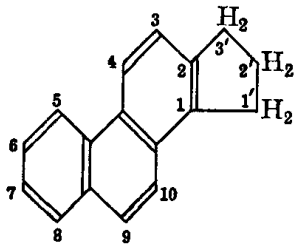
CHAPTER XI

STEROIDS

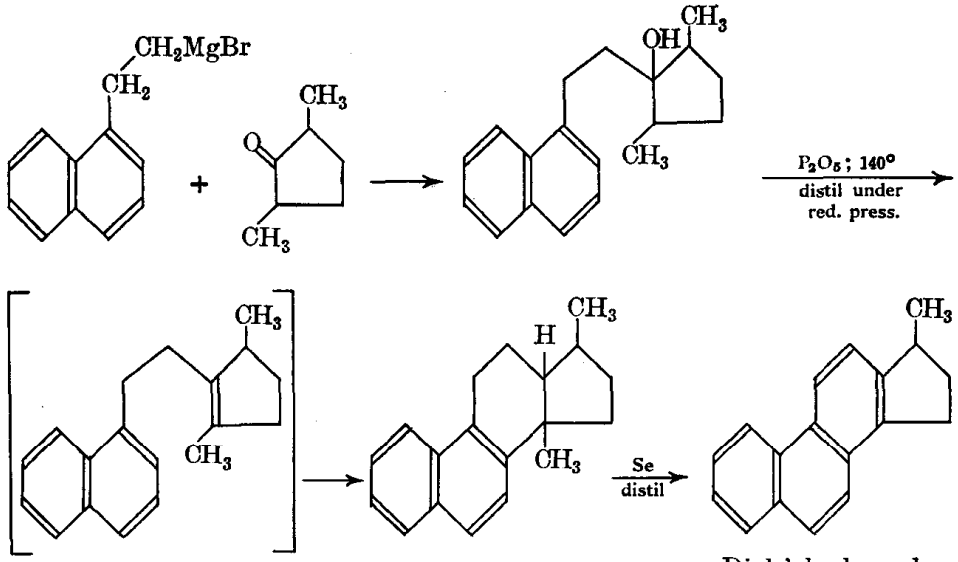
§1. Introduction. The steroids form a group of structurally related compounds which are widely distributed in animals and plants. Included in the steroids are the sterols (from which the name *steroid* is derived), vitamin D, the bile acids, a number of sex hormones, the adrenal cortex hormones, some carcinogenic hydrocarbons, certain sapogenins, etc. The structures of the steroids are based on the 1 : 2-*cyclo*pentenophenanthrene skeleton (Rosenheim and King, 1932; Wieland and Dane, 1932). All the steroids



1:2-*cyclo*pentenophenanthrene

give, among other products, Diels' hydrocarbon on dehydrogenation with selenium at 360° (Diels, 1927). In fact, a steroid could be defined as any compound which gives Diels' hydrocarbon when distilled with selenium. When the distillation with selenium is carried out at 420°, the steroids give mainly chrysene (§4. X) and a small amount of picene (§4a. X).

Diels' hydrocarbon is a solid, m.p. 126–127°. Its molecular formula is $C_{18}H_{16}$, and the results of oxidation experiments, X-ray crystal analysis and absorption spectrum measurements showed that the hydrocarbon is probably 3'-methyl-1 : 2-*cyclo*pentenophenanthrene. This structure for the compound was definitely established by synthesis, *e.g.*, that of Harper, Kon and Ruzicka (1934) who used the Bogert–Cook method [§2 (vi) *e.* X], starting from 2-(1-naphthyl)-ethylmagnesium bromide and 2 : 5-dimethyl*cyclo*pentanone.



Diels' hydrocarbon

STEROLS

§2. Sterols occur in animal and plant oils and fats. They are crystalline compounds, and contain an alcoholic group; they occur free or as esters of the higher fatty acids, and are isolated from the unsaponifiable portion of oils and fats. Cholesterol, cholestanol and coprostanol (coprosterol) are the animal sterols; ergosterol and stigmasterol are the principal plant sterols. The sterols that are obtained from animal sources are often referred to as the *zoosterols*, and those obtained from plant sources as the *phytosterols*. A third group of sterols, which are obtained from yeast and fungi, are referred to as the *mycosterols*. This classification, however, is not rigid, since some sterols are obtained from more than one of these groups.

§3. **Cholesterol,** $C_{27}H_{46}O$, m.p. 149°. This is the sterol of the higher animals, occurring free or as fatty esters in all animal cells, particularly in the brain and spinal cord. Cholesterol was first isolated from human gallstones (these consist almost entirely of cholesterol). The main sources of cholesterol are the fish-liver oils, and the brain and spinal cord of cattle. Lanoline, the fat from wool, is a mixture of cholesteryl palmitate, stearate and oleate.

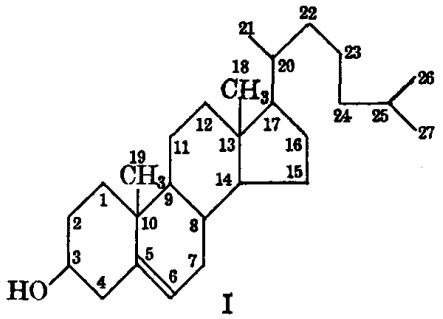
Cholesterol is a white crystalline solid which is optically active (lævorotatory). Cholesterol (and other sterols) gives many colour reactions, *e.g.*,

(i) *The Salkowski reaction* (1908). When concentrated sulphuric acid is added to a solution of cholesterol in chloroform, a red colour is produced in the chloroform layer.

(ii) *The Liebermann–Burchard reaction* (1885, 1890). A greenish colour is developed when a solution of cholesterol in chloroform is treated with concentrated sulphuric acid and acetic anhydride.

