696. *Triterpenoids. Part IV.* Some Observations on the Constitution of Lanostadienol (Lanosterol).*

By D. H. R. Barton, J. S. Fawcett, and B. R. Thomas.

Treatment of diketolanostanol with phosphorus pentachloride leads to the formation of an *iso*lanostenedione, isomerised by acid to an αβ-unsaturated ketone. Similar treatment of triketolanostadienol gives a compound containing the chromophoric system $-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=\overset{|}{C}-CO-\overset{|}{C}=\overset{|}{C}-CO-CO-$, the formulation of which is confirmed by the absorption spectrum of the dicarboxylic acid obtained by fission of the α-diketone grouping with alkaline hydrogen peroxide. The formation of mono-2 : 4-dinitrophenylhydrazones from *iso*lanostenedione and its conjugated isomer distinguishes between the less and more hindered carbonyl groups. These experiments lead to unambiguous partial formulæ for lanostenol and its various oxidation products. Experiments are also described which enable a partial formula to be assigned to *iso*lanostenol.

Dehydrogenation of both "lanosterol" and "lanostene" affords 1 : 2 : 8-trimethylphenanthrene. From this it is concluded that only one of the methyl groups in the latter comes from ring A. On the basis of this and other facts the proven partial formula for lanostenol can be (tentatively) expanded to include two additional angular methyl groups.

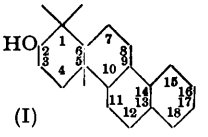
In the last decade important contributions have been made to our knowledge of the chemistry of lanostadienol (lanosterol), the main triterpenoid component (along with its dihydro-derivative) of the non-saponifiable matter of wool fat. For these advances we are indebted to Ruzicka and his collaborators in Switzerland (*Helv. Chim. Acta*, 1950, **33**, 1893 and earlier papers) and to McGhie and his associates in this country (*J.*, 1951, 834 and earlier papers). It has been established that lanostadienol contains a *sec.*-hydroxyl group (in ring A), flanked by $>CH_2$ and $>CMe_2$ groups and that the more reactive ethylenic linkage is present as the grouping $-CH{:}CMe_2$ in a side chain. Having regard to these facts and to dehydrogenation

* Part III, *J.*, 1951, 1444.

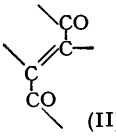
evidence (Schulze, *Z. physiol. Chem.*, 1936, **238**, 35; Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472) it can be concluded that the carbon skeleton comprises three (rings A, B, and C) and, possibly four (rings A, B, C, and D) six-membered rings. The partial formula (I) summarises these conclusions. In this formula a methyl group is almost certainly attached at $C_{(5)}$, as indicated.

The less reactive ethylenic linkage contained within the tetracyclic nucleus is indicated by the infra-red spectrum of lanostene (Roth and Jeger, *Helv. Chim. Acta*, 1949, **32**, 1620) as fully substituted. Our own examination of the infra-red spectrum of lanostenyl acetate in the 12-μ region confirms this conclusion. In addition, the apparent ultra-violet absorption spectrum in the 195—215-mμ region (see Experimental) corresponds to that of cholest-8(9)-enol rather than of cholest-8(14)-enol and indicates that the double bond must lie between rings B and C or C and D. There is further evidence for this conclusion in the behaviour of lanostenyl acetate on chromic acid oxidation. Under fairly vigorous conditions diketolanostenyl acetate results (Ruzicka, Rey, and Muhr, *loc. cit.*; Dorée and McGhie, *Nature*, 1944, **153**, 148; cf. Wieland and Joost, *Annalen*, 1941, **546**, 103). This compound has a characteristic absorption maximum at about 274 mμ which is indicative of the system $-CO-\overset{|}{C}=\overset{|}{C}-CO-$ in the fully *transoid* arrangement (II) (see Campbell and Harris, *J. Amer. Chem. Soc.*, 1941, **63**, 2721). Other relevant arguments in support of this conclusion are presented by Voser, Montavon, Günthard, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1950, **33**, 1893).

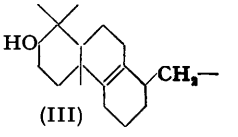
Recently Voser *et al.* (*loc. cit.*) advanced the partial formula (III) for lanostenol. It seemed to us that this was an arbitrary choice and that there was nothing in the published literature to distinguish between the position of the double bond as 9(10) [as in (III)] or as 13(14). Accordingly, our first experiments in this field were directed towards reaching a final distinction between these possible positions for the nuclear double bond. The evidence that we have obtained places the 9(10)-position beyond doubt.



(I)

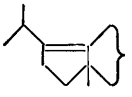


(II)

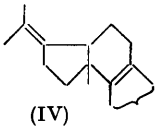


(III)

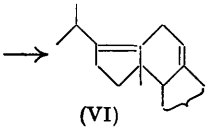
Reaction of lanostenol with phosphorus pentachloride afforded *iso*lanostadiene (IV) (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467; Ruzicka, Montavon, and Jeger, *Helv. Chim. Acta*, 1948, **31**, 818).* It was hoped that acidic reagents would move the two ethylenic linkages into conjugation, for in comparable *iso*propylidene compounds (Ruzicka *et al.*, *ibid.*, 1945, **28**, 380; 1946, **29**, 210) the double bond moves into the five-membered ring [as in (V)] under such conditions. Indeed, treatment with hydrogen chloride in chloroform gave an isomeric hydrocarbon [probably (VI); see below] but the absorption spectrum showed the presence of only isolated double bonds. Similar isomerisation of *iso*-γ-lanostatriene (VII) (Dorée, McGhie, and Kurzer, *loc. cit.*) likewise failed to give a triply conjugated triene.



