122. *Lanosterol. Part VI. Further Dehydrogenation and Oxidation Reactions.*

By Charles Dorée, James F. McGhie, and Frederick Kurzer.

Oxidation of diketodihydrolanosterol (as the acetate) with selenium dioxide has given *triketodihydrolanosterol*. The same reagent dehydrogenates diketolanostanyl acetate to *dehydrodiketolanostanyl acetate*. The conversion of lanosterol compounds into the corresponding *agnosterol* derivatives, previously carried out with selenium dioxide (Dorée and Bellamy, *J.*, 1941, 176), has now been effected with *N*-bromosuccinimide.

Selenium dioxide is widely used as an oxidizing agent in the sterol series. The reaction proceeds along different courses according to the type of compound oxidized, and the experimental conditions. Callow and Rosenheim (*J.*, 1933, 387), for example, report the direct conversion of ergosterol into dehydroergosterol, with the loss of two atoms of hydrogen and the introduction of a new unsaturated linkage. The mechanism of simple dehydrogenations of this type is usually considered to involve the intermediate formation of hydroxy-compounds, which undergo immediate dehydration, an unsaturated bond being formed. These hydroxyl-containing intermediates have been isolated in several instances (Watkins and Clark, *Chem. Reviews*, 1945, **36**, 244, 270).
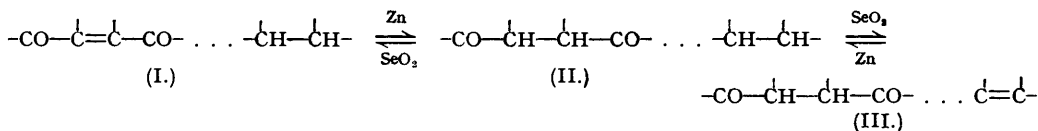
In other cases the action of selenium dioxide on unsaturated hydroxylated sterols leads, in the first place, to α-glycols. For instance, cholesterol (Rosenheim and Starling, *J.*, 1937, 377), sitosterol, and stigmasterol (Marker *et al.*, *J. Amer. Chem. Soc.*, 1938, **60**, 1071) yield the corresponding diols. Similarly, Rosenheim and Stiller have shown (*J.*, 1938, 353) that steroid ketones are oxidized in one stage to α-diketones, as, *e.g.*, cholestanone to cholestane-2 : 3-dione. Among the triterpenes, β-amyrin acetate undergoes simultaneous dehydrogenation and oxidation, to yield β-amyradienedione acetate (Ruzicka *et al.*, *Helv. Chim. Acta*, 1941, **24**, 1236).

The dehydrogenating action of selenium dioxide in the lanosterol series was first described by Dorée and Bellamy (*loc. cit.*), who found that a second double bond could be introduced into dihydrolanosterol, giving good yields of dihydroagnosterol (" γ-lanosterol "). In the present investigation the action of selenium dioxide on ketonic lanosterol derivatives has been examined, the compounds selected being diketodihydrolanosteryl acetate and diketolanostanyl acetate. The former substance, obtained by the oxidation of dihydrolanosteryl or dihydroagnosteryl acetate (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472), is deep yellow and contains the chromophoric grouping ·CO·C̈:C̈·CO·. Reduction of this compound gave the colourless diketolanostanyl acetate (Dorée, McGhie, and Kurzer, *J.*, 1948, 988) in which the chromophoric system had been removed by the hydrogenation of the double bond. Diketodihydrolanosteryl acetate, when treated with selenium dioxide in a mixture of glacial acetic acid and acetic anhydride for 2—5 hours, gave a product from which a triketone, *triketodihydrolanosteryl acetate*, could be isolated by chromatographic separation. The completeness of the oxidation was found to depend on the amount of selenium dioxide used and the time of reaction, but yields of triketone never exceeded 40% of the theoretical. The use of a large excess of oxidizing agent under prolonged drastic conditions gave unfavourable results. A proportion of unchanged starting material was still recovered, but partial destruction of the reactants lowered the yields of the desired product.

