

408. *The Chemistry of the Triterpenes and Related Compounds.*
Part XXXV. Some Non-acidic Constituents of Polyporus pinicola, Fr.*

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Two new trimethyl-steroids, 21-hydroxylanosta-7:9(11)-24-trien-3-one and lanosta-7:9(11):24-triene-3 β :21-diol, have been isolated from the neutral extract of *Polyporus pinicola* Fr. Another component appears to be a mixture of ergost-7-en- and ergosta-7:22-dien-3 β -ol.

NUMEROUS publications describing the neutral constituents of the higher fungi have established that they include a complex mixture of sterols related to ergosterol.^{1,2} In particular, Zellner and his co-workers have frequently recorded the presence of ergosterol and "ergosterol-like" compounds in the extracts of the sporophores of various Basidiomycetes. These sterol mixtures were difficult to separate and it is clear that in many cases the "ergosterol" was impure and inadequately characterised. Compounds other than ergosterol have also been reported.^{3,4}

From *Polyporus pinicola* Hartmann and Zellner isolated a number of neutral substances with high melting points and also recorded the isolation of fungisterol as well as ergosterol.⁵ It is likely, however, that their fungisterol was not pure. Fungisterol, now known to be ergost-7-en-3 β -ol,⁶ is also said to be a constituent of *Calocera viscosa*,⁷ *Polyporus confluens* Fr.,³ *P. sulphureus* (Bull) Fr.,⁸ *Hydnum imbricatum* L.,⁹ and *Geaster fimbriatus* Fr.¹⁰ Ergosta-7:22-diene-3-one has been isolated from *Fomes fomentarius*.¹¹ Schmid and

* Part XXXIV, *J.*, 1959, 1877.

¹ Cf. Rosenthal, *Sitzungsber. Akad. Wiss. Wien*, IIB, 1922, **131**, 189.

² Cf. Elsevier's "Encyclopaedia of Organic Chemistry," 1940, Vol. XIV; 1954 Vol. XIV S.

³ Fröschl and Zellner, *Monatsh.*, 1929, **53**—**54**, 146.

⁴ Sumi, *Chem. Zentr.*, 1931, I, 1773.

⁵ Hartmann and Zellner, *Monatsh.*, 1928, **50**, 193.

⁶ Wieland and Coutelle, *Annalen*, 1941, **548**, 270.

⁷ Fröschl and Zellner, *Monatsh.*, 1928, **50**, 201.

⁸ Zellner and Zikmunda, *ibid.*, 1930, **56**, 200.

⁹ Lukacs and Zellner, *ibid.*, 1933, **62**, 214.

¹⁰ Ruthner and Zellner, *ibid.*, 1935, **66**, 76.

¹¹ Arthur, Halsall, and Smith, *J.*, 1958, 2603.

Czerny^{12,13} recently described the isolation of a sterol mixture, said to be ergosterol and fungisterol, from the light petroleum extract of *P. pinicola*, and also of an oxo-alcohol, m. p. 116°, $[\alpha]_D +71^\circ$, λ_{\max} 2360, 2450, and 2520 Å (ϵ 5400, 6000, and 4200).

Continuing our systematic examination of the wood-rotting fungi we have now examined some of the neutral constituents of *Polyporus pinicola* Fr. The first constituent isolated was a small amount of material with the melting point of ergosterol but with a much smaller negative rotation and a lower intensity of absorption at 2820 Å. The main component (A) isolated had m. p. 157—159° and $[\alpha]_D -13^\circ$ and showed no selective ultra-violet absorption. Its analysis agreed with the formula $C_{28}H_{46(48)}O$. Its constants resemble those reported for "anasterol," a substance isolated as its benzoate by Wieland and Gough¹⁴ from the mother-liquors from the crystallisation of impure ergosteryl benzoate. The constants of component A and its derivatives are compared with those of ergosta-7:22-dien-3 β -ol and the dihydro-derivative, fungisterol (cf. Table). Component A may be a mixture of ergosta-7:22-dien- and ergost-7-en-3 β -ol.

Another constituent was isolated as its acetate, the ultraviolet absorption of which indicated that it contained the 7:9(11)-diene system of a trimethyl-steroid. Hydrolysis gave a ketol, reduction of which yielded lanosta-7:9(11)-24-triene-3 β :21-diol (III) identical both with an authentic sample prepared from pinicolic acid A (I) and with a further constituent of the neutral material from *P. pinicola*.

