considerable interest exists in the nature of the adducts formed between *cis*-Pt and the DNA bases. The identification of these adducts by means of standard chromatographic methods requires their liberation from the DNA in essentially an intact form. This task is complicated by the refractory nature of the DNA in the vicinity of a Pt lesion to enzymatic hydrolysis [2]. It is of interest, therefore, to determine whether acid hydrolysis is a more suitable means of liberating the Pt-base adducts from DNA. In this article we examine by means of ion-exchange chromatography the effects of acid hydrolysis on the adducts formed between dG and *cis*-Pt. The methodology developed here using this simple model system has application to the analysis of *cis*-Pt-treated DNA.

MATERIALS AND METHODS

Materials

Radiolabeled *cis*-Pt (^{195m}Pt, $t_{1/2}$ = 4.02 days) was prepared at Oak Ridge National Laboratory (ORNL) [3] and supplied as a 0.9% NaCl solution. Deoxyguanosine, purchased from Sigma Chemical Co., was used without further purification. Highly soluble salts present in the *cis*-Pt stock solution, such as NaCl, were preferentially removed by drying and repeated extractions with small amounts of water; the yellow undissolved material remaining was then used as "salt-free" *cis*-Pt. *cis*-Pt has the relatively low "solubility" in water of ~2 mg/ml at 25°C.

Reaction

The reaction between *cis*-Pt and dG was carried out at 37°C (unless otherwise specified) in 0.005 M $NaClO_4$ (pH ~ 5.6). Stock solutions containing 1 mg/ml of either *cis*-Pt or dG were mixed together in the appropriate amounts to obtain solutions with r values of 0.25, 0.5, and 1.0. The reaction was judged complete after 48 hr.

Chromatography

An Aminex A6 (BioRad) cation-exchange column (1 × 30 cm) was used to separate the reaction products from the starting material. The column was maintained at a temperature of 48°C, and, following application of a 50 μl sample, was eluted with a freshly prepared K_2CO_3 solution (0.01 M, pH 11). The low buffering capacity of the elution solution required that fresh solutions be prepared daily. The column was run under 400 lb/in.^{2} (which provided a flow rate of 0.5–1.0 ml/min), and the absorbance of the eluted material continuously monitored at 254 and 280 nm by using a flow cell with a 1-cm path length.

Radioactivity

Column fractions were collected, and 0.1 ml aliquots were counted on filter paper discs in toluene-BBOT by means of a Packard scintillation counter (settings: 23% efficiency, windows 0–1000). Recovery of the radioactivity was 100%.

Absorbance

Absorbance measurements on individual fractions were made with a Beckman DU-8 spectrophotometer using a 1-cm path-length cell.

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CHEMISTRY OF THE PODOCARPACEAE—VI*

CONSTITUENTS OF THE HEARTWOOD OF *PODOCARPUS TOTARA* G. BENN.

R. C. CAMBIE and L. N. MANDER
Department of Chemistry, University of Auckland, New Zealand

(*Received* 13 *October* 1961)

Abstract—Totarol (I, R = H), 16-hydroxytotarol (I, R = OH), sugiol (II), podocarpic acid (III, R = H), methyl podocarpate (III, R = CH_3), pododacric acid, β-sitosterol, and two unidentified compounds, the major one of which has been named podototarin, have been isolated from the heartwood of *Podocarpus totara*. The absolute configuration of 16-hydroxytotarol has been determined by the conversion to (+)-totarol.

PREVIOUS investigations have shown that the diterpenoid phenol, totarol (I, R = H), is the major constituent of the heartwood of *Podocarpus totara* G. Benn. ex D. Don.[1] The structure of totarol followed from the work of Short *et al.*[2,3] and was confirmed by synthesis of the racemate by Barltrop and Rogers.[4] Chow and Erdtman[5] have since shown that the absolute configuration of the *trans* A/B ring junction is of the conventional steroid type by optical rotatory dispersion measurements and by direct correlation with dehydroabietic acid. In a preliminary account of the constituents of *P. totara* Brandt and Thomas[6] reported the presence of two further compounds in the wood. One with formula, $C_{20}H_{30}O_2$, m.p. 230–231°, was apparently a hydroxytotarol while the other, a further phenol, $C_{30}H_{44}O_2$, m.p. 222–223°, was reported as probably being triterpenoid, but details of this work have not been published. In the present work a systematic investigation of the heartwood constituents has been carried out and the isolation and investigation of these and further compounds is reported. Since the completion of this work Wenkert and Beak[7] have reported the structure (I, R = OH) of the hydroxytotarol.

As in previous parts of the series[8,9] the residue from an initial extraction with methanol, mixed with Celite, was successively re-extracted with solvents of increasing polarity. Light petroleum and ether extracts, constituting the bulk of the total extractives (51 and 38 per cent, respectively), were fractionated with alkalies of increasing basicity to give "acidic", "phenolic", and "neutral" fractions. The scheme of extraction and fractionation and the products isolated are shown in Table 1.

