520. *The Chemistry of the Triterpenes and Related Compounds. Part XVIII.* Elucidation of the Structure of Polyporenic Acid C.*

By A. Bowers, T. G. Halsall, E. R. H. Jones, and (in part) A. J. Lemin.

Polyporenic acid C has been characterised as a triethenoid tetracyclic hydroxy-keto-carboxylic acid with the formula $C_{31}H_{46}O_4$. Its structure (XXXIV) has been finally established by conversion into a derivative of eburicoic acid which has in turn been converted (Holker, Powell, Robertson, Simes, Wright, and Gascoigne, *J.*, 1953, 2422) into a lanosterol derivative. Eburicoic acid and polyporenic acid C can be regarded as derivatives of trimethylergostane. It is of particular interest that the carbon skeleton of the side chains is identical with that of the major fungal sterol, ergosterol, suggesting some common paths in their biosynthesis.

In 1939 Cross, Eliot, Heilbron, and Jones (*J.*, 1940, 632) carried out a re-investigation of the constituents of the birch tree fungus, *Polyporus betulinus* Fr., which led to the isolation of polyporenic acids A, B, and C. It was suggested that these acids might be triterpenoids and in the case of polyporenic acid C a formula $C_{30}H_{46}O_4$ or $C_{30}H_{48}O_4$ was suggested on the basis of an analysis of methyl polyporenate C, m. p. 192—193°. The work was abandoned in 1940 and it is only recently that it has become possible to examine polyporenic acid C in detail.

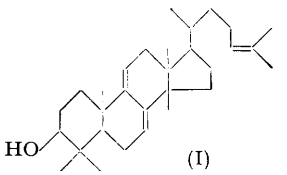
In the interval only one communication relating to polyporenic acid C has appeared. Birkinshaw, Morgan, and Findlay (*Biochem. J.*, 1952, **50**, 509) have described the isolation of an acid ($C_{30}H_{46}O_4$), m. p. 285—290°, from the metabolic products of the wood-rotting fungus *Polyporus benzoinus* (Wahl) Fr. The acid gave colour reactions characteristic of triterpenoid acids and was converted into a neutral monomethyl ester, m. p. 192—193°. Ozonolysis of the acid afforded a 53% yield of formaldehyde. It was suggested that the

* Part XVII, *J.*, 1953, 590.

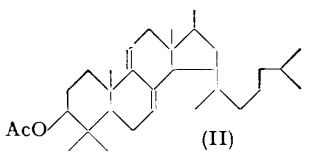
acid was a dihydroxy-monocarboxylic acid possessing a vinylidene group. Further, attention was drawn to the possibility that the acid might be polyporenic acid C and this was enhanced when a sample of methyl polyporenate C showed no depression in melting point on admixture with the methyl ester of the *P. benzoinus* acid. Through the courtesy of Dr. J. H. Birkinshaw it has been possible for us to compare the infra-red spectra of the methyl esters of polyporenic acid C and of the *benzoinus* acid. They are identical and it may be concluded that the *benzoinus* acid is very probably polyporenic acid C. However, for reasons which appear in the sequel, our conclusions about the nature of polyporenic acid C differ from those drawn by Birkinshaw *et al.* (*loc. cit.*).

At first great difficulty was experienced in isolating appreciable quantities of acid C, partly owing to the lack of a suitable method of estimating it in impure samples. This handicap was overcome when methyl polyporenate C was found to contain a conjugated diene system and the purification of the acid could be followed by spectrographic examination. The further discovery that polyporenic acid C contained a keto-group (see below) enabled a satisfactory method for its isolation based on a Girard separation to be developed.

Pure methyl polyporenate C, m. p. 198—199°, analysed for either $C_{31}H_{46}O_4$ or $C_{32}H_{48}O_4$, and showed maximal light absorption at 2360, 2430, and 2510 Å (ε = 14,600, 16,900, and 11,200 respectively). These values, indicating the presence of a heteroannular diene system, are very similar to those of methyl dehydroeburicoate and of dehydrolanosterol (I), and different from those of dehydroeuphenyl acetate (II) (cf. Dawson, Halsall, and Swayne, *J.*, 1953, 590). This suggested that the lanostane ring system (III) might be present in polyporenic acid C. It seemed possible that polyporenic acid C might be dehydropolyporenic acid A, especially in view of the co-existence of eburicoic acid and dehydroeburicoic acid in a number of fungi (Gascoigne, Holker, Ralph, and Robertson, *J.*, 1951, 2346). However, the ultra-violet spectrum of methyl polyporenate C indicated the presence of a keto-group (inflexion at 2760—2820 Å, ε = 57), and a 2 : 4-dinitrophenylhydrazone was prepared. Determination of the infra-red spectrum in carbon tetrachloride revealed a band at 1712 cm.$^{-1}$, indicative of a keto-group in either a six-membered ring or an aliphatic side chain. The infra-red spectrum also had bands at 3641 cm.$^{-1}$, and at 891 and 1639 cm.$^{-1}$, indicative of hydroxyl and vinylidene groups respectively. Thus methyl



(I)



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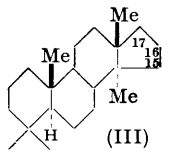


(III)

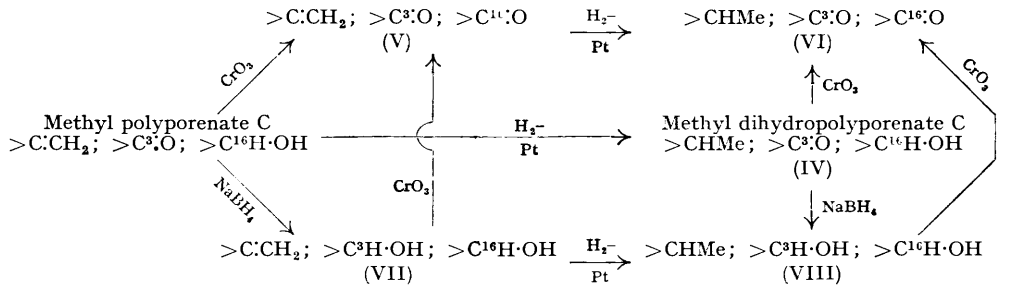
polyporenate C could be formulated as the ester of a hydroxy-keto-acid. The analytical data did not enable a clear-cut decision to be made between $C_{31}H_{46}O_4$ and $C_{32}H_{48}O_4$ for the ester, but, if the presence of three double bonds is assumed, both possibilities indicated the tetracyclic nature of polyporenic acid C. As will become clear in the sequel the correct formula of the acid is $C_{31}H_{46}O_4$ and that of the methyl ester, $C_{32}H_{48}O_4$.

Ozonolysis of methyl polyporenate C gave formaldehyde, isolated in 44% yield as its dimethone. Hydrogenation gave methyl dihydropolyporenate C, showing the same ultra-violet absorption as the parent ester, but no absorption in the infra-red corresponding to a vinylidene group. Thus the latter group was not part of the diene system, and the presence of three double bonds in acid C was confirmed.

Hydrolysis of the methyl ester was not as easy as with methyl polyporenate A, heating under reflux with 10% methanolic potassium hydroxide for 16 hr. being necessary. This indicated some hindrance of the carboxyl group although not as intense as that in, for example, oleanolic acid. Acetylation of the acid gave only a monoacetate and no evidence of the formation of a diacetate was obtained.

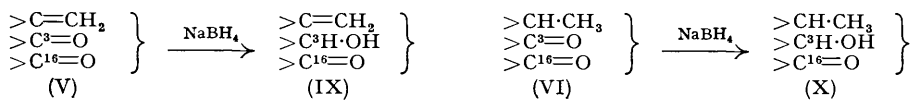
The simple oxidation, reduction, and hydrogenation reactions which have been carried out starting from the methyl ester are summarised schematically below. The diene system remained unaffected during these transformations. The systematic nomenclature

of the various products on the basis of eburicane for the saturated parent hydrocarbon is given in the Experimental section.

