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CHEMISTRY OF THE PODOCARPACEAE—VIII*

MACROPHYLLIC ACID, A BISDITERPENOID FROM *PODOCARPUS MACROPHYLLUS* D. DON

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Abstract—Macrophyllic acid from the heartwood of *Podocarpus macrophyllus* has been shown to be the bisditerpenoid dicarboxylic acid (I, R = H) related to podototarin, and its di-O-methyl dimethyl ester synthesized from 16-hydroxytotarol. Podototarin (IV, R = CH_3) has been prepared by a one-step enzymatic coupling of (+)-totarol and the coupling reaction extended to the preparation of the lithium aluminium hydride reduction product of macrophyllic acid dimethyl ester.

PREVIOUS investigations of *Podocarpus macrophyllus* D. Don (Japanese name "Inumaki") have been restricted to the leaves which contain α- and β- pinenes, camphene, cadinene,[1] the diterpene (−)-kaurene,[1,2] and the biflavonyl kayaflavone.[3] In an earlier communication[4] one of us (T. T.) reported the preliminary isolation of two crystalline compounds from the heartwood, m.ps. 237–238° (dec.) and 251–253° (dec.), designated as compounds A and B, respectively. Compound A, for which we propose the name macrophyllic acid, has now been shown to be a bisditerpenoid (I, R = H) comprized of two units of 16-carboxytotarol linked through the C_{12}–C_{12}' positions.

Extraction of the heartwood with methanol and fractionation of the ether solubles with alkalics of increasing basicity yielded macrophyllic acid in both sodium carbonate and sodium hydroxide fractions. Chromatography on silica gel gave the pure compound (0·015% yield). It formed an acetate, m.p. 240–241°, a methyl ester, m.p. 177–179°, and by prolonged refluxing with dimethyl sulphate and anhydrous potassium carbonate in dry acetone or with excess diazomethane a methyl-O-methyl derivative, m.p. 163–164°. Reduction of the methyl ester with lithium aluminium hydride gave a compound, m.p. 128–133°, containing both phenolic and primary aliphatic hydroxyl groups.

From analytical and I.R. data macrophyllic acid was considered earlier[4] to have

* Part VII: R. C. Cambie, W. R. J. Simpson and L. D. Colebrook, *Tetrahedron* **19**, 209 (1963).

[1] K. Nishida and H. Uota, *J. Agric. Chem. Soc. Japan* **6**, 1078 (1930); **7**, 157, 957 (1931).

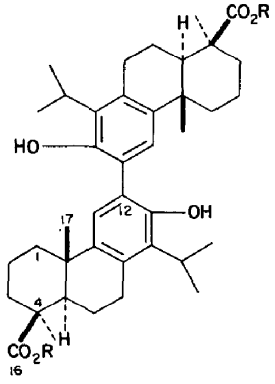
[2] L. H. Briggs and R. W. Cawley, *J. Chem. Soc.* 1888 (1948).

[3] T. Sawada, *J. Pharm. Soc. Japan* **78**, 1023 (1958); W. Baker and W. D. Ollis in *Recent Developments in the Chemistry of Natural Phenolic Compounds* (Ed. W. D. Ollis) Ch. 9, p. 152. Pergamon Press, Oxford (1961).

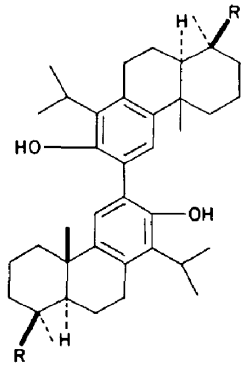
[4] T. Takahashi, *J. Japan Wood Res. Soc.* **5**, 185 (1959).

formula $C_{20}H_{28}O_3$ and it therefore appeared to belong to the phenolic acids of the podocarpic acid type and to possess an isopropyl group. However, both the likely structures of methyl-O-methyl-13-isopropylpodocarpate (II)[5] and 16-carbomethoxy-totaryl methyl ether (III)[6] were not identical with methyl-O-methylmacrophyllate, while a direct comparison of the parent acid itself with 13-isopropylpodocarpic acid, prepared by demethylation of (II) with pyridine hydrochloride, showed significant differences in their spectroscopic data.

