# 435. *Lanosterol. Part VIII. The Action of Perbenzoic Acid on Derivatives of Lanosterol.*

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α-Dihydrolanosteryl acetate absorbed one atom of oxygen per molecule when treated with an excess of perbenzoic acid, and the final product was the doubly unsaturated α-dihydroagnosteryl acetate. The results obtained in perbenzoic acid titrations of a number of lanosterol derivatives are recorded; the number of atoms of oxygen absorbed per molecule approximated in each case to a whole number, but in the agnosterol series was always one less than the number of double bonds present.

Conflicting results have been reported by earlier workers investigating the action of perbenzoic acid on " lanosterol " (Windaus, *Z. physiol. Chem.*, 1930, **190**, 59; Dorée and Garratt, *J. Soc. Chem. Ind.*, 1933, **52**, 360T; Dorée and Petrow, *J.*, 1936, 1561). Recently Ruzicka (*Helv. Chim. Acta*, 1945, **28**, 759) has shown that the " lanosterol " of previous workers was, in fact, a mixture of lanosterol $C_{30}H_{50}O$ and dihydrolanosterol $C_{30}H_{52}O$, which on catalytic hydrogenation yields pure dihydrolanosterol (Marker, *J. Amer. Chem. Soc.*, 1937, **59**, 1368). Some disagreement exists, however, in the values reported for the per-acid titration of dihydrolanosterol. Wieland *et al.* (*Annalen*, 1937, **529**, 68) working on cryptosterol, which subsequent work (Ruzicka *et al.*, *Helv. Chim. Acta*, 1945, **28**, 759; McGhie, Thesis, London, 1947) has shown to be identical with lanosterol, found that dihydrocryptosteryl acetate absorbed one atom of oxygen to give an " oxide," m. p. 142°, $[\alpha]_D^{20}$ +1·7°. On the other hand, Muhr (Thesis, Zurich, 1945) found that dihydrolanosteryl acetate did not absorb oxygen from monoperphthalic acid at −10°. These conflicting results made it seem desirable to reinvestigate the problem, and we now record our findings.

Perbenzoic acid reacts with dihydrolanosteryl acetate to give a labile intermediate, m. p. 142°, $[\alpha]_D^{20}$ +28·5°, provisionally assumed to be an " oxide." This " oxide " on treatment with sulphuric acid in glacial acetic acid, or on long heating with acetic anhydride, gives a product m. p. 167—168°, $[\alpha]_D^{20}$ +86·0°, which is also obtained from the mother-liquors from the perbenzoic oxidation of α-dihydrolanosteryl acetate.

The intermediate is so labile that ready conversion into the diene takes place when recrystallisation is attempted. This is shown by the progressive increase in the intensity of the ultra-violet light absorption at 243 mμ. and by the approach towards the value for the specific rotation of α-dihydroagnosteryl acetate. Thus, it has not been possible to obtain the so-called " oxide " in the pure state for further investigation; a similar example was observed by Windaus and Luttringhaus (*Annalen*, 1930, **481**, 119) in the case of α-ergostenyl acetate.

The compound, m. p. 167—168°, $[\alpha]_D^{20}$ +86·0°, has an ultra-violet absorption spectrum identical with that of α-dihydroagnosteryl acetate, and a close comparison of the two, and of their derivatives (see Table I), established the identity beyond reasonable doubt.

Table I.

| | α-Dihydroagnosteryl acetate.* | | Perbenzoic acid oxidn. product. | |
|---|---|---|---|---|
| Derivative. | M. p. | $[\alpha]_D^{20}$. | M. p. | $[\alpha]_D^{20}$. |
| Acetate | 168—169° | +87·8° | 167—168° | +86·0° |
| Alcohol | 157—158 | +67·5 | 157 | +65·4 |
| Ketone | 131—132 | +47·7 | 128—129 | +44·5 |
| Ultra-violet absorption of acetate. | $\lambda_{max.}$ = 243 mμ. ($E^{1\%}_{1\,cm.}$ = 360);<br>$\lambda_{max.}$ = 251 mμ. ($E^{1\%}_{1\,cm.}$ = 225); | | $\lambda_{max.}$ = 243 mμ. ($E^{1\%}_{1\,cm.}$ = 350).<br>$\lambda_{max.}$ = 251 mμ. ($E^{1\%}_{1\,cm.}$ = 240). | |

* Ruzicka, Denss, and Jeger, *Helv. Chim. Acta*, 1946, **29**, 204.

The introduction of a second olefinic linkage into α-dihydrolanosteryl acetate by means of perbenzoic acid is similar to the conversion of α-ergostenol into dehydroergostenol by perbenzoic acid through the intermediate oxide (Windaus and Luttringhaus, *loc. cit.*; Morrison and Simpson, *J.*, 1932, 1710). As in the case of α-dihydrolanosteryl acetate which is dehydrogenated to α-dihydroagnosteryl acetate by selenium dioxide (Dorée and Bellamy, *J.*, 1941, 176), α-ergostenol yields dehydroergostenol (Callow, *J.*, 1936, 462).

The second nuclear double bond in dihydroagnosterol, like that in dihydrolanosterol, is unreactive: it cannot, for example, be catalytically hydrogenated. α-Dihydroagnosteryl acetate, although containing two double bonds, was found, under the conditions here described, to absorb only one atom of oxygen per molecule. This parallel between lanosterol and agnosterol compounds is maintained in the hydrocarbons obtained by dehydration of the alcohols with phosphorus oxychloride and with phosphorus pentachloride, yielding two series of isomeric hydrocarbons (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467).