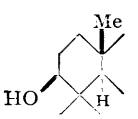


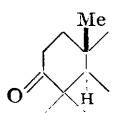
The ultra-violet spectrum of the diketo-ester (V), obtained by oxidation of methyl polyporenate C, showed that the diene system was not conjugated with a keto-group. The original hydroxyl group cannot therefore be in a position α to the diene system. Further evidence about its location was provided by the infra-red spectrum of the diketo-ester which had an additional band at 1743 cm.$^{-1}$ due to the new keto-group derived from the original hydroxyl group. This must therefore be attached to a five-membered ring (Jones *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 2024).

Sodium borohydride treatment of the diketo-esters (V and VI) derived from methyl polyporenate C and methyl dihydropolyporenate C effected preferential reduction of the original, and apparently less hindered, keto-group with the formation of the keto-alcohols (IX) and (X) respectively. Hydrogenation of the alcohol (IX) gave (X), and acetylation of both gave monoacetates. The products of these selective borohydride reductions showed no infra-red absorption band corresponding to a carbonyl group in a six-membered ring. It is therefore the keto-group in the five-membered ring arising from the original (16-)hydroxyl group, which is apparently somewhat more hindered than the original (3-)keto-group in the six-membered ring.



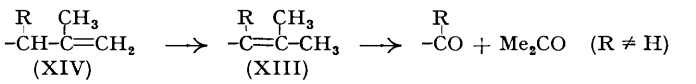
Dehydration of the sodium borohydride reduction product (X) with phosphorus pentachloride in light petroleum gave a product which on ozonolysis yielded acetone, isolated in 75% yield as its 2 : 4-dinitrophenylhydrazone. Analogous dehydrations are well known with triterpenes having partial structure (XI) (cf. Christen, Dünnenberger Roth, Heusser,

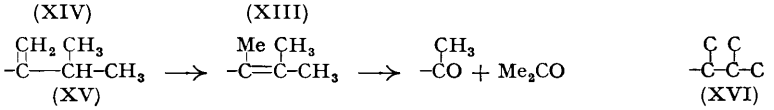
(XI) 



(XII)

and Jeger, *Helv. Chim. Acta*, 1952, **35**, 1757) and it was concluded that polyporenic acid C possessed a typical triterpene ring A but containing a keto-group as in (XII), and that reduction of the keto-group gave a hydroxyl group with the normal β-configuration (*i.e.*, XI).

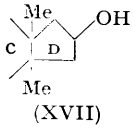




Polyporenic acid C, in contrast to polyporenic acid A, was not decarboxylated on melting, making a βγ-unsaturated acid formula improbable. The unconjugated double bond on the other hand migrated under acidic conditions; treatment of a chloroformic

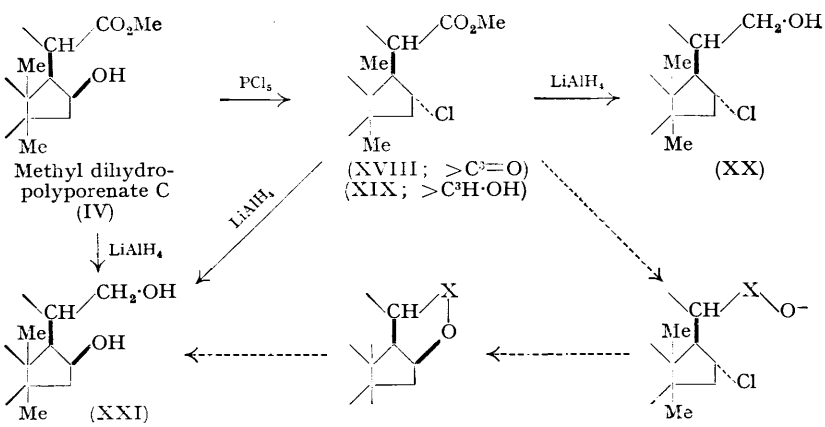
solution of methyl polyporenate C with hydrogen chloride gave an *iso*-compound (methyl *iso*polyporenate C; double bond as in XIII), ozonolysis of which gave acetone and a neutral compound, not oxidised further by chromic acid in acetone. These results suggested that the neutral component was a ketone rather than an aldehyde and were consistent with the structures (XIV) and (XV). Either structure is consistent with the presence in polyporenic acid C of a side chain terminating with the carbon skeleton (XVI).

In an attempt to dehydrate methyl dihydropolyporenate C, it was treated with phosphorus pentachloride. Instead of elimination occurring, the hydroxyl group was replaced by chlorine. This replacement would not occur if either of the carbon atoms adjacent to the CH·OH group was fully substituted, in which circumstances elimination would be expected to occur, as with (XI) above. If the lanostane ring system (III) were present in polyporenic acid C, a possibility indicated earlier, then the only carbon atom in the five-membered ring D to which the hydroxyl group could be attached would be $C_{(16)}$ (cf. XVII).



(XVII)

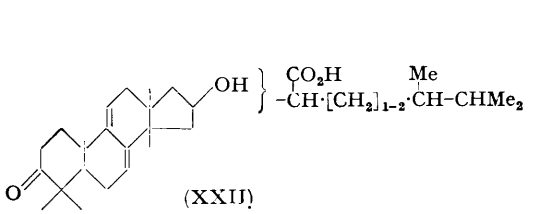
The keto-group in the chloro-compound (XVIII) was reduced with sodium borohydride and the resulting chloro-hydroxy-ester (XIX) was treated with lithium aluminium hydride to reduce the methoxycarbonyl group. Besides the expected chlorodihydroxy-compound (XX), a triol was obtained which was identical with the triol (XXI) resulting from the reduction of methyl dihydropolyporenate C with lithium aluminium hydride. The chlorine atom had thus been replaced by a hydroxyl group: a most unexpected result. One possible explanation appeared to be that the methoxycarbonyl group was in either the γ or the δ position to the chlorine-substituted carbon atom, and that during the reduction a negative ion arising from the carboxyl group displaced the chlorine atom in an S_N2 reaction, and that the resulting intermediate was later hydrolysed. When the possibility of a steroid-type side chain in acid C was borne in mind this explanation suggested the sequence of reactions (IV ⟶ XXI), and the presence in polyporenic acid C of the partial grouping represented in (IV). The lithium aluminium hydride reduction will be discussed more fully in a later publication. When the work already described was complete it was possible to put forward a partial structure (XXII) for dihydropolyporenic acid C which was based on the lanostane ring system and included the groupings (XII) and (XVI), and the 5-membered ring system in (IV). An attractive complete structure was (XXIII) which has a C_{31} formula and bears the same relation to the lanosterol carbon skeleton as that



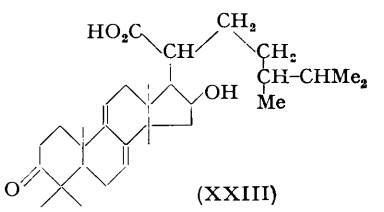
of ergostanol does to the cholestanol skeleton. This somewhat revolutionary C_{31} possibility had been under consideration for some time for polyporenic acid A (cf. Cross, Eliot, Heilbron, and Jones, *J.*, 1940, 632, and the comment on the formula of polyporenic acid A; Curtis, Heilbron, Jones, and Woods, *J.*, 1953, 457) although it suffered from the apparent disadvantage that it did not allow an isoprenoid structure. However, with the elucidation of the structure of lanosterol and the proof that it was a C_{30} trimethyl-steroid, not obeying

the isoprene rule (cf. Voser, Mijovic, Heusser, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1952, **35**, 2414), this inhibition disappeared. In fact the existence of trimethyl-steroids with ergostanol and stigmastanol type side-chains appeared an attractive possibility.

