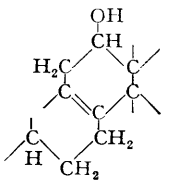
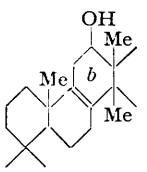
599. *The Chemistry of the Triterpenes and Related Compounds. Part XIX.* Further Evidence concerning the Structure of Polyporenic Acid A.*

By T. G. Halsall, R. Hodges, and E. R. H. Jones.

The nature of the side-chain of polyporenic acid A has been investigated by oxidative degradation, and the environment of the hydroxyl group in ring A has been elucidated. The results obtained, taken in conjunction with earlier work, suggest that the acid has the structure (XXII), *i.e.*, 3α : 12α-dihydroxyeburico-8 : 24(28)-dien-26-oic acid.

Polyporenic acid A has been shown to be a tetracyclic diethenoid dihydroxy-carboxylic acid and to possess partial structure (I) (Curtis, Heilbron, Jones, and Woods, *J.*, 1953, **457**; Jones and Woods, *J.*, 1953, **464**; Halsall, Jones, and Lemin, *J.*, 1953, **468**) which, it was suggested, might be expanded to (II).

(I) 

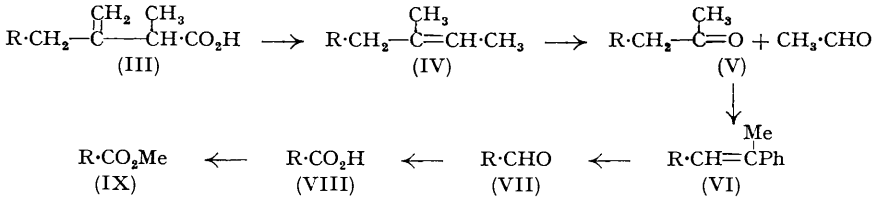


(II)

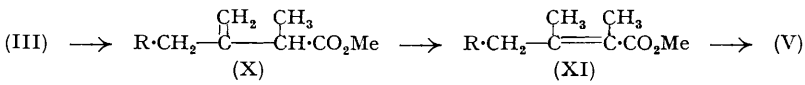
Polyporenic acid A is readily decarboxylated on melting, in a manner typical of αβ-unsaturated acids, giving a decarboxy-compound. By a series of degradations starting from the diacetate of the decarboxy-compound it has now been shown that the grouping (III) is present in polyporenic acid A. The *ab*-diacetate of the decarboxy-compound (IV) gave acetaldehyde and the methyl ketone (V) on ozonolysis. The latter product was treated with phenylmagnesium bromide, followed by acetic anhydride and sodium acetate, and so yielded the styryl derivative (VI). This had an ultra-violet absorption

* Part XVIII, *J.*, 1953, 2548.

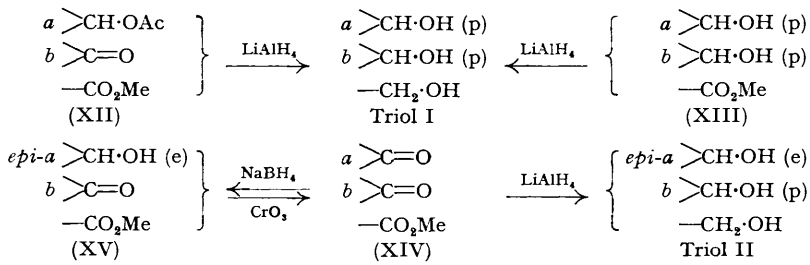
maximum at 2460 Å ($\varepsilon = 15{,}500$) (bands at higher wave-lengths being absent), typical of an α-substituted styrene (Hirschberg, *J. Amer. Chem. Soc.*, 1949, **71**, 3241). On ozonolysis it gave acetophenone and a product which was oxidised directly with chromic acid under mild conditions to an acid (VIII), characterised as its methyl ester (IX). Analysis of the ester indicated that five carbon atoms had been removed during the degradation of polyporenic acid A to (VIII). The initial product of the ozonolysis of the styryl derivative must therefore be the aldehyde (VII).



