

## VOLATILE LEAF OILS OF *EUCALYPTUS* *DELEGATENSIS* SEEDLINGS

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**Abstract**—A study of the volatile leaf oils of *Eucalyptus delegatensis* seedlings with respect to the geographic provenance of the parent seed has shown that oils of mainland Australian origin contained significantly higher proportions of 4-phenylbutan-2-one than oils of Tasmanian origin.

*Eucalyptus delegatensis* R. T. Baker is one of Australia's commercially most important hardwood species. It is a large, relatively fast growing and cold tolerant tree extending from the highlands of southern New South Wales to Victoria and also to the Australian island state of Tasmania. Whilst the considerable body of published work on the volatile leaf oils of the genus *Eucalyptus* deals exclusively with adult trees, seedling oils have, to our knowledge, not been previously investigated.

The foliage of seedlings of *E. delegatensis*, raised from seed obtained from 65 different sites (Fig. 1), 41 from mainland Australia and 24 from Tasmania (precise location details of all sites are given elsewhere[1]), was steam-distilled and the oils analysed by GC/MS. The gas chromatograms indicated some 64 constituents (Table 1). Whilst all oils were qualitatively very similar, quantitative variations were often large, as for example in the cases of  $\alpha$ -phellandrene,  $\beta$ -phellandrene, *p*-cymene, *trans*-piperitol, methyl cinnamate and a compound  $C_{10}H_{12}O$  (MW 148) identified as 4-phenylbutan-2-one by its mass spectrum and the  $^1H$  NMR spectrum of its 2, 4-DNP derivative. The terminal methyl of the 4-phenylbutan-2-one showed up in the NMR spectrum of its 2,4-DNP derivative as two sharp singlets of unequal size totalling 3H, owing to an equilibrium of syn- and anti-isomers in solution. The syn-anti ratio observed, 84/16, was in good agreement with the ratio of 85/15 reported previously in nitrobenzene solution[2].

Furthermore, the chemical composition of the oils appeared to be fairly independent of their geographical origin. The only significant exception was 4-phenylbutan-2-one which showed statistically significant differences between the mainland and

Tasmania. Its respective range (%), mean content (%) and coefficient of variation were 1.1–18.1, 6.8, 73.1 and trace –0.8, 0.2, 128.6.

Oil yields were in all cases, irrespective of geographical origin, very low ranging from less than 0.01% to a maximum of 0.12% (mean 0.04%; s.d.  $\pm 0.02\%$ ) and were considerably less than the values reported for adult foliage (1.3–1.9%)[3]. Previous workers[3] identified only 1- $\alpha$ -phellandrene (main component) together with small amounts of piperitone and 1,8-cineole in adult leaf oil.

### EXPERIMENTAL

**Plant material and isolation of volatile oils.** Seedlings were grown for 19 weeks in a glasshouse with a day/night temp. regime of 24/19°. There were 25 seedlings per site, made up to five seedlings from each of five parent trees. Fresh foliage (50–200 g depending on sample; since some seedlings, less than 5% overall, died during the trial the number of seedlings harvested was in some cases slightly less than 25 per sample) was steam-distilled using the all-glass semi-micro apparatus of Franklin and Keyzer[4]. In all cases ca 0.5 ml Na-dried  $Et_2O$  was added to the receiver in order to facilitate the complete recovery of the very small vols of oils obtained.

**Identification of oil constituents.** Individual seedling oil constituents were identified by co-GC with authentic specimens and by GC/MS as described elsewhere[5].

**4-Phenylbutan-2-one.** The MS of peak no. 43 ( $M^+$ :  $m/z$  148; base peak:  $m/z$  105) was identical with a published spectrum of 4-phenylbutan-2-one[6]. The combined oils of mainland provenance were treated with 2,4-DNP in the usual way to yield orange-red crystals (from MeOH), mp 127.5–129.5° (lit.[7] 131–132°).  $^1H$  NMR of the 2,4-DNP derivative determined after 15 min equilibration at 20° (100 MHz,  $CDCl_3$ , TMS int. standard):  $\delta$  7.20 (5H, *m*,  $C_6H_5$ ), 2.85 and 2.80 (2H each, *m*'s  $-CH_2-CH_2-$ ), 2.05 and 1.47 (3H, *s*'s  $Me-C=N-$ , % ratio syn-anti: 84/16).

