38 *Lanosterol. Part III. The Action of Selenium Dioxide and of Perbenzoic Acid on Lanosterol.*

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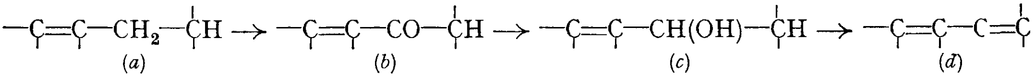
The presence of a methylene group adjacent to the active double bond in lanosteryl acetate is shown by the action of selenium dioxide, which gives a *diol* capable of forming a *diacetate*. With α-dihydrolanosteryl acetate, selenium dioxide gives a *monoacetate*,

$C_{32}H_{52}O_2$, indicating that dehydration has taken place with the introduction of a new double bond. This was confirmed by hydrogenation of the compound, one molecular proportion of hydrogen being absorbed and α-dihydrolanosterol obtained. The properties of the acetate $C_{32}H_{52}O_2$ closely resemble those of α-dihydroagnosterol acetate and the two substances may be identical.

The oxide produced by the action of perbenzoic acid on lanosteryl acetate (J., 1936, 1562) can be hydrolysed to give a *triol*, one of the hydroxyl groups of which is tertiary, indicating that the active double link in lanosterol is of the type >C═CH—. Lanostenone also formed an *oxide*, and on treatment with alcoholic hydrochloric acid simultaneous hydrolysis and dehydration took place, a *dehydro*-derivative, $C_{30}H_{46}O$, being obtained in which three double bonds were present. One of these, only, could be hydrogenated and, as the absorption spectrum of the dihydro-derivative shows that the two inactive double bonds are conjugated, this compound resembles α-dihydroagnosterol. The results throw light on the bond systems of lanosterol and agnosterol.

Selenium Dioxide.—The action of selenium dioxide on the unsaturated members of the sterol group first studied by Rosenheim and Callow (J., 1933, 387) is normally one of two types. In the first, oxidation of a methylene group adjacent to the unsaturated linkage takes place, a hydroxyl group being introduced. Cholesterol, for example, with selenium dioxide gives Δ^5-cholestene-3:4 diol (Rosenheim and Starling, J., 1937, 377), which undergoes in the course of the reaction an allylic rearrangement to Δ^4-cholestene-3 : 6-diol (Petrow, Rosenheim, and Starling, J., 1938, 679). The second type leads to the formation of a new unsaturated centre in the molecule, probably owing to dehydration at the position of a hydroxyl group primarily introduced. The bond originally present sometimes migrates, but in the resulting diene the bonds are always conjugated.

Lanosterol, $C_{30}H_{50}O$, with one active and one inert double bond occurs in association with the structurally similar alcohol agnosterol, $C_{30}H_{48}O$, which has one active and two inert double bonds. Reaction of lanosterol with selenium dioxide might be expected to lead to the formation of agnosterol or its isomers, either by the introduction of a new double linking, or by the production of a hydroxy-compound which could subsequently be dehydrated to produce one. The latter probability is supported by the work of Marker, Wittle, and Nixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368), who by the action of chromic acid on a methylene group adjacent to the inert double bond in α-dihydrolanosteryl acetate obtained a keto-acetate which on reduction and dehydration gave α-dihydroagnosteryl acetate identical with that prepared from natural agnosterol by catalytic hydrogenation (see scheme, p. 179). There is therefore a methylene group adjacent to the inert double bond of α-dihydrolanosterol, and the reactions described may be represented thus :



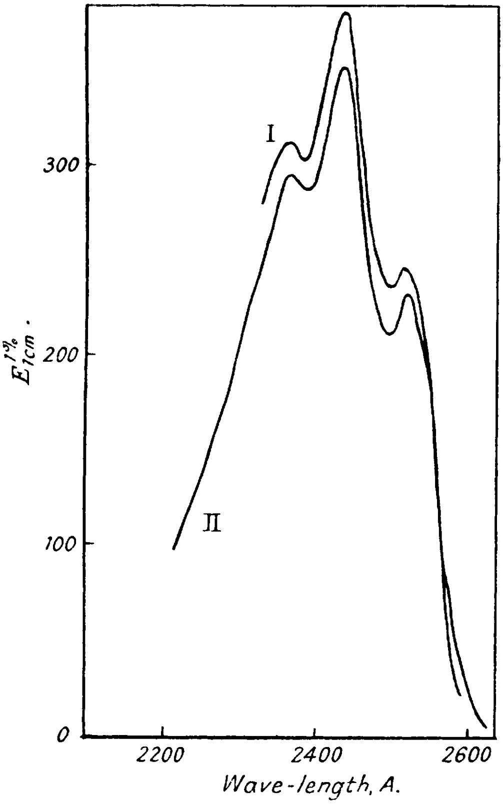
$$\text{—C=C—CH}_2\text{—CH} \rightarrow \text{—C=C—CO—CH} \rightarrow \text{—C=C—CH(OH)—CH} \rightarrow \text{—C=C—C=C}$$