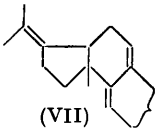
(V)



(IV)



(VI)

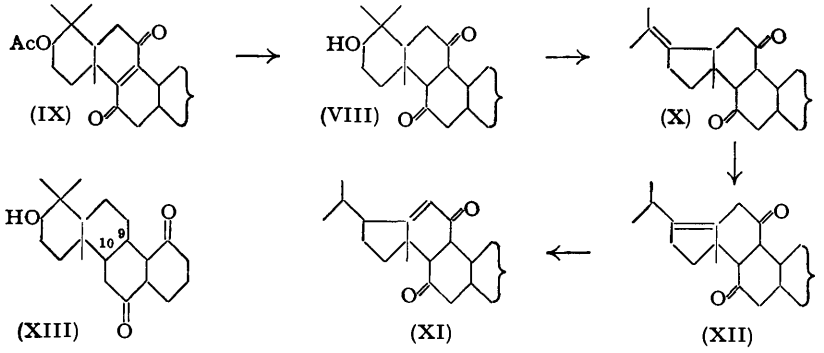


(VII)

Greater success attended experiments based on 2-hydroxylanostane-8 : 11-dione (diketolanostanol) (VIII), which is readily prepared (Dorée, McGhie, and Kurzer, *J.*, 1948, 988) by reduction of 8 : 11-diketolanost-9-en-2-yl acetate (diketolanostenyl acetate; see above) (IX), followed by hydrolysis. On treatment with phosphorus pentchloride, (VIII) afforded an *iso*lanostenedione (X) the absorption spectrum of which (Fig. 1) showed the three chromophoric groups to be isolated from each other. Isomerisation of (X) under mild acid conditions gave an isomer (XI) in which the double bond was in conjugation with one of the ketone groups, as shown by the absorption spectrum (Fig. 1). The formation of (XI) is readily intelligible as proceeding through the $\beta\gamma$-unsaturated isomer (XII). The alternative formula (XIII) for diketolanostanol could hardly explain this isomerisation (on the assumption, of course, that no rearrangement of the carbon skeleton occurs) even if there were no methyl groups at

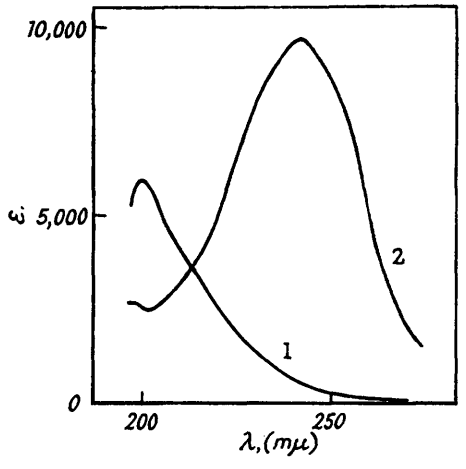
* There seems to be no published proof that *iso*lanostadiene still retains the 9(10)-ethylenic linkage. However, this is very probable, for on similar treatment with phosphorus pentachloride lanostenyl acetate was recovered unchanged almost quantitatively.

$C_{(9)}$ or $C_{(10)}$, for it would proceed through a disecondary ethylenic linkage (at $C_{(7)}$: $C_{(8)}$) which, itself, would be formed through a carbonium ion produced by the improbable non-Markownikoff addition of a proton to the 6 : 7-double bond.



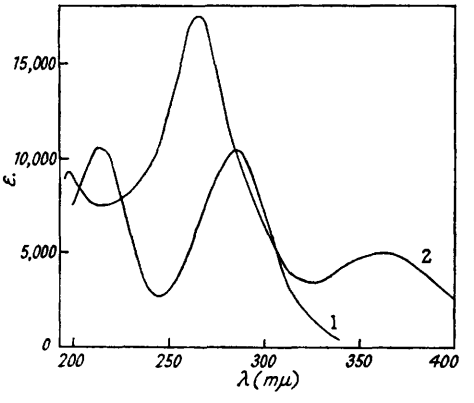
A further, and more rigid, proof for the position of the nuclear double bond at 9(10) was obtained in the following way. Treatment of 2-hydroxylanosta-6 : 9-diene-8 : 11 : 12-trione (lanostadienetrionol) (XIV), itself prepared by hydrolysis of the corresponding acetate (Doree, McGhie, and Kurzer, *J.*, 1949, 570; Voser *et al.*, *loc. cit.*), with phosphorus pentachloride gave *iso*lanostatrienetrione (XV) in which an extra double bond had clearly been placed in

FIG. 1.



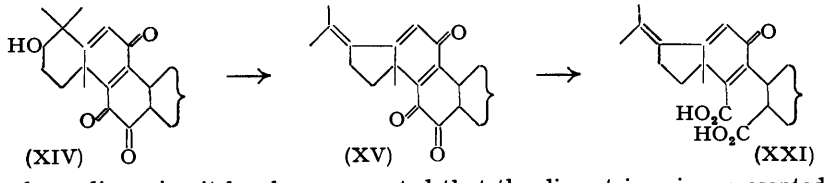
1. iso*Lanostenedione*.
2. *Isomerised* iso*lanostenedione*.