When an ethanolic solution of cholesterol is treated with an ethanolic solution of digitonin (a saponin; see §19. iii), a large white precipitate of cholesterol digitonide is formed. This is a molecular complex containing one molecule of cholesterol and one of digitonin, from which the components may be recovered by dissolving the complex in pyridine (which brings about complete dissociation) and then adding ether (the cholesterol remains in solution and the digitonin is precipitated). Digitonide formation is used for the estimation of cholesterol.

The structure of cholesterol was elucidated only after a tremendous amount of work was done, particularly by Wieland, Windaus and their coworkers (1903–1932). Only a very bare outline is given here, and in order to appreciate the evidence that is going to be described, it is necessary to



I

have the established structure of cholesterol at the beginning of our discussion. I is the structure of cholesterol, and shows the method of numbering. The molecule consists of a *side-chain* and a *nucleus* which is composed of four rings; these rings are usually designated A, B, C and D or I, II, III and

IV, beginning from the six-membered ring on the left (see also (iii) below). It should be noted that the nucleus contains two angular methyl groups, one at C_{10} and the other at C_{13}.

(i) **Structure of the ring system.** Under this heading we shall deal with the nature of the ring system present in cholesterol; the problem of the angular methyl groups is dealt with later [see (iv)].

The usual tests for functional groups showed that cholesterol contains one double bond and one hydroxyl group. Now let us consider the following set of reactions.

$$\underset{C_{27}H_{46}O}{\text{Cholesterol}} \xrightarrow{H_2\text{—Pt}} \underset{C_{27}H_{48}O}{\text{Cholestanol}} \xrightarrow{CrO_3} \underset{C_{27}H_{46}O}{\text{Cholestanone}} \xrightarrow[HCl]{Zn\text{—}Hg} \underset{C_{27}H_{48}}{\text{Cholestane}}$$

I II III IV

The conversion of cholesterol into cholestanol, II, shows the presence of one double bond in I, and the oxidation of II to the ketone cholestanone, III, shows that cholesterol is a secondary alcohol. Cholestane, IV, is a saturated hydrocarbon, and corresponds to the general formula C_nH_{2n-6}, and consequently is tetracyclic; thus cholesterol is tetracyclic.

When cholesterol is distilled with selenium at 360°, Diels' hydrocarbon is obtained (see §1). The formation of this compound could be explained by assuming that this nucleus is present in cholesterol. The yield of this hydrocarbon, however, is always poor, and other products are always formed at the same time, particularly chrysene (see §1). Thus, on the basis of this dehydrogenation, the presence of the *cyclo*pentenophenanthrene nucleus must be accepted with reserve. Rosenheim and King (1932) thought that chrysene was the normal product of the selenium dehydrogenation, and so proposed (on this basis and also on some information obtained from X-ray analysis work of Bernal, 1932; see §4a) that the steroids contained the chrysene skeleton. Within a few months, however, Rosenheim and King (1932) modified this suggestion, as did also Wieland and Dane (1932). These two groups of workers proposed that the *cyclo*pentenophenanthrene nucleus is the one present in cholesterol (*i.e.*, in steroids in general). This structure fits far better all the evidence that has been obtained from a detailed investigation of the oxidation products of the sterols and bile acids. This structure has now been confirmed by the synthesis of cholesterol (see later in this section).

Although an account of the oxidative degradation of the steroids cannot be discussed here, the following points in this connection are of some interest.

(i) The nature of the *nucleus* in sterols and bile acids was shown to be the same, since cholanic acid or *allo*cholanic acid is one of the oxidation products (see §4a for the significance of the prefix *allo*).

(ii) The oxidation of the bile acids led to the formation of products in which various rings were opened. The examination of these products showed that the positions of the hydroxyl groups were limited mainly to three positions, and further work showed that the hydroxyl groups behaved differently towards a given reagent, *e.g.*,

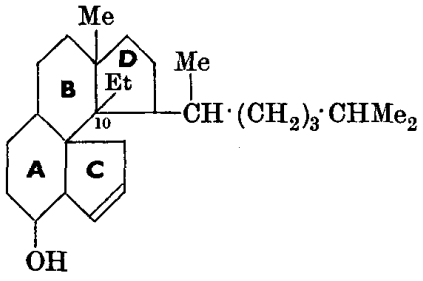
(*a*) The ease of oxidation of hydroxyl groups to keto groups by means of chromic acid is $C_7 > C_{12} > C_3$. More recently, Fonken *et al.* (1955) have shown that *tert.*-butyl hypochlorite apparently oxidises the 3-OH group selectively to the keto group; this reaction, however, failed with cholesterol. Sneedon *et al.* (1955) have also shown that the 3-OH group in steroids is oxidised by oxygen-platinum, but not those at 6, 7 or 12.

(*b*) The three keto groups are not equally readily reduced to a methylene group (by the Clemmensen reduction) or to an alcoholic group (by H_2—platinum). The ease of reduction is $C_3 > C_7 > C_{12}$. This is also the order

for the ease of hydrolysis or acetylation when these positions are occupied by hydroxyl groups (see also testosterone, §13). More recently, it has been shown that the modified Wolff-Kishner reduction of Huang-Minlon (see Vol. I) on steroid ketones reduces keto groups at 3, 7, 12, 17 and 20, but not at 11. Another interesting point in this connection is that lithium aluminium hydride, in the presence of aluminium chloride, does not reduce unsaturated ketones to alcohols, *e.g.*, cholest-4-en-3-one, under these conditions, is reduced to cholest-3-ene (Broome *et al.*, 1956).

Thus a knowledge of (*a*) and (*b*) enabled workers to open the molecule at *different* points by oxidation under the appropriate conditions. This led to a large variety of degradation products, the examination of which enabled the nature of the nucleus to be ascertained.

(*c*) Blanc's rule was also used to determine the sizes of the various rings, but the failure of the rule in certain cases led to an erroneous formula; *e.g.*, ring C was originally believed to be five-membered. Thus Windaus and Wieland (1928) proposed the following formula for cholesterol, and the uncertain point (at that time) was the nature of the two extra carbon atoms. These were *assumed* to be present as an ethyl group at position 10, but Wieland *et al.* (1930) finally proved that there was no ethyl group at this



position. These two " homeless " carbon atoms were not placed until Rosenheim and King first proposed that steroids contained the chrysene nucleus and then proposed the *cyclo*pentenophenanthrene nucleus (see above). Bernal (1932) also showed, from the X-ray analysis of cholesterol, ergosterol, etc., that the molecule was thin, whereas the above structure for the steroid nucleus would be rather thick.

