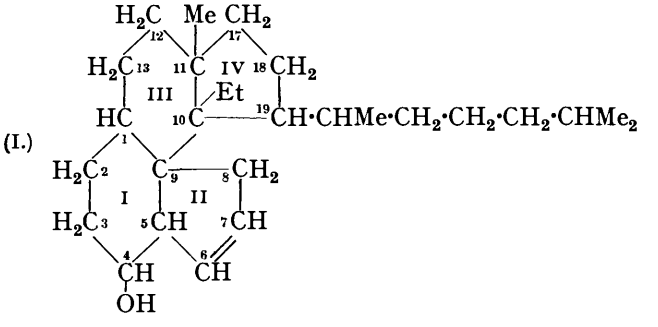
158. *The Structure of the Sterols and Bile Acids.*

(Augmented from a paper read before the Chemical Society for discussion on December 15th, 1932.)

By I. M. Heilbron, J. C. E. Simpson, and F. S. Spring.

The investigations carried out, notably by Borsche, Wieland, and Windaus, during the last two decades have shown that a close relationship exists between cholesterol and the bile acids. A large amount of the evidence bearing on the structure of these compounds is embodied in the excellent account given by Professor C. K. Ingold in the *Annual Reports* for 1927 and 1928, where the argument leading to the adoption of (I) for cholesterol is developed.

(I.)



Since 1928, however, evidence has been accumulated irreconcilable with this representation, and in the present paper this will be summarised and correlated with still more recent work which has resulted in the advancement of an entirely new nuclear structure.

Of the four rings shown in formula (I), rings I and II were postulated by Windaus from a study of the reactions of cholesterol itself. The structures of rings III and IV were, however, deduced by Wieland from investigations of the bile acids, of which the monobasic cholanic acid, $C_{24}H_{40}O_2$, is the prototype. A direct connexion between this compound and cholesterol was first established by Windaus and Neukirchen (*Ber.*, 1919, **52**, 1915),

who converted coprostane, $C_{27}H_{48}$ (the hydrocarbon derived from coprosterol, a naturally occurring dihydrocholesterol), into cholanic acid by oxidation. This, together with the fact that acetone had previously been obtained by drastic oxidation of cholesterol derivatives, showed that the nuclear skeletons of cholesterol and the bile acids were identical, the terminal CO·OH of the latter being replaced by CH_2·$CHMe_2$ in cholesterol. Similarly the oxidation of cholestane, a stereoisomeride of coprostane, produced *allo*cholanic acid (Windaus and Neukirchen, *loc. cit.*). This fundamental relationship was further established by Wieland and Jacobi (*Ber.*, 1926, **59**, 2064), who synthesised coprostane from cholanic ester by means of *iso*propylmagnesium iodide.

It was therefore permissible to combine the knowledge gained from the separate studies of cholesterol and the bile acids in assigning a nuclear structure to these compounds, and the result, up to 1926, is that reproduced in formula (I). The presence of the *iso*octyl side-chain indicated in this formula was determined in two ways: (1) Windaus and Resau (*Ber.*, 1913, **46**, 1246) obtained methyl *iso*hexyl ketone by oxidation of cholesteryl acetate, thus proving that cholesterol must contain in its side-chain at least 8 carbon atoms having the structure (II) CHMe·CH_2·CH_2·CH_2·$CHMe_2$; (2) Wieland, Schlichting, and Jacobi (*Z. physiol. Chem.*, 1926, **161**, 80) proved definitely that the bile-acid side-chain consisted of the group ·CHMe·$[CH_2]_2$·CO_2H. This was achieved by removing one carbon atom at a time from the side-chain by means of the Grignard reaction, followed by oxidation; there were then successively obtained a C_{23} acid, a C_{22} acid, a C_{21} methyl ketone, a C_{20} acid, and finally a C_{19} dibasic acid (ætiobilianic acid) produced by opening of ring IV.

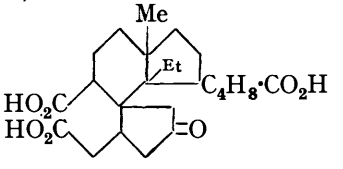
Formula (I) was definitely found to be inadequate to represent the bile-acid structure in its entirety when Wieland and Vocke (*Z. physiol. Chem.*, 1930, **191**, 69) showed that the ethyl group could be attached neither to C_{10} nor to any other carbon atom in ring IV. (It may be mentioned *en passant* that the four alicyclic rings and the side-chain in the bile acids account for only 21 carbon atoms. Of the three remaining, one was placed on C_{11} for reasons indicated below and the other two carbon atoms were provisionally attached to C_{10} as being apparently the only remaining position where they could be accommodated.)

In order to follow the argument for the rejection of C_{10} as carrying the ethyl group it is now necessary to consider in some detail certain bile acid reactions.

