115. *Triterpenoids. Part IX.* The Constitution of Lanostadienol (Lanosterol).*

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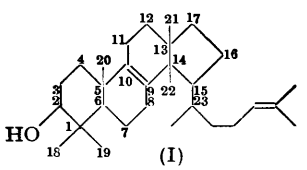
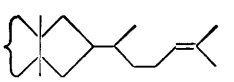
Reduction of 8 : 11-diketolanostan-2-yl acetate with sodium and propanol affords lanostane-2β : 8β : 11α-triol, which is easily acylated to the tribenzoate and to the triacetate. Vigorous chromic acid oxidation of the latter affords (*a*) 2β : 8β : 11α-triacetoxytrisnorlanostanoic acid, the constitution of which was confirmed by partial synthesis (of the methyl ester) from 2β-hydroxy-8 : 11-diketotrisnorlanostanoic acid, (*b*) 2β : 8β : 11α-triacetoxy-23-hydroxytrisnorlanostanoic acid [27→23]-lactone, and (*c*) 2β : 8β : 11α-triacetoxylanan-17-one. The characterisation of the grouping >CMe·CO·[CH_2]$_2$·CMe< in a five-membered ring in this ketone by both chemical and physical methods excludes the possible attachment of the lanosterol side chain at $C_{(16)}$ and confirms the size of ring D.

The present paper describes work designed to confirm the correctness of the isoprenoid formulæ (I) and (II) for lanostadienol (Voser, Montavon, Günthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893; Cavalla, McGhie, and Pradhan, *J.*, 1951, 3142; Barton, Fawcett, and Thomas, *ibid.*, p. 3147; Ruzicka *et al.*, *Helv. Chim. Acta*, 1951, **34**, 1585; 1952, **35**, 66, 503; Barton, McGhie, *et al.*, *Chem. and Ind.*, 1951, 1067), with the added objective of distinguishing between them. A preliminary account of this work (Barnes, Barton, Cole, Fawcett, and Thomas, *Chem. and Ind.*, 1952, 426) has already been published.



(I)



(II)

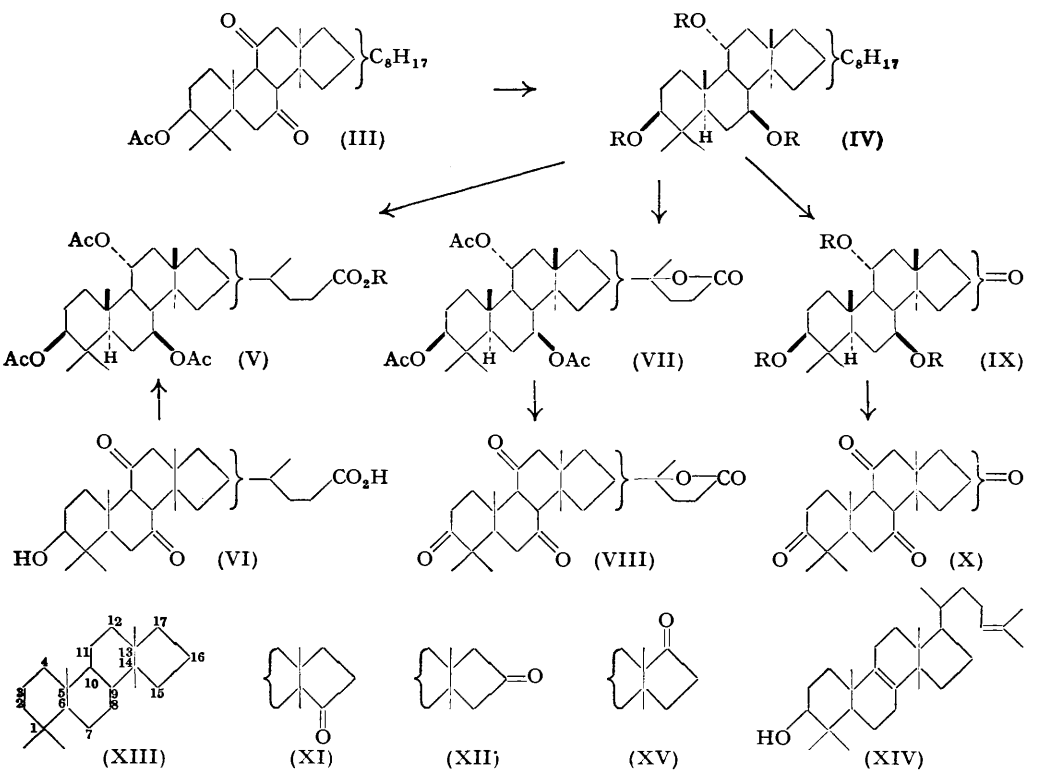
It seemed to us *a priori* that the isolation of 6-methylheptan-2-one in the oxidation of lanostenyl acetate (Barton, McGhie, *et al.*, *loc. cit.*) implied the presence in the total oxidation products of a tetracyclic fragment characteristic of the rest of the molecule. However a search for this fragment proved abortive and it accordingly appeared that, in order to secure a higher concentration of the desired compound, the tetracyclic nucleus must be stabilised against oxidative attack in rings B and C. This was achieved by sodium and propanol reduction of 8 : 11-diketolanostanyl acetate (III) (Dorée, McGhie, and Kurzer, *J.*, 1948, 988) to lanostane-2β : 8β : 11α-triol † (IV; R = H), characterised as the

