545. *Lanosterol. Part XII.* The Constitution of Some Oxidation Products from Lanostadienol and Lanostenol.*

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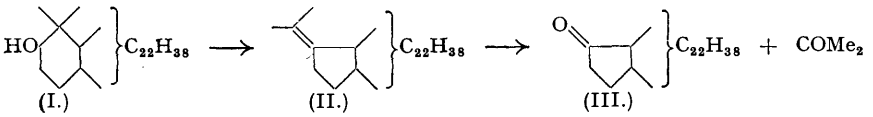
A reinvestigation of the acidic oxidation products reported by Marker, Wittle, and Mixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368) and by Wieland and Joost (*Annalen*, 1941, **546**, 103) has shown that they may be formulated as $C_{27}H_{40}O_5$ and $C_{30}H_{50}O_4$, respectively. Attention is drawn to conflicting reports of acidic degradation products from euphadienyl acetate together with a suggested reinterpretation.

In recent years considerable progress has been made in the elucidation of the structure of the diolefinic alcohol, lanostadienol, owing largely to the work of Ruzicka and Jeger and their co-workers in Switzerland and of Dorée and McGhie and their co-workers in this country. As a result of these investigations the environment of the secondary alcohol grouping has been established, and the reactive ethylenic linkage shown to be present in an exocyclic *iso*propylidene group. These conclusions have been based on the following experimental facts.

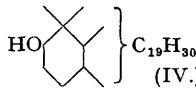
Dehydration of lanostenol ($C_{30}H_{52}O$) with phosphorus pentachloride gave an unsaturated hydrocarbon, *iso*lanostadiene $C_{30}H_{50}$ (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467), which could not be hydrogenated to lanostene; it was postulated that in its formation a retropinacoline change had taken place. Oxidation of this hydrocarbon with osmium tetroxide followed by fission of the two isomeric glycols, so obtained, with lead tetra-acetate gave the A-norketone,† $C_{27}H_{44}O$, together with acetone, which was isolated and identified as the 2 : 4-dinitrophenylhydrazone (Dorée, McGhie, and Kurzer, *Nature*, 1949, **163**, 140; *J.*, 1949, S 167; see also Ruzicka, Montavon, and Jeger, *Helv. Chim. Acta*, 1948, **31**, 818). This sequence of changes may be formulated by the partial formulæ (I)—(III).



(I.) (II.) (III.)

The reactions described above definitely established the presence of a *gem*-dimethyl group in a position vicinal to the hydroxyl group. This grouping is analogous to that observed in the case of the pentacyclic triterpenes (*idem, ibid.*, 1945, **28**, 767, 942, 1628). Furthermore, the formation of hydroxymethylenelanostenone from lanostenone—the latter, on oxidation with alkaline hydrogen peroxide gave a dibasic acid, $C_{30}H_{50}O_4$ (Ruzicka, Rey, and Muhr, *ibid.*, 1944, **27**, 472)—necessitates that a methylene group be placed α to the alcoholic hydroxyl group. It is clear from this evidence that the secondary hydroxyl group is flanked on one side by a *gem*-dimethyl group and on the other by a methylene group, *viz.*, $\cdot CMe_2 \cdot CH(OH) \cdot CH_2 \cdot$. The dibasic acid, $C_{30}H_{50}O_4$, when heated, lost carbon dioxide and cyclised, to give the norketone, $C_{29}H_{48}O$, and from this it follows that the hydroxyl group is present in a ring system which is probably six-membered (Ruzicka *et al.*, *locc. cit.*).

The reactive ethylenic linkage of lanostadienol has been shown to be present in an exocyclic *iso*propylidene group, since oxidative fission of lanostadienol and its derivatives gave acetone together with non-volatile acidic oxidation products (Dorée and Garratt, *J. Soc. Chem. Ind.*, 1933, **52**, 355T; Wieland and Benend, *Z. physiol. Chem.*, 1942, **274**, 215; Ruzicka, Rey, and Muhr, *loc. cit.*). On the basis of these results, the partial formula (IV) may be put forward for lanostadienol.