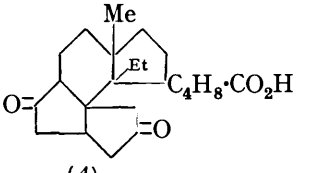
The three most extensively examined members of this series are cholic acid, $C_{24}H_{40}O_5$, deoxycholic acid, $C_{24}H_{40}O_4$, and lithocholic acid, $C_{24}H_{40}O_3$. These all occur naturally, and are hydroxylated cholanic acids substituted in positions 3 : 7 : 12, 3 : 7, and 3 respectively. [The hydroxyl group in position C_{12} in cholic acid was originally placed on C_{13}, but was moved to C_{12} following work carried out on chenodeoxycholic acid (3 : 12-dihydroxycholanic acid) (Windaus and van Schoor, *Z. physiol. Chem.*, 1926, **157**, 1771; Borsche and Frank, *Ber.*, 1926, **59**, 1748).]

When these acids are treated with chromic anhydride they are converted into keto-acids, which on regulated oxidation with nitric acid suffer rupture of ring I, giving the corresponding tribasic acids. Thus deoxycholic acid gives deoxybilianic acid, $C_{24}H_{36}O_7$ (III) (cf. Latschinoff, *Ber.*, 1885, **18**, 3039; Wieland and Kulenkampff, *Z. physiol. Chem.*, 1919, **108**, 295):

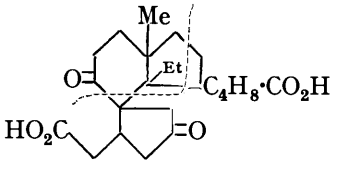
(III.)



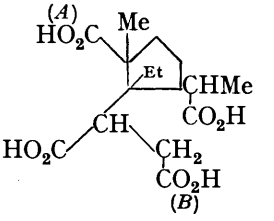
(IV.)



(V.)



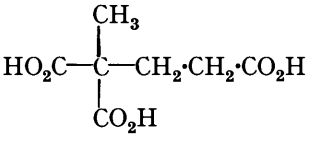
(VI.)



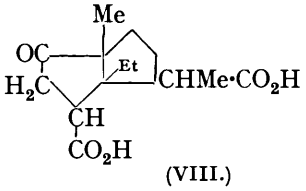
When the latter acid is heated in a vacuum it undergoes pyrolysis with loss of carbon dioxide and water, giving pyrodeoxybilianic acid (IV), which passes on oxidation with potassium permanganate into a diketo-dibasic acid, $C_{23}H_{34}O_6$ (V) (Wieland and Kulenkampff, *loc. cit.*).

When (V) is treated with a mixture of concentrated nitric and sulphuric acids, $C_{16}H_{24}O_8$ (VI) is formed (Wieland and Schlichting, *Z. physiol. Chem.*, 1924, **134**, 276), and in addition, from the mother-liquors of this oxidation, an acid, $C_7H_{10}O_6$, was later isolated and identified as *n*-butane-$\alpha\gamma\gamma$-tricarboxylic acid (VII) (Wieland and Vocke, *Z. physiol. Chem.*, 1928, **177**, 68). This acid supplies the evidence for the attachment of the methyl group to C_{11}, since it can only reasonably be supposed to be derived by rupture of (V) as shown by the dotted line.

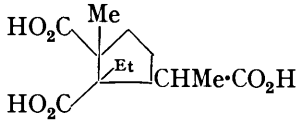
The acid (VI) on pyrolysis loses carbon dioxide and water and forms a keto-dibasic acid, $C_{15}H_{22}O_5$, which on further oxidation gives a tribasic acid, $C_{13}H_{20}O_6$ (Wieland and Schlichting, *loc. cit.*). It follows that, assuming (VI) to be correct for the $C_{16}H_{24}O_8$ acid, the structures of these two new acids must be (VIII) and (IX) respectively, the two carboxyls *A* and *B* of (VI) taking part in the production of (VIII), which on oxidation yields a malonic acid from which two molecules of carbon dioxide are lost.



(VII.)



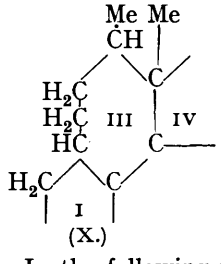
(VIII.)



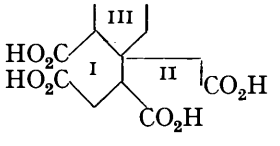
(IX.)

Now the keto-acid (VIII) on reduction by Clemmensen's method gives a dibasic acid, $C_{15}H_{24}O_4$, which on treatment with two mols. of phenylmagnesium bromide, followed by oxidation of the carbinol thus produced, passes into a lower homologous acid, $C_{14}H_{22}O_4$ (Wieland and Vocke, *Z. physiol. Chem.*, 1930, **191**, 69). Clearly an *acid* could not be formed unless a group $CH_2{\cdot}CO_2H$ were present in $C_{15}H_{24}O_4$, and it therefore follows that in the acids $C_{16}H_{24}O_8$, $C_{15}H_{22}O_5$, and $C_{13}H_{20}O_6$ the original side-chain of the bile acid must have been present unimpaired.* The acid $C_{13}H_{20}O_6$ must accordingly contain a five-membered ring, a side-chain containing the group $CHMe{\cdot}[CH_2]_2{\cdot}CO_2H$, and two further carboxyls together with a methyl radical. As all the 13 carbon atoms are accounted for in this manner, the ethyl group cannot possibly reside in any position in ring IV.

