254. *Lanosterol. Part IX. Ketodihydrolanosterol.*

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The main product of the oxidation of dihydroagnosteryl acetate with perbenzoic acid is the $\alpha\beta$-unsaturated ketone, ketodihydrolanosteryl acetate. Dihydrolanosteryl acetate, on oxidation with chromic acid under mild conditions, gave the same ketone, together with dihydroagnosteryl acetate. Ketodihydrolanosteryl acetate was oxidized by selenium dioxide to a more highly unsaturated ketodidehydrodihydrolanosteryl acetate.

In a previous communication (*J.*, 1949, 2038), it was reported that titration of dihydroagnosteryl acetate with perbenzoic acid showed an absorption of 1·2 atoms of oxygen per molecule. The oily oxidation product has now been isolated as a solid by treatment with boiling methanol; this, after two further crystallizations, had physical constants closely resembling those reported by Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 472) for ketodihydrolanosteryl acetate, obtained by the oxidation of dihydrolanosteryl acetate with ozone.

	M. p.	$[\alpha]_D^{18}$.	$\lambda_{max.}$.	log ε.
Ketodihydrolanosteryl acetate	151·5—152·5°	+21·3°	255 mμ.	4·08 (in alcohol)
Perbenzoic acid oxidation product ...	151—152°	+26·0°	252—256 mμ.	4·1 (in chloroform)

Recrystallization of the oxidation product led to a gradual rise in the melting point, indicating that the product was not homogeneous. By chromatography it was resolved into two components, the main fraction, m. p. 151—152°, $[\alpha]_D^{18}$ +24°, gave analytical figures in agreement with the formula $C_{32}H_{52}O_3$ and showed high-intensity absorption in the ultra-violet; the second and minor component, m. p. 184°, also had the formula $C_{32}H_{52}O_3$, but showed no high-intensity ultra-violet absorption.

The major component gave no melting-point depression with ketodihydrolanosteryl acetate prepared according to Ruzicka *et al.* (*loc. cit.*), and the close agreement in the physical constants supports the conclusion that the two substances are identical.

The mother liquors from the perbenzoic acid oxidation of dihydroagnosteryl acetate, after treatment with methanol, gave an uncrystallizable oil, which on treatment with glacial acetic acid and sulphuric acid yielded a colourless crystalline solid, m. p. 205°, $[\alpha]_D^{18}$ +96·3°, transparent to ultra-violet light. Analysis gave figures in agreement with the formula $C_{32}H_{52}O_4$, which indicated that further oxidation of the primary oxidation product probably takes place, but, as would be expected with an αβ-unsaturated ketone, at a much slower rate (cf. Spring and Vickerstaff, *J.*, 1937, 249; Ruzicka *et al.*, *Helv. Chim. Acta*, 1932, **15**, 482, 1285).

It has previously been reported by Marker, Wittle, and Mixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368) that oxidation of dihydrolanosteryl acetate with chromic acid under mild conditions gave rise to two isomeric ketodihydrolanosteryl acetates. The α-isomer was isolated from the less soluble fraction on crystallization from glacial acetic acid; further crystallization gave a product having m. p. 150°, which was reported to be unaffected by boiling acetic anhydride, and to distil unchanged at 200° in a vacuum, the yield of pure substance being 6—7%. The mother liquors gave the β-isomer, m. p. 152°; this compound gave a 10° depression in melting point when mixed with the α-isomer. Both α- and β-ketodihydrolanosteryl acetate were reported to form the same ketodihydrolanosterol, m. p. 134—135°, on hydrolysis, and this on acetylation gave only β-ketodihydrolanosteryl acetate.

Unfortunately, the only constant available for these compounds is the melting point, which is insufficient in itself to allow comparison with the ketodihydrolanosteryl acetate obtained in our experiments with perbenzoic acid.

Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 472) have obtained only one ketodihydrolanosteryl acetate, m. p. 151·5—152·5°, $[\alpha]_D$ +18·2°, in the chromic acid oxidation of dihydrolanosteryl acetate, although the reaction conditions were somewhat different from those described by Marker and his co-workers.

Numerous unsuccessful attempts have been made by us to repeat the preparation of α-ketodihydrolanosteryl acetate following the directions of Marker *et al.* (*loc. cit.*), although no difficulty was experienced in the isolation of the β-ketodihydrolanosteryl acetate.

The less soluble fraction of crude material from the chromic acid oxidation of dihydrolanosteryl acetate was obtained in yields varying from less than 1% to 14% under apparently the same reaction conditions. Exhaustive purification of this fraction, either according to Marker or by repeated chromatography, yielded a substance, m. p. 149—150°, $[\alpha]_D^{18}$ +80·0°, an admixture of which with β-ketodihydrolanosteryl acetate had m. p. 139—141°. Analysis of this compound showed that it was in fact impure dihydroagnosteryl acetate ($[\alpha]_D^{20}$ +87·8°). The ultra-violet absorption spectrum [$\lambda_{max.} = 242$ mμ.; log $\varepsilon = 4·1$ (in *cyclo*hexane)] is very close to that of dihydroagnosteryl acetate ($\lambda_{max.} = 243$ mμ.; log $\varepsilon = 4·2$) as also is the specific rotation.

Furthermore although the m. p. (149—151°) is low compared with that of pure dihydroagnosteryl acetate (m. p. 167·5°), brief treatment with selenium dioxide in glacial acetic acid gave pure dihydroagnosteryl acetate, m. p. 167—168°, $[\alpha]_D^{18}$ +87·0°, a direct comparison between the two confirming their identity.

The compound also gave an intense yellow colour with tetranitromethane, whereas the β-isomer, like that described by Ruzicka *et al.* (*loc. cit.*), gives no colour with this reagent.

When dihydroagnosteryl acetate was oxidized with chromic acid under the conditions described by Marker for dihydrolanosteryl acetate, it was found that very rapid oxidation took place and the sole product isolated was diketodihydrolanosteryl acetate. This simple oxidation probably accounts for the variation in yields of crude dihydroagnosteryl acetate obtained in the oxidation of dihydrolanosteryl acetate, since in the time interval between the completion of the reaction and the dilution of the reaction mixture with water rapid oxidation of the dihydroagnosteryl acetate could take place to give diketodihydrolanosteryl acetate.

The identity of the more soluble compound, obtained from the mother liquors of the oxidation of dihydrolanosteryl acetate (apparently identical with Marker's β-ketodihydrolanosteryl acetate), with the ketodihydrolanosteryl acetate prepared by Ruzicka's (*loc. cit.*) method was indicated by analytical data, mixed melting-point determination, and by a comparison of physical constants.

	M. p.	$[\alpha]_D^{18}$.	$\lambda_{max.}$.	log ε.
Ketodihydrolanosteryl acetate	151·5—152·5°	+21·3°	255 mμ.	4·08 (in alcohol)
Chromic acid oxidation product (β-isomer)	151—152°	+22·9°	257 mμ.	4·1 (in alcohol)

This ketodihydrolanosteryl acetate when treated with selenium dioxide in glacial acetic acid under conditions analogous to those used for the conversion of dihydrolanosteryl acetate into dihydroagnosteryl acetate gave a product, m. p. 195—196°, $[\alpha]_D^{18}$ +67·7°, provisionally designated as a ketodidehydro-acetate. The ketodidehydro-acetate gave a yellow colour with tetranitromethane and from the analytical figures it would appear probable that two more ethylenic linkages have been introduced into the molecule with the formation of a trienone.

