The Chemistry of Fungi. Part XXIII. Tumulosic Acid.*

By L. A. Cort, R. M. Gascoigne, J. S. E. Holker, B. J. Ralph, Alexander Robertson, and J. J. H. Simes.

[Reprint Order No. 5414.]

A mixture of acids, isolated from artificially grown *Polyporus tumulosus* Cooke, *P. australiensis* Wakefield, or *Poria cocos* Wolf, difficult to separate, has been shown to contain a doubly unsaturated dihydroxy-monobasic acid, tumulosic acid, $C_{31}H_{50}O_4$, and its dehydro-derivative, $C_{31}H_{48}O_4$, each of which contains a reactive double bond present in a vinylidene group.

Tumulosic and dehydrotumulosic acid, respectively, have been converted into methyl eburico-7 : 9(11)-dien-21-oate (III; R = H), and 3β : 16α-dihydroxyeburico-7 : 9(11)-dien-21-oate (VII; R = Me, R′ = R″ = H) and its diacetate (VII; R = Me, R′ = R″ = Ac). The position of the reactive vinylidene group in tumulosic acid has been determined and a structure (XII) for the compound deduced.

In an appendix, acids from strains of *Poria cocos* (Schw.) Wolf are reported.

In Part XIV (*J.*, 1950, 3380) the identification of some of the water-soluble metabolites produced by the wood-rotting Basidiomycete fungus *Polyporus tumulosus* Cooke grown on an artificial medium was described and, simultaneously with the subsequent investigations on eburicoic acid (Part XVI, *J.*, 1951, 2346; Part XVII, *J.*, 1953, 1830; Part XVIII, *J.*, 1953, 2414; Part XIX, *J.*, 1953 2422), an examination of the mycelial products of this fungus led to the isolation of ergosterol and a new unsaturated, monobasic, tetracylic, dihydroxy-acid, $C_{31}H_{50}O_4$, for which the name tumulosic acid is provisionally adopted. Like eburicoic acid from some species of Basidiomycete fungi (Part XVI, *loc. cit.*) tumulosic acid is accompanied by a closely related compound characterised by selective ultra-violet absorption maximum at 243 mμ with associated subsidiary peaks at 236 and 251 mμ. The compound which shows this spectral property and occurs together with eburicoic acid in some fungi has been shown to be dehydroeburicoic acid (Part XVII, *loc. cit.*) and it appeared probable that in the case of tumulosic acid the accompanying acid with $\lambda_{max.}$ 243 mμ was dehydrotumulosic acid. Like the natural mixture of eburicoic and dehydroeburicoic acid the mixed acids from *P. tumulosus* Cooke proved extremely difficult to separate. However, by exhaustive chromatography of the mixture of the acetylated acids sufficient pure tumulosic acid † has been obtained for characterisation and for preliminary experiments. The isolation of dehydrotumulosic acid from the natural mixture has not so far been successful. A closely similar mixture of tumulosic and dehydrotumulosic acid has also been isolated from the mycelium of *Polyporus australiensis* Wakefield grown on the artificial medium, and from the naturally occurring sporophores of this species, but a separation of the mixture was not attempted; its identity was established by the preparation of derivatives of the mixed acids and the comparison of their infra-red absorption with the absorption of corresponding derivatives of the mixture obtained from *P. tumulosus*.

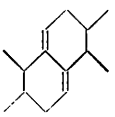
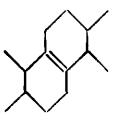
Two hydroxyl groups are present in tumulosic acid since the acid and its methyl ester, respectively, form *O*-diacetyltumulosic acid and methyl *O*-diacetyltumulosate, each of which on catalytic hydrogenation absorbs a mol. of hydrogen, giving the corresponding dihydro-compounds. The dihydro-derivatives give yellow colours with chloroformic tetranitromethane, indicating the presence of a second and unreactive double bond. On oxidation with lead tetra-acetate, the diol formed from methyl *O*-diacetyltumulosate with osmium tetroxide gave formaldehyde, whereas methyl *O*-diacetyldihydrotumulosate was not oxidised under these conditions. Consequently the reactive double bond of tumulosic

* Part XXII, *J.*, 1954, 1432.

† The mixture of tumulosic and dehydrotumulosic acid was first isolated by Dr. B. J. Ralph (Thesis, Liverpool, 1949), and the isolation of tumulosic acid was reported by Dr. R. M. Gascoigne (Thesis, Liverpool, 1951). A. R.

acid is present in a vinylidene group and in agreement with this conclusion the infra-red spectrum of methyl *O*-diacetyltumulosate exhibits a peak at 891 cm.$^{-1}$ whereas the corresponding dihydro-derivative does not show selective absorption in the 12 μ region which could be ascribed to a double bond. Hence it appears that the inert double bond in tumulosic acid and its dihydro-derivative is tetrasubstituted.

On oxidation by the Oppenauer method tumulosic acid gave a diketo-acid, tumulosodionic acid, indicating that the hydroxyl groups are secondary. Oxidation of methyl *O*-diacetyltumulosate with selenium dioxide yielded an intractable mixture, but with this reagent methyl *O*-diacetyldihydrotumulosate furnished a good yield of methyl *O*-diacetyldehydrodihydrotumulosate which on deacetylation gave methyl dehydrodihydrotumulosate. Methyl *O*-diacetyldehydrodihydrotumulosate showed selective absorption in the ultra-violet with a maximum at 234 mμ (log ε 4·25) and subsidiary peaks at 236 and 251 mμ (log ε 4·19, 4·08), and selective absorption in the infra-red with a peak at 817 cm.$^{-1}$, indicating the presence of the transoid heteroannular diene system (I) (cf. Voser, Montavon, Günthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893, and Part XVII, *loc. cit.*). Further, it appears that this diene system has arisen from the partial structure (II) present in methyl *O*-diacetyldihydrotumulosate.

