STUDIES IN THE STEROID GROUP.

THESIS

submitted by

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PREFACE.

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PART I.

EXPERIMENTS IN RELATION TO AN ATTEMPTED
PARTIAL SYNTHESIS OF HYODEOXYCHOLIC ACID FROM CHOLESTEROL.

THE MECHANISM OF ACYL MIGRATION FROM C₃ TO C₄
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\textsuperscript{1}) initiating his classical investigations on cholesterol in 1903, the latter\textsuperscript{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\textsuperscript{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\textsuperscript{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

![Chemical structure]

(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)*. Catalytic hydrogenation of the latter yielded the saturated compound cholestene (IV)*.

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*.

The correlation of the fully hydrogenated compounds was effected by means of an isomer of cholestene (pseudocholestene), obtained* by the elimination of hydrogen

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*The following degradations, 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 hydro-
genation 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

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 C17 or C20 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 coprostone, the parent hydrocarbons of the sterol group, have the molecular formula C27H48, 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 C24H40O2 again suggesting the presence of four rings.
carried out in 1919 by Windaus and Neukirchen\textsuperscript{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\textsuperscript{12} that the odour resembled that of methylhexylketone, Windaus\textsuperscript{13} undertook its isolation. From the product 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:

$$\text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me}$$

$$\text{(AcO} \cdot C_{19}H_{28}) \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH} \quad \rightarrow \quad \text{O=CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}$$

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:

$$\text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me}$$

$$\text{-CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH} \quad \rightarrow \quad \text{-CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH} \quad + \quad \text{CO}$$

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 coprostanone oxidation.

Seven years later, the convergence of the two series was indisputably established\textsuperscript{14} by the reconversion of cholanic acid into coprostanone as follows. Chromic acid oxidation of the carbinol (XII), obtained from ethyl cholanate (XI) and isopropylmagnesium iodide, yielded a ketone (XIII) (coprostanone-24-one), which was reduced to coprostanone (XIV) with zinc amalgam.
It was thus evident that the parent bile acids exhibit precisely the same isomerism as cholestane and coprostanone, 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 ($\alpha$-) 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 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).

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 and of an X-ray crystallographic study of ergosterol and some of its irradiation products, 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 had carried out some dehydrogenation experiments with sterols and bile acids, isolating from the resulting mixtures small amounts of three aromatic hydrocarbons. By 1932 one of these had been identified as chrysene (XVII). Rosenheim and King drew attention to this neglected observation, and working on the assumption that the carbon skeleton of chrysene represented the cyclic moiety 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).

![Formulae (XVII), (XVIII), and (XIX)](image-url)

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 and Wieland and Dane 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, together 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 resulting 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 coprostanone (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\textsubscript{10}-methyl group has been chosen as the reference point\textsuperscript{*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)\textsuperscript{**}.

\begin{center}
\includegraphics[width=0.5\textwidth]{formula.png}
\end{center}

\begin{itemize}
  \item \textsuperscript{*} Originally, Ruzicka referred all configurations to the C\textsubscript{5}-hydrogen, but difficulties were encountered in compounds lacking this reference point. The assumption had to be made that \(\Delta^{\text{II}}\)-steroids were theoretically derived from the coprostanone series, and that \(\Delta^{\text{III}}\)-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\textsubscript{9}-hydrogen as a convenient point of reference.

  \item \textsuperscript{**} 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\textsubscript{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.
\end{itemize}
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 coprostone 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\textsuperscript{23) and chemical grounds\textsuperscript{24)}, that rings A and B in the cholestane series are linked in a trans-decalin manner, while coprostone (and the bile acids) has the corresponding cis-structure.

The evidence for a trans-linking of rings B and C, although somewhat meagre\textsuperscript{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
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 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.

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\textsuperscript{27}, and a few authentic cases of inversion at this centre in "synthetic" products have been reported\textsuperscript{28}. Only one reliable instance of stereoisomerism at C_{20} is on record\textsuperscript{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.

* 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 C₃-hydroxyl group.

Both configurations of the C₃-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 infers, on the basis of the Auwers-Skita hydrogenation rule as applied to the reduction of cholestane-3-one and coprostane-3-one, that sterols have their C₃-OH cis to the C₁₀-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 C₅-H or the C₅-C₆ cyclic component giving exactly opposite configurations in each case, e.g.,

\[
\text{Referring to } \text{C}_5\text{-H} \\
\text{Referring to } \text{C}_5\text{-C}_6
\]

* The following methods of nomenclature are in general use:

I. C₃-OH cis to C₁₀-Me -------- normal or \( \beta \)-configuration.
   C₃-OH trans to C₁₀-Me ------ epi or \( \alpha \)-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 C₃- and C₇-hydroxyl groups trans to the C₁₀-Me and is named 3(\( \alpha \)), 7(\( \alpha \))-dihydroxy-cholanic acid; ursodeoxycholic acid, which is its C₇-epimer, is 3(\( \alpha \)), 7(\( \beta \))-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\textsuperscript{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(\(\beta\))-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\textsuperscript{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\textsuperscript{34}) find that the acetates and benzoates of epimeric sterols having rings A and B trans-fused, 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 coprostan e 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 \(220^\circ\) following the formation of a \(\Delta^*\)-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\textsuperscript{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

Hyodeoxycholic acid

Cholic acid

Lithocholic acid
e.tc.

Cholanic acid
THEORETICAL.

Hyodeoxycholic acid was discovered by Strecker 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 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.

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 allo- cholanic 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"-dehydro- hyodeoxycholic acid; 3,6-diketocholestanic acid). This compound is unstable, however, and rearranges to 3,6-diketoallo- cholanic acid (XXVIII) ("β"-dehydrohyodeoxycholic acid) on
treatment with dilute acid or alkali. It is evident, therefore, that a Clemmensen reduction of the "$\alpha$"-acid (XXVII) must involve its preliminary allomerisation to the "$\alpha$"-isomer (XXVIII), which then reduces normally to allocholanic acid (XXIX).

![Diagram of chemical structures]

Like the other naturally occurring bile acids, the C3-OH in this compound has the customary $\alpha$-configuration, but no degradation yet effected enables the orientation of the C6-OH to be fixed.