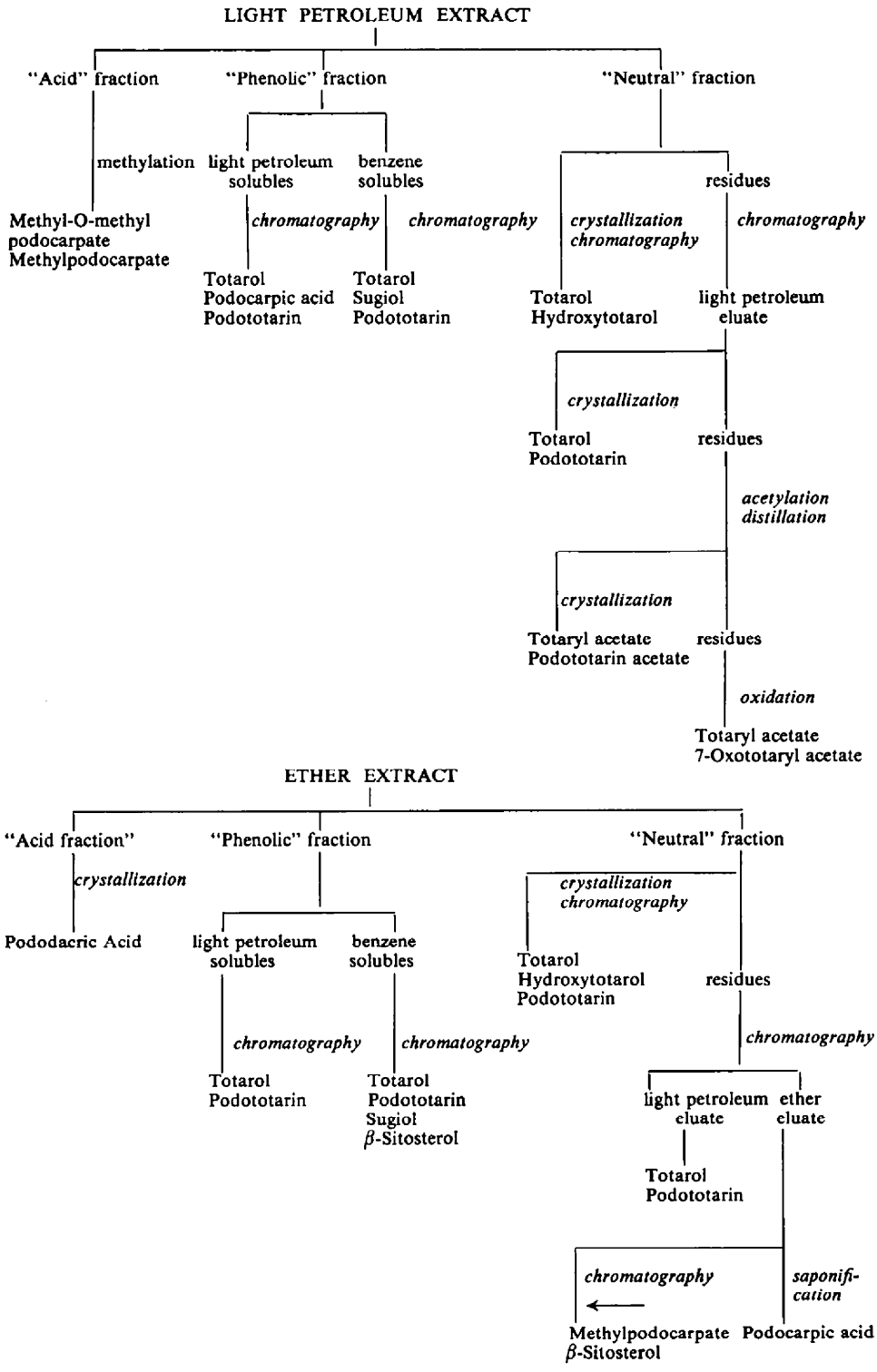
As expected, totarol (I, R = H) comprised the major constituent (5·07 per cent

* Part V: L. H. Briggs and R. C. Cambie, *Tetrahedron* **8**, 356 (1960).

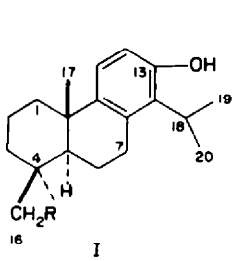
† A preliminary report of this work has been published: R. C. Cambie and L. N. Mander, *Chem. & Ind.* 1877 (1961),

[1] T. H. Easterfield and J. C. McDowell, *Trans. New Zealand Inst.* **43**, 55 (1911); **48**, 518 (1915).
[2] W. F. Short and H. Stromberg, *J. Chem. Soc.* 516 (1937).
[3] W. F. Short and H. Wang, *J. Chem. Soc.* 991 (1950); 2979 (1951).
[4] J. A. Barltrop and N. A. J. Rogers, *J. Chem. Soc.* 2566 (1958).
[5] Y-L. Chow and H. Erdtman, *Acta Chem. Scand.* **14**, 1852 (1960).
[6] C. W. Brandt and B. R. Thomas, *New Zealand J. Sci. and Tech.* **33B**, 950 (1951).
[7] E. Wenkert and P. Beak, *Tetrahedron Letters* 358, (1961).
[8] L. H. Briggs, R. C. Cambie, and J. L. Hoare, *Tetrahedron* **7**, 262 (1959).
[9] L. H. Briggs, R. C. Cambie, R. N. Seelye, and A. D. Warth, *Tetrahedron* **7**, 270 (1959).

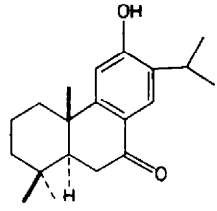
TABLE 1. HEARTWOOD CONSTITUENTS OF PODOCARPUS TOTARA



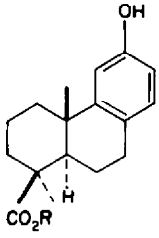
yield) and was readily isolated from "phenolic" and "neutral" fractions of the light petroleum and ether extracts by direct crystallization and chromatography on alumina. A ketone (0·002 per cent yield), isolated from the "phenolic" fraction of the light petroleum extract by chromatography, was identified as sugiol (7-oxo-ferruginol, II) by direct comparison with authentic material. In view of the occurrence of sugiol in *P. totara*, and its co-occurrence with ferruginol and xanthoperol (or its precursor[10]) in both the related species *P. dacrydioides*[8] and *Cryptomeria japonica*[11], an exhaustive search was made for the latter two compounds according to the procedures of Kondo *et al.* [11] However, this search which consisted of distillation, saponification, and oxidation of unidentified residues from "neutral" fractions, was unsuccessful and it is considered most probable that ferruginol and xanthoperol do not occur in the heartwood of *P. totara*. During this search a new derivative of totarol, the 7-oxo compound, was prepared for comparison purposes by chromic acid oxidation[12] of totaryl acetate followed by deacetylation. Reduction of 7-oxototarol or its acetate with potassium borohydride afforded 7-β-hydroxytotarol.