* Part VIII, *J.*, 1952, 3751.

† The stereochemistry of this and other lanostadienol derivatives is discussed in the following paper.

triacetate and the tribenzoate. The tri-equatorial character, and hence ease of acylation, of this triol was predicted from its mode of preparation (cf. Barton, *Experientia*, 1950, **6**, 316; Barton and Rosenfelder, *J.*, 1951, 1048). 2β : 8β : 11α-Triacetoxylanostane (IV; R = Ac) proved suitable for vigorous chromic acid oxidation to the desired tetracyclic fragment.

After oxidation of the triacetate in acetic acid–aqueous sulphuric acid at 26—30° for 14 hours the product was separated into acid and neutral fractions. The acid fraction on methylation and chromatography afforded methyl 2β : 8β : 11α-triacetoxytrisnorlanostanoate (V; R = Me), the constitution of which was confirmed by its preparation by reduction of 2β-hydroxy-8 : 11-diketotrisnorlanostanoic acid (VI) (McGhie, Pradhan, Cavalla, and Knight, *Chem. and Ind.*, 1951, 1165; Voser, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1952, **35**, 497) with sodium and propanol followed by acetylation and methylation. The neutral fraction, on chromatography, furnished two crystalline substances. One of these was a high-melting compound, $C_{33}H_{50}O_8$, which was shown by its infra-red spectrum (band at 1780 cm.$^{-1}$ in CS_2) to be a γ-lactone. Like the corresponding lactone obtained in the cholesteryl acetate dibromide oxidation (see Ryer and Gebert, *J. Amer. Chem. Soc.*, 1952, **74**, 4336; cf. Billeter and Miescher, *Helv. Chim. Acta*, 1949, **32**, 564) the lactone ring of the lanostane derivative must be closed on to the tertiary carbon one removed from the ring carbon atom to which the side chain is attached, and therefore we formulate it as (VII). There was some difficulty in obtaining consistent analyses for the triacetate lactone itself; in consequence it was hydrolysed to the corresponding tetrahydroxy-acid (which at once relactonised) and then converted by chromic acid oxidation into the corresponding triketo-lactone (VIII). The other neutral compound had the composition



$C_{28}H_{42}O_7$ and was shown to be a ketone by the ultra-violet spectrum ($\lambda_{max.}$ 293 mμ; ε, 33) and by the infra-red spectrum (see further below). This ketone is formulated as (IX; R = Ac). Because of the acetate residues the band in the infra-red spectrum

near 1740 cm.$^{-1}$ due to the ketone grouping in a five-membered ring was masked. Alkaline hydrolysis gave the corresponding trihydroxy-ketone (IX; R = H), which gave a tribenzoate (IX; R = Bz). The latter showed the benzoate carbonyl band in the infra-red at 1718 cm.$^{-1}$ and also the expected ketone band at 1747 cm.$^{-1}$. The triacetoxy-ketone (IX; R = Ac) was further characterised by conversion into its oxime acetate, and by hydrolysis and oxidation to the tetraketone (X).