(IV.)

The conclusions summarized in the foregoing section are firmly based on experimental evidence, which is incapable of other interpretations. However, there exist in the literature two apparent discrepancies which are irreconcilable if the above facts are correct, and hitherto these have received no adequate explanation; first, the isolation by Marker, Wittle, and Mixon (*loc. cit.*) of a monobasic acid, $C_{25}H_{46}O_2$, on chromic acid oxidation of lanostadienyl or lanostenyl acetate; secondly, the isolation by Wieland and Joost (*loc. cit.*) of a monobasic acid

* Part XI, *J.*, 1951, 834. † The prefix A-nor denotes contraction of ring A.

formulated as $C_{27}H_{40}O_2$ (or less probably $C_{26}H_{38}O_2$ or $C_{28}H_{42}O_2$) on oxidation of lanostenol. We have reinvestigated these results with the hope of throwing further light on the problem as a whole, and we now record our own results and interpretations. These elucidate these inconsistencies and make possible the general acceptance of the partial formula for lanostadienol given above.

Marker, Wittle, and Mixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368) oxidised lanostadienyl and lanostenyl acetate with chromic acid under conditions analogous to those used for the oxidation of *epi*cholestanyl acetate to androsterone. No volatile products were obtained, but from the acid fraction a monobasic acid, m. p. 81° (methyl ester, m. p. 67°), formulated as $C_{25}H_{46}O_2$, was isolated. This was reported to be unchanged by boiling acetic anhydride, to be inert to ketonic reagents, and to be saturated to bromine in acetic acid, even at 60°. The neutral fraction obtained in the oxidation gave no semicarbazone and was not investigated further.

Basing their conclusions on the assumption that the original acetoxy-group was present in a terminal ring, Marker *et al.* came to the following conclusions. " If the acetate group is in the first ring of these compounds, then the active double bond must also be in that ring to lose five carbon atoms and give a saturated monocarboxylic acid identical to [*sic*] that formed from dihydrolanosteryl acetate. Getting a monocarboxylic acid from this ring system could be explained by a mechanism similar to that of the opening of ring B of ergosterol by ultra-violet light to give calciferol."

The experimental results of Marker *et al.* (*loc. cit.*) were apparently confirmed in subsequent work by Bellamy and Dorée (*J.*, 1941, 172), who found that oxidation of lanostadienol under the conditions used by Marker for the oxidation of the acetate gave a substance, m. p. 81—82° (ethyl ester, m. p. 64°), which was apparently identical with that described by the American workers. The interpretation of these results is at direct variance with more recent investigations as to the relative positions of the reactive olefinic linkage and the hydroxyl group.

Accordingly, we repeated the oxidation of lanostadienyl acetate under the conditions laid down by Marker *et al.* and by the modified method of Bellamy and Dorée (*locc. cit.*) and obtained a yellow amorphous product, from ethyl acetate, having m. p. *ca.* 80°, but giving analyses in fair agreement with the formula $C_{29}H_{42}O_6$. All attempts to obtain the acid, its salts, or its methyl ester in a crystalline form proved fruitless. However, we found that the amorphous oxidation product on treatment with 2% ethanolic potassium hydroxide gave a deep red solution which on being heated for 2 hours developed an emerald-green colour (reversal of these changes occurring on cooling). From the alkaline solution was isolated an acid, in moderate and variable yields, separating from ethyl acetate in fine yellow needles, m. p. 205—206°, $[\alpha]_D$ 104°; this gave analytical figures, after prolonged drying in a high vacuum at 140°, in good agreement with the formulation $C_{27}H_{40}O_5$. A determination of the equivalent of this substance by electrometric titration showed it to be a monobasic acid. The ultra-violet absorption spectra showed a maximum at 275 mμ. (log ε 4·03), which indicated the presence of an unsaturated 1 : 4-diketo-system similar to that present in diketolanostenyl acetate (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472). The fifth oxygen atom was shown as follows to be present in the secondary alcoholic group of the parent lanostadienol. Benzoylation gave a substance which after prolonged drying in a high vacuum had m. p. 218—220°, $[\alpha]_D$ 112°, and gave an analysis agreeing with $C_{34}H_{44}O_6$. This was identical with that obtained by the oxidation of lanostadienyl benzoate. Alkaline hydrolysis regenerated the original substance, m. p. 205—206°; mild oxidation of this substance with chromic acid gave a triketo-acid, isolated as the methyl ester, m. p. 149—150°, $[\alpha]_D$ +172°. These results confirm the conclusion that the yellow substance, $C_{27}H_{40}O_5$, m. p. 205—206°, is a hydroxydiketo-monobasic acid.