FIG. 2.



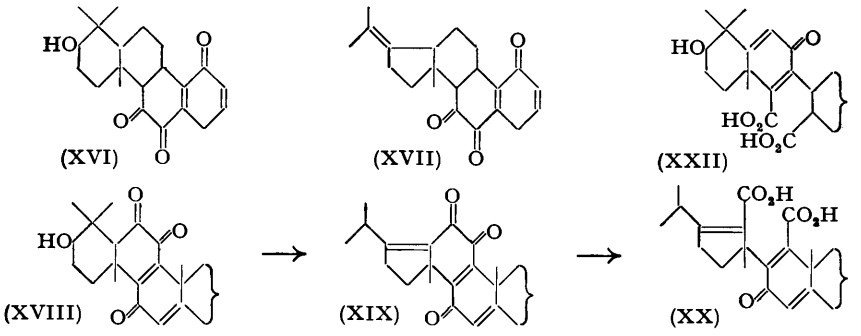
1. 2-*Hydroxylanosta*-6 : 9-*diene*-8 : 11 : 12-*trione*.
2. iso*Lanostatrienetrione*.

conjugation with the original chromophore. This was deduced from the change in absorption spectrum from (XIV) to (XV) (Fig. 2). Obviously if (say) (XVI), with the 13(14)-double bond had been the correct formula for the diene-trione there should have been no change in absorption spectrum on forming the triene-trione, for this would then have been formulated as (XVII). 8 : 11 : 12-Triketolanosta-6 : 9-dien-2-yl acetate, itself, was unchanged by a corresponding treatment with phosphorus pentachloride.



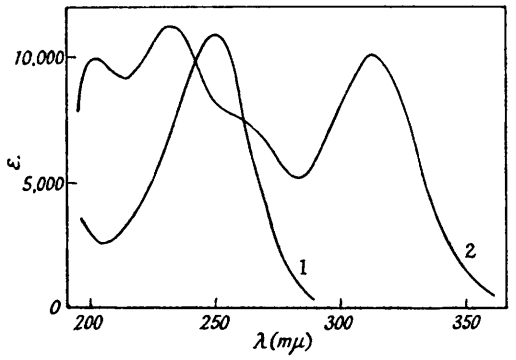
In the above discussion it has been suggested that the diene-trione is represented with the α-diketone grouping in ring C rather than in ring B. This is not altogether unequivocal as it is

formally possible that a diene-trione of formula (XVIII) could give (XIX) on dehydration. The change in chromophoric properties in such a case would not be expected to be so marked as is experimentally observed and in addition it would involve a migration of the double bond



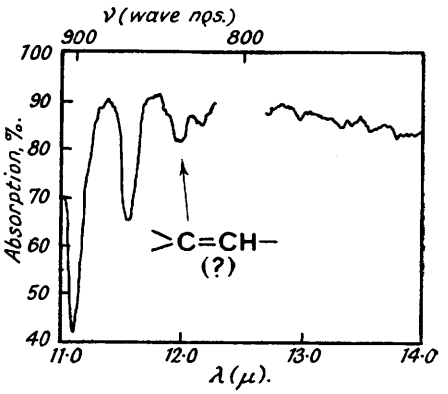
of the *iso*propylidene group into the five-membered ring. Formula (XVIII) was definitely excluded, however, by the following evidence. Oxidation of the diene-trione acetate by alkaline hydrogen peroxide gave a hydroxy-keto-dicarboxylic acid (Cavalla and McGhie, *J.*, 1951, 744) which must contain the chromophoric system $-\overset{|}{C}=\overset{|}{C}-CO-\overset{|}{C}=\overset{|}{C}-CO_2H$. In agreement, it absorbed at 249 mμ (Fig. 3). Similar oxidation of the triene-trione gave a keto-dicarboxylic acid. If the diene-trione had the formula (XVIII) and the triene-trione had formula (XIX), then this dicarboxylic acid (XX) would have (*a*) the same chromophoric system absorbing at 249 mμ. and (*b*) the system $-\overset{|}{C}=\overset{|}{C}-CO_2H$ which absorbs at about 220 mμ. The alternative formulation of the precursor as (XV) would give an acid (XXI) in which the extended chromophoric system $-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=\overset{|}{C}-CO-\overset{|}{C}=\overset{|}{C}-CO_2H$ would be present. The absorption spectrum observed for the trienone-dicarboxylic acid (Fig. 3) shows a band at 311 mμ. Formula (XX) is, therefore, definitely excluded in favour of (XXI). The hydroxy-keto-dicarboxylic acid of Cavalla and McGhie (*loc. cit.*) must be represented by (XXII).

FIG. 3.



1. *Dicarboxylic acid of Cavalla and McGhie.*
2. iso*Lanostatrienetrione oxidation product.*

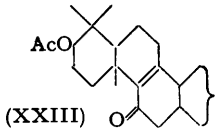
FIG. 4.

