37. *Lanosterol. Part II. The Oxidation of Lanosterol with Chromic Acid.*

By Lionel J. Bellamy and Charles Dorée.

To throw light on the chemical nature of lanosterol, its reactions with chromic acid, selenium dioxide, and perbenzoic acid have been investigated. The results obtained with chromic acid are described in the present communication.

That bond movement takes place during the formation of the ketone lanostenone is shown (*a*) by its reduction to an isomer of lanosterol, lanosterol-E, and (*b*) by the isolation of a second ketone, *lanostenone-B*, which, since it is reducible smoothly to lanosterol, probably has the same bond system. The acid $C_{25}H_{46}O_2$ has been re-examined, but its saturated character and empirical formula remain in doubt.

The unsaponifiable fraction of the fat from the wool of the sheep contains considerable quantities of cholesterol together with a second alcohol of high molecular weight, formerly called *iso*cholesterol (Schulze, *Ber.*, 1872, **5**, 1775). The assignment of this substance to the sterol group was based on its origin as a product of animal metabolism. Its isomerism with cholesterol could not be confirmed, as the composition and properties of different preparations did not agree with one another, and it gradually became evident that *iso*-cholesterol was not a single compound.

This was confirmed by Windaus and Tschesche (*Z. physiol. Chem.*, 1930, **190**, 59), who showed that crude *iso*cholesterol consists of two unsaturated alcohols, which they named lanosterol, $C_{30}H_{50}O$ (about 90%), and agnosterol, $C_{30}H_{48}O$ (about 10%). Variations in these proportions explained the differences previously recorded in the properties of *iso*-cholesterol preparations. Lanosterol was shown to have two double bonds in the molecule, one of which was active and the other inert to catalytic hydrogenation. These results were confirmed by Dorée and Garratt (*J. Soc. Chem. Ind.*, 1933, **52**, 335 T) and by Marker, Wittle, and Nixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368).

The empirical formula of lanosterol suggested a connection with alcohols of the β-amyrin type rather than with the sterols; the results of dehydrogenation experiments supported this view, and showed that lanosterol did not possess the sterol structure. When heated with palladium charcoal, lanosterol gave a partially dehydrogenated phenanthrene hydrocarbon $C_{18}H_{20}$ (Dorée and Petrow, J., 1936, 1562), and with selenium it yielded 1 : 2 : 8-trimethylphenanthrene (Schulze, *Z. physiol. Chem.*, 1936, **238**, 35). The amyrin series of triterpenes usually yield 1 : 2 : 7-trimethylnaphthalene, 1 : 2 : 5-trimethyl-6-naphthol and 1 : 8-dimethylpicene, but the observation of Drake (*J. Amer. Chem. Soc.*, 1936, **58**, 1681), that friedelin, a triterpene from cork, yields some of these compounds in addition to 1 : 2 : 8-trimethylphenanthrene, indicates that the formation of this hydrocarbon is not incompatible with a triterpenoid structure. A minor point of resemblance between lanosterol and the resinoid alcohols is found in the properties of the monolayer formed by spreading on a water surface. Lanosterol forms an unstable film which rapidly changes to the normal " condensed solid " type (Dorée and Petrow, *loc. cit.*). This unusual phenomenon is shown also by β-amyrin and its derivatives.

Windaus and Tschesche (*loc. cit.*), from the results of perbenzoic acid titrations, concluded that lanosterol possessed two unsaturated centres, one of which was reactive and the other inert to catalytic hydrogenation. Later workers (Dorée and Petrow, J., 1936, 1562; Marker, Wittle, and Nixon, *loc. cit.*) have confirmed this view and the existence in the lanosterol molecule of two unsaturated linkages may be considered as satisfactorily proved.

A doubly unsaturated alcohol, $C_{30}H_{50}O$, must be tetracyclic and, if the relationship of lanosterol to the triterpenes is accepted, a formulation such as (I), based on that of β-amyrin with ring *B* open, may be suggested as a working hypothesis to explain the results obtained in this communication. The formula resembles that of basseol, $C_{30}H_{50}O$, which has been shown by Heilbron, Spring, and others (*Nature*, 1938, **142**, 434; *Chem. and Ind.*, 1939, **58**, 58) and by Ruzicka (*Helv. Chim. Acta*, 1937, **20**, 1553) to have a tetracyclic structure.