Triketodihydrolanosteryl acetate forms dark orange needles, m. p. 185—187°, and appears to be identical with the by-product sometimes obtained by Ruzicka (1944, *loc. cit.*) in the chromic acid oxidation of dihydrolanosteryl acetate. Careful hydrolysis under mild conditions gave *triketodihydrolanosterol*, m. p. 169—172°. Oxidation of the hydroxyl group converted this secondary alcohol into the corresponding tetraketone, *triketolanostenone*, m. p. 156—158°. Since they contain three keto-groups these products differ from the corresponding diketo-dihydro-derivatives in being more deeply coloured and showing a marked increase in their solubility, particularly in methanol and ethanol. The position of the keto-group introduced into the lanosterol molecule by selenium dioxide cannot yet be decided with certainty. The absence of a 1 : 2-diketo-system, as found in the sterol series by Rosenheim and Stiller (*loc. cit.*), must be inferred, since neither triketodihydrolanosteryl acetate nor triketolanostenone showed any of the characteristic properties of *o*-diketones. They were insoluble in caustic alkalis, gave no colouration with alcoholic ferric chloride solution, and failed to react with *o*-phenylenediamine to yield quinoxaline derivatives. Reduction of triketodihydrolanosteryl acetate according to Clemmensen gave excellent yields of diketolanostanyl acetate, m. p. 222—224°, identical with the product obtained from diketodihydrolanosteryl acetate under analogous
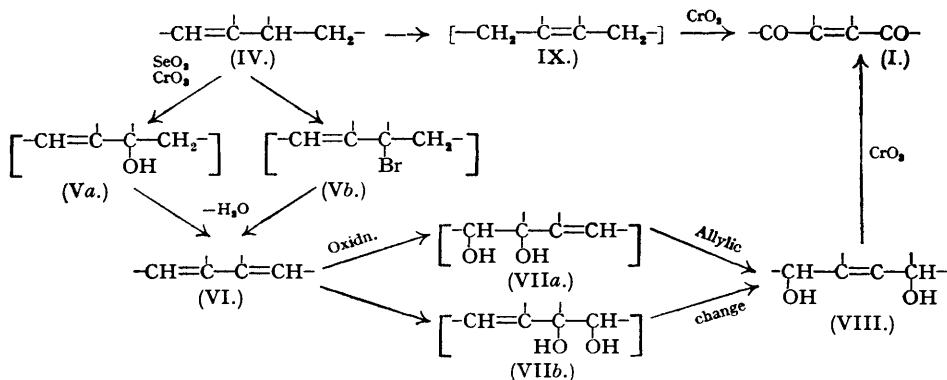
conditions. In this reduction the chromophore has been removed as expected (cf. Dorée, McGhie, and Kurzer, *loc. cit.*), while the third keto-group, newly introduced by selenium dioxide oxidation, has been reduced to methylene. The formation of the same reduction product from both diketo- and triketo-dihydrolanosteryl acetate is thus accounted for.

Diketolanostanyl acetate (II), when acted upon by selenium dioxide under drastic conditions, underwent simple dehydrogenation to yield colourless *dehydrodiketolanostanyl acetate* (III), m. p. 176—178°. Small quantities of diketodihydrolanosteryl acetate (I) (partly oxidized to the *triketo-acetate*), also produced in the reaction, and isolated from the main product by chromatographic separation, indicated that elimination of hydrogen occurred to a limited extent at the carbon atoms situated between the keto-groups, with re-establishment of the chromophore :

$$-CO-\overset{|}{C}=\overset{|}{C}-CO-\ \ldots\ -\overset{|}{C}H-\overset{|}{C}H-\ \underset{SeO_2}{\overset{Zn}{\rightleftharpoons}}\ -CO-\overset{|}{C}H-\overset{|}{C}H-CO-\ \ldots\ -\overset{|}{C}H-\overset{|}{C}H-\ \underset{Zn}{\overset{SeO_2}{\rightleftharpoons}}$$

$$\text{(I.)} \qquad\qquad\qquad\qquad \text{(II.)}$$

$$-CO-\overset{|}{C}H-\overset{|}{C}H-CO-\ \ldots\ \overset{|}{C}=\overset{|}{C}-$$
$$\text{(III.)}$$