At this stage, through the courtesy of Professor A. Robertson, F.R.S., Dr. J. S. E. Holker, and their colleagues at Liverpool, we learned that a relation had been established between

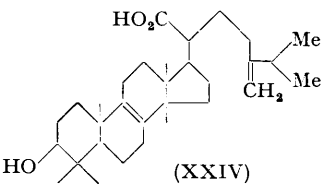


(XXII)



(XXIII)

lanosterol and eburicoic acid and that the latter had now been proved to be a C_{31} compound with structure (XXIV) (Holker, Powell, Robertson, Simes, Wright, and Gascoigne, *J.*, 1953, 2422). It was apparent that if dihydropolyporenic acid C was (XXIII) it should be convertible into a compound derived from eburicoic acid, *e.g.*, the dehydrodihydro-acid (XXV). The sodium borohydride reduction product (X) of the ketone (VI) obtained by oxidising methyl dihydropolyporenate C was reduced by the Wolff–Kishner method. The product was methylated and acetylated and proved to be identical with methyl acetyl-



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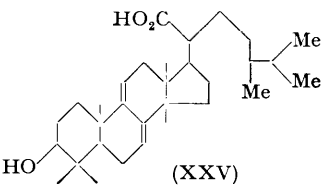
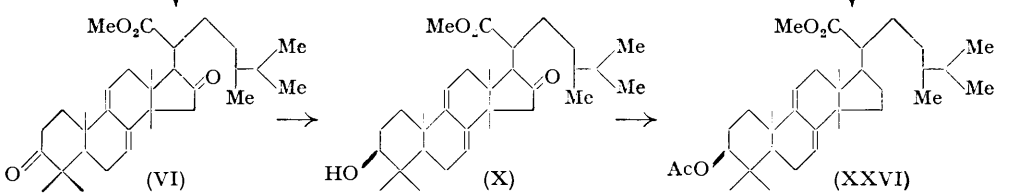


(XXV)

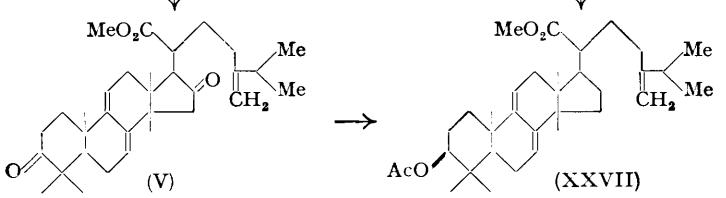
Methyl dihydro-polyporenate C (XXIII)

Eburicoic acid (XXIV)



Methyl polyporenate C

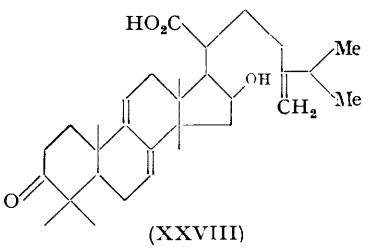
Eburicoic acid (XXIV)



dehydrodihydroeburicoate (XXVI) from eburicoic acid. A similar series of reactions starting from the sodium borohydride reduction product (IX) of the ketone (V) from methyl polyporenate C gave methyl acetyldehydroeburicoate (XXVII). Structure (XXIII) was thus confirmed for dihydropolyporenic acid C, and (XXVIII) followed for polyporenic acid C, the position of the vinylidene group being established by the conversion into methyl acetyldehydroeburicoate (XXVII).

On the assumption that no stereochemical inversion of the carbon atom carrying the carboxyl group occurs during the conversion into an eburicoic acid derivative the parent hydrocarbon of polyporenic acid C is eburicane (XXIX) (Holker *et al.*, *loc. cit.*), the stereochemistry of which is the same as that of lanostane (cf. Voser *et al.*, *loc. cit.*; Barnes, Barton, Fawcett, and Thomas, *J.*, 1953, 576). The systematic naming of polyporenic acid C derivatives described in this paper is based on eburicane which is numbered according to the rules of steroid nomenclature (*J.*, 1951, 3526) [there is no C_{29} as this number is reserved for the additional carbon atom of the stigmastanol side chain (XXX)].

The evidence presented so far for the position (16) of the hydroxyl group in polyporenic acid C has depended on the phosphorus pentachloride reaction and on the interpretation of the lithium aluminium hydride reaction described above (XIX —→ XXI). This is supported by the molecular-rotation difference between the acetate of the 16-oxo-



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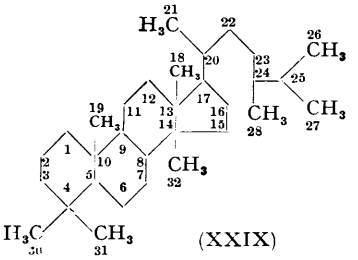
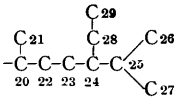


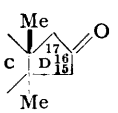
(XXIX)

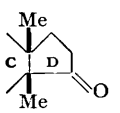


(XXX)

compound (X) and the corresponding deoxo-compound (XXVI). The value (M_D −498°) is characteristic of the groupings (XXXI) and (XXXII) (Klyne, *J.*, 1952, 2916). Structure (XXXII) is additionally excluded, however, as it involves a C/D *cis*-fusion, while the C/D ring fusion in polyporenic acid C must be *trans*, as in lanosterol (XXXIII), as indicated by *X*-ray evidence (Curtis, Fridricksons, and Mathieson, *Nature*, 1952, **170**, 321) and by molecular-rotation data (Barnes, Barton, Fawcett, and Thomas, *loc. cit.*). Partial structure (XXXI) must therefore be present in the 16-oxo-compound (X).

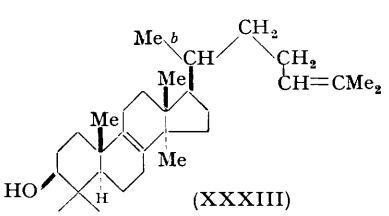
(XXXI)





(XXXII)

The infra-red spectra of the 16-oxo-compounds (IX) and (X) both exhibit a band at 1419 cm.$^{-1}$ in carbon tetrachloride. This is probably due to the bending vibration of the C–H bonds of the $C_{(15)}$ methylene group in the α-position to the carbonyl group. Jones and Cole (*J. Amer. Chem. Soc.*, 1952, **74**, 5648) list the positions of the bands due to the C–H bending vibration of $C_{(16)}$ methylene groups in a series of $C_{(17)}$ keto-steroids and conclude that they lie between 1404 and 1411 cm.$^{-1}$. Barton, Cole, *et al.* (*Chem. and Ind.*,



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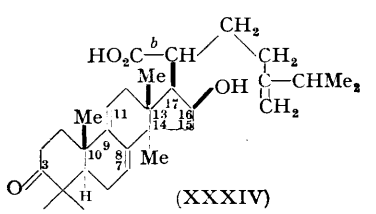

(XXXIV)

1952, 426) find that the analogous band of two 17-oxolanosterol derivatives also lies in this region. On the other hand, the infra-red spectrum of a carbon tetrachloride solution of kryptogenin acetate (kindly supplied by Dr. C. Djerassi), in which there is a $C_{(15)}$ methylene group in the α-position to a 16-oxo-group, as in derivatives of polyporenic acid C, exhibits no band between 1404 and 1411 cm.$^{-1}$. There is a band at 1418 cm.$^{-1}$ but it is not known whether this is due to the $C_{(15)}$ or the $C_{(23)}$ methylene group. These results suggest that it

may be possible to distinguish between 16- and 17-keto-steroids on the basis of infra-red data.

The 3-hydroxyl group formed on reduction of the oxo group of polyporenic acid C must have the β(equatorial)-configuration because of the course of the phosphorus pentachloride dehydration reaction.

The configuration of the $C_{(16)}$-hydroxyl group in polyporenic acid C is more difficult to determine and evidence concerning it is not yet complete. The reactions so far investigated, including the lithium aluminium hydride reduction of the chloro-compound (XIX), are best explained if the $C_{(16)}$-hydroxyl group is assumed to be β.

