437. *Triterpenoids. Part VII.* Some Further Observations on the Constitution of Lanostadienol (Lanosterol).*

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

Chromic acid oxidation of 8 : 11-diketolanost-9-en-2-yl acetate affords in small yield acetone and 6-methylheptan-2-one. The latter corresponds to the side chain of lanostadienol. This result, coupled with arguments previously adduced and assumption of the applicability of the " isoprene rule," restricts the number of possible formulæ for lanostadienol to two.

The action of heat on the hydroxy-dicarboxylic acid obtained by fission (and hydrolysis) of 8 : 11 : 12-triketolanosta-6 : 9-dien-2-yl acetate has been studied.

The Schmidt reaction on 8 : 11-diketolanostan-2-yl acetate furnishes 8 : 11-diketo-8*a*-aza-B-homolanostan-2-yl acetate, also obtained by Beckmann rearrangement of the appropriate monoxime. Selenium dioxide oxidation of this 8*a*-aza-compound gives, successively, 8 : 11 : 12-triketo-8*a*-aza-B-homolanostan-2-yl acetate, further characterised as the corresponding diosphenol acetate, and the acetoxy-7 : 8 : 11 : 12-tetraketone. Fission of these compounds by alkaline hydrogen peroxide has been studied.

The Schmidt reaction on 8 : 11-diketolanost-9-en-2-yl acetate affords a mixture of 8 : 11-diketo-7*a*- and 8*a*-aza-B-homolanost-9-en-2-yl acetates and a tetrazole. The first two products are also obtained by Beckmann rearrangement of the appropriate monoxime. Selenium dioxide oxidation of the 7*a*-aza-amide gives 8 : 11 : 12-triketo-7*a*-aza-B-homolanost-9-en-2-yl acetate, whilst reduction with zinc dust in acetic acid furnishes 8 : 11-diketo-7*a*-aza-B-homolanostan-2-yl acetate. The action of alkaline hydrogen peroxide on the 8 : 11 : 12-triketone has been investigated.

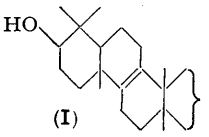
In Part IV of this series (Barton, Fawcett, and Thomas, *J.*, 1951, 3147) cogent arguments were advanced in favour of the partial formula (I) for lanostadienol. Recently Voser, Mijovic, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1951, **34**, 1585) described a stepwise degradation of the lanostadienol side chain from which they concluded that it should be represented by the expression (II). We now report evidence which led us independently to the same conclusion. Our work was briefly summarised in a preliminary communication in association with Dr. J. F. McGhie and his colleagues (Barnes, Barton, Fawcett, Knight, McGhie, Pradhan, and Thomas, *Chem. and Ind.*, 1951, 1067). Recent work by McGhie, Pradhan, Cavalla, and Knight (*ibid.*, p. 1165) and by Curtis (*J.*, 1952, 1187; personal communication) has, by stepwise degradation similar to that already reported by Voser *et al.* (*loc. cit.*), abundantly confirmed the nature of the side chain.

Chromic acid oxidation of 8 : 11-diketolanost-9-en-2-yl acetate (diketolanostenyl acetate) and steam-distillation of the reaction products gave, in small yield, acetone and 6-methylheptan-2-one (III), each characterised as its 2 : 4-dinitrophenylhydrazone. An authentic specimen of the 6-methylheptan-2-one derivative was very kindly provided by Professor D. H. Hey and Dr. D. S. Morris (see *J.*, 1948, 48). The same oxidation product was obtained by Dr. J. F. McGhie and his collaborators (Barnes *et al.*, *loc. cit.*) and identified as the semicarbazone. The expansion of partial formula (I) to include the side chain (II) can only be reasonably accommodated, on the assumed applicability of the " isoprene rule," by the formulæ (IV) and (V). As yet a distinction between these formulæ cannot be made.

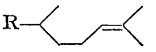
For final clarification of the formula of lanosterol it seemed to us either that the degradation of the side chain already accomplished would have to be extended into ring D, or that the molecule would have to be severed in ring B and C into two fragments which could, in turn, be degraded further. In so far as the latter approach would probably be applicable to a number of tetracyclic triterpenoids it appeared to be worthy of prior investigation.

* Part VI, *J.*, 1952, 1683.

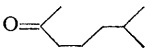
The cleavage of the ring system of the molecule into two fragments requires the rupture of at least two carbon–carbon bonds. We first turned our attention to the dicarboxylic acid (VI) initially prepared by Cavalla and McGhie (*J.*, 1951, 744; cf. Barton, Fawcett, and Thomas, *loc. cit.*), in the hope of effecting pyrolytic scission. When merely melted this



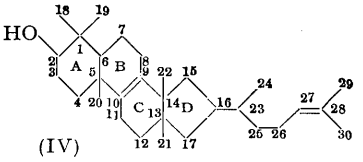
(I)



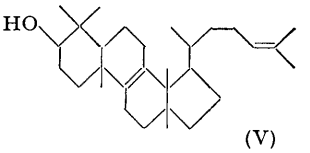
(II)



(III)

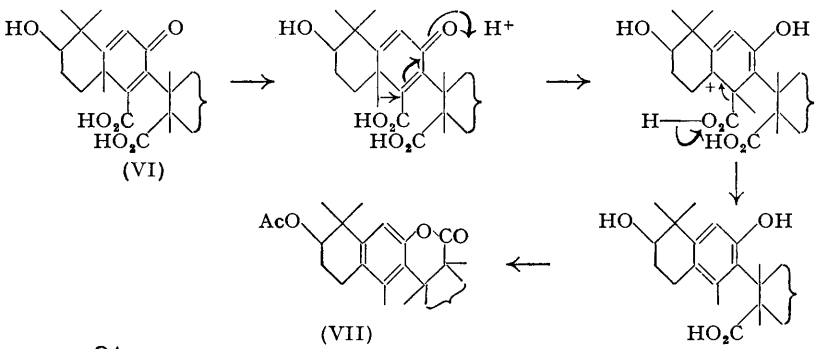


(IV)