While investigating the alleged presence of epimeric bile acids in nature, Kimura isolated from pig bile a new acid which, like hyodeoxycholic acid, yielded "$\alpha$"-dehydro-hyodeoxycholic acid on oxidation with chromic acid. The new acid, named $\beta$-hyodeoxycholic acid, must therefore be a stereoisomer of ($\alpha$-) hyodeoxycholic acid. Applying an analogous reaction sequence, formulae (XXX) to (XXXII), to that employed by Wieland and co-workers in the case of
the α-isomer, formulae (XXXIII) to (XXXV), Kimura proved that β-hyodeoxycholic acid had its C₃-OH in the β-configuration.

On the basis of the above degradations alone, he postulates that the two acids are C₃-epimers, but the possibility of the C₆-OH in the β-acid having a different configuration cannot be excluded.

The formulae employed in the above paper appear to imply a specific (β-) configuration for the C₆-OH in both
the $\alpha$- and $\beta$-hyo acids; their confusing nomenclature, however, offers a possible clue - see formulae (XXXVI) and (XXXVII). Why two groups, C$_3$-OH and C$_6$-OH, situated on opposite sides of the ring system in (XXXVI) should have the same prefix ($\alpha$) can only be explained as follows. While the terms $\alpha$ and $\beta$ as applied to the C$_3$-OH in (XXXVI) and (XXXVII) indicate its specific trans and cis relationship, respectively, to the C$_{10}$-Me; the term $\alpha$ as applied to the C$_6$-OH is presumably to be interpreted as implying an arbitrary (i.e., yet unknown) configuration. The formula for $\alpha$-hyodeoxycholic acid is taken from a paper by Sugiyama 43), where the same nomenclature is again employed.

Until the configuration of the C$_6$-OH has been settled the two acids are preferably named: $\alpha$-hyodeoxycholic acid, 3($\alpha$),6-dihydroxy-cholanic acid; $\beta$-hyodeoxycholic acid, 3($\beta$),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 isolation. The method employed involved the preliminary
removal of \( \alpha \)-hyodeoxycholic acid and 3-hydroxy-6-ketoallocholanic acid (also present in pig bile) by salting out with sodium chloride, followed by precipitation of the crude \( \beta \)-glycohyodeoxycholic acid with hydrochloric acid. The glyco acid was then hydrolysed in the autoclave with aqueous alcoholic potash yielding impure \( \beta \)-hyodeoxycholic acid on acidification. Since the salting out of the \( \alpha \)-acid can hardly be considered to result in its quantitative 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 \( \alpha \)- and \( \beta \)-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 \( \beta \)-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 \( \alpha \)- and \( \beta \)-

* This \( \beta \)-glycohyodeoxycholic acid is undoubtedly identical with the "\( \beta \)-hyoglycocholic acid" isolated from pig bile by Jolin (Z. physiol. Chem., 1887, 11, 417; 1888, 12, 512; 1889, 13, 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 coprostone-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 \( \beta \)-acid from pig bile in such a manner that epimerisation of hydroxyl groups is rendered unlikely; and the indirect method, whereby the \( \alpha \)-acid is subjected to the experimental conditions employed in the isolation of the \( \beta \)-stereoisomer, the product being examined for the presence or otherwise of the latter. A negative result from the indirect procedure would indicate that the \( \beta \)-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 coprostone-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 \( \alpha \)- and \( \beta \)-hyodeoxycholic acid be the same, these acids would be capable of "partial synthesis" from the pair of coprostanediols represented by
either (XXXVIII) or (XXXIX).

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 coprostan-3\((\alpha)\),6\((\beta)\)-diol (XXXIX A) and coprostan-3\((\beta)\),6\((\beta)\)-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 (\(\alpha\)- and \(\beta\)- 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 $C_{4}H_{8}COOH$ 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.,

* In this connection it is worthy of note that in a paper describing the preparation of $6(\alpha)$-hydroxy-progesterone Ehrenstein and Stevens (J. Org. Chem., 1940, 5, 318) state that "it would be interesting to transform hyodeoxycholic acid into a $6$-hydroxyprogesterone and to establish whether the hydroxyl group at carbon atom 6 possesses the $\alpha$ or (3 configuration" but the experimental realisation of this transformation presents difficulties.
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.
The stereochemical relationship of the two isomeric 3(β), 5,6-trihydroxycholestanes (triols-I and -II) has been examined by Ellis and Petrow. Triol-I, which is obtained by oxidation of cholesterol with hydrogen peroxide or by hydrolysis of α-cholesterol oxide, has been shown by Criegee to be a trans-α-glycol (C5-C6). Triol-II is prepared by the oxidation of cholesterol with either potassium permanganate or osmic acid and, as is to be expected from its method of preparation, is a cis-α-glycol. Since oxidation of the isomeric triols yields different 5-hydroxy-3,6-diketocholestanes, it follows that the two triols differ only in orientation around C5. 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 C4 to yield a C4-C6 oxide is excluded because of the stability of the oxide obtained. Oxidation of 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 C4-C6 rupture, a behaviour known to be characteristic of C5-keto-steroids in which rings A and B are trans-fused. 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 C₅-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) C₂⁺C₃ 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 trans-fused. Secondly, the oft-observed fact that cis-hydroxylation of 3(β)-hydroxy-Δ₅-steroids by means of osmic acid is inhibited by acetylation finds an explanation if triol-II is represented by (LIV) (cf. Ehrenstein⁵²) below).

Ehrenstein⁵²) has prepared two stereoisomeric 3,5,6-triols (LV) and (LVII) in the pregnane series; (LV) by treatment of Δ⁵-pregnen-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 same diketo-compound (LVIII), and hence the configuration of the C₅-OH is

![Diagram](https://via.placeholder.com/150)

(LV)

(LVI)

(LVII)

(LVIII)
identical in both. Thus while the cholestanetriols are stereoisomeric with respect to the configuration of the \(\text{C}_{5}\)-OH, the pregnanetriols differ solely in epimerism at the \(\text{C}_{6}\)-OH.