(I)   (II)

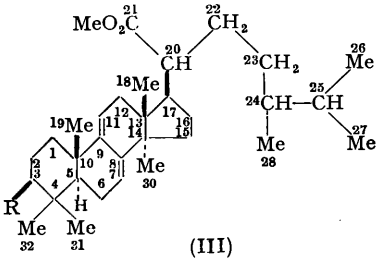
In an attempt to resolve the natural mixture of acids from *P. tumulosus* by the procedure employed for the natural mixed eburicoic and dehydroeburicoic acid (Part XVII, *loc. cit.*) the tumulosic–dehydrotumulosic acid mixture was oxidised by the Oppenauer method, but the resulting mixture of diketo-acids proved intractable. When, however, the mixture of tumulosic and dehydrotumulosic acid was successively acetylated, esterified with diazomethane, and hydrogenated, and the resulting mixture of methyl *O*-diacetyldidihydro- and *O*-diacetyldehydrodihydro-tumulosate was oxidised with selenium dioxide the homogeneous product consisted of methyl *O*-diacetyldehydrodihydrotumulosate, thus supporting the view that the component of the natural mixed acids showing absorption in the ultra-violet is dehydrotumulosic acid. On oxidation with selenium dioxide the product obtained by acetylation and subsequent hydrogenation of the mixed acids gave *O*-diacetyldehydrodihydrotumulosic acid which appeared to be homogeneous, but when this was deacetylated and the product subsequently esterified a somewhat impure methyl dehydrodihydrotumulosate was obtained which could not be readily purified by recrystallisation. Acetylation of this ester, however, gave methyl *O*-diacetyldehydrodihydrotumulosate.

In the course of experiments devised to relate tumulosic acid to compounds of known structure it was found that mild oxidation of methyl dehydrodihydrotumulosate with chromic anhydride gave methyl dehydrodihydrotumulosodionate, characterised as the bis-2 : 4-dinitrophenylhydrazone. The absorption spectrum of this diketone indicated that both keto-groups were isolated. Reduction of the diketone by the Wolff–Kishner method gave methyl dehydrodideoxydihydrotumulosate which was shown to be identical with methyl eburico-7 : 9(11)-dien-21-oate (III; R = H) derived by the oxidation of methyl 3β-hydroxyeburico-7 : 9(11)-dien-21-oate (methyl dehydrodihydroeburicoate; Part XVII, *loc. cit.*) (III; R = OH) with chromic anhydride and subsequent reduction by the Wolff–Kishner method of the resulting methyl 3-oxoeburico-7 : 9(11)-dien-21-oate (IV). This conversion of tumulosic acid into (III; R = H) serves to show that tumulosic and dehydrotumulosic acid contain the carbon skeleton of eburicoic acid and also that the relative positions of the carboxyl groups and inert double bonds are those obtaining in eburicoic and dehydroeburicoic acids respectively (Part XIX, *loc. cit.*).

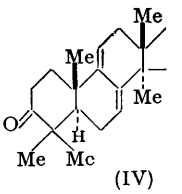
The alcoholic hydroxyl groups in tumulosic acid were readily located. Comparison of methyl dehydrodihydrotumulosodionate with methyl 3 : 16-dioxoeburico-7 : 9(11)-dien-21-oate (V) derived from polyporenic acid C, now believed to be 16α-hydroxy-3-oxoeburico-

7 : 9(11) : 24(28)-trien-21-oic acid (VI) (Bowers, Halsall, and Sayer, *J.*, 1954, 3070; cf. Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548), showed that these compounds were identical, thus proving that the two keto-groups of methyl dehydrodihydrotumulosodionate and hence the two hydroxyl groups in tumulosic acid are located at $C_{(3)}$ and $C_{(16)}$.

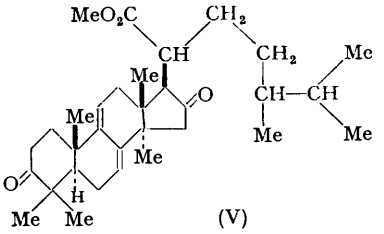
The stereochemical configurations of the hydroxyl groups in tumulosic acid are established by the fact that methyl dehydrodihydrotumulosate and methyl *O*-diacetyldehydrodihydrotumulosate were respectively identical with methyl 3 : 16-dihydroxyeburico-7 : 9(11)-dien-21-oate (VII; R = Me, R′ = R″ = H) and its diacetate (VII; R = Me,



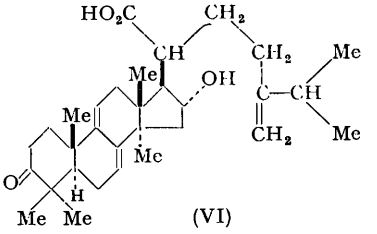
(III)



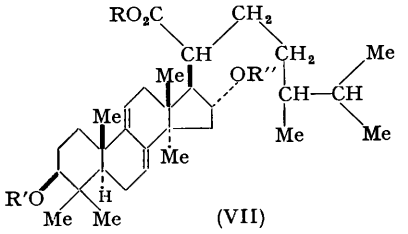
(IV)



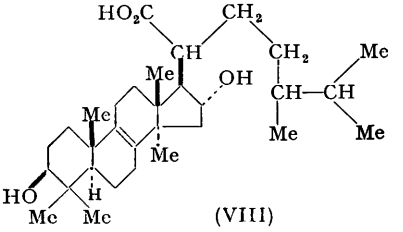
(V)



(VI)



(VII)



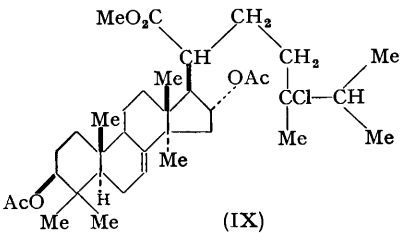
(VIII)

R′ = R″ = Ac) derived from polyporenic acid C (VI) (*idem, loc. cit.*). It is known that the 3-hydroxyl group in (VII) has the β(equatorial)-configuration (*idem, loc. cit.*) and it has recently been shown that the 16-hydroxyl group in polyporenic acid has the α-configuration (Bowers, Halsall, and Sayer, *loc. cit.*). Hence, dihydrotumulosic and dehydrodihydrotumulosic acid are, respectively, 3β : 16α-dihydroxyeburic-8-en-21-oic acid (VIII) and 3β : 16α-dihydroxyeburico-7 : 9(11)-dien-21-oic acid (VII; R = R′ = R″ = H).