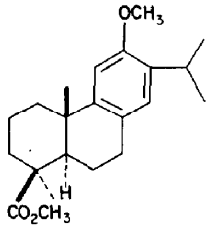
With the recent determination[6] of the structure of podototarin (IV, R = CH_3) it became clear that macrophyllic acid was also a bisditerpenoid, which was probably related to the former compound. Previous[4] and further elemental analyses of the acid and its derivatives are all compatible with a $C_{40}H_{54}O_6$ formula for the parent. Like podototarin the acid gave a negative ferric chloride reaction, failed to couple with



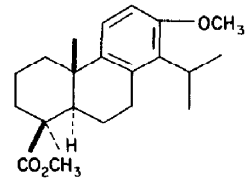
I



IV



II



III

diazotized *p*-nitroaniline or diazotized sulphanilic acid, and was recovered from attempted bromination. Moreover, its ultraviolet spectrum is virtually identical with that of podototarin, exhibiting a band at 254 mμ indicative of a biphenyl system which is absent in the spectra of totarol or the diterpenoid acids.* The I.R. spectra of the acid

* Addition of alkali to ethanolic solutions of macrophyllic acid, its methyl ester, and podototarin causes a bathochromic shift of their longest wave-length maxima (290 mμ) of ca. 28 mμ. The spectrum of the non-phenolic di-O-methyl dimethylmacrophyllate was unaffected by the addition of alkali. Contrary to the report of Short *et al.*[7] addition of alkali to an ethanolic solution of totarol causes a bathochromic shift (14 mμ) of its longest wave-length maximum.

[5] W. P. Campbell and D. Todd, *J. Amer. Chem. Soc.* **62**, 1287 (1940).
[6] R. C. Cambie, W. R. J. Simpson and L. D. Colebrook, *Tetrahedron* **19**, 209 (1963).
[7] W. F. Short, H. Wang (and appendix by J. D. S. Goulden), *J. Chem. Soc.* 2979 (1951).

and its derivatives are also similar to those of podototarin and its derivatives, in particular being marked by the absence of a band due to two adjacent aromatic hydrogens in the 805 cm^{-1} region (cf. Ref. 6). The presence of sterically hindered but non-intermolecularly bonded phenolic groupings in macrophyllic acid and its dimethyl ester are indicated by sharp peaks at 3521 and 3448 cm^{-1}, respectively.

A pK^*_{mcs} determination[8] on macrophyllic acid gave a value of 8·54 in agreement with that calculated (8·41) and observed (8·44–8·68),[9] for C_4-axial carboxylic acids while equivalent weight determinations gave values in excellent agreement with that expected for a dicarboxylic acid with structure (I, R = H). Podocarpic acid, which also possesses a free phenolic group in addition to a C_4-axial carboxyl group, gave a pK^*_{mcs} value of 8·46.

Owing to the low volatility of the compound, initial attempts to determine the molecular weight of macrophyllic acid from mass-spectral analysis, using a standard, all-glass, heated inlet system were unsuccessful. However, the mass-spectrum, determined by the courtesy of Professor Djerassi and using a new inlet system permitting the insertion of the sample directly into the ion source, gave a molecular ion peak of 630 in agreement with the required value. Under the same conditions podototarin dimethyl ether gave a correct molecular ion peak of 598 while its fragmentation pattern was similar to that of totaryl methyl ether, each spectrum showing abundant fragment ions at (M-85), (M-97), (M-111), and (M-127). The nature of the ions causing these peaks is at present speculative and requires confirmation with labelled compounds. In addition to giving abundant molecular ions the mass-spectra of macrophyllic acid, podototarin dimethyl ether, totaryl methyl ether, 16-hydroxytotarol, and totarol all showed strong fragment peaks at (M 15) corresponding to the removal of a methyl group.

