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DITERPENES OF *PODOCARPUS FERRUGINEUS* BARK

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Key Word Index—*Podocarpus ferrugineus*; Podocarpaceae; oxygenated diterpenes; 2-keto ferruginol; 2β-acetoxysugiyl methylether; biogenesis.

Abstract—The following oxygenated diterpenes have been isolated from the bark of *Podocarpus ferrugineus* D. Don: isopimarol, isopimaric acid, sandaracopimaric acid, ferruginol, sugiol, sugiyl methyl ether, xanthoperol, royleanone, 6-dehydroroyleanone, cryptojaponol, 5β-hydroxy-6-oxasugiyl methyl ether, 2-ketoferruginol and 2β-acetoxysugiyl methyl ether.

INTRODUCTION

VARIOUS studies on the terpenic constituents of the New Zealand miro tree (*P. ferrugineus*) have led to the isolation of dipentene, limonene, α-pinene, cineole, cadinene, juniperol, rimuene, hibaene, phyllocladene, kaurene, ferruginol, 6-dehydroferruginol, sugiol, 5-dehydrosugiol and isopimaric acid.[1] In search of some supply of ferruginol we made an exhaustive survey of the terpene content of the bark. The hydrocarbons have been reported[2] to consist of longifolene, calamenene, α-copaene, α-muurolene, γ-muurolene, α-curcumene, α-bergamotene, β-selinene, the norditerpene **1a**,[3] isopimaradiene, and dehydroabietane, while an investigation of the oxygenated diterpenes and a discussion of their possible biosynthetic origin form the basis of the present communication.

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† Rockefeller Foundation Fellowship holder, 1963–1965.

‡ U.S. National Science Foundation Cooperative Fellowship holder, 1962–1965.

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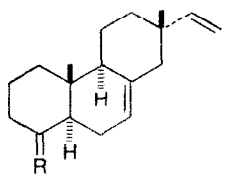
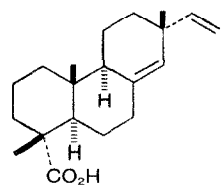
[3] This hydrocarbon remained of unknown constitution.[2] Comparison of its infrared and pmr spectra with those reported recently for **1a** [APPLETON, R. A. and ROERAADE, J. (1969) *Chem. Commun.*, 1407], furnished kindly by Dr. Appleton, indicated the compounds' identity.

RESULTS AND DISCUSSION

The bark constituents isopimarol (**1b**), isopimaric acid (**1c**), sandaracopimaric acid (**2**), ferruginol (**3a**), sugiol (**3b**), sugiyl methyl ether (**3d**),[4] xanthoperol (**4a**) and royleanone (**5**) were recognized by direct comparison with authentic samples. Two others, cryptojaponol (**3c**) and 5β-hydroxy-6-oxasugiyl methyl ether (**4b**), were identified by partial synthesis[5] and 6-dehydroroyleanone (6,7-dehydro-V) by conversion to royleanone on hydrogenation and subsequent air oxidation.[6]



(**1a**) R = CH_2
(**1b**) R = α-CH_2OH, β-Me
(**1c**) R = α-CO_2H, β-Me



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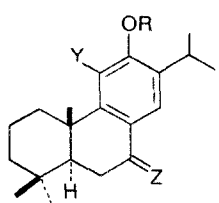
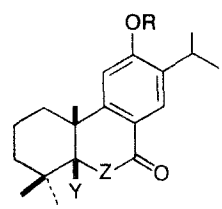
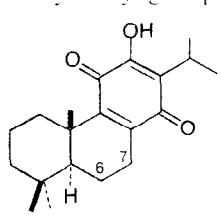


(**3a**) R = Y = H, Z = H_2
(**3b**) R = Y = H, Z = O
(**3c**) R = Me, Y = OH, Z = O
(**3d**) R = Me, Y = H, Z = O
(**3e**) R = Me, Y = H, Z = H_2

