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CHEMISTRY OF THE PODOCARPACEAE—IV*

CONSTITUENTS OF THE HEARTWOOD OF *PODOCARPUS DACRYDIOIDES* A. RICH

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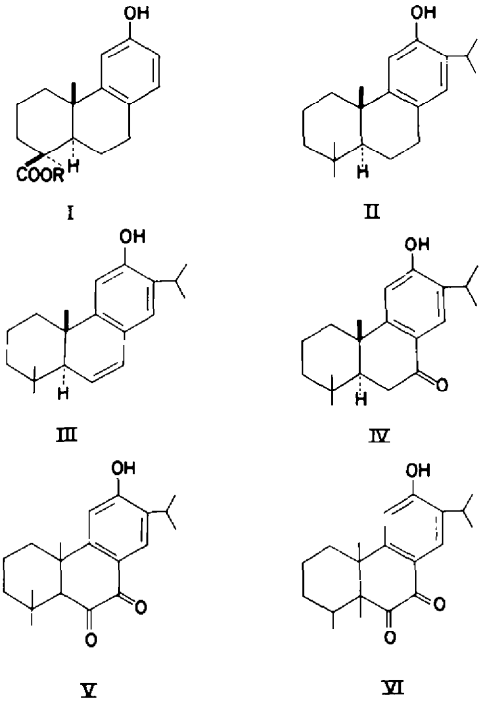
Abstract—The extractives of the heartwood of *Podocarpus dacrydioides* A. Rich. contain the known diterpenoid constituents, podocarpic acid (I, R = H), methyl podocarpate (I, R = CH_3), ferruginol (II), Δ^9-dehydro-ferruginol (III), sugiol (9-ketoferruginol, IV), xanthoperol (V) (in trace amount) and a new resin acid, $C_{20}H_{28}O_6$, for which the name pododacric acid is proposed. A saturated hydrocarbon also isolated is probably n-heptacosane.

PREVIOUS investigations of the constituents of *Podocarpus dacrydioides* A. Rich. (Maori name "kahikatea") have been limited to the essential oil which contains α-pinene,[1,2] β-pinene,[2] and cadinene[2] as the only identified products, and to the heartshake resin, which contains podocarpic acid (I, R = H).[3,4,5]



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[1] H. A. A. Aitken, *J. Soc. Chem. Ind.* **48**, 344 T (1929).
[2] G. J. E. Hunter, *J. Soc. Chem. Ind.* **51**, 394 T (1932).
[3] T. H. Easterfield and B. C. Aston, *Trans. New Zealand Inst.* **43**, 53 (1911).
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[5] C. W. Brandt and B. R. Thomas, *Nature, Lond.* **170**, 1018 (1952).

In a continuation of our studies of the constituents of the New Zealand species of the *Podocarpaceae* a systematic investigation of the constituents of the heartwood of *Podocarpus dacrydioides* has been carried out. Isolation of the constituents was facilitated by re-extraction of the concentrate from an initial methanolic extract mixed with Celite, with light petroleum, ether, ethyl acetate, water and methanol. The extraction scheme and the products identified from each extract are shown in Table 1.

Podocarpic acid (I, R=H) comprised the major constituent (0·37% yield) and was isolated with difficulty from the "acidic" and "phenolic" fractions of the ether extract. It also occurred largely as its magnesium salt in the water extract from which it could be isolated by acidification followed by chromatography.

By chromatography of the "neutral" fraction of the ether extract a phenolic compound, $C_{18}H_{24}O_3$, m.p. 211·5–212°, was isolated (0·02% yield) which, although insoluble in aqueous alkali and giving a negative ferric chloride reaction, readily dissolved in Claisen's cryptophenolic reagent[6] and gave an orange dye with diazotized *p*-nitroaniline. A comparison of the ultra-violet and infra-red spectra with those of podocarpic acid indicated that the compound was methyl podocarpate (I, R=CH_3) a fact confirmed by direct comparison with an authentic sample. Methyl podocarpate has not previously been isolated from natural sources.

