345. *Lanosterol. Part I.*

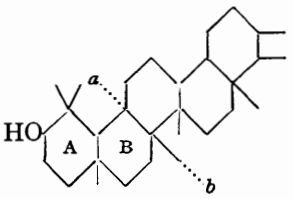
By Charles Dorée and Vladimir A. Petrow.

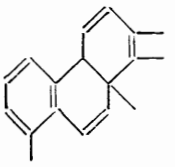
The unsaponifiable portion of the grease from the wool of the sheep contains cholesterol and *iso*cholesterol. The latter has been shown to consist chiefly of lanosterol, $C_{30}H_{50}O$, together with about 10% of a similar substance, agnosterol, $C_{30}H_{48}O$ (Windaus and Tschesche, *Z. physiol. Chem.*, 1930, **190**, 59). The association of lanosterol with cholesterol and the apparent similarity of their reactions caused lanosterol to be regarded as a sterol, although Windaus and Tschesche (*loc. cit.*) suggested that it might be related to the amyrins.

Our own observations tended to the same conclusion, and to determine the question of the existence or otherwise of the sterol ring system in lanosterol, dehydrogenation experiments were carried out. Methyl*cyclo*pentenophenanthrene was not obtained by the action of selenium, or of palladium–charcoal, on pure lanosterol under various conditions. Palladium–charcoal gave an oily hydrocarbon (easily separated by means of its red trinitrobenzene adduct), apparently of the composition $C_{18}H_{20}$. The yield was too small for closer diagnosis and during the preparation of further material Schultze (*Z. physiol. Chem.*, 1936, **238**, 35) described the formation of 1 : 2 : 8-trimethylphenanthrene from *iso*cholesterol by the action of selenium. If this hydrocarbon is derived from lanosterol, its production indicates an amyrin-type of structure for the parent substance.

The oily nature of the hydrocarbon obtained from lanosterol with palladium–charcoal suggests incomplete dehydrogenation. The carbon skeleton (I) recently suggested by Ruzicka *et al.* (*Helv. Chim. Acta*, 1936, **19**, 386) for the triterpenes being accepted, this

(I.)





(II.)

hydrocarbon might be represented by (II), a fission of the isocyclic system taking place along the dotted line *ab*, followed by partial dehydrogenation of the hydrophenanthrene residue.

The Unsaturated Centres of Lanosterol.—The presence of two double linkings in lanosterol

was indicated by Windaus and Tschesche (*loc. cit.*), who found that lanosterol absorbed two atoms of oxygen from perbenzoic acid. Dorée and Garratt (*J. Soc. Chem. Ind.*, 1933, **52**, 355T), using a series of lanosterol derivatives, found an absorption of one atom.

Using perbenzoic acid of the same concentration as that employed by these authors, we find that one atom of oxygen is rapidly absorbed (in 5 hours at 0° by lanosterol), after which the absorption is very slow, the total never exceeding 1·4—1·5 atoms of oxygen. The isomers lanosterol-D (p. 1564) and *iso*lanosterol (p. 1564) behave in the same way (Table II).

TABLE I.

Rate of Consumption of Oxygen from Perbenzoic Acid at 0° by Lanosterol and Lanosteryl Acetate.

Time (hours.).	Atoms of oxygen consumed. Lanosteryl acetate.	Lanosterol.
1	0·65	0·72
3	0·77; 0·79	0·87; 0·89
14	1·10; 1·10	1·24
40	1·26	1·30
70	1·45	1·48
120	1·45; 1·40	1·40; 1·40

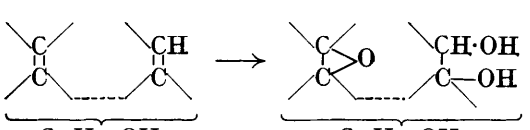
TABLE II.

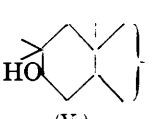
Consumption of Oxygen from Perbenzoic Acid after 120 hours at 0°.