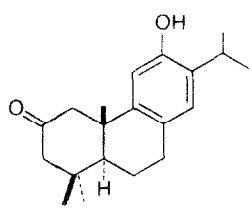


(**4a**) R = Y = H, Z = CO
(**4b**) R = Me, Y = OH, Z = O

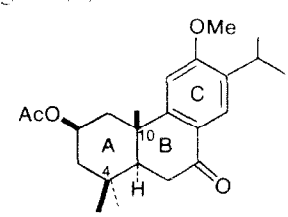
The structures of two extractives, $C_{20}H_{30}O_2$ and $C_{20}H_{28}O_3$ substances, remained unelucidated, while those of two others, $C_{20}H_{28}O_2$ and $C_{23}H_{32}O_4$ substances, were found to be **6** and **7**, respectively. The IR and PMR spectra of the $C_{20}H_{28}O_2$ compound limited it to a ketoferruginol system. While different from sugiol (**3b**) and hinokione, it yielded ferruginol on Wolff–Kishner reduction. Thus it had to be 1-, 2- or 6-ketoferruginol. Its acceptance of four mass units on base-induced deuteration and reprotonation of its phenolic hydroxyl group revealed it as 2-ketoferruginol (**6**).[7]



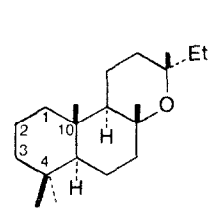
(**5**)



(**6**)



(**7**)



(**8**)

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[6] EDWARDS, O. E., FENIAK, G. and LOS, M. (1962) *Can. J. Chem.*, **40**, 1540.
[7] The acetate of **6** has been reported as an intermediate in a study of the structure of salviol (2α-hydroxyferruginol)[U. HAYASHI, T. HANDA, M. OHASHI, H. KAKISAWA, H.-Y. HSU and Y. P. CHEN, (1971) *Chem. Commun.*, 541].

The IR, UV and PMR spectra of the $C_{23}H_{32}O_4$ compound identified it as a ring A acetoxy derivative of sugiyl methyl ether (**3d**). Presence of the aromatic ketone moiety was confirmed by its hydrogenolysis and the consequent unmasking of a ferruginoid ring C PMR signal pattern. The multiplicity of the acetoxymethine signal indicated coupling to more than two neighboring hydrogens and the line shape was identical with that of the C(2)-H signal of the 2β-acetoxy derivative of dihydromanoyl oxide (**8**). These facts showed the $C_{23}H_{32}O_4$ constituent to be 2β-acetoxysugiyl methyl ether (**7**). The structure was corroborated by analysis of the $\Delta\delta$ values acquired by subtraction of the chemical shifts of the C(4) and C(10) methyl groups in **7**, 7-deoxo-**7** and all ring A acetoxylated dihydromanoyl oxides[8] from those in **3d**, ferruginyl methyl ether (**3e**) and **8**, respectively (Table 1). The $\Delta\delta$ value for the natural compound and its 7-deoxo derivative agreed well with the value for 2β-acetoxydihydromanoyl oxide.

TABLE 1. DIFFERENCES OF CHEMICAL SHIFTS OF METHYL GROUPS IN RING A ACETOXYLATED DITERPENIC COMPOUNDS FROM THOSE IN THEIR RING A UNOXYGENATED RELATIVES

Compounds	$\Delta\delta$ (4α—Me)	$\Delta\delta$ (4β—Me)	$\Delta\delta$ (10—Me)
(1α-Acetoxy-**8**) − (**8**)	0·05	0·04	0·06
(1β-Acetoxy-**8**) − (**8**)	0·01	0·03	0·19
(2α-Acetoxy-**8**) − (**8**)	0·06	0·09	0·10
(2β-Acetoxy-**8**) − (**8**)	0·08	0·18	0·24
(3α-Acetoxy-**8**) − (**8**)	0·02	0·08	0·03
(3β-Acetoxy-**8**) − (**8**)	−0·01	0·06	0·04
(**7**) − (**3d**)	0·07	0·22	0·19
(7-Deoxo-**7**) − (**3e**)	0·07	0·18	0·19