On the basis of the two isoprenoid formulæ (I) and (II) (IX; R = Ac) would be represented by the partial formulæ (XI) and (XII) respectively. The former contains the grouping >CMe·CO·[CH_2]$_2$·CMe< (*A*) and the latter the grouping >CMe·CH_2·CO·CH_2·CMe< (*B*). Decisive evidence in favour of (*A*) was obtained as follows.

(i) The ketone group in (*B*) should not be subject to significant steric hindrance, since it is flanked by two CH_2 groups. In contrast the triacetoxy-ketone (IX; R = Ac) afforded a 2 : 4-dinitrophenylhydrazone with difficulty and only under reflux, the rate of reaction corresponding approximately to that of an 8-keto-group in the lanostane series (Barton, Fawcett, and Thomas, *loc. cit.*). As would be expected, 6- and 7-ketocholestanyl acetate, cholestan-3- and -4-one, 17-ketoandrostan-3β-yl acetate, and A-norcholestanone gave 2 : 4-dinitrophenylhydrazones rapidly under similar conditions but at room temperature.

(ii) In quantitative bromination experiments (see Experimental) the results shown in Table 1 were obtained. Clearly the ketone (IX; R = Ac) contains only two α-hydrogen atoms replaceable by bromine. These results were confirmed by the isolation of 2β : 8β : 11α-triacetoxydibromolananone * after complete bromination of (IX; R = Ac). As would be expected this dibromo-ketone did not consume any more bromine even under vigorous brominating conditions. The 2β : 8β : 11α-triacetoxylananone must therefore contain the grouping (*A*) and not (*B*).

TABLE 1.

Compound	Uptake of Br, mols. 1 day	2 days	3 days	4 days
17-Ketoandrostan-3β-yl acetate	2·0	2·1	2·2	2·2
A-Norcholestanone	2·75	3·0	3·2	3·25
2β : 8β : 11α-Triacetoxylananone (IX; R = Ac)	1·6	1·9	2·1	2·1

(iii) It has recently been shown by R. N. Jones, Cole, *et al.* (*J. Amer. Chem. Soc.*, 1952, **74**, 5648, 5662) that ketones with CH_2 adjacent to the carbonyl group in a five-membered ring have a characteristic absorption maximum near 1410 cm.$^{-1}$ in the infra-red and, since this band is due to a bending vibration of the $-CH_2-$, its intensity (Jones, Ramsay, Keir, and Dobriner, *ibid.*, p. 80) should be proportional to the number of flanking methylene groups. Table 2 shows the application of this principle to various of the compounds described in the present paper. It will be seen that, as would be expected, only A-norcholestanone shows an intensity of absorption corresponding to two α-CH_2 groups. The derivatives of (IX; R = H) must therefore contain the grouping (*A*) and not (*B*).

TABLE 2.

Compound	Infra-red band frequency, cm.$^{-1}$	Apparent integrated absorption intensity,* *I*	No. of α-CH_2 groups, *n*	*I*/*n*
17-Ketoandrostan-3β-yl benzoate	1408	280	1	280
17-Ketoandrostan-3β-yl acetate	1407	320	1	320
A-Norcholestanone	1410	620	2	310
2β : 8β : 11α-Triacetoxylananone (IX; R = Ac)	1412	280	1	280
2β : 8β : 11α-Tribenzoyloxylananone (IX; R = Bz)	1411	300	1	300

* After graphical separation of overlapping bands.

