off, washed with water, dried, and crystallised from ethyl acetate yielding the pure dibenzoate, m.p. 213-214.5°, in the form of elongated leaflets.

Catalytic hydrogenation of 6((β)-acetoxy- $\triangle^4$-cholestene-3-one.

An ethereal solution of the ketone (1 g.) was shaken with hydrogen in the presence of palladium black (0.1 g.). Hydrogen was absorbed fairly rapidly and ceased after 2 hours. The amount taken up corresponded to about 2 molecules. After filtration, the solvent was removed on the water-bath, yielding a colourless gum which could not be crystallised by solvent treatment.

On heating a small portion of the gum in alcohol with semicarbazide hydrochloride and sodium acetate, no evidence of semicarbazone formation was observed.

The remainder of the gum was redissolved in ether and shaken with platinic oxide (0.05 g.) and hydrogen, but no further absorption took place.

The recovered gum was taken up in pyridine (10 c.c.) and treated with benzoyl chloride in the usual manner. After decomposing with water (overnight), the aqueous mixture was extracted with ether and the combined extracts washed successively with dilute acid, sodium carbonate solution and water. Distillation of the dried (sodium sulphate) solution yielded an oil from which no solid material could be isolated.

Reduction of $\triangle^4$-cholestene-3,6-diol with sodium and amyl alcohol.

Sodium (10 g.) was added during one hour to a boiling solution of the diol (2 g., m.p.256-257°) in amyl alcohol (100 c.c.), heating being continued for 3 hours. The product, isolated in the customary manner, was an almost

colourless gum, which after taking up in hot alcohol deposited
long needles (m.p.78-79°) on cooling.   One recrystallisation
from methyl alcohol rendered the substance pure, m.p. 79-
80° (yield 1.4 g.).   A mixed melting point with a specimen
of coprostenone (m.p. 79-80°) (prepared by chromic acid
oxidation of cholesterol dibromide, followed by debromination
with zinc and acetic acid - Windaus, Ber., 1906, 39, 518)
showed a marked depression.   On admixture with authentic
$\triangle^s$-cholestene (m.p. 88-89°) the melting point was again
depressed.   The material rapidly decolorised bromine-water.

Investigation of the compound of m.p. 79-80°.

Attempted acetylation.   The above substance (300 mg.) was
refluxed for one hour with freshly distilled acetic an-
hydride (5 c.c.).   After decomposing with water a solid
(293 mg.) of m.p. 76-78.5° was recovered, which on
crystallisation from alcohol melted at 79-80°, undepressed
with the original material.

Attempted preparation of a 2,4-dinitrophenylhydrazone.   A
suspension of dinitrophenylhydrazine (100 mg.) in alcohol
(10 c.c.) was added to a boiling solution of the substance
(200 mg.) in alcohol (15 c.c.) containing hydrochloric acid
(0.6 c.c.).   After refluxing for one hour, the hot solution
was diluted with a small amount of water (until the formation
of a permanent turbidity) and set aside to crystallise.
Lond needles (170 mg.) of slightly impure starting-material
separated out on cooling.

<u>Hydrogenation</u>.   A solution of the compound (400 mg.) in alcohol-ether (50 c.c.; 1:1) was catalytically reduced with palladium black (100 mg.).   Hydrogen was absorbed rapidly and ceased after 20 minutes.   On the assumption that the substance was a hydrocarbon of molecular formula $C_{27}H_{46}$, i.e., pseudocholestene (coprostene), the amount taken up corresponded to one molecule.   After filtering off the catalyst the solution was distilled and the resultant oil crystallised from alcohol-ether giving leaflets of m.p. 70-72.5°.   Repeated recrystallisation from alcohol yielded a pure compound of m.p. 77-78°, not depressed by an authentic specimen of cholestane (m.p. 79-80°), but depressed to 60° by the starting-material.

Cholesteryl benzoate α- and β-oxides.

A solution of perphthalic acid (24 g.) in ether (1000 c.c.) was added dropwise with stirring during 90 minutes to cholesteryl benzoate (40 g.; m.p. 147-148° and 180.5°) dissolved in a mixture of ether (450 c.c.) and chloroform (300 c.c.) cooled to -5°.   After standing for 12 hours at 0° and 4 days at room temperature, the excess per-acid was removed with sodium carbonate solution.   The crystalline residue obtained on distillation of the washed and dried (sodium sulphate) solution was crystallised several times from ethyl acetate yielding pure cholesteryl benzoate α-oxide (m.p. 167.5-168.5°), identified by its melting point and by its hydrolysis to cholesterol α-oxide, m.p. and mixed m.p. 142-143°.

The combined ethyl acetate mother liquors were evaporated to dryness under diminished pressure and the residue was repeatedly recrystallised from ethyl acetate-methyl alcohol (3:1) yielding finally pure cholesteryl benzoate β-oxide (m.p. 149.5-150.5°), identified as for the α-isomer.

(Yields: α-oxide benzoate, 22 g., 53%; β-oxide benzoate, 10.5 g., 25%).

Reduction of β-cholesterol oxide with sodium and amyl alcohol.

Cholesterol β-oxide (2 g.; m.p. 108-109°), obtained by saponification of the above β-oxide benzoate, was reduced in the usual manner with sodium (10 g.) and amyl alcohol (80 c.c.). From the reaction mixture a yellow gum was isolated, acetone treatment of which yielded a small amount of microcrystalline material (0.07 g.; m.p. 182-192°). After repeated recrystallisation from dilute alcohol this substance was identified by mixed melting point as cholestane-3(β),6(α)-diol.

Benzoylation. Removal of the solvent from the above acetone filtrate yielded the yellow gum (1.97 g.) which was treated with benzoyl chloride (4 c.c.) and dry pyridine (10 c.c.) for 2 days at room temperature. After decomposing the dark-red solution with water (overnight), an oil was isolated by means of ether. Trituration of this with acetone gave a crude substance (0.23 g.; m.p. 195-204°) which on

repeated recrystallisation from ethyl acetate proved to be
cholestane-3(β),6(α)-diol dibenzoate (m.p. and mixed m.p.
213-214.5°).

The gum obtained on distillation of the filtrate from
the acetone trituration was dissolved in petroleum ether
(60 c.c.; b.p. 40-60°) and run through a column of alumina.
Examination of the decolourised solution and the "liquid
chromatogram", obtained by elution with petroleum ether con-
taining alcohol (0.5%) and finally with ether, did not yield
any homogeneous material other than the above dibenzoate.

Attempted catalytic hydrogenation of cholesteryl benzoate β-oxide.

A mixture of the pure β-oxide benzoate (2 g.;
m.p. 149.5-150.5°), platinic oxide (0.05 g.) and absolute
ether (80 c.c.) was shaken in an atmosphere of hydrogen.
After the initial hydrogen uptake, due to the reduction of
the catalyst, no further absorption was observed (over a
period of 5 hours). Unchanged starting-material was re-
covered on evaporation of the filtered solution.

Attempted partial hydrolysis of Δ^4-cholestene-3,6-diol diacetate.

(1)   The diacetate (2 g.; 1 mol.) and potassium hydroxide
(0.23 g.; 1 mol.) were dissolved in a mixture of aqueous
alcohol and ether (100 c.c.) and the homogeneous solution
allowed to stand overnight at room temperature. After

acidifying with a few drops of acetic acid, water was added and the organic material extracted with chloroform.   Distillation of the washed and dried (sodium sulphate) extracts yielded a solid residue which was separated into two fractions on treatment with alcohol.   The more soluble compound was identified by mixed melting point as unchanged starting-material, the less soluble as $\triangle^4$-cholestene-3,6-diol.

(2)   A solution of potassium hydroxide (0.58 g.;   1 mol.) in methyl alcohol (100 c.c.) was added dropwise with mechanical stirring over a period of 8 hours to the diacetate (5 g.;   1 mol.) dissolved in methyl alcohol (900 c.c.). After standing for two days at room temperature the solution was acidified with a few drops of acetic acid and concentrated to small volume in vacuo.   The product, isolated by means of ether, again proved to be a mixture of starting-material and the fully hydrolysed diol.

Selenium dioxide oxidation of carbomethoxy-cholesterol in acetic anhydride solution.

A solution of selenium dioxide (2.5 g.) in water (5 c.c.) was added dropwise with stirring to carbomethoxy-cholesterol (4.6 g.;   m.p. 113.5-114°) dissolved in hot acetic anhydride (100 c.c.;   temp. 105-110°).   After maintaining this temperature for 2 hours the dark-red mixture was cooled, the acetic anhydride decomposed with cold water, and the oxidation product separated from the selenium by extraction with boiling acetone.   The last traces of

selenium were removed by slow addition of the acetone ex-
tract to a well-stirred 10% aqueous potassium cyanide
solution, stirring being continued for 4 hours.   The
organic material was extracted with ether and the washed
and dried (sodium sulphate) ethereal layer distilled,
yielding a dark-brown gum which was triturated with a
small amount of cold alcohol.   Small, apparently homo-
geneous, crystals separated out almost immediately, and
after standing for several hours were filtered off,
m.p. 157-159°.   Two recrystallisations from alcohol rendered
the material (compound A) pure (in microcrystalline form),
m.p. 160.5-161°, depressed on admixture with cis-3,4-
diacetoxy-$\triangle^{5}$-cholestene (m.p. 167.5-168.5°).   Fractional
evaporation of the mother-liquor from the alcohol tri-
turation yielded only further quantities of a substance A
(total yield 0.31 g.) and finally a dark-brown crystallis-
able gum.

Saponification of the uncrystallisable residue.   The gum
was hydrolysed by refluxing for one hour with 5% methyl
alcoholic potash, small crystals separating out after boil-
ing for a few minutes.   These were filtered off, washed
with water and alcohol and dried, m.p. 235-239°.   After
repeated recrystallisation from acetone-chloroform the
characteristic fine needles of $\triangle^{4}$-cholestene-3,6-diol
were obtained, m.p. and mixed m.p. 256-257°.

Selenium dioxide oxidation of carbomethoxy-cholesterol in acetic acid solution.

A solution of carbomethoxy-cholesterol (21 g.) in glacial acetic acid (200 c.c.) was prepared by heating to 100°. Glacial acetic acid (200 c.c.), warmed to the same temperature, was added to selenium dioxide (10 g.) dissolved in water (5 c.c.) and the two solutions were mixed and boiled for 5 minutes. After adding sodium acetate (60 g.) and refluxing for a further 5 minutes the hot solution was filtered from the residual selenium. When cold, the filtrate was poured into half-saturated brine (1000 c.c.) and extracted with ether. The combined extracts were washed with dilute sodium carbonate solution and water, dried over sodium sulphate, and the ether removed on the water-bath. Trituration of the residual dark-brown oil with cold methyl alcohol gave a mixture of leaflets and clusters of micro-crystals, which were filtered off (2.7 g.; m.p. 126-139°) and recrystallised from rectified spirit (100 c.c.). The residue on filtration (m.p. 159-159.5°), after repeated re-crystallisation from alcohol, melted at 160.5-161°, unde-pressed on admixture with compound A (m.p. 160.5-161°) from the previous oxidation. Fractional concentration of the rectified spirit filtrate afforded a number of crops of varying melting point, the more soluble of which crystallised in the form of leaflets. These were combined and repeatedly recrystallised from alcohol yielding finally lustrous leaf-lets of a pure substance (compound B) of m.p. 173-173.5°,

strongly depressed on admixture with compound A and with cis-3,4-diacetoxy- $\triangle^5$ -cholestene.   Further quantities of compounds A and B were obtained from the remaining crops of crystals.