On the basis of the evidence presented polyporenic acid C is formulated as (XXXIV).

Experimental

Rotations were determined in chloroform at room temperature unless otherwise stated. M. p.s were determined on a Kofler block and are corrected. The alumina used for chromatography had an activity I—II. Light petroleum refers to the fraction with b. p. 60—80°. Yields are of once crystallised material.

Extraction of Polyporenic Acid C (XXVIII; XXXIV) *from* Polyporus betulinus *Fr. and Isolation of the Methyl Ester.*—(Measurement of the light absorption at 2430 Å gave a useful indication of the amount of polyporenic acid C present at each stage of its extraction and purification.)

The residual fungus (4 kg.) after the extraction of polyporenic acid A (Curtis *et al., loc. cit.*) was reminced and suspended in boiling acetone for 16 hr. The solvent was decanted, and the last traces were removed by means of a basket centrifuge. Evaporation of the solvent from the combined solutions gave a greasy yellow solid (100 g.). Light absorption in ethanol: Max., 2430 Å; $\varepsilon = 4500$. This was suspended in ether (500 c.c.) and treated with an excess of diazomethane in ether. After several hours the excess of diazomethane was decomposed with acetic acid, and the ethereal solution filtered from an insoluble residue (10 g.). The residue obtained on removal of the ether was adsorbed from benzene (500 c.c.) on alumina (2 kg.). Elution with benzene–ether (9 : 1; 15 l.) gave a crude methyl ester (20 g.) which crystallised as needles, m. p. 175—190°, from methanol. Light absorption in ethanol: Max., 2430 Å; $\varepsilon = 14{,}500$.

Two methods have been adopted for purifying this crude methyl ester: (*a*) The crude methyl ester (8 g.) in ethanol (100 c.c.) containing acetic acid (10 c.c.) was refluxed with Girard's reagent T (7·5 g.) for 1 hr. The cooled mixture was poured into a solution of sodium carbonate (8·0 g.) in ice-water. Ether-extraction removed the non-ketonic material, and the residual aqueous solution was acidified with concentrated hydrochloric acid and kept at 20° for 1 hr. Ether-extraction and crystallisation from methanol gave *methyl polyporenate C* [*methyl* 16β-*hydroxy*-3-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate*] as needles (4·6 g.), m. p. 191—195° raised by repeated crystallisation from methanol, ethanol, or aqueous acetone, to 198—199°, $[\alpha]_D$ +10° (*c*, 0·95) (Found: C, 77·0; H, 9·85. $C_{32}H_{48}O_4$ requires C, 77·35; H, 9·75%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; $\varepsilon = 14{,}600$, 16,900, and 11,200. Inflexion, 2760—2820 Å; $\varepsilon = 57$. The infra-red spectrum determined in carbon tetrachloride had bands at 891, 1639, 1712, 1738, and 3641 $cm.^{-1}$.

(*b*) The crude methyl ester (8 g.) was adsorbed from benzene (200 c.c.) on alumina (400 g.). Elution with benzene–ether (9 : 1; 6 l.) and crystallisation from methanol gave needles of methyl polyporenate C (5·5 g.), m. p. 192—196°, raised by repeated crystallisation to 198—199°.

Isolation of Crude Polyporenic Acid C.—The solid (1 g.) from the acetone extract of the fungus (see above) was adsorbed from benzene (50 c.c.) on alumina (80 g.) which had been deactivated by shaking a suspension in benzene with aqueous acetic acid (8 g.; 10%). Elution with benzene–ether (1 : 1; 200 c.c.) gave a fraction which separated from acetic acid as an amorphous solid (300 mg.), m. p. 245—252°. Light absorption in ethanol: Max., 2430 Å; $\varepsilon = 12{,}000$. Methylation of this acid with diazomethane, followed by chromatographic purification, gave methyl polyporenate C identical with an authentic sample.

Hydrolysis of Methyl Polyporenate C.—Methyl polyporenate C (3·0 g.) was heated under reflux for 16 hr. with methanolic potassium hydroxide (250 c.c.; 10%). After dilution with water (400 c.c.) and two extractions with ether to remove non-acidic material, acidification with acetic acid precipitated the crude acid (2·7 g.), m. p. 237—260°. A solution in ether–dioxan

(7 : 3; 1500 c.c.) was adsorbed on alumina (200 g.) which had been deactivated by shaking a suspension in ether with aqueous acetic acid (15 g.; 10%). Elution with ether–methanol (3 : 1; 4 l.) gave 16β-*hydroxy*-3-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oic acid* (XXXIV) (1·4 g.), m. p. 273—276° (after repeated crystallisation from aqueous dioxan, acetic acid, or *iso*propanol), $[\alpha]_D$ +6° (*c*, 0·80 in pyridine) (Found: C, 76·6; H, 9·4. $C_{31}H_{46}O_4$ requires C, 77·1; H, 9·6%) (Found: equiv., 474, 468. $C_{33}H_{44}O_4$ requires equiv., 469. $C_{31}H_{46}O_4$ requires equiv., 483). Light absorption in ethanol: Max., 2360, 2430, and 2520 Å; ε = 14,550, 16,850, and 11,300.

Methylation of this acid with diazomethane followed by chromatographic purification gave methyl polyporenate C, m. p. 196—198°, undepressed on admixture with an authentic sample, $[\alpha]_D$ +10° (*c*, 1·31).

16β-*Acetoxy*-3-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oic Acid*.—Polyporenic acid C (500 mg.) in pyridine (3 c.c.), treated with acetic anhydride (0·75 c.c.) at 20° for 16 hr., gave the 16-*acetate* as needles (420 mg.) (from aqueous acetic acid), m. p. 193—198°, raised by crystallisation from acetic acid or methanol to 206—210°, $[\alpha]_D$ −22° (*c*, 1·1) (Found: C, 75·5; H, 9·15. $C_{33}H_{48}O_5$ requires C, 75·55; H, 9·2%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 16,350, 18,900, and 12,400.

Methyl 16β-*Acetoxy*-3-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate*.—Methyl polyporenate C (800 mg.) in pyridine (6 c.c.) was treated with acetic anhydride (3 c.c.) at 20° for 16 hr. Dilution with water and extraction with ether yielded a product which was adsorbed from light petroleum (50 c.c.) on alumina (80 g.). Elution with benzene (1 l.) gave the 16β-*acetoxy*-ester as needles (from methanol) (650 mg.), m. p. 122—124° (after repeated crystallisation from methanol or aqueous acetone), $[\alpha]_D$ −29° (*c*, 0·95) (Found: C, 75·45; H, 9·45. $C_{34}H_{50}O_5$ requires C, 75·8; H, 9·35%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 15,100, 17,800, and 11,800. Hydrolysis of the acetate (420 mg.) with methanolic potassium hydroxide (20 c.c.; 10%) at 20° for 16 hr. gave methyl polyporenate C (350 mg.), m. p. 196·5—198·5° (from methanol), undepressed on admixture with an authentic sample, and having $[\alpha]_D$ +11° (*c*, 0·95).

Methyl Polyporenate C 2 : 4-*Dinitrophenylhydrazone*.—Methyl polyporenate C (150 mg.) in ethanol (15 c.c.) was heated under reflux for ½ hr. with 2 : 4-dinitrophenylhydrazine (75 mg.) in ethanol (15 c.c.) containing a few drops of concentrated sulphuric acid. After dilution with water the derivative was isolated and adsorbed from benzene (25 c.c.) on alumina (15 g.). Elution with benzene gave the 2 : 4-*dinitrophenylhydrazone* which separated from ethanol–nitromethane as orange needles, m. p. 195—198°, $[\alpha]_D$ −69° (*c*, 1·00) (Found: C, 67·0; H, 7·95; N, 8·6. $C_{38}H_{52}O_7N_4$ requires C, 67·4; H, 7·75; N, 8·3%). Light absorption in ethanol: Max., 3660 Å; ε = 23,600.