A comparison of the N.M.R. spectrum of macrophyllic acid with those of podototarin, and totarol and its derivatives[6] provides further evidence for the formulation of the acid as a bisditerpenoid. The spectrum shows the presence of four methyl groups per 16-carboxytotaryl unit which can be assigned to an isopropyl group, a C_4-methyl group, and a C_{10}-angular methyl group. As in the case with podototarin the isopropyl methyl groups give rise to two doublets (J = 6·6 c/s) centred at 8·66τ and at 8·70τ, indicating that differential shielding of the two groups resulting from asymmetry of the molecule is not averaged out by rotation. The angular methyl group (8·98τ) is shifted upfield from the corresponding position in the spectra of podototarin and totarol by the shielding effect of the carboxyl group, while the remaining C_4-methyl (8·63τ) as expected[10] has suffered a marked downfield shift.

A multiplet centred at 7·02τ and a septet centred at 6·62τ can be assigned to the C_7-C_7' methylene protons and the isopropyl methine protons, respectively, from their chemical shifts and, in the case of the latter, by the coupling constant (J = 6·6 c/s). A singlet at 4·93τ due to a hydroxyl proton, possesses a chemical shift identical with that of podototarin, while a singlet at 3·11τ is indicative of a single isolated aromatic proton. Since it is the proton giving rise to the higher field doublet of the totarol AB

[8] P. F. Sommers, V. P. Arya and W. Simon, *Tetrahedron Letters* No. 20, 18 (1960).

[9] V. P. Arya, H. Erdtman and T. Kubota, *Tetrahedron* **16**, 258 (1961); E. Mosettig, P. Quitt, U. Beglinger, J. A. Waters, H. Vorbrueggen and C. Djerassi, *J. Amer. Chem. Soc.* **84**, 1990 (1962).

[10] J. C. W. Chien, *J. Amer. Chem. Soc.* **82**, 4762 (1960); J. W. ApSimon, O. E. Edwards and R. Howe, *Canad. J. Chem.* **40**, 630 (1962).

quartet which is absent, the 16-carboxytotaryl nuclei must be linked through carbon atoms *ortho* to the hydroxyl groups.

The N.M.R. spectra of the acetyl, O-methyl, and methyl-O-methyl derivatives of macrophyllic acid are also in agreement with its formulation as a bisditerpenoid. The principal peaks of these spectra and their assignments are recorded in the Table. In the spectrum of methyl-O-methyl macrophyllate the isopropyl resonances show the extra splitting as in the parent acid and as previously observed[6] for the corresponding ethers of totarol and podototarin. An unusual feature of the spectra of macrophyllic acid and its acetate is the apparent absence of a peak at low field[11] assignable to the carboxyl proton. However such a peak is expected to be broad and may be masked by background noise. Alternatively, it is possible that owing to steric hindrance the compounds are monomeric and the resonance appears in the same region as skeletal protons and is therefore masked.

Table. Nuclear magnetic resonance data

Chemical shift (τ)		Assignment
Macrophyllic acid	Macrophyllic acid diacetate	
3·11	3·05	Aromatic protons
4·93		Phenolic hydroxyl protons
6·62 (J = 6·6 c/s)	6·72 (J = 6·5 c/s)	Isopropyl methine protons
7·02	7·15	C_7 and C_7' methylene protons
	8·22	Acetyl methyl protons
8·63	8·67	C_4 methyl protons
8·66 (J = 6·6 c/s) 8·70 (J = 6·6 c/s)	8·72 (J = 6·5 c/s)	Isopropyl methyl protons
8·98	8·87	Angular methyl protons
Dimethylmacrophyllate	Di-O-methyl-dimethylmacrophyllate	
2·98	2·93	Aromatic protons
4·92		Phenolic hydroxyl protons
6·33	6·33	Ester protons
	6·58	Methoxyl protons
6·76 (J = 8·0 c/s)	6·77 (J = 8·0 c/s)	Isopropyl methine protons
7·08	7·09	C_7 & C_7' methylene protons
8·65 (J = 8·0 c/s)	8·61 (J = 8·0 c/s) 8·65 (J = 8·0 c/s)	Isopropyl methyl protons
8·72	8·72	C_4 methyl protons
8·95	8·92	Angular methyl protons