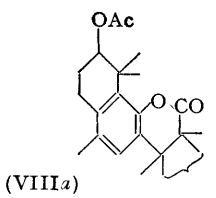


(V)

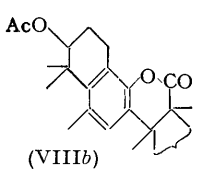
acid was decarboxylated and lost water to give a neutral product characterised as the crystalline acetate, $C_{31}H_{46}O_4$. The absorption spectrum of the acetate ($\lambda_{max.}$ 207 and 275 mμ; ε 30,000 and 900 respectively) showed that aromatisation of ring B had occurred and yet that an acetophenone type system, which might result from normal ketonisation of the two carboxyl groups formed from ring C, had not resulted. The absorption spectrum is in consonance with the presence of a phenolic ester system. Having regard to this, to the total absence of acidic or phenolic properties, and to mechanistic consideration indicated in the formulæ below, we regard (VII) as the most plausible formula for the pyrolysis product. There would be sound analogy for formulæ (VIII*a* or *b*) in the elegant work of Woodward and Singh (*J. Amer. Chem. Soc.*, 1950, **72**, 494) were it not that these formulations do not appear to explain the ready decarboxylation so well on electronic considerations. In agreement with formula (VII) the acid (VI) could be acetylated under mild conditions and the derived non-crystalline acetate pyrolysed at moderate temperature to give the $C_{31}H_{46}O_4$ acetate.



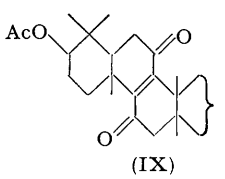
(VI) (VII)



(VIII*a*)



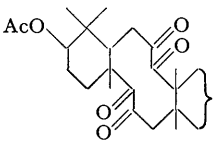
(VIII*b*)

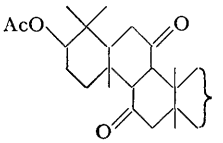


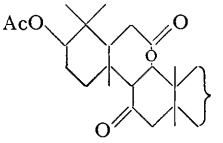
(IX)

The formation of this tetracyclic phenolic lactone on gentle pyrolysis of (VI) made it seem unlikely that more vigorous thermal treatment would prove of interest. Attention was directed therefore to more indirect methods of cleaving the molecule. The obvious approach by ozonolysis of 8 : 11-diketolanost-9-en-2-yl acetate (IX) to a compound such as (X) followed by scission of the α-diketone groupings proved abortive. Similarly we were unable to effect smooth attack upon the 9 : 10-double bond of (IX) by any of

the usual electrophilic or nucleophilic reagents. 8 : 11-Diketolanostan-2-yl acetate (XI) was likewise inert to peracetic acid and the lactone (XII) could not be prepared.


(X)


(XI)

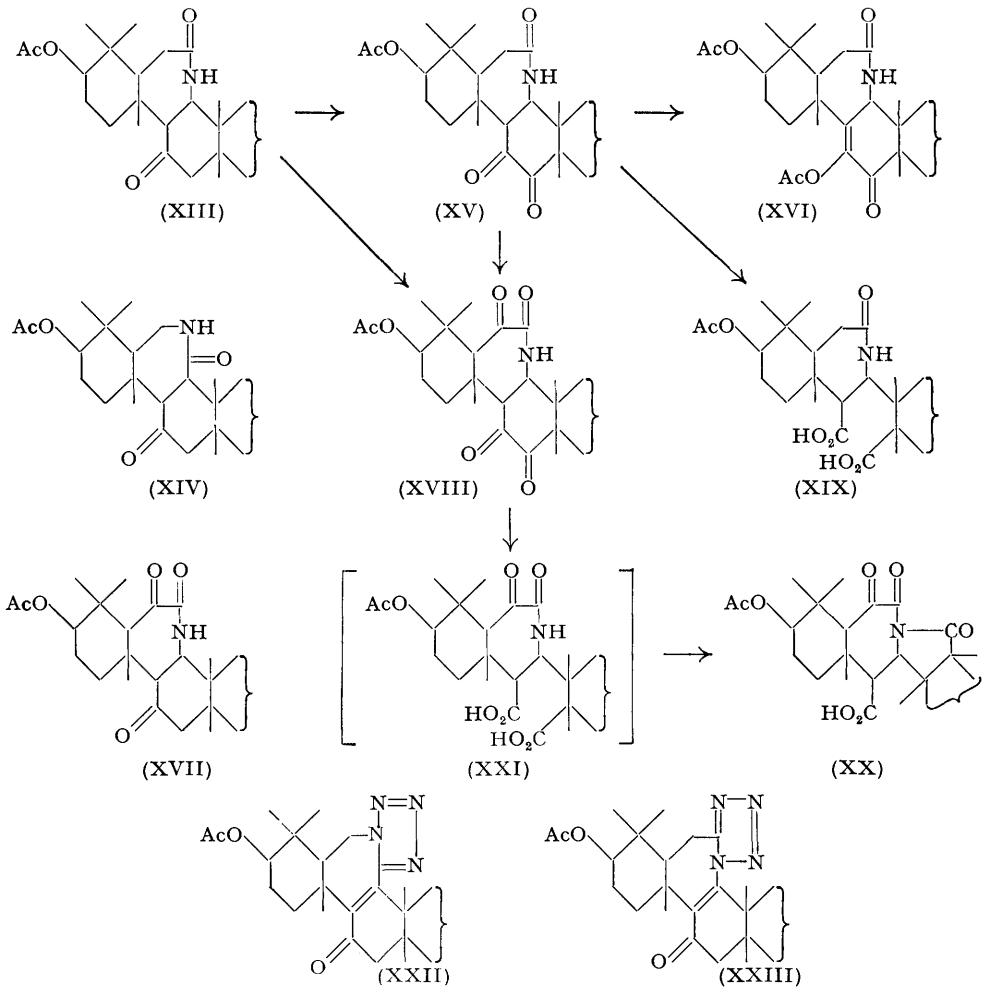

(XII)

