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VARIATION IN THE LEAF OIL OF *EUCALYPTUS PUNCTATA**

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Abstract The steam-volatile leaf oil of *Eucalyptus punctata*, ssp. *punctata* has been shown to contain α-pinene, β-pinene, 1,8-cineole, *p*-cymene and cryptone. Several minor components have been tentatively identified by GLC. The wide variation in the leaf oil composition between individual trees indicated no grounds for the establishment of chemical varieties within the subspecies.

INTRODUCTION

GREY GUM (*Eucalyptus punctata* DC.) leaf is well known in the Sydney area as suitable fodder for the Australian koala, *Phascolarctos cinereus* Goldfuss.[1a] Our investigations of the essential oil metabolites of this endemic marsupial necessitated a thorough re-examination of the leaf oil of *E. punctata* ssp. *punctata*.†

Baker established *E. punctata* var. *didyma* principally on chemical grounds although slender morphological evidence,[2] which was not acknowledged by either Maiden[3] or Blakely,[4] was also cited. The purpose of the division was chiefly economic as the var. *didyma* trees examined yielded a smaller quantity of oil which contained only 10–15% cineole as against 46–64% in the type trees examined.

The presence of aldehydes ('aromadendral') in var. *didyma* and α-pinene in both varieties was also reported.[1b,2] Pryor and Johnson[5] propose four divisions of *E. punctata*: ssp. *punctata*; ssp. *didyma*; ssp. *canaliculata* (*E. canaliculata*) and ssp. *longirostrata* (*E. punctata* var. *longirostrata*). In an attempt to clarify the chemical variation within *E. punctata* we now present the chemical composition of the essential oils from several trees of *E. punctata* ssp. *punctata*.

RESULTS AND DISCUSSION

Forty-five trees growing near Sydney, N.S.W. were examined. Some of these were native to the area and others were grown from seed collected near Mudgee, N.S.W. GLC retention

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* Part VII in the series *The Volatile Oils of the Genus Eucalyptus* (Family Myrtaceae). For Part VI see LASSAK, E. V. and SOUTHWELL, I. A. (1969) *Phytochemistry* **8**, 667.

† Subspecies names used are those currently proposed (see Ref. 5) with formal publication to follow.

[1] PENFOLD, A. R. and WILLIS J. L., (1961) *The Eucalypts*, (a) p. 326, (b) p. 275, Leonard Hill, London.

[2] (a) BAKER R. T. and SMITH H. G. (1902) *Research on the Eucalypts*, 1st Edn, pp. 81, 127, Government Printer, Sydney; (b) *idem.* (1920) 2nd Edn, pp. 179, 209.

[3] MAIDEN, J. H. (1917) *A Critical Revision of the Genus Eucalyptus*, Vol. 3, p. 194, Government Printer, Sydney.

[4] BLAKELY, W. F. (1934) *A Key to the Eucalypts*, p. 101, The Worker Trustees, Sydney.

[5] PRYOR, L. D. and JOHNSON, L. A. S. (1971) *A Classification of the Eucalypts*, pp. 43, 87, Australian National University, Canberra.

times and coinjection with authentic samples on two high resolution columns were used to identify 15 individual components. The identity of the four major components and the most prominent minor component was confirmed by IR spectroscopy. Percentages were calculated by triangulation and are shown in Table 1. Traces (each <0·5%) of 10 other components remained unidentified.

TABLE 1. VARIATION IN THE PERCENTAGE COMPOSITION OF *Eucalyptus punctata* ssp. *punctata* LEAF OILS

Peak No.	Compound	Mean	Range	Standard deviation	Coefficient of variation
1	α-Pinene	18·1	5-48	7·8	0·43
2	β-Pinene	17·4	tr-39	10·4	0·60
3	C_{10} Hydrocarbon	0·7	tr-2	0·7	0·98
4	Myrcene	1·3	tr-17	2·8	2·1
5	α-Phellandrene	0·2	tr-4	0·7	3·6
6	Limonene	2·6	1-6	1·2	0·46
7	1,8-Cineole	21·0	1-70	15·6	0·74
8	γ-Terpinene	1·9	tr-19	4·3	2·37
9	*p*-Cymene	15·7	1-34	7·7	0·49
13	Menthyl acetate	1·3	tr-5	1·3	1·04
15	Terpinen-4-ol	1·1	tr-3	0·8	0·72
17	C_{10} carbonyl	1·5	tr-7	1·6	1·06
18	C_{10} alcohol	2·5	tr-14	2·7	1·10
19	Cryptone	4·2	tr-13	3·0	0·71
20	α-Terpineol	1·5	tr-4	0·9	0·63
23	Phellandral	1·1	tr-4	1·0	0·91
24	Cuminal	2·4	tr-6	1·6	0·67
26	Geraniol	0·6	tr-2	0·7	1·17
27	Higher boiling unknown	0·6	tr-3	0·8	1·30
29	Higher boiling unknown	1·6	tr-6	1·5	0·89