A similar conversion has been carried out with β-amyrenyl benzoate (Beynon, Sharples, and Spring, J., 1938, 1233), a dehydro-β-amyrenyl derivative being ultimately formed which is resistant to catalytic hydrogenation.

Selenium dioxide reacted vigorously with lanosteryl acetate in alcoholic solution. The products gave in small yield the *monoacetate* of a diol, $C_{32}H_{52}O_3$, which on saponification gave the *diol*, $C_{30}H_{50}O_2$, m. p. 143·5°. The diol on acetylation gave a *diacetate*, which proves that dehydration could not have occurred. If the hydroxyl group had occupied the same position as it does in the hydroxydihydrolanosteryl acetate prepared by Marker (*loc. cit.*), it would have participated in dehydration, so the methylene groups attacked in the two cases are not the same. The methylene group concerned in the formation of the diol $C_{30}H_{50}O_2$ is therefore the one adjacent to the active double bond, whereas that oxidised in producing hydroxydihydrolanosterol is adjacent to the inactive double bond. The possibility that selenium dioxide might have reacted with methylene groups activated by each of the double bonds of lanosterol was indicated by the isolation from the mother-liquors, of a second *diol*, m. p. 152°. The yield, however, was so small that no conclusions could be drawn, and to throw further light on the question the action of selenium dioxide on α-dihydrolanosteryl acetate, in which the inactive double bond of lanosterol alone remains, was examined.

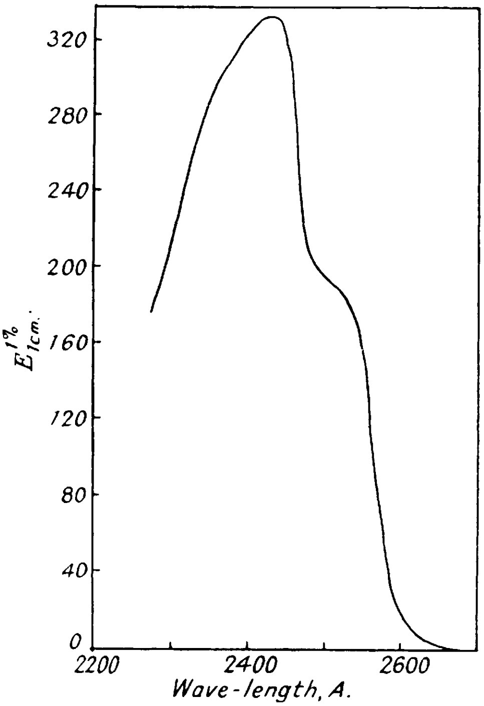
Selenium dioxide did not react with α-dihydrolanosteryl acetate in alcohol or glacial acetic acid at 100°. By using a large excess of the reagent in boiling glacial acetic acid, however, interaction took place and a *monoacetate*, $C_{32}H_{52}O_2$, m. p. 167°, was obtained in the high yield of 40%. Its composition showed that in this case a new double bond had been introduced, which was confirmed by catalytic hydrogenation, hydrogen equivalent to one molecular proportion being absorbed and α-dihydrolanosteryl acetate obtained in quantitative yield. The monoacetate, m. p. 167°, is thus a dehydro-derivative of α-dihydrolanosteryl acetate, and therefore an isomer of lanosteryl acetate. It has accordingly been termed γ-lanosteryl acetate, giving a γ-lanosterol, m. p. 141°.

FIG. 1.



Absorption spectra of (I) *γ-lanosteryl acetate, and* (II) *α-dihydroagnosteryl acetate.*

FIG. 2.



Dehydrolanostenone.