A *potential* opening of ring B was finally achieved in the products of the Schmidt reaction on (IX) and (XI). Treatment of (XI) in chloroform solution with concentrated sulphuric acid and powdered sodium azide gave a single amide, $C_{32}H_{53}O_4N$, m. p. 249—252°, $[\alpha]_D$ +29°, characterised by hydrolysis to the 2-alcohol and by oxidation of the latter to the corresponding 2-ketone. The retention of one ketonic function in the amide was indicated by the absorption spectrum ($\lambda_{max.}$ 302 mμ, ε 50). The amide is formulated as 8 : 11-diketo-8*a*-aza-B-homolanostan-2-yl acetate (XIII) on the basis of the following evidence. Beckmann rearrangement of the monoxime of (XI) (Voser, Montavon, Günthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893) gave the same compound. Since the oxime is known to be formed from the $C_{(8)}$-carbonyl group (see Barton, Fawcett, and Thomas, *loc. cit.*) hydrazoic acid must attack at the same position, not at $C_{(11)}$. Formula (XIII) for the amide, rather than the alternative (XIV), is based on excellent analogy (Schlecter and Kirk, *J. Amer. Chem. Soc.*, 1951, **73**, 3087) and is supported by the behaviour of the amide on selenium dioxide oxidation. Oxidation with a limited time of reflux afforded an α-diketone, 8 : 11 : 12-triketo-8*a*-aza-B-homolanostan-2-yl acetate (XV). On acetylation in pyridine solution this gave a diosphenol acetate (XVI), further characterised by alkaline hydrolysis to 2-hydroxy-8*a*-aza-B-homolanostane-8 : 11 : 12-trione. The absorption spectrum of the diosphenol acetate ($\lambda_{max.}$ 242 mμ; ε 9000) excludes the alternative formulation as an α-keto-amide (XVII). A minor product of this oxidaton was a sparingly soluble trione-amide, $C_{32}H_{49}O_6N$, which we formulate as 7 : 8 : 11 : 12-tetraketo-8*a*-aza-B-homolanostan-2-yl acetate (XVIII). This was further characterised by hydrolysis to the corresponding alcohol. Oxidation of 8 : 11-diketo-8*a*-aza-B-homolanostan-2-yl acetate with selenium dioxide, using an extended period of reflux, gave exclusively (XVIII). Similar oxidation of (XV) with the same reagent likewise furnished (XVIII). The formation of an α-keto-amide grouping in the last two oxidations (for evidence see below) excludes (XIV) from further consideration.

Oxidation of the alcohol corresponding to (XV) under mild conditions with alkaline hydrogen peroxide afforded the corresponding *dicarboxylic acid* (XIX). At this stage of the investigation (XVIII) seemed a particularly promising compound for oxidation, having α-diketone groupings in both rings B and C. However, the action of alkaline hydrogen peroxide on (XVIII) under mild conditions gave an acetate, $C_{32}H_{49}O_7N$, m. p. 217—218° (decomp.), which was shown by electrometric titration to be a *monocarboxylic acid*. Similar treatment of the alcohol corresponding to (XVIII) furnished the analogous acid, which gave the same acetate, m. p. 217—218° (decomp.), on acetylation. We formulate this acetate as (XX), in order to explain the molecular formula, the presence of a ketonic function ($\lambda_{max.}$ 312 mμ; ε 120), and the remarkable fact that it has only one carboxyl group. Alternative ways of eliminating water from the intermediate dicarboxylic acid (XXI) would seem to be excluded by consideration of the absorption spectrum. Unfortunately it was not possible to open ring B of (XX) by further oxidation with hydrogen peroxide. (XX) was inert to this reagent even under energetic conditions, alkaline hydrolysis to the corresponding alcohol being the only defined reaction. The lack of reactivity of the α-keto-amide grouping is not without analogy (Prelog and Spilzfogel, *Helv. Chim. Acta*, 1945, **28**, 1669).

The Schmidt reaction when applied to (IX) gave a mixture of three products which could be separated by chromatography. The most easily eluted compound was a tetrazole, (XXII) or (XXIII). The other two products of reaction were isomeric amides, $C_{32}H_{51}O_4N$, m. p. 264—265°, $[\alpha]_D$ +11°, which comprised the major component, and m. p. 233°, $[\alpha]_D$