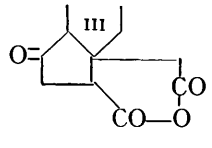
Following upon this, Wieland advanced the suggestion that the two unplaced carbon atoms might reside in ring III, strict proof of the carbon-atom content of which was still lacking. The atoms were introduced as shown in (X) (Wieland and Vocke, *loc. cit.*).



(X.)



(XI.)

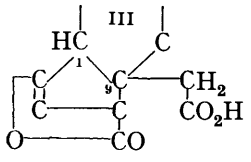


(XII.)

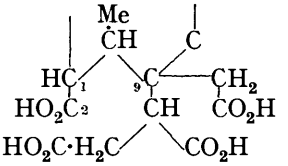
In the following year (1931) this formula also was abandoned by Wieland in view of results obtained by a reinvestigation of the decomposition by heat of choloidanic acid, $C_{24}H_{36}O_{10}$, a pentabasic acid produced by nitric acid oxidation of deoxybilianic acid (III), to which the structure (XI) was given (Wieland, *Z. physiol. Chem.*, 1919, **108**, 306). On pyrolysis choloidanic acid loses CO_2 and $2H_2O$, forming pyrocholoidanic acid, $C_{23}H_{32}O_6$, formerly regarded as a keto-anhydride monobasic acid (XII).

* Analogies supporting this view were found with other acids containing the unshortened side-chain; these on subjection to the conditions used in the production of $C_{16}H_{24}O_8$ suffered no shortening of the side-chain (Wieland and Vocke, *loc. cit.*).

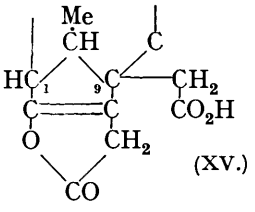
It was found, however (Wieland, Ertel, and Schönberger, *Z. physiol. Chem.*, 1931, **197**, 31), that pyrocholoidanic acid formed a neutral dimethyl ester with diazomethane, and must therefore be the enol-lactone of a tribasic acid, which on the basis of (XI) would be (XIII). Such a formula, however, stands in contradiction to Bredt's rule, and to circum-



(XIII.)



(XIV.)

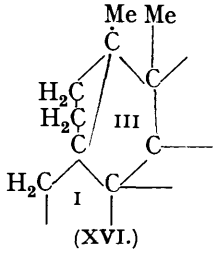


(XV.)

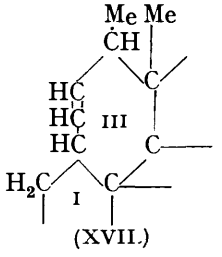
vent this difficulty Wieland placed the two carbon atoms in question as a $CH{\cdot}CH_3$ group between C_1 and C_9, so that the formulæ of choloidanic and pyrocholoidanic acids were represented as (XIV) and (XV), thus making both rings I and III 7-membered.

Further difficulties arose in the investigation of two isomeric acids, *apo*cholic and dihydroxycholenic acids, $C_{24}H_{38}O_4$, produced by the partial dehydration of cholic acid by loss of $1H_2O$. Dihydroxycholenic acid behaves as a normal mono-ethylenic acid, since on hydrogenation it is converted into the naturally occurring deoxycholic acid. *apo*Cholic acid, however, while giving the colour reactions of an unsaturated compound, is completely resistant towards hydrogenation. Furthermore, both *apo*cholic acid and its isomeride react with perbenzoic acid, giving crystalline oxides, both of which on rupture of the oxide ring lose two molecules of water and yield one and the same dihydroxycholadienic acid, $C_{24}H_{36}O_4$. This acid is also produced by direct dehydrogenation of *apo*cholic and dihydroxycholenic acids by means of bromine (and from the former compound with permanganate also), and on catalytic hydrogenation it takes up *two* atoms of hydrogen *only* and is reconverted into *apo*cholic acid.

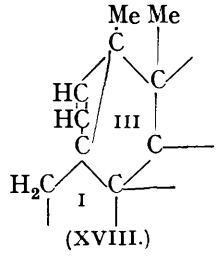
To explain this remarkable series of changes, Borsche and Todd (*Z. physiol. Chem.*, 1931, **197**, 173) rejected Wieland's carbon skeleton indicated in (XIV) and employed his earlier formula (X) in which only ring III was 7-membered. In their opinion *apo*cholic, dihydroxycholenic, and dihydroxycholadienic acids have respectively the formulæ (XVI), (XVII), and (XVIII).



(XVI.)



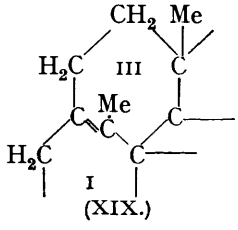
(XVII.)



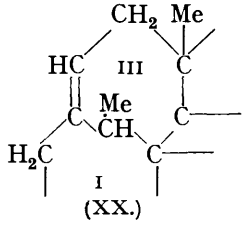
(XVIII.)

This scheme, while offering a possible explanation of the transformations undergone by these acids, entirely fails to account for the formation of pyrocholoidanic acid.