(ii) **Positions of the hydroxyl group and double bond.** Let us consider the following reactions:

$$\underset{\substack{C_{27}H_{46}O\\ III}}{\text{Cholestanone}} \xrightarrow{HNO_3} \underset{\substack{C_{27}H_{46}O_4\\ V}}{\text{Dicarboxylic acid}} \xrightarrow{300^\circ} \underset{\substack{C_{26}H_{44}O\\ VI}}{\text{Ketone}}$$

Since the dicarboxylic acid V contains the same number of carbon atoms as the ketone (III) from which it is derived, the keto group in III must therefore be in a ring. Also, since pyrolysis of the dicarboxylic acid V produces a ketone with the loss of one carbon atom, it therefore follows from Blanc's rule that V is either a 1 : 6- or 1 : 7-dicarboxylic acid. Now we have seen that the nucleus contains three six-membered rings and one five-membered ring. Thus the dicarboxylic acid V must be obtained by the opening of ring A, B or C, and consequently it follows that the hydroxyl group in cholesterol (which was converted into the keto group in cholestanone; see (i) above) is in ring A, B or C.

Actually *two* isomeric dicarboxylic acids are obtained when cholestanone is oxidised. The formation of these two acids indicates that the keto group

in cholestanone is flanked on either side by a methylene group, *i.e.*, the grouping —CH_2·CO·CH_2— is present in cholestanone. Examination of the reference structure I of cholesterol shows that such an arrangement is possible only if the hydroxyl group is in ring A.

Now let us consider the further set of reactions:

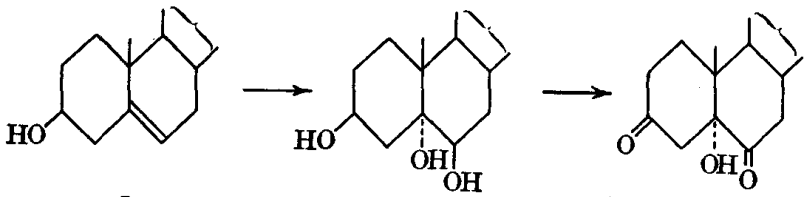
$$\text{Cholesterol } C_{27}H_{46}O \xrightarrow[CH_3\cdot CO_3H]{H_2O_2} \text{Cholestanetriol } C_{27}H_{48}O_3 \xrightarrow{CrO_3} \text{Hydroxycholestanedione } C_{27}H_{44}O_3$$

I VII VIII

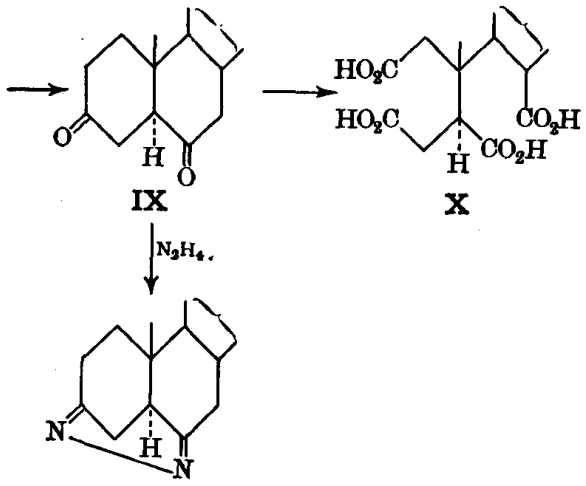
$$\xrightarrow[\text{(ii) } Zn—CH_2\cdot CO_2H]{\text{(i) } -H_2O} \text{Cholestanedione } C_{27}H_{44}O_2 \xrightarrow{CrO_3} \text{Tetracarboxylic acid } C_{27}H_{44}O_8$$

IX X

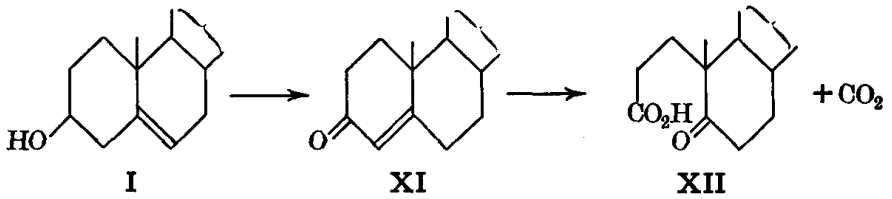
In the conversion of I into VII, the double bond in I is hydroxylated. Since only two of the three hydroxyl groups in VII are oxidised to produce VIII, these two groups are secondary alcoholic groups (one of these being the secondary alcoholic group in cholesterol), and the third, being resistant to oxidation, is probably a tertiary alcoholic group. Dehydration of VIII (by heating *in vacuo*) and subsequent reduction of the double bond forms IX, and this, on oxidation, gives a tetracarboxylic acid *without loss of carbon atoms*. Thus the two keto groups in IX must be in *different* rings; had they been in the *same* ring, then carbon would have been lost and X not obtained. It therefore follows that the hydroxyl group and double bond in cholesterol must be in *different* rings. Furthermore, since IX forms a pyridazine derivative with hydrazine, IX is a γ-diketone. Since we have already tentatively placed the hydroxyl group in ring A, the above reactions can be readily explained if we place the hydroxyl group at position 3, and the double bond between 5 and 6. In the following equations only rings A and B are drawn; this is an accepted convention of focusing attention on any part of the steroid molecule that is under consideration (also note that full lines represent groups lying above the plane, and broken lines groups lying below the plane; see also §§4, 4a, 4b). Noller (1939) has shown that the pyridazine derivative is a polymer, and so the interpretation that IX is a γ-diketone is rendered uncertain. Supporting evidence, however, for the above interpretation is afforded by the fact that when cholesterol is heated with copper oxide at 290°, cholestenone, XI, is produced, and this on oxidation with permanganate forms a keto-acid, XII, with the loss of one carbon atom. The formation of XII indicates that the keto group and the double bond in cholestenone are in the *same* ring. The ultraviolet absorption spectrum of cholestenone shows that the keto group and the double bond are conjugated (Menschick *et al.*, 1932). These results can be explained if we assume that the double bond in cholesterol migrates in the formation of cholestenone, the simplest explanation being that the hydroxyl group



