

Volatile Leaf Oils of some South-western and Southern Australian Species of the Genus *Eucalyptus* (Series I). Part XIII. (Series I). (a) Series Subulatae, (b) Series Curviptera, (c) Series Contortae, (d) Series Incognitae, (e) Series Terminaliptera, (f) Series Inclusae, (g) Series Microcorythae and (h) Series Cornutae

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The volatile leaf oils of *Eucalyptus* 'Species R', *E.* 'Species Q', *E. socialis* F. Muell. ex Miq., *E.* 'Species O', *E. wyolensis* Boomsma, *E. glomerosa* Brooker & Hopper, *E. leptopoda* Benth. subsp. *elevata* K. D. Hill & L. A. S. Johnson, *E. effusa* Brooker subsp. *effusa*, *E. effusa* Brooker subsp. *exsul* L. A. S. Johnson & K. D. Hill, *E. cosmophylla* F. Muell., *E. longifolia* Link, *E. paludicola* D. Nicolle, *E. calophylla* R. Br. ex Lindley, *E. haematoxylon* Maiden, *E. ficifolia* F. Muell., *E. diversicolor* F. Muell., *E. cladocalyx* F. Muell. and *E. gomphocephala* DC., isolated by vacuum distillation, were analysed by GC and by GC-MS. Many species contained α -pinene (0.2-31.1%), β -pinene (0-12.5%), 1,8-cineole (0.2-76.8%), *p*-cymene (0-20.8%), aromadendrene (0-13.6%), bicyclogermacrene (0-43.4%) and spathulenol (0.1-15.2%) as principal leaf oil components: all species contained torquatone.

KEY WORDS: *Eucalyptus* 'Species R'; *Eucalyptus* 'Species Q'; *Eucalyptus socialis* F. Muell. ex Miq.; *Eucalyptus* 'Species O'; *Eucalyptus wyolensis* Boomsma; *Eucalyptus glomerosa* Brooker & Hopper; *Eucalyptus leptopoda* Benth. subsp. *elevata* K. D. Hill & L. A. S. Johnson; *Eucalyptus effusa* Brooker subsp. *effusa*; *Eucalyptus effusa* Brooker subsp. *exsul* L. A. S. Johnson & K. D. Hill; *Eucalyptus cosmophylla* F. Muell.; *Eucalyptus longifolia* Link; *Eucalyptus paludicola* D. Nicolle; *Eucalyptus calophylla* R. Br. ex Lindley; *Eucalyptus haematoxylon* Maiden; *Eucalyptus ficifolia* F. Muell.; *Eucalyptus diversicolor* F. Muell.; *Eucalyptus cladocalyx* F. Muell.; *Eucalyptus gomphocephala* DC.; Myrtaceae; leaf essential oil composition; torquatone; mono- and sesquiterpenoids; GC-MS

INTRODUCTION

Continuing our investigation of indigenous Australian eucalypts¹ we have examined the leaf oils of eighteen species; five from Series Subulatae Blakely; two from Series Curviptera Maiden; two

from Series Contortae Blakely; three from Series Incognitae Nicolle; three from Series Terminaliptera Maiden; and one each from Series Inclusae (Benth.) Chippendale, Series Microcorythae Blakely, and Series Cornutae Blakely (see classification system, *Flora of Australia*²).

Locations of all species which are native to south-western and southern Australia, are listed in Table 1. A more detailed description of the occurrence of these eucalypts has been given elsewhere.^{2,3}

To our knowledge, analyses of the oils of *E. cosmophylla*,⁴ *E. calophylla*,⁴ *E. longifolia*,^{4,5,6} *E. ficifolia*,^{7,8} *E. diversicolor*,^{4,6,9} *E. cladocalyx*,^{4,6} *E. gomphocephala*^{4,6,10} and *E. leptopoda*¹¹ have been published previously.