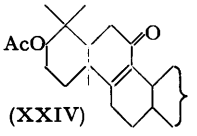


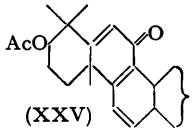
Infra-red spectrum of iso*lanostenyl acetate.*

When lanostenyl acetate is oxidised by chromic acid under controlled conditions it affords a monoketolanostenyl acetate (Marker, Wittle, and Mixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368; Ruzicka, Rey, and Muhr, *loc. cit.*; Birchenough and McGhie, *J.*, 1950, 1249; Cavalla and McGhie, *loc. cit.*) which must be either (XXIII) or (XXIV). Further oxidation by selenium dioxide furnishes a trienone (Birchenough and McGhie, *loc. cit.*) which must have formula (XXV) because it can be oxidised smoothly (as the acetate) by chromic acid to (XIV) Therefore, the monoketone must be represented by (XXIV). This observation does *not*, however, prove that the less hindered keto-group in 2-hydroxylanostane-8 : 11-dione (VIII) is at $C_{(8)}$ rather than at $C_{(11)}$. *Proof* that it was at $C_{(8)}$ was obtained in the following way. The *iso*lanostenedione (X) afforded, without difficulty, a mono-2 : 4-dinitrophenylhydrazone ($\lambda_{max.}$

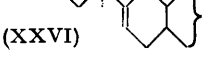
368 mμ in chloroform) in which the double bond was not in conjugation with the hydrazone residue. The isomeric αβ-unsaturated ketone (XI), however, gave a 2 : 4-dinitrophenylhydrazone ($\lambda_{max.}$ 389 mμ in chloroform) in which the double bond *was* in conjugation with the hydrazone residue.

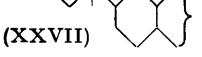

(XXIII)

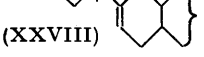

(XXIV)


(XXV)

When lanostenyl acetate is treated with hydrogen chloride in chloroform it is isomerised to an equilibrium mixture of *iso*lanostenyl acetate and (presumably) lanostenyl acetate from which the former compound can be isolated, albeit with some difficulty (Marker, Wittle, and Mixon, *loc. cit.*; McGhie, Thesis, London, 1947; cf. Ruzicka, Rey, and Muhr, *loc. cit.*; Wieland and Benend, *Z. physiol. Chem.*, 1942, **274**, 215). We were interested in this *iso*-compound for it could, conceivably, have had the double bond at 9(14), in which case a methyl group could not have been attached at $C_{(14)}$. However the infra-red spectrum (Fig. 4) indicated that it was triply substituted. On treatment with perbenzoic acid *iso*lanostenyl acetate gave several products. The most important of these was γ-lanostadienyl acetate (dihydroagnosteryl acetate), which must now be formulated as lanosta-8 : 10-dien-2-yl acetate (XXVI). The formation of (XXVI) can best be explained if *iso*lanostenyl acetate is lanost-8-enyl (XXVII) or lanost-10-enyl (XXVIII) acetate. The former of these formulæ was shown to be correct by the characterisation of *iso*lanostenol as the Wolff–Kishner reduction product of (XXIV).


(XXVI)

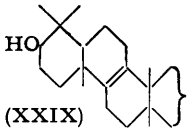

(XXVII)

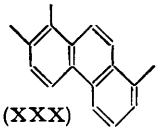

(XXVIII)

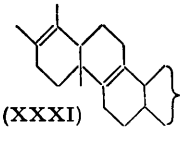
In the latter reaction the double bond shifts from its original position. From its method of preparation *iso*lanostenyl acetate must have the more stable configuration at $C_{(10)}$. Formula (XXVIII) has been correctly assigned to a lanostenyl acetate isomer prepared recently by Voser *et al.* (*loc. cit.*).

The two other products of the action of perbenzoic acid on *iso*lanostenyl acetate which were identified were 8-ketolanost-9-en-2-yl acetate (XXIV), presumably formed by the action of the per-acid on the γ-lanostadienyl acetate (Birchenough and McGhie, *J.*, 1949, 2038), and a saturated ketone which must be formulated as 8-ketolanostan-2-yl acetate. It differed from the 11-ketolanostanon-2-yl acetate of Voser *et al.* (*loc. cit.*).

The failure to obtain evidence for a double-bond isomer at 9(14), which, being tetrasubstituted, would be expected to be favoured thermodynamically [compare the 7(8)-, 8(9)-, and 8(14)-positions in the steroid nucleus], and the failure to extend the unsaturation or substitution beyond the systems represented in (XIV) and (XXV), can be *tentatively* explained by placing methyl groups at $C_{(13)}$ and $C_{(14)}$ as in the lanostenol formula (XXIX). Against such a formulation seemed to be the dehydrogenation evidence. The main dehydrogenation product of lanostadienol was identified by Schulze (*loc. cit.*) as 1 : 2 : 8-trimethylphenanthrene (XXX). This could be interpreted (cf. Voser *et al.*, *loc. cit.*) as arising from a rearrangement in ring A in the sense of (XXXI) giving (XXX). In the particular case of lanostadienol it has now been


(XXIX)

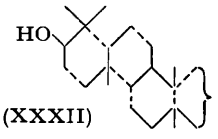

(XXX)


(XXXI)

shown that dehydrogenation of both "lanosterol" itself and of "lanostene" affords 1 : 2 : 8(1 : 7 : 8)-trimethylphenanthrene the yield being superior in the *latter* case. It is very improbable therefore that this phenanthrene can arise by rearrangement of ring A. Thus the dehydrogenation evidence can be reconciled with formula (XXIX) without difficulty.