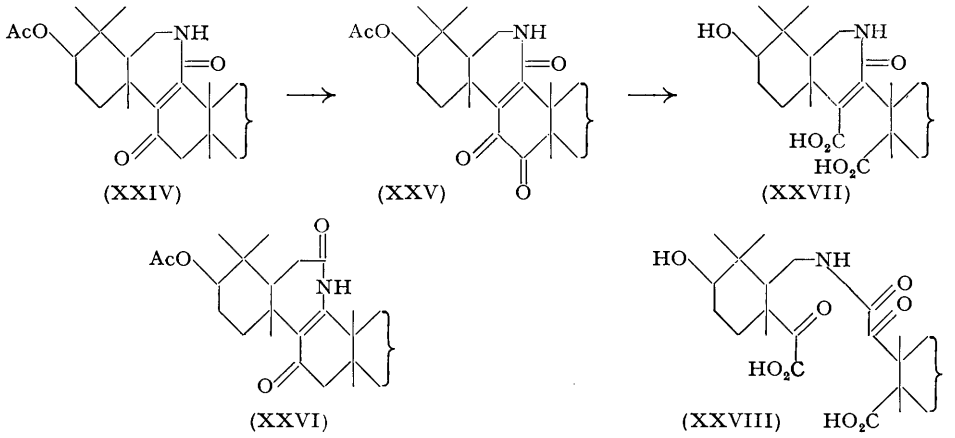
+305°. The same mixture of isomeric amides in approximately the same proportions was obtained by Beckmann rearrangement of the monoxime of (IX). Again we construe this as evidence that it is the carbonyl group at $C_{(8)}$ in (IX) which is attacked by hydrazoic acid, not that at $C_{(11)}$. The higher-melting amide, which was further characterised by hydrolysis to the corresponding alcohol, is formulated as 8 : 11-diketo-7*a*-aza-B-homolanost-9-en-2-yl acetate (XXIV) on the basis of its absorption spectrum ($\lambda_{max.}$ 249 mμ;



ε 7500), its ready reduction by zinc dust and acetic acid to the corresponding dihydro-compound (XIV), and its oxidation by selenium dioxide in acetic acid to an α-diketone (XXV). The last was obtained independently of the excess of selenium dioxide employed. The use of more drastic conditions by carrying out the reaction in refluxing acetic anhydride gave only the *N*-acetate of (XXV); this could also be prepared by direct acetylation of (XXV). The lower-melting amide is formulated as 8 : 11-diketo-8*a*-aza-B-homolanost-9-en-2-yl acetate (XXVI) on the basis of the absorption spectrum ($\lambda_{max.}$ 293 mμ; ε 13,000) and its *failure* to be reduced by zinc dust and acetic acid.

Oxidation of (XXV) by alkaline hydrogen peroxide gave, with hydrolysis, the dicarboxylic acid (XXVII), characterised as the crystalline acetate dimethyl ester. Considerable attention was devoted to attempts to rupture the double bond in (XXVII), for this would conceivably have led to products such as (XXVIII), eminently suited to effecting a final scission of the molecule. The double bond of (XXVII) was, however,

extremely inert both to electrophilic reagents such as ozone and to nucleophilic reagents. Undoubtedly the chemical inertness of both (IX) and (XXVII) is due both to steric and, for the electrophilic reagents, to electronic hindrance of reaction.



(XXIV) (XXV) (XXVII)

(XXVI) (XXVIII)

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 contrary, 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, unless specified to the contrary, measured in ethanol solution using a Unicam S.P. 500 Spectrophotometer.

Electrometric titrations were carried out in 50% aqueous ethanolic solution using a Cambridge pH meter. During titrations precautions were taken to prevent ingress of carbon dioxide. A glass electrode was used in the usual way with a calomel cell as reference electrode.

Isolation of 6-*Methylheptan*-2-*one*.—Lanost-9-en-2-yl acetate (dihydrolanosteryl acetate) (100 g.) in " AnalaR " acetic acid (200 ml.) was oxidised by the addition of chromium trioxide (120 g.) in 50% aqueous acetic acid (200 ml.) at 95—96° during 3 hours. Excess of aqueous sodium hydrogen sulphite was added and the reaction mixture steam-distilled. The distillate (100 ml.) was treated with 2 : 4-dinitrophenylhydrazine (300 mg.) in dilute hydrochloric acid (80 ml.) and left overnight at 0°. The product was filtered off and chromatographed in benzene over alumina. The first band eluted, after recrystallisation from methanol, gave 6-methylheptan-2-one 2 : 4-dinitrophenylhydrazone (25 mg.), m. p. 77°, $[\alpha]_D$ ±0°, $\lambda_{max.}$ 366 mμ (log ε 4·36 in $CHCl_3$) (Found : C, 54·6; H, 6·15; N, 18·2. Calc. for $C_{14}H_{20}O_4N_4$: C, 54·5; H, 6·55; N, 18·2%). There was no depression in m. p. on admixture with an authentic specimen of the same m. p. and absorption spectrum. The second band, eluted also with benzene, gave on crystallisation from methanol, acetone 2 : 4-dinitrophenylhydrazone (130 mg.) (m. p. and mixed m. p.). The residue of steam-distillation was worked up for 8 : 11-diketolanost-9-en-2-yl acetate in the usual way.

Pyrolysis of the Hydroxy-keto-dicarboxylic Acid.—The $C_{30}H_{46}O_6$ dicarboxylic acid (VI), m. p. 244° (decomp.) (Cavalla and McGhie, *J.*, 1951, 744; Barton, Fawcett, and Thomas, *ibid.*, p. 3147) (300 mg.) was heated *in vacuo* at 250° for 10 minutes. The resultant melt was dissolved in ether, and unchanged acid removed by washing with sodium hydroxide. Evaporation of the ethereal solution gave a non-crystallisable residue. This was acetylated with pyridine and acetic anhydride for ½ hour on the steam-bath. The product, isolated in the usual way, was chromatographed over alumina (six fractions). Light petroleum–benzene (4 : 1) eluted a crystalline *acetate* (VII). Recrystallised from methanol this had m. p. 116—118°, $[\alpha]_D$ +146° (*c*, 2·17), $\lambda_{max.}$ 275 and 207 mμ (ε 900 and 30,000 respectively) (Found : C, 77·1; H, 9·45. $C_{31}H_{46}O_4$ requires C, 77·15; H, 9·6%).