With these results and with the assumed applicability of the isoprene rule, formula (XI) (2β : 8β : 11α-triacetoxylanan-15-one) can be deduced for the triacetoxy-ketone and the corresponding formula (I) for lanosterol. Ruzicka, Jeger, and their collaborators have

* We propose that the annexed tetracyclic nucleus (XIII), which is a trimethylandrostane, should be given the trivial name lanane and numbered as indicated.

however always been careful to point out that, if the isoprene rule is *not* applicable to the skeleton of lanosterol, then the side chain may also be attached at $C_{(17)}$. Curtis, Fridrichsons, and Mathieson (*Nature*, 1952, **170**, 321) have recently reported on the *X*-ray diffraction analysis of lanostenyl iodoacetate from which they conclude that the side chain is indeed attached at $C_{(17)}$, so that lanostadienol has the formula (XIV). There is, of course, nothing in the results described here which excludes formula (XIV), for the derived ketone (XV) would also contain the grouping (*A*). On the assumption that chemical verification of the attachment of the side chain at $C_{(17)}$ will eventually be forthcoming,* we accept the *X*-ray evidence. In our opinion, the latter represents an important contribution to the chemistry, and especially to the stereochemistry (see the following paper), of lanostadienol.

Experimental

M. p.s are uncorrected. 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 to the fraction of b. p. 40—60°.

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 measured in ethanol solution with a Unicam S.P. 500 Spectrophotometer. Infra-red spectra were measured in carbon tetrachloride with a Perkin–Elmer Model 12 C Spectrometer (sodium chloride prism). We gratefully acknowledge permission to use the instrument at the Chester Beatty Institute for this purpose.

Lanostane-2β : 8β : 11α-triol (IV; R = H) *and its Derivatives.*—Sodium (20 g.) was added portionwise to a refluxing solution of 8 : 11-diketolanostanyl acetate (20 g.) in propanol (200 ml.) during 2 hours. Decomposition of the excess of sodium by aqueous ethanol, followed by isolation of the product in the usual manner, gave *lanostane-2β : 8β : 11α-triol.* Recrystallised from aqueous propanol this had m. p. 207—208°, $[\alpha]_D$ +3° (*c*, 1·10) (Found: C, 76·95; H, 11·7. $C_{30}H_{54}O_3,0{\cdot}5H_2O$ requires C, 76·4; H, 11·75%).

With pyridine–acetic anhydride on the steam-bath (30 min.) the triol yielded the corresponding *triacetate*, m. p. 156° (from methanol), $[\alpha]_D$ +29° (*c*, 2·18) (Found: C, 73·9; H, 10·35. $C_{36}H_{60}O_6$ requires C, 73·4; H, 10·3%). Benzoyl chloride similarly gave the *tribenzoate*, m. p. 162—164° (from chloroform–methanol), $[\alpha]_D$ +46° (*c*, 1·77), $\lambda_{max.}$ 231, 274, and 281 mμ (ε 42,000, 2800, and 2300 respectively) (Found: C, 74·4; H, 8·3; Cl, 6·8. $C_{51}H_{66}O_6,0{\cdot}5CHCl_3$ requires C, 74·1; H, 8·05; Cl, 6·4%).

Chromic Acid Oxidation of 2β : 8β : 11α-Triacetoxylanostane (IV; R = Ac).—Chromium trioxide (40 g.) in water (40 ml.), acetic acid (200 ml.), and concentrated sulphuric acid (20 ml.) was added during 45 minutes to a stirred solution of 2β : 8β : 11α-triacetoxylanostane (20 g.) in acetic acid (400 ml.) and acetic anhydride (20 ml.), kept throughout at 26—30°. The mixture was then stirred overnight at room temperature, poured into water (4 l.), and extracted with ether. The ethereal extract was separated into acid (9·8 g.) and neutral (6·0 g.) fractions.

The acid moiety was methylated with diazomethane and chromatographed over alumina (Peter Spence, grade H), to give as the only crystalline fractions (eluted with 2 : 1 benzene–ether) *methyl 2β : 8β : 11α-triacetoxytrisnorlanostanoate* (V; R = Me) (1·9 g.), m. p. 195—196° (from ethyl acetate–light petroleum), $[\alpha]_D$ +25° (*c*, 1·19) (Found: C, 68·75; H, 9·55. $C_{34}H_{54}O_8$ requires C, 69·1; H, 9·2%). There was no depression in m. p. on admixture with an authentic specimen of the same m. p. prepared as described below.

