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Chemistry of the Order Podocarpales

I. Heartwood Constituents of the Huon Pine *(Dacrydium franklini* Hook. f.)

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The heartwood of the Huon Pine contains the common C_{16}—C_{26} fatty acids, a wax consisting of fatty acid esters, eugenol methyl ether, elemicin, coniferyl aldehyde methyl ether, coniferyl alcohol methyl ether, C_{20}-C_{26} fatty alcohols, β-sitosterol, eugenol, zanthoxylol, and a new phenol, dacriniol [3-(5-γγ-dimethylallyl-4-hydroxy-3-methoxyphenyl)-propanol] which we have synthesised. Evidence was obtained to show that the aldehyde corresponding to dacriniol, *i.e.* dacrinial was also present.

The conifer genus *Dacrydium* (Podocarpaceae) is essentially southern hemispheric and embraces some twenty-five species. It has long been felt by the taxonomists that this heterogeneous group needs a thorough revision. Although only a few species have been subjected to any chemical investigation the results confirm this opinion.[1] On anatomical, cytological and palynological evidence the genus has been tentatively divided [2] into two groups each of which is further divided into subgroups which sometimes consist of only a single species. Examples of such isolated taxa are *Dacrydium colensoi*, *D. cupressinum* (both New Zealand) and *D. franklini* (Tasmania).

The steam volatile oil of the wood of the latter species, the "Huon pine", is reported [3,4] to consist almost entirely (95—97 %) of eugenol methyl ether together with small amounts of eugenol. Eugenol methyl ether has also been shown to be present in the leaf oil together with (+)-α-pinene, (−)-β-pinene, (+)-limonene, Δ^4-carene, phyllocladene,[5,6] isophyllocladene, and kaurene.[7] The heartwood of all the New Zealand species investigated so far contains diterpenes of the labdane, pimarane, and (*D. cupressinum*) abietane or podocarpane type. Several of these compounds are not steam-volatile and in order to find out whether or not such compounds occur in *Dacrydium franklini* we have now investigated the wood constituents using the extraction technique.

The wood was extracted with light petroleum to give a light yellow oil. On standing a gel-like material precipitated, the infra-red spectrum of which indicated that it was a fatty acid ester. This material was hydrolysed and the products investigated by gas-liquid chromatography (GLC). The acid fraction consisted of varying amounts of the common fatty acids with an even number of carbon atoms, *i.e.* palmitic, stearic, arachidic, behenic, and lignoceric acids. The alcohol fraction contained even numbered monohydric C_{20} to C_{26} alcohols.

The standard technique for separating the extract into acidic, phenolic, and neutral fractions by treatment with sodium bicarbonate and base proved to be unsuitable since stable emulsions were formed which took several days to break down. However, this difficulty was overcome by scaling up Zinkel and Rowe's [8] Sephadex A 25 ion exchanger method.

The acid fraction was investigated by GLC of the methyl esters and was found to consist mainly of palmitic, linoleic, and an unidentified acid, with smaller amounts of oleic, stearic, arachidic, linolenic, behenic, and lignoceric acids. No resin acids could be detected.

The major part of the neutral fraction distilled below 185°/19 mm. Further fractionation and GLC analysis showed that the volatile material was a mixture of methyl eugenol (76 %) and elemicin (14 %) with only traces of other compounds. The distillation residue was investigated by thin-layer chromatography (TLC) for the presence of manoyl derivatives which have been isolated from other *Dacrydium* species, such as manoyl oxide, manool and 2-oxomanoyl oxide from *D. biforme* [9] and more highly oxygenated derivatives from *D. colensoi*.[10] None of these compounds were detected. Coniferyl aldehyde methyl ether together with a lesser amount of β-sitosterol were found to constitute the main bulk of the non-volatile neutral material. A small quantity of a mixture of C_{20}-C_{26} alcohols was also isolated from the distillation residue.

The untreated neutral fraction and the distillation residue were investigated for the presence of diterpene hydrocarbons (*e.g.* phyllocladene); none were detected.