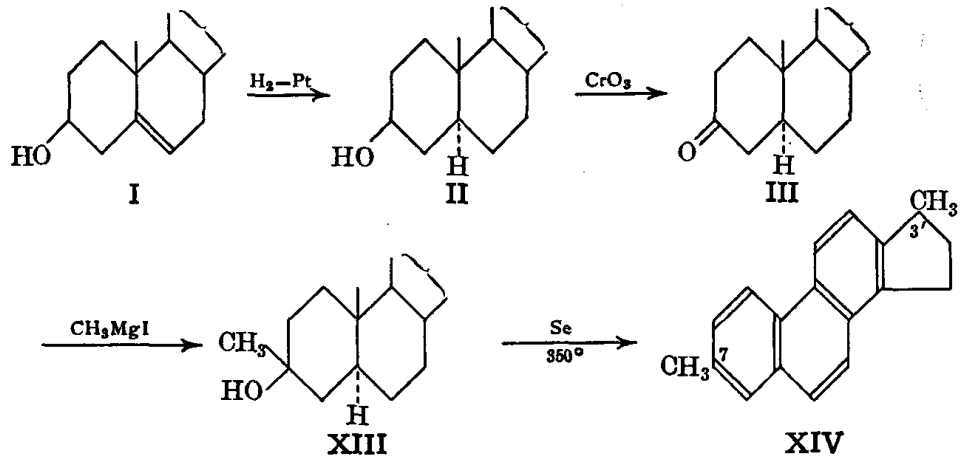
I VII VIII



is in position 3 and the double bond between 5 and 6, *position 5 being common to both rings A and B.* Thus:

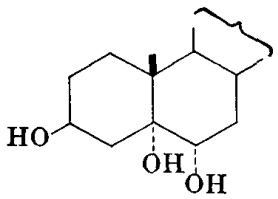


The position of the hydroxyl group at position 3 is definitely proved by the experiments of Kon *et al.* (1937, 1939). These authors reduced cholesterol, I, to cholestanol, II, oxidised this to cholestanone, III, treated this with methylmagnesium iodide and dehydrogenated the product, a tertiary alcohol, XIII, to 3′ : 7-dimethyl*cyclo*pentenophenanthrene, XIV, by means of selenium. The structure of XIV was proved by synthesis, and so the reactions may be formulated as follows, with the hydroxyl at position 3.

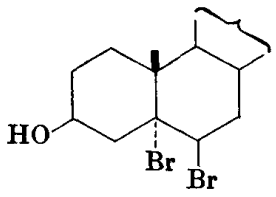


It might be noted here that the orientation of the two hydroxyl groups (introduced across the double bond in cholesterol) depends on the nature of the reagent used. With hydrogen peroxide, or *via* the oxide, the cholestanetriol is *trans*-5 : 6 (VII); with potassium permanganate or osmium

tetroxide, the product is *cis*-5 : 6 (VII*a*; *cf.* §5a. IV). These orientations may be explained as follows. When the addition of the two hydroxyl groups occurs *via* the oxide (the 5 : 6-oxide), the oxide ring will be formed *behind* the plane of the molecule due to the steric effect of the methyl group. Since opening of the epoxide ring occurs by attack on the conjugate acid (§5a. IV), the water molecule will attack from the back of the ring (*i.e.*, from the *front* of the molecule), and also preferably at position 6 due to the steric effect of the methyl group. Thus the orientation of the two hydroxyl groups (*trans*) will be as shown in VII. With permanganate (and osmium tetroxide),