EXPERIMENTAL

Samples of clean, mature leaves were picked from single trees and, after freezing with liquid nitrogen, were reduced to a fine powder using a stainless steel Waring Blender (Model No. SS110). This procedure was necessary to rupture the oil glands. The dry powder was then vacuum-distilled so that the leaf oil condensed on to a gold-plated copper rod maintained at approximately -75°C . Complete details of this procedure have been published previously.¹² All oils obtained were colourless to pale yellow and lighter than water. Table 1 lists the oil yields (wt%, dry weight) for the 20 species studied.

Analytical gas chromatography (GC) was carried out on a Shimadzu GC6 AMP gas chromatograph. A glass SCOT column of SP1000 (85 m \times 0.5 mm) which was programmed from 65°C to 225°C at $3^{\circ}\text{C}/\text{min}$ was used with helium carrier gas. The GC integrations of the peaks were performed on a SMAD electronic integrator. GC analyses were also performed with a HP5890 Series II unit operated in conjunction with a HP3396 Series II integrator. The 'on-column' injection technique was used with a SGE BP20 capillary column of 25 m \times 0.33 mm i.d., and film thickness 0.5 μm . The carrier gas was hydrogen with an inlet pressure of 25 kPa; the flow rate was 2.0 cm^3/min . The oven was programmed to rise from 80°C to 220°C at $5^{\circ}\text{C}/\text{min}$, and the inlet temperature set to 83°C and increased at the same rate as the column. Using these conditions and a 1.0 μl sample of 0.4% solution of oil in purified dry diethyl ether, essentially all the components were recorded by the integrator in 31 minutes. GC-MS was performed on a VG Quattro mass spectrometer operating at 70 eV ionization energy. The GC column in this case was a DB-Wax (60 m \times 0.32 mm). Compounds were identified by their GC retention indices to known

compounds and by comparison of their mass spectra either with known compounds or published spectra.¹³⁻¹⁵

Only five of the species (*E.* 'Species R', *E. socialis*, *E.* 'Species O', *E. haematoxylon* and *E. gomphocephala*) were analysed with GC-MS. The oil components of the rest were identified using normalized retention times. For this purpose the column was calibrated by assuming times for three markers, 1,8-cineole, octadecane (OD, added to the ether) and torquatone. The raw retention times were first normalized to 525 s for OD, and times before and after OD adjusted by assuming linearity and using 99 s for cineole and 997 s for torquatone. Torquatone was found to be present in all oil samples; when this was not the case a sufficient amount was added to the oil solution to obtain that reference point. The normalized retention times of the column were identified with oil components analysed previously by GC-MS for over 75 *Eucalyptus* species: some of these results have been published.¹

All GC analyses were performed in duplicate and the retention times and percentage compositions of each component averaged. Duplicate times were discarded if they differed by more than 1 second. Components which contributed less than 0.06% to the final analyses were not considered (an arbitrary but practical decision).

RESULTS AND DISCUSSION

Freshly isolated oils obtained by vacuum distillation of powdered leaves from *single* trees were analysed by GC and by GC-MS. The results for the 18 species are listed in Table 2; only those components with concentrations greater than 0.05% are reported. The principal components in the oils were the monoterpenes α -pinene (0.2-31.1%), β -pinene (0-12.5%), limonene (0-2.2%), 1,8-cineole (0.2-76.8%) and *p*-cymene (0-20.8%). Apart from 1,8-cineole, the *main* oxygenated monoterpenes detected were *trans*-pinocarveol (0-17.9%), α -terpineol (0-10.5%) and pinocaryone (0-6.2%).