	Alcohol		Acetate		Benzoate	
	M. p.	$[\alpha]_D$	M. p.	$[\alpha]_D$	M. p.	$[\alpha]_D$
Ergosta-7:22-dien-3 β -ol	176° ^a	-19°	178—180° ^a	-19°	200°	-10°
Component A	157—159	-13	167—168	-6	189—192	-2
Ergost-7-en-3 β -ol (fungisterol) ...	148 ^b	-2	157—159 ^b	-4	180-5 ^b	+2
"Anasterol"	157—159 ^c	-8	—	—	180—182 ^c	-14

^a Barton and Cox, *J.*, 1948, 1354. ^b *Idem, ibid.*, p. 783. ^c Ref. 14.

The conversion of pinicolic acid A (I) into the trienediol (III) was carried out by chromic acid oxidation of its methyl ester to methyl 3:7-dioxolanosta-8:24-dien-21-oate (II) which was reduced with lithium aluminium hydride to the intermediate allylic triol. This was dehydrated with acetic anhydride, and the product hydrolysed to the diol (III).

Of the two possible structures (IV and VI) initially considered for the ketol the latter was excluded because oxidation of the ketol gave the keto-aldehyde (V) which had infrared bands at 2700 and 1725 cm^{-1} typical of an aldehyde group and absent from the spectrum of its precursor. The change in rotation on reduction of the carbonyl group in the ketol (IV) ($\Delta M_D + 72^\circ$) corresponds well with that occurring on reduction of agnosterone (VII) ($\Delta M_D + 71^\circ$). The ketol was obtained by Oppenauer oxidation of the diol (III) prepared from pinicolic acid A (I).

These results differ somewhat from those obtained in a preliminary examination carried out some time ago on a small amount of fungus when two products were obtained in addition to crude ergosterol.¹⁵ The first was an oxo-alcohol, m. p. 114—117°, $[\alpha]_D +68.5^\circ$, λ_{\max} 2360 and 2430, inflexion 2510 Å (ϵ 4000, 4500, and 3000). These constants are very similar to those of the oxo-alcohol obtained by Schmid and Czerny¹³ and the two materials are almost certainly identical. These constants indicate a mixture of 21-hydroxy-lanost-8:24-dien-3-one (75%) and the corresponding dehydro-derivative [7:9(11)-diene (IV)]. Reduction of this mixture with sodium borohydride gave a mixture of diols, $[\alpha]_D +57^\circ$, m. p. 189—192°, undepressed* on admixture with pure lanosta-8:24-diene-3 β :21-diol, m. p. 189—192°, $[\alpha]_D +57^\circ$, prepared by reduction of pinicolic acid A with

* One feature of this field of chemistry is the absence of melting-point depression when a significant amount of a 7:9(11)-diene is mixed with the parent 8-ene.

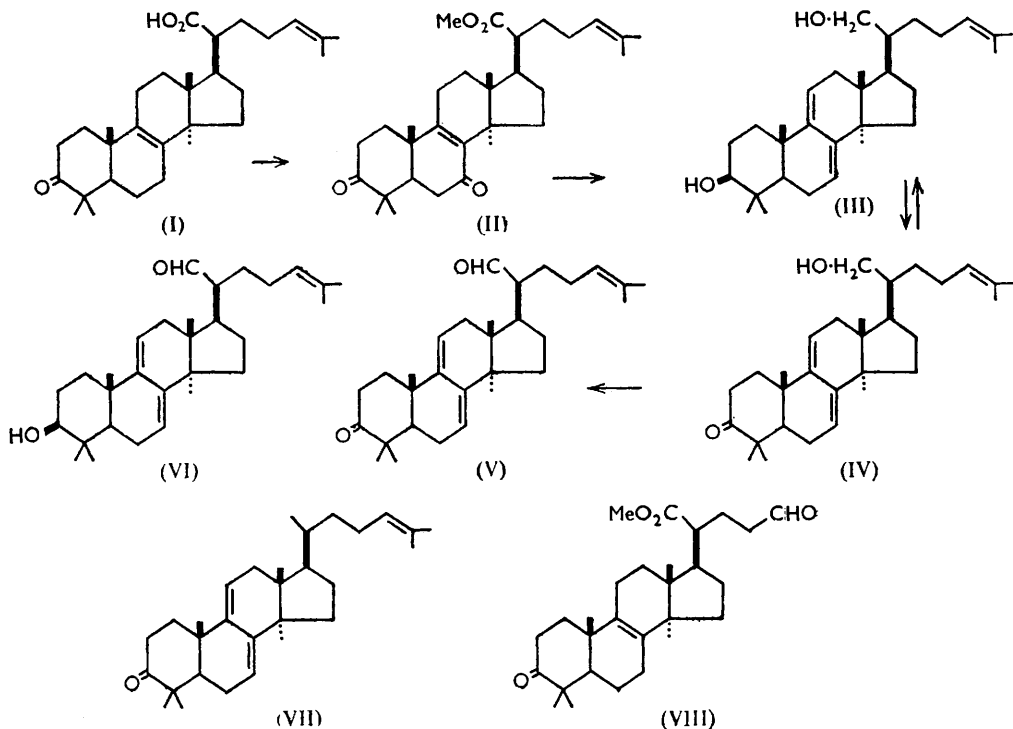
¹² Schmid and Czerny, *Sci. Pharm.*, 1953, **21**, 258.