Saponification of the uncrystallisable residue.   Evaporation of the filtrate from the above initial crystallisation yielded an oil which could not be crystallised.   This was refluxed for one hour with 5% methyl alcoholic potash and the product worked up as described in the previous hydrolysis. $\triangle^4$ -cholestene-3,6-diol (3.6 g.) m.p. and mixed m.p. 256-257$^{\text{O}}$, was again obtained.

<div align="center">Investigation of compound A.</div>

Hydrolysis.   A small quantity of compound A was refluxed for one hour with 5% methyl alcoholic potash, whereupon the cooled hydrolysate was diluted with water and extracted with ether.   The residue obtained on distillation of the washed and dried (sodium sulphate) ethereal solution was crystallised from alcohol giving leaflets of $\triangle^5$ -cholestene-3,4-diol, m.p. and mixed m.p. 174-174.5$^{\text{O}}$.

Attempted acetylation.   Substance A (100 mg.) was refluxed for one hour with freshly distilled acetic anhydride (5 c.c.), when the excess of the latter was decomposed with ice-water. The filtered residue (100 mg.) on crystallisation from alcohol melted at 160.5-161$^{\text{O}}$, undepressed on admixture with the starting-material.

Analysis.

Found: C, 74.13; H, 9.62%

Calculated for 3-carbomethoxy-
4-acetoxy-cholesterol,
$C_{31}H_{50}O_5$: C, 74.13; H, 9.96%

Monocarbomethoxylation of 4-hydroxy-cholesterol.

(3-Carbomethoxy-4-hydroxy-cholesterol.)

Cf. Preparation of carbomethoxy- and carbethoxy-cholesterol (Robberecht, Bull.Soc.chim.Belg., 1938, 47, 597).

4-Hydroxy-cholesterol (1 g.) dissolved in a mixture of anhydrous ether and benzene (40 c.c.; 1:1) was mixed with methyl chloroformate (5 c.c.) and cooled in ice. Dry pyridine (5 c.c.) was added dropwise with shaking, an abundant white precipitate forming immediately, followed by a faint pink colouration of the mixture. After standing for 2 hours at room temperature, closed by a calcium chloride-tube, the pyridine hydrochloride was filtered off and washed with dry ether. The filtrate was concentrated to small volume under reduced pressure and the residue mixed with water and the solid filtered off. The dried product on repeated recrystallisation from absolute alcohol yielded thin plates of 3-carbomethoxy-4-hydroxy-cholesterol, m.p. 157-159.5°. A mixed melting point with the starting-material gave a marked depression.

Found: C, 75.52; H, 10.66%

$C_{29}H_{48}O_4$ requires: C, 75.65; H, 10.43%

3-Carbomethoxy-4-acetoxy-cholesterol.

The above monocarbomethoxy-compound (200 mg.) was refluxed for 1 hour with acetic anhydride (5 c.c.) and the cooled solution was poured into ice-water.   After standing overnight the product was filtered off and dried, m.p. 156-157° (200 mg.).   Two recrystallisations from alcohol gave clusters of micro-crystals of m.p. 160-160.5°, undepressed on admixture with compound A (m.p. 160.5-161°).

3-Carbomethoxy-4-benzoyloxy-cholesterol.

Benzoyl chloride (1 c.c.) was added dropwise to an ice-cooled solution of 3-carbomethoxy-4-hydroxy-cholesterol (200 mg.) in dry pyridine (5 c.c.).   After heating for 2 hours on the water-bath the mixture was cooled and poured into water, the red oil which separated becoming granular on standing (24 hours).   The dark-brown residue on filtration was washed with sodium carbonate solution and water and purified by repeated recrystallisation from acetone-alcohol yielding rectangular platelets of the mixed ester (185 mg.), m.p. 173-174°.

Found: C, 76.49; H, 9.20%

$C_{36}H_{52}O_5$ requires: C, 76.60; H, 9.22%

Investigation of compound B.

Hydrolysis.   Compound B (100 mg.) was hydrolysed in the usual manner with methyl alcoholic potash (15 c.c.; 5%) and the diluted hydrolysate ether-extracted.   Distillation of the washed and dried (sodium sulphate) ethereal solution

yielded a white residue (88 mg.) of m.p. 174-174.5^O, un-
altered on crystallisation from methyl alcohol. A mixed-
melting point with an authentic specimen of 4-hydroxy-
cholesterol (m.p. 174-174.5^O) showed no depression.

Attempted acetylation. The material (100 mg.; m.p. 173-
173.5^O) was refluxed for one hour with freshly distilled
acetic anhydride (5 c.c.). After pouring into ice-water
and allowing to stand overnight the solid was filtered off
and dried (100 mg.), m.p. 172-172.5^O. One recrystallisa-
tion from alcohol raised the melting point to 173-173.5^O,
undepressed on admixture with the starting material.

Attempted benzoylation. 1) To a cooled solution of the
substance (100 mg.) in dry pyridine (5 c.c.) benzoyl
chloride (1 c.c.) was added dropwise with shaking, and the
mixture allowed to stand for 48 hours at room temperature.
After pouring into ice-water a solid was deposited and
filtered off, m.p. 171.5-172°. Repeated recrystallisation
from alcohol yielded lustrous leaflets melting at 173-173.5^O,
either alone or when mixed with the starting-material.
2) A solution of the material (100 mg.) in dry pyridine
(5 c.c.) to which benzoyl chloride (3 c.c.) had been added
was heated on the steam-bath for 2 hours. On working up
the product in the usual manner compound B was recovered
almost quantitatively and identified as before by a mixed
melting point.

Analysis.

Found: C, 78.68;   H, 11.07%

Calculated for 4-hydroxy-
cholesteryl carbonate, $C_{28}H_{44}O_3$:   C, 78.50;   H, 10.28%

(Calculated for 4-hydroxy-
cholesteryl monoacetate, $C_{29}H_{48}O_3$:   C, 78.38;   H, 10.81%)

Experiments with phosgene and 4-hydroxy-cholesterol.

1) Attempted esterification at 70° with one equivalent of phosgene.   To the pure diol (1 g.;   m.p. 174-174.5°) dissolved in a mixture of anhydrous pyridine (6 c.c.) and benzene (10 c.c.) a solution of phosgene in toluene (2.2 c.c. of 12.5%;   S.G. .99) was added and the whole heated in a sealed tube for 2 hours at 70°.   After cooling, the tube was opened and the dark-brown mixture poured into water and extracted with ether.   The combined ethereal extracts were washed free from pyridine with dilute hydrochloric acid, finally with dilute sodium carbonate solution and water. Distillation of the dried (sodium sulphate) solution gave a white residue which proved to be essentially starting-material (mixed melting point).

2) "Condensation" at 70° with 8 equivalents of phosgene. The diol (0.5 g.) in dry pyridine (6 c.c.) and benzene (10 c.c.) was mixed with the solution of phosgene in toluene (8.8 c.c.) and heated in a sealed tube for 2 hours at 70°.   On cooling and working up as before a yellowish crystalline residue was obtained which after repeated re-crystallisation from alcohol (norite) yielded colourless

lustrous leaflets (0.37 g.) of m.p. 173-173.5°, either alone
or when mixed with compound B of the same melting point.

Attempted preparation of the acetone-compound of 4-hydroxy-cholesterol.

The cis-diol (300 mg.) was suspended in acetone
(40 c.c.) containing 1% of dry hydrogen chloride.   After
standing for 24 hours at room temperature the homogeneous
solution was diluted with water (100 c.c.) and ether-
extracted.   Distillation of the washed (sodium carbonate
solution and water) and dried (sodium sulphate) ether
extract yielded a yellowish gum which could not be
crystallised.

Selenium dioxide oxidation of carbethoxy-cholesterol in acetic anhydride solution.

Selenium dioxide (1.25 g.) dissolved in water
(2.5 c.c.) was added dropwise to a well-stirred solution
of carbethoxy-cholesterol (2.38 g.; m.p. 71-71.5° and
105.5°) in acetic anhydride (50 c.c.) maintained at 105-
110°.   After a reaction duration of 2 hours (temp. 105-
110°) the dark-red solution was cooled and the acetic
anhydride decomposed with water.   The selenium was re-
moved as described in the analogous oxidation of carbo-
methoxy-cholesterol, the organic material being finally
isolated by means of ether.

The product, a dark-brown gum, was triturated with a small volume of cold alcohol, and after standing for several hours the crystalline deposit was filtered off (0.86 g.), m.p. 100.5-106.5^{o} clear at 118°. On recrystallisation from the same solvent, long slender needles and rosettes of elongated platelets, obviously a mixture, were obtained (m.p. 104-113°). An attempt to effect a separation by fractional crystallisation from alcohol failed, but acetone subsequently proved to be the best solvent. After one recrystallisation from the latter, needles of m.p. 160-162° were obtained, which on further purification from the same solvent yielded a substance (compound C) in the form of long, slender, somewhat flattened needles of m.p. 163-163.5^{o}.

The residue (m.p. 102.5-105°) obtained on evaporation of the first acetone filtrate was repeatedly recrystallised from alcohol giving finally rosettes of elongated platelets of a pure material (compound D), m.p. 121-122.5^{o}.

Investigation of compound C.

Hydrolysis. A small quantity of the material was refluxed for one hour with 5% methyl alcoholic potash and the hot solution precipitated with water. The filtered residue on crystallisation from alcohol yielded 4-hydroxy-cholesterol, m.p. 173-174°, undepressed on admixture with an authentic specimen.

Attempted acetylation.   A solution of the substance (50 mg.;
m.p. 163-163.5°) in freshly distilled acetic anhydride
(5 c.c.) was boiled for one hour and poured into cold water.
After standing overnight the product was filtered off
(43 mg.; m.p. 162.5-163°) and showed no melting point
depression when mixed with the starting-material.

Analysis.

Found:	C, 73.64;	H, 9.80%
$C_{32}H_{52}O_5$ requires:	C, 74.42;	H, 10.08%

Monocarbethoxylation of 4-hydroxy-cholesterol.
(3-Carbethoxy-4-hydroxy-cholesterol.)

To an ice-cooled solution of the diol (1 g.) in
anhydrous ether, benzene and ethyl chloroformate (45 c.c.;
4:4:1) dry pyridine (5 c.c.) was added dropwise.   After
standing for 2 hours closed by a calcium chloride tube the
product was worked up as described in the previous carbo-
methoxylation of the diol.   The crude material (m.p. 116-
120°) after repeated recrystallisation alternately from
alcohol and ligroin (80-100°) gave elongated rectangular
platelets of 3-carbethoxy-4-hydroxy-cholesterol, m.p. 130.5-
131°.