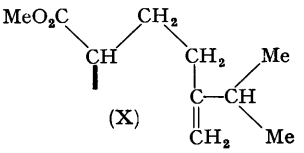
The position of the vinylidene group in tumulosic acid was established by a method analogous to that employed for eburicoic acid (Part XIX, *loc. cit.*). Treatment of methyl *O*-diacetyltumulosate with chloroformic hydrogen chloride gave an adduct containing a lablile chlorine atom. As expected by analogy with methyl 3β-acetoxyeburico-8 : 24(28)-dien-21-oate (cf. Part XIX, *loc. cit.*) this addition was accompanied by migration of the inert double bond from the Δ^8- to the Δ^7-position as shown by the presence of a peak at 817 cm.$^{-1}$ in the infra-red spectrum of the hydrochloride which is therefore formulated as (IX).

By the action of acetic anhydride on (IX), hydrogen chloride was eliminated, giving a mixture of isomeric compounds which differ only in the position of the reactive double bond and are respectively methyl 3β : 16α-diacetoxyeburico-7 : 24(28)-dien-21-oate (X)

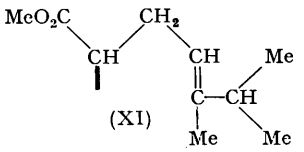
and methyl 3β : 16α-diacetoxyeburico-7 : 23-dien-21-oate (XI). The infra-red absorption spectrum of the mixture exhibited peaks at 890, 828, and 817 cm.$^{-1}$, due, respectively, to the 24 : 28-, 23 : 24-, and 7 : 8-double bonds. This diene mixture could not be separated but on ozonolysis gave formaldehyde and methyl *iso*propyl ketone (characterised as the dimethone and 2 : 4-dinitrophenylhydrazone respectively) as the sole volatile carbonyl products. This is in accordance with the formulæ postulated for the components (X)



(IX)

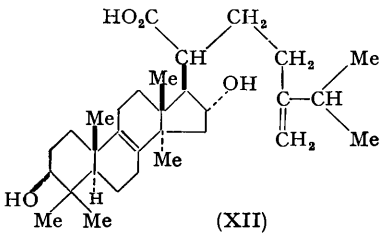


(X)

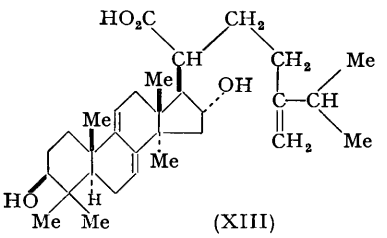


(XI)

and (XI) and hence tumulosic acid and dehydrotumulosic acid are respectively 3β : 16α-dihydroxyeburico-8 : 24(28)-dien-21-oic acid (XII) and 3β : 16α-dihydroxyeburico-7 : 9(11) : 24(28)-trien-21-oic acid (XIII). Owing to lack of material and their intractable nature, the mixture of aldehydic and ketonic non-volatile products of ozonolysis could not be separated.



(XII)



(XIII)

After the present communication had been drafted Professor E. R. H. Jones, F.R.S., of Manchester kindly sent us a copy of a communication on the natural mixed acids " Polyporenic acid B " which he and his collaborators have submitted for publication. They have shown that this " polyporenic acid B " is a mixture of 3β : 16α-dihydroxyeburico-8 : 24(28)-dien-21-oic acid (XII) and its dehydro-derivative (XIII). Since the Manchester authors have not yet isolated from the mixture pure 3β : 16α-dihydroxyeburico-8 : 24(28)-dien-21-oic acid which we had provisionally termed tumulosic acid we have at present, for convenience and brevity, retained this trivial name.

Experimental

Unless stated otherwise, optical rotations were measured in chloroform at room temperature (18—22°) with a 1-dm. tube, ultra-violet absorption spectra in 95% alcohol with a Unicam spectrophotometer, and infra-red spectra in " Nujol " pastes with a Grubb-Parsons single-beam spectrometer. The light petroleum used had b. p. 60—80°.

The fungi were cultivated on the modified Williams and Saunders medium (Part XVII, *loc. cit.*), and the dry pulverised mycelium was exhaustively extracted with light petroleum and then with ether.

Tumulosic Acid from Polyporus tumulosus *Cook and from* P. australiensis *Wakefield.*—The residue left on evaporation of the light petroleum extract from the mycelium of *P. tumulosus* Cooke was repeatedly recrystallised from aqueous alcohol, yielding ergosterol hydrate in colourless laminae, m. p. and mixed m. p. 160—162°, $[\alpha]_D$ −124° (*c*, 2·20), $\lambda_{max.}$ 262·5, 271, 281, 293·5 mμ (ε 7700, 11,100, 11,700, 6700) (Found : C, 81·3; H, 10·8. Calc. for $C_{28}H_{44}O,H_2O$:

C, 81·1; H, 11·2%). The acetate had m. p. and mixed m. p. 174—176°, $[\alpha]_D$ −91·5° (*c*, 1·12) (Found: C, 82·0; H, 10·5. Calc. for $C_{30}H_{46}O_2$: C, 82·1; H, 10·6%).

The product from the ethereal extract of the mycelium of *P. tumulosus* was digested with boiling 15% aqueous sodium carbonate, and a solution of the resulting insoluble sodium salts in boiling alcohol was diluted with 1% aqueous sodium hydroxide solution until the mixture of crystalline sodium tumulosate and dehydrotumulosate began to separate. This solid was recrystallised from methanol–ethyl acetate (1 : 1) (yield of the crude salt was about 3% of the weight of the dry mycelium).

