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THE VOLATILE LEAF OILS OF *EUCALYPTUS YOUMANII* AND *EUCALYPTUS MACRORHYNCHA.*

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Abstract

The compositions of the steam volatile leaf oils of two Eucalyptus *species,* E. youmanii *and* E. macrorhyncha *have been examined by G.L.C. and G.L.C.-M.S. techniques. Only small differences were found, thus adding chemotaxonomic support to the close botanical relationship postulated for these species. Of the approximately thirty compounds present in > 0.1% of the mixtures, twenty three have been identified. In each case 1,8-cineole and the three isomeric eudesmols were the major mono- and sesquiterpenoid components respectively.*

Introduction

Much scientific and commercial interest has been centred on the essential oils from various species of the genus *Eucalyptus* (family Myrtaceae). These oils are often exceedingly complex mixtures and it is only with the advent of G.L.C.-M.S. techniques that their detailed chemical compositions have been determinable.

As part of a continuing chemotaxonomic study of this genus[1,2,3,4], we have now examined the two species *E. youmanii* Blakely & McKie and *E. macrorhyncha* F. Muell. ex Benth. subsp. *macrorhyncha. E. youmanii* occurs as a medium sized tree,

characterised by its variably spreading habit and thick, markedly furrowed strong fibrous bark. It grows throughout the New England district of New South Wales, as far north as Stanthorpe in southern Queensland[5]. *E. macrorhyncha* occurs as a small to medium sized stringybark over a wide region of the temperate region of south-eastern Australia[5]. Although the foliage of these trees has been used extensively as a rich source of the pharmaceutically important flavonoid, rutin[6,7], only a cursory examination of the oil of *E. macrorhyncha* has been reported[8,9].

Both of these species belong to a group generally referred to as "stringybarks" ; stringybarks are also known to readily hybridise and hence lose genetic homogeneity. In the present investigation, the chemotaxonomic validity of the results is ensured since the leaf material used was from trees which were cultivated from seed, and whose progeny were assessed as being botanically pure.

Experimental

Analytical G.L.C. utilised either a Tracor 560 instrument fitted with a WCOT vitreous silica carbowax 20M column (27 m × 0.18 mm i.d.) or a Perkin Elmer 900 gas chromatograph fitted with a FFAP coated glass SCOT column (15 m × 0.5 mm i.d.). In the former case, nitrogen was used as carrier gas, and a temperature range of 80–210°, programmed at 6° per minute following an initial hold of 4 minutes, whilst for the latter, helium was the carrier gas with a temperature programme of 80–170° at 6° per minute with an initial hold of 3 minutes. The relative proportions of the components in the oils were determined with a Hewlett Packard 3370 A integrator.

G.L.C.-M.S. were determined using a Shimadzu GC6-AMP gas chromatograph equipped with a FFAP coated SCOT column (105 m × 0.77 mm i.d.) or OV 17 coated SCOT column (109 m × 0.77 mm i.d.) interfaced to an AEI MS-12 mass spectrometer through an all glass straight split with helium as carrier gas. The gas chromatograph was programmed from 70–230° at 3° per minute and the mass spectrometer operated at 70 eV with the ion source at 150°. Spectra were recorded and processed by a VG Digispec Display data system which produced standard bar graphs for direct comparison with published spectra.

Refractive indices were determined on an Otago refractometer and optical rotations on an Otago manual polarimeter. Density measurements were made as described in the literature[10].

Collection of plant material and isolation of volatile oils

Fresh leaves and terminal branchlets were obtained from genetically pure stands of *E. youmanii* and *E. macrorhyncha* at the Museum of Applied Arts and Sciences' Plantation, Castle Hill, Sydney. The *E. youmanii* was grown from seed collected at

Ebor in the New England district of N.S.W., and *E. macrorhyncha* from seed collected in the Ilford district of N.S.W. Since stringybarks are known to readily hybridise, in the present case, the genetic purity of the trees was verified by raising progeny and observing their morphological characteristics. A tree was assumed genetically pure when all of the progeny examined were homogeneous. The leaves were steam distilled in an all glass Dean and Stark apparatus modified to give lower phase return and the oils obtained as viscous pale yellow liquids in yields of 1.3% and 0.3% (on an air-dried leaf basis) respectively. After drying over sodium sulphate the physical constants of the oils were determined as follows:

E. youmanii : n_D^{20} 1.4878 ; α_D^{20} + 11.3° ; d_{25} 0.9434.

E. macrorhyncha : n_D^{20} 1.4809 ; α_D^{20} + 9.8° ; d_{20} 0.9393.

Identification of components

Unless otherwise specified, compounds were identified by G.L.C. analysis with co-injection of authentic materials on two column types and by G.L.C.-M.S. analysis also on two column types. Resultant mass spectra were either compared directly with those from authentic materials or with published data[11].