Dehydrodiketolanostanyl acetate (III) contains a double bond and two keto-groups and is isomeric with diketodihydrolanosteryl acetate (I). In contrast to the latter compound, however, it is colourless, and its centres of unsaturation are therefore not part of a chromophoric system. Its light-extinction curve shows a high-intensity absorption in the near-ultra-violet region of the spectrum ($\lambda_{max.}$ = 238 mμ. ; log ε = 3·9), characteristic of a system of conjugated double bonds. Assuming the presence of the ·CO·$\overset{.}{C}$H·$\overset{.}{C}$H·CO· grouping in the starting material (II), a distribution of the type ·$\overset{.}{C}$:$\overset{.}{C}$·CO·$\overset{.}{C}$H·$\overset{.}{C}$H·CO· (IIIa) seems not unlikely, and is in agreement with the expected location of absorption maxima due to substituted αβ-unsaturated ketones (Woodward, *J. Amer. Chem. Soc.*, 1941, **63**, 1123). Dehydrodiketolanostanyl acetate is a stable compound, and resisted chromic acid oxidation; its acetoxy-group could not be deacylated by the usual methods of hydrolysis. A migration of this acetoxy-group during selenium dioxide dehydrogenation in the strongly acid medium to a tertiary position, in which the acetyl group is known to be firmly held, is unlikely, since Clemmensen reduction gave once again diketolanostanyl acetate, m. p. 222—224°, whose acetoxy-group undergoes deacetylation without difficulty.
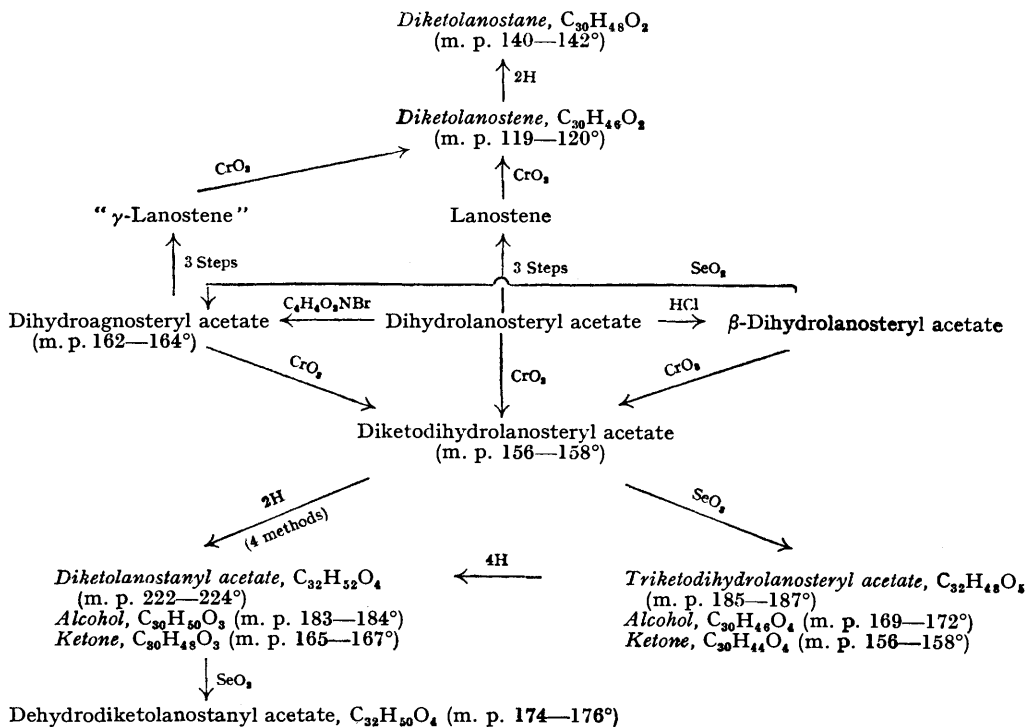
The dehydrogenation of lanosterol compounds to the corresponding agnosterol derivatives, previously effected with selenium dioxide (Bellamy and Dorée, *loc. cit.*), has now been carried out by means of *N*-bromosuccinimide. The action of this reagent, normally introducing a bromine atom into the allylic position (Ziegler *et al., Annalen,* 1942, **551**, 80), is widely applicable to unsaturated compounds and was claimed to brominate cholesteryl esters in almost quantitative yields. With triterpenes of the amyrin type, however, Ruzicka *et al.* (*Helv. Chim. Acta,* 1943, **26**, 1235) obtained halogen-free compounds, into which additional double bonds had been introduced in one step (*e.g.,* β-amyrin acetate to β-amyratrienyl acetate). When applied to compounds of the lanosterol type in the present series of experiments, the procedure gave dehydrogenated products directly. As with the amyrins, the reaction was accompanied by vigorous evolution of hydrogen bromide and the isolation of bromo-substituted intermediates

$$-CH=\overset{|}{C}-\overset{|}{C}H-CH_2-\ \longrightarrow\ [-CH_2-\overset{|}{C}=\overset{|}{C}-CH_2-]\ \overset{CrO_3}{\longrightarrow}\ -CO-\overset{|}{C}=\overset{|}{C}-CO-$$
$$\underset{\substack{SeO_2\\CrO_3}}{}\ \text{(IV.)} \qquad\qquad \text{IX.)} \qquad\qquad\qquad \text{(I.)}$$