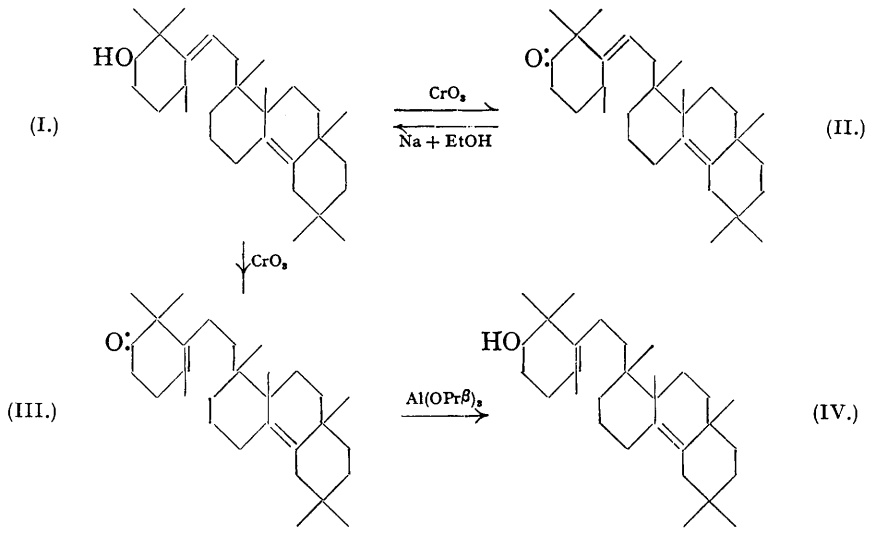
The reactive double link in the tentative lanosterol structure (I) is assigned a position near the hydroxyl group, but not in the same ring. This placing is supported : (*a*) By the observation that the ketones formed from lanosterol are not αβ-unsaturated and therefore the double link cannot be very near to the oxygen atom, even allowing for migration such as occurs during the oxidation of the unsaturated sterol alcohols. (*b*) By the production of the " saturated monobasic non-ketonic acid " $C_{25}H_{46}O_2$ (see p. 174) which results from the oxidation of lanosterol with excess of chromic acid (Marker, Wittle, and Nixon, *loc. cit.*). Its formation, presumably by rupture of the molecule at the position of the active double link, indicates the breakdown of the ring containing the hydroxyl group with the loss of five or perhaps more carbon atoms, and this would be possible on a triterpenoid skeleton if there is adjacent to ring *A* an open ring in which the double link is situated. (*c*) By the analogy of the unimolecular film areas of oxidolanosterol, oxidolanosteryl acetate, and lanostenetriol (Dorée and Petrow, *loc. cit.*), which are of the same order as those of the corresponding derivatives of cholesterol, indicating probably a similar relationship between the position of the hydroxyl group and the double link in both substances.

Agnosterol has been shown by Marker and Wittle (*J. Amer. Chem. Soc.*, 1937, **59**, 2289) to have the same carbon skeleton as lanosterol. It has three unsaturated centres in the molecule, one active and two inert to catalytic hydrogenation. The ultra-violet absorption spectrum indicates that two of these linkings are conjugated (Windaus and Tschesche, *loc. cit.*).

Lanosterol is very sensitive to the action of chromic oxide in acid solution. Windaus and Tschesche (*loc. cit.*) were unable to isolate any crystalline products from normal chromic acid oxidation, but Dorée and Garratt (*loc. cit.*), using specially restrained conditions, isolated in small yield (3%) a ketone, lanostenone, m. p. 115°, together with traces of a second ketone, m. p. 89°, and a non-crystalline monobasic ketonic acid.

Lanostenone on reduction with sodium and ethyl alcohol was said to give an alcohol, lanosterol-A, which was not identical with lanosterol. It was deduced that the bond system of lanostenone differs from that in lanosterol, migration having taken place during oxidation (Dorée and Garratt, *loc. cit.*).