Dehydrogenation of the leaf oil of E. youmanii

The leaf oil of *E. youmanii* (2.5 g) and 10% Pd/C (0.3 g) were heated in an argon atmosphere under reflux at 240° for 10 h. The resultant blue/green oil was filtered then analysed by G.L.C. and G.L.C.-M.S.

Results and Discussion

The G.L.C. fingerprints for the essential oils from *E. youmanii* and *E. macrorhyncha* are presented in Figure 1a and 1b respectively and the structural assignments and percentage composition data summarised in Table 1.

For *E. youmanii,* the oil contains approximately equal proportions of mono- and sesquiterpenoids, with 1,8-cineole (30%) predominating in the former and the isomeric α, β and γ-eudesmols (total 41%) in the latter. The other major components (>1%) include α-pinene, myrcene, α-phellandrene, limonene, *p*-cymene, terpinen-4-ol and α-terpineol.

The oil from *E. macrorhyncha* proved richer in 1,8-cineole, (50%), at the expense of the eudesmols (22%). However, percentage compositions excepted, the constitutions of the two oils are strikingly similar. The occurrence of compounds of molecular formula $C_{15}H_{24}$ in both regions II and III of the gas chromatogram suggests that in some instances, at least, they represent artifacts from thermally induced dehydration.

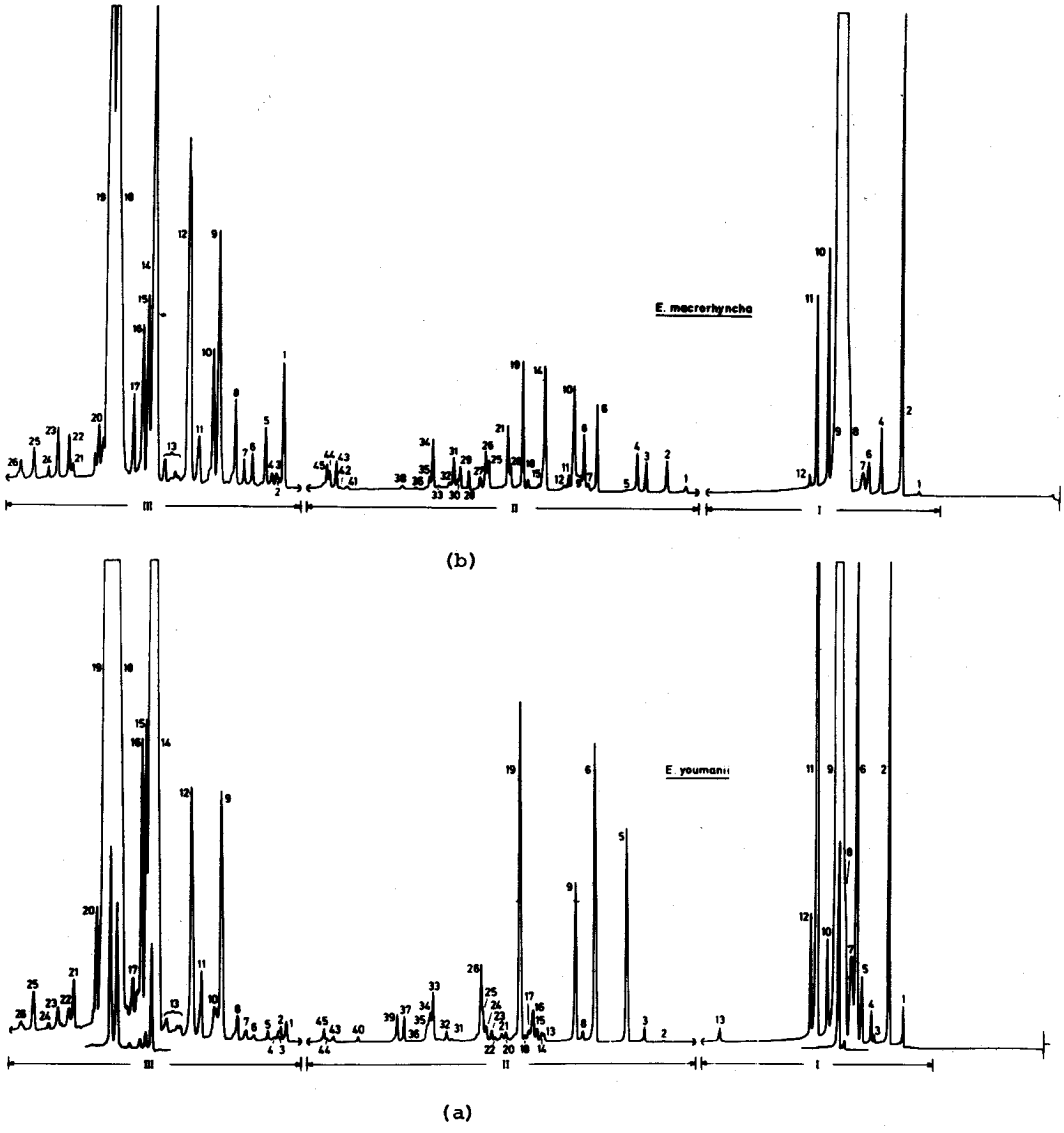

Figure 1 : Gas chromatogram of the volatile leaf oils of

(a) *E. youmanii*