The principal sesquiterpenes encountered in these species were aromadendrene (0-13.6%), *allo*-aromadendrene (0-2.8%) and bicyclogermacrene (0-43.4%), and the related alcohols, globulol (0.2-6.6%), viridiflorol (0.2-5.6%) and spathulenol (0.1-15.2%), as well as γ -eudesmol (0-2.4%), α -eudesmol (0-2.0%) and β -eudesmol

Table 1. Oil yields from several *Eucalyptus* species: Series (a) Subulatae, (b) Curviptera, (c) Contortae, (d) Incognitae, (e) Terminaliptera, (f) Inclusae, (g) Microcorythae, (h) Cornutae

Species and locality ^a	Oil yield (wt%, dry weight)
Series Subulatae Blakely	
<i>Eucalyptus</i> 'Species R' ^{b,c}	3.11
S 27° 31' 42"/E 114° 36' 21"	
<i>E.</i> 'Species Q' ^{b,c}	3.03
S 34° 23' 44"/E 118° 44' 13"	
<i>E. socialis</i> F. Muell, ex. Miq. ^b	2.20
Roseworthy, South Australia	
<i>E.</i> 'Species O' ^{b,c}	1.88
S 28° 06' 04"/E 124° 12' 45"	
<i>E. wyolensis</i> Boomsma ^b	1.36
S 29° 20' 07"/E 130° 13' 15"	
Series Curviptera Maiden	
<i>E. glomerosa</i> Brooker & Hopper ^b	2.19
S 28° 52' 56"/E 130° 27' 43"	
<i>E. leptopoda</i> Benth. subsp. <i>elevata</i> K.D. Hill & L.A.S. Johnson ^b	2.07
S 28° 29' 20"/E 128° 11' 03"	
Series Contortae Blakely	
<i>E. effusa</i> Brooker subsp. <i>effusa</i>	0.24
Currency Creek Arboretum, South Australia	
<i>E. effusa</i> Brooker subsp. <i>exsul</i> L.A.S. Johnson & K.D. Hill	1.28
S 28° 38' 15"/E 118° 47' 40"	
Series Incognitae D. Nicolle	
<i>E. cosmophylla</i> F. Muell. ^b	0.22
Fleurieu Peninsula, South Australia	
<i>E. longifolia</i> Link	0.58
Waite Arboretum, South Australia	
<i>E. paludicola</i> D. Nicolle ^b	0.20
S 35° 22'/E 148° 40'	
Series Terminaliptera Maiden	
<i>E. calophylla</i> R. Br. ex Lindley	tr
Waite Arboretum, South Australia	
<i>E. haematoxylon</i> Maiden	0.15
Highgate, South Australia	
<i>E. ficifolia</i> F. Muell.	tr
Willunga Hill, South Australia	
Series Inclusae (Benth.) Chippendale	
<i>E. diversicolor</i> F. Muell. ^{TR}	tr
Currency Creek Arboretum, South Australia	
Series Microcorythae Blakely	
<i>E. cladocalyx</i> F. Muell.	tr
Waite Arboretum, South Australia	
Series Cornutae Blakely	
<i>E. gomphocephala</i> DC.	tr
Waite Arboretum, South Australia	

^aThe specimens for these species were authenticated by Mr M.I.H. Brooker, Australian National Herbarium, or Dean Nicolle, Currency Creek Arboretum, South Australia.

^bBotanical voucher specimens have been deposited at the South Australian Herbarium by Dean Nicolle, who collected the leaves for these species.

^cFor a description of these three species, see reference 3.

Table 2. (Continued)