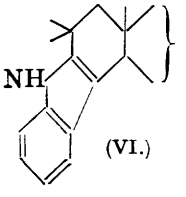
Compound.	Atoms of oxygen consumed.
Lanosterol	1·40
Lanosteryl acetate	1·42
Lanosterol-D	1·40
*iso*Lanosterol	1·40
*iso*Lanostenone	1·53

The results can be explained in either of two ways: (1) Following the rapid absorption of one atom of oxygen by the reactive linking, a second inert linkage may apparently absorb up to half an atom of oxygen, in analogy with the behaviour of the inert double bond of α-pimaric acid (Ruzicka and Frank, *Helv. Chim. Acta*, 1932, **15**, 1994). (2) Dehydrogenation may take place as in the case of methyl dihydro-α-pimarate, which is converted into a dehydro-derivative by the action of perbenzoic acid (Ruzicka and Frank, *loc. cit.*; cf. also Ruzicka, Silbermann, and Fürter, *ibid.*, p. 482).

The results therefore are not conclusive as to the presence of more than one unsaturated linkage in the molecule. More definite evidence for the presence of a second double linking has, however, been obtained from the following results: (i) Lanosterol gives an ultra-violet absorption spectrum (Windaus and Tschesche, *loc. cit.*)—a result we have confirmed. As selective absorption of ultra-violet light is shown only by sterolic compounds with more than one double bond (Heilbron, Morton, and Sexton, J., 1929, 47), lanosterol also should contain more than one. (ii) Treatment of lanosteryl acetate with perbenzoic acid in quantity equivalent to one atom of oxygen gives *lanosteryl acetate oxide*, m. p. 178°, which on saponification yields *lanosteryl oxide*, m. p. 140°. These compounds give yellow colorations with tetranitromethane and are therefore still unsaturated. (iii) Hydrogen peroxide gives with a double bond such as is present in cholesterol an α-glycol (Pickard and Yates, J., 1908, **93**, 1678), and with an inert double bond of the type found in β-amyrin, an oxy-compound (Spring, J., 1933, 1345). Lanosterol (III) by analogy was expected to give an oxytriol derivative with hydrogen peroxide—an assumption justified by the isolation of a fully saturated compound (tetranitromethane), the analysis of which agreed with that required for an *oxylanostanetriol*, $C_{30}H_{52}O_4$ (IV).







These results demonstrate the presence of a reactive and an inert double bond. With regard to their position the fact that lanostenone is not an αβ-unsaturated ketone has been deduced (i) from the ultra-violet absorption spectrum, the bond characteristic of αβ-unsaturated ketones being absent (measurements by Dr. Callow) as shown in the following table:

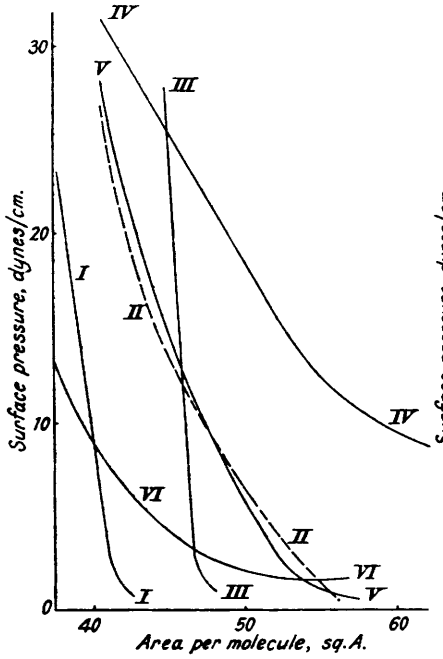
	Wave-length, A.	$E^{1\%}_{1\,cm.}$		Wave-length, A.	$E^{1\%}_{1\,cm.}$
Subsidiary maximum...	2520	37	Minimum	2400	42
Minimum	2500	35·5	Maximum	2355	67
Subsidiary maximum...	2450	56			

and (ii) by its ready conversion into a tetrahydrocarbazole derivative which, on the basis of surface-film measurements, has an angular structure (Dorée and Petrow, J., 1935, 1391). [The triterpene skeleton (V), suggested by Ruzicka (*Helv. Chim. Acta*, 1936, **19**, 114) at the beginning of the year, permits an angular structure (VI) for the tetrahydrocarbazole, but his more recent modification (I) could give only a " linear " compound.]