In analogy with similar reactions in the sterol series it was expected that the double bonds in γ-lanosterol would be conjugated. The ultra-violet absorption curve (Fig. 1) confirmed this, a well-defined maximum at 2450 A. being observed. The inert double bond of γ-lanosterol is almost certainly in its original position, although the reduction to α-dihydrolanosterol is not entirely conclusive, as the addition of hydrogen to conjugate systems in the 1 : 4-position sometimes occurs. γ-Lanosterol, if not identical with α-dihydroagnosterol, must be very closely related to it. The action of selenium dioxide on α-dihydrolanosteryl acetate being assumed to follow the normal course, it would be explained by a similar scheme to that used above in the case of the conversion of α-dihydrolanosteryl acetate into α-dihydroagnosteryl acetate by Marker, Nixon, and Wittle (*loc. cit.*), stage (*b*) being omitted, and γ-lanosterol would be identical with α-dihydroagnosterol. It will be noted (Fig. 1) that the ultra-violet absorption curves of the two substances are practically identical and the following table shows the similarity in the melting points of the two compounds and their derivatives. The acetates of this group have always proved the most suitable material for purification and the melting points of α-lanosteryl and α-dihydroagnosteryl acetates are not only equal, but they give no depression on admixture. The alcohol γ-lanosterol may contain an epimer, which would account for the lower melting point.

	Alcohol.	Acetate.	Ketone.
α-Dihydroagnosterol	150°	166—167°	130°
γ-Lanosterol	141	167	124

Against this apparent identity must be placed the smooth hydrogenation of γ-lanosterol to α-dihydrolanosterol when a palladium catalyst is used. Agnosterol, when reduced with hydrogen in the presence of platinum, gives only the dihydro-derivative with two conjugate double bonds. Thus one only of the double bonds of γ-lanosterol resists catalytic hydrogenation, whereas, apparently, in the case of α-dihydroagnosterol, both do so.

These reactions connecting lanosterol with agnosterol are summarised in the following scheme, which includes the observations of Marker, Nixon, and Wittle (*loc. cit.*) and ourselves.

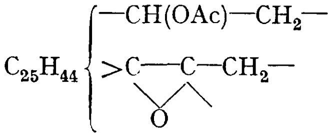
$$\begin{tikzcd}
\text{Lanosteryl acetate, 114}^\circ \arrow[r, "SeO_2"] \arrow[d, "PtO_2\ H_2"] & \text{Diol monoacetate, } C_{32}H_{52}O_3\text{, 111}^\circ \arrow[r, "Ac_2O"] & \text{Diol diacetate, } C_{34}H_{54}O_4\text{, 132}^\circ \\
\alpha\text{-Dihydrolanosteryl acetate, 119}^\circ \arrow[r, shift left, "SeO_2"] \arrow[dd, "CrO_3"] & \gamma\text{-Lanosteryl acetate, 167}^\circ \arrow[l, shift left, "Pd\text{-}H_2"] \arrow[r, "CrO_3"] & \gamma\text{-Lanostenone, 125}^\circ \\
 & \alpha\text{-Dihydroagnosteryl acetate, 166}^\circ \arrow[r, "CrO_3"] & \alpha\text{-Dihydroagnostenone, 130}^\circ \\
\alpha\text{-Ketodihydrolanosteryl acetate, 150}^\circ \arrow[r, "Na + Pr\beta OH"] & \text{Hydroxydihydrolanosterol, 165}^\circ \arrow[u, "Ac_2O"] &
\end{tikzcd}$$

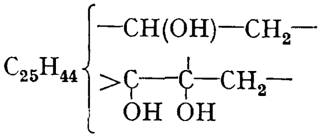
Perbenzoic Acid.—The action of perbenzoic acid has afforded useful evidence regarding the number and nature of the unsaturated linkages present in the sterols and resin alcohols. Cholesterol, for example, gives an oxide which on hydrolysis yields 3 : 5 : 6-trihydroxycholestane. The position of the original double bond is thus made evident. On acetylation a diacetate is obtained, which under the action of suitable dehydrating agents loses the elements of water, giving rise to an unsaturated linking in a new position. Dehydration at one (or both) of the hydroxy-groups of α-glycols such as 3 : 5 : 6-trihydroxycholestane is generally produced by the action of acetic anhydride, but frequently less active reagents will bring about the change. Heilbron (J., 1934, 1577), for example, found that ergostadienetriol I passes into dehydroergosterol on simple crystallisation from glacial acetic acid.