Ehrenstein proposes the above configurational formulae (LV) and (LVII) on the basis of some observations due to Butenandt and Westphal. These workers find that when the \(\text{C}_{3}\)-OH (\(\beta\)-) of dehydroisoandrosterone is esterified, no addition of osmic acid to the \(\text{C}_{5}:\text{C}_{6}\) double bond occurs, and explain this on steric grounds, i.e., in \(\Delta^{5}\)-steroids having a \(\text{C}_{3}\)-OH in the \(\beta\)-position (\(\text{C}_{3}\)-OH cis to \(\text{C}_{10}\)-Me), acylation should sterically hinder the addition of osmic acid, provided that the latter leads to a \text{cis}-linkage of rings A and B.

In this connection Ehrenstein makes the following interesting statement:-

"Ellis and Petrow recently investigated the stereochemical configuration of the cholestane-3,5,6-triols. The triol obtained by treating cholesterol with osmic acid was assigned the coprostan configuration, which is in agreement with the stereochemical considerations of our previous paper" (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 peroxide apparently furnished the coprostan 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 coprostan 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 coprostan epimer showed the greater tendency to crystallise."

(2) Ellis and Petrow obtained an excellent yield (85%) of 6(\(\beta\))-acetoxy-cholestane-3,5-diol (XLII) by treating an
alcoholic solution of $3(\beta), 6(\beta)$-diacetoxy-cholestan-5(\(\alpha\))-ol (trioI-I diacetate) (XLI) with one equivalent of potassium hydroxide at room temperature. Chromic acid oxidation of this monoacetate gave $6(\beta)$-acetoxy-cholestan-5(\(\alpha\))-ol-3-one (XLIII) which was dehydrated to $6(\beta)$-acetoxy-\(\Delta^4\)-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 \(\Delta^4\)-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 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(\(\alpha\))-ol (LXIV) (identical with the diketo-alcohol obtainable from "triol-1") and \(\Delta^+\)-cholestene-3,6-dione (LXIII), a small amount of a compound which Mauthner and Suida\(^{57}\) named "\(\alpha\)-oxycholestenol". This substance has been shown to be a \(\Delta^+\)-cholestene-6-ol-3-one and may be identical with (LXI).

Thus:

\[
\begin{align*}
"\alpha\)-oxycholestenol" \quad & \quad \text{m.p.} \quad 180^\circ \\
\Delta^+\)-cholestene-6(\(\beta\))-ol-3-one & \quad \text{m.p.} \quad 192^\circ
\end{align*}
\]

(3) Extending earlier work on reductions in the sterol group, Ruzicka\(^{30}\) established the stereochemical relationships 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, coprostone-3-one (LXVIII) behaves entirely analogously: hydrogenation in neutral solution yielding epicoprosterol (coprostone-3(α)-ol) (LXXI), and in acid media, coprosterol (coprostone-3(β)-ol) (LXXII).

Coprostenone (LXVII) is readily obtainable by oxidation followed by debromination of cholesterol dibromide, or simply by an Oppenauer oxidation of cholesterol itself. Partial hydrogenation (Pd-catalyst) of this unsaturated ketone (LXVII) yields coprostanone (LXVIII).
(also formed from coprosterol by oxidation).

Since $6(\beta)$-acetoxy-$\Delta^4$-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 $6(\beta)$-acetoxy-coprostane-3-one (LXXIV), the further hydrogenation of which (PtO$_2$), in neutral and acid media, should yield $6(\beta)$-acetoxy-coprostane-3(\(\alpha\))-ol (LXXV) and -3(\(\beta\))-ol (LXXVI), respectively.

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) on treatment with dilute acid or alkali (cf. p.19). By analogy with
the partial oxidations of $\alpha$- and $\beta$-hyodeoxycholic acid to the epimeric 3-hydroxy-6-ketocholanic acids, and the allop-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(5)-hydroxy-cholestan-6-one of Mauthner and Suida.

![Chemical structure](image1)

(LXXIX) \[\rightarrow\] (LXXX)

(4) Hydroxylated C$_{17}$-C$_8$H$_{17}$ steroids are degraded to the corresponding C$_{17}$-C$_4$H$_8$.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- $\Delta^5$-cholenic acid (LXXXII).

![Chemical structure](image2)

(LXXXI) \[\rightarrow\] (LXXXII)
By an analogous procedure Marker \(^{63,64}\) obtained "allohyodeoxycholic acid" \( (\beta,6(\beta)-dihydroxyallocholanic acid) \) (LXXXIII) from \(3(\beta),6(\beta)-\text{diacetoxy-cholestane} \) (LXXXIV). This acid proved to be identical with that of Windaus \(^{24}\), i.e., the acid obtained by catalytic hydrogenation of "\(\beta\)"-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.

![Chemical structures](image1)

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 \(6(\beta)-\text{hydroxy-isoandrosterone} \) (LXXXVII) are isolated together with (LXXXII) and (LXXXIII) respectively.

![Chemical structures](image2)

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(\(\alpha\)),6(\(\beta\))- and 3(\(\beta\)),6(\(\beta\))-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.

\[(\text{LXXXVIII})\]
\[(\text{LXXXIX})\]

It would be interesting to test the physiological properties of these latter compounds.
In 1906 Diels and Abderhalden 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 by catalytic hydrogenation of \( \Delta^+ \)-cholestene-3(β),6(β)-dial*; the other, cholestane-3(β),6(α)-dial (XCI), m.p. 216°, results from a sodium and alcohol reduction of 6-ketocholestanol. The formation of the lower melting diol by sodium and amyl alcohol treatment of 6(β)-acetoxy-coprostenone (XCII) (6(β)-acetoxy-\( \Delta^+ \)-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.

\[
\text{(XC)} \quad \text{(XCI)} \quad \text{(XCII)}
\]