Methyl 16β-*Hydroxy*-3-*oxoeburico*-7 : 9(11)-*dien*-21-*oate* (IV; XXIII).—Methyl polyporenate C (1·0 g.) in ethanol (75 c.c.) was hydrogenated for 45 min. at 20° in presence of Adams's catalyst (50 mg.) [uptake of H_2: 59 c.c. (N.T.P.). Calc. for 1 double bond: 55 c.c. (N.T.P.)]. After filtration and evaporation the product was adsorbed from benzene (50 c.c.) on alumina (100 g.). Elution with benzene–ether (7 : 3; 600 c.c.) gave *methyl* 16β-*hydroxy*-3-*oxoeburico*-7 : 9(11)-*dien*-21-*oate* as needles (900 mg.), m. p. 195—197° (after several crystallisations from acetone and methanol), $[\alpha]_D$ +10° (*c*, 1·12) (Found: C, 75·5; H, 10·0. $C_{32}H_{50}O_4,\frac{1}{2}CH_3{\cdot}OH$ requires C, 75·8; H, 10·2%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 14,750, 16,900, and 11,250. Inflexion, 2760—2820 Å; ε = 67. The infra-red spectrum determined in carbon tetrachloride had bands at 1714, 1738, and 3634 $cm.^{-1}$. The alcohol (450 mg.) in pyridine (20 c.c.) was treated with acetic anhydride (2·0 c.c.) at 20° for 16 hr. After dilution with water, isolation with ether gave the 16β-*acetoxy*-compound as needles (390 mg.), m. p. 131—137° (from methanol), raised by several crystallisations from methanol or aqueous ethanol to 139—141°, $[\alpha]_D$ −24° (*c*, 1·16) (Found: C, 75·7; H, 9·8. $C_{34}H_{52}O_5$ requires C, 75·5; H, 9·7%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 11,500, 13,150, and 8860.

General Method of Oxidation with Chromic Acid.—A cold solution of chromic acid (267 g.) in concentrated sulphuric acid (230 c.c.) and water (400 c.c.) was made up to 1 l. This solution is 8N with respect to oxygen. The compound to be oxidised was dissolved in pure acetone (distilled over potassium permanganate) at 20°, and the reagent added dropwise from a microburette until a persistent orange-brown coloration indicated that oxidation was complete.

Methyl 3 : 16-*Dioxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* (V).—Methyl polyporenate C (500 mg.) was oxidised as described above. After 10 min. dilution with water and isolation with ether yielded a product which was adsorbed from light petroleum (50 c.c.) on alumina (50 g.). Elution with light petroleum–benzene (1 : 1; 800 c.c.) gave *methyl* 3 : 16-*dioxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* as plates (350 mg.), m. p. 164·5—165·5° (after repeated crystallisation from methanol), $[\alpha]_D$ −68° (*c*, 1·00) (Found: C, 77·6; H, 9·4. $C_{32}H_{46}O_4$ requires C, 77·7;

H, 9·35%). Light absorption in ethanol: Max., 2360, 2430, and 2930 Å; ε = 14,700, 16,600, and 71. Inflexion, 2510 Å; ε = 11,000. The infra-red spectrum determined in Nujol had bands at 896, 1639, 1711, 1727, and 1743 cm.$^{-1}$. The *bis*-2 : 4-*dinitrophenylhydrazone* prepared in the usual way separated as needles, m. p. 233—236°, from methanol–nitromethane (Found: C, 62·0; H, 6·1; N, 13·45. $C_{44}H_{54}O_{10}N_8$ requires C, 61·8; H, 6·35; N, 13·1%). Light absorption in chloroform: Max., 3660 Å; ε = 51,200.

Methyl 3 : 16-*Dioxoeburico*-7 : 9(11)-*dien*-21-*oate* (VI).—(*a*) Methyl 3 : 16-dioxoeburico-7 : 9(11) : 24(28)-trien-21-oate (210 mg.) in ethanol (50 c.c.) was hydrogenated in the usual way (uptake of H_2 after 35 min., 1·1 mol.). Removal of the catalyst and evaporation of the filtrate yielded a product which was adsorbed from light petroleum (20 c.c.) on alumina (20 g.). Elution with light petroleum–benzene (1 : 1; 500 c.c.) gave *methyl* 3 : 16-*dioxoeburico*-7 : 9(11)-*dien*-21-*oate* as needles (180 mg.) or plates, m. p. 166—168° (after several crystallisations from methanol), $[\alpha]_D$ −67° (*c*, 0·95) (Found: C, 76·95, 77·45; H, 9·8, 9·65. $C_{32}H_{48}O_4$ requires C, 77·35; H, 9·75%). Light absorption in ethanol: Max., 2360, 2430, and 2930 Å; ε = 14,900, 16,600, and 86. Inflexion, 2510 Å; ε = 11,150. The infra-red spectrum determined in Nujol had bands at 1712, 1729, and 1738 cm.$^{-1}$.

(*b*) Methyl dihydropolyporenate C (200 mg.) in acetone (50 c.c.) was oxidised with chromic acid as described above. Isolation with ether, purification by chromatography, and crystallisation as in (*a*), yielded the same ester, $[\alpha]_D$ −68° (*c*, 0·85), m. p. 167—169° alone or mixed with a specimen prepared as in (*a*). The infra-red spectrum was identical.

Methyl 3β : 16β-*Dihydroxyeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* (VII).—Methyl polyporenate C (1·2 g.) in dioxan (50 c.c.) was treated with sodium borohydride (115 mg.) in aqueous dioxan (1 : 1; 15 c.c.) at 20° for 1 hr. Dilution with water and isolation with ether yielded a product which was adsorbed from benzene (100 c.c.) on alumina (120 g.). Elution with benzene–ether (4 : 1; 1300 c.c.) gave *methyl* 3β : 16β-*dihydroxyeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* as needles (900 mg.), m. p. 177—179° (after several crystallisations from methanol), $[\alpha]_D$ +26° (*c*, 1·14) (Found: C, 74·4; H, 9·9. $C_{32}H_{50}O_4,CH_3{\cdot}OH$ requires C, 74·65; H, 10·25%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 13,870, 16,400, and 10,970.

Methyl 3β : 16β-*Diacetoxyeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate*.—Methyl 3β : 16β-dihydroxyeburico-7 : 9(11) : 24(28)-trien-21-oate (200 mg.) in pyridine (3 c.c.) was treated with acetic anhydride (1 c.c.) at 20° for 16 hr. Dilution with water and isolation with ether yielded a product which was adsorbed from light petroleum (20 c.c.) on alumina (20 g.). Elution with light petroleum–benzene (1 : 1; 500 c.c.) and repeated crystallisation from methanol–nitromethane gave *methyl* 3β : 16β-*diacetoxyeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* as needles, m. p. 110—114°, remelting at 145—147°, $[\alpha]_D$ +24° (*c*, 0·90) (Found: C, 73·15; H, 9·3. $C_{36}H_{54}O_6,\frac{1}{2}CH_3{\cdot}OH$ requires C, 73·2; H, 9·45%). Light absorption in ethanol: Max., 2370, 2440, and 2520 Å; ε = 14,100, 16,400, and 10,800.

Oxidation of Methyl 3β : 16β-*Dihydroxyeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate*.—Methyl 3β : 16β-dihydroxyeburico-7 : 9(11) : 24(28)-trien-21-oate (100 mg.) in acetone (20 c.c.) was oxidised with chromic acid as described above. Isolation with ether yielded a product which was adsorbed from light petroleum (10 c.c.) on alumina (10 g.). Elution with light petroleum–benzene (1 : 1; 150 c.c.) gave methyl 3 : 16-dioxoeburico-7 : 9(11) : 24(28)-trien-21-oate (73 mg.), m. p. 164—165° (after two crystallisations from methanol) undepressed on admixture with an authentic sample, $[\alpha]_D$ −68° (*c*, 0·79).