VII *a*



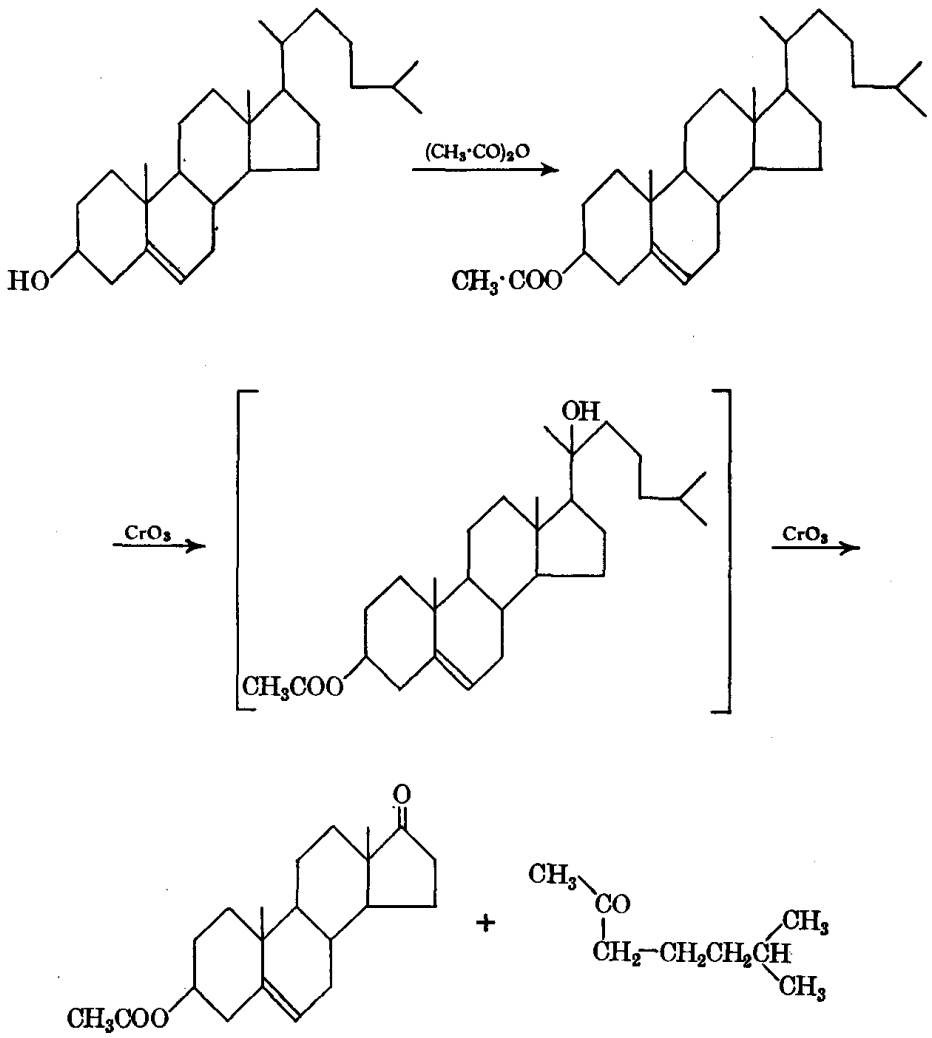
VII *b*

the plane of the cyclic compound will lie at the back of the molecule, again due to the steric effect of the methyl group. Moreover, since in the formation of the dihydroxy compound, *both* glycol oxygen atoms come from the permanganate ion (§5a. IV), it follows that *both* hydroxyl groups will be at the back of the molecule (VII*a*).

The addition of bromine, occurring *via* a brominium ion (§5a. IV), will produce the dibromide VII*b*, the reasons for the orientation being the same as those for the formation of VII (*via* the epoxide).

Since secondary alcoholic groups in steroids are readily oxidised to keto groups, and the latter may be located by mass spectra measurements (see §4b), this offers a very good way of locating secondary hydroxyl groups in the steroid molecule.

(iii) **Nature and position of the side-chain.** Acetylation of cholesterol produces cholesteryl acetate and this, on oxidation with chromium trioxide, forms a steam-volatile ketone and the acetate of a hydroxyketone (which is not steam volatile). The ketone was shown to be *iso*hexyl methyl ketone, $CH_3{\cdot}CO{\cdot}(CH_2)_3{\cdot}CH(CH_3)_2$. Thus this ketone is the side-chain of cholesterol, the point of attachment of the side-chain being at the carbon of the keto group. These results do not show where the side-chain is attached to the nucleus of cholesterol, but if we accept that the position is at 17, then we may formulate the reactions as follows:



The nature of the side-chain has also been shown by the application of the Barbier–Wieland degradation. Since this method also leads to evidence that shows *which ring* of the nucleus is attached to the side-chain, we shall consider the problem of the nature of the side-chain again.

The Barbier–Wieland degradation offers a means of " stepping down " an acid one carbon atom at a time as follows:

$$R{\cdot}CH_2{\cdot}CO_2H \xrightarrow[HCl]{CH_3OH} R{\cdot}CH_2{\cdot}CO_2CH_3 \xrightarrow{2C_6H_5MgBr}$$

$$R{\cdot}CH_2{\cdot}C(OH)(C_6H_5)_2 \xrightarrow{-H_2O} R{\cdot}CH{=}C(C_6H_5)_2 \xrightarrow{CrO_3}$$

$$R{\cdot}CO_2H + (C_6H_5)_2CO$$

Methylmagnesium bromide may be used instead of phenylmagnesium bromide, and the alcohol so obtained may be directly oxidised:

$$R{\cdot}CH_2{\cdot}C(OH)(CH_3)_2 \xrightarrow{CrO_3} R{\cdot}CO_2H + (CH_3)_2CO$$

In the following account, only phenylmagnesium bromide will be used to demonstrate the application of the method to the steroids.

Cholesterol was first converted into coprostane (a stereoisomer of cholestane; see §§4, 4a). If we represent the nucleus of coprostane as Ar, and

the side-chain as C_n, then we may formulate the degradation of coprostane as follows (B–W represents a Barbier–Wieland degradation):

$$\underset{\text{Ar—}C_n}{\text{Coprostane}} \xrightarrow{CrO_3} CH_3{\cdot}CO{\cdot}CH_3 + \underset{\text{Ar—}C_{n-3}}{\text{Cholanic acid}} \xrightarrow{\text{B–W}}$$

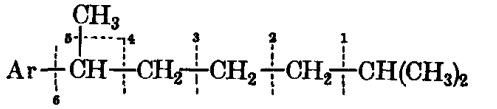
$$(C_6H_5)_2CO + \underset{\text{Ar—}C_{n-4}}{\text{Norcholanic acid}} \xrightarrow{\text{B–W}}$$

$$(C_6H_5)_2CO + \underset{\text{Ar—}C_{n-5}}{\text{Bisnorcholanic acid}} \xrightarrow{\text{B–W}}$$

$$(C_6H_5)_2CO + \underset{\text{Ar—}C_{n-6}}{\text{Ætiocholyl methyl ketone}} \xrightarrow{CrO_3} \underset{\text{Ar—}C_{n-7}}{\text{Etianic acid}}$$

The formation of acetone from coprostane indicates that the side-chain terminates in an *iso*propyl group. The conversion of bisnorcholanic acid into a ketone shows that there is an alkyl group on the α-carbon atom in the former compound. Furthermore, since the ketone is oxidised to etianic acid (formerly known as ætiocholanic acid) with the loss of one carbon atom, the ketone must be a methyl ketone, and so the alkyl group on the α-carbon atom in bisnorcholanic acid is a methyl group.