The observations indicate the presence of the group $CH_2{\cdot}CO{\cdot}CH$ in lanostenone.

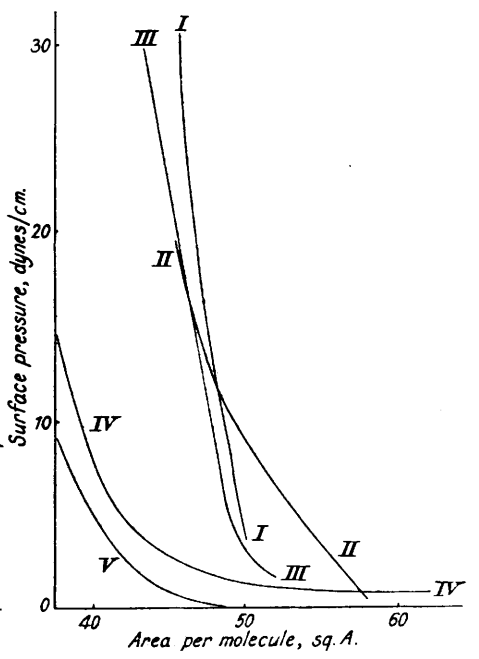
The Isomers of Lanosterol.—To obtain evidence of the position of the " inert " double bond, lanosterol was treated with hydrogen chloride—a reagent that frequently causes an

FIG. 1.



I. *Lanosterol (condensed solid)*; II. *Lanosterol (unstable)*; III. *β-Amyrin*; IV. *Lanosterol acetate oxide*; V. *Lanosterol oxide*; VI. *Lanostenetriol.*

FIG. 2.



I. *Oxylanostanetriol*; II. *Lanostenone*; III. *Tetrahydrocarbazole derivative from lanostenone*; IV. *Lanosteryl acetate*; V. *Acetate of lanosterol-D.*

inert double bond to migrate to a reactive position (Heilbron and Wilkinson, J., 1932, 1708; Reindel, Walter, and Rauch, *Annalen*, 1927, **452**, 34). An isomer was obtained for which the name iso*lanosterol* is suggested. When treated with perbenzoic acid, however, *iso*lanosterol and its derivatives gave values identical with those previously obtained for lanosterol (Tables I and II). The inert double link therefore remains and, if either linkage has shifted, it would appear to be the reactive one.

An attempt was made to characterise the inert double bond by oxidation of lanosteryl acetate with chromic acid, whereby, on analogy with β-amyrin, it was expected that an oxy-compound would be produced (Spring and Vickerstaff, J., 1934, 1859). Lanosteryl acetate was, however, converted by this treatment into another isomer, *lanosterol-D*, characterised by the high melting point of its *acetate*. This change was not due to the action of the acetic acid used as solvent, for lanosteryl acetate was recovered unchanged after analogous treatment with acetic acid alone. Lanosterol-D gave lanostenone on oxidation and thus apparently differs from lanosterol in the arrangement of the hydroxyl group in space (as in cholestanol and epicholestanol)—a conclusion supported by surface-film measurements.