Series	<i>E.</i> 'Species R'	<i>E.</i> 'Species Q'	<i>E. socialis</i> F. Muell, ex Miq.	<i>E.</i> 'Species O'	<i>E. wyolensis</i> Boomsma	<i>E. glomerosa</i> Brooker & Hopper	<i>E. leptopoda</i> Benth. subsp. <i>elevata</i> K.D. Hill & L.A.S. Johnson	<i>E. effusa</i> Brooker subsp. <i>effusa</i>	<i>E. effusa</i> Brooker subsp. <i>exsul</i> L.A.S. Johnson & K.D. Hill	<i>E. cosmophylla</i> F. Muell.	<i>E. longifolia</i> Link	<i>E. paludicola</i> D. Nicolle
	Subulatae					Curviptera		Contortae		Incognitae		
86 γ -Eudesmol	-	0.06	-	0.47	0.15	-	-	0.07	-	0.07	-	-
87 T-Muurolol	0.09	-	-	-	-	-	-	-	-	-	-	-
88 C ₁₅ H ₂₀ O	-	2.81	-	-	-	-	-	0.27	-	-	-	0.11
89 C ₁₅ H ₂₆ O	-	0.71	-	-	-	0.10	0.14	-	-	0.28	0.19	0.24
90 α -Eudesmol	0.15	0.38	-	1.11	0.57	0.10	0.10	0.40	0.13	0.49	1.31	0.64
91 β -Eudesmol	0.89	0.57	-	3.71	0.32	0.73	0.36	0.42	0.08	0.51	0.45	0.73
92 C ₁₅ H ₂₆ O	-	0.24	-	-	-	-	-	0.11	-	-	-	-
93 C ₁₅ H ₂₆ O	0.12	0.27	-	0.09	0.52	0.09	0.07	0.21	0.06	0.11	0.19	0.54
94 Farnesyl acetate	-	-	0.07	-	-	-	-	-	-	-	-	-
95 Component A ^a	-	-	-	0.19	-	-	-	-	-	-	-	0.30
96 Isobicyclogermacral	-	-	0.23	-	-	-	-	-	-	0.18	-	-
97 <i>E,E</i> -Farnesol	-	-	0.16	-	-	-	-	-	-	-	-	-
98 Component B ^a	-	-	-	0.13	-	-	-	0.06	-	-	-	-
99 Torquatone	tr.	tr.	0.15	2.60	0.29	0.43	0.39	0.63	0.17	0.39	0.30	0.31
Total Percentages	96.1	83.8	81.5	94.2	84.2	94.7	95.1	86.9	96.4	95.2	88.7	97.7

^aSee Part IX¹⁸ of this series for tentative structures.

Series	<i>E. calophylla</i> R. Br. ex Lindley	<i>E. haematoxylon</i> Maiden	<i>E. ficifolia</i> F. Muell.	<i>E. diversicolor</i> F. Muell.	<i>E. cladocalyx</i> F. Muell.	<i>E. gomphocephala</i> DC.
	Terminaliptera			Inclusae	Microcorythae	Cornutae
1 α -Pinene	9.49	17.28	1.03	5.31	0.16	0.69
2 α -Fenchene	-	-	-	-	-	-
3 Camphene	-	-	-	0.12	-	0.08
4 β -Pinene	-	-	1.79	0.06	-	-
5 Sabinene	-	0.19	-	-	-	-
6 Myrcene	-	-	0.79	-	-	-
7 α -Phellandrene	-	-	-	-	-	-
8 Isobutyl isovalerate	-	0.13	-	-	-	-
9 Limonene	1.36	1.19	0.41	1.10	-	0.78
10 1,8-Cineole	0.81	0.54	0.17	17.99	0.79	1.53

Table 2. (Continued)