By the same procedure the mixture of salts (yield, 2%) was isolated from *P. australiensis* Wakefield grown on the synthetic medium and from the sporophores of the naturally grown mould (yield, 2%), collected near Bateman's Bay, N.S.W., Australia.

Decomposition of the sodium salts from the mycelium of *P. tumulosus* with boiling acetic acid gave the mixture of tumulosic and dehydrotumulosic acid which separated from ethyl acetate frequently as an amorphous powder or (seldom) in rectangular plates, m. p. 318°, $[\alpha]_D$ +8·6° (*c*, 4·5 in pyridine). Treatment of the mixed sodium salts (1 g.) with excess of methyl iodide in boiling methanol (5 ml.) for 5 hr. and extraction of product with benzene gave a gum (0·8 g.) which on crystallisation from methanol furnished the mixture of methyl esters in truncated prisms, m. p. 163—164·5°, $[\alpha]_D$ +28° (*c*, 2·6) (Found: C, 76·4; H, 10·7; OMe, 6·2%). Acetylation of this yielded the mixture of the diacetates of the esters, forming slender needles, m. p. 155—156·5°, from methanol, $[\alpha]_D$ +7·0° (*c*, 2·2) (Found: C, 73·6; H, 9·4; OMe, 5·5%). The respective mixed diacetates, methyl esters, and diacetyl derivatives of the methyl esters from *P. tumulosus* and *P. australiensis* had identical infra-red absorption spectra (with 25% solutions in CS_2).

Treatment of the mixed acids or salts with hot acetic anhydride–pyridine gave a mixture of the acetylated acids which separated from aqueous alcohol in needles, m. p. 229—230°, $[\alpha]_D$ +6·4° (*c*, 8·5 in pyridine); $E^{1\%}_{1\,cm.}$ at 243 mμ varied from 73 to 110 in material from different batches of mycelium from *P. tumulosus* Cooke, and $E^{1\%}_{1\,cm.}$ 37 at 243 mμ from the mycelium of *P. australiensis* (Found: C, 73·5; H, 9·7%). A solution of this mixture (43·2 g.) from the mycelium of *P. tumulosus* in light petroleum–benzene (3·2 l.; 4 : 1) was poured on a column of neutralised aluminium oxide (60 × 6 cm.) which was eluted with light petroleum–benzene (16·5 l., 4 : 1; then 18 l., 1 : 1), benzene (5 l.), acetone–benzene (21·5 l., 1 : 19; 16·5 l., 1 : 9; 5·3 l., 1 : 4), and finally methanol (2 l.), giving fractions in which $E^{1\%}_{1\,cm.}$ at 243 mμ progressively increased from 6·5 to 175. The fractions of lower intensity were repeatedly rechromatographed, ultimately giving O-*diacetyltumulosic acid* (3·2 g.) which did not show selective absorption in the near ultra-violet and appeared to be dimorphic, separating from dilute alcohol as a mixture of plates and rods, m. p. 217—228° (mechanically separated, the rods had m. p. 214°), and from aqueous acetone in plates, m. p. 228° with sintering at 214°, $[\alpha]_D$ +6·7° (*c*, 2·01) [Found: C, 73·4; H, 9·4; OAc, 20·1, 20·4%; *M* (Rast), 551, 576, 561. $C_{31}H_{48}O_2(OAc)_2$ requires C, 73·6; H, 9·5; OAc, 20·7%; *M*, 571].

O-Diacetyltumulosic acid (210 mg.) was boiled with 5% alcoholic potassium hydroxide (8 ml.) for 1 hr. and the solution diluted with water (20 ml.), concentrated (to 18 ml.), and cooled, giving potassium tumulosate in fine needles. On being cooled, a solution of the dried salt in the minimum amount of boiling acetic acid (*ca.* 10 ml.) deposited amorphous *tumulosic acid* (116 mg.) which was dissolved in boiling aqueous alcohol. When this was concentrated the boiling solution deposited the acid in small needles, m. p. 306° (decomp.), $[\alpha]_D$ +8·1° (*c*, 3·30 in pyridine) (Found: C, 76·3; H, 10·2. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%). Tumulosic acid, which sublimed unchanged in a high vacuum and gave a bright purple-red colour in the Liebermann–Burchard reaction, was insoluble in benzene or light petroleum, very slightly soluble in chloroform, and more soluble in alcohol, methanol, or pyridine. The sodium and potassium salts were readily soluble in alcohol and insoluble in water.

The action of warm methyl iodide on sodium tumulosate or of ethereal diazomethane on tumulosic acid gave *methyl tumulosate*, forming rosettes of colourless needles, m. p. 164—164·5°, $[\alpha]_D$ +26·6° (*c*, 1·69), from methanol (Found: C, 76·6; H, 10·4; OMe, 6·6. $C_{32}H_{52}O_4$ requires C, 76·8; H, 10·5; OMe, 6·2%). Prepared by the acetic anhydride–pyridine method, *methyl* O-*diacetyltumulosate* separated from methanol in colourless needles, m. p. 159—159·5°, $[\alpha]_D$ +6·5° (*c*, 0·70), identical with a specimen formed by the action of ethereal diazomethane on *O*-diacetyltumulosic acid (Found: C, 73·7; H, 9·4: OMe, 5·3. $C_{36}H_{56}O_6$ requires C, 73·9; H, 9·7; OMe, 5·3%).

O-*Diacetyldihydrotumulosic Acid.*—Hydrogenation of *O*-diacetyltumulosic acid (1 g.) in alcohol (100 ml.) with hydrogen (approx. 1·07 mol. absorbed) at atmospheric temperature and

pressure and a palladium–charcoal catalyst was complete in ½ hr., giving O-*diacetyldihydrotumulosic acid*, which separated from aqueous alcohol in needles (0·8 g.), m. p. 231—231·5°, $[\alpha]_D$ +1·5° (*c*, 0·77), with a yellow tetranitromethane reaction in chloroform (Found: C, 73·3; H, 9·6. $C_{35}H_{56}O_6$ requires C, 73·4; H, 9·9%). The use of Adams catalyst in acetic acid gave the same dihydro-derivative.