The crystalline acetate was also obtained in the following way. The $C_{30}H_{46}O_6$ dicarboxylic

acid was treated with pyridine and acetic anhydride at room temperature overnight. Working up in the usual way gave a product which could not be crystallised but it was evident from the absorption spectrum ($\lambda_{max.}$ 245 mμ; ε 8000) that no significant rearrangement had occurred. The amorphous acetate (60 mg.) was heated at 270° *in vacuo* for 5 minutes. After working up and chromatography as above, the crystalline $C_{31}H_{46}O_4$ acetate (30 mg.) was obtained, giving no depression in m. p. on admixture with the specimen prepared by the alternative route above.

8 : 11-*Diketo*-8a-*aza*-B-*homolanostan*-2-*yl Acetate* (XIII).—8 : 11-Diketolanostan-2-yl acetate (5 g.) in chloroform (10 ml.) was stirred with concentrated sulphuric acid (20 ml.) at 0°, and powdered sodium azide (1·5 g.) was added. After 15 minutes' stirring the mixture was poured into ice and water. After extraction with benzene–chloroform, and washing successively with aqueous sodium hydroxide (5%) and 5N-hydrochloric acid, the organic layer was evaporated to dryness *in vacuo*. The residue (4·4 g.) was chromatographed over alumina. Elution with benzene–1% methanol afforded 8 : 11-*diketo*-8a-*aza*-B-*homolanostan*-2-*yl acetate* (2·3 g.). Recrystallised from chloroform–light petroleum or from methanol, this had m. p. 249—252°, $[\alpha]_D$ +29° (*c*, 2·00), $\lambda_{max.}$ 302 mμ (ε 50) (Found : C, 74·1; H, 10·9; N, 2·5. $C_{32}H_{53}O_4N$ requires C, 74·5; H, 10·4; N, 2·7%).

Hydrolysis of the acetate (100 mg.) with methanolic potassium hydroxide gave 2-*hydroxy*-8a-*aza*-B-*homolanostane*-8 : 11-*dione* (60 mg.). Recrystallised from methanol this had m. p. 230—232°, $[\alpha]_D$ +10° (*c*, 1·70) (Found : C, 76·2; H, 10·9. $C_{30}H_{51}O_3N$ requires C, 76·1; H, 10·9%). Reacetylation with pyridine–acetic anhydride overnight at room temperature gave back unchanged 8 : 11-diketo-8*a*-aza-B-homolanostan-2-yl acetate (m. p. and mixed m. p.).

Oxidation of 2-hydroxy-8*a*-aza-B-homolanostane-8 : 11-dione (1·3 g.) in acetic acid (20 ml.) with chromium trioxide (400 mg.) in water (1 ml.) at room temperature for 18 hours afforded, after working up in the usual way, 8a-*aza*-B-*homolanostane*-2 : 8 : 11-*trione*. Recrystallised from aqueous methanol this had m. p. 196—197°, $[\alpha]_D$ +23° (*c*, 2·84) (Found : C, 75·8; H, 10·3; N, 2·7. $C_{30}H_{49}O_3N$ requires C, 76·4; H, 10·45; N, 2·95%).

Beckmann Rearrangement of 8 : 11-*Diketolanostan*-2-*yl Acetate Monoxime*.—The oxime, m. p. 215—216°, $[\alpha]_D$ +21° (*c*, 2·93) (150 mg.), prepared according to the directions of Voser, Montavon, Günthard, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1950, **33**, 1893), in light petroleum (30 ml.) and dry benzene (10 ml.) was treated with phosphorus pentachloride (150 mg.) at room temperature for 1 hour. After being worked up in the usual way the reaction product was chromatographed over alumina. Elution with benzene–ether (1 : 1) furnished 8 : 11-diketo-8*a*-aza-B-homolanostan-2-yl acetate (85 mg.). After recrystallisation from chloroform–methanol this was identified by m. p., mixed m. p., and rotation, $[\alpha]_D$ +30° (*c*, 1·82).

Action of Bromine on 8 : 11-*Diketo*-8a-*aza*-B-*homolanostan*-2-*yl Acetate*.—The amide acetate (100 mg.) in acetic acid (2 ml.) was treated with bromine in acetic acid (0·5 ml.; 4%) at room temperature for 24 hours. Working up in the usual way gave a *monobromo*-derivative. Recrystallised from methanol, this had m. p. 223—224° (decomp.) (Found : C, 64·5; H, 8·5; N, 2·3. $C_{32}H_{52}O_4NBr$ requires C, 64·6; H, 8·8; N, 2·35%). It was not investigated further.

Oxidation of 8 : 11-*Diketo*-8a-*aza*-B-*homolanostan*-2-*yl Acetate with Selenium Dioxide*.—This acetate (1 g.) in acetic acid (30 ml.) containing 1% of acetic anhydride was refluxed with selenium dioxide (2 g.) for 16 hours. The mixture was poured into water and extracted with benzene–chloroform. Filtration from selenium and evaporation of the organic layer *in vacuo* furnished a product which, when recrystallised from methanol or from benzene, gave 7 : 8 : 11 : 12-*tetraketo*-8a-*aza*-B-*homolanostan*-2-*yl acetate* (XVIII) (600 mg.). This had m. p. 311—312° (decomp.), $[\alpha]_D$ −21° (*c*, 2·00), $\lambda_{max.}$ 430 mμ (ε 30), $\lambda_{inflex.}$ *ca.* 280—290 mμ (ε *ca.* 140) (in chloroform) (Found : C, 70·85; H, 8·7; N, 2·7. $C_{32}H_{49}O_6N$ requires C, 70·65; H, 9·1; N, 2·6%). The acetate was unchanged on treatment with pyridine–acetic anhydride for 1 hour at 100°.