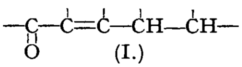
Muhr (Thesis, Zurich, 1945) has described a compound, m. p. 194·5—195·5°, $[\alpha]_D^{16}$ +65·4° (ultra-violet absorption spectrum: $\lambda_{max.} = 260$ and 330—335 mμ.; log $\varepsilon = 3·91$), which was isolated from the mother liquors remaining after the removal of dihydroagnosteryl acetate obtained by the oxidation of dihydrolanosteryl acetate with selenium dioxide. The analytical data recorded for this compound agreed closely with those found for our ketodidehydro-acetate. Surprisingly enough Muhr's compound was formulated as an unsaturated diketo-alcohol ($C_{30}H_{44}O_3$ or $C_{30}H_{46}O_3$). It was tentatively suggested that in the course of chromatographic purification upon alumina deacetylation had occurred, although all attempts to reacetylate the compound failed.

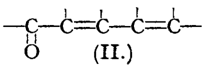
Our experiments have shown that it is possible to convert dihydroagnosteryl acetate into ketodihydrolanosteryl acetate, by perbenzoic acid, and that selenium dioxide oxidizes this further to the ketodidehydro-acetate. The formation of the compound obtained by Muhr can receive a simple explanation by regarding it as the ketodidehydro-acetate, $C_{32}H_{48}O_3$, formed by simultaneous dehydrogenation and introduction of an oxygen atom by the selenium dioxide.

The ultra-violet absorption spectrum of the ketodehydro-acetate is also compatible with the formulation as a trienone, but insufficient spectral data are available for a direct comparison with similar derivatives of known structure in the sterol and triterpene series. It is true that dienones in general have their principal maxima at a lower wave-length, but calculations from the data given by Fieser ("Natural Products related to Phenanthrene" 3rd Edn., 1949, p. 192) show that a dienone having both conjugated double bonds between substituted carbon atoms would possess a similar absorption maximum.

The presence of two maxima in the spectrum of an αβ-unsaturated ketone has previously been reported by Ewen, Gillam, and Spring (*J.*, 1944, 29), α-amyradienone III having maxima at 260 and 292 mμ. These authors suggested that a possible explanation was the formation of mixed crystals of the dienone containing the new double bond with an isomer having the newly introduced ethylenic bond in an isolated position. However, α-amyradienone III could not be resolved into two components by either crystallization or chromatography; the lanosterol derivative similarly behaved as if it was homogeneous.

It seems certain, however, that the original conjugated system in ketodihydrolanosteryl acetate has been extended by the introduction of at least one double bond. One can also infer that the carbon atoms αβ to the original unsaturated centres in ketodihydrolanosteryl acetate must carry at least one hydrogen atom each to allow for the formation of the ketodehydro-acetate. Ketodihydrolanosteryl acetate would, therefore, contain the system (I) which could then react to form the ketodehydro-acetate containing the system (II).


$-\underset{\underset{O}{\|}}{C}-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}H-\overset{|}{C}H-$ (I.)


$-\underset{\underset{O}{\|}}{C}-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=\overset{|}{C}-$ (II.)

EXPERIMENTAL.

[M. p.s are uncorrected. All rotations were determined in chloroform solution. The solvent is specified where the ultra-violet spectrum was not determined in alcohol, the values are then corrected to this solvent by applying the necessary correction as described by Fieser (*loc. cit.*) for αβ-unsaturated ketones.]

Oxidation of Dihydroagnosteryl Acetate.—A solution of dihydroagnosteryl acetate (7 g.) in ice-cold chloroform (30 ml.) was treated at 0° with a chloroform solution of perbenzoic acid (equivalent to 0·4 g. of active oxygen). After 40 hours the excess of oxidizing agent was destroyed by the addition of a solution of potassium iodide, the mixture was acidified, and the iodine was removed by shaking with 0·1N-thiosulphate solution. The chloroform layer was washed twice with 2N-sodium hydroxide solution and then with water, the solution was dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residual oil was dissolved in methanol (50 ml.) and the solution heated under reflux for ten minutes; on cooling, a white powder (4·5 g.), m. p. 145—148°, separated and was filtered off. This, after two further crystallizations from chloroform–methanol, had m. p. 151—152°, $[\alpha]_D^{18}$ +26·0° (*c*, 0·151). Recrystallization led to a gradual rise in the melting point, and a solution of the solid (3 g.) in light petroleum (b. p. 60—80°; 300 ml.) was therefore chromatographed on a column of alumina (150 g.) (Peter Spence, grade H, 100—200 mesh). Elution of the column gave the following fractions:

Fraction.	Eluate.	Product.
(*a*)	200 ml. of light petroleum (b. p. 60—80°) + 50 ml. of benzene	0·2 g. of solid.
(*b*)	200 ml. of light petroleum (b. p. 60—80°) + 50 ml. of benzene	0·1 g. ,,
(*c*)	100 ml. of light petroleum (b. p. 60—80°) + 100 ml. of benzene	0·5 g. ,,
(*d*)	100 ml. of light petroleum (b. p. 60—80°) + 100 ml. of benzene	2·0 g. ,,
(*e*)	200 ml. of benzene	trace of oil.

Fractions (a) *and* (b). These were bulked and on recrystallization from ethyl acetate–methanol gave white needles (0·25 g.), m. p. 184° (Found: C, 79·0; H, 10·8. Calc. for $C_{32}H_{52}O_3$: C, 79·3; H, 10·8%). Ultra-violet spectrum: no absorption above $E_{1\,cm.}^{1\%}$ = 30 in *cyclo*hexane.

Fractions (c) *and* (d). Recrystallization from chloroform–methanol gave long, white needles (2·0 g.), m. p. 151—152°, $[\alpha]_D^{18}$ +24·0° (*c*, 0·0495) (Found: C, 79·3; H, 10·9. Calc. for $C_{32}H_{52}O_3$: C, 79·3; H, 10·8%). Ultra-violet spectrum: $\lambda_{max.}$ = 245 mμ.; log ε = 4·1, in *cyclo*hexane; corresponding to $\lambda_{max.}$ = 256 mμ. in alcohol.

A mixture of this compound with authentic ketodihydrolanosteryl acetate prepared according to Ruzicka *et al.* (*loc. cit.*) had m. p. 151—152°.

Hydrolysis. Saponification of the acetate with 5% alcoholic potash for two hours gave the alcohol ketodihydrolanosterol, m. p. 136°, $[\alpha]_D^{18}$ +28·5° (*c*, 0·0666). Reacetylation gave the acetate, m. p. 151—152°, which gave no depression in m. p. with the original acetate.

Isolation of the Product from the Mother Liquors by Methanol Treatment.—Evaporation of the methanol gave a glassy resin; this was dissolved in glacial acid (50 ml.) and treated at the boiling point with six drops of concentrated sulphuric acid. The mixture was heated for a further 15 minutes on the water-bath and then poured into ice-water. The separated solids (1·5 g.) were filtered off, dried, and recrystallized from ethyl acetate–methanol, and had m. p. 184°. Three further crystallizations from aqueous methanol gave colourless needles, m. p. 205°, $[\alpha]_D^{18}$ +96·3° (*c*, 0·0160) (Found: C, 76·6; H, 10·7. $C_{32}H_{52}O_4$ requires C, 76·8; H, 10·4%). Ultra-violet spectrum: no high intensity absorption in *cyclo*hexane, weak absorption at 267 mμ. due probably to traces of impurity. This *compound* is extremely soluble in methanol, chloroform and ethyl acetate.