Chromatography of the phenolic fraction yielded eugenol and a new phenol $C_{15}H_{22}O_3$, m.p. 52—53°, which we have named dacriniol.

A comparison of the $\Delta\varepsilon$-curve of dacriniol with those of several substituted phenols [11] showed that it was possibly a 4,6-disubstituted catechol monomethyl ether. The IR spectrum showed absorption bands due to an aliphatic —OH group (3640 cm^{-1}), a phenolic —OH group (3540 cm^{-1}), a trisubstituted double bond (840 cm^{-1}) and methyl groups (1375 cm^{-1}). Dacriniol formed a diacetate $C_{19}H_{26}O_5$ which could not be induced to crystallise. Structure *3* was proposed for dacriniol on the basis of the NMR spectra of the phenol (Table 1) and its diacetate (Table 2). Dacriniol shows a triplet centered at $\simeq$ 6.3 τ. That of the diacetate is at 5.9 τ. This confirms that the aliphatic hydroxyl group in dacriniol is primary. Also the upfield shift of the doublet at 6.65 τ to 6.85 τ in the diacetate suggests that the $\gamma\gamma$-dimethylallyl side chain is situated adjacent to the phenolic hydroxyl group. This information, together with the fact that similarly substituted aromatic compounds were present in the neutral fraction, strongly suggested that dacriniol was a $\gamma\gamma$-dimethylallyl derivative

Table 1. NMR spectrum of dacriniol in deuterochloroform solution (TMS internal standard).

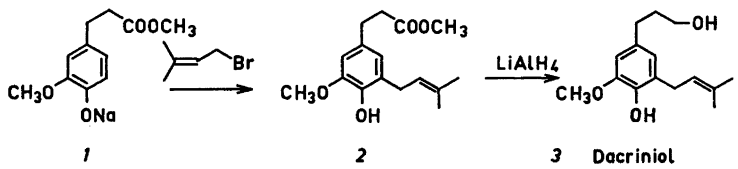
τ-Values	No. of protons	Signal pattern	Assignment
8.3	(6)	Slightly split multiplet, J = 1 cps	$-CH=CH(CH_3)_2$
8.2	(2)	Multiplet	$-CH_2-CH_2-CH_2-$
7.45	(2)	The A part of an A_2B_2 system	$Ar-CH_2-CH_2-$
6.65	(2)	Doublet, J = 8 cps	$Ar-CH_2-CH=C<$
6.31	(2)	Triplet, J = 6.5 cps	$-CH_2-OH$
6.15	(3)	Singlet	$-OCH_3$
4.61	(1)	Broad triplet, J = 8 cps	$-CH_2-CH=C(CH_3)_2$
3.37	(2)	Singlet	Ar-*H* (*meta* oriented)

Table 2. NMR Spectrum of dacriniol diacetate in deuterochloroform solution (TMS internal standard).

τ-Values	No. of protons	Signal pattern	Assignment
8.3	(6)	Slightly split multiplet, J = 1 cps	$-CH=CH(CH_3)_2$
8.03	(3)	Singlet	$-CH_2OCO\,CH_3$
8.0	(2)	Multiplet	$-CH_2-CH_2-CH_2-$
7.78	(3)	Singlet	$ArOCO\,CH_3$
7.45	(2)	The A part of an A_2B_2 system	$Ar-CH_2-CH_2-$
6.85	(2)	Doublet, J = 8 cps	$Ar-CH_2-CH=C<$
6.22	(3)	Singlet	$-OCH_3$
5.92	(2)	Triplet, J = 7 cps	$-CH_2-CH_2-OCOCH_3$
4.75	(2)	Triplet, J = 8 cps	$-CH_2-CH=C(CH_3)_2$
3.42	(2)	Singlet	Ar*H* (*meta* oriented)

of dihydroconiferyl alcohol. Since the two aromatic protons are *meta* oriented structure *3* is fully consistent with the spectroscopic evidence.