Found:	C, 76.04;	H, 10.66%
$C_{30}H_{50}O_4$ requires:	C, 75.95;	H, 10.55%

3-Carbethoxy-4-acetoxy-cholesterol.

3-Carbethoxy-4-hydroxy-cholesterol (200 mg.) was refluxed for one hour with acetic anhydride (5 c.c.) and the cooled solution decomposed with water.   The filtered product (193 mg.; m.p. 159-160°) on repeated recrystallisation from acetone yielded long, slender, somewhat flattened needles of the mixed ester, m.p. 162.5-163°, undepressed on admixture with compound C (m.p. 163-163.5°).

3-Carbethoxy-4-benzoyloxy-cholesterol.

To an ice-cooled solution of the monocarbethoxylate (200 mg.) in dry pyridine (5 c.c.), benzoyl chloride (1 c.c.) was added and the mixture heated on the water-bath for 2 hours.   After pouring into water and allowing to stand for 24 hours the granular product was filtered off and repeatedly recrystallised from alcohol giving elongated rectangular platelets of the benzoate, m.p. 131-131.5°, strongly depressed on admixture with the starting-material (m.p. 130.5-131°).

Found: C, 76.41; H, 9.25%

$C_{37}H_{54}O_5$ requires: C, 76.82; H, 9.34%

Investigation of compound D.

Hydrolysis.   The small crystals which separated out when the substance (100 mg.) was refluxed for one hour with 5% methyl alcoholic potash were filtered off and washed with alcohol (73 mg.; m.p. 243-245°).   Two recrystallisations

from acetone-chloroform yielded $\triangle^4$-cholestene-3,6-diol,
m.p. and mixed m.p. 256-257°.

Analysis.

Found   C, 74.62; H, 10.19%

Calculated for 3,6-dihydroxy-
    $\triangle^4$-cholestene 3-carbethoxylate,
$C_{32}H_{52}O_5$:   C, 74.42; H, 10.08%

Selenium dioxide oxidation of carbethoxy-cholesterol in acetic acid solution.

Carbethoxy-cholesterol (4.28 g.) dissolved in hot
glacial acetic acid (40 c.c.) was mixed and boiled for 5
minutes with a solution of selenium dioxide (2 g.; water,
2 c.c.) in the same solvent (40 c.c.).   Sodium acetate
(12 g.) was added and the mixture was refluxed for a further
5 minutes.   After removal of the selenium by filtration,
the oxidation product was worked up exactly as described
in the analogous oxidation of carbomethoxy-cholesterol.

Considerable difficulty was experienced in separat-
ing the solid fractions from the dark-red gum, this being
achieved by slow crystallisation (6 weeks) from glacial
acetic acid.   The residue on filtration was taken up in
the minimum amount of boiling alcohol and filtered hot
from the inorganic impurity (selenium).   Evaporation of
the filtrate yielded a small amount of crystalline material
(0.11 g.).

Recrystallisation of the above product from acetone
(4 c.c.) gave long slender needles (m.p. 157.5-159°) which

after repeated purification from the same solvent proved to be compound C, m.p. and mixed m.p. 163-163.5°.

The first acetone filtrate was evaporated and the residue (m.p. 135-146°) recrystallised from ligroin (80-100°) giving lustrous leaflets of m.p. 170.5-171°. Two recrystallisations from alcohol yielded 4-hydroxy-cholesteryl carbonate (compound B), m.p. and mixed m.p. 173-173.5°.

The residue from the evaporation of the ligroin filtrate was finely powdered and digested with a few drops of acetone. After evaporation of the acetone extract the process was repeated with ligroin yielding finally a few milligrams of material of m.p. 99-105° which after two recrystallisations from alcohol melted at 115-117.5° (elongated platelets). A mixed melting point with compound D (m.p. 121-122.5°) showed no depression (m.p. 118-120°).

<u>4-Acetoxy-cholesterol.</u>

2) <u>By rearrangement of the 3-acetate in acetic acid solution.</u>
3-Acetyl-4-hydroxy-cholesterol (3 g.; m.p. 189.5-190.5°) was dissolved in the minimum amount of glacial acetic acid previously warmed to 90° and maintained at this temperature for 1 hour. On cooling, crystals separated out and were filtered off (1.17 g.), m.p. 116-135°. After one re-crystallisation from acetic acid (warming to 90°) followed

by repeated recrystallisation from methyl alcohol leaflets of pure 4-acetoxy-cholesterol, m.p. 161-163°, were obtained. Further quantities of the 4-acetate were recovered from the combined acetic acid filtrates by concentrating in vacuo and reheating to 90° for a few minutes (total yield of pure rearrangement product, 1.91 g.). A mixed melting point with the 3-monoacetate showed a depression (m.p. 140-147°).

Benzoate. To an ice-cooled solution of the above 4-mono-ester (200 mg.) in dry pyridine (3 c.c.), benzoyl chloride (1 c.c.) was added with shaking. After heating on the water-bath for 1 hour the mixture was poured into ice-water and allowed to stand overnight. The product, isolated by means of ether, was a viscous oil which on trituration with cold methyl alcohol gave an abundant yield of crystals. These were filtered off (187 mg.) and purified by repeated recrystallisation from acetone. The acetate-benzoate crystallised in small rectangular platelets of m.p. 165.5-166.5°, depressed on admixture with the starting material. A mixed melting point with an authentic specimen of the 3-benzoate 4-acetate (m.p. 165.5-166.5°), prepared by acetylation of the 3-monobenzoate (m.p. 212.5-213.5°) obtained from a selenium dioxide oxidation of cholesteryl benzoate (Rosenheim and Starling, J.C.S., 1937, 380), showed no depression.

b) <u>By rearrangement of the 3-acetate in propionic acid solution (isolation as benzoate)</u>. The 3-monoacetate (1 g.) was heated in propionic acid (5 c.c.) for 1 hour at 100°. After cooling, impure starting-material separated out and was filtered off (m.p. 174-180°), a further quantity being obtained by addition of water to the heated filtrate until a faint permanent turbidity was produced. This latter material (m.p. 139-146°) was recrystallised once from alcohol giving still slightly contaminated 3-acetate. The combined aqueous propionic acid and alcohol filtrates were diluted with ether (50 c.c.) and the acid washed out with sodium carbonate solution. Distillation of the washed and dried (sodium sulphate) ethereal layer gave a yellow gum which was taken up in dry pyridine and benzoylated at room temperature in the usual manner. On pouring the benzoylation mixture into ice-water a red oil separated which showed no tendency to become granular on standing. After extracting with ether and washing successively with dilute hydrochloric acid, sodium carbonate solution and water, the ether was removed on the water-bath and the oily residue crystallised by trituration with a small volume of cold methyl alcohol. A white crystalline powder was deposited and filtered off, m.p. 155-161°. Two re-crystallisations from acetone yielded small rectangular platelets of 3-benzoyl-4-acetoxy-cholesterol (0.37 g.), m.p. and mixed m.p. 165.5-166.5°.

Identity of 4-acetoxy-cholesterol and the alleged 3-monoacetate of Marker and Rohrmann.

Cholesteryl acetate was oxidised with selenium dioxide according to the directions of Marker and Rohrmann (J.A.C.S., 1939, 61, 3022). The crude product on repeated recrystallisation from methyl alcohol gave leaflets of m.p. 161-163°, undepressed on admixture with a pure specimen of the 4-monoacetate of the same melting point, but depressed with the 3-monoacetate (m.p. 189.5-190.5°).

The benzoate of this material, prepared with benzoyl chloride and pyridine in the usual manner, was crystallised from acetone, separating from this solvent in small rectangular platelets of m.p. 165.5-166.5°. A mixed melting point with 3-benzoyl-4-acetoxy-cholesterol (m.p. 165.5-166.5°) showed no depression.

Attempted preparation of a polymorphic modification of the 3- or 4-monoacetate.

The 4-acetate (m.p. 161-163°) was recrystallised from ether-methanol yielding lustrous leaflets of the same melting point (and mixed m.p.), depressed to about 140° as is that of the original material, on admixture with a specimen of the 3-acetate (m.p. 189.5-190.5°).

A pure specimen of the 3-acetate was recrystallised from methyl alcohol yielding small leaflets of m.p. 189.5-190.5°, undepressed on admixture with the material before crystallisation from this solvent, but depressed to about

140° when mixed with the 4-acetate.

While, as stated above, a mixture of equal parts of the 3- and 4-monoacetates melted at about 140-147°, it was observed that a mixture of the same substances, respectively, in the ratio 3:1 melted at 165-179°.

Preparation of the glycol carbonate (compound B) by loss of alcohol from:

a) **3-Carbomethoxy-4-hydroxy-cholesterol in boiling acetic acid.** The monocarbomethoxy-compound (200 mg.) was refluxed for 1 hour with glacial acetic acid (10 c.c.) to which water (0.5 c.c.) had been added. After diluting with water and ether-extracting the combined extracts were deacidified with sodium carbonate solution. The residue (m.p. 140-147°) obtained on distillation of the washed and dried (sodium sulphate) etherial solution was repeatedly recrystallised from alcohol yielding lustrous leaflets (137 mg.) of m.p. 173-173.5°, undepressed on admixture with compound B (m.p. 173-173.5°).

b) **3-Carbethoxy-4-hydroxy-cholesterol in boiling acetic acid.** A solution of the carbethoxy-ester (400 mg.) in glacial acetic acid (20 c.c.; water, 1 c.c.) was refluxed for 30 minutes. Isolated as before, the product, a gum, was crystallised by trituration with alcohol giving crystals of m.p. 168-170.5° (183 mg.). After repeated recrystallisation from alcohol the glycol carbonate, m.p. and mixed m.p. 173-173.5°, was obtained.

c)   3-Carbomethoxy-4-hydroxy-cholesterol in boiling pro-
pionic acid.   After boiling a solution of the monoester
(100 mg.) in propionic acid (10 c.c.; water, 0.5 c.c.)
for 1 hour the product was isolated in the usual manner
and purified from alcohol.   4-Hydroxy-cholesteryl carbon-
ate, m.p. and mixed m.p. 173-173.5°, was again isolated.

Attempted rearrangement of 3-carbomethoxy-cholesterol with-
out loss of methyl alcohol.

The ester (0.6 g.) was dissolved in the minimum
amount of glacial acetic acid at 90° and maintained at this
temperature for 1 hour.   Rosettes of small needles crystall-
ised out on cooling and were filtered off, m.p. 151-155°.
On repeated recrystallisation from alcohol this material was
identified as unchanged starting-material (mixed m.p.).
The acetic acid filtrate was vacuum distilled and the
residue after repeated recrystallisation from alcohol melted
at 172.5-173°, undepressed on admixture with compound B.
Evaporation of the filtrates from this purification gave
small amounts of material which proved to be impure 3-carbo-
methoxylate.

Carbomethoxylation and carbethoxylation of 4-acetoxy-
cholesterol.