Our experimental findings are readily explained if Marker's primary oxidation product retains the acetoxy-group of lanostadienyl acetate and fission of the exocyclic *iso*propylidene group has taken place with simultaneous oxidation, to keto-groups, of the two methylene groups vicinal to the inert ethylenic bond; the subsequent action of the alkali would be a simple hydrolysis of the acetoxy-group. This interpretation was confirmed by the determination of the equivalent weight of Marker's product, which indicated the presence of both an acetoxy- and a carboxyl group. The yields of the hydroxydiketo-acid (diketotrisnorlanostenolic acid) from lanostadienyl acetate are not good (20—30% at the best), probably owing to further oxidation of the nucleus (*e.g.*, fission of ring A) to give more complex non-crystalline acidic products, which we are now studying.

Oxidation of lanostenyl acetate under the conditions used by Marker gave a small acidic fraction from which the diketotrisnorlanostenolic acid could be obtained. Although we have carried out this oxidation many times during the past eight years, the major oxidation product

was always diketolanostenyl acetate; since this may be partly extracted with strong alkali from ether, it may account for the large " acidic " fraction reported by Marker. We think that our own observations render unnecessary the premises of Marker *et al.* to account for the formation of their acidic degradation product.

The diketotrisnorlanostenolic acid described above is identical with an acid obtained by Wieland and Joost (*loc. cit.*) by chromic acid oxidation of cryptostadienyl benzoate, followed by hydrolysis. This acid was formulated by Wieland as $C_{28}H_{42}O_5$, although the analytical data quoted agree equally well with the alternative formulation $C_{27}H_{40}O_5$. A table of comparison of the two series of oxidation products is shown below and leaves little doubt as to their identity,

	Cryptostadienol.	Lanostadienol.	
	M. p.	M. p.	$[\alpha]_D$.
Benzoyloxy-acid	210—220°	218—220°	+112°
Hydroxy-acid	201—203	205—206	+104
Keto-acid (methyl ester)	154—155	149—150	+172

although Wieland and Joost (*loc. cit.*) gave no values for the specific rotations. Cryptosterol has been shown to be identical with lanostadienol (Ruzicka *et al.*, *Helv. Chim. Acta*, 1945, **28**, 759; for further evidence see McGhie, Ph.D. Thesis, London, 1947), and this provides further confirmation of the identity of the two triterpenes.

Our explanation of the mode of formation of these acids has been directly confirmed as follows. Ozonolysis of lanostadienol followed by methylation with diazomethane gave methyl trisnorlanostenolate (Wieland and Joost; Ruzicka, Rey, and Muhr, *locc. cit.*), which was oxidised with chromic acid to methyl diketotrisnorlanostenonate, identified by comparison with the ester obtained from diketotrisnorlanostenolic acid.