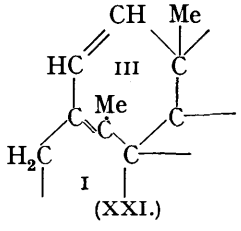
Wieland and Deulofeu (*Z. physiol. Chem.*, 1931, **198**, 127), on the other hand, employ formulæ (XIX), (XX), and (XXI) to represent the three unsaturated acids (cf. also Wieland and Dane, *Z. physiol. Chem.*, 1932, **206**, 243; **212**, 263).



(XIX.)



(XX.)

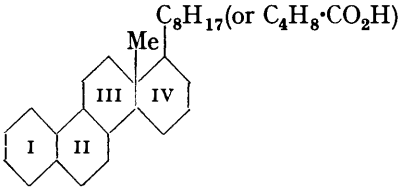


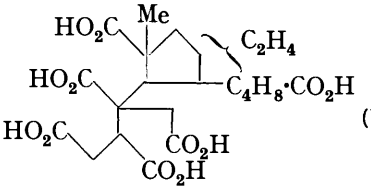
(XXI.)

At this point a revolutionary proposal was advanced by Rosenheim and King (*J. Soc. Chem. Ind.*, 1932, **51**, 464) based on (*a*) the *X*-ray evidence of Bernal (*ibid.*, p. 466), who found that the older accepted formulæ for the sterols and bile acids could not be made to fit into the crystallographic cells, and (*b*) the facts that cholic acid is stated by Diels and Karstens (*Annalen*, 1930, **478**, 129) to give chrysene in good yield on dehydrogenation with selenium, and cholesterol furnishes this hydrocarbon on treatment with palladised charcoal (Diels and Gädke, *Ber.*, 1927, **60**, 140).*

Arising from this, Rosenheim and King suggested that the nuclear skeleton of cholesterol and the bile acids was essentially that of chrysene as indicated in (XXII), which formula accommodates the hitherto " homeless " carbon atoms. Alteration of the position of the side-chain to that shown in this formula was effected to meet the requirements of Bernal's *X*-ray measurements (*Nature*, 1932, **129**, 277).

(XXII.)





(XXIII.)

With this formulation, however, various difficulties are experienced in attempting to explain certain of the bile acid reactions, notably the formation of (*a*) biloidanic acid (XXIII), a hexabasic acid, $C_{22}H_{32}O_{12}$, obtained from pyrocholoidanic acid, or from cholic acid by direct oxidation, and (*b*) the tetrabasic acid $C_{16}H_{24}O_8$ from the diketo-dibasic acid $C_{23}H_{34}O_6$ (V).

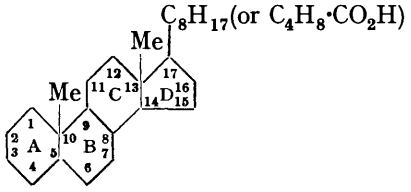
A modification of the Rosenheim–King formula which seems to offer a satisfactory interpretation of all the known bile-acid reactions was shortly afterwards advanced by Wieland and Dane (*Z. physiol. Chem.*, 1932, **210**, 268). The first step in this direction consisted in the preparation of a new bile acid containing a hydroxyl group in ring III only (up to this stage Wieland regarded this ring as 7-membered). On oxidation this acid gave a tribasic acid, $C_{24}H_{38}O_6$, thilobilianic acid, in which scission of ring III had occurred, and this on pyrolysis yielded *an anhydride in place of the confidently anticipated ketone.* Since a substituted pimelic acid could scarcely fail to form a ketone, Wieland was accordingly forced to abandon the idea of a 7-membered ring III. On the other hand, Borsche and Frank (*Ber.*, 1927, **60**, 723) had definitely proved that ring III is at least 6-membered, from which the important deductions emerge (*a*) that the Blanc rule is not universally applicable to acids resulting from rupture of condensed ring-systems, and (*b*) that the two " homeless " carbon atoms cannot be contained in ring III. Arising from this breakdown of the Blanc rule, the evidence in favour of ring II being 5-membered is also negatived. It should be pointed out, however, that this does not apply to rings I and IV, where the carboxyl groups are attached to only one ring (the reasons for this are fully discussed in the original paper).

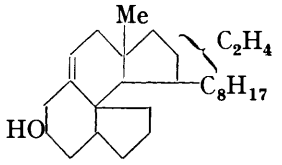
Consequent upon the above work, Wieland and Dane rejected the bile-acid carbon skeleton hitherto employed, and adopted the Rosenheim–King structure in general outline. Certain far-reaching modifications were, however, introduced; ring IV was retained as a 5-membered ring and *a transposition of the functions of rings II and III in the bile acids but not in the sterols was postulated*, a rearrangement of fundamental importance and one clarifying the whole position. The new skeleton is represented as (XXIV), in which rings A, B, C, and D have the functions of rings I, III, II, and IV respectively in the bile acids and of I, II,

* Recent papers by Ruzicka and Thomann (*Helv. Chim. Acta*, 1933, **16**, 216) and Ruzicka, Ehmann, and Mörgeli (*ibid.*, p. 314) throw considerable doubt on Diels and Karstens' claim that chrysene is actually produced from cholic acid. These authors state that the dehydrogenation product is a complex mixture of four hydrocarbons, each apparently containing a 5-membered ring, whose physical properties simulate those of chrysene (see further Diels's reply; *Ber.*, 1933, **66**, 487).