Hydrogenation of methyl *O*-diacetyltumulosate with Adams catalyst and hydrogen (1 mol. absorbed) in acetic acid yielded *methyl* O-*diacetyldihydrotumulosate* which separated from aqueous methanol in needles, m. p. 183—184°, $[\alpha]_D$ +1·4° (*c*, 8·4), giving a yellow colour with tetranitromethane in chloroform (Found: C, 73·4; H, 9·8; OMe, 5·3. $C_{36}H_{58}O_6$ requires C, 73·7; H, 10·0; OMe, 5·3%).

Oxidation of Methyl O-*Diacetyltumulosate with Osmium Tetroxide.*—Osmium tetroxide (0·5 g.) was added to a solution of the ester (1·35 g.) in ether (20 ml.), the mixture kept at room temperature for 6 days, and the solvent evaporated. A solution of the black residue in methanol (20 ml.) was added to sodium sulphite (10 g.), dissolved in water (80 ml.), and the mixture heated under reflux for 1 hr., cooled, and filtered. Extraction of the solid with boiling methanol (15 ml. × 5) gave the *diol* in small needles (105 mg.) which on recrystallisation from the same solvent had m. p. 198—199° (Found: C, 69·8; H, 9·7; OMe, 5·0. $C_{36}H_{58}O_8$ requires C, 69·9; H, 9·5; OMe, 5·0%).

A solution of the diol (317 mg.) and lead tetra-acetate (180 mg.) in acetic acid (5 ml.) was kept at room temperature for ½ hour and poured into water, giving a gel. The volatile products were removed with steam, leaving a precipitate which did not crystallise, formed an amorphous semicarbazone and 2 : 4-dinitrophenylhydrazone, and did not react with sodium hypoiodite. The distillate contained formaldehyde which was isolated as the 2 : 4-dinitrophenylhydrazone (75 mg., 0·70 mol.), m. p. and mixed m. p. 164° (Found: C, 40·2; H, 3·0; N, 26·3. Calc. for $C_7H_6O_4N_4$: C, 40·0; H, 2·9; N, 26·7%). In another experiment the formaldehyde was converted into the dimedone derivative, m. p. and mixed m. p. 187° (Found: C, 69·5; H, 8·5. Calc. for $C_{17}H_{24}O_4$: C, 69·8; 8·3%).

Tumulosodionic Acid [3 : 16-*Dioxoeburico*-8 : 24(28)-*dien*-21-*oic Acid*].—A mixture of tumulosic acid (0·77 g.), aluminium *tert.*-butoxide (1·5 g.), *cyclo*hexanone (1·8 ml.), and dioxan (6·5 ml.) was heated under reflux for 11 hr. cooled, and diluted with an excess of 2N-sulphuric acid. The dioxan and excess of *cyclo*hexanone were removed with steam, and the residual solid was extracted with hot 1% aqueous sodium hydroxide (100 ml.). The filtered extract was concentrated (to 35 ml.) and treated with sodium carbonate (3·5 g.), giving sodium tumulosodionate which on isolation was dissolved in hot water (40 ml.) and decomposed with hydrochloric acid. The resulting *tumulosodionic acid* (0·185 g.) separated from aqueous alcohol as a colourless microcrystalline solid, m. p. 280—281° (decomp.), $\lambda_{max.}$ 285 mμ (log ε 1·87) (Found: C, 77·5; H, 9·8. $C_{31}H_{46}O_4$ requires C, 77·1; H, 9·6%).

Oxidation of mixed tumulosic and dehydrotumulosic acid under the same conditions gave a product which did not crystallise.

Methyl O-*Diacetyldehydrodihydrotumulosate* (*Methyl* 3β : 16α-*Diacetoxyeburico*-7 : 9(11)-*dien*-21-*oate*) (VII; R = Me, R′ = R″ = Ac).—A mixture of methyl *O*-diacetyldihydrotumulosate (methyl 3β : 16α-diacetoxyeburic-8-en-21-oate) (0·5 g.), selenium dioxide (1·3 g.), acetic acid (14 ml.), and water (1 ml.) was heated under reflux for 12 hr., filtered, and diluted with water (200 ml.). After crystallisation from methanol, the resulting product (0·28 g.) was purified by chromatography from benzene on aluminium oxide, followed by crystallisation from methanol, forming colourless needles, m. p. 170—170·5°, undepressed on admixture with methyl 3β : 16α-diacetoxyeburico-7 : 9(11)-dien-21-oate prepared from polyporenic acid C (Bowers, Halsall, Jones, and Lemin, *loc. cit.*); the product had $[\alpha]_D$ +21° (*c*, 1·18), $\lambda_{max.}$ 236, 243, 251 mμ (log ε 4·19, 4·25, 4·08) (Found: C, 74·0; H, 9·5; OMe, 5·2. Calc. for $C_{36}H_{56}O_6$: C, 73·9; H, 9·7; OMe, 5·3%). The infra-red spectrum was identical with that of a sample of methyl 3β : 16α-diacetoxyeburico-7 : 9(11)-dien-21-oate.

Under the same conditions the oxidation of a mixture of methyl *O*-diacetyldihydrotumulosate and methyl *O*-diacetyldehydrodihydrotumulosate (0·5 g.), prepared from the acidic complex isolated from the mycelium of *P. tumulosus*, gave methyl *O*-diacetyldehydrodihydrotumulosate (0·25 g.), m. p. and mixed m. p. 170—171°, $[\alpha]_D$ +22° (*c*, 2·09), having an infra-red spectrum identical with that of a sample of authentic material, measured with 25% solutions in CS_2.

