92. *The Chemistry of the Triterpenes. Part XIII.* The Further Characterisation of Polyporenic Acid A.*

By R. G. Curtis, Sir Ian Heilbron, E. R. H. Jones, and Gilbert F. Woods.

Polyporenic acid A, a diethenoid dihydroxy-monocarboxylic sapogenin from the birch-tree fungus *Polyporus betulinus* Fr., has been further characterised. Both hydroxyl groups are secondary and exhibit different reactivities as do the corresponding ketones. On melting, polyporenic acid A decomposes smoothly and in good yield, to give a decarboxy-compound which has also been characterised. The significance of the molecular-rotation changes involved in the formation of these derivatives is discussed.

In 1939 Cross, Eliot, Heilbron, and Jones (*J.*, 1940, 632) commenced a systematic investigation of the constituents of the higher fungi with a re-investigation of the birch-tree fungus, *Polyporus betulinus* Fr., which resulted in the isolation of polyporenic acids A, B, and C. It was suggested that these acids might be triterpenoid in nature; acids A and B appeared to be isomeric $C_{30}H_{48}O_4$ compounds containing one carboxyl group, two hydroxyl groups, and two ethylenic double bonds. The work was extended by Cross and Jones (*J.*, 1940, 1491), with an investigation of the unsaturated system of polyporenic acid A. Of the two double bonds present only one could be hydrogenated catalytically at ordinary temperature and pressure, and it was concluded from ozonolysis experiments that the reactive double bond was present as a vinylidene group ($CH_2{=}C<$). Owing to the war the work had then to be abandoned with the consequence that the three acids were not characterised unequivocally.

Unknown to Cross *et al.* (*locc. cit.*) a chemical study of the constituents of *Polyporus betulinus* Fr. had been carried out just previously by Frèrejacque (*Reviews Myc.*, 1938, **3**, 95) who succeeded in isolating a new compound, m. p. 185—187° (decomp.), $[\alpha]_D$ +71·5° (in methanol), which he designated ungulinic acid. Subsequently, Locquin, Locquin, and Prevot (*ibid.*, 1948, **13**, 3) repeated the isolation of polyporenic acid A and confirmed its identity with ungulinic acid.

The present communication concerns the isolation and purification of substantial quantities of polyporenic acid A, and an investigation of the chemistry of the acid as a foundation for a more vigorous attack on the problem of its structure. Large quantities of *Polyporus betulinus* Fr. were supplied through the courtesy of Mr. G. D. Rouse of H.M. Forestry Commission (Lyndhurst). The method employed for extracting the acid was similar to that developed by Cross *et al.* (*loc. cit.*) with slight modifications because of the much larger quantities involved and the fact that the exclusive isolation of acid A was aimed at. The latter was entirely successful and only traces of the higher-melting acids were detected. Initially the spore tissue was separated from the fleshy part of the fungus and extracted separately. However, the yield of acid A was the same as from the bulk fungus and subsequently the tedious process of separation was abandoned.

The physical constants of the acid and its methyl ester agreed closely with those reported earlier, the melting point discrepancies being due to our use of a Kofler hot stage. The acid forms a series of salts with amines, but these, of which the *cyclo*hexylamine salt is typical, have characteristic, though poorly defined, melting properties and have not proved useful for the isolation or purification of the acid (cf. Harris and Sanderson, *J. Amer. Chem. Soc.*, 1948, **70**, 334).

The equivalent weight of polyporenic acid has been redetermined and as before corresponds to a formula of $C_{31}H_{50}O_4$. The analytical data, however, fit the formula $C_{30}H_{48}O_4$ rather better than $C_{31}H_{50}O_4$ (cf. following paper) and at the moment no clear decision can be made between these two formulæ. Because of the prevalence of C_{30} and the absence of C_{31} cyclic compounds in Nature the calculated analytical figures in the experimental section and the formulæ quoted in this and the two following papers are based on $C_{30}H_{48}O_4$ for polyporenic acid A, but this does not imply our acceptance of this formula over $C_{31}H_{50}O_4$.

* Part XII, *J.*, 1952, 2868.

The presence of two ethylenic linkages has been confirmed by perbenzoic acid titration of the triol formed by lithium aluminium hydride reduction of the methyl ester. Two hydroxyl groups, designated *a* and *b*, have been characterised in the methyl ester by formation of an *a*-acetate, an *a*-benzoate, and an *a* : *b*-diacetate. Attempts to prepare an *a* : *b*-dibenzoate were unsuccessful. Diacetylation was carried out at 20° by Whitman and Schwenk's method (*J. Amer. Chem. Soc.*, 1946, **68**, 1865), a trace of anhydrous perchloric acid being used as catalyst. Cross *et al.* (*loc. cit.*) described methyl polyporenate A monoacetate, m. p. 112°, $[\alpha]_D$ +88°, which we now consider must have been the *a* : *b*-diacetate, m. p. 118·5—120°, $[\alpha]_D$ +87°, thus explaining the unsuccessful attempts further to acetylate the supposed monoacetate. Further, the monoformate described by Cross and Jones (*loc. cit.*) was most likely the diformate.