III, and IV in cholesterol. It will be noted that the remaining unplaced carbon atom is now attached to C_{10} as a methyl group.

(XXIV.)



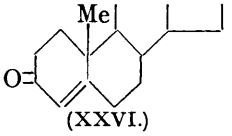


(XXV.)

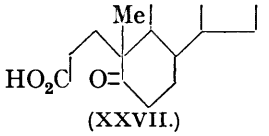
It is now necessary to review the evidence produced by Wieland and his collaborators in support of these changes.

Starting from lithocholic acid, Wieland, Dane, and Scholtz (*Z. physiol. Chem.*, 1932, **211**, 261) obtained by degradation of pyrolithobilianic acid two tetrabasic acids, $C_{22}H_{34}O_8$ and $C_{21}H_{32}O_8$, in which rings I and III of the bile acid molecule had been opened. These two acids were found to be identical in every respect with two acids previously isolated by Windaus (*Ber.*, 1909, **42**, 3770; 1912, **45**, 2421) from cholesterol by opening the two rings of the sterol containing the hydroxyl and the double bond, followed by removal of the *iso*propyl group from the side-chain. Hence the ring containing the double bond of cholesterol corresponds to ring III of the bile acids. If, however, cholesterol be formulated as (XXV), it becomes impossible to represent further degradations undergone by the acid $C_{24}H_{40}O_6$ from cholesterol, from which $C_{21}H_{32}O_8$ was derived by shortening of the side-chain. On the basis of (XXIV), however, these results are readily explicable, cholesterol containing its hydroxyl in ring A and its double bond in ring B.

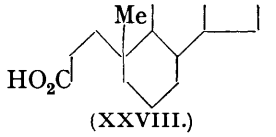
Further important evidence in support of the new formulation has been adduced by Tschesche (*Annalen*, 1932, **498**, 185). Windaus (*Ber.*, 1906, **39**, 2008) had previously found that cholestenone could be oxidised to a keto-monobasic acid $C_{26}H_{44}O_3$, which Diels, Gädke, and Körding (*Annalen*, 1927, **459**, 1) reduced to the monobasic acid $C_{26}H_{46}O_2$ (XXVIII). Starting from the latter acid, Tschesche obtained, by application of the stage-wise Grignard degradation method, a C_{25}- and a C_{24}-monobasic acid successively. Not only are these results completely incapable of interpretation on the old formula, but also definite proof is afforded that C_1 is part of a methylene group and therefore cannot be the point of junction of two rings. Further, the C_{24} acid was found to be extremely difficult to esterify, and it was therefore suggested that the carbon atom adjacent to the CO_2H group (*i.e.*, C_{10}) is quaternary, a result in agreement with the attachment of a methyl group to C_{10}. Cholestenone, and the acids $C_{26}H_{44}O_3$, $C_{26}H_{46}O_2$, $C_{25}H_{44}O_2$, and $C_{24}H_{42}O_2$ are represented as (XXVI), (XXVII), (XXVIII), (XXIX), and (XXX) respectively.



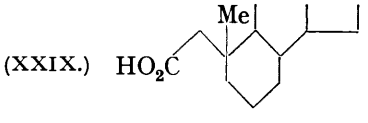
(XXVI.)



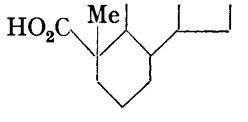
(XXVII.)



(XXVIII.)



(XXIX.)



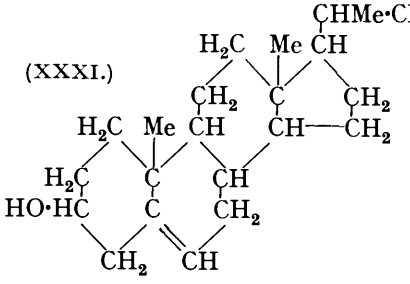
(XXX.)

It will be noticed that in the above scheme cholestenone is represented as an αβ-unsaturated ketone having its keto-group attached to C_3. Spectrographic evidence that cholestenone is actually an αβ-unsaturated ketone had previously been put forward by Page, Menschick, and Bossert (*Annalen*, 1932, **495**, 225). That the hydroxyl group in cholesterol is actually in position 3 and not in position 4 is not only demanded by theoretical and biogenetic

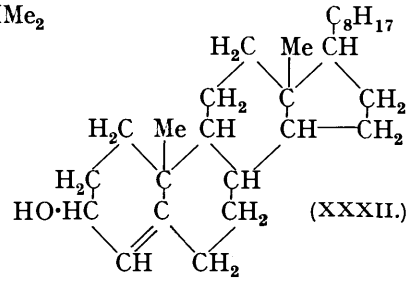
considerations, but is placed beyond all reasonable doubt by the isolation by Wieland and Dane (*Z. physiol. Chem.*, 1932, **212**, 41) of 3-hydroxy*allo*cholanic acid, which is almost certainly identical with the acid obtained by Windaus and Hossfeld (*Z. physiol. Chem.*, 1925, **145**, 177) by removal of the *iso*propyl group from the side-chain of chlorocholestane, followed by hydrolysis of the resulting chloro-acid.