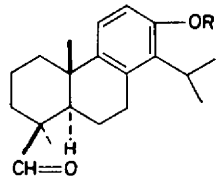
I



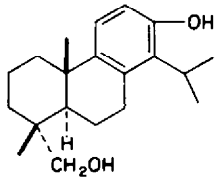
II



III



IV



V

[10] J. B. Bredenberg, *Acta Chem. Scand.* **14**, 385 (1960).
[11] T. Kondo, H. Imamura and M. Suda, *Bull. Chem. Soc. Japan* **23**, 233 (1959).
[12] R. P. Jacobsen, *J. Amer. Chem. Soc.* **73**, 3463 (1951); M. Ohta and L. Ohmori, *Bull. Pharm. Soc. Japan* **5**, 91 (1957); E. Wenkert and B. G. Jackson, *J. Amer. Chem. Soc.* **80**, 211 (1958); R. H. Bible and N. M. Hoehn, *U.S. Pat.* 2,705,725 (1955); *Chem. Abstr.* **50**, 5034 (1956).

Podocarpic acid (III, R = H) which occurred in all "acid" and "phenolic" fractions was isolated (0·001 per cent yield) by chromatography on deactivated alumina of the "phenolic" fraction of the light petroleum extract. Its further identification in the hydrolysed "neutral" fraction of the ether extract led to the isolation of methyl podocarpate (0·002 per cent yield; III, R = CH_3) from the bulk fraction by repeated chromatography and sublimation. A further acid, pododacric acid, which was earlier isolated from *P. dacrydioides*[9] was shown to be present in "acid" fractions by paper chromatography. It was isolated in trace amount from the "acid" fraction of the ether extract.

Chromatography on alumina, followed by sublimation and crystallization of the "neutral" and "phenolic" fractions of the ether extract gave a small yield (0·001 per cent) of β-sitosterol. Although a common plant constituent, β-sitosterol has not previously been reported in the extractives of the *Podocarpus* genus. It does occur, however, in the barks and heartwoods of the related species *Dacrydium cupressinum*[6,13] and D. *biforme*.[13]

Accompanying totarol from "neutral" fractions of the light petroleum and ether extracts was a further cryptophenol, partial separation of which could be effected by the greater solubility of totarol in light petroleum. The less soluble phenol (0·02 per cent yield) was readily separated by chromatography on alumina and, when pure, had melting point 230–231°, identical with that of the hydroxytotarol reported by Brandt and Thomas.[6] The compound has the formula, $C_{20}H_{30}O_2$, and was characterized by the formation of a diacetate and a dibenzoate, thus accounting for both oxygen functions as hydroxyl groups. In addition, a monoacetate which still possessed a free phenolic group could be prepared by heating the compound under reflux with glacial acetic acid. The suggestion by Brandt and Thomas that it is in fact a hydroxytotarol is strongly supported by the marked similarity of both the ultra-violet and infra-red spectra with those of totarol itself. Although the diol gives a negative ferric chloride reaction, it reacts with diazotized *p*-nitroaniline to give an orange derivative, showing that at least one of the hydroxyl groups is phenolic. That only one is phenolic follows from the formation of a monomethyl ether with dimethyl sulphate and anhydrous potassium carbonate in boiling acetone, or better, with methyl iodide and potassium in refluxing benzene. Like totarol the parent compound is unaffected by treatment with an excess of an ethereal solution of diazomethane. Totaryl methyl ether may be prepared from totarol, however, by carrying out the reaction in methylene chloride with the addition of fluoroboric acid.[14]

The infra-red spectrum of the monomethyl ether differs essentially from that of totaryl methyl ether only in possessing additional bands at 3636 and 1014 cm^{-1} indicating that the second hydroxyl function is a primary alcoholic group.[15] Both spectra show strong characteristic absorption at 1054 cm^{-1} in the region which has been assigned to the C—O—C symmetrical stretching vibration of aryl methyl ethers.[16] A further strong band expected in the 1225–1310 cm^{-1} region due to

[13] R. C. Cambie and B. F. Cain, *New Zealand J. Sci.* **3**, 121 (1960); B. F. Cain, S. Scannell, and R. C. Cambie, *New Zealand J. Sci.* **4**, 3 (1961).

[14] M. Neeman, M. C. Caserio, J. D. Roberts, and W. S. Johnson, *Tetrahedron* **6**, 36 (1959).

[15] L. J. Bellamy. *The Infra-red Spectra of Complex Molecules* (2nd Ed.) p. 108. Methuen, London (1958).

[16] L. H. Briggs, L. D. Colebrook, H. M. Fales, and W. C. Wildman, *Analyt. Chem.* 904 (1957); A. R. Katritzky and N. A. Coats, *J. Chem. Soc.* 2062 (1959).

asymmetrical stretching is masked by skeletal absorption of the perhydrophenanthrene nucleus.[3] The possibility of the presence of a second phenolic group is precluded by an examination of the infra-red spectrum of hydroxytotarol and its derivatives in the 800–900 cm^{-1} region which show the absorption expected of a 1:2:3:4 aromatic substitution pattern as in totarol. This possibility is also precluded by nuclear magnetic resonance data (Table 2).

TABLE 2. N.M.R. DATA OF TOTAROL AND 16-HYDROXYTOTAROL

Totarol (τ)	16-Hydroxytotarol (τ)	Assignment‡
2·93	3·06	Symmetrical AB quartet of 2 aromatic protons (C_{11},C_{12})
3·04	3·21	
3·41	3·57	
3·55	3·72	
5·57	3·51*	phenolic proton (sharp)
6·74	†	C_{18} proton (quintet)
—	5·23	C_4 hydroxymethyl proton (quartet)
7·19	†	C_7 methylene protons (multiplet)
8·62	8·65	C_{14} isopropyl methyls (J = 7·2 c.p.s.) each split by high resolution
8·74	8·77	
8·85	8·87	C_{10} angular methyl
9·08	8·99	C_4 methyls
9·10	—	

* The downfield shift is due to hydrogen bonding with dioxan

† Peaks masked by dioxan.

‡ Assignment of peaks follows from unpublished data on natural diterpenoids with A/B *trans* systems.