Series	<i>Eucalyptus</i> species					
	<i>E. calophylla</i> R. Br. ex Lindley	<i>E. haematocylon</i> Maiden	<i>E. ficifolia</i> F. Muell.	<i>E. diversicolor</i> F. Muell.	<i>E. cladocalyx</i> F. Muell.	<i>E. gomphocephala</i> DC.
	Terminaliptera			Inclusae	Microcorythae	Cornutae
11 γ -Terpinene	12.14	16.37	-	-	-	0.07
12 β - <i>trans</i> -Ocimene	-	-	-	-	-	-
13 <i>p</i> -Cymene	1.42	1.27	-	0.74	5.30	0.47
14 Terpinolene	0.27	0.46	-	-	-	0.47
15 Isoamyl isovalerate	-	-	-	0.57	-	0.14
16 α , <i>p</i> -Dimethylstyrene	-	-	-	0.11	-	-
17 δ -Elemene	-	-	0.06	0.08	-	0.25
18 α -Cubebene	0.17	0.06	0.28	-	-	-
19 Bicycloelemene	0.06	-	0.09	-	-	-
20 α -Copaene	-	0.06	0.17	0.26	0.11	0.07
21 α -Campholenic aldehyde	0.09	-	-	0.09	0.15	0.40
22 α -Gurjunene	1.25	0.76	1.16	-	-	0.22
23 β -Cubebene	-	-	-	-	-	-
24 Linalol	-	-	-	-	0.07	-
25 <i>trans-p</i> -Menth-2-en-1-ol	-	-	-	-	-	-
26 Camphor	-	-	-	-	0.15	-
27 Pinocarvone	-	-	0.09	4.13	0.68	1.94
28 C ₁₀ acetate (C ₁₀ H ₁₈ O)	-	1.28	-	-	-	-
29 Fenchol	-	-	-	2.80	0.18	0.37
30 β -Elemene	-	-	0.17	-	0.17	-
31 β -Gurjunene	0.06	-	0.07	-	-	-
32 β -Caryophyllene	3.78	1.85	1.27	0.16	0.59	0.79
33 Terpinen-4-ol	1.30	1.38	1.00	0.07	0.48	-
34 Aromadendrene	3.26	1.16	6.19	0.26	1.62	2.83
35 α -Bulnesene	0.70	0.26	1.09	-	-	0.12
36 <i>cis-p</i> -Menth-2-en-1-ol	-	-	-	-	0.27	-
37 Myrtenal	-	-	0.32	0.09	0.25	-
38 <i>allo</i> -Aromadendrene	1.44	0.71	2.77	0.44	0.87	1.02
39 <i>trans</i> -Pinocarveol	-	0.09	0.77	17.86	1.55	7.25
40 Humulene	-	-	-	0.13	-	0.14
41 δ -Terpineol	-	-	-	-	-	-
42 <i>cis</i> -Piperitol	-	-	-	-	1.25	-
43 Cryptone	-	-	-	-	3.63	0.32
44 Neral	0.65	0.48	0.83	0.07	-	3.11
45 Viridiflorene	1.16	0.42	3.04	-	0.24	-
46 α -Terpineol	0.94	0.65	-	10.54	0.10	4.02
47 Borneol	0.11	-	0.09	3.46	-	0.60
48 Verbenone	-	-	-	-	0.50	-
49 β -Selinene	0.34	0.13	0.48	-	0.18	0.19
50 α -Selinene	0.74	0.29	0.88	-	0.10	-
51 A Muurolene	-	0.31	-	1.04	-	-
52 Piperitone	-	0.97	-	-	-	0.97
53 Bicyclogermacrene	2.33	-	42.78	0.21	3.84	-
54 Carvone	-	0.07	-	-	-	8.95
55 <i>trans</i> -Piperitol	0.19	0.08	-	-	0.16	-
56 δ -Cadinene	0.69	0.66	3.07	0.18	0.26	1.53

Table 2. (Continued)