That both hydroxyl groups are secondary has been established by chromic acid oxidation to the corresponding ketones. The method employed was adapted from that used for the oxidation of acetylenic carbinols (see Bowden, Heilbron, Jones, and Weedon, *J.*, 1946, 39), and involved the addition of a standard aqueous sulphuric acid solution of chromium trioxide to an acetone solution of the compound at 20°. The reagent oxidises a primary hydroxyl group to a carboxyl group. Thus, the triol mentioned above was oxidised to *a* : *b*-diketopolyporenic acid A, identical with the compound produced by similar oxidation of acid A. As was expected the acid and its methyl ester formed diketones and the methyl ester *a*-acetate and *a*-benzoate formed monoketones, thus confirming the presence of a free hydroxyl group in the latter compounds.

The different reactivities of the hydroxyl groups in polyporenic acid A are reflected in the properties of the keto-groups which are formed on oxidation. The *a*-carbonyl group reacted readily with the usual carbonyl reagents whereas the *b*-carbonyl group failed to react even under vigorous conditions. The somewhat sterically hindered location of the *b*-hydroxyl group became evident when attempts to prepare the C_{29}-hydrocarbon by Wolff–Kishner reduction of the decarboxy- *a* : *b*-diketo-compound (see below) resulted in the isolation of two compounds both of which still contained oxygen. This behaviour is analogous to that of diketosumaresinolic acid (Ruzicka, Jeger, Grob, and Hosli, *Helv. Chim. Acta*, 1943, **26**, 2283), the unreactive carbonyl group of which failed to react with carbonyl reagents and resisted reduction under Wolff–Kishner and Clemmensen conditions. The presence of an unreactive carbonyl group has been established by infra-red examination. The infra-red absorption data are discussed in the following paper. *b*-Ketopolyporenic acid A and its methyl ester *a*-acetate also exhibited selective low intensity absorption at 2920—2930 Å ($\varepsilon = 450$) and 2900 Å ($\varepsilon = 270$) respectively. The molecular-extinction coefficients of these two bands are appreciably higher than would be expected for isolated carbonyl groups. This is almost certainly due in each case to the presence of a trace of a conjugated diene, the formation of which will be discussed in a later paper.

The absence of high-intensity absorption at 2200—2600 Å in any of the carbonyl derivatives of polyporenic acid A precludes the presence of αβ-unsaturation. Further, absence of ferric chloride coloration and characteristic α- or β-diketone absorption shows that the hydroxyl groups are separated from the double bonds by at least one saturated carbon atom and that the two hydroxyl groups are separated from each other by at least two carbon atoms. The absence of selective absorption in the parent acid precludes the conjugation of the double bonds either with each other or with the carboxyl group. Finally, the comparative stability of *a* : *b*-diketopolyporenic acid A shows that neither of the hydroxyl groups is β- to the carboxyl group.

When melted in a capillary, polyporenic acid A readily underwent decomposition with frothing and evolution of a volatile component (cf. morolic acid, Barton and Brooks, *J.* 1951, 257). The resultant clear melt solidified on cooling and remelted at a much lower temperature. A quantitative study of the reaction revealed that it involved simply the elimination of carbon dioxide without any concurrent dehydration. Melting the acid in an inert atmosphere gave the decarboxy-compound in good yield, and it was characterised as its *a*-acetate, *a*-benzoate, and *a* : *b*-diacetate. The relative reactivities of the hydroxyl groups and the corresponding carbonyl groups were the same as in the parent acid. Conjugation of the double bonds was again precluded by the absence of selective light absorption

above 2200 Å. The decarboxy-diketo-compound also failed to show any high-intensity absorption, proving the complete absence of any conjugation between the two carbonyl groups and the two double bonds.

The molecular-rotation differences (cf. Barton and Jones, *J.*, 1944, 659) of polyporenic acid A and its derivatives are shown in Tables 1—5. For the purpose of direct comparison the rotation of the acid was determined in chloroform, but for characterisation the more accurate value is that determined in pyridine, because of the comparative insolubility of the acid in chloroform. It will be seen that, in agreement with general experience, simple changes produce regular molecular-rotation differences in polyporenic acid A. The

TABLE 1. *Esterification of acids.*

Compound	$[M]_D$, acid	$[M]_D$, Me ester	Δ
Polyporenic acid A	+350°	+386°	+36°
b-Keto-acid A	+362	+370	+ 8
a : *b*-Diketo-acid A	+494	+507	+13