Alkaline hydrolysis (20 minutes' refluxing in 5% methanolic potassium hydroxide) gave 2-*hydroxy*-8a-*aza*-B-*homolanostane*-7 : 8 : 11 : 12-*tetraone*. Recrystallised from methanol this had m. p. 284—285° (decomp.), $[\alpha]_D$ −54° (*c*, 0·70), $\lambda_{max.}$ 430 mμ (ε 30 in chloroform) (Found : C, 71·3; H, 9·85; N, 2·95. $C_{30}H_{47}O_5N$ requires C, 71·8; H, 9·45; N, 2·8%). The hydrolysis was also effected by refluxing with ethanol containing 15% of concentrated hydrochloric acid for 3 hours. Reacetylation with pyridine–acetic anhydride at room temperature overnight gave back the acetate unchanged (m. p. and mixed m. p.).

8 : 11-Diketo-8*a*-aza-B-homolanostan-2-yl acetate (2 g.) in acetic acid (50 ml.) was heated under reflux with selenium dioxide (2 g.) for 2 hours. After being worked up as above, the product of reaction was digested with benzene (10 ml.). The benzene-insoluble residue, recrystallised from methanol, gave 7 : 8 : 11 : 12-tetraketo-8*a*-aza-B-homolanostan-2-yl acetate

(m. p. and mixed m. p.). The benzene-soluble material was chromatographed over alumina. Elution with ether containing 2% of methanol afforded 8 : 11 : 12-*triketo*-8a-*aza*-B-*homolanostan*-2-*yl acetate* (XV), m. p. 269—273°, $[\alpha]_D$ +88° (*c*, 1·30), $\lambda_{max.}$ 385 and 280 mμ (ε 15 and 90 respectively in chloroform) (Found : C, 72·6; H, 9·35; N, 2·6. $C_{32}H_{51}O_5N$ requires C, 72·6; H, 9·7; N, 2·65%). As an alternative to this method of working up, the crude reaction product, freed from 2-acetoxy-8*a*-aza-B-homolanostane-7 : 8 : 11 : 12-tetraone by digestion with benzene, was acetylated with pyridine–acetic anhydride on the water-bath for 1 hour. After working up in the usual way the reaction product was chromatographed over alumina. Benzene eluted a small amount (200 mg. from 2 g. of 8 : 11-diketo-8*a*-aza-B-homolanostan-2-yl acetate) of a *substance* which, recrystallised from methanol, had m. p. 203°, $[\alpha]_D$ −35° (*c*, 2·70), $\lambda_{max.}$ 320 and 244 mμ (ε 100 and 8500 respectively). This was not investigated further (Found : C, 70·75; H, 9·1; N, 2·55%). Elution with ether furnished 8 : 12-*diketo*-8a-*aza*-B-*homolanost*-10-*ene*-2 : 11-*diol diacetate* (900 mg. from 2 g. of starting amide). Recrystallised from methanol this had m. p. 216—217°, $[\alpha]_D$ −53° (*c*, 1·20), $\lambda_{max.}$ 321 and 242 mμ (ε 100 and 9000 respectively) (Found : C, 71·7; H, 9·0; N, 2·6. $C_{34}H_{53}O_6N$ requires C, 71·4; H, 9·3; N, 2·5%). The same diacetate was also obtained by acetylation of pure 2-hydroxy-8*a*-aza-B-homolanostane-8 : 11 : 12-trione with pyridine–acetic anhydride on the steam-bath for 30 minutes (identified by m. p. and mixed m. p.). Similar acetylation at room temperature overnight gave only the monoacetate (see above). Alkaline hydrolysis of the diosphenol diacetate with 5% methanolic potassium hydroxide gave 2-*hydroxy*-8a-*aza*-B-*homolanostane*-8 : 11 : 12-*trione*. Recrystallised from methanol this had m. p. 227—228°, $[\alpha]_D$ +81° (*c*, 1·70), $\lambda_{max.}$ 382 and 280 mμ (ε 30 and 450 respectively in chloroform) (Found : C, 73·5; H, 10·1; N, 3·65. $C_{30}H_{49}O_4N$ requires C, 73·9; H, 10·15; N, 2·85%).

8 : 11 : 12-Triketo-8*a*-aza-B-homolanostan-2-yl acetate (100 mg.) in acetic acid (5 ml.) was refluxed with selenium dioxide (200 mg.) for 16 hours. Working up as before and recrystallisation from benzene gave 7 : 8 : 11 : 12-tetraketo-8*a*-aza-B-homolanostan-2-yl acetate (60 mg.), identified by m. p. and mixed m. p. In a similar experiment 2-hydroxy-8*a*-aza-B-homolanostane-8 : 11 : 12-trione (10 mg.) in acetic acid (20 ml.) was heated under reflux with selenium dioxide (50 mg.) for 30 hours. Working up as before and recrystallisation from methanol gave 2-hydroxy-8*a*-aza-B-homolanostane-7 : 8 : 11 : 12-tetraone (2 mg.), identified by m. p. and mixed m. p.

Action of Alkaline Hydrogen Peroxide on 2-*Hydroxy*-8a-*aza*-B-*homolanostane*-8 : 11 : 12-*trione*.—The amide (250 mg.), dissolved in methanolic potassium hydroxide (20 ml.; 2%), was treated with hydrogen peroxide (2 ml.; 30%) at 0° for 2 hours. After being worked up in the usual way, the acidic fraction (270 mg.) was recrystallised from ethyl acetate–methanol, to give the *dicarboxylic acid* (XIX). This sintered at 175°, decomposed at 195°, and had $[\alpha]_D$ +10° (*c*, 1·30) and no absorption max. in the ultra-violet region (Found : C, 66·7; H, 9·55; N, 3·2. $C_{30}H_{51}O_6N,CH_3{\cdot}OH$ requires C, 67·2; H, 10·0; N, 2·5%). Electrometric titration showed 2·17 carboxyl groups per mol.