3-Carbomethoxy-4-acetoxy-cholesterol.   To the 4-monoacetate
(0.33 g.; m.p. 161-163°) (prepared, as above, by rearrange-
ment of the 3-acetate in acetic acid) in a mixture of

benzene (7 c.c.) and methyl chloroformate (3.5 c.c.), an-
hydrous pyridine (6 c.c.) was added dropwise at 0°.   After
standing for 3 hours at room temperature (closed by a
calcium chloride-tube) the product was worked up in the
usual manner yielding an oil which was crystallised by
means of cold alcohol.   On filtration, the crude material
(0.21 g.;   m.p. 156-158°) was repeatedly recrystallised from
alcohol, separating from this solvent in microcrystalline
form, m.p. 160-160.5°.   The melting point of the carbo-
methoxylate-acetate was depressed when it was mixed with
the starting-material, but no depression was observed on
admixture with compound A (m.p. 160.5-161°).

3-Carbethoxy-4-acetoxy-cholesterol.   Using the same quanti-
ties the 4-acetate was acylated with ethyl chloroformate as
described above, and the resultant oil triturated with cold
alcohol.   The product (0.27 g.) on repeated recrystallisa-
tion from acetone gave long, slightly flattened needles of
the mixed-ester, m.p. 163-163.5°.   A mixed melting point
with compound C (m.p. 163-163.5°) showed no depression.

Action of methylmagnesium iodide of the glycol carbonate.

        The diol carbonate (0.5 g.;   1 mol.) dissolved in
absolute ether (20 c.c.) was treated with an ethereal
solution of methylmagnesium iodide (approx. 0.38 g.;
2 mols.) in an atmosphere of hydrogen, and allowed to stand
overnight.   After pouring into ice-water and acidifying
with dilute hydrochloric acid the product was extracted

with ether.   Distillation of the washed (sodium carbonate solution and water) and dried (sodium sulphate) ethereal solution yielded a gum which was crystallised from hot alcohol.   The needles (m.p. 75-77°) which separated out on cooling were repeatedly recrystallised from methyl alcohol from which a pure compound of m.p. 77-78° was finally obtained.   A mixed melting point with an authentic specimen of coprostenone (m.p. 79-80°) showed a marked depression, none being obtained on admixture with the product (m.p. 79-80°; probably pseudocholestene) from the sodium and amyl alcohol reduction of $\triangle^{4}$-cholestene-3,6-diol.

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PART II.

THE PHYTOSTEROLS OF CALYCANTHUS OIL.

DISCUSSION.

In 1888, Eccles[1] isolated from the seeds of Caly-
canthus glaucus (a shrub growing in Georgia, North Carolina
and Tennessee) 39% of fixed oil together with an alkaloid
which he named calycanthine.   Wiley[2] also had been examin-
ing both the oil and the alkaloid and reported the content
of the former to be even higher (47%).   A second alkaloid,
isocalycanthine, was isolated from Calycanthus glaucus by
Gordin[3].

Calycanthus floridus, another member of the botanical
family of Calycanthaceae, was also found to contain calycan-
thine[4], and in 1938 Barger and coworkers[5] obtained from
this source a third alkaloid, calycanthidine.

A by-product from the latter work was a considerable
amount of the plant oil, the seeds having been initially
defatted before extraction of the alkaloids.   Since no
examination of the phytosterol fraction of this oil appeared
to have been made, a preliminary investigation of this matter
was considered desirable.

By analogy with other highly evolved plants, i.e.,
phanerogams, the presence in this oil of some of the following
commonly occurring phytosterols was to be expected[6]:-

a) the usual sitosterol mixture (α_1-, α_2-, α_3-, β-, γ-,
   and possibly δ- and ϵ-),

b) sitostanol (dihydrositosterol), and

c) stigmasterol.

As described on p.120 extraction of the seeds with cold benzene yielded about 33% of a pale yellow oil of low alkaloid content.   The larger supply of oil in hand had been separated by means of hot benzene and possessed a dark-brown colour, the latter probably originating from the highly coloured hulls.   Moreover, it contained appreciably more alkaloid, the preliminary removal of which was deemed advisable. This was accomplished by taking the oil up in petroleum ether and washing with dilute mineral acid.   The crude calycanthine thus obtained amounted to some 4% of the weight of the oil.

Hydrolysis of the oil was effected in the normal manner with methyl alcoholic potash, and after troublesome distillations and extractions, a small supply of the unsaponifiable components was eventually accumulated.   This material was obtained as an oily crystalline mass composing about 1.2% of the original oil.   Treatment with petroleum ether removed the unsaponifiable oil, yielding pure white crystals of the phytosterol mixture.

Fractionation of the phytosterols from alcohol afforded a number of crops (A to E, see table, p.123), which differed but slightly in melting point, indicating little or no separation.   Subsequent work showed, however, that a partial separation had in fact occurred, this being all that was originally intended.

Since a positive Tortelli-Jaffé colour reaction with phytosterols indicates the presence of the α-sitosterol

mixture[7]), each of the crops was tested according to the procedure adopted by Westphal[8]. Only in the case of the most soluble fraction (F-G) did a faint green colour develop, suggesting the probable presence, in low concentration, of one or more of the α-sitosterols[*]. In view of the fact that the latter represent the most soluble portion of phytosterol mixtures their concentration in this fraction was to be expected.

The presence or otherwise of stigmasterol and sitostanol in crop A, i.e., the least soluble fraction, was next investigated; these two compounds being most likely to occur here. Stigmasterol is conveniently separated via its sparingly soluble acetate tetrabromide[9][**], but the observed non-formation of a well-definted precipitate on bromination of an acetic acid solution of the phytosteryl acetates would appear to indicate its absence from Calycanthus oil.

Anderson and Nabenhauer[10) successfully applied a modified Liebermann-Burchard reaction to the removal of unsaturated from saturated sterols. A portion of crop A (recovered from the previous bromination by dehalogenation) treated according to this method yielded a minute amount of

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[*] Since α_1-sitosterol contains an inert $C_8:C_{14}$ olefinic linkage (Bernstein and Wallis, J.A.C.S., 1939, 61, 2308), the positive Tortelli-Jaffé reaction may be ascribed to it (see Westphal, loc. cit.). The other α-sitosterols (of as yet uncertain structure) may, however, react similarly.

[**] Brassicasterol (Windaus and Welsch, Ber., 1909, 42, 612) and poriferasterol (Bergmann et al., J. Org. Chem., 1941, 6, 452; 1942, 7, 341, 428) also give sparingly soluble acetate tetrabromides.

material sufficient only for a melting point determination.
This latter indicated that the substance was probably dihydro-
sitosterol.

It having been established that phytosterol mixtures
are more completely separated by crystallisation of the
benzoates (and 3,5-dinitrobenzoates)[11] rather than the
acetates[12], the subsequent study of the sterol fractions
was directed to an examination of the benzoylation products.

Benzoylation of the remaining portion of crop A
afforded a crystalline ester, the melting point of which
corresponded closely with that reported for β-sitosteryl
benzoate. This fact appeared somewhat curious since γ-sito-
sterol, a common plant product, had been expected to be
isolated from this fraction, and the further characterisation
of the compound was therefore undertaken.

Hydrolysis yielded the free sterol, which was con-
verted into its acetate and 3,5-dinitrobenzoate. The melting
points of these substances, together with the specific rota-
tion of the phytosterol itself, indicated with a high degree
of probability, if not certainty, that the compound was β-
sitosterol. The observed and reported values are tabulated
on the following page.

The nature of the compound was later confirmed by
direct comparison with an authentic specimen of β-sitosterol.

Similar treatment of the remaining crops again led to
the isolation of β-sitosteryl benzoate, and only in the case
of the most soluble fraction did the mother liquors yield any

Sterol m.p.	$[\alpha]_D$	Acetate m.p.	Benzoate m.p.	3,5-dinitro-benzoate m.p.	Refer-ences.
136 - 137	-36.6	125- 126	146- 147	202- 203	13
135- 135.5	-34.2	126- 127	145- 146	207- 209	14
136- 137	-31.5	122- 123	146- 147	---	15
137.5- 138.5	-34.0 (CHCl$_3$)	126.5- 127.5	145.5- 146.5	208- 209	Values obtd.

other compound.   In this instance a small amount of material was obtained, hydrolysis of which gave crystals of lower melting point than that of β-sitosterol.   Since the substance gave an intense Tortelli-Jaffé reaction, a clear indication of the presence of α-sitosterol was obtained, but unfortunately no more material was available for further characterisation.

From the results obtained in this investigation it would therefore appear that the phytosterol fraction of Calycanthus oil consists mainly of β-sitosterol (at least 70% can be safely assumed), together with a small amount of α-sitosterol, and possibly sitostanol.   In view of the small amount of phytosterol examined, however, the presence in low concentration of γ-sitosterol and other compounds cannot be disregarded.

Calycanthus oil therefore resembles cottonseed oil[13] in having a high content of β-sitosterol.   Soya bean[7] and

wheat germ[12] oils, on the other hand, contain γ-sitosterol
as their principal component.

The interesting[16] fact has recently come to light
that β-sitosterol, which is 24-ethyl-cholesterol, can be
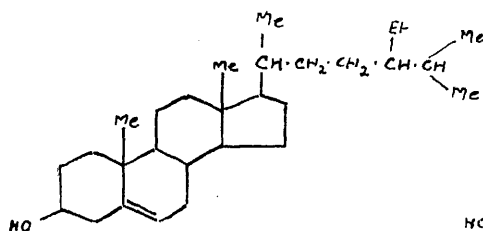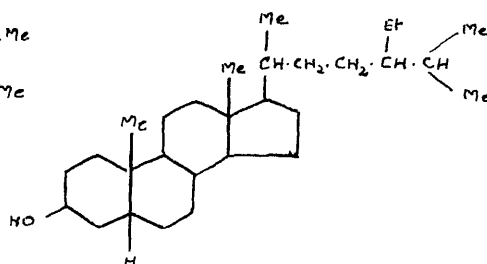
β-Sitosterol

Coprositostanol
(24-Ethyl-coprostanol)

metabolised by the animal organism yielding a homologue of
coprosterol, i.e., coprositostanol.

EXPERIMENTAL.

Extraction of the fixed oil from Calycanthus seeds.

a) Small scale.

The following procedure was based on that employed by Gordin (J.A.C.S., 1905, 27, 144).

The roughly ground seeds (kernels and hulls, 250 g.) were covered with benzene (800 c.c.) and allowed to stand at room temperature for one week with occasional agitation, after which the solution was filtered and the residue washed with a small amount of the solvent. A pale yellow oil (81.5 g.) was obtained on distillation (reduced pressure) of the combined filtrates. The deoleated material was ground to a fine powder and the extraction repeated giving a further small quantity (1.5 g.) of the oil, the extraction then being considered to be complete.

This oil only contained traces of alkaloid.

b) Large scale.

The above yield of oil (33.2%) corresponded very closely to that obtained (32.4%) by Roche Products Ltd. in an extraction employing 43.8 kg. of the seeds and 90 kg. of benzene. Owing to a misunderstanding in the latter, however, the seeds had been deoleated with hot solvent with the result that appreciable quantities (3.8%, see below) of the alkaloidal fraction passed into the oil. In addition, the oil was opaque and dark-brown in colour, due presumably to extraction of the colouring matter from the pigmented hulls.

This dark-brown oil was mainly employed in the present investigation, the preliminary removal of its alkaloidal content being considered advisable.