Wieland and Joost (*loc. cit.*) have also reported the oxidation of lanostenol by chromic acid to a crystalline monobasic acid, m. p. 195°, to which the formula $C_{27}H_{40}O_2$ (or less probably $C_{26}H_{38}O_2$) was assigned. They interpreted their results (with caution) on the assumptions that, in the production of the monobasic acid, the original hydroxyl group had been lost, that this hydroxyl group was present in a side chain, and that one of the four rings of the tetracyclic system had become aromatic. Since, as a result of their experiments on lanostenyl hydrochloride, they had concluded that the reactive double bond of lanostadienol is $\beta\gamma$ to the alcoholic hydroxyl group, it appeared likely that *both* the reactive double bond and the hydroxyl group were present in the side chain. This conclusion is in direct opposition to that of later workers.

A repetition of Wieland and Joost's investigations resulted in the isolation of a substance, $C_{30}H_{50}O_4$, m. p 195°, $[\alpha]_D$ +87° (Wieland and Joost give no value for the specific rotation), the nature of which as a dibasic acid was disclosed by its equivalent weight and by preparation of a dimethyl ester which, although it could not be obtained crystalline, contained two methoxyl groups (Zeisel). Although our experiments were hampered by very poor yields of this acid, we were able to confirm its identity with a dibasic acid isolated by Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 472) by oxidation of hydroxymethylenelanostenone. The formation of the dibasic acid in the oxidative degradation of lanostenol is easily explained by cleavage of the terminal hydroxyl-bearing ring, thus :

We are of the opinion that a similar misinterpretation of experimental results exists in regard to the constitution of the acidic oxidation products obtained from the acetate of the closely related tetracyclic triterpene alcohol, euphadienol. Dupont, Dulou, and Vilkas (*Bull. Soc. chim.*, 1949, **16**, 809) have reported the oxidation of euphadienyl acetate with potassium permanganate to give a monobasic acid, $C_{27}H_{40}O_5$, m. p. 231—231·5°, $[\alpha]$ 14·2°. The corresponding methyl ester had m. p. 160·5—161°, 167·5—169·5°, and 190—190·5° depending on the solvent used for crystallisation. The acid was stated to possess an ultra-violet absorption maxima at 270 mμ. (log ε 3·89), which is very close to that of diketotrisnorlanostenolic acid, $\lambda_{max.}$ 275 mμ. (log ε 4·03). The acid was reported to be unaffected by acetic anhydride, to give no quinoxaline derivative with *o*-phenylenediamine, and to form an oxime, m. p. 207·5—208·5°. In a subsequent communication, Dupont *et al.* (*ibid.*, p. 813) stated that further oxidation of the acid, $C_{27}H_{40}O_5$, gave the nor-acid $C_{26}H_{38}O_5$, m. p. 191·5—192° (methyl ester, m. p. 140·5—141·5°). This

acid was also obtained from the mother-liquors obtained in the preparation of euphadienone by oxidation of euphadienol.

On the other hand, Krusi (*J.*, 1950, 2864) has reported that euphadienyl acetate, on dehydrogenation with *N*-bromosuccinimide followed by oxidation with chromic acid, gave an acid, isolated as the methyl ester, which on subsequent hydrolysis gave the acid, m. p. 233—234° (after sublimation, m. p. 231—233°); the methyl ester had m. p. 185—186°. The acid was assigned the molecular formula, $C_{25}H_{36}O_5$, and Krusi interpreted his results on the basis of this formula. It should be noted that Krusi assumed that in the course of dehydrogenation with *N*-bromosuccinimide a conjugated diene system had been formed, the new double bond being in conjugation with that originally present in the *iso*propylidene group, and that on oxidation it was this new double bond which was ruptured. The evidence for such a conjugated