It is now found that by working at room temperature with chromic acid, lanostenone (III) is obtained as a main product (up to 40%). On reduction with aluminium *iso*-



propoxide, a reagent with which bond movement is unlikely, lanostenone gives a new isomer of lanosterol (IV), here designated lanosterol-E.* With sodium and ethyl or propyl alcohol, reduction of lanostenone proceeds further to give α-dihydrolanosterol, the active double bond becoming saturated. This observation proves that the inert double link in lanostenone is in the same position as it is in lanosterol, since an identical product is obtained by the hydrogenation of either substance.

Marker (*loc. cit.*) has also demonstrated the immobility of the inert double bond during oxidation by converting α-dihydrolanosterol into the corresponding ketone and showing that on reduction the original alcohol is obtained. It is therefore the active double bond of lanosterol that migrates, and the fact that it is not adjacent to the hydroxyl group is supported by our observation that lanosterol is unaffected by aluminium *tert.*-butoxide in acetone solution (Oppenauer). The negative result indicates that at least an αβ-position to the hydroxyl group is unlikely. The formation of the acid $C_{25}H_{46}O_2$ by severe oxidation of lanosterol or α-dihydrolanosterol with chromic acid (Marker, Wittle, and Nixon, *loc. cit.*) is also of importance in this connection. The acid is described as monobasic, saturated and non-ketonic. The difficulty of accounting for the formation of an acid with such properties and a formula containing 25 carbon atoms, led us to re-examine the composition and reactions of this substance, but we can only confirm the original statements. The acid proved indifferent to bromine at 0°, to hydrogen (palladium charcoal), and to perbenzoic acid. No carbonyl reactions were given, and the ultra-violet absorption spectrum (Figure) showed general absorption, whereas, had an αβ-unsaturated ketonic system been present (which in some triterpenes gives negative reactions), selective absorption would have been expected. The substance requires further critical examination, but its existence indicates a centre in the lanosterol molecule which is open to oxidative attack.

From the mother-liquors of the preparation of lanostenone a second isomeric ketone, m. p. 78°, has been obtained, which is provisionally designated *lanostenone-B*. This ketone on reduction gives lanosterol (I), from which it would appear that lanostenone-B (II) is the ketone corresponding in structure to lanosterol, the mild conditions of oxidation employed allowing a fraction of the alcohol to be converted without bond movement.

The oxidation of lanosteryl acetate with chromic acid was undertaken by Dorée and

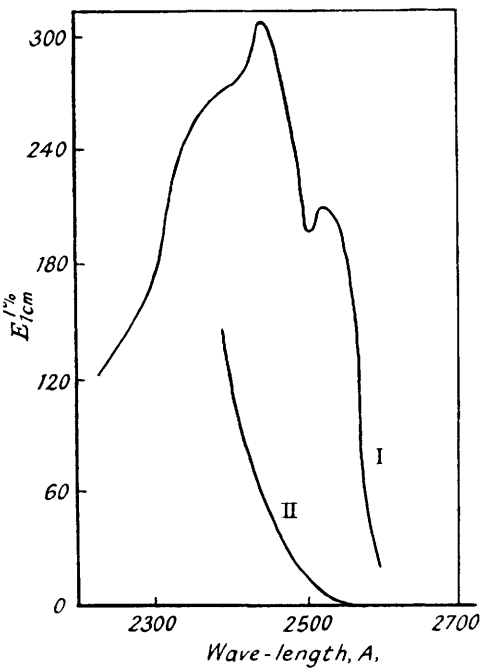
* A series of these isomers has been described. Lanosterol-A is no doubt a mixture of lanosterol-E with α-dihydrolanosterol. Isomers B and C are doubtful. For lanosterol-D, see next page.