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-ketocholestanol 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(\(\beta\)),6(\(\alpha\))-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, a recent finding of Urushibara proved to be of assistance. In a paper which admirably summarises the configurations and inter-relationships of the \(\alpha\)- and \(\beta\)-oxides of cholesterol, the \(\Delta^\pi\)-cholestene-3,6-diols and the cholestane-3,6-diols; he mentions the conversion of cholesterol \(\alpha\)-oxide (XCIII) into cholestane-3(\(\beta\)),6(\(\alpha\))-diol (XCIV) by the action of sodium and amyl alcohol. For this he gave no experimental details, but the procedure seemed quite straightforward.

\[
\begin{align*}
\text{(XCIII)} & \quad \rightarrow \quad \text{(XCIV)}
\end{align*}
\]

Cholesterol \(\alpha\)-oxide was prepared from cholesterol and monoperphthalic acid in ether solution. The yield obtained (87%) compares very favourably with that of Ruzicka and Bosshard (75%), who employed the less stable perbenzoic acid. Sodium and amyl alcohol reduction of the pure \(\alpha\)-oxide gave a small yield of the diol (XCIV), m.p. 218-219.5°, the dibenzoate of which melted at 213-214.5°.
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 C6-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 Δ5-cholestene-3(β),6(α)-diol yields mixtures of cholestane, coprostone, and cholestanol, and cholestane, cholestanol and cholestane-3(β),6(β)-diol, on hydrogenation with palladium and platinum catalysts respectively68), 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 Δ5-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
\( \Delta^5 \)-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 (\( \Delta^4 \)-cholestene)\(^{71} \), which the compound is in all probability.

Since the above catalytic hydrogenation of \( 6(\beta) \)-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 coprostone-3,6-diols was investigated.

The reduction, by means of sodium and amyl alcohol or catalytically, of the oxide ring in cholesterol \( \beta \)-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 \( 6(\beta) \)-hydroxy-coprostenone with a Raney nickel catalyst, however, may be well worth investigating.
(XCV) offered a possible means of preparing coprostan-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.

\[
\begin{align*}
\text{(XCV)} & \quad \rightarrow \quad \text{(XCVI)} & \quad \rightarrow \\
\text{(XCVII)} & \quad \rightarrow \quad \text{(XCVIII)} & \quad \rightarrow \\
\text{(XCIX)} & \quad \rightarrow
\end{align*}
\]

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\(_{10}-\text{Me};\) the discontinuous lines, the trans.
yielded (CVII) and (CII) respectively; while the methyl derivative (CVI) was obtained from the free (\(\beta\)-oxide (CIII, \(R=H\)).

\[
\begin{align*}
\text{(C)} & \xrightarrow{R=Ac} \text{(CI)} & \quad \text{(CII)} & \xrightarrow{R=Ac} \text{(CIII)} \\
\text{R=Ac} & \quad \text{R=H} & \quad \text{R=Ac} & \quad \text{R=Ac} \\
\text{(CIV)} & \quad \text{(CV)} & \quad \text{(CVI)} & \quad \text{(CVII)}
\end{align*}
\]

The fission of oxide rings by means of hydrogen chloride had also been studied by Spring and Swain. Employing cholesteryl benzoate \(\alpha\)- and \(\beta\)-oxides an entirely analogous series of changes resulted, see formulae above.

* Hattori's fission experiments with the \(\beta\)-oxide were carried out with a sample (m.p. 136\(^{\circ}\)) prepared by the action of alcoholic potash on the 5-chloro-derivative of "trioI-I". The lower melting point (112-113\(^{\circ}\)) quoted elsewhere in the literature and confirmed in the present investigation (see p. 8\(^{\circ}\)) appears to be a peculiar property of the \(\beta\)-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 \(\beta\)-oxide, the other being due to association with the \(\alpha\)-oxide as a molecular compound. It is curious, nevertheless, that both "forms" yield the same acetate of m.p. 112-113\(^{\circ}\).

This may explain the isolation of cholestane-3(\(\beta\)), 6(\(\alpha\))-diol from sodium and amyl alcohol reductions of both the \(\alpha\)- and \(\beta\)-oxides (pp. 38 and 44).
It is evident from the above formulae that the oxide ring in cholesterol $\alpha$-oxide tends to split between C$_6$ and the oxide oxygen atom; the $\beta$-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 $\alpha$-oxide towards reduction with sodium and amyl alcohol would appear anomalous, see p.38). Furthermore, the production of a coprostone 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 $\alpha$- and $\beta$-oxide benzoates in slightly different yield from those reported by Spring and Swain in an analogous preparation employing perbenzoic acid (see below). This difference, however, is to be expected.

<table>
<thead>
<tr>
<th></th>
<th>$\alpha$-isomer</th>
<th>$\beta$-isomer</th>
<th>Total yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perphthalic acid</td>
<td>53%</td>
<td>25%</td>
<td>78%</td>
</tr>
<tr>
<td>Perbenzoic acid</td>
<td>46%</td>
<td>38%</td>
<td>84%</td>
</tr>
</tbody>
</table>

A sodium and amyl alcohol reduction of cholesterol $\beta$-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 $\alpha$-oxide benzoate by perphthalic acid treatment of cholesteryl benzoate, no $\beta$-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 $\alpha$-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 C5 and C6 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 hyodeoxycholic acids probably requires a different approach.

* 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 elimination of the C5-OH. "Triol-I" diacetate is probably formed by direct acetalolysis 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 acetalolysis 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, 873) have reduced the β-oxide of 3(α)-acetoxy-Δ"-cholenic acid
Concurrently with the above attempts to reduce 6-acetoxy-Δ^4-cholestene-3-one to the (C5-) epimeric coprostan-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 C5-epimeric diacetate (CXIV) does not yield a monoacetate but merely a mixture of unchanged material and fully hydrolysed triol\(^{52)55}\).

\[
\begin{align*}
\text{(CVIII)} & \quad \text{(CIX)} & \quad \text{(CX)} & \quad \text{(CXI)} \\
\text{(CXII)} & \quad \text{(CXIII)} & \quad \text{(CXIV)}
\end{align*}
\]

(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 3-oxide may yield coprostan-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-\({\Delta}_{4}^{+}\)-cholestene\(^*\) (CXV) (readily obtainable in 25\% yield by selenium dioxide oxidation of cholesteryl acetate, (see pp.47-49) to 6(\(\beta\))-acetoxy-\({\Delta}_{4}^{+}\)-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\(^{58}\).