Petrow (J., 1939, 1000) also reports that during the hydrolysis of 5-methylnorcholestane-3 : 6-diol 8 : 9-oxide with alcoholic hydrochloric acid, dehydration of both hydroxyl groups takes place, giving a dehydro-derivative, 5-methyl-$\Delta^{9:11,\,8:14}$-norcholestadiene-3 : 6-diol. The reactions of lanosterol and lanostenone with perbenzoic acid can be explained on similar lines. They are summarised in the scheme given below.

$$\begin{tikzcd}
\text{Lanosteryl acetate, } C_{32}H_{52}O_2\text{, 114}^\circ \arrow[d, "BzO_2H"] & \text{Lanostenone, } C_{30}H_{48}O\text{, 115}^\circ \arrow[d, "BzO_2H"] \\
\text{Lanosteryl acetate oxide, } C_{32}H_{52}O_3\text{, 185}^\circ \arrow[d, "HCl\ EtOH"] & \text{Oxidolanostenone, } C_{30}H_{48}O_2\text{, 92}^\circ \arrow[d, "HCl\ EtOH"] \\
\text{Lanostenetriol, } C_{30}H_{50}O_3\text{, 130}^\circ \arrow[d, "Ac_2O"] & \text{Dehydrolanostenone, } C_{30}H_{46}O\text{, 126}^\circ \arrow[d, "Pd\text{-}H_2"] \\
\text{Lanostenetriol diacetate, } C_{34}H_{54}O_5\text{, 104}^\circ & \text{Dihydro-derivative, } C_{30}H_{48}O\ (\alpha\text{-}\textit{iso}\text{dihydroagnostenone), 124}^\circ
\end{tikzcd}$$

Lanosteryl acetate, when treated with perbenzoic acid, gave the lanosteryl acetate oxide described by Dorée and Petrow (J., 1936, 1562). The melting point, however, proved to be 185°, instead of 178° as recorded by them. Treatment with alcoholic hydrochloric acid opened the oxide ring with the production of an α-glycol, thus :—





A *lanostenetriol*, $C_{30}H_{50}O_3$, was obtained, which, on acetylation, gave a *diacetate*. The two hydroxyl groups acetylated must be of the secondary type, one being that originally present in lanosterol, and the second, one of those introduced at the position of the double bond. The other non-reactive hydroxyl introduced must be tertiary, and in consequence the double bond at which the hydroxyls were situated is of the type >C=CH—.

Lanostenone, when treated with perbenzoic acid equivalent to one atom of oxygen per molecule, reacted very slowly to give an *oxide*, $C_{30}H_{48}O_2$, m. p. 92°. Hydrolysis with alcoholic hydrochloric acid was expected to yield a keto-diol, but instead a *dehydro*-derivative of lanostenone, $C_{30}H_{46}O$, was obtained, a result indicating that hydrolysis and dehydration had taken place and that the new compound should contain three ethylenic linkages. Two of these are conjugated (Fig. 2) and one only reacted with hydrogen in the presence of palladium charcoal, giving a dihydrodehydrolanostenone, m. p. 124°. The ultra-violet absorption measurements of this substance were very different from those of dehydrolanostenone (Fig. 2), showing a maximum $E^{1\%}_{1\,\mathrm{cm.}}$ of 60 at 2400 A. As this compound, m. p. 124°, apparently contains two double bonds which resist catalytic hydrogenation, it resembles α-dihydroagnosterol (Marker, Nixon, and Wittle, *J. Amer. Chem., Soc.*, 1937, **59**, 2289) and the name α-*iso*dihydroagnostenone is suggested for it.

EXPERIMENTAL.

Action of Selenium Dioxide on Lanosteryl Acetate.—To lanosteryl acetate (4 g.), dissolved in boiling ethyl alcohol (100 ml.), was added selenium dioxide (1·1 g.) in a little water. After being refluxed for 1 hour and filtered hot, the liquid was poured into water and extracted with ether. Residual selenium was removed by treatment with potassium cyanide solution, and the ethereal solution washed with water and dried. After removal of the ether, the product was repeatedly crystallised from methyl alcohol, and gave a small quantity of the *diol monoacetate*, in needles, m. p. 110° (Found : C, 79·5; H, 10·7. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8%). The acetate was saponified, giving the *diol*, which crystallised from acetone–methyl alcohol in felted needles, m. p. 143—144° (Found : C, 81·8; H, 11·9. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·3%). The diol on acetylation gave the *diol diacetate*, which formed large plates from acetone–methyl alcohol, m. p. 132° (Found : C, 76·6; H, 10·0. $C_{34}H_{54}O_4$ requires C, 77·5; H, 10·2%).

Preparation of the Diol, $C_{30}H_{50}O_2$, *m. p.* 152°.—The mother-liquors from the first five crystallisations of the initial product were bulked and saponified. Crystallisation from ethyl acetate gave a very small quantity of a *diol*, m. p. 152° (Found : C, 81·6; H, 11·7. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·3%).