Isolation of the alkaloidal fraction from Calycanthus oil.

The oil was diluted and rendered more mobile with twice its volume of petroleum ether (b.p. 40-60°), a small deposit of amorphous material, probably protein, being filtered off. The liquid was then repeatedly extracted with dilute hydrochloric acid (concentrated acid, water ; 1:4) until caustic soda solution no longer gave a precipitate. After rendering the combined acid extracts alkaline, the precipitated alkaloids were filtered off, washed with water and dried (yield, 3.8%). A small sample of the crude material was repeatedly crystallised from acetone-hexane and methyl alcohol yielding pure calycanthine, m.p. 245.5-247°.[*]

Saponification of the alkaloid-free oil.

The above alkaloid-free liquor was deacidified with sodium carbonate solution, and after washing with water the oil was recovered by distillation of the solvent. Saponification of the oil (580 g.) was effected by refluxing with methyl alcoholic potash (190 g. in 2,500 c.c.) for 8 hours, when the orange-red homogeneous solution was vacuum distilled to about quarter volume. During the later stages of the distillation considerable bumping occurred owing to the separation of potassium salts. This was avoided to some extent by the

[*] All melting points are corrected.

addition of water, but the resultant frothing also proved
troublesome.   After diluting with water (2,000 c.c.) the
unsaponifiable components were exhaustively extracted with
ether and the combined extracts washed with dilute hydro-
chloric acid to remove traces of alkaloid.   Distillation of
the washed and dried (sodium sulphate) ether solution yielded
an oily crystalline residue (6.6 g.;   yield, 1.14%).

The yield of unsaponifiable material obtained in a
similar manner from the sample of pale yellow oil was slight-
ly higher (1.6%).

Isolation of the phytosterol mixture.

The combined crystalline residues (9.7 g.) from
several saponifications were taken up in the minimum amount
of boiling petroleum ether (b.p. 40-60°) and filtered hot
from traces of amorphous material.   Crystals separated out
on cooling, and after standing for 24 hours at 0°, were
filtered off, m.p. 135-137°.   Fractional concentration of
the petroleum ether filtrate afforded further crops of
crystalline phytosterols (which were added to the initial
crop), and finally a reddish-brown uncrystallisable oil
(yields: phytosterols, approx. 60%;   oil, 40%).

Fractional crystallisation of the phytosterols.

The phytosterol mixture (5.83 g.) was submitted to a
triangle scheme of fractional crystallisation from absolute
alcohol involving 36 crystallisations.   The melting points
and yields of the final series of crops are tabulated below:

			more soluble fractions →			
Crop	A	B	C	D	E	F & G
Melting point	134-135.5°	134.5-135.5°	133-135°	135-136.5°	132.5-135°	132-134.5°
Yield	1.33 g.	0.66 g.	0.39 g.	0.09 g.	0.32 g.	0.13 g.
Total yield, 2.92 g.						

Crops A to G all had the same crystalline form, i.e., elongated leaflets.

Tortelli-Jaffé colour reaction.

Approximately 0.5 mg. of each of the fractions A to F-G was dissolved in 0.2 c.c. of glacial acetic acid and carefully "underlayered" with 0.1 c.c. of a 2% solution of bromine in chloroform, the results being compared with similarly executed positive and negative controls using α-cholestenol and cholesterol respectively. The reaction was negative with the test portions A to E, but in the case of the combined fraction F-G, a faint green colour developed after a few minutes.

Acetylation and bromination of crop A.

Crop A was acetylated by refluxing with freshly distilled acetic anhydride (10 c.c.) for one hour, after which the residual anhydride was decomposed with water and the product filtered off. A solution of the dry acetate (1.45 g.), m.p. 118.5-123.5°, in ether (15 c.c.) was mixed

with bromine (0.3 c.c.) in glacial acetic acid (20 c.c.) and
the mixture allowed to stand at room temperature for a few
hours.   Only a slight trace of material separated out, in-
sufficient for a melting point determination.

Debromination.

Zinc dust (1.5 g.) was added to the above bromination
mixture and the ether was distilled off on the water-bath.
After adding glacial acetic acid (50 c.c.) debromination was
completed by refluxing for 3 hours, when the solution was
filtered hot from the residual zinc.   The phytosteryl
acetate (m.p. 118-122°) was recovered in crystalline con-
dition by addition of water to the heated filtrate.

Examination for the presence of dihydrositosterol.

The recovered acetate was hydrolysed with 10% methyl
alcoholic potash and the product, isolated by means of ether,
was crystallised from alcohol giving elongated leaflets
(0.85 g.) of m.p. 133.5-135°.   The latter (0.5 g.) were
dissolved in a mixture of carbon tetrachloride (30 c.c.) and
acetic anhydride (10 c.c.), contained in a separating funnel,
and concentrated sulphuric acid (10 c.c.) was run in slowly
with cooling.   A transient reddish-purple colour quickly
turned to dark green.   After standing for a few minutes
water was added, a few drops at a time, while shaking and
cooling.   Separation into two layers soon occurred, when the
slightly coloured carbon tetrachloride solution was drawn off
and washed thrice with water.   The small residue obtained on

distillation of the solvent was saponified with methyl
alcoholic potash, the isolated sterol after 2 crystallisa-
tions from alcohol forming leaflets of (probably) dihydro-
sitosterol, m.p. 137-138°.

Isolation of β-sitosteryl benzoate from crop A.

The remainder of the free sterol (0.35 g.) was
dissolved in dry pyridine (5 c.c.) and a few drops of benzoyl
chloride were added with cooling in ice-water.   After heating
on the water-bath for one hour the dark-red solution was
poured into ice-water and allowed to stand for one day.
Crystallisation of the granular filtration residue from
alcohol-benzene afforded platelets (0.41 g.) of m.p.143-144°.
Two recrystallisations from the same solvent yielded pure
β-sitosteryl benzoate, m.p. 145.5-146.5°.

Saponification.   The pure benzoate was refluxed for 2 hours
with 5% methyl alcoholic potash, when the hydrolysate was
diluted with water and ether extracted.   Distillation of
the washed and dried (sodium sulphate) extracts yielded a
crystalline residue of m.p. 126.5-129°, which after repeated
recrystallisation from absolute alcohol gave elongated
leaflets of pure β-sitosterol, m.p. 137.5-138.5°;
$[\alpha]_D^{18°} = -34.0°$ (ℓ = 1, c = 2.267 in chloroform).

Acetylation.   The above β-sitosterol was refluxed for one
hour with acetic anhydride, the acetyl derivative crystalli-
ing out on cooling, m.p. 126-127°.   After one recrystallisa-
tion from absolute alcohol pure β-sitosteryl acetate,

m.p. 126.5-127.5°, was obtained.

Esterification with 3,5-dinitrobenzoyl chloride.   The pure
sterol[*] was heated for one hour at 100° with 3,5-dinitro-
benzoyl chloride (Saunders et al., Biochem. J., 1942, 36,
268) in pyridine solution, the ester being worked up in the
customary manner.   Repeated recrystallisation of the crude
product from cyclohexane yielded β-sitosteryl 3,5-dinitro-
benzoate, m.p. 208-209°, depressed on admixture with a pure
specimen (m.p. 202.5-204.5°) of 3,5-dinitrobenzoic acid.

Benzoylation of crops B, C, D, and E.

Crop B was benzoylated as before yielding platelets
(0.71 g.) of m.p. 145-146°, undepressed on admixture with
β-sitosteryl benzoate (m.p. 145.5-146.5°) from crop A.

Similar treatment of crops C, D, and E afforded
further quantities of the same benzoate; m.ps. 145-146°
(0.4 g.), 144.5-145.5° (0.11 g.) and 144.5-145.5° (0.37 g.)
respectively.

The above samples were combined and recrystallised
twice from alcohol-benzene yielding pure β-sitosteryl
benzoate (m.p. 145.5-146.5°).

Examination of the mother liquors from all the above
crystallisations afforded no substance other than the above
ester.

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[*]   The β-sitosterol here employed was derived from
crops B to F; having been isolated and purified by way of
its benzoate (see below).

Examination of crop F-G for the presence of the α-sitosterols.

β-Sitosterol was removed from crop F-G by benzoylation followed by one crystallisation of the crude esters from alcohol-benzene (yield of β-sitosteryl benzoate 0.14 g., m.p. 144.5-145.5°). Distillation of the filtrate afforded a small amount of crystalline material (m.p. 101-123°) which was saponified with methyl alcoholic potash and the free sterol crystallised from dilute alcohol. Small crystals of m.p. 130-132.5° were obtained, sufficient only for a colour test. The Tortelli-Jaffé reaction gave an intense green colour indicating the presence of the α-sitosterol mixture.

Final identification of β-sitosterol.

The free sterol obtained by hydrolysis of the above (purified) benzoates was crystallised to constant melting point (137.5-138.5°) from absolute alcohol. When mixed with an authentic specimen of β-sitosterol (from tall oil), m.p. 137.5-138.5°, no depression in the melting point was observed.

Benzoylation of the uncrystallisable oil and the combined filtrates from the initial fractionation of the crude phytosterol mixture yielded a further quantity of β-sitosteryl benzoate (1.47 g.). Assuming fraction A to consist almost entirely of β-sitosterol, the total amount of the latter actually isolated represents about 70% of the original phytosterol mixture.

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     Gloyer and Schuette, ibid., 1939, 61, 1901;
     Bernstein and Wallis, ibid., 1939, 61, 1903.

12). Anderson, ibid., 1924, 46, 1450;
     Nabenhauer and Anderson, ibid, 1926, 48, 2972;
     Anderson and Shriner, ibid., 1926, 48, 2976;
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PART III.

THE PROBABLE TRANS-FUSION OF RINGS C AND D IN STEROIDS.

THEORETICAL.

When this work was commenced some doubt had arisen regarding the proposed trans-linkage of rings C and D in steroids. The trans-hydrindane structure had been inferred from the fact that 12-keto-cholanic acid[1] and deoxycholic acid[2] had been degraded to the tricarboxylic acid (I), the anhydride (II) of which had yielded an isomeric acid (III) on hydrolysis. The first acid must therefore have its "C_{13}-" and "C_{14}-" carboxyl groups on opposite sides of the plane of the ring, the second acid (III) being its cis-isomer. It had hence been argued that steroids have the same configuration about C_{13} and C_{14} as the acid (I).

(I)   (II)   (III)
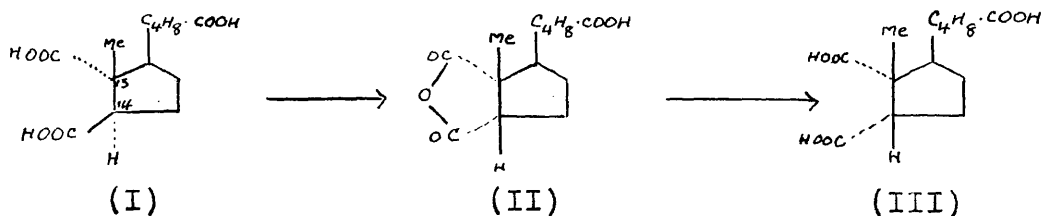
Peak[3] suggested, however, that the configuration of the compound (I) might bear no relationship to that in the original starting-materials, since the possibility of inversion in some of the intermediate steps leading to the formation of the former was not excluded.