The relationship of the compound to totarol was established by oxidation of the alcoholic group followed by Wolff-Kishner reduction of the derived oxo-compound. Attempted oxidation of hydroxytotarol by the Oppenauer method was unsuccessful while chromic acid oxidation, although leading to the desired aldehyde, did so in low yield and, like totarol, gave a mixture of products by attack on the phenolic nucleus. Careful oxidation of the monomethyl ether with chromic acid[17], however, gave an aldehyde (IV, R = CH_3) as an oil which was characterized as the crystalline 2,4-dinitrophenylhydrazone. The identification of the product as an aldehyde followed from a positive silver mirror test and a comparison of the infra-red spectrum with those of O-methyl podocarpinal and O-methyl-13-isopropylpodocarpinal which show characteristic C—O vibrations at 1718 and 1750 cm^{-1} and C—H stretching at 2737 and 2766 $cm.^{-1}$ Using totaryl methyl ether as a model it was found that demethylation of (IV, R = CH_3) could be smoothly effected by heating under reflux with pyridine hydrochloride.[18] The resulting oxo-phenol (IV, R = H) was identical with an aldehyde which co-occurs with the hydroxytotarol in the related species *P. hallii*.[19] Wolff-Kishner reduction of the semicarbazone of (IV, R = CH_3) yielded (+)-totaryl methyl ether thus establishing the original diol as a hydroxytotarol.

As already discussed by Wenkert and Beak[7] a comparison of the nuclear magnetic resonance spectra of totarol and hydroxytotarol, also carried out in the present work (Table 2), eliminates all possible structures with the exception of the epimers (I, R =

[17] R. H. Bible, *Tetrahedron* **11**, 22 (1960).
[18] R. H. Bible, *U.S. Pat.* 2,854,474 (1958), *Chem. Abstr.* **53**, 8096 (1959).
[19] R. C. Cambie and W. R. J. Simpson, unpublished results.

OH) and (V). Furthermore, from a comparison of model compounds with *axial* and *equatorial* groups at C_4 Wenkert and Beak have distinguished between the systems and have shown that hydroxytotarol possesses an *axial* hydroxymethylene group. The same result is also obtained from a comparison of the hydroxymethyl C—O stretching vibrations of hydroxytotarol (1033 cm^{-1}) which is in the region of those shown by compounds in the podocarpinol series (1033–1035 cm^{-1}) and is in contrast with that shown by abietinol and dehydroabietinol (1053 cm^{-1}). Attempts to determine the stereochemistry about C_4 from a comparison of optical rotatory dispersion measurements of the aldehyde (IV, R = H) with O-methylpodocarpinal, O-methyl-13-isopropylpodocarpinal and dehydroabietinal* has led to inconclusive results. The final point of doubt (A/B *trans* versus A/B *cis*[7]) of the absolute configuration of the hydroxytotarol is, however, resolved by our conversion to totarol of established configuration.[5] The structure (I, R = OH) has also been confirmed by a comparison of the mass spectrum of our compound with that of totarol.[20]

Also isolated from the heartwood by chromatography of "phenolic" and "neutral" fractions of the light petroleum and ether extracts was an additional phenol (0·2 per cent yield), m.p. 224–225°, which could be separated from totarol by chromatography on active alumina or by its lower solubility in cold aqueous alcohol. It is identical by direct comparison with the "triterpene phenol" of Brandt and Thomas, a small sample of which was available in this Department. Only a preliminary examination of this compound, for which the name podototarin is proposed, is reported in the present work. Analyses of podototarin, dried at 100°, support the formula $C_{30}H_{44}O_2$ proposed by Brandt and Thomas, but analyses of the derived acetate, methyl ether, and monobenzoate are incompatible with this formula. Analyses of samples dried to constant weight correspond to the formula $C_{35}H_{48}O_2$, in agreement with molecular weight determination of the methyl ether by the X-ray method. On the available analytical data, however, other formulae are still permissible and the close similarity of the infra-red spectrum of totarol and podototarin suggests they are closely related compounds. Further work is in progress.

A further compound, designated as A, was isolated in trace amount by extraction of residues of the combined original ethyl acetate, acetone, and water extracts with hot water. The substance has m.p. 246–247° and analyses support the assignment of formulae $C_{15}H_{22-24}O_8$. It gives a positive Molisch test and its formulation as a glucoside followed from the identification of glucose by comparative paper chromatography after acid hydrolysis. The infra-red spectrum shows strong absorption at 3380 cm^{-1} and in the 1100–1000 cm^{-1} region as expected for a glucoside. A further strong band at 1760 cm^{-1} and light absorption in the ultra-violet (220 mμ) suggests the presence of an $\alpha\beta$-unsaturated γ-lactone. The spectrum shows marked similarity to that of a compound, $C_{17}H_{18}O_8$, m.p. 251–253°, from the heartwood of the related species *P. macrophylla* D. Don.[21] but direct comparison showed their non-identity.

EXPERIMENTAL

Analyses were by Dr. A. D. Campbell and associates, University of Otago, New Zealand. Infra-red spectra were measured as KBr disks, unless otherwise stated, with a Beckman IR2 instrument.

* The authors are indebted to Professors C. Djerassi and A. K. Bose for these determinations.

[20] C. Enzell and R. Ryhage, person. communication.
[21] T. Takahashi, *J. Japan Wood Res. Soc.* 5, 185 (1959).

and ultra-violet spectra for EtOH solutions with a Beckman DU instrument. Light petroleum was of b.p. 50–60°.

Nuclear magnetic resonance spectra were measured with a Varian V-4300 B spectrophotometer, equipped with flux stabilizer and sample spinner and operating at 60 m.c. Spectra were determined in $CDCl_3$ as solvent with the addition of a little dioxan, in the case of hydroxytotarol to effect solution, and with tetramethylsilane as internal reference. Peak positions are expressed in Tiers' (τ) values.[22]

Circular paper chromatography of resin acids was carried out as previously described.[9] Chromatography on alumina (Spence and Co., type H), unless otherwise stated, was carried out by eluting columns (packed in light petroleum), successively, with light petroleum, light petroleum-benzene (1:1), benzene, benzene–ether (10:1, 5:1, 1:1), ether, ethyl acetate, and methanol until no further material was eluted with each solvent. Deactivated alumina refers to alumina treated with 5% of 10% aqueous acetic acid.

