

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

### IV.† THE LEAF OILS OF *E. AMPLIFOLIA* NAUDIN AND *E. KITSONIANA* MAIDEN

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*Eucalyptus amplifolia* Naudin (cabbage gum) is classified by Blakely<sup>1a</sup> in his section Macrantherae, series Exsertae. It is a distinctive species of wide distribution and of fairly common occurrence along the coast and highlands of eastern Australia where it is found chiefly on alluvial soils in the vicinity of water-courses and on damp soils generally. Observations on *E. amplifolia* on a variety of sites and in varied associations in New South Wales from the Shoalhaven River to Walcha lead to the view that this species exhibits great constancy of morphology, and that it does not display any evidence of hybridism, even with closely related species (such as *E. tereticornis* Sm.) with which it comes in contact. Fresh foliage collected at Riverstone, N.S.W., where this species was associated only with *E. moluccana* Roxb. (formerly known as *E. hemipholia* F. Muell. ex Benth.<sup>2</sup>), yielded an oil for which the following composition was found (percentages determined by gas chromatography): ( $\pm$ )-limonene (57%); 1,8-cineole (20%);  $\alpha$ -pinene (6%);  $\alpha$ -terpineol (5%); aromadendrene (4%);  $\alpha$ -eudesmol (1%);  $\beta$ -eudesmol (6%); isovaleric aldehyde and unidentified compounds (1%).

*E. kitsoniana* Maiden is placed by Blakely<sup>1b</sup> in his section Macrantherae, series Semiunicolores, and is a dwarf, smooth-barked species of restricted distribution, being known only from three disjunct coastal areas of southern Victoria: between Wonthaggi and Foster (the type locality), between Apollo Bay and Cape Otway, and on the lower Glenelg River between Dartmoor and Kentbruck.<sup>3</sup> Fresh foliage collected half a mile east of Foster yielded an oil which was found to consist of the following (percentages determined by gas chromatography): 1,8-cineole (52%); ( $\pm$ )-limonene (27%);  $\alpha$ -pinene (5%); aromadendrene (5%); sesquiterpene alcohols (5%); unidentified constituents (6%). Although *E. kitsoniana* is not known to hybridize with other species, nevertheless a progeny of 50 trees was raised at Castle Hill, N.S.W., from open-pollinated seed from the parent material. This progeny was morphologically uniform, and gas chromatography of each individual oil failed to reveal any qualitative chemical differences amongst them or from the oil of the parent tree.

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<sup>1</sup> Blakely, W. F., "A Key to the Eucalypts." (a) p. 136; (b) p. 167. (Forestry and Timber Bureau: Canberra 1955.)

<sup>2</sup> Johnson, L. A. S., *Contrib. N.S.W. nat. Herb.*, 1962, **3**, 115.

<sup>3</sup> Willis, J. H., private communication.

### Experimental

All melting points are uncorrected. Infrared spectra were determined on a Perkin-Elmer Infracord 137 instrument. Gas chromatography was carried out with a Bodenseewerk Perkin-Elmer 116 Fractometer using a 2 mm by 4 mm copper column packed with Celite, mesh 80-100, impregnated with Silicone DC550; methoxy polyethylene glycol was used for separations of  $\alpha$ -terpineol; helium was used as carrier gas. Where a compound was separated by gas chromatography and the infrared spectrum of the fraction collected at the outlet of the column determined, the homogeneity of each fraction was determined by reintroducing a portion of the collected material into the apparatus. Microanalyses were performed by the Australian Microanalytical Service, Melbourne.

#### (a) Oil of *E. amplifolia*

**Isolation of Essential Oil.**—Fresh leaves and terminal branchlets were collected from three different localities in New South Wales (Seven Hills, Riverstone, and Coopernook), and on steam distillation pale amber-coloured oils were obtained in yield ranging from 0.9 to 1.2%, and whose characteristics were as follows:  $d_{4}^{20}$  0.8921-0.9063;  $n_D^{20}$  1.4726-1.4794;  $\alpha_D^{20}$  +2.5° to +13.8°; acid number, 1.0-2.5; ester number, 2.5-12.5; ester number after acetylation, 44.5-70.0; soluble in 0.8-7.5 volumes of 80% (w/w) alcohol. The Riverstone oil was used in the identification of constituents.

**Identification of Constituents.**—The crude oil was fractionated through a Widmer-type column under 10 mm of pressure and the following compounds identified from the fractions obtained:

(i) *Isovaleric aldehyde*.—Treatment of the cold-trap fraction with Brady's reagent yielded an orange precipitate which was identified as the 2,4-dinitrophenylhydrazone of isovaleric aldehyde, m.p. and mixed m.p. 123-124°.

(ii) (+)- *$\alpha$ -Pinene*.—A fraction (b.p. 39-42°/10 mm,  $d_{4}^{20}$  0.8664;  $n_D^{20}$  1.4666;  $\alpha_D$  +28.7°) when treated with nitrosyl chloride yielded the nitrosochloride of (+)- $\alpha$ -pinene, m.p. and mixed m.p. 109.5-110°. A gas chromatography fraction had an infrared spectrum identical with that of an authentic specimen.

(iii) *1,8-Cineole*.—The lower boiling fractions (b.p. 70°/10 mm) were extracted with 50% aqueous resorcinol solution to yield cineole, identified by the *o*-cresol addition compound, m.p. and mixed m.p. 56°. The cineole content of the crude oil was found to be 19.5%, as determined by the *o*-cresol method on the monoterpene fraction, and calculating back to the crude oil.