O-*Diacetyldehydrodihydrotumulosic Acid* [3β : 16α-*Diacetoxyeburico*-7 : 9(11)-*dien*-21-*oic Acid*) (VII; R = H, R′ = R″ = Ac].—The acidic mixture from the mycelium was hydrogenated with the aid of Adams catalyst, and the product acetylated. The resulting mixture of dihydrodiacetates (80 g.) was oxidised with selenium dioxide by the procedure described above and a

solution of the crude product in benzene (4 l.) poured on to neutralised aluminium oxide (80 cm. × 6 cm.). Eluted with benzene followed by acetone–benzene (1 : 19), the compound (33·6 g.) was contaminated with selenium. A mixture of this material (2·1 g.), potassium hydroxide (4 g.), and alcohol (80 ml.) was heated under reflux for 1 hr., diluted with water (200 ml.), and concentrated (to 180 ml.). Potassium dehydrodihydrotumulosate then separated in fine needles and was decomposed with boiling acetic acid (100 ml.), giving *dehydrodihydrotumulosic acid* (VII; R = R′ = R″ = H) (1·4 g.) which separated from aqueous alcohol in short colourless needles, m. p. 324° (decomp.), $[\alpha]_D$ +26° (*c*, 2·1 in EtOH; 4-dm. tube), $\lambda_{max.}$ 236, 243, and 251 mμ (log ε 4·17, 4·24, 4·04) (Found: C, 76·2; H, 10·3. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%). By the pyridine–acetic anhydride method, this acid gave the *diacetyl* derivative which formed colourless needles, m. p. 231°, from alcohol, $[\alpha]_D$ +21·5° (*c*, 2·18), $\lambda_{max.}$ 236, 243, and 251 mμ (log ε 4·19, 4·24, 4·07) (Found: C, 73·2; H, 9·7. $C_{35}H_{54}O_6$ requires C, 73·6; H, 9·5%).

Methyl Dehydrodihydrotumulosate [*Methyl* 3β : 16α-*Dihydroxyeburico*-7 : 9(11)-*dien*-21-*oate*] (VII; R = Me, R′ = R″ = H).—(*A*) A solution of methyl *O*-diacetyldehydrodihydrotumulosate (11 g.) in methanol (600 ml.), containing potassium hydroxide (60 g.), was heated under reflux for 50 min., cooled, and poured into water (1·5 l.). Isolated with ether, the resulting methyl dehydrodihydrotumulosate (5·9 g.) separated from aqueous methanol in colourless needles, m. p. 184·5—185·5°, $[\alpha]_D$ +27° (*c*, 2·08), $\lambda_{max.}$ 236, 243, and 251 mμ (log ε 4·17, 4·24, 4·07) (Found: C, 76·7; H, 10·7; OMe, 6·4. Calc. for $C_{32}H_{52}O_4$: C, 76·7; H, 10·5; OMe, 6·2%). On being kept, the aqueous liquor deposited a further quantity of material which was collected 3 days later and on treatment with ethereal diazomethane gave more methyl ester (1·9 g.), m. p. 183—185°. Thus obtained, methyl dehydrodihydrotumulosate was identical with methyl 3β : 16α-dihydroxyeburico-7 : 9(11)-dien-21-oate (m. p. 185—187°, $[\alpha]_D$ +27°), prepared from polyporenic acid C (Bowers, Halsall, Jones, and Lemin, *loc. cit.*) (mixed m. p. and infra-red spectrum).

(*B*) Dehydrodihydrotumulosic acid (1 g.) was esterified with ethereal diazomethane, to give methyl dehydrodihydrotumulosate (0·85 g.), m. p. 167—168°, $[\alpha]_D$ +33° (*c*, 2·50), which on repeated crystallisation from methanol had m. p. 179° (with sintering at 168°), $[\alpha]_D$ +27° (*c*, 0·82), $\lambda_{max.}$ 236, 243, and 251 mμ (log ε 4·17, 4·24, 4·07). Mixed with authentic methyl dehydrodihydrotumulosate, this had m. p. 179—184°. With acetic anhydride–pyridine the methyl ester, m. p. 167—168°, gave methyl *O*-diacetyldehydrodihydrotumulosate, m. p. and mixed m. p. 170°.

Methyl Dehydrodihydrotumulosodionate [*Methyl* 3 : 16-*Dioxoeburico*-7 : 9(11)-*dien*-21-*oate*] (V). —A solution of chromic anhydride (0·46 g.) in acetic acid (14 ml.) was added dropwise to methyl dehydrodihydrotumulosate (1·5 g.) in acetic acid (42·5 ml.) at 60° during 1 hr. and after being kept at 60° for 1 hr. the mixture was treated with a little methanol to destroy unchanged anhydride, and diluted with water (375 ml.) and saturated aqueous sodium carbonate (*ca.* 30 ml.). A solution of the dried precipitate in light petroleum–benzene (30 ml.; 1 : 1) was poured on aluminium oxide (20 × 1·2 cm.). Elution with the same solvent mixture gave methyl dehydrodihydrotumulosodionate (0·5 g.) which separated from methanol in colourless needles or from acetone in plates, m. p. 169—169·5°, $[\alpha]_D$ −67° (*c*, 0·98; 0·5-dm. micro-tube), $\lambda_{max.}$ 236, 243, 251, and 295 mμ (log ε 4·18, 4·24, 4·07, 1·86) (Found: C, 77·1; H, 9·5; OMe, 6·4. Calc. for $C_{32}H_{48}O_4$: C, 77·4; H, 9·7; OMe, 6·3%). Thus obtained, this diketo-ester was identical with a specimen of methyl 3 : 16-dioxoeburico-7 : 9(11)-dien-21-oate m. p. 166·5—168·5°, $[\alpha]_D$ −68°, prepared from polyporenic acid C (Bowers, Halsall, Jones, and Lemin, *loc. cit.*) (mixed m. p. and infra-red spectra).

The *bis*-2 : 4-*dinitrophenylhydrazone* separated from aqueous dioxan in orange aggregates, m. p. 264—266° (decomp.) (Found: C, 62·3; H, 6·9; N, 12·7; OMe, 3·6. $C_{44}H_{56}O_{10}N_8$ requires C, 61·7; H, 6·6; N, 13·1; OMe, 3·6%).