Methyl 3β : 16β-*Dihydroxyeburico*-7 : 9(11)-*dien*-21-*oate* (VIII).—(*a*) Methyl dihydropolyporenate C (200 mg.) in dioxan (35 c.c.) was treated with sodium borohydride (20 mg.) in aqueous dioxan (1 : 1; 10 c.c.) at 20° for 1 hr. Dilution with water and isolation with ether yielded a product which was adsorbed from benzene (20 c.c.) on alumina (20 g.). Elution with benzene–ether (6 : 1; 300 c.c.) gave *methyl* 3β : 16β-*dihydroxyeburico*-7 : 9(11)-*dien*-21-*oate* as needles (175 mg.), m. p. 188—190° (after several crystallisations from methanol), $[\alpha]_D$ +24° (*c*, 0·86) (Found: C, 74·05; H, 10·4. $C_{32}H_{52}O_4,CH_3{\cdot}OH$ requires C, 74·4; H, 10·6%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 13,900, 16,300, and 11,000.

(*b*) Methyl 3β : 16β-dihydroxyeburico-7 : 9(11) : 24(28)-trien-21-oate (480 mg.) in glacial acetic acid (30 c.c.) was hydrogenated in the usual way [uptake of H_2 after 1·5 hr., 21 c.c. (N.T.P.). Calc. for one double bond: 22 c.c.]. Filtration and evaporation yielded a product which was adsorbed from benzene (50 c.c.) on alumina (50 g.). Elution with benzene–ether (6 : 1; 500 c.c.) gave methyl 3β : 16β-dihydroxyeburico-7 : 9(11)-dien-21-oate as needles (430 mg.), m. p. 188—190° (after repeated crystallisation from methanol or ethanol) undepressed on admixture with a specimen prepared as in (*a*), $[\alpha]_D$ +27° (*c*, 0·85).

Methyl 3 : 16-*Diacetoxyeburico*-7 : 9(11)-*dien*-21-*oate*.—Methyl 3 : 16-diacetoxyeburico-

7 : 9(11) : 24(28)-trien-21-oate (219 mg.) in ethanol (50 c.c.) was hydrogenated in the usual way. Filtration and evaporation yielded a product which was adsorbed from light petroleum–benzene (4 : 1; 20 c.c.) on alumina (20 g.). Elution with light petroleum–benzene (1 : 1; 500 c.c.) gave *methyl* 3 : 16-*diacetoxyeburico*-7 : 9(11)-*dien*-21-*oate* as needles (200 mg.), m. p. 169—171° (after several crystallisations from methanol), $[\alpha]_D$ +20° (*c*, 1·06) (Found: C, 74·05; H, 9·7. $C_{36}H_{56}O_6$ requires C, 73·95; H, 9·65%). Light absorption in ethanol: Max. 2360, 2430, and 2510 Å; ε = 13,900, 16,100, and 10,600.

Oxidation of Methyl 3β : 16β-*Dihydroxyeburico*-7 : 9(11)-*dien*-21-*oate*.—Methyl 3β : 16β-dihydroxyeburico-7 : 9(11)-dien-21-oate (200 mg.) in acetone (50 c.c.) was oxidised with chromic acid as described above. Isolation with ether yielded a product which was adsorbed from light petroleum (20 c.c.) on alumina (20 g.). Elution with light petroleum–benzene (1 : 1; 500 c.c.) gave methyl 3 : 16-dioxoeburico-7 : 9(11)-dien-21-oate as needles (160 mg.), m. p. 165·5—167·5° (after repeated crystallisation from methanol and aqueous acetone) undepressed on admixture with an authentic sample. $[\alpha]_D$ −67° (*c*, 0·74). The infra-red spectrum was identical with that of an authentic sample.

Methyl 3β-*Hydroxy*-16-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* (IX).—Methyl 3 : 16-dioxoeburico-7 : 9(11) : 24(28)-trien-21-oate (480 mg.) in dioxan (30 c.c.) was treated with sodium borohydride (90 mg.) in aqueous dioxan (1 : 1; 30 c.c.) at 20° for 1 hr. Dilution with water and isolation with ether then yielded a product which was adsorbed from light petroleum (50 c.c.) on alumina (50 g.). Elution with benzene (900 c.c.) gave *methyl* 3β-*hydroxy*-16-*oxoeburico*-7 : 9(11)-24(28)-*trien*-21-*oate* as flat needles (330 mg.), m. p. 156—158° (after several crystallisations from aqueous methanol), $[\alpha]_D$ −52° (*c*, 1·00) (Found: C, 76·9; H, 9·75. $C_{32}H_{48}O_4$ requires C, 77·35; H, 9·75%). Light absorption in ethanol: Max., 2430 Å; ε = 16,600. Inflexions, 2370, 2510, and 2890 Å; ε = 15,100, 11,300, and 51. The infra-red spectrum determined in carbon tetrachloride had bands at 893, 1648, and 1743 cm.$^{-1}$. The 1743-cm.$^{-1}$ band was broad and due to two carbonyl groups, the methoxycarbonyl C=O group and the 5 membered-ring C=O group.

Oxidation of Methyl 3β-*Hydroxy*-16-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate*.—Methyl 3β-hydroxy-16-oxoeburico-7 : 9(11) : 24(28)-trien-21-oate (70 mg.) in acetone (20 c.c.) was oxidised with chromic acid as described above. Isolation with ether yielded a product which was purified by chromatography on alumina, to give methyl 3 : 16-dioxoeburico-7 : 9(11) : 24(28)-trien-21-oate (50 mg.) as plates (from methanol), m. p. 164—165° undepressed on admixture of the sample with an authentic specimen, $[\alpha]_D$ −69° (*c*, 0·87). The infra-red spectrum was identical with that of an authentic sample.

Methyl 3β-*Acetoxy*-16-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate*.—Methyl 3β-hydroxy-16-oxoeburico-7 : 9(11) : 24(28)-trien-21-oate (235 mg.) in pyridine (10 c.c.) was treated with acetic anhydride (1·5 c.c.) at 20° for 16 hr. Dilution with water and extraction with ether then yielded a product which was adsorbed from light petroleum (20 c.c.) on alumina (20 g.). Elution with benzene (400 c.c.) gave *methyl* 3β-*acetoxy*-16-*oxoeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* as needles (200 mg.), m. p. 186—188° (after repeated crystallisation from methanol), $[\alpha]_D$ −18° (*c*, 1·25) (Found: C, 75·35; H, 9·3. $C_{34}H_{50}O_5$ requires C, 75·8; H, 9·35%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 13,400, 15,000, and 9900.

Methyl 3β-*Hydroxy*-16-*oxoeburico*-7 : 9(11)-*dien*-21-*oate* (X).—(*a*) Methyl 3β-hydroxy-16-oxoeburico-7 : 9(11) : 24(28)-trien-21-oate (315 mg.) in ethanol (50 c.c.) was hydrogenated in the usual way [uptake of H_2 after 1 hr., 19·5 c.c. (N.T.P.). Calc. for one double bond: 21 c.c.]. Filtration and evaporation yielded a product which was adsorbed from light petroleum (30 c.c.) on alumina (30 g.). Elution with benzene–ether (4 : 1; 600 c.c.) gave *methyl* 3β-*hydroxy*-16-*oxoeburico*-7 : 9(11)-*dien*-21-*oate* as plates (250 mg.), m. p. 174—177° (after repeated crystallisation from aqueous methanol), $[\alpha]_D$ −54° (*c*, 1·03) (Found: C, 77·05; H, 10·1. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%). Light absorption in ethanol: Max., 2430 Å; ε = 16,800. Inflexions, 2360, 2510, and 2940 Å; ε = 14,900, 11,300, and 35.