¹³ *Idem, Monatsh.*, 1954, **85**, 1307.

¹⁴ Wieland and Gough, *Annalen*, 1930, **482**, 36.

¹⁵ Guider, Ph.D. Thesis, Manchester, 1954.

lithium aluminium hydride.¹⁶ The second product, m. p. 189–192°, $[\alpha]_D +60^\circ$, λ_{\max} 2360 and 2430, inflexion 2510 Å (ϵ 8500, 9700, and 6500), was apparently a mixture of the corresponding diol and its dehydro-derivative [7:9(11)-diene] (III)]. It is probable that the percentage of the trimethyl-steroid fraction in the oxidised 7:9(11)-diene form differs



in different samples of fungus, possibly varying with the conditions of growth and time of collection.

The preparation of a trisnor-derivative (VIII) of pinicolic acid A (I) needed as a reference compound is described below.

EXPERIMENTAL

M. p.s were determined on a Kofler block and are corrected. Rotations were determined for chloroform solution at room temperature. Alumina for chromatography was usually alumina (activity II) which had been deactivated with 5% of 10% aqueous acetic acid. Light petroleum refers to the fraction with b. p. 60–80°. Infrared spectra were determined for CS₂ solution unless otherwise stated.

Extraction of Polyporus pinicola and Isolation of the Neutral Fraction.—The fungus (9.5 g.) extracted with alcohol, and the extract separated into acidic and neutral fractions in the usual way. An ethereal solution of the neutral fraction was repeatedly extracted with cold 5% aqueous potassium hydroxide and then kept with cold 10% methanolic potassium hydroxide for several days after which it was again separated into neutral and acidic fractions. The neutral fraction (93.5 g.) in methanol (500 c.c.) was heated under reflux for 24 hr. with 1% methanolic potassium hydroxide (2 l.). The solution was cooled to 20° and diluted with ether (2 l.) and water (2 l.). The ethereal layer was separated and the aqueous layer twice more extracted with ether (2 l.). Working up the ethereal extracts in the usual manner gave the non-saponifiable neutral fraction as a dark-brown gum (86.5 g.).

Chromatographic Separation of the Neutral Fraction.—This fraction (86 g.) was adsorbed

¹⁶ Guider, Halsall, and Jones, *J.*, 1954, 4471.

from light petroleum-benzene (3 : 1; 1.5 l.) on alumina (2 kg.; deactivated with 10% of 10% acetic acid) and eluted as shown:

Fraction	Eluent	Vol. (l.)	Wt. (g.)	Appearance	$\lambda_{\max.}$ (principal)
1	Light petroleum	18	53.5	Waxy brown solid	2430, 2820 Å
2	Benzene	12	13	Pale brown solid	2430 Å
3	Ether	15	19.8	Brown gum	—

Examination of fraction 1. This fraction crystallised from methanol as plates (fraction 1A) (6.78 g.), m. p. 157—163°. Evaporation of the mother-liquor gave a dark gum (fraction 1B) (46 g.) which slowly gave a crystalline mass (needles).