Chromic acid Oxidation of Dihydrolanosteryl Acetate according to Marker et al.—To a solution of dihydrolanosteryl acetate (15 g.) in glacial acetic acid (450 ml.) at 80° was added a solution of chromic acid (4·5 g.) in 90% acetic acid (50 ml.). The mixture was heated at 80° for a further 10 minutes and then poured into water (150 ml.); this was extracted with ether, and the ethereal extract was washed twice with 10% sodium carbonate solution and twice with water, and dried (Na_2SO_4). The residue, after removal of the ether, was dissolved in glacial acetic acid (200 ml.); on cooling to room temperature a small amount of gelatinous material separated and was filtered off; overnight the filtrate deposited a crystalline solid (2 g.), m. p. 130° (Marker *et al.* reported that 4 g. separated from 250 ml. of acetic acid). Repeated crystallization from ethyl acetate, methanol, acetone, and ethyl alcohol caused a gradual rise in m. p. to the value 149—150° recorded by Marker *et al.* Purification by this process is very wasteful and chromatography gave no better results, the overall yield being 0·4—0·5 g. (Found : C, 82·15; H, 11·5. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). The material had $[\alpha]_D^{18}$ +80·0° (*c*, 0·0472). Ultra-violet spectrum : $\lambda_{max.}$ = 242 mμ., log ε = 4·1 (in *cyclo*hexane).

The acetic acid filtrate from the first crystallization was evaporated to dryness in a vacuum and the residue recrystallized from ethyl acetate to give a product (2 g.), m. p. 148°. This was recrystallized from acetone until the m. p. had risen, after four further crystallizations, to 151—152°, giving 1·5 g. of product, $[\alpha]_D^{18}$ +22·9° (*c*, 0·0532) (Found : C, 78·9; H, 10·8. Calc. for $C_{32}H_{52}O_3$: C, 79·3; H, 10·8%). Ultra-violet spectrum : $\lambda_{max.}$ = 246 mμ.; log ε = 4·1 (inflexion at 252 mμ.) in *cyclo*hexane; corresponding to $\lambda_{max.}$ = 257 mμ. in alcohol.

From the mother liquors pure diketodihydrolanosteryl acetate (1 g.), m. p. 157·5°, $[\alpha]_D^{18}$ +90·0° (*c*, 0·0491), was obtained together with some unchanged dihydrolanosteryl acetate.

Dehydrogenation of Ketodihydrolanosteryl Acetate.—Selenium dioxide (0·5 g.) was added to a solution of ketodihydrolanosteryl acetate (1 g.) in glacial acetic acid (100 ml.). The solution was refluxed for 2½ hours and then filtered from the black selenium which had separated, and the filtrate poured into water. The precipitated solids were extracted with ether (2 × 200 ml.), and the ether extract was washed successively with 2N-sodium hydroxide solution and water. The ether extract, after being dried (Na_2SO_4), was evaporated, and the residual solid in light petroleum (b. p. 60—80°; 200 ml.) was percolated through a column of alumina (40 g.). Evaporation of the filtrate gave no solid residue.

The column was eluted :

(*a*) 200 ml. of light petroleum (b. p. 60—80°) gave a trace of yellow oil insoluble in methanol; (*b*) 500 ml. benzene gave 0·75 g. of solid; (*c*) 200 ml. chloroform gave 0·1 g. of oil.

Fraction (*b*) was crystallized from acetone (20 ml.)–methanol (50 ml.) and gave glistening needles (0·65 g.), m. p. 195°. Further crystallization gave a pure *product* (0·5 g.), m. p. 195—196°, $[\alpha]_D^{18}$ +67·7° (*c*, 0·0293) (Found : C, 80·0; H, 10·0. $C_{32}H_{48}O_3$ requires C, 79·9; H, 10·1. $C_{32}H_{50}O_3$ requires C, 79·6; H, 10·45%). Ultra-violet spectrum : $\lambda_{max.}$ = 256 and 327 mμ.; log ε = 3·9, in alcohol. The compound is readily soluble in chloroform and acetone, but sparingly soluble in methanol. It gives an intense yellow colour with tetranitromethane.

The authors are indebted to Dr. E. A. Braude for the ultra-violet absorption spectra, and to Mr. P. Barker for the specific rotations. They gratefully acknowledge the advice and criticism of Professor H. Burton.

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[*Received, January 27th*, 1950.]