The large array of oxygenated, ferruginoid substances in *P. ferrugineus* indicates a high activity of oxidizing enzymes in the plant. Perhaps the most significant finding in the survey of terpenic constituents of the miro tree is the discovery of dehydroabietane (**9**) as a natural product for the first time.[2] The appearance of this hydrocarbon, upon whose molecular skeleton the structures of all ring C aromatic diterpenic constituents of *P. ferrugineus* are based, lends a clue to the biogenetic origin of the naturally abundant, diterpenic phenols ferruginol (**3a**), sempervirol (**10**) and totarol (**11**). In contrast to the earlier view of ferruginol being the first aromatic intermediate, from which totarol and podocarpic acid (**12**) are derived by acid-catalyzed isomerization and dealkylation, respectively,[9] it is reasonable to assume now that the plant oxidases act upon dehydroabietane instead. Not only can oxidation be expected at the benzylic carbon 7 and subsequently at C(6) and C(5) (e.g. **3b–d** and **4**), but also oxidation on the benzene ring can be envisaged. Thus the natural phenols can be expected to be the end products of isomerization and hydration-dehydration processes on dehydroabietane oxide (**13**) or its oxygen position isomers.[10] The biogenetic pathways are depicted in Scheme 1.

[8] The authors are indebted to Professor P. Grant (University of Otago, Dunedin, New Zealand) for a gift of samples of these compounds.

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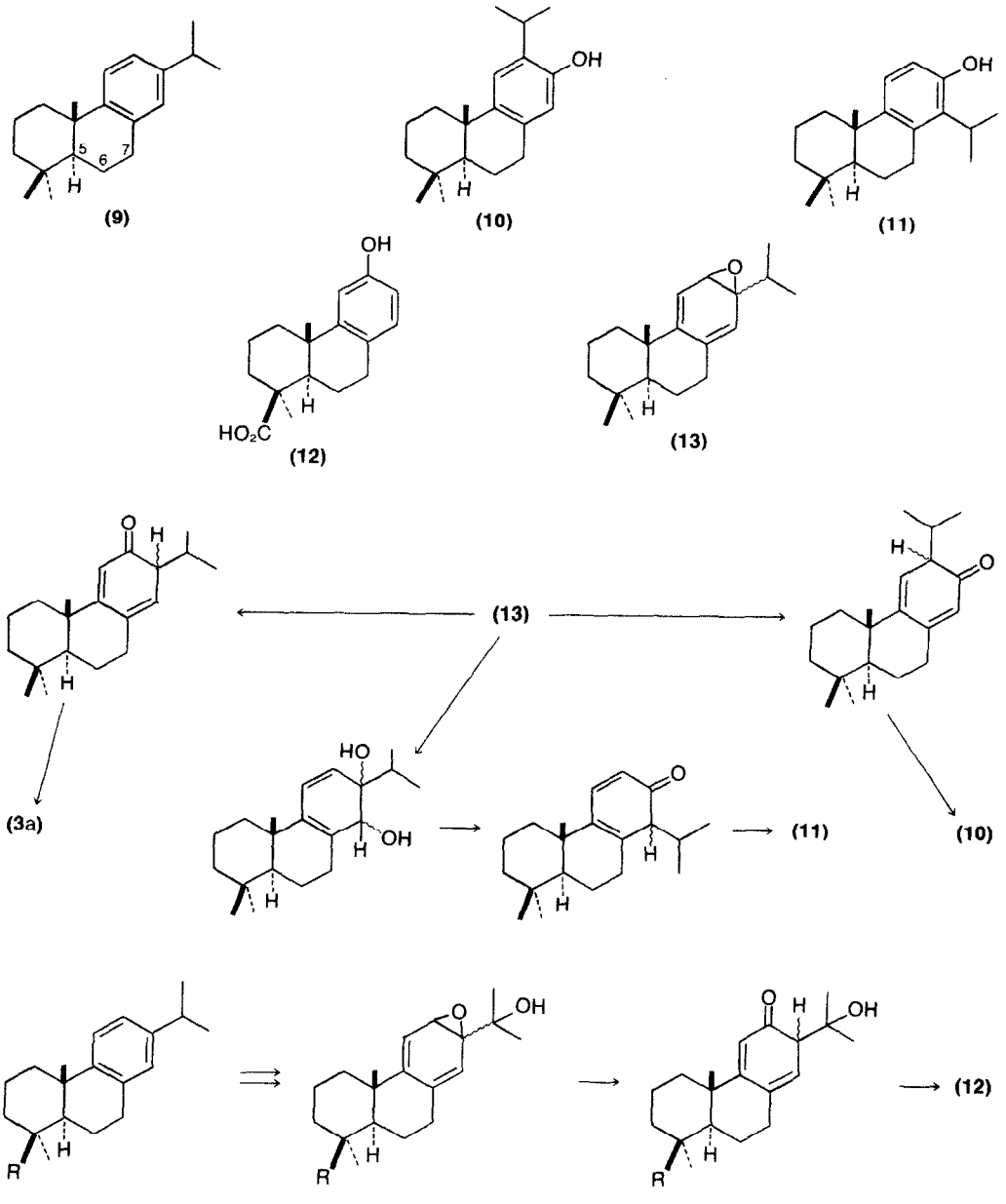