Methyl Dehydrodideoxydihydrotumulosate [*Methyl Eburico*-7 : 9(11)-*dien*-21-*oate*] (III; R = H).—(*A*) A mixture of methyl dehydrodihydrotumulosodionate (0·3 g.), potassium hydroxide (0·3 g.), 90% hydrazine hydrate (0·5 ml.), and diethylene glycol (5 ml.) was heated under reflux for ½ hr. and then at 195° for 4 hr., cooled, and poured into water (100 ml.). The washed, dried precipitate was treated with ethereal diazomethane, and the product purified by chromatography from light petroleum–benzene (1 : 1) on a small column of aluminium oxide and then by crystallisation from aqueous methanol, giving *methyl dehydrodideoxydihydrotumulosate* in colourless plates, m. p. 134°, $[\alpha]_D$ +45° (*c*, 0·91; 0·5-dm. micro-tube), $\lambda_{max.}$ 236, 243, and 251 mμ (log ε 4·17, 4·25, 4·07) (Found: C, 81·8; H, 11·2; OMe, 6·5. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2; OMe, 6·6%).

(*B*) Methyl *O*-acetyldihydroeburicoate (methyl 3β-acetoxyeburic-8-en-21-oate) (from eburicoic acid; Part XVI, *loc. cit.*) was oxidised with selenium dioxide under the usual conditions, giving methyl *O*-acetyldehydrodihydroeburicoate [methyl 3β-acetoxyeburico-7 : 9(11)-dien-21-oate] (Part XVII, *loc. cit.*) which, on hydrolysis with hot 5% methanolic potassium hydroxide, furnished methyl dehydrodihydroeburicoate [methyl 3β-hydroxyeburico-7 : 9(11)-dien-21-oate] (Part XVII, *loc cit.*) subsequently oxidised with chromic anhydride under the usual conditions to methyl dehydrodihydroeburiconate [methyl 3-oxoeburico-7 : 9(11)-dien-21-oate; Part XVII, *loc. cit.*].

Reduction of this compound (0·77 g.) by the Wolff–Kishner procedure described above gave methyl eburico-7 : 9(11)-dien-21-oate (0·22 g.) which separated from methanol in colourless plates, m. p. 134°, $[\alpha]_D$ +46° (*c*, 1·0; 0·5-dm. micro-tube), $\lambda_{max.}$ 236, 243, and 251 mμ (log ε 4·17, 4·25, 4·07) (Found: C, 81·9; H, 11·2; OMe, 6·6%), and was identical with methyl dehydrodideoxydihydrotumulosate (mixed m. p. and infra-red spectra).

Isomerisation of Methyl O-*Diacetyltumulosate.*—A stream of hydrogen chloride was led into a solution of this ester (1·3 g.) in chloroform (15 ml.) at room temperature for 7 hr., and next day the solvent was distilled in a vacuum and a solution of the residue in light petroleum (25 ml.) was poured on aluminium oxide (10 × 1·2 cm.). Eluted with the same solvent, the *hydrochloride* formed colourless needles (0·5 g.), m. p. 164—166°, from methanol, $[\alpha]_D$ +3·8° (*c*, 2·69) (Found: C, 69·0; H, 9·4; Cl, 5·2; OMe, 5·3. $C_{36}H_{56}O_6$,HCl requires C, 69·6; H, 9·3; Cl, 5·7; OMe, 5·0%). A solution of this hydrochloride (0·5 g.) in acetic anhydride (6·5 ml.) was boiled for 10 hr., diluted with water (65 ml.), and kept for 16 hr. Repeated crystallisation of the resulting precipitate from methanol gave a mixture of methyl 3β : 16α-diacetoxyeburico-7 : 23-dien-21-oate and methyl 3β : 16α-diacetoxyeburico-7 : 24(28)-dien-21-oate, m. p. 179—180° (Found: C, 73·7; H, 9·9; OMe, 5·4. Calc. for $C_{36}H_{56}O_6$: C, 73·9; H, 9·7; OMe, 5·3%).

Ozonolysis of this mixture (1·6 g.) in acetic acid (50 ml.), followed by removal of the volatile products with steam, gave a distillate which on treatment with ½% aqueous dimedone (200 ml.) furnished the formaldehyde derivative (0·15 g.), m. p. and mixed m. p. 187°, of dimedone. Distillation of the aqueous filtrate from the dimedone reaction mixture and treatment of this distillate with 1% aqueous 2 : 4-dinitrophenylhydrazine sulphate (80 ml.) yielded the 2 : 4-dinitrophenylhydrazone (0·46 g.) of methyl *iso*propyl ketone which was purified by chromatography on neutralised aluminium oxide and then from methanol, forming orange prisms, m. p. 120°, identical with an authentic specimen (Found: C, 49·3; H, 5·2; N, 21·1. Calc. for $C_{11}H_{14}O_4N_4$: C, 49·6; H, 5·3; N, 21·0%). No other volatile carbonyl compound was detected.

Appendix

In continuation of studies on the metabolic products of Basidiomycete fungi the mycelial products from strains of *Poria cocos* (Schw.) Wolf (syn. *Pachyma Hoelen* Rumph.) grown artificially have been examined. From the naturally occurring sclerotium of this species Nakanisi, Yamamoto, and Ikeda (*J. Pharm. Soc. Japan*, 1939, **59**, 725) isolated a hydroxy-lactonic acid, $C_{30}H_{44}O_5$, named pachymic acid. Through the kindness of Dr. R. W. Davidson, Division of Forest Pathology, United States Department of Agriculture, seven strains of this species were obtained, *viz.*, Nos. 71693, 71692-R, 71730-S, 72152, 90852-R, 90886-T, and 94401, and have been grown on the artificial medium. The mycelium from strains 71693, 71692-R, and 90852-R contained the mixture of eburicoic and dehydroeburicoic acid (Part XVII, *loc. cit.*), strain No. 94401 furnished the mixture of tumulosic and dehydrotumulosic acid, whilst the mycelium of the remaining three species gave quantities of material insufficient for examination. Pachymic acid did not appear to be produced by the strains thus examined.