$C_{19}H_{33}$ { $-CH_2\cdot CH_2\cdot CH{:}CMe_2$; $-CH_2\cdot C{:}C\cdot CH_2-$; $>CH\cdot OH$ } $C_{19}H_{33}$ { $-CH{:}CH\cdot CH{:}CMe_2$; $-CH_2\cdot C{:}C\cdot CH_2-$; $>CH\cdot OH$ } $C_{19}H_{33}$ { $-CO_2H$; $-CO\cdot C{:}C\cdot CO-$; $>CH\cdot OH$ }

system was the ultra-violet absorption spectra which showed maxima at 232 mμ. (log ε = 3·50), 238·5 mμ. (log ε = 3·52), and 247 mμ. (log ε = 3·33), these values being very similar to those reported by Bellamy and Dorée (*J.*, 1941, 176) for γ-lanostadienyl acetate, with maxima at 236, 244, and 250 mμ., which is known to contain a nuclear system of conjugated double bonds. It appears to us that Krusi ignored the alternative possibility that the introduction of a conjugated system could have involved the inert nuclear double bond; furthermore, since Dorée, McGhie, and Kurzer (*J.*, 1949, 570) have reported that dehydrogenation of lanostenyl acetate with *N*-bromosuccinimide gave γ-lanostadienyl acetate identical with that described by Bellamy and Dorée (*loc. cit.*), and this reaction must of necessity involve the endocyclic double bond to produce a conjugated system (cf. Birchenough and McGhie, *J.*, 1949, 2038), this possibility seems more plausible to us. Further, oxidation of γ-lanostadienyl acetate with chromic acid is known to yield diketolanostenyl acetate (Muhr, Thesis, Zurich, 1945; Cavalla and McGhie, *J.*, 1951, 834) showing an absorption maximum in the ultra-violet region at 275 mμ. (log ε = 3·94), which is very close to the values reported by Krusi and by Dupont *et al.* [$\lambda_{max.}$ 272 mμ. (log ε = 3·99) and 270 mμ. (log ε = 3·89), respectively] for their acid oxidation products.

It appears that the alternative explanation, which was not considered by Krusi, is the more probable, especially as the length of the side chain in the tetracyclic triterpenes, lanostadienol and euphadienol, is not known with certainty. Accordingly, we suggest that dehydrogenation with *N*-bromosuccinimide involves the introduction of a nuclear conjugated system $\cdot CH{:}\overset{|}{C}\cdot\overset{|}{C}{:}CH\cdot$ (cf. Dorée *et al.*, *loc. cit.*) which on further oxidation leads to the 1 : 4-unsaturated diketo-group situated in the nucleus and the simultaneous fission of the *iso*propylidene side chain resulting in the formation of the carboxyl group. On this hypothesis, Krusi's acidic oxidation product, and that of Dupont *et al.*, may both be formulated on the basis of a parent monobasic diketotrisnor-acid, $C_{27}H_{40}O_5$ (cf. diketotrisnorlanostenolic acid), the fifth oxygen atom being present in the secondary alcoholic hydroxyl group. The following table summarises the results of Krusi and of Dupont *et al.*

	Dupont *et al.*		Krusi.
	M. p.	[α].	M. p.
Acid	231—231·5°	+14·2°	233—234°
	245·5—246·5 †	+22·9	231—233 ‡
Methyl ester	160·5—161	+13·0	170—171
	167·5—169·5 *		168—170 ‡
	190—190·5 †		
Methyl ester of hydrolysed product			185—186

* Crystallised from acetic anhydride. † Crystallised from aqueous alcohol.
‡ Resublimed.

A comparison of these two products shows their close similarity and it appears on this evidence that the two acids are identical although certain discrepancies have still to be explained. For instance, Krusi isolated his acid by hydrolysis of the methyl acetoxy-ester, whereas Dupont *et al.* obtained theirs by direct oxidation of euphadienyl acetate. It is possible that in Dupont's work hydrolysis occurred during isolation, or that Krusi incompletely hydrolysed his compound.

The reported oxidation by Dupont of his acid to the nor-acid, $C_{26}H_{38}O_5$, probably involves a simple oxidation of the secondary alcoholic group to give a triketo-acid, $C_{27}H_{38}O_5$ (cf. formation of methyl diketotrisnorlanostenonate from diketotrisnorlanostenolic acid). In favour of our tentative suggestions, it should be noted that the analytical data recorded by both groups of workers may be fairly satisfactorily accommodated on our assumption that the parent acid possesses the molecular formula, $C_{27}H_{40}O_5$. We hope soon to report experiments designed to prove conclusively the correctness of this assumption.