(i) Fraction 1A. Repeated recrystallisation of this fraction from methanol gave a small amount of plates, m. p. 163—166°, undepressed on admixture with ergosterol monohydrate, $[\alpha]_D -18^\circ$ (c 1.15), $\lambda_{\max.}$ 2710, 2810, and 2930 Å (ϵ 6300, 6600, and 3700). The residue (6 g.) from the combined mother-liquors from these crystallisations was examined by chromatography, 1 g. of it being adsorbed from light petroleum-benzene (1 : 1; 85 c.c.) on alumina (100 g.). Elution with benzene (600 c.c.) gave compound A (185 mg.), m. p. 157—159° (needles from methanol; leaflets from acetone), $[\alpha]_D -13^\circ$ (c 1.04) (Found: C, 82.45; H, 11.85. Calc. for $C_{28}H_{46}O_1.5CH_3.OH$: C, 82.55; H, 11.65%). Compound A showed no significant ultraviolet light absorption but infrared max. (in Nujol) at 3350, 1640, 870, 845, 827, and 795 cm^{-1} .

Compound A (80 mg.) in pyridine (1.5 c.c.) was acetylated with acetic anhydride (0.5 c.c.) at 20° for 24 hr. Isolation with ether gave compound A acetate, m. p. 167—168° (needles from methanol and acetone), $[\alpha]_D -6^\circ$ (c 1.02) (Found: C, 81.65; H, 10.9. Calc. for $C_{30}H_{48}O_2$: C, 81.75; H, 11.0%), $\nu_{\max.}$ (in Nujol) 1730, 1640, 840, 825, and 795 cm^{-1} . Hydrolysis of the acetate with methanolic potassium hydroxide gave back compound A, m. p. and mixed m. p. 156—158°.

Compound A (75 mg.) in pyridine (1.5 c.c.) was treated with benzoyl chloride (0.5 c.c.) at 20° for 24 hr. Isolation with ether yielded a product (105 mg.) which was adsorbed from light petroleum (25 c.c.) on alumina (10 g.). Elution with light petroleum (50 c.c.) gave compound A benzoate, m. p. 189—192° (plates from methanol-ethyl acetate and ethyl acetate), $[\alpha]_D -2^\circ$ (c 1.03) (Found: C, 83.65; H, 10.4. Calc. for $C_{35}H_{50}O_3$: C, 83.6; H, 10.0%), $\nu_{\max.}$ 1710, 840, 825, and 795 cm^{-1} .

(ii) Isolation of 21-hydroxylanosta-7 : 9(11) : 24-trien-3-one (IV) from fraction 1B. This fraction (1 g.) in light petroleum-benzene (3 : 1; 100 c.c.) was adsorbed on alumina (100 g.). Elution with light petroleum-benzene (1 : 1; 300 c.c.) gave a gum (265 mg.) which slowly crystallised in contact with methanol to give the impure ketol, m. p. 114—117°, $\lambda_{\max.}$ 2430 Å (ϵ 8900). Only small and impure quantities of crystalline material were obtained from other fractions, some of which showed low intensity maxima at 2430 and 2810 Å.

The impure ketol (240 mg.) in pyridine (5 c.c.) was treated with acetic anhydride (1.5 c.c.) at 20° for 24 hr. Isolation with ether gave a product (230 mg.) which was adsorbed from light petroleum (25 c.c.) on alumina (20 g.). Elution with light petroleum-benzene (3 : 1; 350 c.c.) gave 21-acetoxylanosta-7 : 9(11) : 24-trien-3-one, m. p. 111—113° (from methanol and aqueous methanol), $[\alpha]_D +81^\circ$ (c 1.08) (Found: C, 79.65; H, 9.85. $C_{32}H_{48}O_3$ requires C, 79.95; H, 10.1%), $\lambda_{\max.}$ 2360, 2430, and 2510 Å (ϵ 15,000, 16,800, and 11,400).

21-Acetoxylanosta-7 : 9(11) : 24-trien-3-one (100 mg.) in methanol (10 c.c.) was treated with 10% methanolic potassium hydroxide (10 c.c.) at 20° overnight. Isolation with ether gave the ketol as a gum which was adsorbed from light petroleum (20 c.c.) on alumina (10 g.). Elution with light petroleum-benzene (1 : 1; 150 c.c.) gave 21-hydroxylanosta-7 : 9(11) : 24-trien-3-one as needles (from dilute methanol or ethanol), m. p. 119—121°, $[\alpha]_D +56^\circ$ (c 0.97) (Found: C, 82.4; H, 10.4. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%), $\lambda_{\max.}$ 2360, 2430, and 2510 Å (ϵ 10,400, 12,300, and 7800), $\nu_{\max.}$ 1710 and 3600 cm^{-1} .