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Table 1. Percentage composition of *E. delegatensis* seedling leaf oils

Peak no.	Compound	Range (%)	Mean (%)	Coefficient of variation
1	3-Methylbutanal	tr-6.2	1.2	115
2	$\alpha$ -Pinene	tr-2.9	1.0	73
3	$\beta$ -Pinene	tr-1.5	0.3	104
4	Myrcene	tr-1.6	0.9	48
5	$\alpha$ -Phellandrene	0.2-26.9	14.3	53
6	$\alpha$ -Terpinene	tr-4.7	2.3	58
7	Limonene	tr-2.8	0.7	75
8	$\beta$ -Phellandrene	1.2-18.7	8.9	45
9	1,8-Cineole	0-0.5	0.1	130
10	$\gamma$ -Terpinene	tr-7.6	3.6	47
11	<i>p</i> -Cymene	0.8-10.0	4.4	53
12	Terpinolene	tr-3.3	1.9	44
13	un	0-0.7	0.1	146
14	un	0-1.5	0.2	147
15	un	0-0.9	0.2	128
16	un	0.1.0	0.3	85
17	un	0-0.8	0.1	117
18	Linalool	0-1.8	0.2	148
19	un	0-0.7	0.1	136
20	un	0-0.6	0.2	133
21	un	0-0.6	0.1	130
22	Citronellal	0-0.9	0.1	162
23	un	tr-1.0	0.3	73
24	un	0.2-2.2	0.9	55
25	<i>trans-p</i> -Menth-2-en-1-ol	0.1-3.2	1.4	44
26	Terpinen-4-ol	0.3-3.7	1.7	42
27	<i>cis-p</i> -Menth-2-en-1-ol	0.4-4.0	1.5	49
28	$\beta$ -Caryophyllene	tr-3.8	0.9	79
29	C <sub>10</sub> H <sub>18</sub> O (alcohol)	0-2.8	0.5	126
30	<i>cis</i> -Piperitol	0.1-4.4	1.2	70
31	un	0-1.7	0.8	67
32	$\alpha$ -Terpineol	tr-3.4	1.1	58
33	$\alpha$ -Terpinyl acetate	0-2.3	0.3	182
34	Piperitone	tr-3.9	1.4	60
35	<i>trans</i> -Piperitol	1.3-23.9	7.6	60
36	Aromadendrene	tr-4.2	1.4	78
37	un	0-6.5	0.7	131
38	un	0-3.9	0.3	254
39	un	0-3.0	0.4	190
40	Cuminal	0-5.0	1.0	105
41	C <sub>10</sub> H <sub>14</sub> O (alcohol)	0-1.6	0.2	137
42	C <sub>10</sub> H <sub>14</sub> O (alcohol)	0-0.7	0.1	131
43	4-Phenylbutan-2-one	tr-18.1	4.6	111
44	un	0-1.5	0.2	142
45	un	0-1.2	0.2	125
46	un	0-1.6	0.2	178
47	un	0.1-13.9	3.5	94
48	un	0-3.8	0.8	123
49	un	0.1-7.2	1.8	107
50	un	0-1.2	0.2	126
51	un	tr-2.5	0.5	117
52	un	0.2-3.3	1.2	74
53	Methyl cinnamate	1.2-21.3	8.5	53
54	un	0-4.0	1.2	93
55	C <sub>15</sub> H <sub>26</sub> O (alcohol)	tr-5.4	1.3	82
56	C <sub>15</sub> H <sub>24</sub> O (alcohol)	0.8-11.0	4.2	65
57	un	0-2.3	0.4	219
58	un	0-4.2	0.5	150
59	un	0-4.4	0.7	147
60	$\gamma$ -Eudesmol	0-3.8	0.3	262
61	$\alpha$ -Eudesmol	0-4.0	1.0	92
62	$\beta$ -Eudesmol	0-3.4	0.6	137
63	un	0-12.0	0.7	227
64	un	0-6.6	0.5	247

tr, &lt; 0.1%; un, unknown.