Tr—trace, i.e < 0·5%

Elution from a silica gel column separated the oil into hydrocarbon, cineole and other oxygenated component fractions. Isolation of the carbonyl fraction of the oil was achieved by regeneration from the bisulphite adduct. Cineole content was confirmed by the *o*-cresol adduct method where sufficient oil was available.

The four major components α-pinene, β-pinene, cineole, and *p*-cymene constitute on average 72%, and cryptone, 4% of the oil. Variation of these individual components was considerable but so random that division into varieties based on cineole or cryptone content was not warranted. These variations could not be correlated with seasonal, geographical, soil-type or tree-age variations. In particular many oils contained cineole in proportions intermediate to Baker's divisions.[2] Thus Baker's var. *didyma* appears to have included low-cineole variants of Pryor and Johnson's ssp. *punctata* as well as their ssp. *didyma* which is distinguished on morphological grounds.

Amplification of GLC signals from oils which at first appeared devoid of some components showed that variation was quantitative rather than qualitative. This finding is consistent with the quantitative variation within *Eucalyptus dives* where the oils divide the species into four distinct chemical varieties.[6]

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[6] HELLYER, R. O., LASSAK, E. V., MCKERN, H. H. G. and WILLIS, J. L. (1969) *Phytochemistry* **8**, 1513.

EXPERIMENTAL

Sample collection. A total of 71 samples of leaf were collected in autumn from 32 8-year-old trees cultivated at Castle Hill, N.S.W. (Museum of Applied Arts and Sciences Herbarium Nos. 72–103 to 106 and 146–153) from seed collected 250 km to the north-west near Mudgee, and in spring from 13 mature native trees growing within 80 km of Castle Hill at Berowra (No. 72–155), Beacon Hill (Nos. 72–158, 172, 173), Castle Hill (No. 72–162), Kariong (No. 72–174), Patonga (72–177), Yarramundi (Nos. 72–180 to 182) and Bilpin (Nos. 72–183 to 185) on both sandstone and shale soils.

Isolation of volatile material. 400–500 g fr. wt, leaf only samples were steam-distilled with cohobation in all-glass apparatus[7] for 7 hr. Yields and physical constants of volatile oils from different trees varied as shown in Table 2.

TABLE 2. VARIATION IN YIELD AND PHYSICAL CONSTANTS OF *E. punctata* ssp. *punctata* LEAF OILS

	Yield	n_D^{20}	$\alpha_D{}^0$	d_4^{20}
Mean	1·3	1·484	−16·7	0·910
Range	0·2–2·3	1·465–1·491	−26·0–+2·8	0·893–0·931
Standard deviation	0·6	0 005	7·0	0·011
Coefficient of variation	0·43	0·003	0·42	0·012

Identification of constituents. GLC. The volatile oil components were analysed using a model 900 Perkin Elmer instrument with high resolution F.F.A.P. and Castorwax support coated open tubular (SCOT) columns (15 m × 0·5 mm i d.). The components recorded in Table 1 were identified by their retention times and co-injection with authentic samples on both columns. *Chemical separation.* Several oil samples (1·0 ml) were eluted from a silica gel (60 g) column with petrol (40–60°, 250 ml), Et_2O–petrol (1:9, 250 ml) and Et_2O (250 ml). IR spectroscopy showed some of the hydrocarbon fractions to be principally α-and β-pinene, others principally *p*-cymene or a sesquiterpene hydrocarbon or a mixture of these. The Et_2O–petrol. eluted cineole (IR) and the Et_2O, cryptone (IR) and other carbonyl and alcohol components. Regeneration of the bisulphite shakings of several oils by the method of Berry[8] revealed four carbonyl components. The major component, cryptone, was identified by IR spectroscopy and GLC, and phellandral and cuminal by GLC retention times and coinjection with authentic material. Cineole contents were confirmed chemically by the *o*-cresol method of Cocking.[9]

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[7] HUGHES, A. (1970) *Chem. Ind.* (*London*) **48,** 1536.

[8] BERRY, P. A. (1947) *Australian Chem. Inst. J. Proc.* **14,** 176.

[9] COCKING, T. T. (1927) *Analyst* **52,** 276.