In support of a cis-fusion of rings C and D he[3] quoted work on derivatives of cis- and trans-hydrindane[4], indicating that the cis form is the more stable, while the other structure has a moderate degree of strain.

Now, β-ergostenol (V) had been obtained[5] from

ergosterol (IV) (by a somewhat similar series of reactions to that illustrated on p.132 in the case of the analogous cholesterol derivatives), its structure having been subsequently confirmed by Laucht[6]. Catalytic hydrogenation of this substance had afforded a compound which had been found to be identical with the direct hydrogenation product (VI), ergostanol, of ergosterol[7].

(IV)   (V)   (VI)
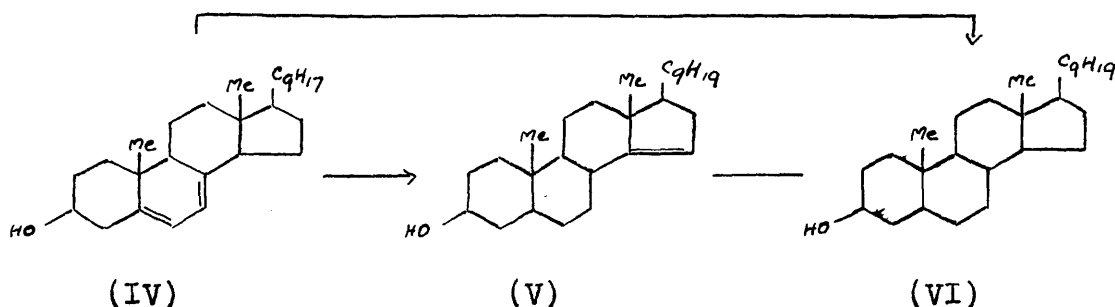
Since one would expect the more stable (cis) configuration to be formed on saturation of the C_{14}-C_{15} olefinic linkage in (V), Peak proposed a cis-fusion of rings C and D in the natural product (IV)[*].

Schenck and his collaborators[8] reduced 7-dehydrocholesterol (VII, R = H) with sodium and alcohol and obtained γ-cholestenol (X). The latter was isomerised to the α-isomer (VIII, R = H) in the presence of a platinum or palladium catalyst, the product being very resistant to hydrogenation. α-Cholestenyl acetate (VIII, R = Ac) was also obtained by direct catalytic hydrogenation of 7-dehydro-cholesteryl

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[*]   A similar inference can be drawn from the results of a degradation of digoxigenin (Steiger and Reichstein, Helv. Chim. Acta, 1938, 21, 828).

acetate (VII, R = Ac). When α-cholestenyl benzoate (VIII,

R = C_6H_5.CO) was treated with dry hydrogen chloride in chloro-

form solution a further isomerisation occurred, the double-

(VII)   (VIII)   (IX)

(X)   (XI)   (XII)
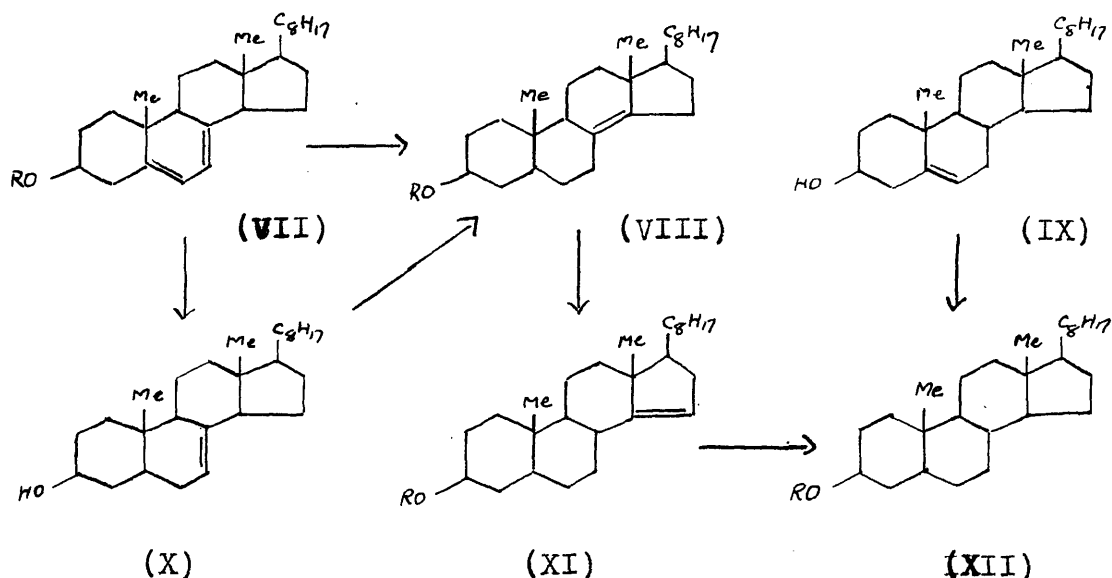
bond migrating to the C_{14}-C_{15} position yielding β-cholestenyl

benzoate (XI, R = C_6H_5.CO). The corresponding acetate (XI,

R = Ac) underwent a relatively facile hydrogenation (with a

platinum catalyst) yielding a saturated compound (XII, R =

Ac), the melting point of which was undepressed on admixture

with a specimen of cholestanyl acetate prepared directly from

cholesterol (IX).

Trans-linkage of rings C and D in the natural pro-

ducts, however, seemed probable, and since hydrogenated β-

stenols were considered to have the cis-arrangement (in con-

formity with the relative stabilities of cis- and trans-

hydrindanes) the two cholestanols obtained ought to have been

stereoisomers. It therefore appeared desirable to investi-

gate the validity of this supposed identity.

7-Hydroxy-cholesterol (XIV, R = H) was prepared as described by Windaus and his collaborators[9], i.e., by aluminium isopropylate reduction of 7-keto-cholesteryl acetate (XIII).   The crude diol thus obtained was purified in a similar manner by benzoylation, repeated recrystallisation of the product from acetone-methyl alcohol yielding pure 7-hydroxy-cholesteryl dibenzoate (XIV, R = C_6H_5·CO).   The overall yield was slightly lower than that reported by the above workers, this probably being due to incomplete reduction (see below) and to the employment of undistilled aluminium isopropylate.

(XIII)   (XIV)   (XV)
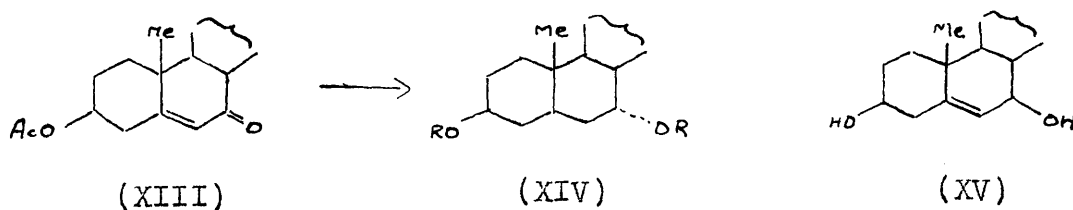
Since a new centre of asymmetry is formed by hydrogenation of steroid cyclic keto-groups, a mixture of two epimeric alcohols may result; but depending on the configuration of the molecule and on variations in the reduction procedure adopted, the formation of one of the epimers is generally favoured.   A Meerwein-Ponndorff reduction of 7-keto-cholesterol, therefore, may reasonably be expected to yield some 7(β)-hydroxy-cholesterol (XV) in addition to the above (α-) isomer (XIV).

A search for the β-compound was accordingly made; the acetone-methyl alcohol mother liquors from the purification of 7(α)-hydroxy-cholesteryl dibenzoate being examined.   Concentration of these liquors led to the isolation of a small

amount of colourless well-formed needles which melted at 159.5-161° giving liquid crystals. This anisotropic phase disappeared at 183.5°, a curious pea-green colour appearing over the last degree. Barr and co-workers[10] prepared 7(β)-hydroxy-cholesterol by permanganate oxidation of cholesteryl hydrogen phthalate, and gave the melting point of the dibenzoate as 150-151°. The higher melting point of the isolated compound therefore indicated either a more highly purified specimen of 7(β)-hydroxy-cholesteryl dibenzoate, or the presence of a different substance. Some experiments were hence carried out with a view to settling this point.

Treatment of the by-product with dimethylaniline according to the method of Haslewood[11] resulted in the material being recovered unchanged. Since the dibenzoate of the α-isomer afforded under the same conditions a good yield of 7-dehydro-cholesteryl benzoate (see p.143), this fact alone strongly indicated that the substance was other than expected.

At this stage a review of the experimental procedure was made, and it was concluded that the material was in all probability the previously undescribed benzoate of 7-keto-cholesterol (XVII, $R = C_6H_5 \cdot CO$). Thus, 7-keto-cholesteryl acetate remaining after an incomplete reduction would be hydrolysed to the free keto-alcohol in the subsequent treatment with cold methyl alcoholic potash (to decompose the aluminium compound of 7-hydroxy-cholesterol). The 7-hydroxy-cholesterol and 7-keto-cholesterol thus formed would furnish the mixture of benzoates in the final esterification.

Depending on the conditions, 7-keto-cholesteryl acetate yields either of two products on treatment with methyl alcoholic potash[18]. By refluxing gently with dilute sodium methylate (or by allowing a hydrolysis with methyl alcoholic potash to take place at room temperature, see p.142) the reaction follows the normal course yielding the keto-alcohol (XVI). When, however, a moderately concentrated solution of methyl alcoholic potash is employed at the boiling point, acetic acid is split off and $\triangle^{3,5}$-cholestadiene-7-one* (XVIII) is formed.

(XVI)   (XVII)   (XVIII)
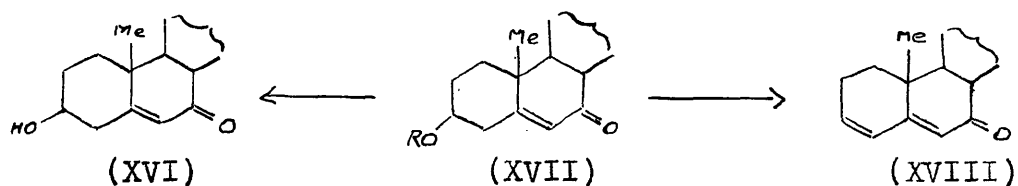
It was to be expected that 7-keto-cholesteryl benzoate would show a similar behaviour, and hydrolyses in cold and boiling methyl alcoholic potash were accordingly attempted. Boiling 3% methyl alcoholic potash yielded a product which when pure melted at 112-113°. A mixed melting point with a specimen of $\triangle^{3,5}$-cholestadiene-7-one prepared by similar treatment of 7-keto-cholesteryl acetate, and having the same melting point, showed no depression. Hydrolysis in the cold with ethereal methyl alcoholic potash, on the other hand, yielded

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*   Jackson and Jones (J.C.S., 1940, 659) have prepared this compound in almost quantitative yield by treating 7-keto-cholesteryl acetate with a hot solution of hydrobromic acid in acetic acid.

a product of melting point 166-167.5°, which was undepressed
on admixture with a specimen of 7-keto-cholesterol prepared
by an analogous saponification of the corresponding acetate.
Thus the formation of 7-keto-cholesterol and $\triangle^{3,5}$-cholesta-
diene-7-one proved conclusively that the by-product was
7-keto-cholesteryl benzoate. The latter was subsequently
obtained by benzoylation of 7-keto-cholesterol.