Action of Alkaline Hydrogen Peroxide on 2-*Hydroxy*-8a-*aza*-B-*homolanostane*-7 : 8 : 11 : 12-*tetraone and its Acetate*.—The hydroxy-amide (200 mg.) in methanolic potassium hydroxide solution (50 ml.; 2%) was treated with hydrogen peroxide (10 ml.; 30%) for 30 minutes at 0°. After being worked up in the usual way the acidic fraction (175 mg.) was recrystallised from methanol, to give the *monocarboxylic acid*. This sintered at 160° and then decomposed and had $[\alpha]_D$ −95° (*c*, 1·30) and $\lambda_{max.}$ 311 mμ (ε 115) (Found : C, 67·5; H, 9·55; N, 4·15, 3·3, 1·75. $C_{30}H_{47}O_6N,CH_3{\cdot}OH$ requires C, 67·7; H, 9·35; N, 2·6%). Acetylation with pyridine–acetic anhydride at room temperature overnight afforded the corresponding acetate (see below) identified by m. p. and mixed m. p.

The amide acetate (250 mg.) in methanolic potassium hydroxide solution (100 ml.; 2%) was treated with hydrogen peroxide (5 ml.; 30%) at 0° for 10 minutes. After being worked up in the usual way the acidic fraction (230 mg.) was recrystallised from methanol, to give the *monocarboxylic acid acetate* (XX), m. p. 217—218° (decomp.), $[\alpha]_D$ −55° (*c*, 2·68), $\lambda_{max.}$ 312 mμ (ε 120) (Found : C, 68·5; H, 9·25; N, 2·9. $C_{32}H_{49}O_7N$ requires C, 68·65; H, 8·85; N, 2·5%). Electrometric titration showed 0·97 carboxyl group per mol.

Schmidt Reaction on 8 : 11-*Diketolanost*-9-*en*-2-*yl Acetate*.—The acetate (20 g.) in chloroform (300 ml.) and concentrated sulphuric acid (100 ml.) was stirred at 0° during the addition of powdered sodium azide (5 g.) (15 minutes). The mixture was stirred for a further 30 minutes and then poured into ice and water and worked up as for the saturated analogue (see above). Digestion of the crude reaction product with light petroleum (b. p. 60—80°) gave nearly pure 8 : 11-*diketo*-7a-*aza*-B-*homolanost*-9-*en*-2-*yl acetate* (XXIV) (10 g.). Recrystallised from

chloroform–methanol this had m. p. 264—265°, $[\alpha]_D$ +11° (*c*, 1·02), +13° (*c*, 1·30), $\lambda_{max.}$ 249 mμ (ε 7500) (Found : C, 75·05; H, 10·15; N, 2·9. $C_{32}H_{51}O_4N$ requires C, 74·8; H, 10·0; N, 2·7%).

The portion of the reaction product which was soluble in light petroleum and the mother-liquors from the crystallisation of the 7*a*-aza-amide were combined and evaporated to dryness *in vacuo*, and the residue was chromatographed in benzene over alumina. Elution with benzene gave a small amount of unchanged starting material. Elution with benzene–ether (20 : 1) furnished the *tetrazole* (XXII or XXIII). Recrystallised from chloroform–methanol this had m. p. 254—256°, $[\alpha]_D$ −92° (*c*, 0·60), $\lambda_{max.}$ 265 mμ (ε 6500) (Found : C, 71·95; H, 9·4; N, 10·7. $C_{32}H_{50}O_3N_4$ requires C, 71·3; H, 9·4; N, 10·4%).

Elution with benzene–ether (9 : 1) afforded 8 : 11-*diketo*-8a-*aza*-B-*homolanost*-9-*en*-2-*yl acetate* (XXVI). Recrystallised from chloroform–methanol this had m. p. 233°, $[\alpha]_D$ +305° (*c*, 1·00), +300° (*c*, 0·90), $\lambda_{max.}$ 293 mμ (ε 13,000) (Found : C, 75·2; H, 10·2; N, 2·55. $C_{32}H_{51}O_4N$ requires C, 74·8; H, 10·0; N, 2·7%).

Elution with benzene–ether (6 : 1 and 3 : 1) afforded a further quantity of 8 : 11-diketo-7*a*-aza-B-homolanost-9-en-2-yl acetate.

Alkaline hydrolysis of the last-mentioned acetate furnished the corresponding *alcohol*. Recrystallised from methanol this had m. p. 210—212°, $[\alpha]_D$ +15° (*c*, 1·57), $\lambda_{max.}$ 253 mμ (ε 7000) (Found : C, 75·95; H, 10·3. $C_{30}H_{49}O_3N$ requires C, 76·35; H, 10·5%).

Beckmann Rearrangement of 8 : 11-*Diketolanost*-9-*en*-2-*yl Acetate Monoxime*.—This acetate (100 mg.) and hydroxylamine hydrochloride (200 mg.) were heated in ethanol (20 ml.) and pyridine (5 ml.) under reflux for 6 hours. Concentration *in vacuo* and working up in the usual way afforded the *monoxime* (60 mg.). Purified by chromatography over alumina and recrystallisation from light petroleum this had m. p. 150—152°, $[\alpha]_D$ +69° (*c*, 1·05), $\lambda_{max.}$ 290 mμ (ε 12,500) (Found : C, 74·45; H, 10·05. $C_{32}H_{51}O_4N$ requires C, 74·8; H, 10·0%). The oxime was also prepared by treating the acetate (200 mg.) with hydroxylamine hydrochloride (400 mg.) in pyridine (5 ml.) at room temperature for 24 hours. The oxime, isolated as above, had m. p. 150—151° (140 mg.). In a further experiment this oxime was obtained in a higher-melting *form*. After purification by chromatography and crystallisation from chloroform–light petroleum this had m. p. 190°, $[\alpha]_D$ +67° (*c*, 1·39) (Found : C, 74·6; H, 9·8; N, 2·7%). The mixed m. p. of the higher- with the lower-melting form was 189—190° on slow heating from 145°.