Confirmation of the structure and stereochemistry of macrophyllic acid (I, R = H) was obtained by synthesis of its di-O-methyl dimethyl ester. Bromination of (+)-16-carbomethoxytotaryl methyl ether gave a 12-bromo-derivative which on Ullmann coupling yielded di-O-methyl dimethylmacrophyllate, identical in all respects with the fully methylated derivative from the natural product.

Recently an oxidase capable of forming extended quinones by the enzymatic coupling of phenols was isolated from the wood-rotting fungus *Polyporus versicolor*.[12]

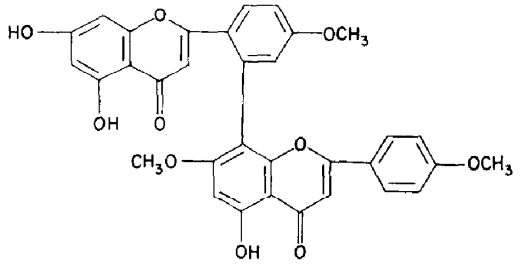
[11] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 71. Pergamon Press, London (1959).

[12] S. M. Bocks, B. R. Brown and A. H. Todd, *Proc. Chem. Soc.* 117 (1962).

Further use of the enzyme has shown that it is capable of coupling a phenol in the *ortho* position when the *para* position is blocked and that with certain molecules the extended unsaturated dione rather than the quinone is the end product of reaction.[13] The latter method has now been applied to the preparation of podototarin (IV, R = CH_3). Treatment of a dilute aqueous methanol solution of (+)-totarol, buffered to pH 5, with the enzyme from *P. versicolor* at 30° and spectroscopic investigation at intervals showed the rapid formation of a compound possessing the chromophore of podototarin. Working-up of the product from a large scale reaction and separation from unchanged totarol by alumina chromatography gave podototarin in 20% yield, identical in all respects with the compound from *Podocarpus totara*. At the present time there appears to be no previous example in the literature of an enzymically catalyzed reaction leading to the oxidative coupling of terpenoid compounds.

Replacement of totarol by 16-hydroxytotarol in the above procedure resulted in the formation of 16,16′-dihydroxypodototarin (IV, R = CH_2OH) which was identical with the product obtained by reduction of dimethyl macrophyllate with lithium aluminium hydride. In addition, spectroscopic examination showed that other derivatives of totarol (e.g. 16-acetoxytotarol and 7-oxototarol) possessing a free phenolic group also underwent coupling in a similar manner. While the appearance of a shoulder at 254 mμ in the UV spectra indicated that both podocarpic acid and methylpodocarpate underwent coupling to some extent under the same conditions, unchanged material was the only pure product isolated from a large scale attempt to form a dimer of methylpodocarpate or of podocarpic acid. Attempts to prepare a bisditerpenoid by Ullman coupling of the 13-bromo derivative of methyl-O-methylpodocarpate were either unsuccessful or resulted in extensive decomposition of the starting material.