When methyl polyporenate A (partial formula X) was heated with 5% methanolic potassium hydroxide, part of it was hydrolysed to polyporenic acid A. In the unhydrolysed ester the double bond situated βγ- to the methoxycarbonyl group moved into conjugation, and an αβ-unsaturated ester, methyl *iso*polyporenate A (partial formula XI), resulted. This formulation of the *iso*-ester was supported by light-absorption data and confirmed by ozonolysis of its diacetate, the methyl ketone (V) described above being obtained. A similar partial conversion into the corresponding *iso*-compound was obtained with methyl *b*-oxopolyporenate A *a*-acetate. The simultaneous formation of polyporenic acid A and methyl *iso*polyporenate is explained by assuming that methyl polyporenate A can either undergo hydrolysis to the carboxylate ion of acid A which does not rearrange, or be converted into the *iso*-ester which is much more difficult to hydrolyse than its precursor. With the isolation of methyl *iso*polyporenate A many puzzling features of the effect of hot alkali on derivatives of methyl polyporenate A, which had hitherto been encountered, were explained. Methyl polyporenate A can be satisfactorily hydrolysed to acid A by cold methanolic potassium hydroxide (cf. Curtis, Heilbron, Jones, and Woods, *loc. cit.*).

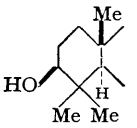


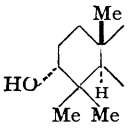
The reduction of methyl *b*-oxopolyporenate A *a*-acetate (XII) was attempted with both lithium aluminium hydride and sodium borohydride. The former gave a triol (triol I), identical with that already obtained by similar reduction of methyl polyporenate A (XIII) (Curtis, Heilbron, Jones, and Woods, *loc. cit.*). Hence reduction of the *b*-keto-group gives a hydroxyl group with the same configuration as the *b*-hydroxyl group originally present in polyporenic acid A. No reduction occurred with sodium borohydride. This confirms the hindered nature of the *b*-keto-group already indicated by earlier evidence, and suggests that the *b*-hydroxy-group arising from the lithium aluminium hydride reduction is polar (cf. Barton and Holness, *J.*, 1952, 83).

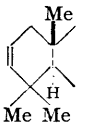


When methyl *a* : *b*-dioxopolyporenate A (XIV) was reduced with lithium aluminium hydride a different triol (triol II) was obtained. Therefore reduction of the *a*-keto-group leads to a hydroxyl group with a configuration *different* from that of the original *a*-hydroxyl

group of polyporenic acid A. Since the *a*-keto-group is not hindered (cf. oxime formation), the *a*-hydroxyl group in triol II is probably equatorial, and hence the parent *a*-hydroxyl group in polyporenic acid A is probably polar. Reduction of the *ab*-diketo-ester with sodium borohydride gave a *new* hydroxyketo-ester (XV), not identical with methyl *a*-hydroxy-*b*-oxopolyporenate A, which also must have an epimeric *a*-hydroxyl group, *i.e.*, it is methyl *epi-a*-hydroxy-*b*-oxopolyporenate A. Treatment of this compound with phosphorus pentachloride resulted in the retropinacolinic dehydration characteristic of

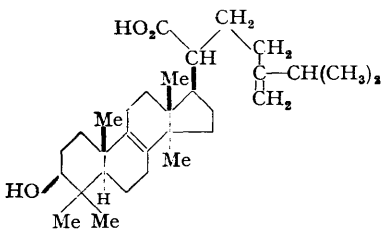

(XVI)

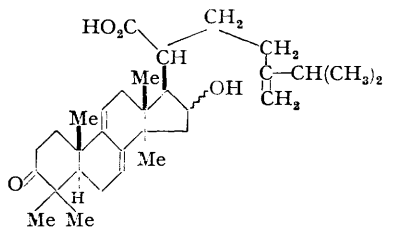

(XVII)


(XVIII)

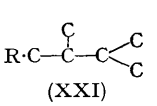
normal triterpenes with partial structure (XVI), since the product gave acetone on ozonolysis. The grouping (XVI) is therefore likely in the *epi-a*-acid, and the abnormal arrangement (XVII) in polyporenic acid A itself. The fact that dehydration of methyl *b*-oxopolyporenate A with phosphorus oxychloride yielded a product (XVIII), which gave only formaldehyde (from the unsaturated side-chain) on ozonolysis, supports this conclusion.