TABLE 2. *Acetylation.*

	$[M]_D$					
Compound	Diol	*a*-Ac	*a* : *b*-Diac	Δ(*a*)	Δ(*b*)	Δ(*a* : *b*)
Acid A methyl ester	+386°	+175°	+497°	−211°	+322°	+111°
Dihydro-(I)-acid A methyl ester ...	+374	+170	+458	−204	+288	+ 84
Dihydro-(II)-acid A methyl ester	+379	+207	+498	−172	+291	+119
Decarboxy-compound	+296	+104	+405	−192	+301	+109
b-Keto-acid A methyl ester	+370	+237	—	−133	—	—

Δ(*a*) = $[M]_D$, *a*-acetate − $[M]_D$, diol. Δ(*b*) = $[M]_D$, *a* : *b*-diacetate − $[M]_D$, *a*-acetate.
Δ(*a* : *b*) = $[M]_D$, *a* : *b*-diacetate − $[M]_D$, diol.

TABLE 3.—*Benzoylation of the* a*-hydroxyl group.*

Compound	$[M]_D$, Diol	$[M]_D$, *a*-Monobenzoate	Δ
Acid A methyl ester	+386°	+ 74°	−312°
b-Keto-acid A methyl ester	+370	+147	−223
Decarboxy-compound	+296	− 27	−323

TABLE 4. *Oxidation.*

	$[M]_D$					
Compound	Alcohol	*b*-Ketone	*a* : *b*-Dione	Δ(*b*)	Δ(*a*)	Δ(*a* : *b*)
Polyporenic acid A	+350°	+362°	+494°	+12°	+132°	+144°
Acid A methyl ester	+386	+370	+507	−16	+137	+121
Methyl ester *a*-acetate	+175	+237	—	+62	—	—
Methyl ester *a*-benzoate	+ 74	+147	—	+73	—	—
Decarboxy-compound	+296	—	+427	—	—	+131

Δ(*b*) = $[M]_D$, *b*-ketone − $[M]_D$, alcohol. Δ(*a*) = $[M]_D$, *a* : *b*-dione − $[M]_D$, *b*-ketone.
Δ(*a* : *b*) = $[M]_D$, *a* : *b*-dione − $[M]_D$, alcohol.

TABLE 5. *Decarboxylation.*

Compound	$[M]_D$, Acid or Me ester	$[M]_D$, Decarboxy-compound	Δ
Polyporenic acid A	+350°	+296°	− 54°
Acid A methyl ester	+386	+296	− 90
Methyl ester *a*-acetate	+175	+104	− 71
Methyl ester *a* : *b*-diacetate	+497	+405	− 92
Methyl ester *a*-benzoate	+ 74	− 27	−101
a : *b*-Diketo-acid A	+494	+427	− 67
a : *b*-Diketo-methyl ester	+507	+427	− 80

changes resulting from the oxidation of the *b*-hydroxyl group reveal the only irregularities, the probable cause of which will be discussed in a later paper.

The very marked changes in molecular rotation ($[M]_D$) on acetylation and benzoylation of the *a*-hydroxyl group, *i.e.*, −200° and −300° respectively, and acetylation of the *b*-hydroxyl group (+300°) find no parallel in any of the groups of triterpenes at present described.

As only a limited number of acidic derivatives of polyporenic acid A are known, the

decarboxy-compounds have been compared with the corresponding derivatives of the methyl ester. This is considered justifiable on the ground that, as is general with triterpene acids, $[M]_D$ does not change appreciably when the acid is esterified (cf. Barton and Jones, *loc. cit.*). Despite the presence of a carboxyl group and two hydroxyl groups the molecular-rotation differences show that the presence of hydrogen bonding between the carboxyl group or its ester and either of the hydroxyl groups in polyporenic acid A is very unlikely. Thus, in contrast to the α- and β-boswellic acids (Barton and Jones, *loc. cit.*) polyporenic acid A and its decarboxy-compound (in which hydrogen bonding involving the carboxyl group is impossible) show the same regular molecular-rotation differences. Such absence of hydrogen bonding is further evidence for the isolated nature of the functional groups in the acid. Further, the regular molecular-rotation difference (−85°) on the decarboxylation of polyporenic acid A and its derivatives shows that the process of decarboxylation does not cause any fundamental change in the polyporenic acid A skeleton (see, however, following paper).

Experimental

Rotations were determined in chloroform. M. p.s were determined on a Kofler block and are corrected unless otherwise stated. Analytical specimens were dried at a suitable temperature for 5—10 hours in a high vacuum. Alumina of activity I—II was employed for all chromatograms, and except where specified light petroleum refers to the fraction with b. p. 60—80°.