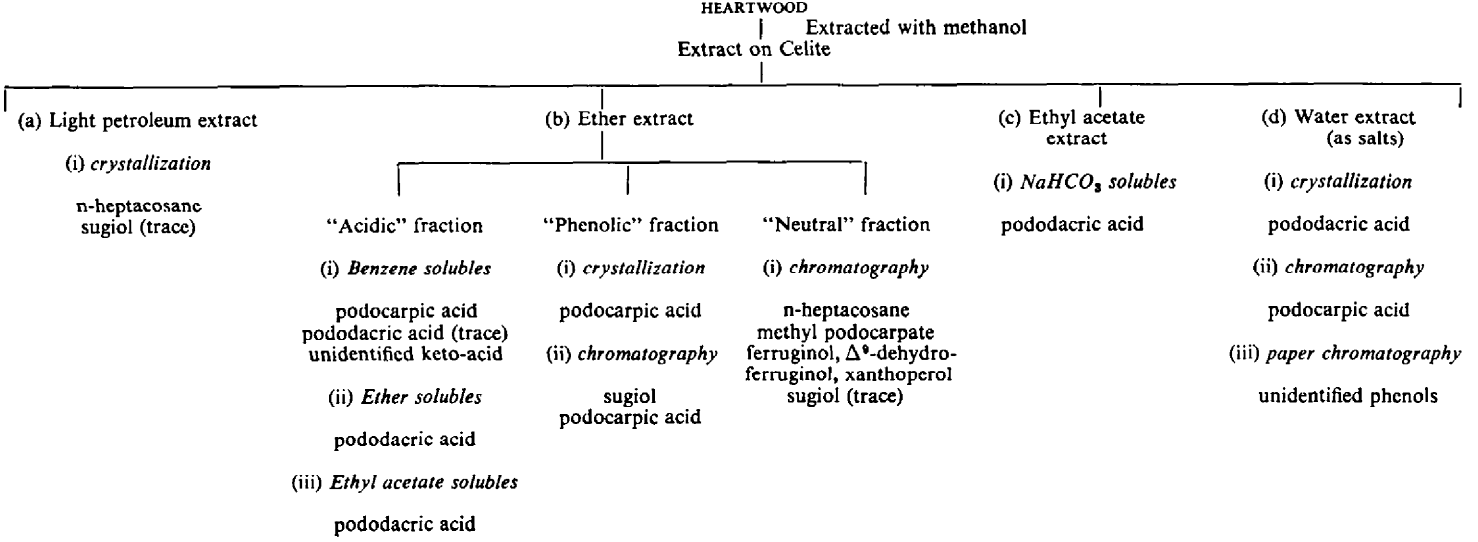
Acetylation and re-chromatography of the "neutral" fraction of the ether extract after the separation of the bulk of methyl podocarpate led to the isolation of an acetate whose properties were identical with those of ferruginyl acetate isolated by Brandt and Thomas from *Dacrydium cupressinum*.[7] The identity was confirmed by de-acetylation to ferruginol (II) and characterization as the benzoate. Bredenberg,[8] when investigating the acetate obtained from *Juniperus communis* L.[9] has shown that ferruginyl acetate forms mixed crystals with its dehydroderivative (III) and also that ferruginol isolated from *Podocarpus ferrugineus*[10] and from *Dacrydium cupressinum*[7] contains small amounts of Δ^9-dehydroferruginol by a comparison of the ultra-violet spectra of the acetates with that of pure ferruginyl acetate. From a comparison of the spectra of our acetate and its hydrogenation product it would appear that it contained about 3 per cent of the dehydro derivative.

A phenolic ketone (0·01% yield) isolated from the "phenolic" fraction of the ether extract by chromatography was identified as sugiol (9-keto-ferruginol, IV)[11] by comparison with an authentic sample. Its occurrence together with ferruginol and Δ^9-dehydroferruginol is of interest in view of their co-occurrence in the related species *Dacrydium cupressinum*.[7,8] It has been shown that the C_9 methylene group of dehydroabietic acid and its derivatives undergo facile oxidation to the 9-oxo compounds[12,13] and that sugiol on reduction with aluminium isopropoxide undergoes simultaneous reduction and dehydration to the dehydroderivative.[8]

A small amount of a yellow crystalline compound eluted from the column after the above acetate possessed properties identical with those of xanthoperol, an artefact

[6] L. Claisen, *Liebigs Ann.* **418**, 69 (1919).
[7] C. W. Brandt and B. R. Thomas, *New Zealand J. Sci. and Tech.* **33 B**, 950 (1951).
[8] J. B. Bredenberg, *Acta Chem. Scand.* **11**, 932 (1957).
[9] J. B. Bredenberg and J. Gripenberg, *Acta Chem. Scand.* **10**, 1511 (1956).
[10] C. W. Brandt and L. G. Neubauer, *J. Chem. Soc.* 1031 (1939).
[11] C. W. Brandt and B. R. Thomas, *J. Chem. Soc.* 2642 (1952).
[12] R. P. Jackson, *J. Amer. Chem. Soc.* **73**, 3463 (1951).
[13] P. F. Ritchie, T. F. Sanderson and L. F. McBurney, *J. Amer. Chem. Soc.* **75**, 2610 (1953).