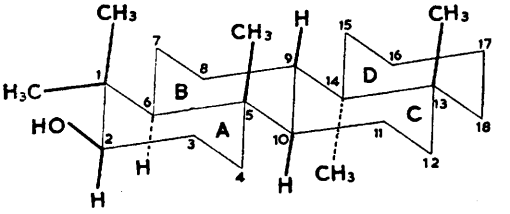
It remains to be pointed out that the part formula (XXIX) obeys the isoprene rule [see (XXXII)]. The differing degrees of steric hindrance of the ketonic groups, in 8 : 11-diketo-

lanostan-2-ol (VIII) could be explained by the stereochemistry shown for lanostanol in Fig. 5. The keto-group at $C_{(11)}$ would then correspond to $C_{(11)}$ in the steroid series (hindrance by *two* polar type C–CH_3 bonds) whilst that at $C_{(8)}$ would correspond to $C_{(4)}$ or $C_{(6)}$ in the steroid series (hindrance by *one* polar type C–CH_3 bond).*



(XXXII)

FIG. 5.



After the experiments described above had been concluded, we learnt that Cavalla, McGhie, and Pradhan had independently reached similar conclusions as to the position of the inert double bond in lanostadienol and had also carried out some analogous experiments on the constitution of *iso*lanostenyl acetate (see preceding paper). The results reported in the two papers are in excellent agreement. We thank Dr. J. F. McGhie for his courtesy in informing us of his results before their general publication.

EXPERIMENTAL.

M. p.s are uncorrected. Unless specified to the contrary rotations were determined in chloroform solution at room temperature, which varied from 15° to 25°. Values of $[\alpha]_D$ have been approximated to the nearest degree.

Light petroleum refers, unless specified to the contary, to the fraction of b. p. 40—60°.

In the test below the phrase " in the usual way " refers to dilution with water, extraction with ether, washing successively with aqueous potassium hydroxide solution, aqueous hydrochloric acid, and water, followed by evaporation of the ethereal solution *in vacuo*. Where necessary, water was removed from the residue by azeotropic distillation *in vacuo* with benzene as entrainer.

Alkaline hydrolyses were effected by using several equivalents of potassium hydroxide and refluxing the reactants for 30—60 minutes in methanolic, ethanolic, or dioxan–methanolic solution, depending on the solubility requirements of the ester.

Ultra-violet absorption spectra were, unless specified to the contrary, measured in ethanol solution, with a Unicam S.P. 500 Spectrophotometer.

Infra-red absorption spectra were kindly determined, in chloroform solution, by Dr. Hans Heymann (Harvard University) using a Baird Associates self-recording double-beam instrument.

Isomerisation of iso*Lanostadiene.*—*iso*Lanostadiene (530 mg.) (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467; Ruzicka, Montavon, and Jeger, *Helv. Chim. Acta*, 1948, **31**, 818) in chloroform (20 ml.) at 0° was treated with a vigorous stream of dry hydrogen chloride for 20 minutes. Removal of the chloroform and dissolved hydrogen chloride *in vacuo* and recrystallisation of the residue from chloroform–methanol afforded an isomeric *hydrocarbon*, m. p. 71°, $[\alpha]_D$ +94° (*c*, 0·83), $\lambda_{max.}$ 201 mμ ($\varepsilon_{max.}$ 10,000) (*c*, 0·0046) (Found: C, 87·8; H, 12·5. $C_{30}H_{50}$ requires C, 87·8; H, 12·2%). When lanostenyl acetate was treated with phosphorus pentachloride under the conditions employed in the preparation of the *iso*lanostadiene, it was recovered unchanged in almost quantitative yield. Isomerisation of the nuclear double bond very probably does not, therefore, occur in the preparation of the latter hydrocarbon.

iso-γ-Lanostatriene, m. p. 135—136°, $[\alpha]_D$ +38° (*c*, 1·96), $\lambda_{max.}$ 235, 243, and 253 mμ ($\varepsilon_{max.}$ 14,000, 17,000, and 11,000 respectively) (720 mg.) (Dorée, McGhie, and Kurzer, *loc. cit.*), was treated similarly with dry hydrogen chloride. Working up as before gave a crude product (from chloroform–methanol), m. p. 75—80°, whose absorption spectrum was similar to that of the starting material. There was no indication of a band corresponding to three double bonds in conjugation.

Dehydration of 2-*Hydroxylanostane*-8 : 11-*dione.*—8 : 11-Diketolanostan-2-yl acetate was prepared according to the directions of Dorée, McGhie, and Kurzer (*J.*, 1948, 988). Alkaline hydrolysis furnished 2-hydroxylanostane-8 : 11-dione which, recrystallised from methancl, had m. p. 184—187°, $[\alpha]_D$ +58° (*c*, 1·66). Dorée, McGhie, and Kurzer (*loc. cit.*) recorded m. p. 183—184°, $[\alpha]_D$ +26°, for this compound.

2-Hydroxylanostane-8 : 11-dione (500 mg.) and phosphorus pentachloride (500 mg.) were suspended in light petroleum of b. p. 60—80° (20 ml.) and left at 0° for 1 hour with occasional agitation. The reaction product, after being worked up in the usual way, was chromatographed over alumina (Birlec). Elution with 1 : 5 benzene–light petroleum and recrystallisation from methanol furnished iso*lanostenedione* (X), m. p. 145—146°, $[\alpha]_D$ +76° (*c*, 1·84) (Found: C, 82·0; H, 10·9. $C_{30}H_{48}O_2$ requires

* In Fig. 5 we have *arbitrarily* assumed that rings A and B are *trans*. The differing degrees of steric hindrance for ketonic groups at $C_{(8)}$ and $C_{(11)}$ can equally well be explained in the same way if rings A and B are *cis*. At present we have no preference for either *cis*- or *trans*-fusion of these rings.