$$\left[-CH=\overset{|}{\underset{OH}{C}}-\overset{|}{C}-CH_2-\right] \qquad \left[-CH=\overset{|}{C}-\overset{|}{\underset{Br}{C}}-CH_2-\right]$$
$$\text{(Va.)} \qquad\qquad\qquad \text{(Vb.)}$$

$$-CH=\overset{|}{C}-\overset{|}{C}=CH-$$
$$\text{(VI.)}$$

$$\left[-\overset{|}{\underset{OH}{C}}H-\overset{|}{\underset{OH}{C}}-\overset{|}{C}=CH-\right]$$
$$\text{(VIIa.)}$$

$$\left[-CH=\overset{|}{C}-\overset{|}{\underset{HO\ \ OH}{C}}-CH-\right]$$
$$\text{(VIIb.)}$$

$$-\overset{|}{\underset{OH}{C}}H-\overset{|}{C}=\overset{|}{C}-\overset{|}{\underset{OH}{C}}H-$$
$$\text{(VIII.)}$$

was not possible. The halogenation of the allyl position by *N*-bromosuccinimide and certain other *N*-halogen compounds in substances of known structure (Ziegler *et al., loc. cit.*) suggests

that the dehydrogenation takes place in two stages, *viz.*, bromination at the carbon atom adjacent to the double bond, followed by the removal ot hydrogen bromide with the formation of a new unsaturated linkage. The ready loss of the bromine atom (and hence the corresponding hydroxyl group introduced in the identical position during selenium dioxide dehydrogenation; see V*a*) under the mild conditions of the experiment indicates its probable attachment to a tertiary carbon atom. The " inert " double bond of dihydrolanosterol is therefore situated in, or migrates under the influence of the acid media during dehydrogenation into, a position adjacent to a tertiary carbon atom. The formation of dihydroagnosteryl acetate can thus be represented by partial formulæ (IV)—(VI), accounting for the establishment of the required system of conjugated double bonds distributed over two rings. The scheme is extended to show the observed production of diketodihydrolanosteryl acetate (I) by the chromic acid oxidation of both dihydrolanosteryl and dihydroagnosteryl acetate (VI) (Ruzicka *et al.*, 1944, *loc. cit.*). In the latter case the reaction mechanism may involve preliminary attack at either double bond of the conjugated system, followed by an allylic rearrangement, and final oxidation to (I) The oxidation of dihydrolanosteryl acetate to the same product may take place, by preliminary bond movement, *via* (IX). The identity of the oxidation product obtained from compounds of the lanosterol and agnosterol series (see also " α "-lanostene, " γ "-lanostene, to diketolanostene, Dorée, McGhie, and Kurzer, *loc. cit.*) appears significant, and the possibility of the intermediate formation of agnosterol derivatives in the oxidation of lanosterol compounds (IV ⟶ VI ⟶ VIII ⟶ I) should perhaps not be overlooked.

Scheme summarising reactions described in Parts V and VI.

Diketolanostane, $C_{30}H_{48}O_2$
(m. p. 140—142°)

↑ 2H

Diketolanostene, $C_{30}H_{46}O_2$
(m. p. 119—120°)

CrO₃ ↗   ↑ CrO₃

" γ-Lanostene "   Lanostene

↑ 3 Steps   3 Steps   SeO₂

Dihydroagnosteryl acetate ⟵ $C_4H_4O_2NBr$ — Dihydrolanosteryl acetate ⟶ HCl ⟶ β-Dihydrolanosteryl acetate
(m. p. 162—164°)

CrO₃   ↓ CrO₃   CrO₃

Diketodihydrolanosteryl acetate
(m. p. 156—158°)

2H (4 methods) ↙   4H ⟵   SeO₂ ↘

Diketolanostanyl acetate, $C_{32}H_{52}O_4$ *Triketodihydrolanosteryl acetate*, $C_{32}H_{48}O_5$
(m. p. 222—224°)   (m. p. 185—187°)
Alcohol, $C_{30}H_{50}O_3$ (m. p. 183—184°)   *Alcohol*, $C_{30}H_{46}O_4$ (m. p. 169—172°)
Ketone, $C_{30}H_{48}O_3$ (m. p. 165—167°)   *Ketone*, $C_{30}H_{44}O_4$ (m. p. 156—158°)

↓ SeO₂

Dehydrodiketolanostanyl acetate, $C_{32}H_{50}O_4$ (m. p. 174—176°)

Dihydrolanosteryl acetate, on treatment with *N*-bromosuccinimide according to Ziegler, gave a reaction product from which dihydroagnosteryl acetate, m. p. 160—162°, was isolated. Although the m. p. of the specimens obtained by this method was consistently 3—4° too low, the product was identified by conversion into derivatives and by its characteristic ultra-violet-absorption curve. The observed intensities (below) indicated the presence of 5—6% of