Extraction of Podocarpus totara *heartwood.* The finely ground heartwood (15·14 kg) was continuously extracted (Soxhlet) in two batches, each with methanol for 24 hr. Removal of solvent, *in vacuo*, and intimate mixing of the concentrate with Celite (1·5 kg) gave a mixture which was air dried to a dark brown friable solid (3·2 kg). The powdered solid was then successively re-extracted (Soxhlet) with (a) light petroleum (20 hr), (b) ether (27 hr), (c) ethyl acetate (27 hr), (d) acetone (27 hr), and (e) water (45 hr).

The residues from the light petroleum and ether extracts (950 g and 645 g, respectively) in ether solution were fractionated, in batches, between saturated sodium hydrogen carbonate, 10% sodium carbonate, and 3·5% sodium hydroxide solutions. Acidification of the sodium carbonate extracts, in each case, gave small residues from which no crystalline products were obtained after chromatography on deactivated alumina or sublimation at 10^{-4} mm. Acidification of other alkaline extracts gave "acidic" and "phenolic" fractions as brown solids when dry, while "neutral" fractions were obtained from the remaining ether solutions.

Podocarpic acid (III, R = H). Comparative circular paper chromatography with authentic material indicated the presence of podocarpic acid in all "acid" and "phenolic" fractions. The "phenolic" fraction of the light petroleum extract (a), which appeared to contain the bulk of podocarpic acid, was chromatographed on deactivated alumina. Further purification of residues from the ether eluate by sublimation at 10^{-4} mm gave podocarpic acid (200 mg), m.p. and mixed m.p. 192–193° (identical infra-red spectrum).

Methylation of the total "acid" fraction (450 mg) of the light petroleum extract (a), in absolute methanol, with excess of an ethereal solution of diazomethane and trituration of the resulting oil with methanol at 0° gave methyl-O-methylpodocarpate (33 mg) which crystallized from methanol as needles m.p. and mixed m.p. 127–128°. Chromatography of the mother liquors on alumina (grade III) yielded further methyl-O-methylpodocarpate (14 mg) from the benzene eluate. Methylpodocarpate (15 mg), m.p. and mixed m.p. 209–211°, was eluted from the same column with benzene–ether (1:1).

Pododacric acid. The brown powder (3·8 g) from the "acid" fraction of the ether extract (b) was successively extracted with benzene (20 cc), ether (20 cc), and ethyl acetate (20 cc). Comparative circular chromatography indicated the presence of pododacric acid in the ether and ethyl acetate solubles. The residues from these were combined, dissolved in hot ethanol (20 cc), and boiling water added dropwise. The solution was cooled slowly with repeated decantation from dark oils to finally yield a pale yellow solution from which pododacric acid slowly crystallized. Recrystallization from 30% aqueous ethanol gave prisms (15 mg), m.p. and mixed m.p. 213–214° (identical infra-red spectrum).

Separation of constituents from "phenolic" fractions*

(a) *Light petroleum extract.* The "phenolic" fraction (49 g) was further extracted, successively, with light petroleum (300 cc), benzene (2 × 300 cc), and ethyl acetate (300 cc). Light petroleum solubles (5 g) were chromatographed on alumina (200 g, grade III) yielding podototarin (60 mg, initial light petroleum eluate), totarol (500 mg, late light petroleum eluate), and podocarpic acid (ether eluate). The benzene solubles (30 g) on similar chromatography gave podototarin (30 mg), totarol (15 g), and sugiol (145 mg, benzene eluate). No crystalline products were isolated from the ethyl acetate solubles when residues were chromatographed before or after acetylation.

(b) *Ether extract.* Chromatography of the "phenolic" fraction (150 g) as above gave podototarin (400 mg), totarol (7 g) and sugiol (130 mg). Sublimation of residues from late ether eluates at 10^{-4} mm followed by crystallization from benzene–light petroleum yielded β-sitosterol (3 mg).

* Identification of compounds obtained from "phenolic" and "neutral" fractions was made by mixed melting point determination and infra-red spectra in all cases.

[22] G. V. D. Tiers, *J. Phys. Chem.* **62,** 1151 (1958).

Separation of constituents from "neutral" fractions

(a) *Light petroleum extract.* Slow crystallization of the "neutral" concentrate (900 g) from light petroleum at 0° yielded mixed crystals of totarol and 16-hydroxytotarol (220 g), separated by fractional crystallization from light petroleum or by chromatography. In a typical experiment 120 g of the mixture, dissolved in the minimum amount of benzene, was chromatographed on alumina (2·5 kg, grade III) to yield totarol (116 g) from benzene–light petroleum (1:1) eluates and 16-hydroxytotarol (500 mg) from benzene–light petroleum (14:1) eluates. Trial chromatography of non-crystalline residues on alumina (grade I–II) gave podototarin (initial light petroleum eluate) and totarol (light petroleum–benzene eluates) as the only crystalline products. Bulk residues were chromatographed in 50 g portions on alumina (1 kg, grade III) and two fractions eluted from the columns with light petroleum and ether (A) respectively.

The concentrate (450 g) from the light petroleum fraction crystallized slowly from ethyl acetate at 0° to give mixed crystals of totarol and podototarin (310 g) which were separated by dissolving the totarol in cold 90% aqueous methanol. The mother liquor was concentrated and heated under reflux with acetic anhydride (100 cc) and pyridine (5 cc) for 3 hr at 100°. The crude acetates were extracted with light petroleum (2 × 600 cc), washed with water (3 × 300 cc), and the dried concentrate distilled, *in vacuo*. A fraction (22 g), b.p. 200–250°/0·16 mm, on crystallization from ethyl acetate gave podototarin acetate (1·2 g). A fraction (84 g), b.p. 160–170°/0·15 mm, on crystallization from aqueous ethanol gave totaryl acetate (28 g). Non-crystalline residues were redistilled and a portion (5 g), b.p. 160–170°/0·15 mm, dissolved in anhydrous acetone (20 cc), oxidized with 8 N-chromic acid–sulphuric acid reagent (2 g).[23] Isolation of the products followed by chromatography on alumina (200 g, grade I–II) gave totaryl acetate (1·2 g, light petroleum eluate) and 7-oxototaryl acetate (120 mg, benzene eluate).

Rechromatography of the fraction (A), before and after hydrolysis with 2 N-alcoholic potassium hydroxide, gave no crystalline products.