C, 81·75; H, 11·0%). The 2 : 4-dinitrophenylhydrazone was obtained on refluxing the reactants for 15 minutes. Purified by chromatography and recrystallisation from chloroform-methanol it had m. p. 215—220°, $\lambda_{max.}$ 368 mμ ($\varepsilon_{max.}$ 20,000) (in chloroform) (Found: N, 9·4. $C_{36}H_{52}O_5N_4$ requires N, 9·05%).

Isomerisation of iso*Lanostenedione.*—*iso*Lanostenedione (200 mg.) was heated under reflux with acetic acid (20 ml.) and concentrated hydrochloric acid (10 ml.) for 12 hours. Working up in the usual way and crystallisation from methanol afforded the αβ-unsaturated *isomer* (XI), m. p. 129—130°, $\lambda_{max.}$ 241 mμ ($\varepsilon_{max.}$ 9500) (Found: C, 81·2; H, 10·9%). The 2 : 4-*dinitrophenylhydrazone* (prepared as for *iso*lanostenedione, see above) purified by chromatography over alumina (Birlec) and recrystallisation from chloroform–methanol, had m. p. 233°, $\lambda_{max.}$ 389 mμ ($\varepsilon_{max.}$ 29,000) (in chloroform) (Found: N, 10·0%). When the isomerisation was carried out in dioxan, with refluxing for only 4 hours, a mixture resulted. This was partly resolved on chromatography, the more easily eluted fractions furnishing, after crystallisation from ethanol, colourless plates, m. p. 142—146°, $\lambda_{max.}$ 238 mμ ($\varepsilon_{max.}$ 2800). This substance, which clearly contained about 30% of the αβ-unsaturated isomer, gave a 20° depression in m. p. with the starting material and it is possible that it was mainly the βγ-unsaturated diketone (XII).

Action of Phosphorus Pentachloride on 2-Hydroxylanosta-6 : 9-diene-8 : 11 : 12-trione.—8 : 11-Diketolanost-9-en-2-yl acetate (5 g.) (Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472; Dorée and McGhie, *Nature*, 1944, **153**, 148) and selenium dioxide (5 g.) were heated under reflux in acetic anhydride (30 ml.) for 3 hours. The precipitated selenium was filtered off and the filtrate diluted with water and worked up in the usual way. Chromatography over alumina afforded 8 : 11 : 12-triketolanosta-6 : 9-dien-2-yl acetate (2·5 g.) which recrystallised from methanol as yellow needles, m. p. 191—192°, $[\alpha]_D$ −68° (*c*, 2·76). This acetate (1·3 g.) was heated under reflux with ethanolic potassium hydroxide (30 ml. of 3%) for 30 minutes and the product worked up in the usual way. Recrystallisation from methanol and then from benzene–light petroleum afforded 2-hydroxylanosta-6 : 9-diene-8 : 11 : 12-trione, m. p. 194—195°, $[\alpha]_D$ −74° (*c*, 2·20) (Found: C, 77·0; H, 10·0. Calc. for $C_{30}H_{44}O_4$: C, 76·9; H, 9·5%). These physical constants are different from those, m. p. 169—172°, $[\alpha]_D$ +37°, reported by Dorée, McGhie, and Kurzer, (*J.*, 1949, 570). In a personal communication Dr. McGhie has kindly informed us that his corrected constants are m. p. 190—191°, $[\alpha]_D$ −71° (*c*, 2·00). The absorption spectrum of the alcohol (see Fig. 2) with $\lambda_{max.}$ 213 and 286 mμ ($\varepsilon_{max.}$ 10,500 in both cases)(*c*, 0·0093) is in good agreement (for the 286-mμ band) with the spectrum for the corresponding acetate (compare Dorée, McGhie, and Kurzer, *loc. cit.*; Voser *et al.*, *Helv. Chim. Acta*, 1950, **33**, 1893). On reacetylation with acetic anhydride and pyridine (0·5 hour at 100°) 8 : 11 : 12-triketolanosta-6 : 9-dien-2-yl acetate was obtained, identical with the starting acetate.

2-Hydroxylanosta-6 : 9-diene-8 : 11 : 12-trione (400 mg.) was dissolved by warming it in 100 ml. of light petroleum (b. p. 60—80°), the solution concentrated *in vacuo* to one-half its volume, then cooled to 0°. Phosphorus pentachloride (1 g.) was added to the suspension, and the mixture set aside at 0° for 5 hours. All the alcohol was by then in solution. After working up in the usual way and chromatography over alumina (Birlec), elution of the bright yellow band with benzene and recrystallisation from methanol gave iso*lanostatrienetrione* (50 mg.), m. p. 154°, $\lambda_{max.}$ 197, 265, and 363 mμ ($\varepsilon_{max.}$ 9,000, 17,500 and 5,000 respectively) (*c*, 0·0040) (Found: C, 80·2; H, 9·3. $C_{30}H_{42}O_3$ requires C, 80·0; H, 9·4%).