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.

\(^*\) The configurations assigned to the acetoxy 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 $\Delta^4$-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:

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 $\Delta^5$-cholesten-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 reaction was found to take a different course. The principal crystalline product from a selenium dioxide oxidation of cholesteryl acetate was the diacetate of an isomeric compound, together with a small amount of a monoacetate of the previously 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 configurations (CXXIV) and (CXXVI).
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\(^{0}\) melting compound, but that it was the isomeric \(\Delta^2\)-cholesten-3,6-diol, and they suggested its formation from the 3,4-diol by allylic rearrangement. Finally, since the diols were
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.\textsuperscript{59}

The oxidation of cholesteryl benzoate (in acetic
acid solution) with selenium dioxide was also studied by
Rosenheim and Starling\textsuperscript{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.

\begin{align*}
\text{(CXXVII)} & \quad \text{(CXXVIII)}
\end{align*}

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, $R = \text{Ac}$) or a mixture of this with the 3-carbo-
methoxylate (CXXIX, $R = \text{H}$), 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-
atives of cholesterol used in this investigation were
prepared according to the method of Robberecht\textsuperscript{77}), i.e., by

\textsuperscript{59} 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-\textgon\textsuperscript{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.

\[ \text{(R = Ac and/or H)} \]

An acetic anhydride solution of carbomethoxy-cholesterol was oxidised with selenium dioxide and the product was worked up exactly as described by Butenandt and Hausmann 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\(^\circ\)-161\(^\circ\), yielded cis-\(\Delta^5\)-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 compound 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 carbomethoxylate yielded two derivatives of cis-$\Delta^5$-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 decided to settle the matter by synthetic means.

A cooled solution of the cis-$\alpha$-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,

* The mixed esters of the cis-3,4-diol are more conveniently described by this nomenclature. Thus, the "trivial" and systematic names, 4-hydroxy-cholesterol and cis-$\Delta^5$-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 \( ^{68}73 \) \( ^{78} \) (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 undescribed 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\textsuperscript{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 formation 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).

\begin{align*}
\text{(CXXXI)} & \quad \text{(CXXXII)} \\
\text{(CXXXIII)} & \quad \text{(CXXXIV)}
\end{align*}
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 (CXXXII) --- 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-
6-acetate of the 3,6-diol and the unacetylated 3-monobenzoate of the 3,4-isomer. The medium employed now appears to influence 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 \textit{aliphatic}-3-monoester (Note 2, p.74), tending to rearrange 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-formation of the 3,6-diol, or a derivative thereof, from cholesterol itself (see p.67), i.e.,

1) the formation of the non-acylated 3,4-diol in the oxidation 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 cholesteryl 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 isomeric 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.

* 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.

The "intra-annular" mechanism for the rearrangement of 3-carbomethoxy-4-hydroxy-cholesterol, formulae (CXXXIX) to (CXLII), similarly failed to yield a substance other than the 4-carbomethoxylate (CXLII), 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 conditions of the oxidation (i.e., boiling acetic acid). The product from the fission of methyl alcohol is the intramolecular \( \alpha \)-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\(^{\circ} \), 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

\[
\text{(CXLIV)} \\
\text{(CXLV)} \\
\text{(CXLVI)}
\]
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. An attempt to prepare a similar derivative of the cis-diol (CXLVIII) yielded an uncrystallisable gum which was not further investigated.

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 carbethoxycholesterol —– 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 carbemethoxy- and carbethoxy-cholesterol, respectively).

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\degree; the other, compound D, melted at 121-122.5\degree.

The higher melting substance yielded 4-hydroxy-cholesterol on hydrolysis and proved to be fully acylated, i.e., incapable of acetylation with boiling acetic anhydride. An analysis of the material supported the contention that it was the carbethoxy analogue of compound A (i.e., 3-carbethoxy-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 $\Delta^4$-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)

![Chemical structure](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-\(\Delta^4\)-cholestene-3-ol (compound D), and the 3-carbethoxylate-4-acetate (compound C) and carbonate (compound B) of cis- \(\Delta^5\)-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\(^\circ\)) was prepared by treatment at room temperature of a pyridine solution of 4-hydroxy-cholesterol with one equivalent of acetic anhydride. A saturated solution of the monoacetate in acetic acid at 90\(^\circ\)
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\(^6\) (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\(^8\).

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 mechanisms; 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 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 \( \Delta^7 \)-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-carbomethoxy 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-derivatives 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 previously 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 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) → (CLIX) → (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 Δ⁴-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 cis-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-Δ⁴-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, \( R = \text{Me} \& \text{Et} \), see extending chart between pp. 73 and 74) in acetic anhydride solution yield the respective 3-carbonate-4-acetates (CLXI, \( R = \text{Me} \& \text{Et} \)) of 4-hydroxy-cholesterol, together with the analogous 3-carbonate-6-acetates (CLXII, \( R = \text{Me} \& \text{Et} \)) of the isomeric 3,6-diol. When acetic acid is substituted for its anhydride in these reactions there is formed, in addition, the intramolecular 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, \( R = \text{Me} \& \text{Et} \)) from their preparation by carbomethoxylation and carbethoxylation of the same compound (CLXIX), followed by acetylation of the resulting 3-monoesters (CLXV, \( R = \text{Me} \& \text{Et} \)). 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, \( R = \text{Me} \& \text{Et} \)) 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 intermediate 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).
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 cholesterol 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 cholesterol 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 Δ\(^{\alpha}\)-cholestene-3,6-diol (CLXXXIII) by Ponndorff reduction of 3(Δ\(^{\alpha}\))-acetoxy-Δ\(^{\alpha}\)-cholestene-6-one (CLXXII). The latter obtained from 3(Δ\(^{\alpha}\))-acetoxy-cholestane-6-one (CLXX) by way of the monobromide (CLXXI). Unless an inversion has occurred at the C\(_9\)-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\(_8\)-hydroxyl group.