Now the carboxyl group in etianic acid is directly attached to the nucleus; this is shown by the following fact. When etianic acid is subjected to one more Barbier–Wieland degradation, a ketone, ætiocholanone, is obtained and this, on oxidation with nitric acid, gives a dicarboxylic acid, ætiobilianic acid, *without loss of any carbon atoms*. Thus ætiocholanone must be a *cyclic* ketone, and so it follows that there are *eight* carbon atoms in the side-chain, which must have the following structure in order to account for the foregoing degradations (see also the end of this section iii):



In addition to the Barbier–Wieland degradation, there are also more recent methods for degrading the side-chain:

(i) Gallagher *et al.* (1946) have introduced a method to eliminate *two* carbon atoms at a time:

$$Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H \xrightarrow[\text{(ii) } CH_2N_2]{\text{(i) } SOCl_2} Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CHN_2 \xrightarrow{HCl}$$

$$Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CH_2Cl \xrightarrow[AcOH]{Zn} Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CH_3 \xrightarrow[\text{(ii) } -HBr]{\text{(i) } Br_2}$$

$$Ar{\cdot}CHMe{\cdot}CH{=}CH{\cdot}CO{\cdot}CH_3 \xrightarrow{CrO_3} Ar{\cdot}CHMe{\cdot}CO_2H$$

(ii) Miescher *et al.* (1944) have introduced a method to eliminate *three* carbon atoms at a time:

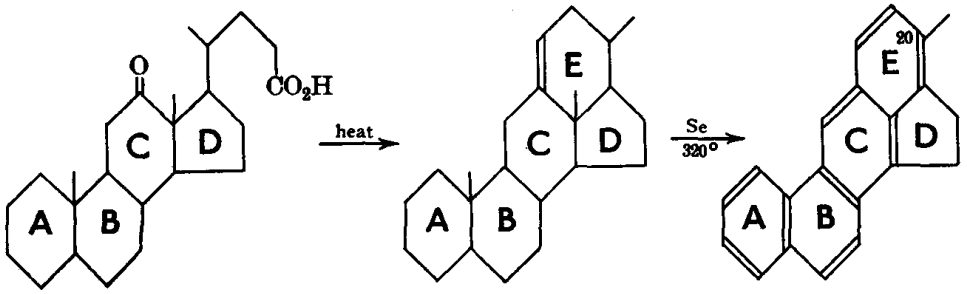
$$Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Me \xrightarrow{2PhMgBr} Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}C(OH)Ph_2 \xrightarrow{-H_2O}$$

$$Ar{\cdot}CHMe{\cdot}CH_2{\cdot}CH{=}CPh_2 \xrightarrow[\text{succinimide}]{N\text{-bromo-}} Ar{\cdot}CHMe{\cdot}CHBr{\cdot}CH{=}CPh_2 \xrightarrow{-HBr}$$

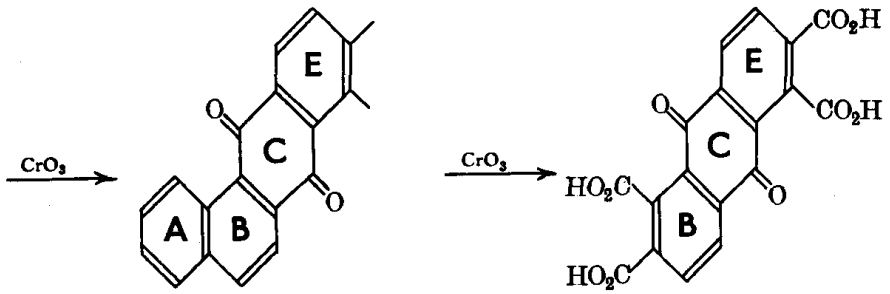
$$Ar{\cdot}CMe{=}CH{\cdot}CH{=}CPh_2 \xrightarrow{CrO_3} Ar{\cdot}COMe$$

(iii) Jones *et al.* (1958) have carried out the fission of a steroid side-chain with an acid catalyst and have then subjected the volatile products to chromatography. This method has been used with as little as 30 mg. of material.

The problem now is: Where is the position of this side-chain? This is partly answered by the following observation. The dicarboxylic acid, ætiobilianic acid, forms an anhydride when heated with acetic anhydride. Thus the ketone (ætiocholanone) is probably a five-membered ring ketone (in accordance with Blanc's rule), and therefore the side-chain is attached to the five-membered ring D. The actual point of attachment to this ring, however, is not shown by this work. The formation of Diels' hydrocarbon (§1) from cholesterol suggests that the side-chain is at position 17, since selenium dehydrogenations may degrade a side-chain to a methyl group (see §2 vii. X). Position 17 is also supported by evidence obtained from X-ray photographs and surface film measurements. Finally, the following chemical evidence may be cited to show that the position of the side-chain is 17. As we have seen above, cholanic acid may be obtained by the oxidation of coprostane. Cholanic acid may also be obtained by the oxidation of deoxycholic acid (a bile acid; see §8) followed by a Clemmensen reduction. Thus the side-chains in cholesterol and deoxycholic acid are in the same position. Now deoxycholic acid can also be converted into 12-ketocholanic acid which, on heating to 320°, loses water and carbon dioxide to form dehydronorcholene (Wieland *et al.*, 1930). This, when distilled with selenium, forms 20-methylcholanthrene, the structure of which is indicated by its oxidation to 5 : 6-dimethyl-1 : 2-benzanthraquinone which, in turn, gives on further oxidation, anthraquinone-1 : 2 : 5 : 6-tetracarboxylic acid (Cook, 1933). Finally, the structure of 20-methylcholanthrene has been confirmed by synthesis (Fieser *et al.*, 1935; see §3f. X). The foregoing facts can be explained only if the side-chain in cholesterol is in position 17; thus:



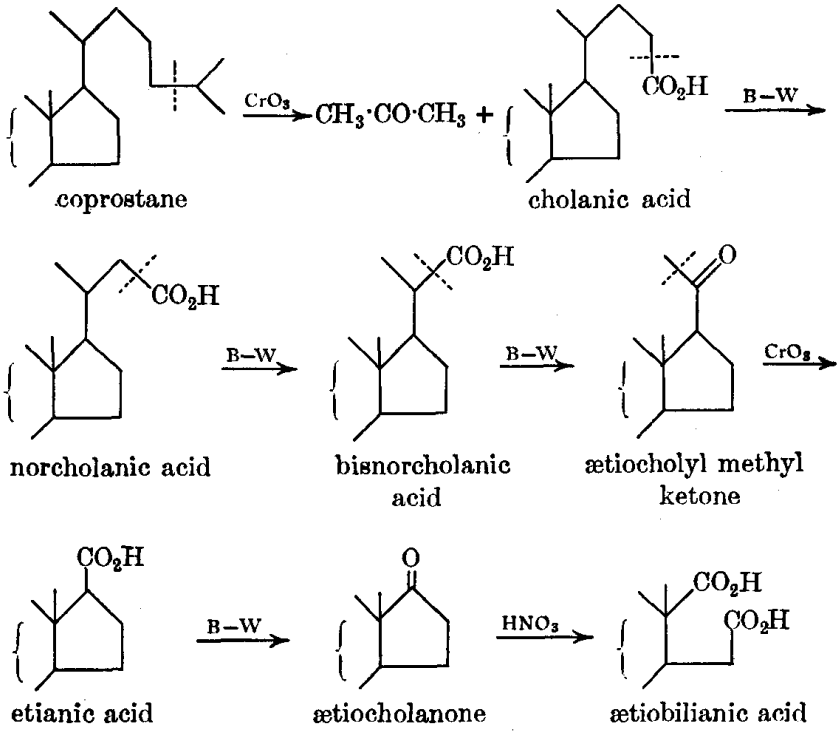
12-ketocholanic acid dehydronorcholene 20-methylcholanthrene



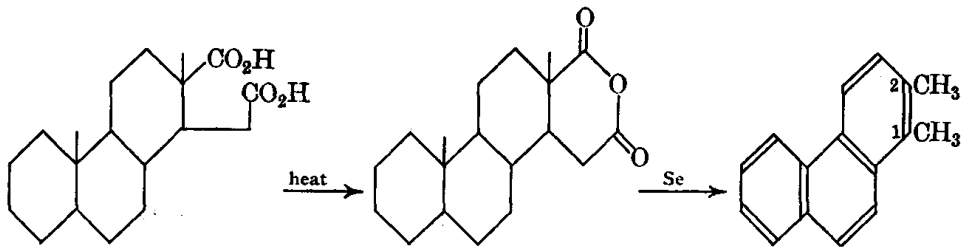
5:6-dimethyl-1:2-benzanthraquinone anthraquinone-1:2:5:6-tetracarboxylic acid

It should be noted that the isolation of methylcholanthrene affords additional evidence for the presence of the *cyclo*pentenophenanthrene nucleus in cholesterol.

Thus, now that we know the nature and position of the side-chain, we can formulate the conversion of coprostane into ætiobilianic acid as follows:



A point of interest in this connection is that when the anhydride of ætiobilianic acid is distilled with selenium, 1 : 2-dimethylphenanthrene is obtained (Butenandt *et al.*, 1933). This also provides proof for the presence of the phenanthrene nucleus in cholesterol, and also evidence for the position of the C_{13} angular methyl group (see iv).



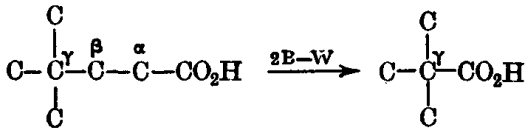
ætiobilianic acid
XV

anhydride

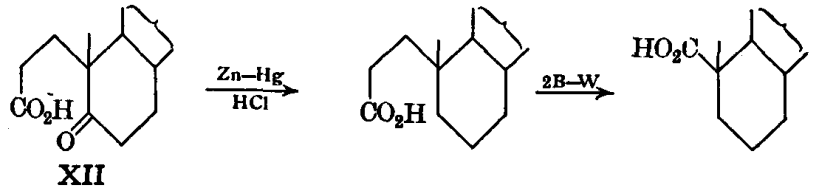
1:2-dimethyl-phenanthrene
XVI

(iv) **Positions of the two angular methyl groups.** The *cyclo*pentenophenanthrene nucleus of cholesterol accounts for seventeen carbon atoms, and the side-chain for eight. Thus twenty-five carbon atoms in all have been accounted for, but since the molecular formula of cholesterol is $C_{27}H_{46}O$, two more carbon atoms must be fitted into the structure. These two carbon atoms have been shown to be angular methyl groups.

In elucidating the positions of the hydroxyl group and double bond, one of the compounds obtained was the keto-acid XII. This compound, when subjected to the Clemmensen reduction and followed by two Barbier–Wieland degradations, gives an acid which is very difficult to esterify, and evolves carbon monoxide when warmed with concentrated sulphuric acid (Tschesche, 1932). Since these reactions are characteristic of an acid containing a carboxyl group attached to a tertiary carbon atom (*cf.* abietic acid, §31. VIII), the side-chain in XII must be of the type

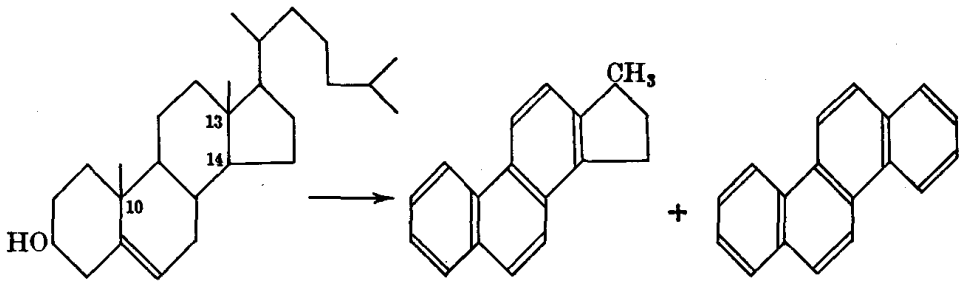


Thus there must be an alkyl group at position 10 in XII. This could be an ethyl group (as originally believed by Windaus and Wieland) or a methyl group, provided that in the latter case the second " missing " carbon atom can be accounted for. As we shall see later, there is also a methyl group at position 13, and so the alkyl group at position 10 must be a methyl group. On this basis, the degradation of XII may be formulated:



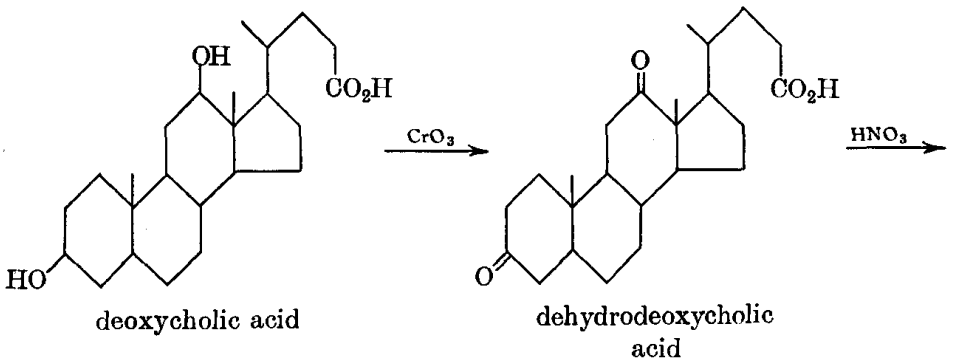
XII

The position of the other angular methyl group is indicated by the following evidence. When cholesterol is distilled with selenium, chrysene is obtained as well as Diels' hydrocarbon (see §1). How, then, is the former produced if the latter is the ring skeleton of cholesterol? One possible explanation is that there is an angular methyl group at position 13, and on selenium dehydrogenation, this methyl group enters the five-membered ring D to form a six-membered ring; thus:



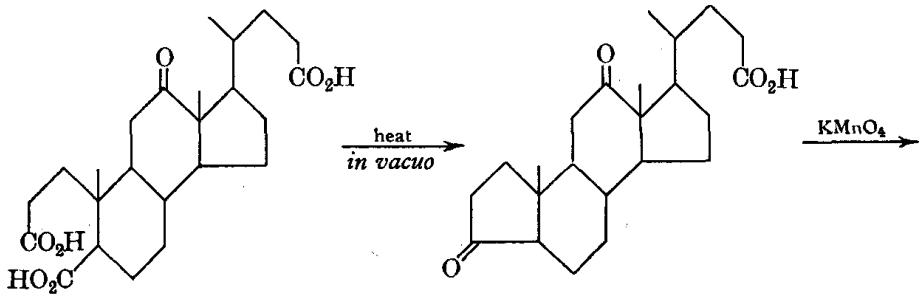
cholesterol Diels' hydrocarbon chrysene

This evidence, however, is not conclusive, since ring expansion could have taken place had the angular methyl group been at position 14. Further support for the positions of the two angular methyl groups is given by the following degradative experiments (Wieland *et al.*, 1924, 1928, 1933) (see overleaf).



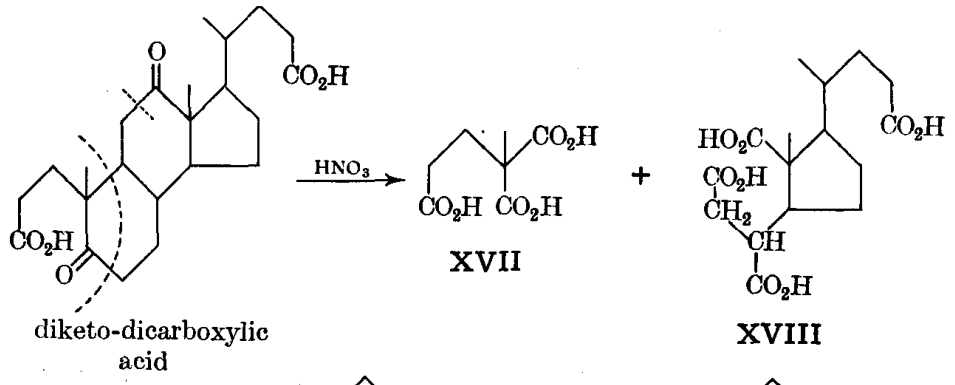
deoxycholic acid

dehydrodeoxycholic acid



deoxybilianic acid

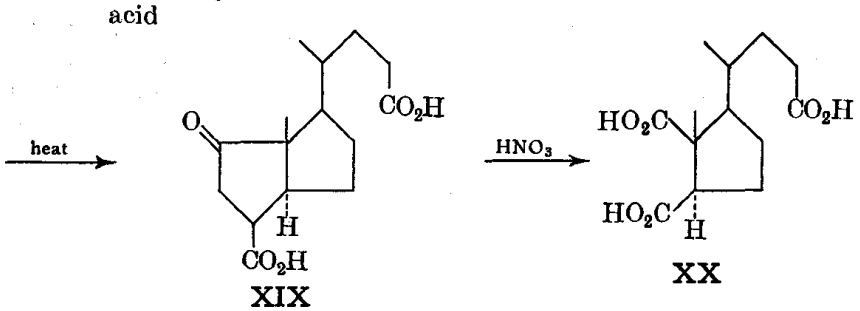
pyrodeoxybilianic acid



diketo-dicarboxylic acid

XVII

XVIII



XIX

XX

XVII was shown to be butane-2 : 2 : 4-tricarboxylic acid; thus there is a methyl group at position 10. XVIII was shown to be a tetracarboxylic acid containing a *cyclo*pentane ring with a side-chain

$$—CH(CH_3)\cdot CH_2\cdot CH_2\cdot CO_2H.$$

Thus this compound is derived from ring D. XX was also shown to be a tricarboxylic acid containing a *cyclo*pentane ring. Furthermore, one carb-