8 : 11 : 12-Triketolanosta-6 : 9-dien-2-yl acetate was treated with phosphorus pentachloride under comparable conditions. Working up in the usual way gave back the starting material unchanged.

Action of Alkaline Hydrogen Peroxide on 8 : 11 : 12-Triketolanosta-6 : 9-dien-2-yl Acetate.—This acetate (450 mg.) in dioxan (20 ml.) and methanol (20 ml.) containing potassium hydroxide (300 mg.) was treated at 0° with stirring with " Perhydrol " (5 ml.). After being allowed to warm to room temperature the mixture was poured into water and worked up in the usual way. From the acid fraction there was obtained a product, m. p. 238—239°. This was refluxed with 2% methanolic potassium hydroxide to effect complete hydrolysis and then extracted in the usual way. The resulting hydroxydicarboxylic acid was recrystallised from methanol, and had m. p. 244° (decomp.), $[\alpha]_D$ −46° (*c*, 1·79), $\lambda_{max.}$ 249 mμ (ε_{max} 11,000) (Found: C, 71·4; H, 9·25. Calc. for $C_{30}H_{46}O_6$: C, 71·65; H, 9·25%). Cavalla and McGhie (*J.*, 1951, 744) record m. p. 244° (decomp.), $[\alpha]_D$ −58° (*c*, 0·022) in alcohol, $\lambda_{max.}$ 251 mμ ($\varepsilon_{max.}$ 10,700).

Action of Alkaline Hydrogen Peroxide on iso*Lanostatrienetrione.*—A solution of *iso*lanostatrienetrione (120 mg.) in methanolic potassium hydroxide solution (30 ml.; 2%) was treated with " Perhydrol " (3 ml.) at 0° for 3 hours until the colour was almost discharged. After being worked up in the usual way the acidic fraction was rubbed with methanol and water, to give a fine white powder (65 mg.), m. p. 155—170°. This was triturated with ethyl acetate and then recrystallised from the same solvent, to give the *dicarboxylic acid*, m. p. 222° (decomp.), $\lambda_{max.}$ 202, 231, and 312 mμ ($\varepsilon_{max.}$ 10,000, 11,500, and 10,000 respectively) (*c*, 0·0040) (Found: C, 74·1; H, 9·5. $C_{30}H_{44}O_5$ requires C, 74·4; H, 9·2%).

Action of Perbenzoic Acid on Lanost-8-enyl Acetate.—Lanost-8-enyl acetate (500 mg.), m. p. 145—146°, $[\alpha]_D$ +28° (*c*, 1·20), prepared according to the method of Marker, Wittle, and Mixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368), in chloroform (15 ml.) was treated with 0·74N-perbenzoic acid (8 ml.) in the same solvent. After 4 days the reaction mixture was worked up in the usual way. Chromatography over alumina afforded four compounds: (*a*) Elution by 1 : 1 benzene–light petroleum and recrystallisation from chloroform–methanol gave lanosta-8 : 10-dien-2-yl acetate (dihydroagnosteryl acetate) (105 mg.), m. p. 165—166°, $[\alpha]_D$ +87° (*c*, 2·09), $\lambda_{max.}$ 234, 243, and 252 mμ ($\varepsilon_{max.}$ 15,000, 17,500, and 11,500 respectively) (Found: C, 82·1; H, 11·3. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%), undepressed in m. p. on admixture with an authentic specimen of the same m. p. (*b*) Elution with benzene and recrystallisation from alcohol gave 8-*ketolanostan-2-yl acetate* (30 mg.), m. p. 172°, $[\alpha]_D$ +35° (*c*, 1·81), $\lambda_{max.}$ 288 mμ ($\varepsilon_{max.}$ 40) (Found: C, 79·4; H, 11·2. $C_{32}H_{54}O_3$ requires C, 78·9; H, 11·2%); its 2 : 4-*dinitrophenylhydrazone* had m. p. 244—245°, λ_{max} 365 mμ ($\varepsilon_{max.}$ 21,000) (Found: N, 8·6.

$C_{38}H_{58}O_6N_4$ requires N, 8·4%); this ketone gave no colour with tetranitromethane. (*c*) Further elution with benzene afforded 8-ketolanost-9-en-2-yl acetate which, recrystallised from methanol, had m. p. 155° $[\alpha]_D$ +24° (*c*, 0·83), undepressed in m. p. on admixture with an authentic specimen, m. p. 155—156°. (*d*) Elution with 9 : 1 benzene–methanol and recrystallisation from alcohol furnished a further compound (120 mg.), m. p. 169—170°, $[\alpha]_D$ +17° (*c*, 1·75) (Found : C, 76·8; H, 10·4%). This showed no significant absorption in the ultra-violet region and gave no colour with tetranitromethane. There was a marked depression in m. p. on admixture with the ketone of comparable m. p. referred to above.

After attempted hydrogenation of lanost-8-enyl acetate (100 mg.) in 50 ml. of 10% acetic acid in ether, using a platinum catalyst for 16 hours at room temperature, the product was unchanged starting material, m. p. 146—147°, $[\alpha]_D$ +28° (*c*, 1·20), undepressed in m. p. on admixture therewith.