Cholesterol must now be given the structure (XXXI) (the position $\Delta^{4:5}$ as present in cholestenone is excluded on account of the production from cholesterol of a saturated C_{27} tetrabasic acid; see *Ann. Reports*, 1927), while *allo*cholesterol (Windaus, *Annalen*, 1927, **453**, 101) will be (XXXII), as has been shown by Heilbron and MacFarlane (forthcoming publication) by its conversion through *allo*cholesteryl chloride into *pseudo*cholestene (XXXIII), which is a position isomeride of cholestene (XXXIV).

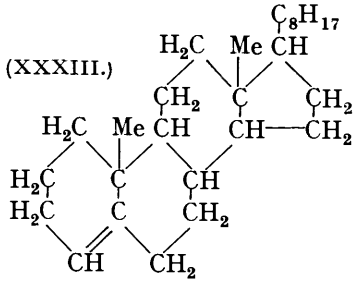
(XXXI.)



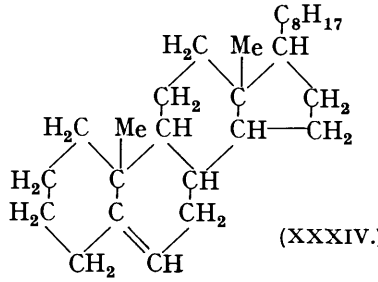
(XXXII.)



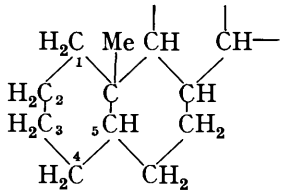
(XXXIII.)



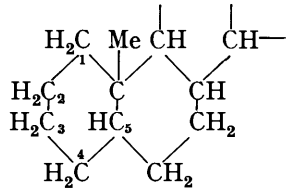
(XXXIV.)



A detailed account of the resultant modification in the structures of the many derivatives of cholesterol has recently been published by Windaus (*Z. physiol. Chem.*, 1932, **213**, 147). Attention will here be drawn simply to the fact that the stereoisomerism existing between the *allo*- and the normal series in the bile acid group (corresponding to the cholestane and coprostane series; see p. 627) is now satisfactorily accounted for by the presence of the asymmetric centre C_5, as shown in (XXXV) and (XXXVI):



(XXXV.) Cholestane and *allo*cholanic acid.



(XXXVI.) Coprostane (pseudocholestane) and cholanic acid.

A point until recently incapable of explanation on the basis of the existence of only one asymmetric centre in the cholesterol molecule concerned the existence of three isomeric dibasic acids, $C_{27}H_{46}O_4$, formed respectively from dihydrocholesterol and coprosterol by oxidation with chromic anhydride and by the hydrogenation of Diels's acid, which latter is prepared from cholesterol by direct oxidation with hypobromite. For a long time these

three acids were considered to be stereoisomerides (cf. Windaus, *Nach. Ges. Wiss. Göttingen*, 1925, 159), thus necessitating the introduction of a second centre of asymmetry, the two being C_1 and C_5 (formula I). Recent work has shown, however, that the oxidation of dihydrocholesterol leads to rupture of ring A (XXXI) between C_2 and C_3, whereas the other two acids originate by scission of ring A between C_3 and C_4, and are stereoisomerides, as shown by their pyrogenic conversion into the same ketone $C_{26}H_{44}O$ (Windaus, *loc. cit.*).

Ergosterol.