Table 1.—Heartwood constituents of *podocarpus dacrydioides*



obtained along with sugiol from the wood of *Juniperus communis* L.[9] The original structure proposed for xanthoperol (V)[9] was subsequently changed[14] to VI mainly on the evidence of its failure to enolize spontaneously and the formation of a ketone different from sugiol on Clemmenson reduction. Wenkert and Chamberlin,[15] however, have recently pointed out that resistance to enolization of V would be caused by unfavourable non-bonded interaction between the equatorial C_1 methyl group and the C_{10} hydroxyl group of the enol form (phenanthrene numbering), while the failure to form sugiol on Clemmenson reduction implies that reduction, preceded by enolization, led to the more stable *cis* isomer of sugiol. The normal structure (V) can therefore be retained. The low yield of xanthoperol from *Podocarpus dacrydioides* and the failure to isolate further material in repeated attempts indicate that here too, it is an artefact.

The analysis of a crystalline hydrocarbon (0·004% yield) isolated from the light petroleum extract and by chromatography of the "neutral" fraction of the ether extract was in agreement with n-heptacosane, one of the more common hydrocarbons from heartwood species.

A keto-acid present in the "acidic" fraction of the ether extract in trace amount was separated from benzene solution by formation of its Girard derivative. All attempts to isolate the compound in a pure state as the oxime, semicarbazone or by chromatography of the crude 2:4-dinitrophenylhydrazone, however, have been unsuccessful.

Accompanying podocarpic acid in the ether extract was a small amount of a further resin acid, partial separation of which could be affected by the greater solubility of podocarpic acid in benzene. The less soluble acid (0·01% yield) could be isolated with difficulty by crystallization and from tarry residues by countercurrent distribution. It is a monocarboxylic acid, $C_{20}H_{28}O_5$, m.p. 213–214°, which can be characterized by the formation of a triacetate and a tribenzoate, thus accounting for all the oxygen functions. The presence of the carboxylic acid grouping and the hydroxyl groups can be confirmed by bands in the infra-red spectrum at 1701 cm^{-1} and at 3425 cm^{-1} and 3145 cm^{-1}, respectively. A positive ferric chloride reaction and the formation of a bright red dye with diazotized *p*-nitroaniline as well as aromatic bands at 1560 cm^{-1} and 1504 cm^{-1} in the infra-red spectrum shows that at least one of the hydroxyl groups is phenolic. With the assumption that only a single aromatic ring is present the formula leads to a tricyclic structure and it seems reasonable, therefore, to infer that this is a further diterpenoid acid of the podocarpic acid type. Such an acid possessing three hydroxyl groups has not been previously reported from natural sources and we propose the name pododacric acid. Further work on its structure is in progress.

EXPERIMENTAL

Analyses were by Dr. A. D. Campbell and associates, University of Otago, New Zealand. Infra-red spectra were measured as KBr disks with a Beckmann IR2 instrument (NaCl prism) and ultra-violet spectra for EtOH solutions with a Beckmann DU instrument. Light petroleum was of b.p. 50–60°.

Circular paper chromatography of resin acids was carried out on a Whatman No. 1 paper with the following solvent systems: (A) 25% aqueous acetic acid, (B) butan-1-ol–ammonium hydroxide–water (20 : 3 : 10). The position of spots was shown by spraying (a) successively with 5% aqueous

[14] J. B. Bredenberg, *Acta Chem. Scand.* **11**, 927 (1957).
[15] E. Wenkert and J. W. Chamberlin, *J. Amer. Chem. Soc.* **81**, 688 (1959).

sodium hydroxide, 1% alcoholic diazotized *p*-nitroaniline and 10% aqueous hydrochloric acid or with (b) a mixture of 1% ferric chloride and 1% potassium ferricyanide. Podocarpic acid has R_f (A) 0·7, (B) 0·8 and pododacric acid R_f (A) 0·8, (B) 0·7, the spots being coloured orange and red respectively, using spray reagent (a).