	Maximum I.		Maximum II.		Maximum III.	
	$\lambda_{max.}$	$E_1^1.$	$\lambda_{max.}$	$E_1^1.$	$\lambda_{max.}$	$E_1^1.$
Dihydroagnosteryl acetate.						
By selenum dioxide	238	300	245	380	250	240
By *N*-bromosuccinimide	236	285	243	360	251	225

unchanged dihydrolanosteryl acetate, which could not be removed by continued crystallisation. The more soluble fractions of the reaction product gave an uncrystallisable yellow oil, from which no well-defined compound could be isolated. The position of its absorption maximum (at $\lambda = 223$ mμ., $E = 320$) suggested the absence of material containing a system of three double bonds as found in the trienol obtained from β-amyrin.

Dehydrogenation by *N*-bromosuccinimide took place smoothly in cases where selenium dioxide, on account of its more severe action, proved less suitable or inapplicable. Thus, dihydrolanosterol gave good yields of *dihydroagnosterol*, m. p. 154—156°, while dihydrolanostenone (which, with selenium dioxide yields yellow compounds containing conjugated systems; authors' unpublished work) was readily converted into *dihydroagnostenone*, m. p. 126—128°. Dehydrogenations requiring the use of selenium dioxide under severe conditions did not take place with *N*-bromosuccinimide; for instance, diketodihydrolanosteryl and diketolanostanyl acetate were recovered unchanged after prolonged treatment. The interaction of *N*-bromosuccinimide with a lanosterol hydrocarbon has been previously reported (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467). Although the reagent is not superior, from the preparative point of view, to selenium dioxide, if applicable, it is valuable for introducing double bonds directly into more sensitive compounds of lanosterol, and gives information of the position of the centres of unsaturation already present in the molecule.

A summary of the reactions discussed in this and the previous communication (*J.*, 1948, 988) is given in the accompanying scheme ($C_4H_4O_2NBr = N$-bromosuccinimide).

<div align="center">EXPERIMENTAL.</div>

All m. p.s are uncorrected. Specific rotations were measured in chloroform solution at 20°. Aluminium oxide used in chromatographic work was from Messrs. Peter Spence Ltd. (" Grade H ").

Triketodihydrolanosteryl Acetate.—Diketodihydrolanosteryl acetate (30 g.; 1 mol.), dissolved in a mixture of glacial acetic acid and acetic anhydride (1 : 1; 500 ml.), was treated with excess of selenium dioxide (30 g.; 4·5 mols.; saturated aqueous solution) and refluxed vigorously with mechanical stirring for 4 hours. The selenium was filtered off, the filtrate concentrated to 100 ml. in a vacuum and diluted with ether (600 ml.), and the deep orange liquid washed with sodium hydroxide solution and water until neutral, and no more black tarry matter separated at the ether–water interface. After evaporation of the neutral ethereal extracts in a vacuum, the orange solid remaining was taken up in light petroleum (b. p. 60—80°; 800 ml.), completely absorbed on an aluminium oxide column (300 g., dimensions 4·5 × 16 cm.), and eluted as follows :

Eluant.	Eluate.	Weight, g.
(1) Light petroleum (400 ml., b. p. 60—80°)	Fraction 1 : Yellow crystalline needles, m. p. 158—164°; mainly diketodihydrolanosteryl acetate	4·90
(2) Light petroleum (b. p. 60—80°)—benzene (1 : 1, 400 ml.)	Fraction 2 : Deep yellow crystalline compact needles, m. p. 168—173°; mixed crystals of diketo- and triketo-dihydrolanosteryl acetate (approx. 1 : 1)	4·05
(3) Benzene (600 ml.)	Fraction 3 : Orange needles, m. p. 184—187°; triketodihydrolanosteryl acetate	7·75
(4) Benzene (200 ml.)	Fraction 4 : Orange oily material, uncrystallisable	1·2
(5) Chloroform (2 × 500 ml.)	Fractions 5 and 6 : Deep brown viscous oil, uncrystallisable	2·1

On removal of the solvent from eluate (3) a deep orange crystalline residue was obtained, which, after two crystallisations from chloroform—methanol (I : 12, 50 c.c.; II : 10, 40 c.c., resp.), gave orange needles of *triketodihydrolanosteryl acetate* (7·25 g.), m. p. 184—187° (Found : C, 75·0; H, 9·1. $C_{32}H_{48}O_5$ requires C, 75·0; H, 9·4%); $[\alpha]_D - 50·4°$ (*c*, 0·952). Ultra-violet absorption spectrum : Maximum absorption at 280 mμ.; $E_{1\text{ cm.}}^{1\%}$ 205.