Structure *3* was confirmed by the synthesis of dacriniol by the following route:



Sodium methyl dihydroferulate *1* was alkylated with γγ-dimethylallyl bromide in benzene to yield a phenolic material and an ether which were separated by chromatography. The spectroscopic properties of the phenolic material, which showed only one spot on TLC, were consistent for the material

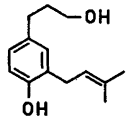
having the structure *2*. The NMR spectrum of the phenolic material indicated the presence of small amounts of the conjugated double bond isomer. Lithium aluminium hydride reduction of *2* yielded two products one of which was identical with dacriniol (IR, NMR, m.p., mixed m.p. of natural and synthetic material).

The wood remaining after the light petroleum extraction was then extracted with acetone and the ether soluble, light petroleum insoluble part of this extract investigated. No difficulty was encountered during the separation of the extract into acidic, phenolic, and neutral compounds using the standard sodium bicarbonate and sodium hydroxide extraction technique.

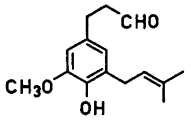
The acidic fraction was a complex mixture of polyphenolic compounds together with a very small quantity of fatty acids. No resin acids could be detected.

The neutral fraction contained eugenol methyl ether, elemicin, coniferyl aldehyde methyl ether, and the corresponding alcohol.

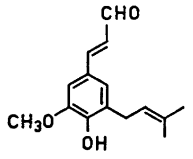
The main constituent of the phenol fraction was dacriniol. Two other compounds were isolated; one was identified as zanthoxylol which was recently isolated from *Fagara zanthoxyloides* (Rutaceae),[12] the other was an aldehydic phenol. The latter material was homogeneous by TLC and its NMR spectrum showed that it contained the following groups; $\gamma\gamma$-dimethylallyl, methoxyl, *meta* oriented aromatic protons, and an aldehyde group. Reduction of this phenol with sodium borohydride in methanol gave a mixture of five products the main component of which was shown to be dacriniol by TLC and the NMR spectrum. Hence the aldehydic phenol first isolated was a mixture comprising mainly dacrinial (*5*).



4 Zanthoxylol



5 Dacrinial



6 Parvifloral

Another phenol, parvifloral (*6*), closely related to dacriniol (*3*) and zanthoxylol (*4*), has recently been isolated from *Xanthoxylum parviflorum* (Rutaceae).[13] Like dacriniol, parvifloral was synthesised by *ortho* prenylation of vanillin, etc.

The absence of diterpenes and the pattern of aromatic constituents of the heartwood of *Dacrydium franklini* seem to indicate that in *Dacrydium sensu lato* this species occupies a unique position. In preliminary investigations of the wood of *D. elatum* Wallich, *D. gibbsiae* Stapf., and probably a new species with affinity to *D. beccarii* Parl., (collected on Mt. Kinabalu, Sabah, Borneo) no eugenol or eugenol methyl ether could be found.

EXPERIMENTAL

NMR Spectra ($CDCl_3$; TMS as internal standard), IR and UV spectra were recorded on a Varian A60 spectrometer, a Perkin-Elmer 237, and a Beckman DK2 spectrophotometer, respectively. GLC was carried out using a Wilkens Aerograph instrument, flame ionisation detector. Identifications were made by comparison of IR, NMR spectra, chromatographic behaviour, m.p./b.p., with those of authentic samples unless stated otherwise.

Extraction. The air-dried heartwood (20 kg) was extracted with hot light petroleum (b.p. 40—60°) for 5 days. Removal of the solvent gave a clear yellow oil (1.02 kg) which after standing for a few days deposited a gel-like material. This was filtered off, washed with light petroleum and on attempted crystallisation from acetonitrile gave a soft amorphous solid (5.6 g) melting at 68—72° which did not change on further "recrystallisation" from propionitrile. 200 mg of this material was refluxed in methanolic KOH (25 ml 3 %) for 3 h. After acidification (dil. HCl), the products were isolated by extraction with ether and then treated with excess diazomethane in ether. The alcohols (100 mg) and fatty acid methyl esters (100 mg) were separated by column chromatography and then analysed by GLC. The alcohols were analysed as trimethylsilyl ethers (for preparation and column conditions see Ref. 14). The analyses of the esters were carried out using a 1 metre 5 % BDS column at 205°. Identifications were made by comparison of retention data with those of authentic samples.