Experimental.

All m. p.s are uncorrected. Specific rotations were measured in chloroform solution at 20°. Aluminium oxide used in chromatographic work was from Mesrs. Peter Spence Ltd. (Grade " O "). All analyses are by Drs. Weiler and Strauss, Oxford.

Oxidation of Lanostadienyl Acetate according to Marker et al. (loc. cit.).—Lanostadienyl acetate (5 g.) in glacial acetic acid (100 ml.) was treated at 90° with chromic acid (10 g.) in 90% acetic acid (50 ml.), added dropwise during 3 hours with constant mechanical stirring; this temperature was maintained for a further 3 hours, and the mixture then set aside at room temperature overnight. The excess of chromic acid was destroyed by addition of ethyl alcohol, and the solution evaporated to dryness under reduced pressure. The residue was dissolved in ether, and the ethereal solution washed with water and sodium hydrogen carbonate solution, then extracted with 10% sodium hydroxide solution. The alkaline extracts were washed with ether to remove any neutral fraction and acidified to Congo-red with dilute hydrochloric acid, and the precipitated solids extracted with ether. The ethereal layer was washed with water and dried (Na_2SO_4), and the solvent removed to leave a dark yellow gum (4 g.). This was dissolved in ethyl acetate, and the solution on long storage deposited a yellow amorphous product (3 g.), m. p. 80—81° (Found : C, 72·2; H, 9·1. Calc. for $C_{25}H_{46}O_2$: C, 79·3; H, 12·3. Calc. for $C_{29}H_{42}O_6$: C, 71·6; H, 8·6%). Attempts to obtain crystalline salts were unsuccessful; methylation, using diazomethane, followed by chromatography gave no crystalline material.

Oxidation of lanostenyl acetate (5 g.) with chromic acid, under the same conditions as for lanostadienyl acetate, gave a small non-crystalline acid fraction (0·2 g.), the main product being the neutral diketo-lanostenyl acetate, m. p. 158—159°, $[\alpha]_D$ +91·6° (*c*, 0·024), identical with an authentic specimen.

Modified Method for the Preparation of Marker's Oxidation Product.—Lanostadienyl acetate (5 g.) in glacial acetic acid (300 ml.) was treated at 90° with a solution of chromic acid (2·5 g.) in 90% acetic acid (50 ml.) added dropwise, with stirring, during an hour. Stirring was continued at the same temperature for a further hour, after which the mixture was poured on crushed ice, and the excess of chromic acid destroyed by addition of sulphurous acid. The precipitated solid was filtered off and taken up in ether, and the ethereal solution extracted with 10% sodium hydroxide solution. The alkaline extracts were washed with ether and acidified to Congo-red with dilute hydrochloric acid, and the precipitated acid was extracted with ether in the usual manner to give a yellow gum (3 g.) on the removal of the ether. Crystallisation of this product from ethyl acetate gave an amorphous yellow material, m. p. 79—81°, identical with that prepared from lanostadienyl acetate according to Marker's procedure (Found : equiv., 400, 420. Calc. for $C_{29}H_{42}O_6$: equiv., 486. An acetyl determination showed the presence of 0·95 acetoxy-group).

Treatment of Marker's Oxidation Product with Alkali.—The oxidation product (2 g.) was heated under reflux with a 2% solution of alcoholic potassium hydroxide (200 ml.) for 3 hours, and the mixture then poured into water and extracted with ether in the usual manner. Removal of the ether gave no residue. The alkaline solution was acidified to Congo-red with dilute hydrochloric acid, and the precipitated solids were extracted with ether, the ethereal layer was washed and dried (Na_2SO_4), and the solvent removed to leave a pale yellow solid, which, crystallised from ethyl acetate, had m. p. 201—203°. Two further crystallisations from ethyl acetate gave pale yellow needles of *diketotrisnorlanostenolic acid*, m. p. 205—206°, $[\alpha]_D$ +104° (*c*, 0·026) (Found : C, 73·2, 73·1; H, 8·9, 8·9%; equiv., 440, 438. $C_{27}H_{40}O_5$ requires C, 73·0; H, 9·0%; equiv., 444).