The biflavonyl, kayaflavone (V), has been isolated from the leaves of three *Podocarpus* species, including those of *P. macrophyllus*,[3] and it has been suggested that biflavonyls are probably produced in the plant by oxidative coupling of a flavonoid precursor such as apigenin or a closely related compound. From the symmetry of the structures of the known natural bisditerpenoids, podototarin and macrophyllic acid, it is probable that they are also formed biosynthetically from a totaryl precursor in the same manner involving an enzymic coupling of the type demonstrated above.*



V

* A third and more complex bisditerpenoid, maytenone, from the heartwood of *Maytenus dispermus* has been suggested to arise from oxidized ferruginyl units followed by dimerization.[14]

[13] B. R. Brown and S. M. Bocks, *Plant Phenolics Symposium* Liverpool, April, 1962, to be published; S. M. Bocks, B. R. Brown and A. H. Todd, unpublished results.

[14] C. P. Falshaw, A. W. Johnson and T. J. King, *Proc. Chem. Soc.* 265 (1961); A. W. Johnson, T. J. King and R. J. Martin, *J. Chem. Soc.* 4420 (1961).

It was therefore of interest to examine members of the Podocarpaceae and related gymnosperms for the presence of an enzyme capable of oxidative coupling. By using 2,6-dimethoxyphenol as the test substrate crude leaf extracts of a number of species have been found to catalyze the formation of 3,5,3′,5′-tetramethoxydiphenoquinone and the results of this survey will be reported in detail elsewhere. While bisditerpenoids have yet to be isolated from leaf samples it may be significant that the leaves of *P. macrophyllus* and *P. totara* each gave a positive test for the presence of an oxidase while those of *P. hallii* which is closely related to the latter species gave a negative test. The absence of bisditerpenoids in the heartwood of *P. hallii* has already been commented upon in Part VII.[6]

EXPERIMENTAL

Analyses were by Mrs. N. Taneka and Mr. K. Sakurai, Govt. Forest Expt. Station, Meguro, and by Drs. Weiler and Strauss, Dyson Perrins Laboratory, Oxford. I.R. spectra of solutions were measured with a Perkin-Elmer 21 instrument, and for nujol mulls with a Koken DS 301 instrument. U.V. spectra were determined for EtOH solutions on a Cary recording Spectrophotometer Model 14 M or on a Hitachi EPS-2 recording Spectrophotometer, and optical rotations for EtOH solutions on an ETL-NPL automatic polarimeter. Alumina for chromatography was P. Spence grade "H", deactivated by treatment with 5% of 10% acetic acid. Light petroleum was of b.p. 60–80° M.p.'s were determined on a Kofler block and are uncorrected.

The N.M.R. spectra in $CDCl_3$ as solvent were measured with a Varian V-4300 B spectrometer operating at 60 mc/s. Positions of major peaks were measured relative to tetramethylsilane as internal reference by the audio frequency sideband method.

Extraction of Podocarpus macrophyllus *heartwood.* The finely ground heartwood (2 kg) was extracted twice with boiling methanol for 5 hr. The residue (40 g) obtained after removal of solvent from the combined extracts was then successively re-extracted with light petroleum, ether, and acetone. Ether solubles (15 g), which constituted the bulk of the extractives (38%), were fractionated in batches between 5% sodium hydrogen carbonate, 3% sodium carbonate, and 5% sodium hydroxide solutions. Material (7 g) recovered from the sodium carbonate fraction after acidification was further extracted with ether–hexane (1:1; 200 cc) and the solution chromatographed on silica gel. Fractions eluted with the same solvent yielded macrophyllic acid (200 mg). A further yield (100 mg) was isolated by similar treatment of the sodium hydroxide fraction.

Macrophyllic acid (I, R = H)

Macrophyllic acid crystallized from ether–hexane or benzene–ligroin as silky needles, m.p. 237–238° (dec.), $[\alpha]_D^8$ +79° (*c* 1·05) (Found: C, 76·15, 76·2; H, 8·7, 8·7. $C_{40}H_{54}O_6$ requires: C, 76·15; H, 8·6% Equiv (by titration) 316, 319, 322. $C_{38}H_{52}O_2(CO_2H)_2$ requires: equiv 315) λ_{max} 290 (log ε 3·85), 254 (log ε 4·18), and 220 mμ (log ε 4·68), $\lambda_{max}^{0.01N\ alc\ KOH}$ 318 (log ε 3·95), 260 (sh, log ε 4·07) and 226 mμ (log ε 4·32), $\nu_{max}^{CS_2}$ 3521 (OH), 2700–2550 (broad, OH of acid), 1704 (CO_2H), and 1182 cm^{-1} (phenolic C—OH), ν_{max}^{nujol} 3510 (OH), 2700–2550 (broad, OH of acid), 1696 (CO_2H), and 1180 cm^{-1} (phenolic C—OH).

