

THE VOLATILE OILS OF THE GENUS *EUCALYPTUS* (FAMILY MYRTACEAE)*

III. THE LEAF OIL OF *E. CRENULATA* BLAKELY & DE BEUZEVILLE

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The biological factors discussed in Part I of this series¹ were taken into consideration in this investigation of the composition of the volatile leaf oil of *Eucalyptus crenulata* Blakely & de Beuzeville.² This is a very rare species, and in Blakely's classification³ is placed in Section Macrantherae, Series Globulares. It is a small, glaucous, straggly tree, found only on swampy or low-lying ground, and is of very restricted distribution, being known from only two small occurrences—near Buxton (the type locality), and near Yering, both in Victoria.

The foliage yielded 0.9% of steam-volatile material, of which about 30% was a crystalline solid extracted from the distillation water. The oil which separated had d_{15}^{20} 0.9101; n_D^{20} 1.4905; $[\alpha]_D^{22}$ -0.25° ; acid number 2.4; ester number 62.2; ester number after acetylation 82.3. It was found to consist chiefly of γ -terpinene, *p*-cymene, and methyl eudesmate (methyl ester of 3,4,5-trimethoxybenzoic acid) together with small quantities of α -pinene, terpinolene, isovaleric aldehyde, and terpin-1-en-4-ol. The solid recovered from the distillation waters consisted almost entirely of methyl eudesmate, so that the ester content of the total distillate amounted to approximately 47%. Since γ -terpinene was a major component, some or all of the *p*-cymene may be an artefact formed during isolation or vacuum distillation of the oil. The composition of this oil is unusual for the genus, and differs markedly from the oils of closely related species such as *E. pulverulenta* Sims, *E. cordata* Labill., and *E. perriniana* F. Muell. ex Rodway. Eudesmic acid (later shown by Birch and Elliott⁴ to be 3,4,5-trimethoxybenzoic acid) was isolated by Smith⁵ as a saponification product of a fraction from the leaf oil of *E. aggregata* Deane & Maiden, in which the acid was considered by Smith to be present as an amyl ester.

The population at Yering shows an occasional tree having features indicating hybridism with *E. ovata* Labill., coextensive with *E. crenulata* on this site.⁶ However, in a progeny of 20 trees raised from open-pollinated seed from the experimental material from the type locality, no evidence of segregation could be detected, from

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¹ Willis, J. L., McKern, H. H. G., and Hellyer, R. O., *J. Roy. Soc. N.S.W.*, 1963, **96**, 59.

² Blakely, W. F., and Beuzeville, W. A. W. de, *Contr. N.S.W. Nat. Herb.*, 1939, **1**, 37.

³ Blakely, W. F., "A Key to the Eucalypts." 2nd Ed. p. 159. (Forestry and Timber Bureau: Canberra 1955.)

⁴ Birch, A. J., and Elliot, P., *J. Chem. Soc.*, 1953, 355.

⁵ Smith, H. G., *J. Roy. Soc. N.S.W.*, 1900, **34**, 72.

⁶ Willis, J. H., private communication, 1963.

both visual inspection of morphology and from gas chromatographic examination of the oils.

Experimental

All melting points are uncorrected. Vacuum distillations were carried out in a Podbielniak "Mini-cal" Series 3475 fractionating column using "Heli-grid" packing. Gas chromatography was conducted in a Bodenseewerk Perkin-Elmer Vapor Fraktometer, Model 116, with a 2 m by 4 mm aluminium column packed with "Celite" impregnated with methoxypolyethylene glycol, using helium as carrier gas. Infrared spectra were obtained from a Perkin-Elmer "Infracord" spectrophotometer, model 137. Microanalyses were carried out by the CSIRO and University of Melbourne Microanalytical Laboratory.

(a) *Isolation of Volatile Material*.—Fresh foliage (13.2 kg) of *E. crenulata* collected between Buxton and Narbethong, Vic., was steam-distilled in a tin-lined apparatus to yield a pale amber mobile oil (85.5 g; 0.65%). Petrol extraction of the aqueous distillate yielded a crystalline solid, m.p. 75–80° (35 g; 0.24%).

(b) *Isovaleric Aldehyde*.—The liquid condensed in the dry ice-acetone cold trap during vacuum fractional distillation of the oil yielded a yellow 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. with an authentic specimen of the 2,4-dinitrophenylhydrazone of isovaleric aldehyde 123°.

(c) *α -Pinene*.—The first runnings from the fractional distillation of the oil were submitted to gas chromatography. A fraction corresponding to a peak having the retention time of α -pinene was condensed and found to give an infrared spectrum identical with that of an authentic specimen. The fraction was homogeneous to further gas chromatography.

(d) *p-Cymene*.—A fraction of the oil, b.p. 58–62° at 15.5 mm, was submitted to gas chromatography (column packed with "Celite" 545, 80–120 mesh, impregnated with picric acid (5%) and methoxypolyethylene glycol 750 (15%)). A fraction corresponding to a peak with the retention time of *p*-cymene was condensed and found to give an infrared spectrum identical with that of an authentic specimen of *p*-cymene. The fraction was homogeneous to further gas chromatography.

(e) *γ -Terpinene*.—A fraction, b.p. 59–60° at 10 mm, yielded a nitrosochloride, m.p. and mixed m.p. 111°. Ice-cold alkaline permanganate oxidation gave *p*-menthane-1,2,4,5-tetrol, m.p. and mixed m.p. 239°. The infrared spectrum of a gas chromatography fraction (from fraction boiling at 65° at 15.5 mm) was identical with that of an authentic specimen of γ -terpinene.

(f) *Terpinolene*.—A fraction, b.p. 78–80° at 16 mm, was submitted to gas chromatography and a fraction corresponding to a peak having the retention time of terpinolene was collected. Resubmission of the fraction to gas chromatography indicated homogeneity and the infrared spectrum of the fraction was found to be identical with that of terpinolene.

(g) *Terpin-1-en-4-ol*.—Gas chromatography of the still-pot residue, b.p. > 80° at 16 mm, from a vacuum fractionation of the oil showed one major peak due to methyl eudesmate, together with three minor peaks. Two of these were due to γ -terpinene and terpinolene. The fraction corresponding to the third peak was collected. Resubmission to gas chromatography indicated homogeneity and its infrared spectrum was identical with that of a specimen of 1-terpinen-4-ol isolated from oil of *Melaleuca alternifolia* Cheel.

(h) *Methyl Eudesmate (Methyl 3,4,5-Trimethoxybenzoate)*.—The solid residues from the fractional distillations, as well as the solid extracted by petrol from the steam-distillation water, were recrystallized from methanol to m.p. 82° (Found: C, 58.6; H, 6.2; O, 35.5; CH₃O, 54.0%. Calc. for C₇H₂O(CH₃O)₄: C, 58.4; H, 6.2; O, 35.4; CH₃O, 54.9%). Saponification: 1.829 g required 16.2 ml 0.5N KOH; C₁₁H₁₄O₆ (monobasic acid, monohydric alcohol) requires 16.17 ml. From the saponification product an acid, m.p. 169.5° from benzene, was isolated. It did not depress the m.p. of a specimen of synthetic 3,4,5-trimethoxybenzoic acid of m.p. 169°. The m.p. of a specimen of synthetic methyl eudesmate prepared by the method of Birch and Elliott⁴ was not depressed by admixture with the natural product.

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