Petrow (J., 1936, 1562) in the expectation of converting a methylene group adjacent to an unsaturated linking into a carbonyl group, as examples of this reaction in the triterpene series are well known (Spring and Vickerstaff, J., 1934, 1859; 1937, 249). They obtained unexpectedly the acetate of an alcohol, lanosterol-D, which was stated to be isomeric with lanosterol and to give lanostenone on further oxidation. A repetition of the work shows that lanosteryl-D acetate, m. p. 164°, is certainly formed and that, in the absence of chromic acid, neither glacial acetic acid nor chromic acetate has any effect on lanosteryl acetate.

Professor Marker (private communication) informs us that he has confirmed this result, and has prepared lanosteryl-D acetate, m. p. 164°, but that lanosterol-D itself after extensive purification shrinks at 139—141°, and melts between 155° and 160°, indicating that the limit of purification is not yet reached. Lanosterol-D obtained by us had m. p. 145° and on oxidation with chromic acid gave a ketone, m. p. 105° (tetrahydrocarbazole derivative, m. p. 128°), values which are very different from those given by lanostenone (115° and 202° respectively).

Lanosteryl-D acetate on hydrogenation absorbs one molecular proportion of hydrogen per molecule, giving a dihydrolanosteryl-D acetate, m. p. 218°, differing sharply from α-dihydrolanosteryl acetate (m. p. 119°). The ultra-violet absorption curve given by the acetate of lanosterol-D (Figure) shows strong absorption in the region of 2400—2500 A., indicating that two conjugate double bonds are present.

The action of chromic acid on lanosteryl acetate may possibly bring about the formation of a second inert double bond, in which case lanosterol-D with three double linkings would be an isomer, not of lanosterol, but of agnosterol.



Absorption spectra of (I) *the acetate of lanosterol-D and* (II) *the acid* $C_{25}H_{46}O_2$ (*lanosterol and β-lanostenone similar*).

Experimental.

Analyses by Dr. Weiler, Oxford. Melting points are uncorrected.

Lanostenone: Improved Method of Preparation.—18·5 G. of chromic acid in 190 ml. of water were mixed with 300 ml. of glacial acetic acid, 21 g. of lanosterol in 300 ml. of benzene added, and the whole shaken for 6 hours. After dilution with water the benzene layer was removed, extracted with potassium hydroxide and with water, and dried (sodium sulphate). The benzene solution gave a crystalline mass, which was repeatedly fractionated from acetone–methyl alcohol. Yield, up to 40% of lanostenone.

Lanostenone-B.—The residues from the mother-liquors of the first five crystallisations of the above crude product were fractionated from ethyl alcohol, giving *lanostenone-B* in long needles, m. p. 78°, easily soluble in acetone, benzene and ethyl acetate, less soluble in methyl alcohol (Found: C, 84·3; H, 11·2. $C_{30}H_{48}O$ requires C, 84·8, H, 11·4%). The 2 : 4-dinitrophenylhydrazone formed yellow plates, m. p. 191°. The tetrahydrocarbazole derivative was prepared by warming 0·08 g. of the ketone in 25 ml. of glacial acetic acid with 0·16 g. of phenylhydrazine in 10 ml. of the same solvent for 1 hour on the water-bath. On cooling, colourless crystals separated; these were recrystallised from glacial acetic acid and then had m. p. 178°.

Reduction of Lanostenone-B to Lanosterol.—0·35 G. of lanostenone-B was dissolved in 30 ml. of boiling ethyl alcohol, 1 g. of sodium added as quickly as possible, the solution diluted with 15 ml. of alcohol, and 0·5 g. more of sodium added. The solution was refluxed for 2 hours, poured into water, and extracted with ether. The extract was dried (sodium sulphate), the ether removed, and the product recrystallised from acetone–methyl alcohol (2 : 1), giving lanosterol, m. p. 140—141°, showing no depression on admixture with an authentic specimen.

The acetate, m. p., 114°, and the benzoate, m. p. 187°, of this product were prepared and confirmed its identity with lanosterol.