(b) *Ether extract.* The concentrate from the bulk "neutral" fraction, after separation of podototarin (2·2 g), gave a dark red glass (472 g) which yielded mixed crystals of totarol and 16-hydroxytotarol (95 g) from light petroleum. Separation was effected as in the previous manner. Mother liquors were chromatographed as for the light petroleum extract (a). Working up of the bulk light petroleum eluate as above gave totarol (163 g) and podototarin (17 g).

A portion (2 g) of the ether eluate (53 g) was rechromatographed on alumina (150 g, grade I–II). Sublimation of the residue from initial ether eluates gave methyl podocarpate (13 mg). Rechromatography of later ether eluates on alumina (grade I–II) gave β-sitosterol (22 mg). The remainder of the bulk ether eluate (51 g) was heated under reflux with 10% alcoholic potassium hydroxide (200 cc) and water (20 cc) for 5 hr. A portion of the acid fraction from hydrolysis was chromatographed on alumina (200 g, grade III) to yield podocarpic acid (15 mg) from the ether eluate and after repeated sublimation at 10^{-4} mm.

Totarol (I, R = H). Repeated crystallization of crude material from light petroleum gave totarol as prisms (total yield 768 g), m.p. and mixed m.p. 132°, $[\alpha]_D^{17}$ + 41·4° (*c* 1·6 in EtOH) (identical infra-red spectrum). Totaryl acetate, prisms from aqueous ethanol, had m.p. and mixed m.p. 124–125°. Totaryl methyl ether, prepared by Short and Stromberg's method (95% yield),[2] or by treating totarol (100 mg), dissolved in methylene chloride (10 cc) with diazomethane (140 mg) in methylene chloride (10 cc) and a drop of fluoroboric acid[14] (62% yield), crystallized from methanol as needles, m.p. 91·5–92·5° (lit.[2] m.p. 92–92·5°). Totaryl methyl ether (500 mg) was demethylated by heating under reflux with pyridine hydrochloride (1·5 g) at 210° for 1 hr and the product isolated by pouring into water and extraction with ether. Chromatography on alumina gave totarol (410 mg, 85% yield), m.p. and mixed m.p. 132° (identical infra-red spectrum).

7-Oxototaryl acetate: (a) A solution of chromic acid (7·11 g) in 80% aqueous acetic acid (16·53 g) was added dropwise, with stirring, to a cooled solution of totaryl acetate (9·44 g) in glacial acetic acid (104·4 g) over a period of 15 min. The mixture was stirred for a further 5 days at room temp and poured into water (700 cc). The product after thorough washing crystallized from ethanol to yield *7-oxototaryl acetate* (7·01 g, 74·6%) as needles, m.p. 168–169° (lit[5] m.p. 169–170°) (Found: C, 77·1; H, 9·1. $C_{22}H_{30}O_3$ requires C, 77·1; H, 8·8%) γ_{max} 1751 (aryl acetate) and 1669 (aryl C:O) cm^{-1}.

(b) Totaryl acetate (1·2 g) in acetone (20 cc) was treated with 8 N-chromic acid[23] and the mixture stood for 12 hr at room temp. Isolation of the product in the usual manner and chromatography on alumina (200 g, grade I–II) gave totaryl acetate (700 mg), m.p. 122–124°, from light petroleum eluates and 7-oxototaryl acetate (350 mg, 28%) from benzene–light petroleum eluates.

7-Oxototarol. 7-Oxototaryl acetate (3 g) was heated under reflux with potassium hydroxide (1 g) in 90% aqueous methanol (100 cc) for 1 hr. The mixture was poured into water and extracted with ether (2 × 30 cc), the ether extract washed with water (2 × 20 cc), dried, and the solvent removed.

[23] A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.* 2548 (1953).

The residue crystallized from aqueous ethanol to yield 7-*oxototarol* (2·2 g, 83%) as rods, m.p. 240–241° (Found: C, 79·3; H, 9·0. $C_{20}H_{28}O_2$ requires C, 79·95; H, 9·4%) γ_{max} 3225 (OH) and 1653 (aryl C:O) cm.$^{-1}$

7-*Oxototaryl methyl ether* (340 mg, 91·6%) was prepared by heating 7-oxototarol (350 mg) in dry acetone (30 cc), with anhydrous potassium carbonate (3 g) and methyl iodide (20 cc) under reflux for 6 hr. Crystallization from methanol gave needles, m.p. 159–160° (Found: C, 80·3; H, 9·4. $C_{21}H_{30}O_2$ requires: C, 80·2; H, 9·6%).

Treatment of 7-oxototarol (332 mg) with benzoyl chloride (3 cc) and pyridine (4 drops) with warming followed by storage for 12 hr at room temp gave 7-*oxototaryl benzoate* as an oil, which was washed with saturated aqueous sodium hydrogen carbonate and crystallized from methanol as prisms, m.p. 146–147°, and then as needles (200 mg, 50%), m.p. 152–153° (Found: C, 80·3; H, 7·8. $C_{27}H_{34}O_3$ requires: C, 80·2; H, 8·0%).

7-*β-Hydroxytotarol*. 7-Oxototaryl acetate (50 mg) in methanol (5 cc) was treated with potassium borohydride (45 mg) in methanol (10 cc). Water (2 cc) was added, the mixture heated under reflux for 1 hr and poured into water (50 cc). The mixture was extracted with ether (2 × 30 cc), the ether solution washed with water (2 × 10 cc), dried, and solvent removed. The residue crystallized from benzene-light petroleum to yield needles of 7-*β-hydroxytotarol*, m.p. 200–201° (Found: C, 79·3; H, 10.3. $C_{20}H_{30}O_2$ requires: C, 79·4; H, 10·0%) ν_{max}. 3390 (OH), 1186 (phenolic C-OH), 959 (alcoholic C-OH) cm^{-1}. Similar reduction of 7-oxototarol gave the same product.

16-*Hydroxytotarol* (I, R = OH). 16-Hydroxytotarol, from the heartwood, sublimed, *in vacuo* to yield colourless needles, m.p. 209–211°. Crystallization from benzene–light petroleum or chloroform–light petroleum gave fine needles (total yield 2·7 g), m.p. 230–231°, $[\alpha]_D^{17}$ +41·9° (*c* 2·0 in EtOH) (Found: C, 79·2; H, 9·7. $C_{20}H_{30}O_2$ requires: C, 79·4; H, 10·0%) λ_{max} 256 mμ (log ε 3·21) and 283 mμ (log ε 3·39). ν_{max} 3390 (OH), 1189 (phenolic C—OH), 1014 (alcoholic C—OH) cm^{-1}.