The neutral fraction was chromatographed over alumina (Peter Spence, grade H) to give two crystalline products. (i) Elution with 4 : 1 benzene–ether afforded *2β : 8β : 11α-triacetoxylanan-17-one* (IX; R = Ac) (750 mg.), m. p. 190—191° (from aqueous methanol), $[\alpha]_D$ +41° (*c*, 1·52), $[M]_D$ +201°, $\lambda_{max.}$ 293 mμ (ε, 33) (Found: C, 68·45; H, 8·6. $C_{28}H_{42}O_7$ requires C, 68·55; H, 8·6%). (ii) Elution with ether gave *2β : 8β : 11α-triacetoxy-23-hydroxytrisnorlanostanoic acid* [27→23]-*lactone* (VII) (500 mg.), m. p. 272—274° (from methanol), $[\alpha]_D$ +37° (*c*, 1·47); it was sublimed for analysis (Found: C, 68·4; H, 8·6. $C_{33}H_{50}O_8$ requires C, 68·95; H, 8·75%).

Methyl 2β : 8β : 11α-Triacetoxytrisnorlanostanoate (V; R = Me) *from 2β-Hydroxy-8 : 11-diketotrisnorlanostanoic Acid* (VI).—The hydroxy-diketo-acid (1·0 g.) in refluxing propanol (50 ml.) was reduced by the addition of sodium until the solution was saturated. After being

* *Added, December 12th*, 1952.—Voser, Mijovic, Heusser, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1952, **35**, 2414) have now provided convincing *chemical* evidence for a $C_{(17)}$ side chain.

worked up in the usual way the trihydroxy-acid was acetylated with pyridine–acetic anhydride on the steam-bath for 1 hour and then methylated with diazomethane. Chromatography of the product over alumina and elution with 2 : 1 benzene–ether afforded methyl 2β : 8β : 11α-triacetoxytrisnorlanostanoate, m. p. 195—196°, $[\alpha]_D$ +23° (*c*, 1·76).

Derivatives of 2β : 8β : 11α-*Trihydroxylanan*-17-*one* (IX; R = H).—Alkaline hydrolysis of the triacetoxy-ketone afforded 2β : 8β : 11α-*trihydroxylanan*-17-*one*, m. p. 235—256° (from aqueous methanol) (Found: C, 71·45; H, 9·75. $C_{22}H_{36}O_4,0{\cdot}5CH_3{\cdot}OH$ requires C, 71·05; H, 10·05%). Repeated fractional crystallisation failed to alter the wide m. p. range.

2β : 8β : 11α-Trihydroxylanan-17-one with benzoyl chloride in pyridine on the steam-bath (30 min.) gave the *tribenzoate*, m. p. 172—175° (from chloroform–methanol), $[\alpha]_D$ +56° (*c*, 1·25), $\lambda_{max.}$ 231, 274, and 281 mμ (ε, 40,000, 2750, and 2200 respectively) (Found: C, 75·7; H, 7·2. $C_{43}H_{48}O_7$ requires C, 76·3; H, 7·15%).

2β : 8β : 11α-Triacetoxylanan-17-one (100 mg.) was treated with 2 : 4-dinitrophenylhydrazine (50 mg.) in refluxing ethanolic hydrochloric acid for 40 minutes. After the usual working up the product was re-acetylated with pyridine–acetic anhydride overnight at room temperature. Chromatography over alumina washed with ethyl acetate gave some unchanged triacetoxy-ketone and then the 2 : 4-*dinitrophenylhydrazone* (20 mg.). Recrystallised from chloroform–methanol this had m. p. 277—279°, $\lambda_{max.}$ 362 mμ (ε, 25,000) (Found: C, 60·55; H, 7·05; N, 8·4. $C_{34}H_{46}O_{10}N_4$ requires C, 60·85; H, 6·90; N, 8·35%).