Surface-film Measurements on Lanosterol and its Derivatives.—By means of the standard technique the lanosterol molecule has been found to occupy an area of 42 sq. A. (Fig. 1), a value falling within the limits required by the sterols and amyrins. When spread on water, it differs from the sterols in first forming an " unstable film " (Fig. 1, curve II), which rapidly changes to the " condensed solid " type. This abnormal behaviour is shown by other derivatives of lanosterol and also by β-amyrin and β-amyrone (private communication from Dr. F. A. Askew). Lanostenone, on the other hand, behaves normally and its surface pressure–area curve is almost identical with that given for coprostenone (Adam, Askew, and Danielli, *Biochem. J.*, 1935, **29**, 1786).

Measurements have also been made on lanosteryl acetate oxide, lanosterol oxide, lanostenetriol (Fig. 1), and oxylanostanetriol (Fig. 2) with a view to obtaining evidence for the position of the reactive double bond in lanosterol. These compounds behave like lanosterol, the initial film rapidly contracting to a solid film. The areas given by these solid films (Figs. 1 and 2) are of the same order as those given by cholestanetriol (56 sq. A.), cholestanetriol 3-acetate (67 sq. A.), and β-ergostadienetriol (46 sq. A.) and are consistent with a second water-attracting group in ring B (I). If this is so, the facts that lanosterol does not give toluenetetracarboxylic acid on oxidation with nitric acid (following Reindel and Niederländer, *Annalen*, 1930, **482**, 264) and does not form a maleic anhydride adduct (following Windaus and Lüttringhaus, *Ber.*, 1931, **64**, 850), can only be taken to imply that the other double bond is situated elsewhere than in ring B.

The curve for the tetrahydrocarbazole derivative is shown in Fig. 2. It gives a stable, relatively incompressible, film occupying an area of 50 sq. A. at zero pressure. This area indicates that the water-attracting group (NH) is in such a position that, like the hydroxyl group of cholesterol, it is at the end of the molecule and will be able to reach the water when the molecule is situated with its long dimension vertical.

The surface area–pressure curve of the acetate of lanosterol-D, and, for comparison, that of lanosteryl acetate, are given in Fig. 2. The films are much more compressible and have larger limiting areas than the parent compound owing to the weakening effect of acetylation on the water-attracting power of the hydroxyl group. Lanosteryl acetate occupies a larger area (44·7 sq. A.) at zero pressure than the acetate of lanosterol-D (39·7 sq. A.). Adam, Askew, and Danielli (*loc. cit.*) have shown that with the sterols the epi-derivatives occupy the larger areas, so the isomerism of lanosterol and lanosterol-D may be of a similar type.

Experimental.

Dehydrogenation of Lanosterol with Palladium–Charcoal.—10 G. of lanosterol and 15 g. of palladium–charcoal (Diels and Gädke, *Ber.*, 1925, **58**, 1231) were heated together for 55 hours at 330—360°. The product was extracted with benzene, distilled at 1 mm., and the fractions, b. p. (i) 80—110°; (ii) 110—170°; (iii) 170—250°, collected.

Fraction (i) was a pale yellow, mobile oil which did not react with picric acid or with trinitrobenzene (Found: C, 88·1; H, 11·1; *M*, 220. Calc. for $C_{16}H_{24}$: C, 88·9; H, 11·1%; *M*, 216).

Fraction (ii) was purified by chromatographic adsorption and treated with trinitrobenzene. The adduct, after recrystallisation, had m. p. 147—148°. The hydrocarbon was regenerated and, after further purification by chromatographic adsorption, formed a pale yellow glass (Found: C, 92·0; H, 8·0. Calc. for $C_{18}H_{26}$: C, 91·5; H, 8·5%). On treatment with trinitrobenzene and purification from benzene–alcohol the adduct was obtained in dark red needles, m. p. 186—187° [Found: C, 65·1; H, 4·7; N, 8·6. Calc. for $C_{18}H_{26},C_6H_3(NO_2)_3$: C, 64·1; H, 5·1; N, 9·3%].

Titrations with Perbenzoic Acid.—0·2 G. of lanosterol was mixed with 5 ml. of a chloroform solution of perbenzoic acid equivalent to 0·019 g. of active oxygen and kept at 0°. The results are in Table I.