Isolation of Polyporenic Acid A from Polyporus betulinus *Fr.* (*cf.* Cross *et al.*, *loc. cit.*).—The minced fungus (204 kg.) was kept under alcohol at room temperature for 48 hours; the solvent was decanted and the fungus pressed out through muslin. The last traces of solvent were removed by means of a basket centrifuge, and the combined solutions were evaporated under reduced pressure at 30—40° until the bulk of the saponins had been deposited. Dilution with water precipitated the residual saponins, and the crude product was filtered off on muslin. The air-dried saponins were hydrolysed by 4 hours' refluxing with methanolic potassium hydroxide (15 c.c. of 5% methanolic potassium hydroxide for each g. of saponin). Then the solution was concentrated to a small bulk under reduced pressure at 30° and the residue was poured into excess of water. The non-saponifiable material was extracted with ether, and the saponified portion was saturated with salt and heated on a steam-bath to remove ether and some methanol; the impure sodium polyporenate A was deposited as a brown, granular, slightly gummy mass, which was filtered off on muslin. The air-dried salt was then digested at 30° for 2 days with sufficient glacial acetic acid to form a thick paste which was then filtered off and washed during the course of 3—4 days with acetic acid (6 × 50 c.c.) to remove most of the gummy impurities. A single crystallisation from *iso*propyl alcohol gave fine needles, m. p. 193—195° (decomp.; capillary uncorr.).

Polyporenic Acid A.—Repeated crystallisation of the impure acid, alternately from *iso*propyl alcohol and nitromethane–methanol, gave polyporenic acid A as needles, m. p. 199—200° (decomp.; preheated block), $[\alpha]_D^{20}$ +64° (*c*, 1·28 in pyridine) [Cross *et al.*, *loc. cit.*, gave m. p. 194° (capillary uncorr.), $[\alpha]_D^{20}$ +69° (*c*, 1·0 in pyridine)]. A further pure specimen of polyporenic acid A was prepared by the hydrolysis of chromatographically pure methyl ester [m. p. 148·5—149·5°, $[\alpha]_D^{20}$ +79·5° (*c*, 1·45) (see below)]. The ester (500 mg.) in methanol (10 c.c.) at 30°, was treated with cold methanolic potassium hydroxide (10 c.c.; 40%) at 20° for 72 hours. Addition of water and acidification with dilute hydrochloric acid gave a flocculent precipitate which crystallised from *iso*propyl alcohol and nitromethane–methanol giving fine needles of polyporenic acid A (300 mg.), m. p. 199—200° (decomp.; preheated block), $[\alpha]_D^{20}$ +64° (*c*, 2·2 in pyridine), $[\alpha]_D^{20}$ +74° (*c*, 0·93) (solution in chloroform was only effected by warming) (Found: C, 76·4; H, 10·1. Calc. for $C_{30}H_{48}O_4$: C, 76·2; H, 10·25%).

Methyl Polyporenate A.—The acid (10 g.) in acetone was treated with a slight excess of diazomethane in ether. Removal of the solvent and crystallisation of the residue from aqueous methanol gave stout needles (9 g.), m. p. 144—146°. A portion (500 mg.) of the product was dissolved in benzene (10 c.c.) and adsorbed on alumina (50 g.). The fraction eluted with benzene–ether (3 : 2) gave the methyl ester (380 mg.) as needles, m. p. 148·5—149·5°, $[\alpha]_D^{20}$ +79·5° (*c*, 1·45), from nitromethane–methanol (Found: C, 76·5; H, 10·4. Calc. for $C_{31}H_{50}O_4$: C, 76·5; H, 10·4%) [Cross *et al.*, *loc. cit.*, gave m. p. 142° (capillary, uncorr.), $[\alpha]_D^{20}$ +77° (*c*, 3·0)]. A chloroform solution gave a yellow colour with tetranitromethane.

cyclo*Hexylamine Salt of Polyporenic Acid A*.—Polyporenic acid A (500 mg.) in a minimum of boiling ethyl acetate was treated with an excess of *cyclo*hexylamine, and the solution heated under

reflux for several minutes; fine needles were deposited on cooling. Recrystallisation from ethyl acetate–alcohol gave long flat needles of the *cyclo*hexylamine *salt* (490 mg.), $[\alpha]_D^{20}$ +47° (*c*, 2·06 in alcohol) (Found: N, 2·75. $C_{36}H_{61}O_4N$ requires N, 2·45%). The m. p. of this compound was characteristic though not well defined; the flat needles became opaque and serrated at 120—130°, and decomposed at 175—177°. Globules of liquid then formed and fresh needles appeared. These were most probably polyporenic acid A since complete melting took place at 190—192°.