The compound is insoluble in light petroleum but soluble in more polar organic solvents. It gave a negative ferric chloride reaction and did not couple with diazotized *p*-nitroaniline or diazotized sulphanilic acid. It was recovered from attempted hydrogenation in acetic acid or alcoholic solutions over 5% palladium charcoal or Adams' platinum oxide catalyst at room temp and atmospheric pressure.

Macrophyllic acid diacetate

Acetylation of macrophyllic acid (90 mg) with acetic anhydride (1 cc) and pyridine (1 cc) for 3 hr at 100° gave the *diacetate* (70 mg) which crystallized from ether as leaflets, m.p. 240–241° with softening at 225° (Found: C, 74·2; H, 8·5. $C_{44}H_{58}O_8$ requires: C, 73·9; H, 8·2%) λ_{max} 278 (log ε 3·25), 245 (sh, log ε 4·03), and 214 mμ (log ε 4·75), $\nu_{max}^{CHCl_3}$ 2700–2550 (broad, OH of acid), 1751 (aryl acetate), 1693 cm^{-1} (CO_2H), ν_{max}^{nujol} 1755 (aryl acetate) and 1696 cm^{-1} (CO_2H); no hydroxyl peaks.

The same product was also prepared by treatment of macrophyllic acid with acetic anhydride—sulphuric acid for 2 hr at room temp.

Dimethylmacrophyllate (I, R = CH_3)

(a) Macrophyllic acid (50 mg) in ether (10 cc) was treated with 2 moles/mole of an ethereal solution of diazomethane and the product chromatographed on alumina. Elution with benzene gave *dimethylmacrophyllate* (50 mg) which crystallized from aqueous methanol as small needles, m.p. 177–179°, with softening at 170°, $[\alpha]_D^{22}$ +98·5° (*c* 1·0) (Found: C, 76·6, 76·6; H, 9·0, 9·1. $C_{42}H_{58}O_6$ requires: C, 76·6; H, 8·9%) λ_{max} 288 (log ε 3·86), 253 (log ε 4·23), and 216 mμ (log ε 4·75), $\lambda_{max}^{0\cdot01N\ KOH}$ 317, 258, and 224 mμ, $\nu_{max}^{CS_2}$ 3448 (sharp, OH), 1724 (ester C:O), and 1189 cm^{-1} (phenolic C—OH), ν_{max}^{nujol} 3510 (OH) and 1726 cm^{-1} (ester C:O).

(b) Dimethyl sulphate (0·02 cc) was added to a stirred solution of macrophyllic acid (80 mg) in 1 cc of a mixture of 0·25N-alcoholic potassium hydroxide and water (1:1). The solution was warmed on the water-bath for a few min, and the hexane soluble portion of the product chromatographed on alumina. Fractions eluted with hexane yielded the diester (30 mg) which formed needles, m.p. 177–179°, with softening at 170° and undepressed by the product from (a) (identical I.R. spectrum).