The ketol (50 mg.) in purified dioxan (5 c.c.) was reduced with sodium borohydride (15 mg.) in aqueous dioxan (1 : 1; 5 c.c.) at 20° for 1 hr. After dilution with water isolation with ether yielded a product which was adsorbed from benzene (5 c.c.) on alumina (10 g.). Elution with benzene-ether (9 : 1; 50 c.c.) gave lanosta-7 : 9(11) : 24-triene-3 β : 21-diol, m. p. 194—196° (from methanol), identical with an authentic sample of the diol (see below).

The ketol (80 mg.) in acetone (20 c.c.) was oxidised with chromic acid in the usual manner.¹⁷ Isolation with ether gave a product which was adsorbed from light petroleum (7 c.c.) on alumina

¹⁷ Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2555.

(10 g.). Elution with light petroleum-benzene (9 : 1; 100 c.c.) gave lanosta-7 : 9(11) : 24-triene-3 : 21-dione, m. p. 141—142° (from methanol), identical with an authentic sample (see below).

Examination of fraction 2. This fraction (13 g.) crystallised from methanol as needles (fraction 2A) (3.85 g.), m. p. 185—190°. Evaporation of the mother-liquors gave a resinous solid (fraction 2B).

(a) Isolation of lanosta-7 : 9(11) : 24-triene-3 β : 21-diol (III). Fraction 2A was adsorbed from benzene (200 c.c.) on alumina (400 g.). Elution with benzene-ether (9 : 1; 2 l.) gave lanosta-7 : 9(11) : 24-triene-3 β : 21-diol, which was obtained as needles (from methanol), m. p. 194—197°, $[\alpha]_D^{25} + 72^\circ$ (*c* 1.06) (Found: C, 81.5; H, 11.15. C₃₀H₄₈O₂ requires C, 81.75; H, 11.0%), λ_{\max} 2360, 2430, and 2510 Å (ϵ 12,900, 15,300, and 10,300). The diol (200 mg.) was acetylated in pyridine (5.5 c.c.) with acetic anhydride (2.5 c.c.) at 20° for 24 hr. to give 3 β : 21-diacetoxylanosta-7 : 9(11) : 24-triene as needles (from methanol), m. p. 122°, $[\alpha]_D^{25} + 73^\circ$ (*c* 0.8) (Found: C, 78.0; H, 10.0. C₃₄H₅₂O₄ requires C, 77.8; H, 10.0%).

(b) Examination of fraction 2B. Fraction 2B (1 g.) in benzene was adsorbed on alumina (100 g.). Elution with benzene-ether (3 : 1; 500 c.c.) gave several fractions (350 mg.) which crystallised from methanol as needles, having m. p.s within the range 175—190°. These fractions were not pure and contained up to 25% of conjugated dienes.

Examination of fraction 3. Chromatography of this fraction did not yield any crystalline fractions.

Oxidation of Lanosta-7 : 9(11) : 24-triene-3 β : 21-diol.—The diol (300 mg.) in acetone (100 c.c.) was oxidised with chromic acid in the usual manner. Isolation with ether gave a product which was adsorbed from light petroleum (35 c.c.) on alumina (20 g.). Elution with light petroleum-benzene (9 : 1; 400 c.c.) gave lanosta-7 : 9(11) : 24-triene-3 : 21-dione (V) (175 mg.) as stout needles (from methanol), m. p. 142—143°, $[\alpha]_D^{25} + 49^\circ$ (*c* 0.99) (Found: C, 82.7; H, 9.9. C₃₀H₄₄O₂ requires C, 82.5; H, 10.15%), λ_{\max} at 2360, 2430, and 2510 Å (ϵ 15,300, 17,200, and 11,500), ν_{\max} 2700, 1725, and 1710 cm.⁻¹.

Oppenauer Oxidation of Lanosta-7 : 9(11)-24-triene-3 β : 21-diol.—The diol (200 mg.) and aluminium *tert.*-butoxide (106 mg.) in dioxan (15 c.c.) were heated to the b. p., treated with cyclohexanone (5 c.c.), and heated under reflux for 24 hr. After dilution with water and acidification, isolation with ether yielded a gum which was adsorbed from light petroleum-benzene (2 : 1; 40 c.c.) on alumina (20 g.).