SCHEME 1.

EXPERIMENTAL

M.ps were determined on a Reichert micro hot stage and are uncorrected. PMR spectra of $CDCl_3$ soln (unless otherwise noted) with internal TMS were recorded on Varian Associates A-60 and HA-100 spectrometers. ORD measurements were recorded with the use of a 1-cm quartz cell on a Durram-Jasco ORD/UV-5 spectrophotometer. MS were determined in the direct inlet system of an AEI MS-9 spectrometer. Column chromatography was performed on G. F. Smith. 50–200 mesh silica gel or neutral, activity I Woelm alumina and TLC on Merck silica gel.

The previously described "acid fraction" from the extraction of *P. ferrugineus* D. Don bark[2] was acidified to pH 6 with 0·1 N HCl and extracted with CH_2Cl_2 (3 × 500 ml). The extract was dried and evaporated yielding 65 g of dark red gum. Furthermore, the alumina chromatography of the "neutral fraction" of the original bark extract was continued beyond the elution with light petrol.–Et_2O (9:1) which had led to a "hydrocarbon fraction" previously.[2]

Continuation of the elution of the alumina chromatographic column with petroleum–ether (4:1) gave 3·7 g of a mixture. Elution with a 3:2 solvent mixture yielded 63·7 g of ferruginol (**3a**) as viscous oil [acetate (Ac_2O-pyridine), colorless needles; m.p., m.m.p. 81–82°; $[\alpha]_D^{25}$ + 58° (c = 0·1, MeOH)], with light petrol.–Et_2O (1:1) 46·5 g of a mixture, and with a 1:4 combination 9·5 g of a mixture. Chromatography of the 4:1 eluate on 150 g of silica gel and elution with light petrol.–Et_2O (9:1) gave 1·6 g of oily ferruginol (**3a**), 600 mg of sugiyl methyl ether (**3d**); m.p., m.m.p. 136–137°; $[\alpha]_D^{25}$ + 32° (c = 0·1, MeOH), and 20 mg of 2-acetoxysugiyl methyl ether (**7**); m.p. 168–170°; UV λ_{max}^{EtOH} 230 nm (ε 17,500), 282 (14,500); IR (KBr) CO 5·76 (s), 6·00 (s) μ; PMR δ 1·00, 1·21, 1·43 (s, 3, Me each), 1·21, 1·24 (*d*, 3, *J* 7 Hz, Me each), 2·10 (s, 3, Ac Me), 3·90 (s, 3, OMe), 4·69 (m, 1, OCH), 6·69 [s, 1, H(11)], 7·91 [s, 1, H(14)]; o.r.d. (c = 0·05, MeOH) $[\alpha]_{700}^{25}$ + 10°, $[\alpha]_{600}$ + 15°, $[\alpha]_{589}$ + 15°, $[\alpha]_{500}$ + 45°, $[\alpha]_{400}$ + 100°, $[\alpha]_{350}$ + 680°, $[\alpha]_{330}$ + 1100°, $[\alpha]_{318}$ 0°, $[\alpha]_{308}$ − 1200°, $[\alpha]_{300}$ − 1000°, $[\alpha]_{250}$ − 1000°, $[\alpha]_{240}$ 0°; *m/e* 372·2301 (calc. for $C_{23}H_{32}O_4$: 372·2300). (Hydrogenation over Pd/C in EtOH gave a deoxo product spectrally identical with ferruginyl methyl ether in rings B and C; IR 6·00 μ CO band missing; PMR aromatic H signals shifted to 6·67 and 6·84 ppm.) Chromatography of the 1:1 eluate on 2 kg of silica gel and elution with light petrol.–Et_2O (4:1) yielded 10·1 g of isopimarol (**1b**); m.p., m.m.p. 86–87°; $[\alpha]_D^{25}$ − 22° (c = 0·09, MeOH), 150 mg of cryptojaponol (**3c**); m.p., m.m.p.[5] 204–206°; $[\alpha]_D^{25}$ + 51° (c = 0·003, MeOH), and 110 mg of a substance of unknown constitution; m.p. 200–202°; *m/e* 316·2038 (calc. for $C_{20}H_{28}O_3$: 316·2038). Chromatography of the 1:4 eluate on 500 g of silica gel and elution with light petrol.–Et_2O (1:1) afforded 1·5 g of sugiyl methyl ether (**3d**) and 75 mg of a substance of unknown constitution; m.p. 180–182°; *m/e* 302·2247 (calc. for $C_{20}H_{30}O_2$: 302·2246).