16-*Hydroxytotaryl diacetate*. 16-Hydroxytotarol (82 mg) was heated under reflux with acetic anhydride (5 cc) and pyridine (2 drops) for 2 hr at 100°. The cooled mixture was poured into ice-water yielding a colourless gum which was chromatographed on alumina (10 g, grade III). Crystallization from ethanol of the residue from benzene–light petroleum (1:1) eluates gave the *diacetate* (50 mg, 74%) as prisms, m.p. 133–134° (Found: C, 74·5; H, 9·1. $C_{24}H_{34}O_4$ requires: C, 74·6; H, 8·9%) ν_{max} 1761 (aryl acetate), 1742 (alkyl acetate) cm^{-1}; no hydroxyl peaks.

16-*Hydroxytotaryl monoacetate* (I, R = OAc). (a) 16-Hydroxytotarol (60 mg) was heated under reflux with glacial acetic acid (5 cc) for 5 hr. Isolation of the product by contact with water and crystallization from aqueous methanol gave the *monoacetate* (54 mg, 71·9%) as needles, m.p. 148–149° (Found: C, 76·4 H, 8·8. $C_{22}H_{32}O_3$ requires: C, 76·7; H, 9·4%) ν_{max} (CCl_4) 3600 (OH), 1173 (phenolic C—OH), 1739 (alkyl acetate) cm^{-1}. The monoacetate showed a slight but definite solubility in 20% aqueous sodium hydroxide and coupled with diazotized *p*-nitroaniline. Further acetylation with acetic anhydride–pyridine (2 hr, 100°) gave the diacetate, m.p. and mixed m.p. 133–134° (identical infra-red spectrum).

(b). Hydroxytotarol (196 mg) in anhydrous benzene (15 cc) was treated with acetic anhydride (0·8 cc) and pyridine (2 drops) in anhydrous benzene (15 cc), the mixture stood at room temp for 24 hr, and then heated under reflux for 15 min. Removal of solvent and chromatography of the residue on alumina (grade III) gave the mono-acetate (86 mg, 45·5%), m.p. and mixed m.p. 148–149°, from light petroleum eluates and hydroxytotarol (96 mg), m.p. and mixed m.p. 230–231°, from benzene eluates.

16-*Hydroxytotaryl dibenzoate*. 16-Hydroxytotarol (60 mg) was warmed with benzoyl chloride (3 cc) and pyridine (1 drop) for 24 hr at 35°. The mixture was poured into ice-water and the product washed with saturated sodium hydrogen carbonate solution and chromatographed on alumina (10 g, grade III). Crystallization from aqueous ethanol of residues from the light petroleum eluate gave needles of the *dibenzoate* (22 mg, 35%), m.p. 193–194° (Found: C, 79·1; H, 7·5. $C_{32}H_{36}O_4$ requires: C, 79·9; H, 7·5%) ν_{max} 1745 (aryl Bz), 1727 (alkyl Bz) cm^{-1}; no hydroxyl peaks.

16-*Hydroxytotaryl monomethyl ether*. (a) Hydroxytotarol (140 mg), potassium metal (100 mg), and anhydrous benzene (70 cc) were heated under reflux for 3 hr, methyl iodide (20 g) added, and and the refluxing continued for a further 3 hr. Solvent was removed from the filtered solution and the resultant gum sublimed, *in vacuo*, to yield the *monomethyl ether* (140 mg, 96%) as needles, m.p. 83–85° (Found: C, 79·6; H, 10·2. $C_{21}H_{32}O_2$ requires: C, 79·7; H, 10·2%) ν_{max} 1054 (aryl methyl ether), 1014 (alcoholic C-OH) cm^{-1}.

(b) Hydroxytotarol (80 mg) was heated under reflux with dimethylsulphate (2 cc), anhydrous potassium carbonate (500 mg), and dry acetone (20 cc), for 8 hr. The mixture was filtered and solvent removed from the filtrate, The resultant gum was chromatographed on alumina (grade III). Residues from fractions eluted from the column with light petroleum were sublimed, *in vacuo*, to give the monomethyl ether (53 mg, 62%), m.p. and mixed m.p. 83–85° (identical infra-red spectrum).

16-*Oxototaryl methyl ether* (IV, R = CH_3). Hydroxytotaryl methyl ether (90 mg), dissolved in acetone (10 cc), was treated with an oxidizing reagent (100 mg), prepared from chromic acid (13·62 g),

water (50 cc), and conc sulphuric acid (11 cc). The mixture was poured into water (100 cc), extracted with ether (2 × 50 cc) and the ether solution washed with water (2 × 20 cc), dried, and the solvent removed. The residue was chromatographed on alumina (100 g, grade II). *16-Oxototaryl methyl ether* (75 mg, 83%), obtained as a colourless oil from benzene–light petroleum eluates, could not be crystallized before or after rechromatography. ν_{max} 1718 (C:O) cm^{-1}; 1706 (CCl_4; C:O) cm^{-1}. Hydroxytotaryl methyl ether (8 mg) was eluted from the column with benzene–ether(10:1).

The 2,4-*dinitrophenylhydrazone* crystallized from chloroform–methanol as yellow plates, m.p. 236–237° (Found: C, 65·3; H, 6·95; N, 11·1. $C_{27}H_{34}O_5N$ requires: C, 65·6; H, 6·9; N, 11·3%).