Methyl Ester a-*Acetate*.—Acid A methyl ester (1 g.) in pyridine (6 c.c.) was treated with acetic anhydride (3 c.c.) at 20° for 72 hours, and the reaction mixture was then poured on ice. Crystallisation of the product from aqueous ethanol gave fine needles (800 mg.), m. p. 133—135°. The latter material, dissolved in benzene (20 c.c.), was adsorbed on alumina (100 g.), and the fraction eluted with benzene–ether (4 : 1) afforded *methyl polyporenate A* a-*acetate* (600 mg.), crystallising from nitromethane–methanol as long flat needles, m. p. 137—138°, $[\alpha]_D^{20}$ +33° (*c*, 1·15) (Found: C, 74·9; H, 9·75; OAc, 7·5, 7·7. $C_{33}H_{52}O_5$ requires C, 74·95; H, 9·9; OAc, 8·15%). A chloroform solution gave a yellow colour with tetranitromethane.

Methyl Ester a : b-*Diacetate*.—(*a*) Acid A methyl ester (1 g.) in glacial acetic acid (10 c.c.) and acetic anhydride (3 c.c.) was cooled below 18° and treated with 5N-anhydrous perchloric acid in acetic acid (0·1 c.c.), the temperature being kept below 35° with external cooling (cf. Whitman and Schwenk, *loc. cit.*). After 35 minutes the solution was poured on ice, and the product isolated with ether. Crystallisation from aqueous ethanol gave needles (800 mg.), m. p. 115—117°. Adsorption from benzene (20 c.c.) on alumina (100 g.) and elution with benzene–ether (9 : 1) gave *methyl polyporenate A* a : b-*diacetate* (700 mg.), which crystallised from methanol as needles, m. p. 118·5—120°, $[\alpha]_D^{20}$ +86·5° (*c*, 2·99) (Found: C, 73·85; H, 9·25; OAc, 15·4. $C_{35}H_{54}O_6$ requires C, 73·65; H, 9·55%; OAc, 15·1%). A chloroform solution gave a yellow colour with tetranitromethane.

(*b*) Acid A methyl ester *a*-acetate (500 mg.) was acetylated as in (*a*) above. The product crystallised from aqueous ethanol in needles (408 mg.), m. p. 114—115·5°. Repeated crystallisation from aqueous methanol raised the m. p. to 117—118·5° undepressed on admixture with a specimen prepared as in (*a*). This sample had $[\alpha]_D^{20}$ +87° (*c*, 1·32) (Found: C, 74·05; H, 9·8. Calc. for $C_{35}H_{54}O_6$: C, 73·65; H, 9·55%).

Hydrolysis of the Methyl Ester a : b-*Diacetate*.—The diacetate (1 g.) in methanol (20 c.c.) was treated with methanolic potassium hydroxide (20 c.c.; 20%) at 20° for 72 hours. The solution was concentrated under reduced pressure, diluted with water, and extracted with ether. Acidification and evaporation of the aqueous portion afforded a flocculent precipitate of acid (600 mg.). Several crystallisations from *iso*propyl alcohol yielded fine needles, m. p. 198—199° (decomp.; preheated block) undepressed on admixture with an authentic specimen of polyporenic acid A, $[\alpha]_D^{20}$ +63° (*c*, 1·3 in pyridine).

Methyl Ester a-*Benzoate*.—Acid A methyl ester (2 g.) in pyridine (12 c c.) was treated with benzoyl chloride (1·4 g.) at 0°, and the mixture set aside at 20° for 48 hours. It was then poured on ice, and the product isolated. Crystallisation from aqueous methanol gave *methyl polyporenate A* a-*benzoate* (1·8 g.) as stout blunt needles, m. p. 139—140° raised by repeated crystallisation from methanol to 142—143°, $[\alpha]_D^{20}$ +12·5° (*c*, 1·84) (Found: C, 77·55, 77·1; H, 9·15, 9·3. $C_{38}H_{54}O_5$ requires C, 77·25; H, 9·2%).

Hydrolysis. The benzoate (250 mg.) was hydrolysed with methanolic potassium hydroxide (20%) at 20° for 72 hours. The acidic product (150 mg.), isolated as described above, crystallised from *iso*propyl alcohol as fine needles of polyporenic acid A, m. p. and mixed m. p. 198—199° (decomp.; preheated block).

Chromium Trioxide Oxidising Reagent.—A cold solution of chromium trioxide (266·7 g.) in concentrated sulphuric acid (230 c.c.) and distilled 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 a minimum of pure acetone (distilled over potassium permanganate) at 20°, and the reagent added dropwise from a microburette until an orange-brown colour persisted. External cooling was applied to keep the temperature below 30°.

a : b-*Diketopolyporenic Acid A*.—Acid A (500 mg.) was treated with chromium trioxide solution (0·57 c.c.; theory for 4 equivs., 0·53 c.c.) and set aside for 30 minutes. Dilution with water caused the product to crystallise. Three crystallisations from methanol gave the a : b-*diketopolyporenic acid A* (390 mg.) as stout needles, m. p. 206·5—207·5° (decomp.; preheated block), $[\alpha]_D^{20}$ +105·5° (*c*, 1·05), $[\alpha]_D^{20}$ + 103·5° (*c*, 3 in pyridine) (Found: C, 77·2, 77·15; H, 9·55, 9·7. $C_{30}H_{44}O_4$ requires C, 76·9; H, 9·45%). The diketone readily formed a gelatinous 2 : 4-dinitrophenylhydrazone.