Eburicoic acid was obtained from the mycelium of *Polyporus hispidus* accompanied by only a trace of dehydroeburicoic acid.

Experimental

Eburicoic and Tumulosic Acid from Strains of Poria cocos, *Wolf.*—The light petroleum extract of the mycelium from the seven strains supplied by Dr. R. W. Davidson showed selective ultra-violet absorption with peaks at 260, 271, 281, and 295 mμ, indicating the presence of ergosterol or an ergosterol-like substance. On extraction with ether the mycelium from No. 71693 gave a mixture of eburicoic and dehydroeburicoic acid, m. p. and mixed m. p. 281—283°,

$[\alpha]_D$ +44° (*c*, 0·12; 4 dm. tube), $\lambda_{max.}$ 235, 243, and 251 mμ ($E^{1\%}_{1cm.}$ 100, 116, and 77 respectively) (Found: C, 79·1; H, 10·6. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7. Calc. for $C_{31}H_{48}O_3$: C, 79·4; H, 10·3%) (cf. Part XVII, *loc. cit.*). The acetyl derivative of this mixture had m. p. and mixed m. p. 251—253°, $[\alpha]_D$ +53° (*c*, 1·1) (Found: C, 77·6; H, 10·0. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·2. Calc. for $C_{33}H_{50}O_4$: C, 77·6; H, 9·9%). The mixture of the same acids from No. 71692-R had m. p. and mixed m. p. 282—283°, $[\alpha]_D$ +43° (*c*, 0·09; 4-dm. tube), $\lambda_{max.}$ 235, 243, and 252 mμ ($E^{1\%}_{1cm.}$ 194, 229, and 153 respectively) (Found: C, 78·9; H, 10·5%), giving an acetyl derivative, m. p. and mixed m. p. 250—251°, $[\alpha]_D$ +60·5° (*c*, 2·02) (Found: C, 77·2; H, 9·9%), and from No. 90852-R had m. p. and mixed m. p. 283—285°, $[\alpha]_D$ +38° (*c*, 0·27; 4-dm. tube), $\lambda_{max.}$ 234, 243, and 252 mμ ($E^{1\%}_{1cm.}$ 123, 143, and 99 respectively), giving an acetyl derivative, m. p. and mixed m. p. 248—250°, $[\alpha]_D$ +58·5° (*c*, 0·6) (Found: C, 77·5; H, 9·9%). The acetyl derivatives of the acid mixtures isolated from the 3 strains had infra-red spectra identical with that of an authentic mixture of *O*-acetyleburicoic and *O*-acetyldehydroeburicoic acid (3 : 1).

From strain No. 94401 a mixture of tumulosic and dehydrotumulosic acid was isolated, having m. p. and mixed m. p. 308—310° (decomp.) (Found: C, 76·1; H, 10·1%), which gave the *O*-diacetyl derivative, m. p. and mixed m. p. 224—227°, $[\alpha]_D$ +7·5° (*c*, 2·01), $\lambda_{max.}$ 236, 243, and 252 mμ ($E^{1\%}_{1cm.}$ 83, 86, and 57 respectively) (Found: C, 73·4; H, 9·6%); the mixed methyl ester had m. p. and mixed m. p. 166—167°, $[\alpha]_D$ +26° (*c*, 2·01) (Found: C, 76·2; H, 10·3%), and the diacetate of the mixed methyl ester, m. p. and mixed m. p. 156—158°, $[\alpha]_D$ +9·6° (*c*, 1·83) (Found: C, 73·7; H, 9·8%). The infra-red spectrum of the acetyl derivative was identical with that of acetylated tumulosic–dehydrotumulosic acid from *P. tumulosus*.

Eburicoic Acid from P. hispidus *Bull (Fr.)*.—After the removal of the ergosterol fraction from the mycelium with light petroleum, extraction with ether gave a pale brown, crystalline product which on recrystallisation from methanol, alcohol, and finally methanol–benzene (19 : 1) furnished eburicoic acid in colourless needles, m. p. and mixed m. p. 288—290° (decomp.), $[\alpha]_D$ +48° (*c*, 2·0; chloroform–alcohol, 4 : 1), showing very weak selective absorption in the ultra-violet at 243 mμ ($E^{1\%}_{1cm.}$ 2·3) with subsidiary peaks at 236 and 251 mμ (Found: C, 78·9; H, 10·7. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%). Methyl eburicoate had m. p. and mixed m. p. 140—141°, $[\alpha]_D$ +47° (*c*, 0·57) (Found: C, 79·1; H, 10·7; OMe, 6·2. Calc. for $C_{32}H_{52}O_3$: C, 79·3; H, 10·9; OMe, 6·4%); *O*-acetyleburicoic acid had m. p. and mixed m. p. 255—257°, $[\alpha]_D$ +47° (*c*, 2·0) (Found: C, 77·3; H, 10·2. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·2%), and methyl *O*-acetyleburicoate m. p. and mixed m. p. 153—155°, $[\alpha]_D$ +47° (*c*, 1·19) (Found: C, 77·5; H, 10·5; OMe, 6·0. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3; OMe, 5·9%); the infra-red absorption spectra of these derivatives were identical with those of authentic specimens (Part XVI, *loc. cit.*).

The authors are grateful to Professor E. R. H. Jones, F.R.S., for making available samples of methyl 3β : 16α-dihydroxy-, methyl 3β : 16α-diacetoxy-, and methyl 3 : 16-dioxo-eburico-7 : 9(11)-dien-21-oate prepared from polyporenic acid C. They also thank J. G. Reynolds of the Spectroscopic Section, Thornton Research Centre, Shell Petroleum Co. Ltd., for measuring infra-red spectra of methyl 3β : 16α-diacetoxyeburico-7 : 9(11)-dien-21-oate derived from tumulosic acid and polyporenic acid C. One of them (L. A. C.) is indebted to the Department of Scientific and Industrial Research for a Maintenance Grant.

UNIVERSITY OF LIVERPOOL. [*Received, May 26th*, 1954.]