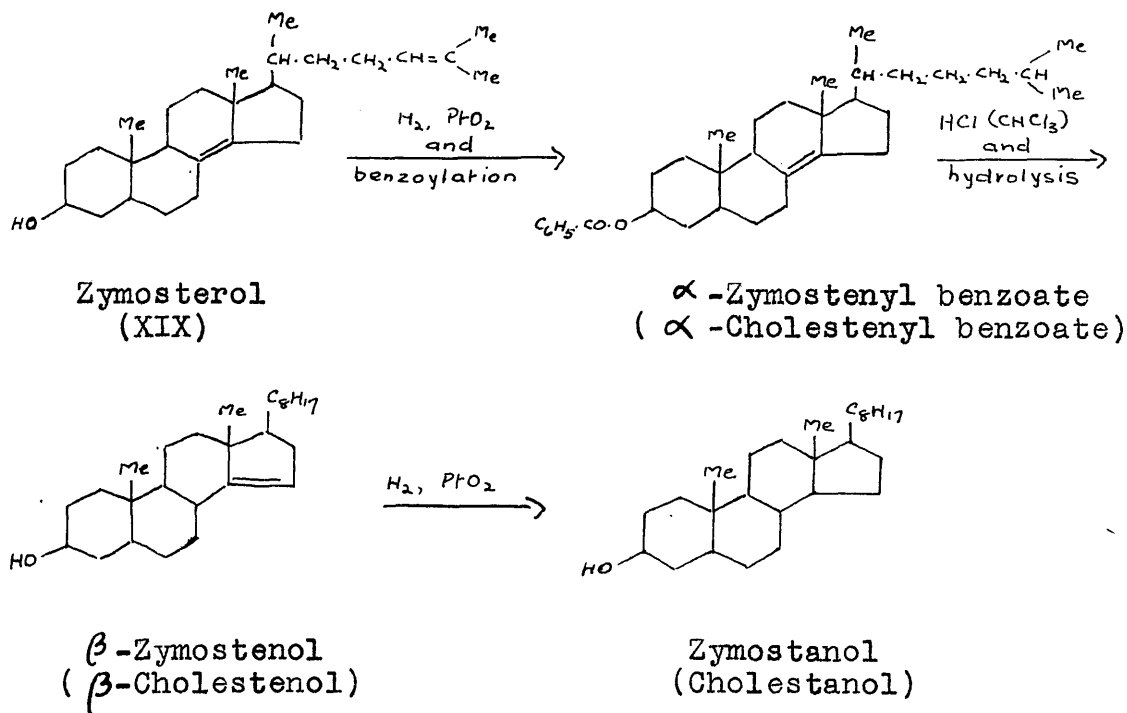The search for 7(β)-hydroxy-cholesterol was not
pursued any further.

7-Dehydro-cholesteryl benzoate was prepared from
7(α)-hydroxy-cholesteryl dibenzoate by Haslewood's method[11],
the yield (71%) being considerably higher than that obtained
(58%) by the older process of vacuum pyrolysis[9]. The
benzoate was then converted directly into α-cholestenyl
benzoate by hydrogenation in acetone solution using a pallad-
ium catalyst (Schenck employed the acetate), the subsequent
isomerisation to β-cholestenyl benzoate being carried out
as described in the literature[8]. The latter compound was
dissolved in ethereal acetic acid and hydrogenated in the
presence of platinic oxide, the amount of hydrogen taken up
corresponding to the saturation of four double bonds. It
therefore appeared that both the $C_{14}:C_{15}$ olefinic linkage
and the benzoyl radical had been reduced, this being supported
by the analytical data. Confirmation of this view was
obtained when the compound was hydrolysed and the acid fraction
isolated and examined. The latter proved to be a low melting

(29-30°) acid, the calcium salt of which crystallised in the characteristic needles of hexahydrobenzoic acid[12].

The neutral fraction of the above saponification afforded a compound, which after a rigorous process of puri-fication <u>proved to be identical with cholesterol-prepared cholestanol</u>, thus:

	$[\alpha]^{19°}_{5500}$ (CHCl$_3$)	M.p.	Mixed m.p.
Dihydro-β-cholestenol:	+26.7°	141.5-142.5°	
Cholestanol:	+25.0°	141.5-142.5°	141.5-142.5°

Shortly before this work had reached its final stages a paper[13] appeared describing the constitution of zymosterol. This compound was shown to be $\triangle^{8,24}$-cholestadiene-3(β)-ol (XIX) and during the course of its investigation the reaction sequence illustrated below was effected:

Zymosterol
(XIX)

α-Zymostenyl benzoate
(α-Cholestenyl benzoate)

β-Zymostenol
(β-Cholestenol)

Zymostanol
(Cholestanol)

By direct comparison of the melting points and optical rotations of a large number of derivatives, zymostanol was shown to be identical with (cholesterol-prepared) cholestanol. For the free alcohols the following data were given[*]:-

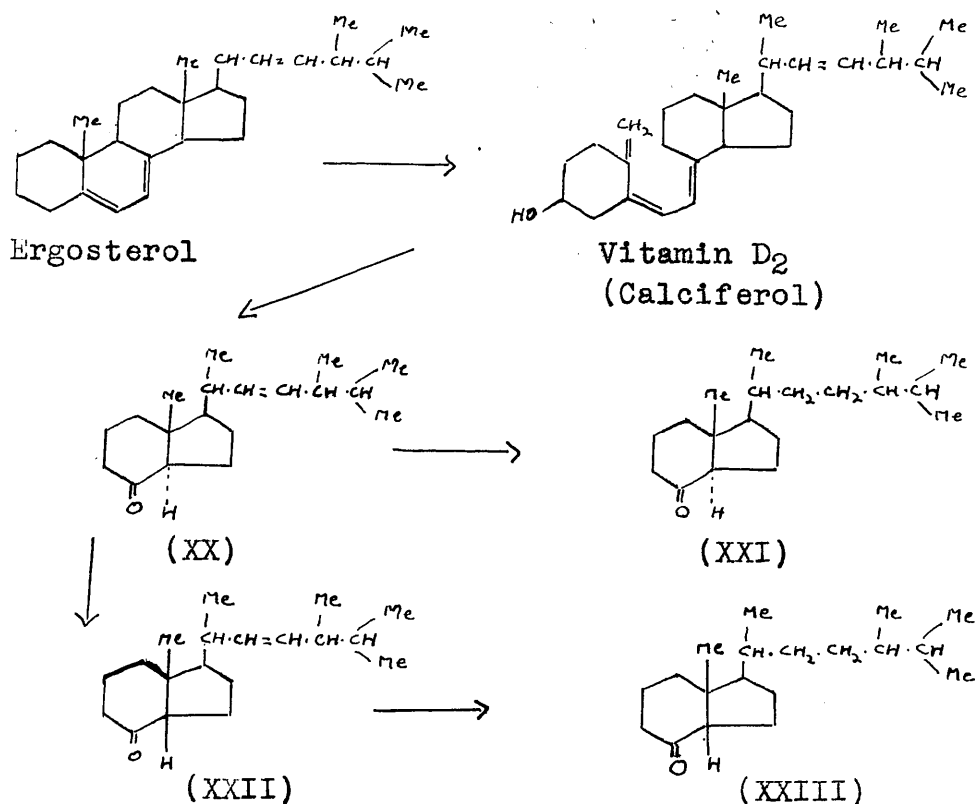|	M.p. (uncorr.)	$[\alpha]_D^{20°}$
Zymostanol :	140-141°	+24.8°
Cholestanol :	140-141°	+24.1°

(ℓ = 1, c = 0.6 in chloroform).

No doubt can therefore be entertained regarding the identity of the compounds prepared by hydrogenation of cholesterol and β-cholestanol.

In 1941 Dimroth and Jonsson[14] published a paper describing certain experiments which render highly probable the postulated trans-fusion of rings C and D in steroids (see also Giacomello, p.12). Their evidence is briefly as follows:-

The ketone (XX) (having been obtained previously by permanganate oxidation of calciferol[15]) underwent a facile and irreversible rearrangement in the presence of acid or alkali (cf. p.20) yielding the compound (XXII). Catalytic hydrogenation of the latter afforded a saturated ketone (XXIII) which differed from that obtained (XXI) in a similar manner from (XX)[15], or by hydrogenation of the maleic anhydride adduct of vitamin D_2 acetate followed by treatment with ozone[16].

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[*]   Shriner and Ko (J.Biol.Chem., 1928, 80, 6) gave m.p. 142-143° (corr.) and $[\alpha]_D^{25°}$ = +26.35° ($CHCl_3$) for a specimen of cholestanol prepared by catalytic hydrogenation (PtO_2) of cholesterol in ethyl acetate. Since this sample gave no Liebermann-Burchard reaction they considered it to represent the completely reduced sterol.

Ergosterol → Vitamin D₂ (Calciferol)

Me CH·CH₂·CH·CH·CH Me Me Me

(XX)

(XXI) Me CH·CH₂·CH₂·CH·CH Me Me Me

(XXII)

(XXIII)

In view of the relative stabilities of cis- and trans-hydrindanes it is clear that the isomerisation of (XX) can only be expected to occur _if it is a trans-compound_, and since calciferol has been shown to have the same spatial orientation of rings C and D as its precursor ergosterol (see below), the latter must have these rings _trans_-fused.

The relating, stereochemically, of ergosterol and calciferol was due to Windaus and Dimroth[17], and was based

Calciferol → "Pyrovitamin" (XIV) → Dehydroergosterol (XXV)
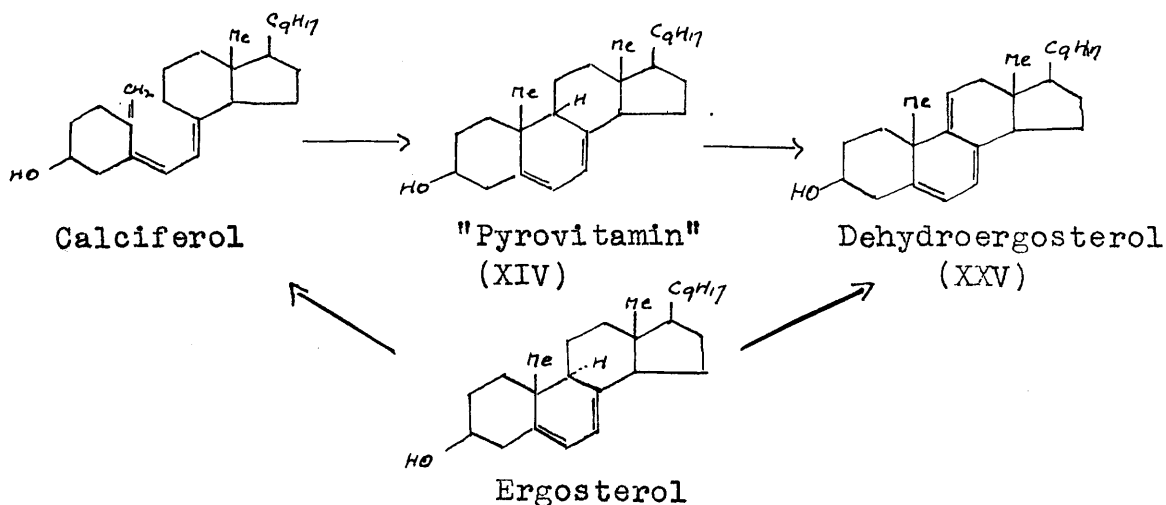
Ergosterol

on the observation that isopyrocalciferol (XXIV) (one of two products derived from thermal treatment of vitamin D_2) and ergosterol yield the same dehydro-compound (XXV).