a-*Oxime*. The diketo-acid (500 mg.) in ethanol (3 c.c.) was boiled for 5 hours with hydroxylamine hydrochloride (200 mg.) and fused sodium acetate (500 mg.). The product, isolated by addition of water to the cold solution, was repeatedly crystallised from aqueous ethanol giving a : b-*diketopolyporenic acid A* a-*oxime* as stout prismatic needles, m. p. 212—214° (decomp.; preheated block), $[\alpha]_D^{20}$ +34° (*c*, 1·49) (Found : C, 74·35; H, 9·4; N, 2·9. $C_{30}H_{45}O_4N$ requires C, 74·5; H, 9·4; N, 2·9%).

Methyl a : b-*Diketopolyporenate A*.—(*a*) Methyl polyporenate A (500 mg.) was oxidised with chromium trioxide solution (0·55 c.c.; theory for 4 equivs., 0·52 c.c.). After 30 minutes water was added. *Methyl* a : b-*diketopolyporenate A* (340 mg.) formed stout needles (from methanol), m. p. 129—130°, $[\alpha]_D^{20}$ +106° (*c*, 0·89) (Found : C, 76·85, 77·45; H, 9·5, 9·8. $C_{31}H_{46}O_4$ requires C, 77·1; H, 9·6%).

(*b*) *a* : *b*-Diketo-acid A (200 mg.) was treated with a slight excess of diazomethane in ether. Evaporation of the solvent gave a gum which crystallised from methanol as stout needles (160 mg.), m. p. 126—127°, which was raised by repeated crystallisation from methanol to 129—130°, undepressed on admixture with a specimen prepared as in (*a*), $[\alpha]_D^{20}$ +105° (*c*, 1·37). The diketone readily formed a gelatinous 2 : 4-dinitrophenylhydrazone.

a-*Oxime*. (*a*) Methyl *a* : *b*-diketopolyporenate A (250 mg.) in ethanol (1·5 c.c.) was boiled for 5 hours with hydroxylamine hydrochloride (100 mg.) and fused sodium acetate (250 mg.). Dilution with water and filtration yielded a product which was crystallised several times from aqueous ethanol and ethanol, giving *methyl* a : b-*diketopolyporenate A* a-*oxime* as fine needles, m. p. 159—160° (capillary; uncorr.), remelting at 170—171° if the melt was allowed to cool and solidify. A specimen dried for analysis had m. p. 163—164° remelting at 168—169° (Found: C, 74·3; H, 9·4; N, 2·8. $C_{31}H_{47}O_4N$ requires C, 74·8; H, 9·5; N, 2·8%).

(*b*) Methyl *a* : *b*-diketopolyporenate A (250 mg.) and hydroxylamine hydrochloride (250 mg.) in pyridine (0·25 c.c.) and ethanol (2·5 c.c.) were heated under reflux for 15 hours. The product, isolated in the usual way and crystallised several times from aqueous alcohol, gave the *a*-oxime as long hairlike needles, m. p. 163—164°, remelting at 169—170° [the m. p. was undepressed on admixture with a specimen prepared as in (*a*)], $[\alpha]_D^{20}$ +59° (*c*, 0·96) (Found : C, 74·7; H, 9·6; N, 2·95. Calc. for $C_{31}H_{47}O_4N$: C, 74·8; H, 9·5; N, 2·8%).

Methyl b-*Ketopolyporenate A* a-*Acetate*.—Methyl polyporenate A *a*-acetate (500 mg.) was oxidised with chromium trioxide solution (0·27 c.c.; theory for 2 equivs., 0·24 c.c.) in the usual way. The product was crystallised from methanol giving *methyl* b-*ketopolyporenate A* a-*acetate* as stout needles (350 mg.), m. p. 159·5—161°, raised by repeated crystallisation from methanol and ethanol to 162—163°, $[\alpha]_D^{20}$ +45° (*c*, 1·2) (Found : C, 75·55; H, 9·8. $C_{33}H_{50}O_5$ requires C, 75·25; H, 9·55%). Light absorption in alcohol : Max. 2900 Å; ε = 270. The compound did not form a 2 : 4-dinitrophenylhydrazone or an oxime.