![Chemical Structures]

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 condensation 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 rearrangement to the 6-acyl compound (see Haworth and Sheldrick, J.C.S., 1934, 864).

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.).

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.
ADDENDUM.

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, 147, 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 C₃ to C₄ 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 monoesters undergo an allylic rearrangement, the acetyl group migrating to C₆, and yield the esters of 14°-cholestene-3,6-diol (CLXXXIV). 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, 70, 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.

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.

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-Δ^5-cholestan 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 Δ^5-cholestan-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.
78.

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 crystallisation 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.


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-Δ4-cholestone-3-one with sodium and amyl alcohol.

6(β)-acetoxy-Δ4-cholestone-3-one was prepared from "triol-l" 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°) 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 (overnight) the yellow layer was separated and the aqueous portion extracted twice with ether. Addition of the ethereal extracts 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°. One recrystallisation from acetone-chloroform followed by two from ethyl acetate gave elongated leaflets (0.12 g.), m.p. 213-214.5°. A mixed melting point with authentic cholestane-3(β),6(α)-diol dibenzoate, m.p. 213-214.5°, (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) ethereal 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° (see below).

**Cholesterol α-oxide.**

An ether solution of paraphthalic 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-Δ⁴-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)-dil 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(3)-acetoxy-Δ⁴-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 Δ⁴-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 Δ5-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 anhydride (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. Long needles (170 mg.) of slightly impure starting-material separated out on cooling.
Hydrogenation. 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 \(\alpha\)- and \(\beta\)-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 \(\alpha\)-oxide (m.p. 167.5-168.5°), identified by its melting point and by its hydrolysis to cholesterol \(\alpha\)-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 (30 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(\(\beta\)),6(\(\alpha\))-diol dibenzoate (m.p. and mixed m.p. 213-214.5\(^\circ\)).

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

**Attempted catalytic hydrogenation of cholesteryl benzoate \(\beta\)-oxide.**

A mixture of the pure \(\beta\)-oxide benzoate (2 g.; m.p. 149.5-150.5\(^\circ\)), 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 recovered on evaporation of the filtered solution.

**Attempted partial hydrolysis of \(\Delta^4\)-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 $\Delta^\text{II}$-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 extract 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 homogeneous, 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-Δ5-cholestene (m.p. 167.5-168.5°). Fractional evaporation of the mother-liquor from the alcohol trituration yielded only further quantities of a substance A (total yield 0.31 g.) and finally a dark-brown crystallisable 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 boiling 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 Δ5-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 recrystallisation from alcohol, melted at 160.5-161°, undepressed 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 leaflets 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-$\Delta^5$-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. $\Delta^4$-cholestene-3,6-diol (3.6 g.) m.p. and mixed m.p. 256-257°, was again obtained.

Investigation of compound A.

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 $\Delta^5$-cholestene-3,4-diol, m.p. and mixed m.p. 174-174.5°.

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°, undepressed on admixture with the starting-material.
Analysis.

Found: C, 74.13; H, 9.62%

Calculated for 3-carbomethoxy-4-acetoxy-cholesterol,
\( \text{C}_{31}\text{H}_{50}\text{O}_5 \):
C, 74.13; H, 9.96%

Monocarbomethoxylation of 4-hydroxy-cholesterol.

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


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%

\( \text{C}_{29}\text{H}_{48}\text{O}_4 \) 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°, unaltered on crystallisation from methyl alcohol. A mixed-melting point with an authentic specimen of 4-hydroxycholesterol (m.p. 174-174.5°) showed no depression.

Attempted acetylation. The material (100 mg.; m.p. 173-173.5°) 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°. One recrystallisation from alcohol raised the melting point to 173-173.5°, 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°, 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:  
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 removed 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° 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°.

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°.

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.
Atmospted 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%

C₃₂H₅₂O₅ 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%

C₃₀H₅₀O₄ 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°).

\[
\text{C}_{37}\text{H}_{54}\text{O}_5 \quad \text{requires:} \quad \text{C}, \ 76.82; \ \text{H}, \ 9.34% \\
\text{Found:} \quad \text{C}, \ 76.41; \ \text{H}, \ 9.25%
\]

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 $ \Delta''$-cholestene-3,6-diol, m.p. and mixed m.p. 256-257°.

**Analysis.**

<table>
<thead>
<tr>
<th>Found</th>
<th>C, 74.62; H, 10.19%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated for 3,6-dihydroxy-$ \Delta''$-cholestene 3-carbethoxylate,</td>
<td>C, 74.42; H, 10.08%</td>
</tr>
<tr>
<td>$C_{32}H_{52}O_{5}$</td>
<td></td>
</tr>
</tbody>
</table>

**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 separating 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-hydroxycholesteryl 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°).

4-Acetoxy-cholesterol.

2) By rearrangement of the 3-acetate in acetic acid solution. 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 recrystallisation 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-monoester (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) By rearrangement of the 3-acetate in propionic acid solution (isolation as benzoate). 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 recrystallisations 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 de-acidified with sodium carbonate solution. The residue (m.p. 140-147°) obtained on distillation of the washed and dried (sodium sulphate) ethereal 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 propionic 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 carbonate, m.p. and mixed m.p. 173-173.5°, was again isolated.

Attempted rearrangement of 3-carbomethoxy-cholesterol without 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 crystallised 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-carbomethoxylate.

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 rearrangement of the 3-acetate in acetic acid) in a mixture of
benzene (7 c.c.) and methyl chloroformate (3.5 c.c.), anhydrous 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 carbomethoxylate-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 quantities 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 recrystallisation 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 $\Delta^4$-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 Calycanthus glaucus (a shrub growing in Georgia, North Carolina and Tennessee) 39% of fixed oil together with an alkaloid which he named calycantheine. Wiley\(^2\) also had been examining both the oil and the alkaloid and reported the content of the former to be even higher (47%). A second alkaloid, isocalycantheine, was isolated from Calycanthus glaucus by Gordin\(^3\).