Wolff-Kishner reduction of 16-oxototaryl methyl ether

16-Oxototaryl methyl ether (75 mg) in pyridine solution was treated with a solution of semicarbazide acetate, prepared from semicarbazide hydrochloride (150 mg) and sodium acetate (150 mg) in 95% aqueous ethanol (5 cc). The solution was stood overnight, diluted with water (20 cc), and extracted with ether (2 × 20 cc). The ether solution was washed with water (1 × 10 cc), dried, and the solvent removed. The crude semicarbazone was heated with sodium (50 mg) in absolute ethanol (3 cc) for 15 hr at 200–210°. The mixture was diluted with water (20 cc) and extracted with ether (2 × 20 cc). The ether solution was washed with water (1 × 10 cc), dried, the solvent removed, and the residue chromatographed on alumina (100 g, grade I–II). Fractions eluted with light petroleum crystallized from methanol to yield needles of totaryl methyl ether (31 mg, 46%), m.p. and mixed m.p. 91–92°, $[\alpha]_D^{17}$ +42·0 (*c* 1·1 in EtOH) (identical infra-red spectrum).

16-Oxototarol (IV, R = H). The aldehyde (IV, R = CH_3; 50 mg) was heated under reflux with pyridine hydrochloride (1 g) for 1 hr and the product isolated in the usual manner. Chromatography on alumina and crystallization from benzene–light petroleum of fractions eluted from the column with benzene–light petroleum (4:1) gave *16-oxototarol* (10 mg), m.p. 177–178° (Found: C, 80·3; H, 9·6. $C_{30}H_{28}O_3$ requires: C, 79·95; H, 9·4%). The m.p. was undepressed on admixture with an oxototarol isolated from *P. hallii* and the samples possessed identical infra-red spectra.

Podototarin. Repeated crystallization of crude material from benzene–alcohol, chloroform–ethyl acetate, or light petroleum gave *podototarin* as colourless needles (total yield 30·5 g), m.p. 224–225°, $[\alpha]_D^{20}$ +136° (*c* 2·0 in $CHCl_3$) (Found: C, 82·8; H, 10·2. $C_{30}H_{44}O_3$ requires: C 82·5; H, 10·2%; Found for sample dried to const. wt.: C, 83·9; H, 9·2. $C_{35}H_{48}O_3$ requires: C, 83·95; H, 9·7%) λ_{max} 254 mμ (log ε 3·50) and 290 mμ (log ε 3·29): ν_{max} 3509, 3425 (OH), 1188 (phenolic C–OH) cm.$^{-1}$

Podototarin diacetate. Podototarin (100 mg) was heated under reflux with acetic anhydride (5 cc) and pyridine (2 drops) for 3 hr at 100°. The *diacetate*, isolated on contact with ice-water, crystallized from ethyl acetate as fine needles (96 mg), m.p. 237–238·5° (Found: C, 79·9, 80·6; H, 9·1, 9·6; Ac, 15·25. $C_{39}H_{52}O_4$ requires: C, 80·1; H, 9·0; 2Ac, 14·7%) ν_{max} 1764 (aryl acetate) cm^{-1}.

Podototarin monobenzcate. The *monobenzoate* was prepared by treatment of podototarin (250 mg) with benzoyl chloride (5 cc) and pyridine (1 drop) for 5 hr at 40°. The product, in ether solution, was washed with saturated aqueous sodium hydrogen carbonate, water, and the solvent removed. Crystallization from ethyl acetate–ethanol–water gave prisms (54 mg), m.p. 252·5–253° (Found: 82·9; H, 8·9; $C_{43}H_{52}O_3$ requires: C, 83·4; H, 8·8%) ν_{max} 3509 (OH), 1724(Bz) cm^{-1}.

Podototarin dimethyl ether. Podototarin (100 mg) was treated with diazomethane (120 mg) in ether solution and the mixture stood for 24 hr at room temp. The product crystallized from ethyl acetate to yield the *dimethyl ether* (98 mg) as needles, m.p. 293–294° (Found: C, 84·2, 84·25; H, 10·1, 9·8; O, 6·0, 6·3%: M, by X-ray method, 509 ± 19. $C_{37}H_{52}O_3$ requires: C, 84·0; H, 9·9; O, 6·05%; M, 529); no hydroxyl peaks in the infra-red spectrum.

Sugiol. Repeated crystallization from benzene or acetic acid gave sugiol as rods (total yield 274 mg), m.p. and mixed m.p. 292–293° (identical infra-red spectrum). Sugiol acetate, needles from aqueous ethanol, had m.p. and mixed m.p. 164–165°, and sugiol methyl ether, plates from aqueous methanol, had m.p. and mixed m.p. 145–146°.

Methyl podocarpate. Methyl podocarpate (total yield 344 mg) crystallized from methanol as needles, m.p. and mixed m.p. 209–211° (identical infra-red spectrum). Hydrolysis of the crude compound gave podocarpic acid, m.p. and mixed m.p. 192–193° (identical infra-red spectrum).

β-Sitosterol. β-Sitosterol crystallized from ethanol as plates (total yield 25 mg), m.p. 127°, or from benzene-light petroleum as flakes, m.p. and mixed m.p. 136–137° (Found, dried to const. wt.: C, 83·8; H, 12·0. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·15%) (identical infra-red spectrum).

Compound A. The combined residues (100 g) from the ethyl acetate (c), acetone (d), and water (e) extracts were extracted with hot butan-1-ol (500 ml) and the concentrate re-extracted with boiling water (2 × 300 cc). Evaporation of the water, *in vacuo*, at 35° gave a dark solid which was further extracted with acetone (2 × 50 cc) and the solvent removed. Repeated crystallization of the residue from 50% aqueous ethanol at 0° gave fine colourless needles of *compound* A (22 mg), m.p. 245–246°

decomp (Found: C, 54·3, 54·4; H, 7·0, 6·0. $C_{18}H_{22}O_8$ requires: 54·5; H, 6·7. $C_{18}H_{14}O_8$ requires: C, 54·2; H, 7·3%). λ_{max} 220 mμ (log ε 4·04). Attempts to form a crystalline acetate were unsuccessful.

Microhydrolysis with 2 N-hydrochloric acid and circular paper co-chromatography with authentic sugars showed the presence of glucose (phenol, saturated with water).

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Note added in proof: D. A. H. Taylor, *Chem. and Ind.* 1712 (1961), has recently reported the isolation of 16-hydroxytotarol (I, R = OH), 16-oxotarol (IV, R = H), and, as its methyl ester, the corresponding acid from the related species *P. mannii*.

R. Hodges, *J. Chem. Soc.* 4247 (1961) has reported a similar preparation of 7-oxototarol to that described above.