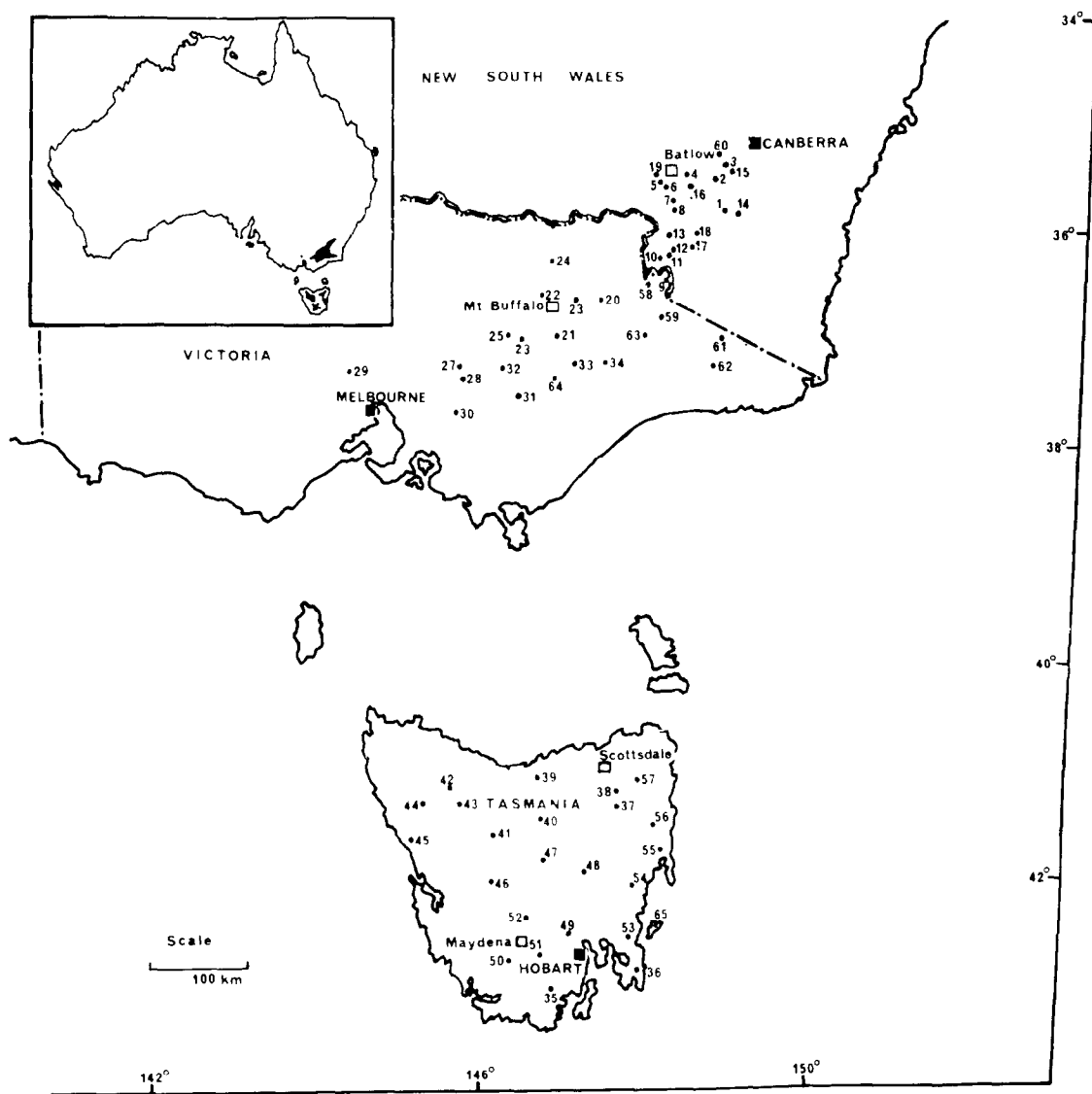


Fig. 1. *E. delegatensis* seed collection sites (insert shows the overall distribution of the species).

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