The values obtained for the titration of these derivatives with perbenzoic acid are summarised in Table II. In all the cases where dehydrogenation by perbenzoic acid has taken place, the parent compound has contained an " inert " double bond; thus, for this type of compound, dehydrogenation rather than formation of a stable oxide may be the predominant reaction. It was suggested in an earlier communication (Dorée, McGhie, and Kurzer, this vol., p. 570) that the dehydrogenation of dihydrolanosteryl acetate to dihydroagnosteryl acetate by selenium dioxide and *N*-bromosuccinimide might well be represented by the scheme outlined in the partial formulæ :

TABLE II.

| Derivative. | Atoms of oxygen absorbed per molecule.<br>30 mins. | 5 hrs. | 24 hrs. | 48 hrs. | 7 days. |
|---|---|---|---|---|---|
| α-Dihydrolanosteryl acetate | 0·7 | 1·0 | 1·0 | 1·0 | 1·1 |
| Lanostadiene | — | 2·0 | 2·1 | 2·1 | 2·1 |
| *iso*Lanostadiene | 1·7 | 1·9 | 2·0 | 2·0 | 2·0 |
| α-Dihydroagnosteryl acetate | 1·0 | 1·0 | 1·2 | 1·2 | 1·2 |
| Lanostatriene | — | — | 2·2 | 2·2 | 2·2 |
| *iso*Lanostatriene | — | — | 2·0 | 2·1 | 2·1 |

$$>\dot{C}{:}\dot{C}\cdot CH\cdot CH< \;\longrightarrow\; >\dot{C}{:}\dot{C}\cdot CX\cdot CH< \;\longrightarrow\; >\dot{C}{:}\dot{C}\cdot C{:}C<,$$

where X = OH or Br. The isolation of the same product after reaction with perbenzoic acid makes it probable that this reaction follows a similar course, the isolation of the labile intermediate being attributable to the extremely mild conditions of the experiment.

The results here reported afford a further example of non-correspondence between the number of double linkages known to be present and the number indicated by titration with perbenzoic acid. They add emphasis to Ruzicka's views (*Helv. Chim. Acta*, 1932, **15**, 1294) on the need for caution in interpreting the results obtained with this reagent.

## EXPERIMENTAL.

All m. p.s are uncorrected. Analyses and rotations by Drs. Weiler and Strauss, Oxford. All rotations in chloroform at 20°.

*Titrations with Perbenzoic Acid.—General procedure.* To a solution of the substance in chloroform at 0°, a 50% excess of perbenzoic acid in chloroform was added, the mixture being kept at 0° throughout. Aliquot portions were withdrawn at intervals and treated with an excess of potassium iodide, the solution was acidified, and the liberated iodine titrated with standard sodium thiosulphate solution. The results are recorded in Table II.

*Dehydrogenation of α-Dihydrolanosteryl Acetate.*—To a solution of α-dihydrolanosteryl acetate (5 g.) in chloroform (20 ml.) was added a solution of perbenzoic acid in chloroform (equivalent to 0·28 g. of active oxygen). After 40 hours the excess of oxidising agent was decomposed by potassium iodide, the mixture acidified, and the liberated iodine removed by shaking with 0·1N-thiosulphate. The chloroform layer was separated and washed twice with 2N-sodium hydroxide and then twice with water. The solution was dried ($Na_2SO_4$), the chloroform removed under reduced pressure, and the residue crystallised from chloroform–methanol to give lustrous plates (4 g.), m. p. 142°, $[\alpha]_D^{20}$ +28·5° (*c*, 0·97) (Found : C, 79·4; H, 10·7. Calc. for $C_{32}H_{54}O_3$ : C, 78·9; H, 11·2%). This product contains approx. 20% of α-dihydroagnosteryl acetate as calculated from the ultra-violet absorption data. The mother-liquors yielded 0·75 g. of impure α-dihydroagnosteryl acetate, m. p. 162°.

*Conversion of the " Oxide," m. p. 142°, into α-Dihydroagnosteryl Acetate.*—A solution of 1 g. of the " oxide " in glacial acetic acid (20 ml.) was treated at the boiling point with 3 drops of concentrated sulphuric acid. On cooling, a product (0·8 g.), m. p. 167—168°, crystallised (Found : C, 81·9; H, 10·9. Calc. for $C_{32}H_{52}O_2$ : C, 82·0; H, 11·2%) ; it showed no depression in m. p. on admixture with α-dihydroagnosteryl acetate, $[\alpha]_D^{20}$ +86·0° (*c*, 1·0). Hydrolysis with 5% alcoholic potash gave the alcohol, m. p. 157° (Found : C, 84·25; H, 11·6. Calc. for $C_{30}H_{50}O$ : C, 84·4; H, 11·8%), $[\alpha]_D^{20}$ +65·4° (*c*, 0·70). Oxidation of the alcohol by Kiliani's chromic acid mixture gave the ketone, m. p. 128—129° (Found : C, 84·6; H, 11·20. Calc. for $C_{30}H_{48}O$ : C, 84·8 : H, 11·4%), $[\alpha]_D^{20}$ +44·5° (*c*, 1·0).

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