Di-O-methyl dimethylmacrophyllate

Macrophyllic acid (60 mg) was heated under reflux with dimethyl sulphate (1·0 cc) and anhydrous potassium carbonate (500 mg) in dry acetone (20 cc) for 10 hr. The hot solution was filtered, the salts were washed well with hot acetone, and the solvent was removed *in vacuo*. The resulting solid was chromatographed from light petroleum on alumina (50 mg). Fractions eluted with light petroleum–benzene (1:1) gave *di-O-methyl dimethylmacrophyllate* (47 mg) which crystallized from aqueous methanol as small beads, m.p. 163–164° with softening at 159°, $[\alpha]_D^{25}$ +88° (*c* 0·9 in EtOH) (Found: C, 76·8, 76·6; H, 9·0, 9·2. $C_{44}H_{62}O_6$ requires: C, 76·9; H, 9·1%) λ_{max} 287 (log ε 3·45), 254 (log ε 3·88), and 217 mμ (log ε 4·42), $\lambda_{max}^{0\cdot01N\ alc\ KOH}$ no shift, $\nu_{max}^{CS_2}$ 1730 (ester C:O), and 1031 cm^{-1} (OCH_3).

The same product (80 mg) was obtained by treatment of macrophyllic acid (100 mg) with an excess of an ethereal solution of diazomethane for 24 hr at room temp, or by heating under reflux with potassium and methyl iodide in dry benzene.

16,16′-*Dihydroxypodototarin* (IV, R = CH_2OH)

A solution of dimethylmacrophyllate (50 mg) in dry ether (20 cc) was heated under reflux with lithium aluminium hydride (200 mg) for 8 hr. The resin obtained on working-up in the usual manner was chromatographed from benzene on alumina (50 g). Fractions eluted with ether contained 16,16′-*dihydroxypodototarin* which was obtained as a colourless amorphous powder (20 mg), m.p. 128–133°, after repeated purification from aqueous methanol (Found: C, 79·4; H, 9·8. $C_{40}H_{58}O_4$ requires: C, 79·7; H, 9·7%) λ_{max} 289 (log ε 3·81), 254 (log ε 4·12), and 215 mμ (log ε 4·62), $\nu_{max}^{CS_2}$ 3584 (OH), 3484 (OH), 1193 (phenolic C—OH), and 1025 cm^{-1} (CH_2—OH).

13-*Isopropylpodocarpic acid*

O-Methyl 13-isopropylpodocarpic acid (60 mg), prepared from methyl-O-methylpodocarpate by Campbell and Todd's method,[5,15] was added to freshly prepared pyridine hydrochloride (1·0 g) and the mixture heated to 214° for 40 min, cooled, and poured into water. The product was washed with water, dil. hydrochloric acid, and again with water. Crystallization from aqueous methanol gave 13-*isopropylpodocarpic acid* (30 mg) as needles, m.p. 246–248°, $[\alpha]_D^{22}$ +118·5° (*c* 1·0) (Found: C, 75·8; H, 8·9. $C_{20}H_{28}O_2$ requires: C, 75·9; H, 8·9%) λ_{max} 284 (log ε 3·66), 218 mμ (log ε 4·01), ν_{max}^{KBr} 3358 (OH), 2700–2500 (broad, OH of acid), 1691 (CO_2H), 1420 and 1192 (phenolic C—OH), and 893 cm^{-1} (isolated aromatic H).

The same product was also prepared in 84% yield by similar treatment of methyl-O-methyl 13-isopropylpodocarpate (400 mg) with pyridine hydrochloride (3·0 g).

Methyl 13-*isopropylpodocarpate*

The above acid (30 mg) in absolute methanol was treated with an excess of an ethereal solution of diazomethane. Crystallization of the product from aqueous methanol gave *methyl* 13-*isopropylpodocarpate* as needles (28 mg), m.p. 178–180° (Found: C, 76·6; H, 9·0. $C_{21}H_{30}O_2$ requires: C, 76·3; H, 9·15%) λ_{max} 282 mμ (log ε 3·58), $\nu_{max}^{CS_2}$ 3534 (OH), 1725 (ester C:O), 1190 (phenolic C—OH), and 889 cm^{-1} (isolated aromatic H).

[15] W. P. Campbell and D. Todd, *J. Amer. Chem. Soc.* **64**, 928 (1942).