Dehydrogenation of "Lanostene" with Selenium.—"Lanosterol" (25 g.) (obtained from the non-saponifiable matter of wool fat and consisting of a mixture of lanostenol and lanostadienol) was oxidised by chromium trioxide (4 g.) in suspension in acetic acid (250 ml.) at room temperature for 15 hours, to give "lanostenone" (14 g.). This ketone (30 g.) in acetic acid (1 l.) was reduced with amalgamated zinc (200 g.) and concentrated hydrochloric acid (200 ml.) under reflux for 2 hours. The product was filtered through alumina (Birlec) in light petroleum, to give "lanostene" (22 g.) which was shown to be free from alcohols. "Lanostene" (25 g.) was heated with selenium (20 g.) at 350° for 48 hours and the product extracted with light petroleum and filtered through alumina (Birlec). The resultant oil was chromatographed in light petroleum over alumina, and the solid fractions obtained were combined (500 mg.; m. p. 110—120°), rechromatographed, purified through the picrate, sublimed *in vacuo*, and recrystallised (three times) from alcohol, to give 1 : 2 : 8-trimethylphenanthrene, m. p. 145—146° (Found : C, 92·3; H, 7·4. Calc. for $C_{17}H_{16}$: C, 92·7; H, 7·3%). There was no depression in m. p. on admixture with an authentic specimen of 1 : 2 : 8-trimethylphenanthrene of the same m. p. The absorption spectrum, $\lambda_{max.}$ 211, 223, 262, 282, 293, and 306 mμ, $\varepsilon_{max.}$ 36,500, 19,500, 58,000, 12,500, 12,500, and 15,500 respectively, was also characteristic of 1 : 2 : 8-trimethylphenanthrene. The hydrocarbon was converted into its picrate, m. p. 167—168° (Found : C, 62·6; H, 4·3; N, 9·15. Calc. for $C_{23}H_{19}O_7N_3$: C, 61·4; H, 4·2; N, 9·35%), which gave no depression in m. p. on admixture with an authentic specimen of the same m. p.

"Lanosterol" (5 g.) was heated with selenium (5 g.) at 350—360° for 40 hours. Working up as above gave 20 mg. of phenanthrenoid hydrocarbon, m. p. 115—120°. This was converted into the picrate, m. p. 165—166°, which gave no depression in m. p. with authentic 1 : 2 : 8-trimethylphenanthrene picrate (see above). The yield of the 1 : 2 : 8-trimethylphenanthrene was considerably greater in the dehydrogenation of the "lanostene."

Absorption Spectrum of Lanostenyl Acetate.—In the region 195—215 mμ lanostenyl acetate showed the following apparent absorption spectrum : $\lambda_{max.}$ 200 mμ, $\varepsilon_{max.}$ 4600, ε_{215} 2000 (*c*, 0·0047). According to data kindly provided by Dr. H. B. Henbest (Manchester) this is indicative of a tetrasubstituted double bond as in cholest-8-enol rather than as in cholest-8(14)-enol.

Absorption Spectrum of Lanost-8-enyl Acetate.—In the region 195—215 mμ lanost-8-enyl acetate showed the following apparent absorption spectrum : $\lambda_{max.}$ 200 mμ, $\varepsilon_{max.}$ 3800, ε_{215} 1100. This is quite an acceptable spectrum for a triply substituted double bond (personal communication from Dr. H. B. Henbest).

Preparation of Lanostane (with T. Bruun).—The fundamental hydrocarbon of the lanosterol series, lanostane, was prepared in the following way. Lanost-9-en-2 : 8 : 11-trione (0·7 g.) (Wieland and Joost, *Annalen*, 1941, **546**, 103; McGhie, Thesis, London, 1947) in dioxan (120 ml.) was added to amalgamated zinc wool (from 28·5 g. of zinc and 28 g. of mercuric chloride in 200 ml. of 50% aqueous ethanol), and the mixture refluxed for 3·5 hours. During the first 2 hours 100 ml. of concentrated hydrochloric acid were added portionwise. The reaction product was extracted with light petroleum and chromatographed over alumina. The fraction easily eluted by light petroleum was treated with chromium trioxide (350 mg.) in AnalaR acetic acid (25 ml.) on the water-bath for ½ hour. The reaction product was again filtered through alumina and then crystallised from chloroform–methanol, to give lanostane, m. p. 95—96° (Found : C, 86·9; H, 13·05. Calc. for $C_{30}H_{54}$: C, 86·9; H, 13·1%). For this hydrocarbon Voser *et al.* (*loc. cit.*) reported m. p. 98—99° (corr.).

Wolff–Kishner Reduction of 8-Ketolanost-9-en-2-yl Acetate.—8-Ketolanost-9-en-2-yl acetate (600 mg.) was reduced in three batches, each being heated with 200 mg. of sodium dissolved in 2 ml. of ethanol and 1 ml. of anhydrous hydrazine at 190° for 12 hours. The reaction product, worked up in the usual way, was acetylated and chromatographed over alumina. The more difficultly eluted fractions were enriched in γ-lanosteryl acetate. The more easily eluted fractions were combined and oxidised by chromic acid as for the preparation of *iso*lanostenyl acetate according to the directions of Marker, Wittle, and Mixon (*loc. cit.*). The reaction product, worked up in the usual way and chromatographed over alumina, gave *iso*lanostenyl acetate (lanost-8-en-2-yl acetate), m. p. 145°, $[\alpha]_D$ +32° (*c*, 1·67), undepressed in m. p. on admixture with an authentic specimen of the same m. p. and comparable rotation.

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