(iv) ( $\pm$ )-*Limonene*.—A fraction (b.p. 53-54°/10 mm,  $d_{4}^{20}$  0.8658,  $n_D^{20}$  1.4704,  $\alpha_D$  -46.5°) when treated with bromine yielded the tetrabromide of ( $\pm$ )-limonene, m.p. and mixed m.p. 124.5°. The dibromide of optically active limonene could not be isolated from the reaction mixture.

(v)  *$\alpha$ -Terpineol*.—A fraction (b.p. 92-106°/10 mm,  $d_{15}^{25}$  0.9436,  $n_D^{20}$  1.4867,  $\alpha_D$  -19.7°) on treatment with phenyl isocyanate yielded the phenylurethan of  $\alpha$ -terpineol, m.p. and mixed m.p. 109-109.5°.

(vi) *Aromadendrene*.—A fraction (b.p. 122-140° at 10 mm,  $d_{4}^{20}$  0.9281,  $n_D^{20}$  1.4986,  $\alpha_D$  -2.62°) on oxidation with  $\text{KMnO}_4$  yielded a glycol, m.p. 117°, identical with the oxidation product of aromadendrene. Another portion of this fraction was treated with nitrosyl chloride to yield a nitrosochloride, m.p. 112-112.5°; treatment of this product with benzylamine yielded the *nitrobenzylamine* derivative, m.p. 139.5° (Found C, 77.6; H, 9.5; N, 8.2.  $\text{C}_{22}\text{H}_{32}\text{N}_2\text{O}$  requires C, 77.5; H, 9.5; N, 8.1%). The infrared spectrum of a gas chromatography fraction was identical with that of aromadendrene.

(vii)  *$\alpha$ - and  $\beta$ -Eudesmol*.—The solid residue (9% of the crude oil) was recrystallized from aqueous alcohol to yield a solid, m.p. 80-81°,  $\alpha_D^{20}$  +30° (c, 10; ethanol). A portion of the recrystallized solid was resolved by gas chromatography into two components which were shown by their respective retention times and their infrared spectra to be the isomeric  $\alpha$ - and  $\beta$ -eudesmols, with the  $\beta$ -isomer being the major component.

(b) *Oil of E. kitsoniana*

*Isolation of Essential Oil.*—Leaves and terminal branchlets (9.2 kg) collected at Foster, Vic., on steam distillation yielded 206 g (2.1%) of oil having  $d_{4}^{25}$  0.9118;  $n_D^{20}$  1.4706;  $\alpha_D + 2.2^\circ$ ; acid number 2; ester number 3.5, after acetylation 36.5. Foliage distilled from 10 trees of an open-pollinated progeny of 50 trees raised from the Foster trees grown at Castle Hill, N.S.W., yielded an oil having  $d_{4}^{25}$  0.9150;  $n_D^{20}$  1.4715;  $\alpha_D + 1.04^\circ$ , apparent cineole content 56% (*o*-cresol method); soluble in 2 vols. 70% w/w ethanol.

*Identification of Constituents.*—The crude oil from the Foster material was fractionated through a Widmer-type column under 10 mm of pressure and the following compounds identified in the fractions obtained:

(i) (+)-*a*-Pinene.—A fraction (b.p. 36–41°/10 mm;  $d_{4}^{25}$  0.8612;  $n_D^{20}$  1.4670;  $\alpha_D + 32^\circ$ ) on treatment with nitrosyl chloride yielded the nitrosochloride of (+)-*a*-pinene, m.p. and mixed m.p. 107°. The infrared spectrum of a gas chromatography fraction was identical with that of *a*-pinene.

(ii) (±)-*Limonene*.—A fraction (b.p. 52–56°/10 mm;  $d_{4}^{25}$  0.8602;  $n_D^{20}$  1.4766;  $\alpha_D + 8^\circ$ ) on treatment with bromine yielded the tetrabromide of (±)-limonene, m.p. and mixed m.p. 124.5°.

(iii) 1,8-Cineole.—The lower boiling fractions (b.p. 60°/10 mm) were extracted with 50% aqueous resorcinol solution to yield cineole which was identified by the preparation of the *o*-cresol addition compound, m.p. and mixed m.p. 56°.

(iv) *Aromadendrene*.—A fraction (b.p. 110–124°/10 mm;  $d_{4}^{25}$  0.9344;  $n_D^{20}$  1.4947;  $\alpha_D - 1.3^\circ$ ) on treatment with nitrosyl chloride yielded a nitrosochloride, m.p. 124°, which when treated with piperidine yielded a *nitrolpiperidide*, m.p. 132–133° (Found: C, 74.8; H, 10.7; O, 8.7.  $C_{26}H_{38}N_2O$  requires C, 75.2; H, 11.0; O, 8.8%). The product was identified by infrared analysis and mixed melting point determination with that from aromadendrene.

(v) *Sesquiterpene Alcohols*.—A fraction (b.p. 101–103°/3 mm;  $d_{4}^{25}$  0.9782;  $n_D^{20}$  1.5009;  $\alpha_D - 18.6^\circ$ ) showed by its infrared spectrum the presence of hydroxyl. Dehydration with 98% formic acid gave a product having  $d_{4}^{25}$  0.9183;  $n_D^{20}$  1.5032;  $\alpha_D - 4.8^\circ$ . Gas chromatography resolved the fraction into two major components, probably sesquiterpene alcohols, which were not further investigated.

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