Calycanthus floridus, another member of the botanical family of Calycanthaceae, was also found to contain calycantheine\(^4\), 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 (\(\alpha, -, \alpha_2, -\), \(\alpha_3, -\), \(\beta, -, \gamma, -\), and possibly \(\delta -\) and \(\epsilon -\)),

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 \( \alpha \)-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 \(\alpha\)-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-defined 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

\* Since \(\alpha\)-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 \(\alpha\)-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) rather than the acetates, 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 $\beta$-sitosteryl benzoate. This fact appeared somewhat curious since $\gamma$-sitosterol, 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 converted into its acetate and 3,5-dinitrobenzoate. The melting points of these substances, together with the specific rotation of the phytosterol itself, indicated with a high degree of probability, if not certainty, that the compound was $\beta$-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 $\beta$-sitosterol.

Similar treatment of the remaining crops again led to the isolation of $\beta$-sitosteryl benzoate, and only in the case of the most soluble fraction did the mother liquors yield any
other compound. In this instance a small amount of material was obtained, hydrolysis of which gave crystals of lower melting point than that of \(\beta\)-sitosterol. Since the substance gave an intense Tortelli-Jaffé reaction, a clear indication of the presence of \(\alpha\)-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 \(\beta\)-sitosterol (at least 70% can be safely assumed), together with a small amount of \(\alpha\)-sitosterol, and possibly sitostanol. In view of the small amount of phytosterol examined, however, the presence in low concentration of \(\gamma\)-sitosterol and other compounds cannot be disregarded.

Calycanthus oil therefore resembles cottonseed oil\(^{13}\) in having a high content of \(\beta\)-sitosterol. Soya bean\(^{7}\) and

<table>
<thead>
<tr>
<th>Sterol m.p.</th>
<th>([\alpha]D)</th>
<th>Acetate m.p.</th>
<th>Benzoate m.p.</th>
<th>3,5-dinitrobenzoate m.p.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>136-137</td>
<td>-36.6</td>
<td>125-126</td>
<td>146-147</td>
<td>202-203</td>
<td>13</td>
</tr>
<tr>
<td>135-135.5</td>
<td>-34.2</td>
<td>126-127</td>
<td>145-146</td>
<td>207-209</td>
<td>14</td>
</tr>
<tr>
<td>136-137</td>
<td>-31.5</td>
<td>122-123</td>
<td>146-147</td>
<td>--</td>
<td>15</td>
</tr>
<tr>
<td>137.5-138.5 (CHCl(_3))</td>
<td>126.5-127.5</td>
<td>145.5-146.5</td>
<td>208-209</td>
<td>Values obtd.</td>
<td></td>
</tr>
</tbody>
</table>
wheat germ oil\textsuperscript{12}) oils, on the other hand, contain \(\gamma\)-sitosterol as their principal component.

The interesting\textsuperscript{16}) fact has recently come to light that \(\beta\)-sitosterol, which is 24-ethyl-cholesterol, can be metabolised by the animal organism yielding a homologue of coprosterol, i.e., coprositostanol.

\begin{center}
\begin{tikzpicture}
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\draw[thick,black] (0,0) -- (1.5,1.5);
\draw[thick,black] (2.5,0) -- (1.5,-1.5);
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\draw[thick,black] (2.5,0) -- (2.5,0);
\draw[thick,black] (0,0) -- (1.5,0);
\draw[thick,black] (2.5,0) -- (1.5,0);
\end{tikzpicture}
\end{center}

\textit{\(\beta\)-Sitosterol}

\begin{center}
\begin{tikzpicture}
\draw[thick,black] (0,0) circle [radius=1.5cm];
\draw[thick,black] (2.5,0) circle [radius=1.5cm];
\draw[thick,black] (0,0) -- (2.5,0);
\draw[thick,black] (0,0) -- (1.5,-1.5);
\draw[thick,black] (0,0) -- (1.5,1.5);
\draw[thick,black] (2.5,0) -- (1.5,-1.5);
\draw[thick,black] (2.5,0) -- (1.5,1.5);
\draw[thick,black] (0,0) -- (0,-1.5);
\draw[thick,black] (2.5,0) -- (2.5,-1.5);
\draw[thick,black] (0,0) -- (0,1.5);
\draw[thick,black] (2.5,0) -- (2.5,1.5);
\draw[thick,black] (0,0) -- (1.5,0);
\draw[thick,black] (2.5,0) -- (1.5,0);
\draw[thick,black] (0,0) -- (0,0);
\draw[thick,black] (2.5,0) -- (2.5,0);
\draw[thick,black] (0,0) -- (1.5,0);
\draw[thick,black] (2.5,0) -- (1.5,0);
\end{tikzpicture}
\end{center}

\textit{Coprositostanol (24-Ethyl-coprostanol)}
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 hydrochloric 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 slightly 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

<table>
<thead>
<tr>
<th>Crop</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F &amp; G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>134-135.5°</td>
<td>134.5-135.5°</td>
<td>133-135°</td>
<td>135-136.5°</td>
<td>132.5-135°</td>
<td>132-134.5°</td>
</tr>
<tr>
<td>Yield</td>
<td>1.33 g.</td>
<td>0.66 g.</td>
<td>0.39 g.</td>
<td>0.09 g.</td>
<td>0.32 g.</td>
<td>0.13 g.</td>
</tr>
</tbody>
</table>

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, insufficient 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 condition 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 crystallisations 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 crystallising out on cooling, m.p. 126-127°. After one recrystallisation from absolute alcohol pure (β-sitosteryl acetate,
Esterification with 3,5-dinitrobenzoyl chloride. The pure sterol* was heated for one hour at 100° with 3,5-dinitrobenzoyl 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-dinitrobenzoate, 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.

* 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 \( \alpha \)-sitosterols.

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

Final identification of \( \beta \)-sitosterol.