Chromatography of the gummy "acid fraction" on 2 kg of silica gel and elution with light petrol. yielded 630 mg of an orange solid whose crystallization from heptane and sublimation gave 139 mg of 6-dehydroroyleanone (6,7-dehydro-5); m.p. 167–168°; spectra identical with those previously recorded.[6] (Hydrogenation over Pd/C in EtOH yielded an unstable tetrahydro product whose air oxidation[6] gave royleanone). Elution with light petrol.–Et_2O (9:1) led to 1·1 g of isopimarol (**1b**) and 5·2 g of light yellow gum whose crystallization from heptane afforded isopimaric acid (**2c**). Elution with 4:1 petrol–ether yielded 1·2 g of ferruginol (**3a**) and 2·3 g of a yellow gum whose crystallization from heptane produced sandaracopimaric acid (**2c**); m.p. m.m.p. 171–172°; $[\alpha]_D^{25}$ − 25° (c = 0·13, MeOH). Elution with a 2:1 solvent mixture gave 1·1 g of sugiol; m.p., m.m.p. 283–284° [acetate; m.p., m.m.p. 164–165°; $[\alpha]_D^{25}$ + 35° (c = 0·003, MeOH)]. Elution with light petrol.–Et_2O (3:2) gave 248 mg of a pale yellow, amorphous solid whose crystallization from heptane–acetone yielded 25 mg of xanthoperol (**4a**); m.p., m.m.p. 265–270° (sealed tube); $[\alpha]_D^{25}$ + 110° (c = 0·07, MeOH). Elution with a 1:1 solvent pair produced 440 mg of a red oil whose rechromatography on 30 g of silica gel and elution with benzene led to 90 mg of golden plates of royleanone (**5**); m.p. m.m.p. 182–183°; $[\alpha]_D^{25}$ + 27° (c = 0·07, MeOH). Elution with light petrol.–Et_2O (2:3) gave 470 mg of pale red oil whose trituration with benzene and crystallization from heptane yielded 23 mg of 2-ketoferruginol (**6**); m.p. 232–234°; UV λ_{max}^{EtOH} 227 nm (ε 8600), 284 (4300); IR (KBr) OH 2·91 (m), CO 5·85 (s), C=C 6·20 (m) μ; PMR δ 0·98, 1·11, 1·20 (s, 3, Me each), 1·23 (d, 6, *J* 7 Hz, Me_2), 6·58, 6·88 (s, 1, aromatic H each); o.r.d. (c = 0·09, MeOH) $[\alpha]_{700}^{25}$ + 17°, $[\alpha]_{600}$ + 33°, $[\alpha]_{589}$ + 50°, $[\alpha]_{500}$ + 117°, $[\alpha]_{400}$ + 233°, $[\alpha]_{350}$ + 500°, $[\alpha]_{320}$ + 1330°, $[\alpha]_{304}$ + 2470°, $[\alpha]_{290}$ 