Portions of the filtrate were separated into acidic, phenolic, and neutral fractions in the following manner. Sephadex A25 ion exchanger (30 g) was obtained in the free base form as described by Zinkel and Rowe [8] except that 200 ml portions of acid, base, and water were used for the washing. A chromatography tube (35 × 2 cm) was then packed with the Sephadex suspended in ether-methanol-water (89:10:1) and the heartwood extract (25 g) in the same solvent (≃ 80 ml) applied to the column. The neutral materials were washed through the column using 500 ml of the same solvent. The phenols were eluted from the column with ether-methanol (90:10) saturated with carbon dioxide (≃ 900 ml) followed immediately by the acids (≃ 300 ml). The extract consisted of 81 % neutrals, 15 % phenols, and 4 % acids.

Acid fraction. The mixture of acids was analysed as methyl esters by GLC as described above. It was found to consist of 43 % linoleic, 29 % palmitic, 9 % of an unidentified acid (possibly a C_{20} acid containing two double bonds), 7 % oleic, 5 % arachidic, 4 % linolenic, and 2 % stearic acid.

Neutral fraction. Most of the neutral fraction (82%) distilled in the range 126—185°/19 mm. GLC showed that the distillate consisted (99 %) of two components in the ratio 76:14. Several distillations using a spinning band column gave pure samples of the two components boiling at 133°/20 mm (n_D^{20} 1.5347) and 144°/10 mm (n_D^{20} 1.5288) which were identified by their IR spectra as methyl eugenol (lit. b.p. 128—129°/11 mm, n_D^{20} 1.532) [15] and elemicin (lit. b.p. 143—6°/10 mm, n_D^{20} 1.527),[15] respectively. TLC of the distillation residue showed three significant spots. Repeated column chromatography (silica gel) of 4 g of this mixture separated the corresponding compounds as follows: an unsaturated fatty alcohol (60 mg) m.p. 74—75° (from diisopropyl ether), $\nu_{max}^{CHCl_3}$ 3561, 3010 cm^{-1}, analysed as described above; C_{20}-C_{26} alcohols; β-sitosterol (300 mg) m.p. 136—137.5° (from methanol), mixed m.p. with authentic sample 136—137°; coniferyl aldehyde methyl ether, m.p. and mixed m.p. 83—84° (from methanol-water).

Phenol fraction. Part of the phenol mixture (4.1 g) was chromatographed on 300 g silica gel; elution with cyclohexane yielded first a colourless oil (3.2 g) which was further purified by distillation, b.p. 127°/15 mm, and identified as eugenol (IR). Elution with cyclohexane-benzene yielded dacriniol (0.306 g) which after vacuum distillation and recrystallisation from cyclohexane gave colourless needles, m.p. 52—53°; $\nu_{max}^{CCl_4}$ 3640, 3540, 1605, 1500, 1375, 1290, 840 cm^{-1}; λ_{max}^{EtOH} 279 mμ (ε 2500). (Found: C 72.1; H 8.8; CH_3O 12.5. Calc. for $C_{15}H_{22}O_3$: C 72.0; H 8.9; CH_3O 12.4).

The dacriniol diacetate was prepared using the pyridine-acetic anhydride method, purified by chromatography on silica gel and distilled, bath temp. 140°/1 mm; $\nu_{max}^{thin\ film}$ 1760, 1730, 1595 cm^{-1}. (Found: C 68.39; H 7.89. Calc. for $C_{19}H_{26}O_5$: C 68.25; H 7.83) n_D^{22} 1.5082.