The monoxime (400 mg.) (see above) in benzene (10 ml.) was treated with phosphorus pentachloride (300 mg.) at 0° for 20 minutes. After being worked up in the usual way the reaction product was chromatographed over alumina. Elution with benzene–ether (4 : 1) gave 8 : 11-diketo-8*a*-aza-B-homolanost-9-en-2-yl acetate (55 mg.), identified by m. p. and mixed m. p. Elution with ether furnished 8 : 11-diketo-7*a*-aza-B-homolanost-9-en-2-yl acetate (125 mg.), identified similarly.

Refluxing the monoxime (200 mg.) with acetic acid (6 ml.) and concentrated hydrochloric acid (2 ml.) for 1 hour gave back unchanged 8 : 11-diketolanost-9-en-2-yl acetate (90 mg.) (m. p. and mixed m. p.).

8 : 11-*Diketo*-7a-*aza*-B-*homolanostan*-2-*yl Acetate*.—The unsaturated 7*a*-aza-amide acetate (see above) (100 mg.) in boiling acetic acid (5 ml.) was treated with zinc dust (100 mg.) and the refluxing continued for 1 hour. Working up in the usual way and chromatography over alumina gave 8 : 11-*diketo*-7a-*aza*-B-*homolanostan*-2-*yl acetate*. Recrystallised from methanol this had m. p. 184—185°, $[\alpha]_D$ +111° (*c*, 1·40), +110° (*c*, 1·20), $\lambda_{max.}$ 290 mμ (ε 65) (Found : C, 73·75; H, 10·3. $C_{32}H_{53}O_4N$ requires C, 74·5; H, 10·4%). Under the same reaction conditions 8 : 11-diketo-8*a*-aza-B-homolanost-9-en-2-yl acetate was recovered unchanged (m. p. and mixed m. p.).

8 : 11 : 12-*Triketo*-7a-*aza*-B-*homolanost*-9-*en*-2-*yl Acetate*.—8 : 11-Diketo-7*a*-aza-B-homolanost-9-en-2-yl acetate (1 g.) in acetic acid (20 ml.) was refluxed with selenium dioxide (1·0 g.) for 2 hours. After working up in the usual way, chromatography over alumina afforded 8 : 11 : 12-*triketo*-7a-*aza*-B-*homolanost*-9-*en*-2-*yl acetate* (800 mg.). Recrystallised from chloroform–methanol this had m. p. 272—273°, $[\alpha]_D$ −9° (*c*, 1·20), $\lambda_{max.}$ 272 mμ (ε 4500) (Found : C, 73·25; H, 9·25; N, 3·5. $C_{32}H_{49}O_5N$ requires C, 72·85; H, 9·4; N, 2·7%). On further refluxing in acetic acid solution with an equal weight of selenium dioxide for 8 hours the 8 : 11 : 12-triketo-7*a*-aza-B-homolanost-9-en-2-yl acetate was recovered unchanged in almost quantitative yield.

A similar oxidation, carried out with the 8 : 11-dione amide acetate (500 mg.) in acetic anhydride (10 ml.) with selenium dioxide (1·0 g.), refluxing being for 2 hours and working up in the usual way, furnished 7*a*-*acetyl*-8 : 11 : 12-*triketo*-7a-*aza*-B-*homolanost*-9-*en*-2-*yl acetate* which, recrystallised from methanol, had m. p. 213—214°, $[\alpha]_D$ −150° (*c*, 1·07), $\lambda_{max.}$ 262 mμ (ε 6500)

(Found: C, 72·2; H, 9·1; N, 2·4. $C_{34}H_{51}O_6N$ requires C, 71·65; H, 9·05; N, 2·45%). The same compound (m. p. and mixed m. p.) was obtained by heating 8 : 11 : 12-triketo-7*a*-aza-B-homolanost-9-en-2-yl acetate with acetic anhydride under reflux.

Action of Alkaline Hydrogen Peroxide on 8 : 11 : 12-*Triketo*-7a-*aza*-B-*homolanost*-9-*en*-2-*yl Acetate.*—The amide (4·0 g.) in dioxan (200 ml.) and methanol (50 ml.) with potassium hydroxide (2 g.) was treated with hydrogen peroxide (30%; 10 ml.), and the mixture left for 1 hour at room temperature. Separation into neutral (negligible) and acid fractions afforded the *hydroxy-dicarboxylic acid* (XXVII) as a glass which could not be crystallised. From aqueous methanol it had m. p. *ca.* 218° (decomp.), $[\alpha]_D$ −38° (*c*, 1·07), $\lambda_{max.}$ 214 mμ (ε 6500) (Found: C, 67·3; H, 9·0; N, 2·6. $C_{30}H_{49}O_6N,H_2O$ requires C, 67·1; H, 9·5; N, 2·6%). Electrometric titration showed the presence of 2·03 carboxyl groups per mol.

Treatment of the hydroxy-dicarboxylic acid with pyridine–acetic anhydride overnight at room temperature gave an acetate which was also an uncrystallisable glass. Methylation with diazomethane afforded the *acetate dimethyl ester*. This, after *slow* crystallisation from aqueous methanol, formed rhombs, m. p. 140°, $[\alpha]_D$ −37° (*c*, 1·04), $\lambda_{max.}$ 216 mμ (ε 8000) (Found: C, 69·05; H, 9·4; N, 2·25. $C_{34}H_{55}O_7N$ requires C, 69·35; H, 9·5; N, 2·25%).

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