Methyl b-*Ketopolyporenate A* a-*Benzoate*.—Methyl polyporenate A *a*-benzoate (500 mg.) was oxidised with chromium trioxide solution (0·25 c.c.; theory for 2 equivs., 0·21 c.c.) at 25°. After 30 minutes water was added and the product isolated. Several crystallisations from methanol gave *methyl* b-*ketopolyporenate A* a-*benzoate* (320 mg.) as long flat needles, m. p. 103—104°, $[\alpha]_D^{20}$ +25° (*c*, 1·8) (Found : C, 77·2; H, 8·8. $C_{38}H_{52}O_5$ requires C, 77·5; H, 8·9%). The ketone did not form a 2 : 4-dinitrophenylhydrazone or an oxime.

b-*Ketopolyporenic Acid A*.—Methyl *b*-ketopolyporenate A *a*-acetate (450 mg.) in methanol (25 c.c.) was treated with methanolic potassium hydroxide (25 c.c.; 20%) for 72 hours at 20°. The yellow solution was diluted with water and extracted with ether. Acidification of the aqueous portion and removal of ether and some methanol under reduced pressure yielded a flocculent precipitate of acid (350 mg.). Repeated crystallisation from aqueous *iso*propyl alcohol and aqueous methanol gave b-*ketopolyporenic acid A* as blunt needles, m. p. 202—204° (decomp.; preheated block), $[\alpha]_D^{20}$ +77·5° ± 2° (*c*, 0·72, 1·07) (Found : C, 76·45; H, 9·75). $C_{30}H_{46}O_4$ requires C, 76·55; H, 9·85%). Light absorption in alcohol : Max. 2920—2930 Å; ε = 450.

Methyl b-*Ketopolyporenate A*.—*b*-Keto-acid A (500 mg.) was treated with a slight excess of diazomethane in ether. Removal of the solvent and repeated crystallisation of the product from aqueous methanol gave *methyl* b-*ketopolyporenate A* as silky needles, m. p. 160—161·5°, $[\alpha]_D^{20}$ +76·5° ± 1° (*c*, 0·59, 1·42) (Found: C, 77·15; H, 10·0. $C_{31}H_{48}O_4$ requires C, 76·8; H, H, 10·0%).

Oxidation of Methyl b-*Ketopolyporenate A*.—The ester (60 mg.) was oxidised in the usual manner. The product, after several crystallisations from methanol, afforded methyl *a* : *b*-diketopolyporenate A (35 mg.) as stout needles, m. p. and mixed 128—129°.

Decarboxylation of Polyporenic Acid A.—Polyporenic acid A (269 mg.), recrystallised eight times from *iso*propyl alcohol and dried for 4 hours at 100°/0·05 mm., was heated for 80 minutes

at 220° in a slow stream of pure dry nitrogen. The issuing gases were passed through an absorption train, and carbon dioxide (25·3 mg.), but no water, was absorbed (theory: CO_2 for one carboxyl group, 25·1 mg.). Polyporenic acid A (1 g.) was decarboxylated at 200—220° in an inert atmosphere, the residual clear brittle resin dissolved in ether, and the solution washed with 5% aqueous methanolic alkali, dilute hydrochloric acid, and water and dried. Evaporation yielded a solid which was excessively soluble in all solvents except light petroleum (b. p. 40—60°) and nitromethane. A single crystallisation from light petroleum (b. p. 40—60°) gave hair-like needles (700 mg.), m. p. 142—145°, raised by repeated crystallisation from nitromethane containing a trace of methanol to 148—150°, $[\alpha]_D^{20}$ +68° (*c*, 1·1). The crude decarboxylated material (900 mg.) was dissolved in benzene (25 c.c.) and adsorbed on alumina (90 g.). Elution with benzene–ether (9 : 1) gave the *decarboxy-compound* (650 mg.), m. p. 149—152°, which afforded long flat needles (from nitromethane), m. p. 151—153°, $[\alpha]_D^{20}$ +69° (*c*, 2·6) (Found: C, 81·55; H, 11·25. $C_{29}H_{48}O_2$ requires C, 81·25; H, 11·3%). A solution in chloroform gave a yellow colour with tetranitromethane.

a-*Acetate of Decarboxy-compound.*—The decarboxy-compound (500 mg.) in pyridine (4 c.c.) was treated with acetic anhydride (2 c.c.) at 20° for 72 hours. The uncrystallisable product was adsorbed on alumina (50 g.) from light petroleum–benzene (5 : 1; 20 c.c.). Elution with light petroleum–benzene (3 : 2) gave the a-*acetate* (420 mg.), as long needles (from methanol), m. p. 154—155·5°, $[\alpha]_D^{20}$ +22° (*c*, 2·92) (Found: C, 79·2; H, 10·6. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·7%). A chloroform solution gave a yellow colour with tetranitromethane.

a : b-*Diacetate of Decarboxy-compound.*—The decarboxy-compound (750 mg.) in acetic anhydride (6 c.c.) was heated under reflux for 1 hour with fused sodium acetate (250 mg.). The product, isolated in the usual way, was dissolved in light petroleum–benzene (4 : 1; 20 c.c.) and adsorbed on alumina (70 g.). The fraction eluted with light petroleum–benzene (2 : 3) crystallised from methanol and ethanol giving the a : b-*diacetate* (455 mg.) as flat needles, m. p. 115—116°, $[\alpha]_D^{20}$ +79° (*c*, 2·67) (Found C, 77·0; H, 10·0. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·25%).