2β : 8β : 11α-Triacetoxylanan-17-one (200 mg.) in pyridine was heated on the steam-bath for 1 hour with hydroxylamine hydrochloride. The derived oxime melted over a range (155—170°) which was not improved by crystallisation or careful chromatography. In the latter fractionation, however, the oxime behaved as a pure compound. Acetylation with pyridine–acetic anhydride overnight at room temperature gave the sharp-melting 2β : 8β : 11α-*triacetoxylanan*-17-*one oxime acetate*, m. p. 212—214° (from ethyl acetate–light petroleum), $[\alpha]_D$ +4° (*c*, 2·00) (Found: C, 65·6; H, 8·4; N, 2·65. $C_{50}H_{45}O_8N$ requires C, 65·8; H, 8·5; N, 2·55%).

2β : 8β : 11α-*Triacetoxy*-16 : 16-*dibromolanan*-17-*one and Bromination Experiments*.—2β : 8β : 11α-Triacetoxylanan-17-one (49 mg.), A-norcholestanone (Windaus and Uibrig, *Ber.*, 1914, **47**, 2384; Windaus and Dalmer, *ibid.*, 1919, **52**, 162) (39 mg.), and 3β-acetoxyandrostan-17-one (34 mg.) were each dissolved in "AnalaR" acetic acid (10 ml.), containing approx. 19 g. of bromine per 100 ml., with addition of hydrogen bromide [0·1 ml.; 50% (w/v) in acetic acid] and left at 40° in a thermostatically controlled oven. At suitable time intervals 1-ml. portions were removed and the bromine remaining (see Table 1) was determined in the usual way. A suitable control was also run.

The fully brominated lanane derivative, from an analogous experiment with 125 mg. of the ketone, was isolated in the usual way. Recrystallisation from aqueous methanol afforded 2β : 8β : 11α-*triacetoxy*-16 : 16-*dibromolanan*-17-*one*, m. p. 239—240°, $[\alpha]_D$ +43° (*c*, 0·74) (Found: Br, 24·8. $C_{28}H_{40}O_7Br_2$ requires Br, 24·7%). The dibromide was resistant to further bromination.

Lanane-2 : 8 : 11 : 17-*tetraone* (X).—2β : 8β : 11α-Trihydroxylanan-17-one (350 mg.) in "AnalaR" acetic acid (5 ml.) was treated with chromium trioxide (23 mg.) in a little aqueous "AnalaR" acetic acid and left overnight at room temperature. Isolation of the product in the usual way afforded *lanane*-2 : 8 : 11 : 17-*tetraone*, m. p. 293—295° (from chloroform–methanol), $[\alpha]_D$ +74° (*c*, 0·92), $[M]_D$ +265°, $\lambda_{max.}$ 292 mμ (ε, 133) (Found: C, 73·2; H, 8·15. $C_{22}H_{30}O_4$ requires C, 73·7; H, 8·4%).

2 : 8 : 11-*Triketo*-23-*hydroxytrisnorlanostanoic Acid* [27→23]-*Lactone* (VIII).—The corresponding triacetoxy-lactone (see above) (500 mg.) was hydrolysed by refluxing alcoholic potassium hydroxide (10%; 10 ml.) for 30 minutes, the solution evaporated to dryness under reduced pressure, and the residue dissolved in water. The clear solution was extracted repeatedly with ether, but there was no neutral fraction. The alkaline solution was acidified and extracted with ethyl acetate, and part of the extract acetylated with pyridine–acetic anhydride. Recrystallisation gave back the initial triacetoxy-lactone, identified by m. p. and mixed m. p. The remainder of the extract (2β : 8β : 11α-trihydroxy-lactone; 60 mg.) in acetic acid (2 ml.) was treated with chromium trioxide (50 mg.) and left overnight at room temperature. Isolation of the product in the usual way and crystallisation from chloroform–methanol furnished 2 : 8 : 11-*triketo*-23-*hydroxytrisnorlanostanoic acid* [27→23]-*lactone*, m. p. 280—283°, $[\alpha]_D$ +70° (*c*, 0·53) (Found: C, 73·2; H, 8·7. $C_{27}H_{38}O_5$ requires C, 73·25; H, 8·65%).

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