(*b*) Methyl 3 : 16-dioxoeburico-7 : 9(11)-dien-21-oate (300 mg.) in dioxan (40 c.c.) was treated with sodium borohydride (55 mg.) in aqueous dioxan (1 : 1, 15 c.c.) for 1 hr. at 20°. Dilution with water and isolation with ether then yielded a product which was adsorbed from benzene (30 c.c.) on alumina (30 g.). Elution with benzene–ether (4 : 1; 600 c.c.) gave methyl 3β-hydroxy-16-oxoeburico-7 : 9(11)-dien-21-oate as plates (225 mg.), m. p. 174—177° (after several crystallisations from aqueous methanol) undepressed on admixture with a specimen prepared as in (*a*), $[\alpha]_D$ −51° (*c*, 0·93). The infra-red spectrum was identical with that of a specimen prepared as in (*a*).

Methyl 3β-*Acetoxy*-16-*oxoeburico*-7 : 9(11)-*dien*-21-*oate*.—Methyl 3β-hydroxy-16-oxoburico-

7 : 9(11)-dien-21-oate (450 mg.) in pyridine (15 c.c.) was treated with acetic anhydride (3·0 c.c.) at 20° for 16 hr. After dilution with water, isolation with ether gave a product which was adsorbed from light petroleum (30 c.c.) on alumina (20 g.). Elution with light petroleum–benzene (1 : 1; 600 c.c.) gave the 3β-*acetate* as needles (400 mg.) (from methanol), m. p. 185—189° raised by several crystallisations from ethanol to 191—192°, $[\alpha]_D$ −24° (*c*, 1·18) (Found: C, 75·7; H, 9·6. $C_{34}H_{52}O_5$ requires C, 75·5; H, 9·7%).

Ozonolysis of Methyl Polyporènate C.—Methyl polyporenate C (540 mg.) in glacial acetic acid (20 c.c.) was treated with ozonised oxygen at 20° for 2 hr. Water was added and the mixture steam-distilled, the distillate being passed into a solution of dimedone (300 mg.) in aqueous ethanol (4 : 1; 10 c.c.). After 16 hr. at 0° the precipitate was collected, dried (143 mg.), and recrystallised from ethanol; it had m. p. 189—190°, undepressed on admixture with an authentic sample of the formaldehyde derivative, m. p. 189—190° (Found: C, 69·7; H, 8·25. Calc. for $C_{17}H_{24}O_4$: C, 69·8; H, 8·2%).

Dehydration of Methyl 3β-*Hydroxy*-16-*oxoeburico*-7 : 9(11)-*dien*-21-*oate.*—A suspension of methyl 3β-hydroxy-16-oxoeburico-7 : 9(11)-dien-21-oate (530 mg.) in light petroleum (50 c.c.) was shaken for 10 min. at 20° with phosphorus pentachloride (300 mg.). After addition to water, isolation with light petroleum gave a product which was adsorbed on alumina (30 g.) and eluted with light petroleum–benzene (1 : 1; 600 c.c.), to give a colourless oil (330 mg.) which has not yet crystallised.

Ozonolysis of Phosphorus Pentachloride Dehydration Product.—The dehydration product (230 mg.) in glacial acetic acid (20 c.c.) was treated with ozonised oxygen for 45 min. at 20°. Water was added and the mixture steam-distilled, the distillate being passed into a solution of dimedone (300 mg.) in methanol (15 c.c.). No precipitate resulted. The dimedone solution was in turn steam-distilled, the distillate being passed into a solution of 2 : 4-dinitrophenylhydrazine (300 mg.) in methanol (15 c.c.) containing a few drops of concentrated hydrochloric acid. Extraction of this solution with benzene gave a product which, after purification by chromatography, was crystallised from methanol and from ethanol to give acetone 2 : 4-dinitrophenylhydrazone (89 mg.) as plates, m. p. 124—125°, undepressed on admixture of the sample with an authentic specimen (Found: N, 23·75. Calc. for $C_9H_{10}O_4N_4$: N, 23·5%).

Methyl 16β-*Hydroxy*-3-*oxoeburico*-7 : 9(11) : 24(25)-*trien*-21-*oate* (XIII).—Hydrogen chloride was passed into a solution of methyl polyporenate C (911 mg.) in chloroform (250 c.c.) for 3 hr. at 20°. The solution was then poured on ice-water. Isolation with chloroform gave *methyl* 16β-*hydroxy*-3-*oxoeburico*-7 : 9(11) : 24(25)-*trien*-21-*oate* as needles (780 mg.), m. p. 174—177° (after several crystallisations from ethanol or aqueous acetone), $[\alpha]_D$ +3° (*c*, 1·19) (difficulty was experienced in the analysis of this compound. The least unsatisfactory analysis was: Found: C, 76·65; H, 9·65. $C_{32}H_{48}O_4$ requires C, 77·35; H, 9·75%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 13,000, 15,100, and 9950.

Ozonolysis of Methyl 16β-*Hydroxy*-3-*oxoeburico*-7 : 9(11) : 24(25)-*trien*-21-*oate.*—Methyl 16β-hydroxy-3-oxoeburico-7 : 9(11) : 24(25)-trien-21-oate (250 mg.) in ethyl acetate (30 c.c.) was treated with ozonised oxygen for 1 hr. at 20°. Ferrous sulphate solution was added (5%; 20 c.c.) and the mixture steam-distilled. Treated as above, the distillate gave no dimedone derivative but afforded acetone 2 : 4-dinitrophenylhydrazone as needles (103 mg.), m. p. and mixed 123—124° (Found: N, 23·55. Calc. for $C_9H_{10}O_4N_4$: N, 23·5%). The non-volatile product was isolated by ether-extraction and then shaken with aqueous sodium carbonate solution. No acid product could be isolated. Oxidation with excess of chromic acid in acetone in the usual way did not produce an acid product. The neutral product did not crystallise.

Methyl 16α-*Chloro*-3-*oxoeburico*-7 : 9(11)-*dien*-21-*oate* (XVIII).—A suspension of methyl 16β-hydroxy-3-oxoeburico-7 : 9(11)-dien-21-oate (500 mg.) in light petroleum (50 c.c.) was shaken with phosphorus pentachloride (350 mg.) at 20° for 10 min., the alcohol dissolving completely. Water was added and the product extracted with light petroleum. Removal of the solvent gave *methyl* 16α-*chloro*-3-*oxoeburico*-7 : 9(11)-*dien*-21-*oate* as needles (430 mg.), m. p. 176—178° (after repeated crystallisation from ethanol or acetone), $[\alpha]_D$ +35° (*c*, 2·50) (Found: C, 74·4; H, 9·6; Cl, 7·25. $C_{32}H_{49}O_3Cl$ requires C, 74·3; H, 9·55; Cl, 6·9%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 15,600, 17,800, and 11,900.

Methyl 16α-*Chloro*-3β-*hydroxyeburico*-7 : 9(11)-*dien*-21-*oate* (XIX).—Methyl 16α-chloro-3-oxoeburico-7 : 9(11)-dien-21-oate (330 mg.) in dioxan (50 c.c.) was treated with sodium borohydride (32 mg.) in aqueous dioxan (1 : 1, 10 c.c.) at 20° for 1 hr. After dilution with water, isolation with ether yielded a product which was adsorbed from benzene (50 c.c.) on alumina (30 g.). Elution with benzene–ether (7 : 3; 400 c.c.) gave *methyl* 16α-*chloro*-3β-*hydroxyeburico*-7 : 9(11)-*dien*-21-*oate* as needles (304 mg.), m. p. 209—210° (after repeated crystallisation from

methanol or ethanol), $[\alpha]_D$ +47° (*c*, 0·95) (Found: C, 73·7; H, 10·0; Cl, 6·85. $C_{32}H_{51}O_3Cl$ requires C, 74·0; H, 9·9; Cl, 6·85%). Light absorption in ethanol: Max.: 2360 and 2430 Å; ε = 13,800 and 16,100. Inflexion, 2510 Å; ε = 10,600.