Extraction of Podocarpus dacrydioides *heartwood.* Continuous extraction (Soxhlet) of the finely ground wood (7·6 kg) with methanol for 25 hr and removal of the solvent gave a brown tar (670 g). The friable solid obtained after mixing with Celite and drying was successively re-extracted (Soxhlet) with (a) light petroleum, (b) ether, (c) ethyl acetate, (d) water and (e) methanol and the solvent removed from each extract. The residue from the ether extract (428 g) was redissolved in ether and fractionated in batches between saturated sodium hydrogen carbonate and 3% sodium hydroxide solutions. It was found necessary to use a large volume of sodium hydrogen carbonate solution to effect reasonable fractionation. Small quantities of resin acids were being extracted after use of 5 l. and extraction was still incomplete after 9 l. Little material was obtained by further trial extraction with 10% aqueous sodium carbonate solution. Acidification of the alkaline extracts gave an "acidic" fraction (169 g) and a "phenolic" fraction (154 g) as brown tars and a "neutral" fraction (58 g) remained as an oil. Similar fractions were obtained by treating the residue (26 g) from the ethyl acetate extract in the same manner. No identified products were obtained from the "phenolic" and "neutral" fractions of the ethyl acetate extract or from the methanol extract.

n-Heptacosane. On long standing the residue (2·6 g) from the light petroleum extract (a) gave a gum which contained crystalline material, separation of which was effected by trituration with acetone. After mechanical separation of a trace of sugiol (*vide infra*), the major solid was purified by washing with conc H_2SO_4 followed by water and crystallized from methanol–acetone to yield small glistening plates of n-heptacosane (50 mg), m.p. 59–60°. Further hydrocarbon (260 mg), m.p. 58·5° was obtained by chromatography of the "neutral" fraction of the ether extract (b) (*vide infra*). (Found: C, 85·3; H, 14·6; M (Rast), 392. Calc. for $C_{27}H_{56}$: C, 85·2; H, 14·8%; M, 381). The purified hydrocarbon gave negative tests for unsaturation and possessed an infra-red spectrum typical of saturated hydrocarbons.

Podocarpic acid (I, R = H). Podocarpic acid was expected to be present in the "acidic" fraction of the ether extract (b) in significant proportions. However, although it could be crystallized directly from the resin of the tree it could not be readily separated from the resins present in the "acidic" fraction. Trial attempts to effect separation by fractional crystallization of various amine salts by Harris and Sanderson's method[16] were unsuccessful. Partial separation (as shown by paper chromatography) was achieved by extraction of the dried residue with benzene followed by ether, leaving a residue soluble in ethyl acetate. Podocarpic acid was concentrated in the benzene solution but was contaminated by a keto-acid and a further acid, subsequently identified as pododacric acid. The ether solution contained a mixture of podocarpic and pododacric acids while pododacric acid was concentrated in the ethyl acetate solution. After extraction with 30% aqueous methanol which removed pododacric acid and separation of the keto-acid by formation of the water-soluble Girard derivative, podocarpic acid (1·8 g) slowly crystallized from the benzene solution on addition of light petroleum.

Podocarpic acid (17·7 g) was also isolated from the "phenolic" fraction of the ether extract (b) by repeated solution in ethyl acetate and precipitation with light petroleum, followed by slow crystallization from aqueous acetic acid.

On standing, the water extract (d) deposited a small amount of an acid magnesium salt, decomposition of which with hydrochloric acid, followed by extraction with ethyl acetate, gave a trace of pododacric acid. Acidification of the aqueous filtrate precipitated the bulk of the organic material, paper chromatography of which showed the presence of podocarpic acid and pododacric acid (trace) and unidentified phenolic compounds. Podocarpic acid was finally isolated by chromatography of a portion of the precipitated solid (450 mg from 4 g) on alumina (Brockmann III) from fractions eluted with methanol.

Podocarpic acid crystallized from 80% ethanol, or 60% acetic acid as long needles, m.p. and mixed m.p. 193–194°, $[\alpha]_D^{25}$ +139° (*c*, 2·2 in $CHCl_3$), λ_{max} 225 mμ (log ε 3·60) and 282 mμ (log ε 3·4). Methyl podocarpate, prepared by the use of an ethereal solution of diazomethane, had m.p. and mixed m.p. 211°. Methyl-O-methyl podocarpate, m.p. and mixed m.p. 128·5°, was prepared from podocarpic

[16] G. C. Harris and T. F. Sanderson, *J. Amer. Chem. Soc.* **70**, 339, 2079 (1948).

acid by treatment with dimethyl sulphate and anhydrous potassium carbonate in boiling acetone or from methylpodocarpate in methanolic solution by further treatment with diazomethane.