Methylation of this acid by means of diazomethane, and crystallisation of the product from aqueous methanol, gave the *methyl* ester, m. p. 175—176° (Found : C, 73·0; H, 9·2. $C_{28}H_{42}O_5$ requires C, 73·3; H, 9·2%).

Benzoylation. Diketotrisnorlanostenolic acid (1 g.) in pyridine (25 ml.) was treated with benzoyl chloride (1·5 ml.) at room temperature for 16 hours. The mixture was poured into water, filtered, washed with dilute hydrochloric acid, and crystallised from acetic acid, giving a product, m. p. 200°; two further crystallisations from the same solvent gave yellow prisms of *diketotrisnorlanostenolic acid benzoate*, m. p. 218—220°, $[\alpha]_D$ +112° (*c*, 0·04) (Found : C, 74·3; H, 8·2. $C_{34}H_{44}O_6$ requires C, 74·5; H, 8·0%). Methylation of this acid by diazomethane, and crystallisation from ethyl acetate–methanol, gave the *methyl* ester, m. p. 197—199° (Found : C, 75·0; H, 8·0. $C_{35}H_{46}O_6$ requires C, 74·7; H, 8·2%).

Oxidation. Diketotrisnorlanostenolic acid (0·25 g.) in glacial acetic acid (25 ml.) was treated at 45—50° with a solution of chromic acid (0·15 g.) in 90% acetic acid (5 ml.). The temperature was maintained for 3 hours, and the excess of chromic acid was then destroyed with sulphurous acid, and the mixture poured into ice-water. The precipitated solids were extracted with ether and worked up in the usual manner, to give a yellow oil which could not be crystallised. This was taken up in ether and treated with an ethereal solution of diazomethane for 4 hours. The product on removal of the ether was crystallised repeatedly from methanol, giving yellow needles of *methyl diketotrisnorlanostenonate*, m. p. 149—150°, $[\alpha]_D$ +172° (*c*, 0·036) (Found : C, 73·8; H, 8·9. $C_{28}H_{40}O_5$ requires C, 73·7; H, 8·8%).

Hydrolysis of this ester with 3% alcoholic potassium hydroxide gave only intractable yellow gums.

Oxidation of Lanostadienyl Benzoate.—Lanostadienyl benzoate (2 g.) in glacial acetic acid (300 ml.) was stirred mechanically at 80—90° while a solution of chromic acid (4 g.) in 90% acetic acid (60 ml.) was added during an hour. At the end of this time the suspended benzoate had passed completely into solution; the temperature was maintained at 80—90°, with stirring, for a further 2 hours. The mixture was then poured into ice-water, the excess of chromic acid destroyed with sulphurous acid, and the precipitate filtered off, washed, and air-dried, and then dissolved in ether. The ethereal layer was washed and separated into a neutral and an acidic fraction in the usual manner. The oily neutral product (0·1 g.) was not further investigated. The acid fraction, crystallised from acetic acid, had m. p. 210°, raised by two further crystallisations to 218—220°, $[\alpha]_D$ +112°. This was diketotrisnorlanostenolic acid benzoate, identical with that prepared by benzoylation of diketotrisnorlanostenolic acid (Found: C, 74·5, 74·3; H, 8·1, 8·1%).