α-Dihydrolanosteryl Acetate from Lanostenone.—2 G. of lanostenone in 75 ml. of boiling ethyl alcohol were treated rapidly with 3 g. of sodium; 25 ml. of alcohol, and a further 1 g. of sodium were then added. After boiling for 2 hours, the solution was poured into water and extracted with ether. The extract, after drying and removal of the ether, gave a product, which was acetylated by heating with acetic anhydride on the water-bath. The acetate after five crystallisations from ethyl acetate had m. p. 118°, not depressed by authentic α-dihydrolanosteryl acetate kindly supplied by Professor Marker. After saponification α-dihydrolanosterol, m. p. 148°, was obtained.

Lanosterol-E.—4·5 G. of lanostenone were dissolved in 70 ml. of dry *iso*propyl alcohol, and 6 g. of aluminium *iso*propoxide added. The mixture was distilled at the rate of one drop per minute, the distillate giving a positive acetone reaction with sodium nitroprusside in acetic acid. After 8 hours, 200 ml. of 5% methyl-alcoholic potash were added, and the mixture warmed for 1 hour. After pouring into water, an ethereal extract gave a crude product, which was acetylated. It required 20 crystallisations from ethyl acetate and from glacial acetic acid to yield the *acetate* of lanosterol-E, in small needles, m. p. 164° (Found: C, 82·0; H, 11·6. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%). After saponification lanosterol-E was obtained, m. p. 143°.

The Acid $C_{25}H_{46}O_2$.—50 G. of lanosterol in 1 l. of glacial acetic acid were heated to 90° on the water-bath; 100 g. of chromic acid, dissolved in 500 ml. of glacial acetic acid containing 25 ml. of water, were added in portions during 3 hours. On cooling, the solid product was separated, dried in a vacuum, and extracted with ether (Soxhlet). The extract after removal of the ether gave the acid, which was crystallised from methyl ethyl ketone. A further yield was obtained by dilution of the original reaction liquid to turbidity and repetition of the process. This method of isolation was found preferable to that of Marker (*loc. cit.*) owing to the sparing solubility of the acid in ether and the comparative insolubility of the sodium and potassium salts in water. The acid formed a microcrystalline powder, m. p. 81—82° (Found: C, 79·0; H, 12·6; *M*, 388. Calc. for $C_{25}H_{46}O_2$: C, 79·2; H, 12·8%; *M*, 379). The ethyl ester, prepared in the usual way and crystallised from aqueous acetone, had m. p. 64°.

Acetate of Lanosterol-D.—Lanosteryl acetate (10 g.) in glacial acetic acid (150 ml.) was treated with a solution of chromic acid (9 g.) in water (5 ml.), which was added with stirring during 1 hour at 90°. The mixture was diluted with water and extracted with ether. The ethereal solution was washed with dilute acid and dilute alkali. The residue obtained on evaporation was repeatedly crystallised from acetone, ethyl acetate, and glacial acetic acid and gave the acetate of lanosterol-D, m. p. 164°, $[\alpha]_D^{20°}$ (chloroform) + 44°.

Saponification with 5% alcoholic potassium hydroxide gave lanosterol-D, which, after exhaustive purification from acetone–methyl alcohol, had m. p. 145°.

Lanostenone-D.—This was prepared by oxidation with chromic acid as in the preparation of lanostenone. It formed small plates, m. p. 105° (tetrahydrocarbazole derivative, white plates, m. p. 128°).

Acetate of Dihydrolanosterol-D.—The acetate of lanosterol-D (0·2 g.) in glacial acetic acid (25 ml.) was shaken with hydrogen at 20° for 6 hours, palladium charcoal (0·05 g.) being used as catalyst. Hydrogen equivalent to one double bond was absorbed. The product crystallised from glacial acetic acid in small needles, m. p. 218°, slightly soluble in methyl alcohol and glacial acetic acid.

Non-action of the Oppenauer Reagent on Lanosterol.—10 G. of lanosterol in 100 ml. of hot acetone were mixed with a solution of 12 g. of crystalline aluminium *tert.*-butoxide in 300 ml. of acetone and refluxed for 10 hours. The product, isolated in the usual way, consisted of unchanged lanosterol.

CHELSEA POLYTECHNIC, LONDON, S.W. 3. [*Received, August 13th*, 1940.]