Action of Perbenzoic Acid on Lanosteryl Acetate.—To a solution of 5 g. of lanosteryl acetate in 50 ml. of chloroform at 0° was added, during 30 minutes, a solution of perbenzoic acid equivalent to 0·19 g. of active oxygen. After 12 hours at 0° the solvent was removed. The residue, in ether, was washed with sodium carbonate solution and dried, and the ether removed. The product, after purification, consisted of *lanosteryl acetate oxide*, glistening plates, m. p. 178° (Found: C, 79·2; H, 10·6. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8%), easily soluble in ethyl acetate and acetone and sparingly in alcohol. After saponification (1½ hours with 5%

alcoholic potassium hydroxide), *lanosterol oxide* was obtained in needles, m. p. 139—140° (Found: C, 80·4; H, 11·9. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·3%).

Action of Hydrogen Peroxide on Lanosteryl Acetate.—4 G. of lanosteryl acetate in 20 ml. of glacial acetic acid were treated with 4 ml. of 100-vol. hydrogen peroxide. The mixture, heated on a water-bath, at once separated into two layers; the upper oily layer gradually disappeared. After 3 hours the whole was poured into water, and the precipitated gum deacetylated by refluxing for 2 hours with excess of sodium methoxide in methyl alcohol. Crystallisation of the product from methyl alcohol gave 0·2 g. of *oxylanostanetriol* in silky needles, m. p. 120—121° (Found: C, 75·9; H, 10·8. $C_{30}H_{52}O_4$ requires C, 75·6; H, 10·9%). Acetylation of the oxylanostanetriol, followed by purification from methyl alcohol, gave silky plates of a *monoacetate*, m. p. 162—163° (Found: C, 76·7; H, 10·0. Oxylanostenediol monoacetate, $C_{32}H_{52}O_4$, requires C, 76·8; H, 10·4%. A diacetate would require C, 75·3; H, 10·0%).

Preparation of Lanostenone.—The following procedure gives a better yield than that of Dorée and Garratt (*loc. cit.*): 1 G. of lanosterol in 200 ml. of acetic acid was mixed with 0·43 g. of chromic acid in 20 ml. of acetic acid and 1 ml. of water and kept at room temperature for 9 hours with mechanical stirring. The product was poured into water, and the ketone purified from methyl alcohol.

Tetrahydrocarbazole Derivative from Lanostenone.—1 G. of lanostenone in 30 ml. of acetic acid was treated for 30 minutes on the water-bath with 2 g. of phenylhydrazine in 10 ml. of acetic acid. The tetrahydrocarbazole *derivative* crystallised on cooling and, after purification, formed white plates, m. p. 201—202° (yield, 60%) (Found: N, 2·9. $C_{36}H_{51}N$ requires N, 2·8%), sparingly soluble in alcohol and acetic acid. It gave a picrate, bronze needles from benzene–alcohol, m. p. 201°.

Maleic Anhydride and Isomeric Lanosterols.—The acetates of lanosterol, *iso*lanosterol, and lanosterol-D were heated with maleic anhydride in xylene at 135° for 8 hours. The products were treated by the method of Windaus and Lüttringhaus (*loc. cit.*), but in each case the original substance was recovered unchanged.

Lanosteryl Acetate Monohydrochloride.—Dry hydrogen chloride was passed for 2 hours through a cooled solution of 2 g. of lanosteryl acetate in 20 ml. of chloroform. The solvent was removed under reduced pressure, and the residue crystallised from acetone–methyl alcohol. After removal of a first crop, m. p. 131—132° (Found: Cl, 9·4, 9·5. Calc. for $C_{32}H_{52}O_2$,2HCl: Cl, 13·1%), needles (0·4 g.) of *lanosteryl acetate monohydrochloride* were obtained, m. p. 126—127° (Found: Cl, 7·2. $C_{32}H_{52}O_2$,HCl requires Cl, 7·0%).