12-Bromo-16-carbomethoxytotaryl methyl ether

A solution of bromine in acetic acid (10%) was added dropwise with shaking to 16-carbomethoxytotaryl methyl ether[6] (50 mg), $[\alpha]_D^{20}$ +116° (*c* 1·0), in dry ether (1 cc) until a positive starch–iodide test was obtained. Solvents were removed *in vacuo* at 50° and the residue was purified from methanol containing a few drops of chloroform to yield *12-bromo-16-carbomethoxytotaryl methyl ether* (48 mg) as an amorphous powder, m.p. 98–102° (Found: C, 62·5; H, 7·2; Br, 18·0. $C_{22}H_{31}O_3Br$ requires: C, 62·4; H, 7·4; Br, 18·45%).

Ullman coupling of 12-bromo-16-carbomethoxytotaryl methyl ether

A close-packed mixture of the above bromo-ether (45 mg) and activated copper bronze was heated in a sealed flask at 250–280° for 1 hr. The cooled mixture was extracted with ether to yield di-O-methyl dimethylmacrophyllate (5 mg), m.p. and mixed m.p. 163–164° (identical I.R. spectrum).

13-Bromo-methyl-O-methylpodocarpate

Methyl-O-methylpodocarpate (3·02 g), $[\alpha]_D^{22}$ +127·5° (*c* 1·0), in dry ether solution (10 cc) was treated dropwise with shaking with a solution of bromine in acetic acid (10%, 5·5 cc), the solution kept overnight, and solvents were removed *in vacuo* at 50°. Crystallization from ether yielded *13-bromo-methyl-O-methylpodocarpate* (3·72 g) as large needles, m.p. 144–145°, $[\alpha]_D^{23}$ +118° (*c* 1·2) (Found: C, 60·15; H, 6·2; Br, 21·3. $C_{19}H_{25}O_3Br$ requires: C, 59·85; H, 6·6; Br, 21·0%) λ_{max} 292 (log ε 3·48) and 283 mμ (log ε 3·51), $\nu_{max}^{CS_2}$ 1727 cm^{-1} (ester C:O). The spectrum exhibited no bands in the 805 cm^{-1} region, characteristic of two adjacent aromatic hydrogens.

Enzymatic coupling of diterpenoids

For large scale coupling the diterpenoid substrate (100 mg) was dissolved in 10% aqueous ethanol (4 l.) and the solution buffered with sodium acetate to pH 5·0. The enzyme solution, prepared from *Polyporus versicolor* as described,[12] was added in portions (10 cc) to the incubated (30°) substrate solutions at 24 hr intervals and the formation of dimerized products was followed by spectroscopic examination of ether extracts of aliquots of the mixture. The formation of a biphenylic system was indicated by the appearance of a peak at 254–256 mμ. When the peak had reached a maximum *E* value (ca. 2 weeks) the solutions were continuously extracted with ether, the solvent was removed, and the residues were chromatographed on alumina (50 g).

(a) With totarol as substrate, fractions eluted from the column with light petroleum yielded podototarin (10 mg), needles, m.p. and mixed m.p. 224–225° (correct U.V. and I.R. spectra). Fractions eluted with light petroleum–benzene (1:1) yielded a mixture of podototarin and totarol (ca. 2 mg) and then pure totarol (40 mg).

(b) With 16-hydroxytotarol as substrate, fractions eluted from the column with benzene–ether (10:1) contained 16-hydroxytotarol (45 mg). Fractions eluted with benzene–ether (10:2) and with benzene containing increasing amounts of ether, contained a mixture of 16-hydroxytotarol and 16,16′-dihydroxypodototarin. Further chromatography of the latter mixture yielded 16,16′-dihydroxypodototarin (8 mg, 16% yield), m.p. and mixed m.p. 127–130° (correct U.V. and I.R. spectra).

(c) With methylpodocarpate as substrate, fractions eluted from the column with light petroleum–benzene gave unchanged material (90 mg).

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