The fractions obtained from eluates (1) and (2) showed absorption maxima in the ultraviolet, and carbon and hydrogen values corresponding to mixtures of diketodihydro- and triketodihydro-lanosteryl acetate (fraction 1, approx. 4:1; fraction 2, approx. 1:1). Maximum absorption at 275 mμ. ($E_1^{1\%}$ 210) and 285 mμ. ($E_1^{1\%}$ 190) (Found : C, 76·5; H, 9·5%). On combination with material obtained from the mother-liquors of fraction (3), and chromatographic separation as described above, a further crop of triketodihydrolanosteryl acetate (2·1 g.) was obtained.

Triketodihydrolanosterol.—Triketodihydrolanosteryl acetate (4 g.) was refluxed with alcoholic potash solution (7·5 g. KOH in 250 ml. EtOH) for one hour. The solution, which underwent brilliant colour change from yellow to dark-red and deep bluish-green during the boiling, was neutralised with concentrated hydrochloric acid, concentrated to 30 ml., diluted with water, and extracted with ether. The solvent was removed, and the alcohol purified by filtration of its light petroleum solution through an aluminium oxide column (15 g.). Three crystallisations from methanol (15, 10, and 8 ml., respectively) gave yellow, silky needles of *triketodihydrolanosterol*, m. p. 169—172° (Found : C, 76·6; H, 9·35. $C_{30}H_{46}O_4$ requires C, 76·6, H, 9·8%); $[\alpha]_D + 36·9°$ (*c*, 1·225).

Triketodihydrolanostenone.—Triketodihydrolanosterol (4 g.; 1 mol.) was dissolved in a mixture of benzene (120 ml.) and glacial acetic acid (20 ml.). To the cooled solution, Kiliani's 10% chromic acid mixture [40 ml.; prepared by dissolving sodium dichromate (60 g.) in water (270 ml.) and adding concentrated sulphuric acid (80 g.)] was added dropwise with cooling below 8° and mechanical stirring during 10

minutes. After vigorous shaking for another 15 minutes, the excess of the oxidising agent was removed by means of sulphur dioxide, the aqueous layer extracted with light petroleum, the combined benzene–light petroleum extracts washed till neutral with sodium hydroxide solution and water, and filtered through aluminium oxide (20 g.). After removal of the solvents in a vacuum, the yellow residual oil was crystallised three times from chloroform–methanol and gave *triketodihydrolanostenone*, m. p. 156—158°, in bright yellow needles (Found : C, 76·8; H, 9·3. $C_{30}H_{44}O_4$ requires C, 76·6; H, 9·8%); $[\alpha]_D + 105°$ (c, 0·870). Ultra-violet absorption spectrum : Maximum absorption at 272 and 278 mμ. (both $E_{1\ cm.}^{1\ \%}$ 285).

Triketodihydrolanostenone was insoluble in caustic alkalis, and did not give a colour with alcoholic ferric chloride solution, indicating the absence of a 1 : 2-diketo-system capable of undergoing enolization. When boiled with phenylenediamine in ethanol or glacial acetic acid for one hour, the tetraketone was recovered unchanged.

Diketolanostanyl Acetate.—A solution of triketodihydrolanosteryl acetate (2·5 g.) in glacial acetic acid (120 ml.) was added to excess of amalgamated zinc (15 g.), and to the boiling reaction mixture concentrated hydrochloric acid (20 ml.) was slowly added within 20 minutes. Refluxing was continued for 1½ hours, during which the yellow liquid was decolorised. The solution was poured off from the zinc and diluted with water (300 ml.), and the white reaction product isolated by extraction with ether. The product was purified by filtration of its light petroleum solution (b. p. 60—80°, 250 ml.) through alumina (20 g.), and was crystallised three times from chloroform–methanol, diketolanostanyl acetate, m. p. 222—224°, being obtained in white lustrous plates. There was no depression of the m. p. in admixture with diketolanostanyl acetate obtained from diketodihydrolanosteryl acetate (Dorée, McGhie, and Kurzer, A., 1948, 988) (Found : C, 76·7; H, 10·5. Calc. for $C_{32}H_{52}O_4$: C, 76·8; H, 10·4%); $[\alpha]_D + 54·6°$ (c, 0·471). The identity of the product with diketolanostanyl acetate was further established by hydrolysis to diketolanostanol, which on oxidation under restrained conditions gave diketolanostanone, m. p. 166—168°.