The clear indication that steroids have rings C and D trans-linked, together with the identity of the cholestanols, discussed above, must therefore mean that contrary to expectation a trans-hydrindane structure is produced when β - stenols are hydrogenated. The explanation for this apparent anomaly must lie in the fact that the strain effects produced by rings A and B on the remaining cyclic portion of the molecule is such as to stabilise the trans-linkage of these latter rings (C and D).

(XXVI)

$(R = C_8H_{17}$ or $C_9H_{19})$
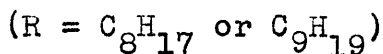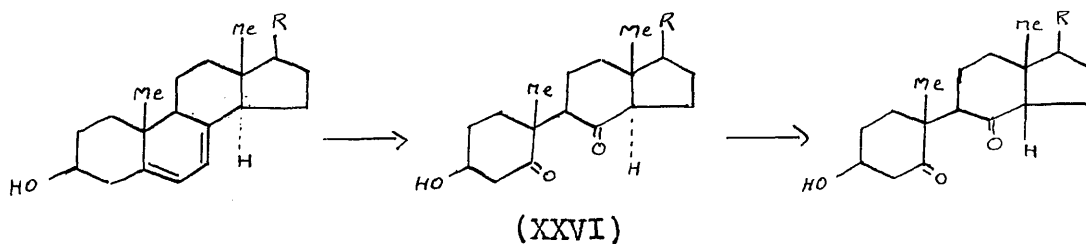
If this be correct then fission of one of the bonds in ring B adjacent to ring C, ought to eliminate this strain and allow the molecule to conform to the requirements of hydrindane structures. Hence it would be interesting to prepare by oxidative degradation of 7-dehydro-cholesterol or dihydro-ergosterol the compound (XXVI) and to ascertain whether it would rearrange on treatment with dilute acid or alkali.

<u>EXPERIMENTAL.</u>

<u>The by-product from the Meerwein-Ponndorff reduction of</u>

<u>7-keto-cholesteryl acetate.</u>

Concentration of the combined mother liquors from
the initial acetone-methyl alcohol crystallisation of
successive batches of 7-hydroxy-cholesteryl dibenzoate
yielded colourless well-formed needles (2.4 g.), m.p.* 135-
138°, which gave a marked depression of the melting point on
admixture with the above dibenzoate (m.p. 170-170.5°).
After repeated recrystallisation from acetone and absolute
alcohol the substance was obtained pure, melting at 159.5-
161° to an opaque liquid, which suddenly became pea-green in
colour at 182.5°, and finally yielded a clear, colourless
liquid at 183.5°. On cooling this transient colour was
again observed.

<u>Investigation of the by-product.</u>

<u>Treatment with dimethylaniline.</u>

The material (0.3 g.) was refluxed for 9 hours with
freshly distilled dimethylaniline (8 c.c.) and the solution
cooled. After pouring into dilute hydrochloric acid and
ether-extracting, the ethereal layer was washed, dried over
sodium sulphate, and the solvent removed on the water-bath.
The residue on recrystallisation from acetone melted at
159-160.5°, undepressed on admixture with the starting-material.
Further quantities of the substance were recovered from the

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* All melting points are corrected.

filtrate, a nearly quantitative return being obtained (0.295 g.).

Saponification.

a) With boiling methyl alcoholic potash. The substance
(0.5 g.) was refluxed for one hour with methyl alcoholic
potash (30 c.c.; 3%), whereupon the solution was diluted
with water and extracted with ether. The washed and dried
ethereal layer was evaporated to dryness on the water-bath,
and the yellow residue repeatedly recrystallised from methyl
alcohol, yielding light yellow prisms of m.p. 112-113°. A
mixed melting point with an authentic specimen of $\triangle^{3,5}$-
cholestadiene-7-one, m.p. 112-113° (prepared by a similar
hydrolysis of 7-keto-cholesteryl acetate; Mauthner and Suida,
Monatsh., 1896, 17, 579), showed no depression.

b) **With ethereal methyl alcoholic potash at room temperature.**
The by-product (0.5 g.) was dissolved in ether (15 c.c.) and
a solution of potassium hydroxide (0.3 g.) in methyl alcohol
(10 c.c.) added. After allowing the clear solution to stand
overnight at room temperature it was poured into water and
extracted with ether. Evaporation of the washed and dried
extract yielded a residue (0.45 g.) which was repeatedly
recrystallised from 70% methyl alcohol giving a felt of fine
needles, m.p. 166-167.5°, depressed on admixture with the
starting-product. A mixed melting point with a specimen of
7-keto-cholesterol (m.p. 166-167.5°) prepared from the
acetate in a similar manner, showed no depression. The
authenticity of the latter was ascertained by its reacetylation

to 7-keto-cholesteryl acetate, m.p. and mixed m.p. 159-160°.

7-Keto-cholesteryl benzoate from 7-keto-cholesterol.

7-Keto-cholesterol (1 g.) (prepared by mild hydrolysis of the acetate as indicated above) was dissolved in anhydrous pyridine (15 c.c.) and benzoyl chloride (0.5 g.; 50% excess) added at 0°. On standing overnight at room temperature the mixture was poured into ice-water, and the filtered residue digested with cold alcohol (10 c.c.). The filter residue was taken up in ether and the benzoic acid was removed with dilute sodium carbonate solution. After washing and drying, the solvent was removed on the water-bath. Repeated recrystallisation of the residue from alcohol and acetone yielded pure 7-keto-cholesteryl benzoate in the form of long colourless needles melting at 159.5-161° to an opaque liquid, becoming pea-green at 182.5°, and clearing at 183.5°. A mixed melting point with the "by-product" showed no depression.

Found: C, 80.98; H, 9.68%

$C_{34}H_{48}O_3$ requires: C, 80.95; H, 9.52%.

7-Dehydro-cholesteryl benzoate.

7-Hydroxy-cholesteryl dibenzoate was refluxed for 8 hours with dry freshly distilled dimethylaniline in the ratio of 1 g. of the dibenzoate to 25 c.c. of the solvent. The cooled solution was poured into dilute hydrochloric acid and extracted with choloroform. After washing, the dried (calcium chloride) extract was evaporated and the residue

recrystallised twice from the minimum amount of boiling
chloroform by precipitation with acetone.   Small plates
of 7-dehydro-cholesteryl benzoate, m.p. 136-137°, crystallised
out, further quantities being recovered by concentration and
reprecipitation with acetone (yield, 71%).

α-Cholestenyl benzoate.

7-Dehydro-cholesteryl benzoate (1 g.) dissolved in
pure acetone (500 c.c.; see Analyst, 1933, 58, 335) was
shaken with palladium black (200 mg.) in an atmosphere of
hydrogen.   Absorption occurred steadily and was complete in
4 hours, one molecule of hydrogen being taken up.   The
solution was decanted from the catalyst through an acid-
hardened filter paper, and concentrated until crystallisation
commenced.   When cold the α-cholestenyl benzoate was
filtered off, m.p. 111.5-112.5° (yield, 0.89 g.).

β-Cholestenyl benzoate.

The isomerisation of α-cholestenyl benzoate (5 g.)
in chloroform solution at 0° with dry hydrogen chloride, was
carried out exactly as described in the literature (Schenck
et al., loc. cit.).   After washing the chloroform solution
with dilute sodium bicarbonate and water, it was dried over
calcium chloride and evaporated on the water-bath.   Two
crystallisations of the residue from acetone yielded long
needles of pure β-cholestenyl benzoate, m.p. 165.5-166°
(yield, 3.1 g.;   62%).

Catalytic hydrogenation of β-cholestenyl benzoate.

A suspension of platinic oxide (0.1 g.) in glacial acetic acid (50 c.c.) was reduced with hydrogen; β-cholestenyl benzoate (3 g.) was then added together with sufficient ether to give a clear solution, and the whole shaken in an atmosphere of hydrogen until absorption ceased (2.5 hours). Approximately 4 molecules of hydrogen were absorbed, no break in the absorption curve being apparent after the addition of 2, 4 and 6 atoms. After removal of the catalyst the ether was distilled off on the water-bath, and the acetic acid under reduced pressure, yielding finally a white crystalline residue of m.p. 158-158.5°. The substance was obtained pure on recrystallisation from absolute alcohol, separating out as colourless needles of m.p. 158.5-159°; $[\alpha]_{5500}^{19} = +26.7°$ ($\ell = 1$, c = 0.6 in chloroform) (yield, 2.91 g.), strongly depressed on admixture with the starting material.

Found:	C, 82.17;	H, 11.66%
Cholestanyl hexahydro-benzoate, $C_{34}H_{58}O_2$, requires:	C, 81.93;	H, 11.65%

Saponification of the hydrogenation product from β-cholestenyl benzoate.

The above product (2.5 g., m.p. 158.5-159°) was dissolved in a hot mixture of ether and methyl alcohol (200 c.c.) containing potassium hydroxide (0.5 g.) and refluxed for 2 hours on the water-bath, when the cooled solution was diluted with water and ether-extracted. The hydrolysate was examined

for both the neutral and acid components.

The neutral fraction.   Evaporation of the washed and dried ether extract yielded a residue, which after recrystallisation from dilute alcohol melted at 141-142° (yield, 1.31 g.).   The last traces of unsaturated sterol were removed from the latter according to the method of Anderson and Nabenhauer (loc. cit.) yielding finally leaflets of pure cholestanol, m.p. 141.5-142.5° (i.e., after recrystallisation from dilute alcohol), undepressed on admixture with an authentic specimen of cholestanol (m.p. 141.5-142.5°) prepared as described below.

The acid fraction.   The alkaline solution from the hydrolysis of the reduction product was rendered acid with dilute hydrochloric acid, and the organic acid extracted with ether. Evaporation of the washed extract yielded a small amount of gum which showed a tendency to crystallise.   On recrystallisation from water, pure hexahydrobenzoic acid, m.p. 29-30°, was obtained.   The acid was further characterised by conversion into the calcium salt.

Preparation of authentic cholestanol.

Cholesterol was hydrogenated in the usual manner with palladium black, and the isolated product, purified as described previously, gave leaflets of m.p. 141.5-142.5°; $[\alpha]_{5500}^{19} = +25.0°$ ($\ell = 1$, c = 0.6 in chloroform).

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