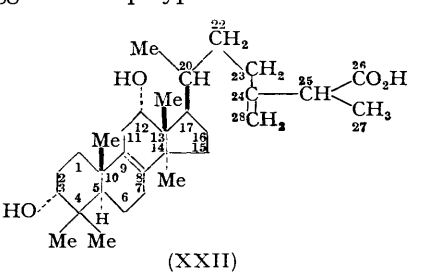
In the previous publications on polyporenic acid A, $C_{30}H_{48}O_4$ has been used as the provisional working formula although, as explained by Curtis, Heilbron, Jones, and Woods (*loc. cit.*), this did not imply our acceptance of it over the formula $C_{31}H_{50}O_4$, for which substantial evidence existed. The structures of two obviously related fungal acids, eburicoic acid and polyporenic acid C have now been elucidated. Both have been shown to be C_{31} acids, eburicoic acid having structure (XIX) (Holker, Powell, Robertson, Simes, Wright, and Gascoigne, *J.*, 1953, 2422) and polyporenic acid C structure (XX) (Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548). The identical side-chains of these acids


(XIX)


(XX)

contain the six-carbon grouping (XXI) known to be present in polyporenic acid A. On the assumption that polyporenic acid A is synthesised by the same general biogenetic route as eburicoic acid and polyporenic acid C, combination of the partial structures (II), (III), and (XVII) leads to structure (XXII) [3α : 12α-dihydroxyeburico-8 : 24(28)-dien-26-oic acid] which is provisionally suggested for polyporenic acid A.


(XXI)


(XXII)

Experiments designed to convert polyporenic acid A into a derivative of lanosterol are in progress.

Experimental

Rotations were determined in chloroform. M. p.s were determined on a Kofler block and are corrected. Alumina of activity I—II was employed for all chromatograms, and the light petroleum used for elution was the fraction of b. p. 60—80°. The derivatives of polyporenic acid A used as starting materials were prepared according to the methods described by Curtis, Heilbron, Jones, and Woods (*loc. cit.*).

Ozonolysis of the ab-*Diacetate of Decarboxylated Polyporenic Acid A*.—Ozonised oxygen (6%) was passed through a solution of the diacetate (1·9 g.) in acetic acid (15 c.c.) for 75 min. After addition of water (30 c.c.) half of the reaction mixture was distilled into a solution of 2 : 4-dinitrophenylhydrazine hydrochloride in methanol. The 2 : 4-dinitrophenylhydrazone formed (420 mg.) was isolated, dissolved in benzene, and chromatographed on alumina. It was then repeatedly crystallised from methanol, to give orange-yellow needles of acetaldehyde 2 : 4-dinitrophenylhydrazone (mixed dimorphic forms), m. p. 146—160°. On admixture with the higher-melting dimorphic form it began to melt at 146°, partly resolidified, and finally melted at 168° (Found : N, 24·75. Calc. for $C_8H_8O_4N_4$: N, 25·0%). Ether-extraction of the solution remaining after distillation yielded a product which was washed with alkali, and then adsorbed from light petroleum on alumina (150 g.). The fraction eluted with benzene–ether (1 : 1) crystallised from methanol, giving needles of the *methyl ketone* (V) (1·26 g.), m. p. 196—198°, raised by further crystallisation from methanol to 201—202°, $[\alpha]_D^{18}$ +85° (*c*, 0·7) (Found : C, 74·85; H, 9·85. $C_{32}H_{50}O_5$ requires C, 74·65; H, 9·8%).