Dehydrodiketolanostanyl Acetate.—Diketolanostanyl acetate (10 g. ; 1 mol.) was dissolved in a mixture of glacial acetic acid (120 ml.) and acetic anhydride (120 ml.), and the hot solution slowly treated with excess of selenium dioxide (14 g., 6 mols., saturated aqueous solution). After vigorous refluxing for 3 hours, the black selenium was filtered off, extracted once again with boiling glacial acetic acid, and the orange-red filtrate concentrated to 80 ml. in a vacuum. The residual dark liquid was poured into water (800 ml.), and the resulting yellow solid extracted with benzene. The collected extracts (approx. 400 ml.) were washed till neutral with 10% sodium hydroxide solution and water, and purified by filtration through an alumina column (50 g.), which was subsequently eluted with pure benzene (2 × 100 ml.). The alumina retained a brown uncrystallisable oil as a deep orange zone, as well as the last traces of selenium. On evaporation of the eluate an orange crystalline mass was obtained, which gave, after two crystallisations from chloroform–methanol, *dehydrodiketolanostanyl acetate*, m. p. 176—178° in white lustrous plates (3·5 g.) (Found : C, 76·7; H, 10·1. $C_{32}H_{50}O_4$ requires C, 77·1; H, 10·1%); $[\alpha]_D 4·1°$, 5·0° (c, 1·07, 0·502). Ultraviolet absorption spectrum : Maximum absorption at λ 238 mμ. ($E_{1\ cm.}^{1\ \%}$ 286) and 308 mμ. ($E_{1\ cm.}^{1\ \%}$ 1·05).

Dehydrodiketolanostanyl acetate (1 g.), when oxidised in glacial acetic acid solution (25 ml.) with excess of chromium trioxide (1 g.) at 100° for 2 hours, was recovered almost quantitatively (0·85 g.). Hydrolysis did not occur when it (3 g.) was refluxed with 15% alcoholic caustic potash for 2 hours or on prolonged refluxing with ethanolic hydrogen chloride.

The mother-liquors from which the colourless dehydrodiketolanostanyl acetate had crystallised were deep orange. The ultra-violet absorption spectrum (maximum absorption at 278 and 280 mμ.) suggested the presence of diketo- and triketo-dihydrolanosteryl acetate. Further chromatographic purification and repeated crystallisation from acetone–methanol gave orange needles (0·4 g.), m. p. 166—170°, consisting of mixed crystals of the acetates of diketo- and triketo-dihydrolanosterol (Found : C, 76·5; H, 9·5. Calc. for $C_{32}H_{50}O_4$: C, 77·1; H, 10·1%. Calc. for $C_{32}H_{48}O_5$: C, 75·0; H, 9·4%). Ultraviolet absorption spectrum : maximum absorption at 274 mμ. ($E_{1\ cm.}^{1\ \%}$ 225) and 279 mμ. ($E_{1\ cm.}^{1\ \%}$ 120), corresponding to approximately equal quantities of the two constituents. The final mother-liquors gave an uncrystallisable oil (maximum absorption at 221 mμ., E_1^1 310; and 257, E_1^1 175).

Diketolanostanyl Acetate.—Dehydrodiketolanostanyl acetate (1 g.), dissolved in glacial acetic acid (50 ml.), was added to amalgamated zinc (8 g.). The boiling reaction mixture was slowly treated with concentrated hydrochloric acid (6 ml.) and refluxed for 2 hours. The resulting clear liquid was decanted into water (200 ml.), and the product isolated and purified as before, diketolanostanyl acetate, m. p. 220—224° being obtained in lustrous plates (Found : C, 77·1; H, 10·3. Calc. for $C_{32}H_{52}O_4$: C, 76·8; H, 10·4%); $[\alpha]_D + 54·1°$ (c, 0·57). Ultra-violet absorption spectrum : Maximum absorption at 298 mμ., $E_{1\ cm.}^{1\ \%}$ 1·3.

Dehydrogenation.—Dihydroagnosteryl acetate. (i) From β-Dihydrolanosteryl acetate. A solution of β-dihydrolanosteryl acetate (Marker *et al., J. Amer. Chem. Soc.*, 1937, **59**, 1368) (2 g., 1 mol.) in glacial acetic acid (40 ml.) was treated with excess of selenium dioxide (2 g., 4 mols. ; saturated aqueous solution) and refluxed for 4 hours. The selenium was filtered off, the filtrate poured into water (150 ml.), and the product extracted with benzene. The neutral extracts were filtered through alumina (20 g.) and the white residue obtained on removal of the solvent was crystallised twice from chloroform–methanol, whereupon dihydroagnosteryl acetate, m. p. 164—167°, was obtained (1·7 g.) (Found : C, 82·0; H, 10·75. Calc. for $C_{32}H_{52}O_2$: C, 82·05; H, 11·1%).