a-*Benzoate of Decarboxy-compound.*—The decarboxy-compound (500 mg.) in pyridine (6 c.c.) was treated with benzoyl chloride (0·5 c.c.) at 0°, and set aside at 0° for 24 hours and at 20° for a further 24 hours. The product, isolated in the usual way, was dissolved in light petroleum–benzene (3 : 2; 25 c.c.) and adsorbed on alumina (50 g.). Elution with light petroleum–benzene (1 : 4) gave the a-*benzoate* (250 mg.), as stout needles (from ethanol), m. p. 166—167°, $[\alpha]_D^{20}$ −5° (*c*, 1·71) (Found: C, 81·15; H, 10·0. $C_{36}H_{52}O_3$ requires C, 81·15; H, 9·85%).

Decarboxy-a : b-*diketo-compound.*—(*a*) *a* : *b*-Diketo-acid A (1 g.) was decarboxylated at 210—220° in an inert atmosphere for 1·5 hours. The cold melt, which solidified on treatment with methanol, was crystallised several times from methanol giving the *decarboxy*-a : b-*diketo*-compound (500 mg.) as plates, m. p. 148—149° raised by repeated crystallisation from methanol–chloroform to 150—151°, $[\alpha]_D^{20}$ +100° (*c*, 2·23) (Found: C, 82·0; H, 10·2. $C_{29}H_{44}O_2$ requires C, 82·05; H, 10·45%). The diketone readily formed a gelatinous 2 : 4-dinitrophenylhydrazone.

(*b*) Decarboxylated acid A (250 mg.) was oxidised with chromium trioxide solution (0·60 c.c.; theory, 0·55 c.c.). The diketone, isolated in the usual way, crystallised from methanol and ethanol as plates, m. p. 149—150° undepressed on admixture with a specimen prepared as in (*a*), $[\alpha]_D^{20}$ +100° (*c*, 0·93).

a-*Oxime.*—The decarboxy-*a* : *b*-diketo-compound (500 mg.) and hydroxylamine hydrochloride (500 mg.) in pyridine (0·5 c.c.) and ethanol (5 c.c.) were heated under reflux for 18 hours. The product, which crystallised on cooling, was isolated by dilution with water and filtration. Several crystallisations from methanol–chloroform and ethanol gave fine needles of the a-*oxime*, m. p. 199—200°, $[\alpha]_D^{20}$ +34·5° (*c*, 2·79) (Found: C, 79·2; H, 10·5; N, 3·55. $C_{29}H_{45}O_2N$ requires C, 79·2; H, 10·3; N, 3·2).

Lithium Aluminium Hydride Reduction of Acid A Methyl Ester.—Methyl polyporenate A (900 mg.) in a minimum of dry ether was stirred at 0° and an excess of an ethereal solution of lithium aluminium hydride (16 c.c.; 0·48M) was added dropwise. After 15 minutes water was added to decompose excess of reagent, and dilute sulphuric acid (20 c.c.; 10%) to decompose the metal complex. The ethereal solution was washed thoroughly with dilute sulphuric acid, sodium hydrogen carbonate solution and water, dried, and evaporated. The residue was crystallised from nitromethane or aqueous methanol giving small prisms of the *triol* (650 mg.), m. p. 172—173·5°, $[\alpha]_D^{20}$ +65° (*c*, 3·42) (Found: C, 78·45; H, 10·7. $C_{30}H_{50}O_3$ requires C, 78·55; H, 11·0%). A chloroform solution gave a yellow colour with tetranitromethane. On titration with perbenzoic acid in chloroform solution the triol had consumed 1·98 equivs. when the uptake of oxidant was complete.

Oxidation of the Triol.—The triol (650 mg.) was carefully oxidised with a slight excess of chromium trioxide solution. Crystallisation of the product from methanol gave *a* : *b*-diketopolyporenic acid A, m. p. and mixed m. p. 205—206°, $[\alpha]_D^{20}$ +104° (*c*, 1·06).

The authors thank H.M. Forestry Commission and its foresters in the New Forest for assistance in securing the *Polyporus betulinus* used in the work described in this and the following paper. One of them (R. G. C.) is indebted to the Commonwealth Scientific and Industrial Research Organisation for making possible his participation in this research and another (G. F. W.) thanks the Department of Scientific and Industrial Research for a maintenance grant. The authors thank Mr. E. S. Morton and Mr. H. Swift for the microanalyses and Miss E. Fuller for the ultra-violet absorption spectra.

IMPERIAL COLLEGE, LONDON, S.W.7.
THE UNIVERSITY, MANCHESTER, 13.

[*Received, August 2nd,* 1952.]