Preparation of the Styryl Derivative (VI).—The methyl ketone (V) (3·0 g.) in ether (100 c.c.) was slowly added to phenylmagnesium bromide (6 g.) in ether (150 c.c.), and then the mixture was heated under reflux for 30 min. The product was worked up in the usual way, chromatographed on alumina in benzene, and then heated under reflux with acetic anhydride (15 c.c.) and sodium acetate (0·5 g.) for 1 hr. After dilution with water, ether-extraction yielded a product which was adsorbed on alumina (200 g.) from light petroleum. Elution with light petroleum–benzene (3 : 7) gave the *styryl* derivative (VI) (1·1 g.) which crystallised from methanol as flat prisms, m. p. 173—174°, $[\alpha]_D^{19}$ +80° (*c*, 1·8) (Found : C, 79·5; H, 9·4. $C_{38}H_{54}O_4$ requires C, 79·4; H, 9·45%). Light absorption in EtOH : Max., 2460 Å; ε = 15,500.

Ozonolysis of the Styryl Derivative (VI).—Ozonised oxygen (6%) was passed through a solution of the styryl derivative (1·0 g.) in acetic acid (30 c.c.) for 35 min. After addition of water (80 c.c.) half of the reaction mixture was distilled into a solution of 2 : 4-dinitrophenylhydrazine hydrochloride in methanol. The 2 : 4-dinitrophenylhydrazone formed (310 mg.) was chromatographed in benzene on alumina, and then crystallised three times from benzene, to give acetophenone 2 : 4-dinitrophenylhydrazone as dark-red needles, m. p. 247°, undepressed on admixture with an authentic sample (Found : N, 18·3. Calc. for $C_{14}H_{12}O_4N_4$: N, 18·65%). The solution remaining after distillation was extracted with ether. The extract was washed with alkali and water, dried, and evaporated. The residue was oxidised in acetone with chromic acid (8N) in sulphuric acid (25% v/v) at 30°. The acidic product (150 mg.) was separated, methylated with diazomethane, and purified by chromatography on alumina (15 g.). Elution with benzene–ether (1 : 1) followed by crystallisation from aqueous methanol gave flat needles of the *methyl* ester (IX), m. p. 197°, $[\alpha]_D^{17}$ +90° (*c*, 0·55) (Found : C, 70·9; H, 9·2. $C_{31}H_{48}O_6,\frac{1}{2}CH_3{\cdot}OH$ requires C, 71·05; H, 9·45%).

Methyl iso*Polyporenate A*.—A solution of methyl polyporenate A (10 g.) in 5% methanolic potassium hydroxide (200 c.c.) was heated under reflux for 25 min. After dilution with water, ether-extraction yielded a product (4·4 g.) which was adsorbed from benzene on alumina (400 g.). The fraction eluted with benzene–ether (1 : 4) was crystallised from methanol and then repeatedly from nitromethane, to give *methyl* iso*polyporenate A* as needles (1·11 g.), m. p. 164—165°, $[\alpha]_D^{18}$ +52° (*c*, 0·98) (Found : C, 76·7; H, 10·45. $C_{32}H_{52}O_4$ requires C, 76·75; H, 10·45%). Light absorption in EtOH : Max., 2260 Å; ε = 8000.

Methyl iso*Polyporenate A* ab-*Diacetate*.—Methyl *iso*polyporenate A (800 mg.), acetic anhydride (10 c.c.), and sodium acetate (250 mg.) were heated under reflux for 1 hr. The product was worked up in the usual manner and crystallised from ethanol, to give *methyl* iso*polyporenate A* ab-*diacetate* as needles, m. p. 126·5—127·5°, $[\alpha]_D^{18}$ +64° (*c*, 1·9) (Found : C, 73·75; H, 9·7. $C_{36}H_{56}O_6$ requires C, 73·95; H, 9·65%). Light absorption in EtOH : Max., 2260 Å; ε = 8900.

Ozonolysis of Methyl iso*Polyporenate A* ab-*Diacetate*.—Ozonised oxygen (6%) was passed through a solution of the diacetate (400 mg.) in acetic acid (10 c.c.) for 20 min. Water (20 c.c.) was then added and the solution heated on the steam-bath for 10 min. Ether-extraction

yielded a product which was adsorbed from benzene on alumina (40 g.) and eluted with benzene–ether (1 : 1), to give the methyl ketone (V) (210 mg.) which crystallised from methanol as needles, $[\alpha]_D^{19}$ +83° (*c*, 0·5), m. p. 198—199° undepressed on admixture with an authentic sample.