Series	Terminaliptera	Inclusae	Microcorythae	Cornutae		
	<i>E. calophylla</i> R. Br. ex Lindley	<i>E. haematoxylon</i> Maiden	<i>E. ficifolia</i> F. Muell.	<i>E. diversicolor</i> F. Muell.	<i>E. cladocalyx</i> F. Muell.	<i>E. gomphocephala</i> DC.
57 γ -Cadinene	-	0.08	0.60	0.24	-	-
58 Cuminol	-	-	-	-	-	-
59 Myrtenol	-	-	-	0.38	0.31	-
60 Cadina-1,4-diene	0.18	0.06	0.07	-	-	-
61 <i>trans-p</i> -Mentha-1(7),8-dien-2-ol	-	-	-	1.65	0.28	-
62 Calamenene	-	-	0.50	0.12	0.18	-
63 <i>trans-p</i> -Mentha-1,8-dien-6-ol	-	-	-	0.65	0.50	0.53
64 <i>p</i> -Cymen-8-ol	0.18	-	0.07	0.28	1.78	1.51
65 <i>cis-p</i> -Mentha-1,8-dien-6-ol	-	-	-	0.18	-	-
66 <i>cis-p</i> -Mentha-1(7),8-dien-2-ol	-	-	-	2.09	0.15	0.40
67 Calacorene	-	-	-	0.12	-	0.14
68 Palustrol	0.20	0.10	0.25	0.08	0.73	1.53
69 Caryophyllene oxide	0.11	-	0.23	0.17	13.69	1.77
70 β -Phenylethyl propionate	-	0.20	0.07	0.20	0.41	0.13
71 C ₁₅ H ₂₆ O	0.15	-	0.10	0.13	0.37	0.19
72 C ₁₅ H ₂₆ O	0.32	0.18	0.34	-	0.41	0.95
73 C ₁₅ H ₂₆ O	0.28	0.21	0.33	0.12	1.13	0.93
74 C ₁₅ H ₂₆ O	1.10	0.52	-	-	0.27	0.38
75 C ₁₅ H ₂₆ O	0.19	0.40	-	0.07	0.30	0.40
76 C ₁₅ H ₂₆ O	-	0.07	0.39	-	-	-
77 C ₁₅ H ₂₆ O	-	-	-	-	0.52	-
78 Globulol	6.57	4.90	1.74	0.70	3.80	5.88
79 Viriflorol	0.49	0.21	0.79	0.31	1.49	1.50
80 Guaiol	0.14	0.14	0.77	0.08	0.21	0.15
81 C ₁₅ H ₂₆ O	0.42	0.17	0.32	-	1.72	0.87
82 C ₁₅ H ₂₆ O	0.49	0.16	0.56	0.21	0.47	1.14
83 Spathulenol	0.68	0.50	3.39	1.86	10.30	4.32
84 C ₁₅ H ₂₄ O	-	-	1.11	-	0.55	0.20
85 T-Cadinol	-	-	-	-	-	-
86 γ -Eudesmol	0.58	0.28	0.32	0.20	2.44	0.88
87 T-Muurolol	-	-	-	-	-	-
88 C ₁₅ H ₂₆ O	-	-	-	-	-	-
89 C ₁₅ H ₂₆ O	-	-	0.14	-	0.39	0.22
90 α -Eudesmol	1.39	1.11	1.07	0.44	1.86	1.99
91 β -Eudesmol	1.98	1.14	1.40	2.61	4.33	4.24
92 C ₁₅ H ₂₆ O	-	-	-	-	-	-
93 C ₁₅ H ₂₆ O	-	-	0.11	0.15	0.28	0.52
94 Farnesyl acetate	5.02	3.81	-	-	0.36	-
95 Component A ^a	0.23	0.24	0.10	0.25	-	0.27
96 Isobicyclogermacral	-	-	-	-	0.90	-
97 <i>E,E</i> -Farnesol	21.25	27.76	0.24	-	1.41	-
98 Component B ^a	-	-	0.10	0.18	-	0.75
99 Torquatone	1.04	2.24	2.00	1.85	5.75	6.35
Total Percentages	87.2	91.9	87.9	82.7	80.9	76.6

(0–4.3%). The aromatic ketone torquatone was detected (tr–6.4%) in all of the 18 species.

Our oil analyses agreed, when comparison was possible, with those of some previous workers^{4,7} but not with others:^{5,6,8–11} in general many more components were identified.

The appearance of 34% of benzaldehyde reported by Dellacassa *et al.*⁶ in the oil of *E. cladocalyx* indicates the problems of using hydrodistillation. The leaves of *E. cladocalyx* contain¹⁶ large amounts of a glycoside of the cyanohydrin of benzaldehyde which in aqueous solution hydrolyses to yield benzaldehyde, hydrogen cyanide and glucose. Somewhat similar problems were experienced by Koedam and Looman¹⁷ when distilling oil from the leaves of *Juniperus sabina*. In their study they were able to show that, depending on the pH, sabinene decomposed into mixtures of α -terpinene, γ -terpinene, terpinolene and terpinen-4-ol.

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