Methyl 2-(5-γγ-dimethylallyl-4-hydroxy-3-methoxyphenyl)-dihydrocinnamate (2). To methyl dihydroferulate (2 g) in dry benzene (25 ml), clean sodium (0.22 g) was added and the mixture stirred under reflux for 20 h. After this time a very small quantity of metallic sodium that remained was removed: redistilled γγ-dimethylallyl bromide (1.5 g) in dry benzene (10 ml) was added and the mixture refluxed with stirring for a further 12 h. The reaction mixture was then washed with water and dried (anhyd. $MgSO_4$). Removal of the benzene yielded 2.65 g of crude product which was chromatographed on silica gel (265 g) to give 0.59 g of mainly *2*. 10 % of the double bond isomer in this fraction was indicated by NMR signals at 8.66 τ (doublet, $J = 7$ cps) and 6.18 τ (singlet). Characteristic NMR signals assigned to the main constituent are 3.47 τ (2 H, singlet), two *meta* substituted aromatic protons; 6.22 τ (3 H, singlet), methoxyl group; 6.38 τ (3 H, singlet), methyl ester; four proton multiplet centered at 7.3 τ, Ar $CH_2CH_2COOCH_3$; 4.61 τ (1 H, broad singlet), 6.66 τ (2 H, doublet, $J = 8$ cps), 8.3 τ (6 H, slightly split multiplet, $J \simeq 1$ cps), γγ-dimethylallyl group. $\nu_{max}^{thin\ film}$ 3540, 1730, 1600, 1585, 1365, 850 cm^{-1}.

Lithium aluminium hydride reduction of 2. Compound *2* (400 mg) was reduced with lithium aluminium hydride (70 mg) in ether. The reaction product was chromatographed thrice on silica gel (70 g, diisopropyl ether) to yield 132 mg of material, m.p. 49–51° (mixed m.p. with natural dacriniol 48–50°). The IR and NMR spectra and TLC behaviour of this product were identical with those of natural dacriniol.

Acetone extract. The wood remaining after the light petroleum extraction was then extracted with acetone for 3 days. The dark red acetone solution was concentrated and then poured into a large volume of vigorously agitated ether. The precipitate (160 g) was removed and the filtrate concentrated to a small volume and poured into light petroleum (40–60°). The dark red oil which separated (360 g) was washed with light petroleum and then separated into acidic, phenolic, and neutral fractions (4, 66, and 30 %, respectively) using the standard sodium bicarbonate and sodium hydroxide extraction technique.

Acid fraction. TLC showed this to be a mixture of at least 15 components. After treatment with CH_2N_2 the mixture still exhibited a considerable -OH absorption in the IR. No resin acids could be detected by TLC.

Neutral fraction. Chromatography of 2 g of this mixture on 100 g silica gel with diisopropyl ether gave 1.15 g of a mixture of eugenol methyl ether and elemicin (9:2 by GLC), coniferyl aldehyde methyl ether (300 mg), coniferyl alcohol methyl ether (300 mg, identified by IR and NMR spectra), and small quantities of more polar materials which were not investigated further.

Phenol fraction. The phenol mixture (3 g) was chromatographed on silica gel (130 g) to give the following materials; eugenol (50 mg), 'phenol 1' (100 mg), 'phenol 2' (300 mg), dacriniol (1.05 g), and a very polar residue. 'Phenol 2' was identified as zanthoxylol by comparison of the IR and NMR spectra with those of an authentic sample. The IR spectrum of 'phenol 1' showed characteristic aldehyde absorption at 1720 and 2730 cm^{-1}. The NMR spectrum showed the bands assignable to γγ-dimethylallyl, methoxyl and *meta* oriented protons at 8.3, 6.7, 4.6 τ; 6.2 τ; and 3.45 τ, respectively, together with bands at 7.2 and 8.8 τ. Sodium borohydride (100 mg) reduction of 'phenol 1' (100 mg) in methanol (10 ml) yielded a mixture of 5 products. Comparative TLC studies showed that the major component of this mixture was dacriniol. Also the NMR spectrum of the mixture exhibited all the bands characteristic of dacriniol and other minor bands mainly at $\simeq$ 3.2 τ and 8.7 τ.

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