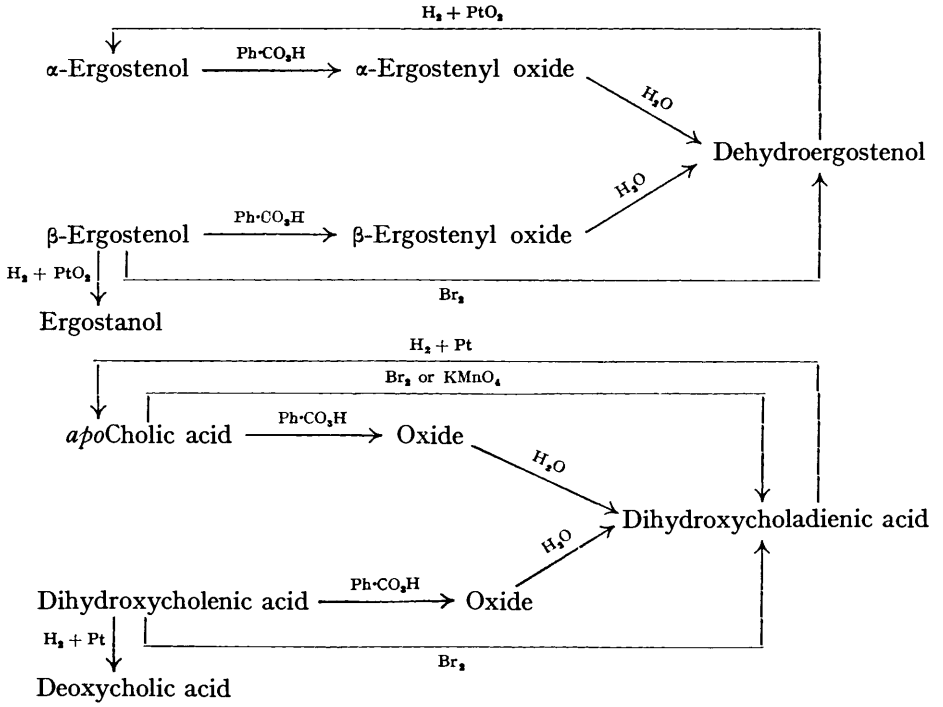
Until recently ergosterol was considered to have the molecular formula $C_{27}H_{42}O\overline{|}_3$ originally assigned to it by Tanret (*Compt. rend.*, 1908, **147**, 75) and to differ from cholesterol simply in respect of the number of its double bonds. That the carbon skeletons of the two sterols are, if not identical, certainly closely related would appear to result from the work of Diels and Karstens (*Annalen*, 1930, **478**, 129), who found that ergosterol on dehydrogenation with selenium gave the same two hydrocarbons, $C_{25}H_{24}$ and $C_{18}H_{16}$, as had previously been obtained from cholesterol by the same method (Diels, Gädke, and Körding, *Annalen*, 1927, **459**, 1).

In 1932, however, Windaus and Lüttringhaus (*Nach. Ges. Wiss. Göttingen*, 1932, 4) suggested that ergosterol might possibly have the formula $C_{28}H_{44}O$, a suggestion based on the analytical values of nitrogen-containing esters of ergosterol derivatives. Further evidence in support of this contention was presented by Windaus, Werder, and Gschaider (*Ber.*, 1932, **65**, 1006) and definitely confirmed by Heilbron and Simpson (J., 1932, 2400) by analyses of certain bromo-ketones derived from ergosterol.

That the extra carbon atom is contained in the side-chain has been proved by the work of Guiteras, Nakamiya, and Inhoffen (*Annalen*, 1932, **494**, 116), who, by oxidation of hexahydroergosterol, isolated a methyl ketone, $C_9H_{18}O$, seemingly identical with dihydrothujaketone, as distinct from the methyl *iso*hexyl ketone, $C_8H_{16}O$, obtained from cholesterol under similar conditions. The same ketone was also isolated by Heilbron, Simpson, and Wilkinson (J., 1932, 1699) from α-ergostenol (tetrahydroergosterol). The side-chain of ergosterol also contains one of the three double bonds, for ozonisation of ergosterol yields methyl*iso*propylacetaldehyde (Reindel and Kipphan, *Annalen*, 1932, **493**, 181), from which it follows that its structure must be $-CHMe{\cdot}CH{:}CH{\cdot}CHMe{\cdot}CHMe_2$ (XXXVII).

Of the three double bonds in ergosterol, only two are capable of saturation by direct catalytic hydrogenation. In the formation of its first hydrogenation product, α-dihydroergosterol, a nuclear double bond is involved, since ozonisation of this compound furnishes methyl*iso*propylacetaldehyde (Guiteras, Nakamiya, and Inhoffen, *loc. cit.*). The formation of the tetrahydro-derivative, α-ergostenol, involves further the saturation of the side-chain double bond, as it fails to give the above aldehyde on ozonisation. The remaining double bond in α-ergostenol is completely resistant towards further direct hydrogenation, but on treatment of its acetate with dry hydrogen chloride in chloroform solution an isomeric " β-acetate " is produced (Reindel, Walter, and Rauch, *Annalen*, 1927, **452**, 34), from which the saturated ergostanol can be obtained by hydrogenation with a platinum catalyst. This " β-acetate " was later shown to be a mixture containing unchanged α-acetate (Reindel and Walter, *Annalen*, 1928, **460**, 212; Hart, Speer, and Heyl, *J. Amer. Chem. Soc.*, 1930, **50**, 2016), but pure β-ergostenol in good yield can be obtained by means of the very sparingly soluble benzoate (Heilbron and Wilkinson, J., 1932, 1708), and the β-compound can be transformed almost quantitatively into ergostanol by hydrogenation by means of Adams's platinum oxide. Windaus and Lüttringhaus (*Annalen*, 1930, **481**, 119) observed that treatment of α-ergostenol with perbenzoic acid yielded dehydroergostenol, $C_{28}H_{46}O\overline{|}_2$, which on hydrogenation again passed into α-ergostenol. β-Ergostenol was also found by Morrison and Simpson (J., 1932, 1710) to yield dehydroergostenol (*a*) *via* a crystalline oxide and (*b*) directly, on treatment with bromine. These authors also isolated the hitherto unknown α-ergostenyl oxide, which was different from the β-isomeride. These reactions, as shown in the following scheme, are wholly analogous to those undergone by *apo*cholic and dihydroxycholenic acids, to which brief reference has already been made (p. 629) and

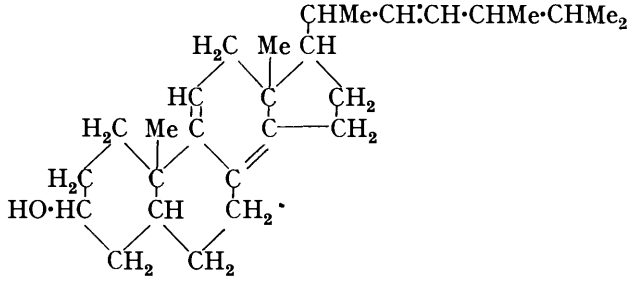
supply further evidence in support of the nuclear identity between ergosterol and the bile acids.