Action of Selenium Dioxide on α-Dihydrolanosteryl Acetate.—α-Dihydrolanosteryl acetate (2 g.) in glacial acetic acid (200 ml.) was treated with selenium dioxide (2 g.) in a little water. The mixture was refluxed for 3 hours, sodium acetate then added, and the solution filtered hot. Residual selenium was removed as before, and the product finally purified from acetone, giving *γ-lanosteryl acetate* in long needles, m. p. 167° (Found : C, 81·9; H, 11·2. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%). There was no m. p. depression in admixture with α-dihydroagnosteryl acetate, m. p. 166°.

Preparation of γ-Lanosterol.—γ-Lanosteryl acetate (1 g.) was saponified with alcoholic potassium hydroxide. The *product* crystallised from acetone–methyl alcohol in soft needles, m. p. 141° (Found : C, 83·9; H, 12·0. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%).

Preparation of γ-Lanostenone.—γ-Lanosterol was oxidised with chromic acid as in the preparation of lanostenone. Crystallised from acetone–methyl alcohol, the *ketone* formed large plates, m. p. 124° (Found : C, 84·4; H, 11·4. $C_{30}H_{48}O$ requires C, 84·9; H, 11·4%). The tetrahydrocarbazole derivative, prepared in the usual way, formed white plates, m. p. 228°, from benzene–alcohol.

Formation of α-Dihydrolanosteryl Acetate from γ-Lanosteryl Acetate.—A mixture of γ-lanosteryl acetate (0·2 g.) in purified glacial acetic acid (25 ml.) with stabilised palladium charcoal (0·05 g.) was shaken for 6 hours with hydrogen at atmospheric pressure. After filtering, the product was isolated by dilution to turbidity; on cooling, α-dihydrolanosteryl acetate separated in long needles, m. p. 118°. It gave no m. p. depression on admixture with an authentic specimen and after saponification α-dihydrolanosterol, m. p. 148°, was obtained showing similar identity.

Preparation of Lanosteryl Acetate Oxide.—Lanosteryl acetate (5 g.) in chloroform (50 ml.) was treated with a chloroform solution of perbenzoic acid equivalent to 0·2 g. of active oxygen during ½ hour at 0°. After 24 hours at 0° the solvent was removed, and the oxide taken up in ether. Repeated purification from ethyl acetate gave lanosteryl acetate oxide, m. p. 185°.

Lanostenetriol.—Lanosteryl acetate oxide (0·5 g.) in ethyl alcohol (30 ml.) was treated with hydrochloric acid (2 ml.) and water (2 ml.) and refluxed for 3 hours. After dilution to turbidity *lanostenetriol* crystallised on cooling, and was purified from acetone solution. It formed white plates, m. p. 130°, easily soluble in benzene and ether, and slightly soluble in methyl alcohol (Found: C, 78·8; H, 11·3. $C_{30}H_{50}O_3$ requires C, 79·0; H, 10·9%).

Diacetate of Lanostenetriol.—Lanostenetriol (0·2 g.) in acetic anhydride (10 ml.) was heated for 1 hour. The *product*, recrystallised from glacial acetic acid, gave white plates, m. p. 104° (Found: C, 75·5; H, 10·3. $C_{30}H_{54}O_5$ requires C, 75·3; H, 9·9%).

The Action of Perbenzoic Acid on Lanostenone.—Lanostenone (5 g.) was treated with a solution of perbenzoic acid as above. The product was fractionally crystallised from methyl alcohol to remove unchanged lanostenone, which formed the least soluble fraction. The mother-liquors gave *lanostenone oxide*, m. p. 92°, readily soluble in benzene, acetone, and ethyl acetate (Found: C, 81·6; H, 11·5. $C_{30}H_{48}O_2$ requires C, 81·7; H, 10·9%).

Dehydrolanostenone.—Lanostenone oxide (0·2 g.) was hydrolysed with alcoholic hydrochloric acid as described above. The *product*, crystallised from acetone–methyl alcohol, gave white plates, m. p. 126° (Found: C, 84·5; H, 11·2. $C_{30}H_{46}O$ requires C, 85·0; H, 11·0%). The tetrahydrocarbazole derivative had m. p. 228°, and the oxime m. p. 183°.

iso*Dihydroagnostenone.*—Dehydrolanostenone (0·2 g.) in glacial acetic acid (25 ml.) was shaken with hydrogen for 6 hours at 20°, palladium charcoal (0·05 g.) being used as catalyst. Hydrogen equivalent to one double bond was absorbed. The compound crystallised from glacial acetic acid in plates, m. p. 124°, and formed a tetrahydrocarbazole derivative, m. p. 202°, and an oxime, m. p. 164°.

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