Hydrolysis of Methyl b-*Oxopolyporenate A* a-*Acetate*.—Methyl *b*-oxopolyporenate A *a*-acetate (230 mg.) was heated under reflux in 5% methanolic potassium hydroxide (10 c.c.) for 20 min. After dilution with water, ether-extraction yielded a product which was separated into neutral (93 mg.) and acidic (131 mg.) fractions. The acidic fraction on methylation with diazomethane gave methyl *b*-ketopolyporenate A, m. p. 160—161°, undepressed on admixture with an authentic sample. The neutral fraction after four recrystallisations from methanol yielded *methyl* b-*oxoisopolyporenate A* as needles, m. p. 171—171·5°, $[\alpha]_D^{19}$ +47° (*c*, 0·62) (Found : C, 76·7; H, 10·3. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%). Light absorption in EtOH : Max. 2260 Å; ε = 9200.

Methyl ab-*Dioxoisopolyporenate A*.—Methyl *iso*polyporenate A in acetone (20 c.c.) was oxidised with chromic acid (8N) in sulphuric acid (25% v/v) at 30°. The product was adsorbed from benzene on alumina (15 g.) and eluted with benzene–ether (1 : 4), to give *methyl* ab-*dioxoisopolyporenate A*, which crystallised from methanol as plates, m. p. 144·5—145·5°, $[\alpha]_D^{18}$ +72° (*c*, 1·5) (Found : C, 77·3; H, 9·85. $C_{32}H_{48}O_4$ requires C, 77·35; H, 9·75%). Light absorption in EtOH : Max., 2260 Å; ε = 9400. Oxidation of methyl *b*-oxo*iso*polyporenate A (40 mg.) in acetone (10 c.c.) in the same manner also gave methyl *ab*-dioxo*iso*polyporenate A, m. p. 145°, undepressed on admixture with a sample prepared from methyl *iso*polyporenate A.

Reduction of Methyl b-*Oxopolyporenate A* a-*Acetate*.—A solution of methyl *b*-oxopolyporenate A *a*-acetate (525 mg.) in ether (20 c.c.) was slowly added to lithium aluminium hydride (250 mg.) in ether (20 c.c.). The mixture was heated under reflux for 15 min., poured into dilute sulphuric acid, and extracted with chloroform. The extracted product was adsorbed from chloroform–benzene (1 : 1) on alumina (50 g.), eluted with chloroform–benzene (4 : 1), and crystallised from methanol as short prisms (385 mg.), $[\alpha]_D^{20}$ +61° (*c*, 0·65), m. p. 172—173, undepressed on admixture with a sample of the triol (" triol I ") prepared by Curtis, Heilbron, Jones, and Woods (*loc. cit.*) from methyl polyporenate A.

Reduction of Methyl ab-*Dioxopolyporenate A*.—Methyl *ab*-dioxopolyporenate A (1 g.) was reduced by lithium aluminium hydride (650 mg.) in the manner described above. " Triol II " was recrystallised four times from methanol, forming fine needles (680 mg.), m. p. 216—216·5°, $[\alpha]_D^{19}$ +86° (*c*, 0·88 in pyridine) (Found : C, 78·6; H, 10·6. $C_{31}H_{52}O_3$ requires C, 78·75; H, 11·1%).

Reduction of Methyl ab-*Dioxopolyporenate A by Sodium Borohydride*.—A solution of methyl *ab*-dioxopolyporenate A (4 g.) and sodium borohydride (375 mg.) in 1% aqueous dioxan (100 c.c.) was kept at 20° for 1 hr. Dilution with water yielded a precipitate which was filtered off and then adsorbed from benzene on alumina (200 g.). Elution with benzene–ether (1·4) gave *methyl* epi-a-*hydroxy*-b-*oxopolyporenate A* (3·8 g.), m. p. 172—175°, raised by crystallisation from methanol and from nitromethane to 175·5—176·5°, $[\alpha]_D^{19}$ +93° (*c*, 1·05) (Found : C, 77·0; H, 9·95. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%). Reduction of methyl *epi-a*-hydroxy-*b*-oxopolyporenate A with lithium aluminium hydride in the manner described above gave " triol-II," m. p. 216°.