Elution with light petroleum-benzene (2 : 1; 350 c.c.) yielded a gum (100 mg.) which was dissolved in pyridine (2 c.c.) and acetylated with acetic anhydride (0.6 c.c.). Isolation with ether afforded a product which was adsorbed on alumina (10 g.) from light petroleum (10 c.c.). Elution with light petroleum-benzene (3 : 1; 150 c.c.) gave 21-acetoxylanosta-7 : 9(11) : 24-trien-3-one as needles (from methanol), m. p. 112—114°, $[\alpha]_D^{25} + 81^\circ$ (*c* 1.0), identical with that prepared from the ketol from the fungus.

Conversion of Methyl Pinicolate A into Lanosta-7 : 9(11) : 24-triene-3 β : 21-diol (III).—Methyl pinicolate A (500 mg.) in acetic acid (10 c.c.) was treated with recrystallised chromic acid (150 mg.; 2.1 mol. with respect to oxygen) in acetic acid (10 c.c.) at 85° for 10 min. Dilution with water and isolation with ether yielded a product (λ_{\max} 2520 Å) which was adsorbed from light petroleum (30 c.c.) on alumina (50 g.). Elution with light petroleum-benzene (2 : 1; 300 c.c.) gave unchanged methyl pinicolate A (200 mg.), m. p. 119—121° (from methanol).

Elution with benzene (400 c.c.) afforded a gum (250 mg.) which slowly formed a sticky solid which was extremely soluble in organic solvents. It showed maximal light absorption at 2520 Å (ϵ 9800) indicative of the presence of methyl 3 : 7-dioxolanosta-8 : 24-dien-21-oate.

The crude methyl 3 : 7-dioxolanosta-8 : 24-dien-21-oate (250 mg.) described above in ether (50 c.c.) was heated under reflux with lithium aluminium hydride (520 mg.) for 3 hr. The excess of reagent was destroyed with ethyl acetate, and the complex decomposed with dilute hydrochloric acid. Isolation with ether gave a solid which was heated under reflux with acetic anhydride (30 c.c.) for 1 hr. Dilution with water and extraction with ether afforded a product which was heated under reflux with 10% methanolic potassium hydroxide (35 c.c.) for 30 min. Isolation with ether gave the crude diol which was adsorbed from benzene (25 c.c.) on alumina (20 g.). Elution with light petroleum-benzene (9 : 1; 400 c.c.) gave lanosta-7 : 9(11) : 24-triene-3 β : 21-diol (145 mg.) which crystallised from methanol as needles, m. p. 196—197°, identical with the diol from the fungus.

Lanosta-8 : 24-diene-3 β : 21-diol [with Miss J. M. GUIDER].—Methyl pinicolate A (150 mg.)

in ether (50 c.c.) was heated under reflux with lithium aluminium hydride (100 mg.) for 1 hr. After decomposition of the excess of hydride, the complex was decomposed with an aqueous solution of tartaric acid. Extraction with ether gave *lanosta-8:24-diene-3 β :21-diol*, m. p. 189—192° (needles from methanol and from acetone), $[\alpha]_D +57^\circ$ (*c* 0.94 in pyridine) (Found: C, 81.6; H, 11.3. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%).

Conversion of Methyl Pinicolate A into Methyl 3:24-Dioxotrisnorlanost-8-en-21-oate.—Methyl pinicolate A (280 mg.) in dry ether (5 c.c.) was treated with osmium tetroxide (160 mg.) at 20° for 3 days. The solution was evaporated to dryness under reduced pressure and the residue in chloroform (50 c.c.) was shaken with a solution of mannitol (2.5 g.) and potassium hydroxide (2.5 g.) in water (50 c.c.) for 24 hr. Isolation with chloroform gave the crude diol as a gum (260 mg.) which was dissolved in ethanol (50 c.c.) and treated with a solution of sodium metaperiodate (260 mg.) in water (5 c.c.). A little water was added to clarify the solution which was kept at 20° for 2 days. Isolation with ether afforded a product which was adsorbed from light petroleum-benzene (9:1; 55 c.c.) on alumina (15 g.). Elution with light petroleum-benzene (1:1; 400 c.c.) gave a gum (65 mg.) which crystallised from methanol to give *methyl 3:24-dioxotrisnorlanost-8-en-21-oate* as needles, m. p. 138—139°, $[\alpha]_D +67^\circ$ (*c* 1.19) (Found: C, 76.15; H, 9.65. $C_{28}H_{42}O_4$ requires C, 75.95; H, 9.55%), ν_{max} 2700, 1730, and 1708 cm^{-1} .

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