(ii) From dihydrolanosteryl acetate. A hot solution of dihydrolanosteryl acetate (5 g., 1 mol.) in anhydrous carbon tetrachloride (60 ml.) was treated with N-bromosuccinimide (2 g. ; 0·9 mol.) and refluxed on the steam-bath for 2 hours. The reaction mixture, initially deep orange, turned pale yellow, and hydrogen bromide was evolved throughout the refluxing. The succinimide was filtered off from the cold solution, and the filtrate washed successively with sodium hydroxide solution and water. Removal of the solvent gave a yellow viscous oil, which was taken up in light petroleum and filtered through

alumina (40 g.), a nearly colourless eluate being obtained. The white solid resulting on distillation of the petroleum was crystallised three times from acetone–methanol, and twice from acetone, and dihydroagnosteryl acetate, m. p. 161—163°, was obtained in lustrous plates (2·7 g.). Beilstein reaction slightly positive (Found : C, 81·8; H, 11·5%); $[a]_D$ 87·5° (c, 0·692). Ultra-violet absorption spectrum : Maximum absorption at 236, 243, and 251 mμ. ($E_{1\ cm.}^{1\ \%}$ 285, 360, and 225, respectively).

The mother-liquors from the crystallisation of the dihydroagnosteryl acetate gave, on further fractionation, mixed crystals of dihydrolanosteryl and dihydroagnosteryl acetates (approx. 1 : 1), m. p. 152—156°. The most soluble fractions gave an uncrystallisable orange oil (ultra-violet absorption spectrum : maximum absorption at 223 mμ.).

The identity of dihydroagnosteryl acetate obtained by the use of N-bromosuccinimide was further established by hydrolysis to dihydroagnosterol, m. p. 154—156°, which, on oxidation under mild conditions by Kiliani's 10% chromic acid mixture, as previously described, gave dihydroagnostenone, m. p. 126—128°. Its *semicarbazone* melted at 242—244° (Found : C, 77·2; H, 11·1; N, 8·6. $C_{31}H_{51}ON_3$ requires C, 77·3; H, 10·7, N, 8·7%).

(iii) *From β-dihydrolanosteryl acetate.* β-Dihydrolanosteryl acetate (2 g., 1 mol.) was dissolved in anhydrous carbon tetrachloride (100 ml.). The solution, treated with excess of N-bromosuccinimide (1·5 g.; 1·8 mols.), was refluxed for 2 hours and the product isolated as described above, dihydroagnosteryl acetate, m. p. 160—162°, being obtained (Found : C, 81·8; H, 11·0%). The m. p.s of the resulting specimens were again somewhat low, owing to the presence of a small proportion of unchanged starting material. The product was readily identified as dihydroagnosteryl acetate (authentic m. p. 167—169°) by its characteristic ultra-violet absorption spectrum ($\lambda_{max.}$ 235, 245, and 251 mμ.; $E_{1\ cm.}^{1\ \%}$ 289, 350, and 320 respectively).

Dihydroagnosterol. Dihydrolanosterol (4 g.; 1 mol.), dissolved in anhydrous carbon tetrachloride (100 ml.), was treated with N-bromosuccinimide (2·3 g.; 1·4 mols.), refluxed for 1 hour, and the product isolated in the usual manner. After filtration of the light petroleum solution through alumina (15 g.) and four crystallisations from acetone–methanol, dihydroagnosterol, m. p. 154—156°, was obtained in soft felted needles (Found : C, 83·9; H, 11·4. Calc. for $C_{30}H_{50}O$: C, 84·5; H, 11·7%).

Dihydroagnostenone. To a solution of dihydrolanostenone (5 g.; 1 mol.) in carbon tetrachloride (120 ml.), N-bromosuccinimide (3·3 g.; 1·5 mols.) was added, and the reaction mixture refluxed on the water-bath for 2 hours. Reaction took place in the familiar manner, although less vigorously than in the case of the acetate and alcohol. After isolation of the product as before, dihydroagnostenone, m. p. 125—128° was obtained in white flat needles from chloroform–acetone–methanol (Found : C, 84·6; H, 11·6. Calc. for $C_{30}H_{48}O$: C, 84·9; H, 11·7%). Reduction by sodium and *iso*propyl alcohol yielded dihydroagnosterol, m. p. 152—156° (acetate, m. p. 164—167°)

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