iso*Lanosterol.*—The hydrochloride was refluxed for 2 hours with alcoholic potassium hydroxide. The product, purified from acetone–methyl alcohol, gave iso*lanosterol* in felted needles, m. p. 131—132° (Found: C, 83·8; H, 12·0. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). This compound is more soluble in the usual solvents than lanosterol.

iso*Lanosteryl acetate* formed large plates, m. p. 130—131°, from acetone–methyl alcohol (Found: C, 81·2; H, 11·4. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%).

iso*Lanostenone.*—Prepared as described under lanostenone, this *compound* formed large irregular plates, from methyl alcohol, m. p. 138—139° (Found: C, 84·1; H, 11·5. $C_{30}H_{48}O$ requires C, 84·8; H, 11·4%), soluble in ethyl acetate and sparingly soluble in methyl alcohol. The semicarbazone formed white silky needles, m. p. 210°, from alcohol.

The *tetrahydrocarbazole* derivative from *iso*lanostenone, prepared as described under lanostenone, crystallised from benzene–alcohol in large plates, m. p. 224—225° (Found: N, 3·3. $C_{36}H_{51}N$ requires N, 2·9%). It formed an unstable picrate.

iso*Lanostene.*—1 G. of *iso*lanostenonesemicarbazone was heated for 20 hours at 180° with a solution of 1 g. of sodium in 20 ml. of ethyl alcohol. The product was poured into water, and the solid purified from methyl alcohol. It formed small plates, m. p. 80—81° (Found: C, 87·0; H, 12·2. $C_{30}H_{50}$ requires C, 87·8; H, 12·2%).

Lanostene.—This compound, prepared from lanostenonesemicarbazone, formed white plates, m. p. 76—77°, from acetone–methyl alcohol (Found: C, 87·4; H, 12·1. $C_{30}H_{50}$ requires C, 87·8; H, 12·2%).

iso*Lanosterol-A.*—*iso*Lanostenone (0·3 g.) was refluxed in alcohol (50 ml.), sodium (4 g.) added in the course of 30 minutes, and heating continued for a further ½ hour. The product, crystallised from acetone–methyl alcohol, gave iso*lanosterol-A* in felted needles, m. p. 130—131° (Found: C, 83·8; H, 12·0. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). On admixture with *iso*lanosterol a m. p. depression of 6° was obtained.

The *acetate* formed large plates, m. p. 138° (Found: C, 81·3; H, 11·3. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%).

Acetate of Lanosterol-D.—To 2 g. of lanosteryl acetate in 60 ml. of acetic acid at 80° was added 0·6 g. of chromic acid in 10 ml. of 66% acetic acid. After 15 minutes the whole was poured into water, and the solids extracted with ether. Removal of the ether and crystallisation of the residue from dilute acetic acid, acetone–alcohol, and glacial acetic acid gave the *acetate* of lanosterol-D in white plates, m. p. 164° (Found: C, 82·0, 82·2; H, 11·0, 11·0. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%).

Lanosterol-D.—Saponification of the acetate and crystallisation from acetone–methyl alcohol gave *lanosterol-D* in felted needles, m. p. 137—138° (Found: C, 83·9; H, 12·0. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%), sparingly soluble in methyl alcohol. Oxidation of lanosterol-D with chromic acid gave lanostenone, m. p. 115·5—116° (tetrahydrocarbazole derivative, m. p. 202°) alone or in admixture with an authentic specimen.

SUMMARY.

1. Lanosterol, dehydrogenated with palladium–charcoal, gives a hydrocarbon $C_{18}H_{20}$. The formation of this can be explained on the basis of an amyrin type of structure for lanosterol.

2. Surface-film measurements support its analogy to the amyrins.

3. The presence of a reactive and an inert double bond is shown by its reactions with perbenzoic acid and with hydrogen peroxide. The reactive bond is probably situated in ring B (I).

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