Complete proof of the correctness of this conception has now been supplied by Chuang (*Annalen*, 1933, **500**, 270), who has succeeded in converting ergostane into *allo*norcholanic acid, $C_{23}H_{38}O_2$, by oxidation. A similar degradation of ergostanyl chloride, $C_{28}H_{49}Cl$, into a chloro-norcholanic acid $C_{23}H_{37}O_2Cl$ has also been carried out by Heilbron and Simpson (*Nature*, 1933, **131**, 438).

Turning now to the question of the location of the two nuclear double bonds, it would appear, from the fact that ergosterol can only be directly hydrogenated to its tetrahydro-derivative, that the ethenoid linkage in the latter compound is almost certainly situated between two quaternary carbon atoms. That the same double bond is present in ergosterol and is not formed during the hydrogenation of this sterol by 1 : 4-addition to a conjugated system arises from the work of Heilbron, Morrison, and Simpson (J., 1933, 302) on the reactions of ergostadienetriol and methoxyergostadienediol. These authors conclude further

(XXXVIII.)*



that the reactive nuclear double bond in ergosterol is of the type >C=CH–. The latter must be conjugated with the inert double bond, for, as shown by Windaus (*Nach. Ges. Wiss. Göttingen*, 1929, 159; *Ber.*, 1931, **64**, 850), ergosterol combines with maleic anhydride.

* The reasons for abandoning the formula for ergosterol previously suggested by the authors (*J. Soc. Chem. Ind.*, 1932, **51**, 1061) are discussed in detail by Heilbron, Morrison, and Simpson (*loc. cit.*).

Taking these facts into consideration, together with the specific production from ergosterol of a methylbenzenetetracarboxylic acid (Guiteras, Nakamiya, and Inhoffen, *loc. cit.*) indicating the presence of both nuclear double bonds in one ring, Heilbron, Morrison, and Simpson (*loc. cit.*) conclude that the structure of ergosterol is, on the evidence so far available, best expressed by formula (XXXVIII), a formulation also favoured by Windaus on theoretical grounds (*Nach. Ges. Wiss. Göttingen*, 1933, 92).

In this formula the hydroxyl is shown attached to C_3. That it is probably present in ring A follows from the pyrolysis of the acid $C_{28}H_{48}O_4$ obtained by oxidation of ergostanol (Reindel, *Annalen*, 1928, **466**, 131), a ketone $C_{27}H_{46}O$ then being produced (cf. p. 630). Although the position of the hydroxyl has not yet been fully demonstrated, its attachment to C_3 is attractive on biogenetic grounds.

Stigmasterol.

Recent work has shown that the molecular formula of this doubly unsaturated sterol, first isolated by Windaus and Hauth (*Ber.*, 1906, **39**, 4378) and represented as $C_{30}H_{50}O$ (Windaus and Brunken, *Z. physiol. Chem.*, 1924, **140**, 48), is actually $C_{29}H_{48}O$ (Sandquist and Gorton, *Ber.*, 1930, **63**, 1935; Windaus, Werder, and Gschaider, *loc. cit.*), thus containing two carbon atoms more than cholesterol. The site of these carbon atoms has now been found in the side chain, which moreover contains one of the ethylenic linkages, since ozonisation of stigmasterol gives rise to ethyl*iso*propylacetaldehyde (Guiteras, *Z. physiol. Chem.*, 1933, **214**, 89). From this there can be little doubt that the side-chain has the formula ·CHMe·CH:CH·CHEt·$CHMe_2$ and that the nuclear skeleton of the sterol conforms to that of cholesterol and ergosterol.

In the above review only such compounds have been referred to as have a specific bearing on constitutional problems related to the bile acids and the sterols. Arising from this, many important derivatives of both groups, such as cheno- and hyo-deoxycholic acids, the numerous isomerides of ergosterol and its irradiated products, including the important isomeride vitamin D, dehydroergosterol, etc., have been omitted from the present discussion.

In the main the structure of the bile acids and sterols would seem to have been elucidated, but the *apo*cholic–dihydroxycholenic acids and the α–β-ergostenol dehydrogenation reactions still await satisfactory explanation. Attention must also be drawn to a paper by Criegee (*Ber.*, 1932, **65**, 1770) suggesting that ring B is actually 5-membered, a suggestion in which, however, Windaus (*Z. physiol. Chem.*, 1932, **213**, 147) does not concur.

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