Pododacric acid. The ether extract of the "acidic" fraction from (b) above, on standing with benzene for 2 months with frequent trituration, deposited crystals mixed with tar. Dilution with an equal volume of methyl isobutyl ketone followed by rapid filtration gave crude pododacric acid (600 mg). Paper chromatography showed that the tar contained a mixture of podocarpic and pododacric acids, separation of which was achieved by countercurrent distribution in the system, light petroleum–n-butyl acetate–methanol–water (4 : 6 : 4 : 2). Twenty-three transfers concentrated pododacric acid in tubes 6–15 while podocarpic acid was concentrated in tubes 18–21 (paper chromatographic determination). Twelve further transfers with double withdrawal and removal of solvent gave crude pododacric acid (300 mg) from tubes 6–25.

Further crude pododacric acid (210 mg) was isolated from the ethyl acetate soluble residue of the "acidic" fraction from (b) and from the "acidic" fraction of the ethyl acetate extract (c) by slow crystallization from 30% methanol. Pure pododacric acid crystallized from 30% acetic acid or 30% methanol as colourless needles,* m.p. 213–214°, $[\alpha]_D^{25}$ +118°[*c*, 0·9 in $CHCl_3$–MeOH (5 : 1)] (Found: C, 68·8; H, 8·0. $C_{20}H_{28}O_5$ requires: C, 68·9; H, 8·1%). λ_{max} 225 mμ (log ε 3·74) and 284 mμ (log ε 3·4).

Tri-O-acetyl pododacric acid. Acetylation of pododacric acid with acetic anhydride–pyridine (100°; 3 hr) and isolation of the product by pouring onto crushed ice gave the *triacetate*, which crystallized from 80% methanol as granules, m.p. 160–160·5°, $[\alpha]_D^{23}$ +82° (*c*, 0·64 in EtOH) (Found: C, 66·3; H, 7·1; Ac, 26·6. $C_{26}H_{34}O_8$ requires: C, 65·8; H, 7·2; 3 Ac, 27·2%).

Tri-O-benzoyl pododacric acid. Benzoylation of pododacric acid with benzoyl chloride–pyridine (100°; 1 hr) and isolation of the product in the above manner gave the *tribenzoate*, needles (from ethanol), m.p. 135–136°, $[\alpha]_D^{22}$ +47° (*c*, 0·4 in EtOH) (Found: C, 75·3; H, 6·2; Bz. 44·8. $C_{41}H_{40}O_8$ requires: C, 74·5; H, 6·1; 3 Bz, 47·7%).

Sugiol (IV). Portions (35 g) of the "phenolic" fraction of the ether extract (b) were repeatedly chromatographed in benzene–ether (5 : 2) on activated magnesia, the initial fractions eluted with the same solvent and re-chromatographed on alumina (Brockman III). Fractions eluted with benzene–ether (3 : 1) gave sugiol (total yield 630 mg) on standing with benzene–light petroleum. Further elution of the columns with ether gave podocarpic acid (8·16 g). Sugiol was also isolated in trace amount from the light petroleum extract (a) and by chromatography of the "neutral" fraction of the ether extract (b). After repeated crystallization from acetic acid it was obtained as long colourless rods, m.p. and mixed m.p. 292·5–293°, $[\alpha]_D^{25}$ +136° [*c*, 0·4 in $CHCl_3$-pyridine (5 : 1)] (Found: C, 79·7; H, 9·1. Calc. for $C_{20}H_{28}O_2$: C, 80·0; H, 9·4%). λ_{max} 233 mμ (log ε 4·22) and 288 mμ (log ε 4·15) (identical infra-red spectrum). The acetate, prepared by the use of acetic anhydride–pyridine (100°; 2 hr) formed needles, m.p. 163–164°, from aqueous methanol (lit[17] m.p. 164–165°) (Found: C, 76·9; H, 8·6. Calc. for $C_{22}H_{30}O_3$: C, 77·15; H, 8·8%).