The free sterol obtained by hydrolysis of the above (purified) benzoates was crystallised to constant melting point (137.5-138.5\(^\circ\)) from absolute alcohol. When mixed with an authentic specimen of \( \beta \)-sitosterol (from tall oil), m.p. 137.5-138.5\(^\circ\), 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 \( \beta \)-sitosteryl benzoate (1.47 g.). Assuming fraction A to consist almost entirely of \( \beta \)-sitosterol, the total amount of the latter actually isolated represents about 70\% of the original phytosterol mixture.
4. Späh and Stroh, Ber., 1925, 58, 2131.
8. Westphal, Ber., 1939, 72, 1243.
11. Wallis and Fernholz, ibid., 1936, 58, 2446; 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 Shiner, ibid., 1926, 48, 2976; Anderson et al., ibid., 1926, 48, 2987.
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).

Peake\(^3\) 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, \(\phi\)-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\(^7\).

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 \(\gamma\)-cholestenol (X). The latter was isomerised to the \(\alpha\)-isomer (VIII, \(R = H\)) in the presence of a platinum or palladium catalyst, the product being very resistant to hydrogenation. \(\alpha\)-Cholestenyl acetate (VIII, \(R = \text{Ac}\)) 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 \( \alpha \)-cholestenyl benzoate (VIII, 
\( R = C_6H_5.CO \)) was treated with dry hydrogen chloride in chloro-
form solution a further isomerisation occurred, the double-

\[
\begin{align*}
(VII) & \xrightarrow{\text{Me}} (VIII) \\
(X) & \xrightarrow{\text{RO}} (XI) \\
& \xrightarrow{\text{RO}} (XII)
\end{align*}
\]

bond migrating to the \( C_{14}-C_{15} \) position yielding \( \beta \)-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 (\( \beta \)-
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-hydroxycholesteryl dibenzoate (XIV, R = C\(_6\)H\(_5\).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.

\[
\begin{align*}
\text{(XIII)} & \quad \rightarrow \quad \text{(XIV)} \\
& \quad \rightarrow \quad \text{(XV)}
\end{align*}
\]

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(\(\beta\))-hydroxy-cholesterol (XV) in addition to the above (\(\alpha\)-) isomer (XIV).

A search for the \(\beta\)-compound was accordingly made; the acetone-methyl alcohol mother liquors from the purification of 7(\(\alpha\))-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 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 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 = C₆H₅·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. 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 $\Delta^{3,5}$-cholestadiene-7-one* (XVIII) is formed.

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 $\Delta^{3,5}$-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

* 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 \( \Delta^{3,5} \)-cholesta-diene-7-one proved conclusively that the by-product was 7-keto-cholesteryl benzoate. The latter was subsequently obtained by benzylation of 7-keto-cholesterol.

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

7-Dehydro-cholesteryl benzoate was prepared from 7(\( \alpha \))-hydroxy-cholesterol dibenzoate by Haslewood's method, the yield (71\%) being considerably higher than that obtained (58\%) by the older process of vacuum pyrolysis. The benzoate was then converted directly into \( \alpha \)-cholestenyl benzoate by hydrogenation in acetone solution using a palladium catalyst (Schenck employed the acetate), the subsequent isomerisation to \( \beta \)-cholestenyl benzoate being carried out as described in the literature. 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\textsuperscript{12}).

The neutral fraction of the above saponification afforded a compound, which after a rigorous process of purification proved to be identical with cholesterol-prepared cholestanol, thus:

\[
\begin{array}{ccc}
\text{Dihydro-\(\beta\)-cholestenol:} & \alpha \rightarrow 19^\circ \\
\text{(CHCl}_3\text{)} & 5500 & \text{M.p.} \\
\text{Mixed} & 141.5-142.5^\circ & 141.5-142.5^\circ \\
\text{Cholestanol:} & +25.0^\circ & 141.5-142.5^\circ \\
\end{array}
\]

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

\[\text{Zymosterol (XIX)} \xrightarrow{\text{H}_2, \text{PO}_4^+ \text{and benzoylation}} \text{\(\alpha\) -Zymostenyl benzoate (\(\alpha\) -Cholesteryl benzoate)}\]

\[\text{\(\beta\) -Zymostenol (\(\beta\) -Cholestenol)} \xrightarrow{\text{H}_2, \text{PO}_4^+} \text{Zymostanol (Cholestanol)}\]

\[\text{Zymostanol (Cholestanol)} \xrightarrow{\text{HCl (CHCl}_3\text{)} \text{ and hydrolysis}} \text{\(\alpha\) -Zymostenyl benzoate (\(\alpha\) -Cholesteryl benzoate)}\]
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*:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting Point (°C)</th>
<th>[α]_D^20°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zymostanol</td>
<td>140-141°</td>
<td>+24.8°</td>
</tr>
<tr>
<td>Cholestanol</td>
<td>140-141°</td>
<td>+24.1°</td>
</tr>
</tbody>
</table>

(*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 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) 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), or by hydrogenation of the maleic anhydride adduct of vitamin D_2 acetate followed by treatment with ozone.

---

*Shriner and Ko (J. Biol. Chem., 1928, 80, 6) gave m.p. 142-143° (corr.) and [α]_D^20° = +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.
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\(^\text{17}\), and was based
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 $\beta$-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).

\[
\text{(XXVI)}
\]

\[R = \text{C}_6\text{H}_{17} \text{ or } \text{C}_9\text{H}_{19}\]

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.
EXPERIMENTAL.

The by-product from the Meerwein-Ponndorff reduction of 7-keto-cholesteryl acetate.

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.

Investigation of the by-product.

Treatment with dimethylaniline.

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 *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 \( \Delta^{5} \)-cholestenadiene-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-cholesterol 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 $\beta$-cholestenyl benzoate.

A suspension of platinic oxide (0.1 g.) in glacial acetic acid (50 c.c.) was reduced with hydrogen; $\beta$-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°; $[\alpha]_{5500}^{19} = +26.7^\circ$ ($\ell = 1$, $c = 0.6$ in chloroform) (yield, 2.91 g.), strongly depressed on admixture with the starting material.

Found: C, 82.17; H, 11.66%

Cholestanyl hexahydrobenzoate, $C_{34}H_{58}O_2$, requires: C, 81.93; H, 11.65%

Saponification of the hydrogenation product from $\beta$-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°; 

\[
[\alpha]_{5500}^{19} = +25.0^\circ \quad (l = 1, c = 0.6 \text{ in chloroform}).
\]
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