Methyl 3β-*Acetoxy*-16α-*chloroeburico*-7 : 9(11)-*dien*-21-*oate*.—Methyl 16α-chloro-3β-hydroxyeburico-7 : 9(11)-dien-21-oate (160 mg.) in pyridine (10 c.c.) was treated with acetic anhydride (2 c.c.) at 20° for 48 hr. After dilution with water extraction with ether yielded a product which was adsorbed from light petroleum (20 c.c.) on alumina (15 g.). Elution with benzene (400 c.c.) gave *methyl* 3β-*acetoxy*-16α-*chloroeburico*-7 : 9(11)-*dien*-21-*oate* as plates (149 mg.), m. p. 184—187°, remelting at 191—193° (after several crystallisations from methanol), $[\alpha]_D$ +74° (*c*, 0·95) (Found: C, 72·25; H, 9·65; Cl, 6·6. $C_{34}H_{53}O_4Cl$ requires C, 72·75; H, 9·5; Cl, 6·3%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 14,500, 17,000, and 11,200.

3β : 16β : 21-*Trihydroxyeburico*-7 : 9(11)-*diene* (XXI).—Methyl 3β : 16β-dihydroxyeburico-7 : 9(11)-dien-21-oate (228 mg.) in ether (100 c.c.) was heated under reflux for 1 hr. with lithium aluminium hydride (400 mg.). The excess of reagent was destroyed with ethyl acetate and the complex decomposed with 2N-hydrochloric acid (100 c.c.). Isolation with ether gave 3β : 16β : 21-*trihydroxyeburico*-7 : 9(11)-*diene* as needles or plates (200 mg.), m. p. 241—244° (after several crystallisations from ethanol or methanol), $[\alpha]_D$ +35° (*c*, 0·78 in pyridine) (Found: C, 79·1; H, 11·3. $C_{31}H_{52}O_3$ requires C, 78·75; H, 11·1%). Light absorption in ethanol: Max., 2370 and 2440 Å; ε = 15,250 and 17,950. Inflexion, 2520 Å; ε = 11,650.

This triol (150 mg.) in pyridine (10 c.c.) was treated with acetic anhydride (4 c.c.) at 20° for 72 hr. Dilution with water, extraction with ether, adsorption from benzene (15 c.c.) on alumina (15 g.), and elution with benzene (300 c.c.) gave the 3β : 16β : 21-*triacetate* as needles (136 mg.), m. p. 139—141° (after five crystallisations from methanol or ethanol), $[\alpha]_D$ +21° (*c*, 1·03) (Found: C, 73·85; H, 9·7. $C_{37}H_{58}O_6$ requires C, 74·2; H, 9·75%). Light absorption in ethanol: Max., 2360, 2430, and 2510 Å; ε = 15,400, 18,000 and 11,900.

Reduction of Methyl 16α-*Chloro*-3β-*hydroxyeburico*-7 : 9(11)-*dien*-21-*oate with Lithium Aluminium Hydride*.—Methyl 16α-chloro-3β-hydroxyeburico-7 : 9(11)-dien-21-oate (320 mg.) in ether (125 c.c.) was treated with lithium aluminium hydride (400 mg.) at 20° for 48 hr. The excess of reagent was destroyed with ethyl acetate and the complex decomposed with 2N-hydrochloric acid (100 c.c.). Isolation with ether gave a product which was adsorbed from benzene (50 c.c.) on alumina (25 g.). Elution with benzene–ether (7 : 3; 600 c.c.) gave 16α-*chloro*-3β : 21-*dihydroxyeburico*-7 : 9(11)-*diene* (XX) as needles (190 mg.), m. p. 208—210° (after several crystallisations from methanol and ethanol), $[\alpha]_D$ +78° (*c*, 0·82) (Found: C, 75·1; H, 10·6; Cl, 7·15. $C_{31}H_{51}O_2Cl$ requires C, 75·75; H, 10·45; Cl, 7·2%). Light absorption in ethanol: Max.: 2370, 2440, and 2520 Å; ε = 14,200, 16,600, and 10,050.

Elution with benzene–ether (1 : 1) gave 3β : 16β : 21-trihydroxyeburico-7 : 9(11)-diene as needles or plates (100 mg.) from ethanol, m. p. and mixed m. p. 241—244° (after crystallisation from ethanol), $[\alpha]_D$ +35° (*c*, 0·79 in pyridine). The infra-red spectrum was identical with that of an authentic sample.

The diol (176 mg.) in pyridine (10 c.c.) was treated with acetic anhydride (3 c.c.) at 20° for 72 hr. After dilution with water isolation with ether yielded a product which was adsorbed from light petroleum on alumina (15 g.). Elution with light petroleum–benzene (4 : 1; 500 c.c.) gave 3β : 21-*diacetoxy*-16α-*chloroeburico*-7 : 9(11)-*diene* as needles (155 mg.), m. p. 147—149° (after several crystallisations from methanol or ethanol), $[\alpha]_D$ +87° (*c*, 1·05) (Found: C, 73·1; H, 9·4. $C_{35}H_{55}O_4Cl$ requires C, 73·05; H, 9·6%). Light absorption in ethanol: Max., 2350, 2430, and 2520 Å; ε = 13,800, 15,600, and 10,600.

Methyl 3β-*Acetoxyeburico*-7 : 9(11)-*dien*-21-*oate* (XXVI).—Methyl 3β-hydroxy-16-oxoeburico-7 : 9(11)-dien-21-oate (2·2 g.) in diethylene glycol (150 c.c.) was heated at 100° for 1½ hr. with hydrazine hydrate (5 c.c.; 90%); then the excess of hydrazine and water was removed by distillation. Potassium hydroxide (2 g.) was added and the solution heated under reflux for 5 hr. After acidification with acetic acid the cooled mixture was diluted with water, and the product was isolated with ether, suspended in acetone (150 c.c.), and treated with excess of ethereal diazomethane at 20° for 16 hr. The crude methyl ester was dissolved in pyridine (75 c.c.) and treated with acetic anhydride (3 c.c.) for 1½ hr. at 100°. Isolation with ether gave a product which was adsorbed from light petroleum–benzene (1 : 1) on alumina (150 g.). Elution with benzene (1800 c.c.) gave methyl 3β-acetoxyeburico-7 : 9(11)-dien-21-oate as needles (850 mg.) (from methanol), m. p. 163—166°, raised by several crystallisations from ethanol to 174—175°, undepressed on admixture with a sample of methyl acetyldehydrodihydroeburicoate; the product had $[\alpha]_D$ +70° (*c*, 0·96) (Found: C, 77·1; H, 10·4. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·35%). The infra-red spectrum was identical with that of a sample of methyl acetyl-

dehydrodihydroeburicoate. Light absorption in ethanol: Max.: 2360, 2440, and 2520 Å; ε = 15,900, 18,450, and 12,100.

Methyl 3β-*Acetoxyeburico*-7 : 9(11) : 24(28)-*trien*-21-*oate* (XXVII).—Methyl 3β-hydroxy-16-oxoeburico-7 : 9(11) : 24(28)-trien-21-oate (2·0 g.) in diethylene glycol (125 c.c.) was heated with hydrazine hydrate (5 c.c.; 90%), then with potassium hydroxide (6 hr.), and esterified and chromatographed, all as in the preceding experiment. Elution with benzene–ether (19 : 1; 1500 c.c.) gave methyl 3β-acetoxyeburico-7 : 9(11) : 24(28)-trien-21-oate as needles (790 mg.), m. p. and mixed m. p. 161—163° (after several crystallisations from methanol or ethanol), $[\alpha]_D$ +65° (*c*, 0·97) (Found: C, 77·55; H, 10·1. Calc. for $C_{34}H_{52}O_4$: C, 77·8; H, 10·0%). The infra-red spectrum was identical with that of methyl acetyldehydroeburicoate. Light absorption in ethanol: Max. 2360, 2430, and 2510 Å; ε = 11,500, 13,900, and 9000.

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