Oxidation of Methyl epi-a-*Hydroxy*-b-*oxopolyporenate A*.—Methyl *epi-a*-hydroxy-*b*-oxopolyporenate A (200 mg.) in acetone (10 c.c.) was oxidised with chromic acid solution (8N) in the usual manner. The product was adsorbed from benzene on alumina (20 g.), eluted with benzene–ether (1 : 1), and crystallised from methanol, to give needles of methyl *ab*-dioxopolyporenate A (150 mg.), $[\alpha]_D^{19}$ +103° (*c*, 0·78), m. p. 128—130°, undepressed on admixture with an authentic specimen.

Dehydration of Methyl b-*Oxopolyporenate A*.—Phosphorus oxychloride (0·5 c.c.) was added to a solution of methyl *b*-oxopolyporenate A (142 mg.) in pyridine at 0°. After being kept at 20° for 7 days the mixture was poured on ice. Ether-extraction yielded a *product* which crystallised from methanol as plates (78 mg.), m. p. 109—111°, raised by further crystallisation from ethanol to 117—117·5°, $[\alpha]_D^{18}$ +152° (*c*, 0·73) (Found : C, 79·9; H, 9·95. $C_{32}H_{48}O_3$ requires C, 79·95; H, 10·05%).

Ozonolysis of the Phosphorus Oxychloride Dehydration Product of Methyl b-*Oxopolyporenate A*.—Ozonised oxygen (6%) was passed through a solution of the dehydration product (400 mg.) in acetic acid (25 c.c.) for 30 min. After addition of water the volatile fragments were steam-distilled, first into a solution of dimedone in aqueous methanol, and then into a solution of 2 : 4-dinitrophenylhydrazine hydrochloride in methanol. Formaldehyde dimethone (55 mg.)

m. p. 189°, undepressed on admixture with an authentic specimen, separated from the dimedone solution. No 2 : 4-dinitrophenylhydrazone was formed.

Dehydration of Methyl epi-a-*Hydroxy*-b-*oxopolyporenate A*.—Phosphorus pentachloride (150 mg.) was shaken with a suspension of methyl *epi-a*-hydroxy-*b*-oxopolyporenate A (250 mg.) in dry light petroleum (25 c.c.) for 15 min., by which time only the excess of phosphorus pentachloride remained undissolved. The solution was washed with water and alkali, dried, and evaporated to dryness. The product was adsorbed from benzene–light petroleum (1 : 1) on alumina (25 g.). Elution with benzene–ether (9 : 1) gave an oil which crystallised from methanol. The crystals (180 mg.) were crystallised four times from methanol, to give the dehydration *product* as needles, m. p. 102·5—103·5°, $[\alpha]_D^{18}$ +110° (*c*, 2·6) (Found : C, 79·75; H, 10·2. $C_{32}H_{48}O_3$ requires C, 79·95; H, 10·05%).

Ozonolysis of the Phosphorus Pentachloride Dehydration Product of Methyl epi-a-*Hydroxy*-b-*oxopolyporenate A*.—Ozonised oxygen (6%) was passed through a solution of the dehydration product (750 mg.) in acetic acid (20 c.c.) for 1 hr. After addition of water the volatile products were steam-distilled into a solution of dimedone in aqueous methanol. No derivative separated. The dimedone solution was then steam-distilled and the distillate passed into a solution of 2 : 4-dinitrophenylhydrazine hydrochloride in methanol. After dilution with water, the 2 : 4-dinitrophenylhydrazone formed was extracted with benzene and then chromatographed on alumina with benzene. Acetone 2 : 4-dinitrophenylhydrazone was eluted and crystallised from ethanol as flat needles (264 mg., 72% yield), m. p. and mixed m. p. 126° (Found : N, 23·3. Calc. for $C_9H_{10}O_4N_4$: N, 23·5%).

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