Methyl podocarpate (I, R = CH_3). The "neutral" fraction of the ether extract (b) was chromatographed in benzene on alumina (Brockman II). Initial fractions eluted with benzene gave n-heptacosane. Repeated rechromatography of fractions eluted with benzene–ether mixtures on alumina (Brockmann IV) gave crystalline material (1·34 g), m.p. 200°. Repeated crystallization from methanol gave methyl podocarpate as needles, m.p. and mixed m.p. 211·5–212° (Found: C, 75·2; 75·0; H, 7·9, 8·0. Calc. for $C_{18}H_{24}O_3$: C, 75·0; H, 8·4%). λ_{max} 222 mμ (log ε 3·74) and 282 mμ (log ε 3·41) (identical infra-red spectrum). The methyl ether, prepared by the use of dimethylsulphate and anhydrous potassium carbonate in boiling acetone, formed prisms, m.p. 128·5–129°, undepressed by methyl-O-methyl podocarpate (Found: C, 75·5, 75·5; H, 8·75, 8·5. Calc. for $C_{19}H_{26}O_3$: C, 75·5, H, 8·7%). λ_{max} 221 mμ (log ε 3·77) and 280 mμ (log ε 3·29) (identical infra-red spectrum).

Methyl-O-acetyl podocarpate. Acetylation of methyl podocarpate (43 mg) with acetic anhydride–pyridine (100°; 2 hr) and isolation of the product by pouring onto crushed ice gave methyl-O-acetyl podocarpate (50 mg), which crystallized from 50% methanol as needles, m.p. 122–123·5° (lit[18] m.p. 125–125·5°).

Ferruginol (II) *and* Δ^9*-dehydroferruginol* (III). The recombined fractions from the columns after separation of methyl podocarpate were acetylated and chromatographed in light petroleum on

* Traces of impurity inhibit crystallization giving oils on standing.

[17] P. Sengupta, S. Choudbury and H. Khastgir, *Chem. & Ind.* 861 (1958).
[18] E. Wenkert and B. G. Jackson, *J. Amer. Chem. Soc.* **80**, 217 (1958).

alumina (Brockmann II). Fractions eluted with light petroleum slowly yielded a crystalline acetate, which on repeated crystallization from ethanol gave mixed crystals of ferruginyl and Δ^9-dehydroferruginyl acetates (120 mg), m.p. 80–81°, $[\alpha]_D^{22}$ +53° (*c*, 1·4 in $CHCl_3$) (Found: C, 80·8; H, 9·7. Calc. for $C_{22}H_{32}O_3$: C, 80·4; H, 9·8%). λ_{max} 269 mμ (log ε 3·14) and 277 mμ (log ε 3·18). The infra-red spectrum was identical with that of a sample of the acetate of ferruginol from *Dacrydium cupressinum*.[7] The infra-red and ultra-violet spectra of fractions eluted from the column with benzene–light petroleum (3 : 10) indicated that further acetate was present but the greater part could not be crystallized even on seeding. The brown oil obtained, had $[\alpha]_D^{22}$ +50° (*c*, 1·0 in EtOH), λ_{max} 272 mμ (log ε 3·17). Catalytic hydrogenation of the crystalline acetate with a palladium–charcoal catalyst gave pure ferruginyl acetate, which crystallized from ethanol as rods, m.p. 81–82°, $[\alpha]_D^{23}$ +60·5° (*c*, 0·9 in EtOH), λ_{max} 269 mμ (log ε 3·08) and 278 mμ (log ε 3·12).

Deacetylation of the crystalline acetate (100 mg) with 5% methanolic potassium hydroxide solution for 1 hr gave ferruginol as a pale yellow resin (80 mg), b.p. 140–145° (bath temp)/0·5 mm. Benzoylation of the resin (70 mg) with benzoyl chloride–pyridine (100°; 1 hr) and crystallization of the product from ethanol gave ferruginyl benzoate (40 mg) as rods, m.p. and mixed m.p. 154–155°.

Xanthoperol (V). After elution of the acetate from the column above, a fraction eluted with benzene gave yellow needles (2 mg), m.p. 250–260° (decomp). The infra-red spectrum and the properties of the compound were identical with those reported by Bredenberg and Gripenberg[9] for xanthoperol. Attempts to isolate further material were unsuccessful. Fractions eluted from the column with benzene–ether gave a mixture (29 mg) of sugiol and methyl podocarpate.

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