0°, $[\alpha]_{270}$ − 2220°, $[\alpha]_{260}$ − 2930°, $[\alpha]_{248}$ 0°, $[\alpha]_{238}$ − 3330°; *m/e* 300·212 (calc for $C_{20}H_{28}O_2$: 300·209). Elution with a 1:4 solvent mixture gave 653 mg of a mixture of fatty acids, while elution with a 1:9 mixture led to 360 mg of a colorless, amorphous solid whose rechromatography on 25 g of silica gel, elution with benzene and crystallization from ethanol gave 46 mg of lactol **4b**[5] m.p. 168–169·5°; UV λ_{max}^{EtOH} 222 nm (ε 5200), 270 (2600); IR (KBr) OH 3·00 (m), CO 5·89 (s), C=C 6·24 (m) μ; PMR δ 0·70, 1·19, 1·39 (s, 3, Me each), 1·22 (d, 6, *J* 7 Hz, Me_2), 3·92 (s, 3, OMe), 6·80 [s, 1, H(11)], 7·90 [s, 1, H(14)]; o.r.d. (c = 0·42, MeOH) $[\alpha]_{700}^{25}$ + 43°, $[\alpha]_{600}$ + 52°, $[\alpha]_{589}$ + 57°, $[\alpha]_{500}$ + 81°, $[\alpha]_{400}$ + 158°, $[\alpha]_{350}$ + 285°, $[\alpha]_{310}$ + 800°, $[\alpha]_{290}$ 0°, $[\alpha]_{270}$ − 80°, $[\alpha]_{248}$ 0°, $[\alpha]_{220}$ − 150°; *m/e* 332·202 (calc. for $C_{20}H_{28}O_4$: 332·199).

Deuteration and reduction of 2-ketoferruginol (**6**). A solution of 4 mg of 2-ketoferruginol (**6**) and 50 mg of NaOMe in 3 ml of D_2O was refluxed for 15 min and evaporated to dryness under vacuum. The procedure was repeated twice and the residue extracted with CH_2Cl_2 (3 × 25 ml). The extract was washed with 10 ml water and dried. Evaporation of the solvent gave 2 mg of material whose mass spectrum revealed strong suppression of the *m/e* 300 peak and appearance of a peak at *m/e* 304.

A solution of 4 mg of 2-ketoferruginol semicarbazone (m.p. 153–155° from EtOH) and 200 mg of KOH in 5 ml of ethylene glycol was refluxed for 18 hr. It then was brought to pH 6 with 0·1 N HCl and extracted with 75 ml of CH_2Cl_2 3 ×. The extract was washed with 10% $NaHCO_3$, dried and evaporated. A solution of the residual oil and 1 ml of Ac_2O in 1 ml of pyridine was kept at room temp. for 12 hr and then evaporated. Fractional sublimation of the residue yielded 1 mg of ferruginyl acetate, m.p. 80–82°, m.m.p. 79–82°.

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