Ozonolysis of Lanostadienol.—Lanostadienol (1 g.), dissolved in absolute chloroform (25 ml.), was treated with a dry stream of ozonised oxygen (3% v/v) for 3 hours. The solvent was removed under reduced pressure, and the glassy residue boiled under reflux with water (200 ml.) for 1 hour; 75 ml. of the liquid were then distilled into a solution of 2 : 4-dinitrophenylhydrazine in 10% hydrochloric acid. The yellow precipitate was filtered off and crystallised from alcohol, giving orange needles of acetone 2 : 4-dinitrophenylhydrazone, m. p. 128° (40%) (Found: C, 45·4; H, 4·3; N, 23·3. Calc. for $C_9H_{10}O_4N_4$: C, 45·4; H, 4·2; N, 23·5%). The non-volatile residue was separated into a neutral and an acidic fraction. The latter was worked up in one of two ways. Repeated crystallisation from acetic acid gave trisnorlanostenolic acid, m. p. 257—259°, $[\alpha]_D$ +52° (*c*, 0·065) (Found: C, 77·7; H, 10·7. Calc. for $C_{27}H_{44}O_3$: C, 77·8; H, 10·6%).

Methylation (diazomethane) gave the methyl ester, m. p. 154—155° (Found: C, 78·0; H, 10·9. Calc. for $C_{28}H_{46}O_3$: C, 78·1; H, 10·8%). Alternatively, the product was methylated with diazomethane, this product was adsorbed on alumina (12 × 1·3 cm.) from benzene–hexane (1 : 1; 100 ml.), and the column eluted with the same solvent mixture (250 ml.). Removal of the solvent and crystallisation of the residue from chloroform–methanol gave methyl trisnorlanostenolate, m. p. 154—155°. Hydrolysis gave the acid, m. p. 257—259°, identical with that obtained above.

Oxidation of Methyl Trisnorlanostenolate.—Oxidation of methyl trisnorlanostenolate, by the method described above for the oxidation of diketotrisnorlanostenolic acid, gave methyl diketotrisnorlanostenonate, m. p. 149—150°, $[\alpha]_D$ +170° (*c*, 0·04), identical with an authentic specimen. This ester could be obtained directly from lanostadienol in poor yield by following Bellamy and Dorée's conditions (*J.*, 1941, 172). The acidic oxidation product was methylated with diazomethane, and the crude methyl ester dissolved in benzene (150 ml.) and adsorbed on a column of alumina (12 × 1·3 cm.), and the column eluted with benzene (200 ml.). Removal of the solvent and crystallisation of the residue from methanol gave methyl diketotrisnorlanostenonate, identical with that prepared above.

Oxidation of Lanostenol.—Lanostenol (4·8 g.) in "AnalaR" acetic acid (400 ml.) was treated at 80—85° with a solution of chromic acid (8 g.) in 90% acetic acid (160 ml.), added dropwise during 2 hours with mechanical stirring. The mixture was poured on crushed ice, the excess of oxidant destroyed with sulphurous acid, and the solution extracted with ether. The ethereal layer was washed repeatedly with 5% sodium hydroxide solution until the alkaline washings were tinged faintly with red (this indicated that all the acetic acid had been removed). Further extraction gave a reddish-brown alkaline solution, from which, when kept overnight, crystals of the sodium salt separated. These were filtered off, suspended in ether, and added to dilute hydrochloric acid, in which they dissolved; the ethereal solution was washed and dried (Na_2SO_4), and the solvent removed to leave a small solid residue (50 mg.), which on crystallisation from acetic acid gave a colourless solid, m. p. 195—196°, $[\alpha]_D$ +87° (*c*, 0·05) (Found: C, 76·0; 76·1; H, 10·6, 10·5%; equiv., 240, 244. Calc. for $C_{30}H_{50}O_4$: C, 75·9; H, 10·6%; equiv., 237). Methylation in the usual manner gave a methyl ester as an uncrystallisable oil [Found: (Zeisel's method), OMe, 11·8, 11·6. Calc. for $C_{32}H_{54}O_4$: OMe, 12·28%].

This acid was identical with the dicarboxylic acid obtained by oxidation of hydroxymethylenelanostenone (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472).

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