(b) *E. macrorhyncha*

run on an FFAP column

TABLE 1.

Peak[1]	Assignment	*E. youmanii*[2]	*E. macrorhyncha*[2]
Region 1			
1.	acetone (solvent)	—	—
2.	α-pinene	1.7	2.9
3.	$C_{10}H_{16}$	t	a
4.	β-pinene	0.2	0.2
5.	myrcene	0.4	a
6.	α-phellandrene	3.0	0.3
7.	α-terpinene	0.8	5.6
8.	limonene	2.6	t
9.	1,8-cineole	30.0	50.2
10.	γ-terpinene	0.3	1.0
11.	p-cymene	3.9	0.6
12.	terpinolene	0.4	a
13.	α-p-dimethylstyrene	0.1	t
Region II			
1.	$C_{15}H_{24}$	a	t
2.	$C_{15}H_{24}$	t	0.1
3.	linalool	0.1	0.1
4.	$C_{15}H_{24}$	a	0.1
5.	3 *trans*-p-menth-2-en-1-ol	0.9	—
	3 $C_{15}H_{24}$	—	t
6.	terpinen-4-ol	1.4	1.2
7.	$C_{15}H_{24}$	a	t
8.	$C_{15}H_{24}$	t	0.3
9.	3 *cis*-p-menth-2-en-1-ol	0.7	—
	3 $C_{15}H_{24}$	—	t
10.	$C_{15}H_{24}$	t	1.3
11.	$C_{15}H_{24}$	a	t
12.	u	a	t
13.	$C_{15}H_{24}$	t	a
14.	$C_{15}H_{24}$	t	1.4
15.	$C_{10}H_{18}O$	t	t
16.	*cis*-piperitol	0.3	a
17.	$C_{15}H_{24}$	t	a

TABLE 1 (Cont.)

Peak[1]	Assignment	*E. youmanii*[2]	*E. macrorhyncha*[2]
18.	$C_{15}H_{24}$	t	t
19.	α-terpineol	1.7	1.5
20.	$C_{15}H_{24}O$	t	0.1
21.	u	t	0.2
22.	$C_{15}H_{24}$	t	a
23.	$C_{15}H_{26}O$	t	a
24.	u	t	a
25.	$C_{15}H_{24}$	t	0.2
26.	[3] *trans*-piperitol	0.4	—
	[3] $C_{15}H_{26}O$	—	0.2
27.	$C_{15}H_{26}O$	a	t
28.	$C_{15}H_{24}$	a	0.1
29.	$C_{15}H_{24}$	a	0.1
30.	u	a	t
31.	[3] $C_{15}H_{24}$	t	—
	[3] $C_{15}H_{22}$	—	0.2
32.	[3] $C_{15}H_{24}O$	t	—
	[3] $C_{15}H_{20}$	—	t
33.	[3] $C_{10}H_{16}O$	0.3	—
	[3] $C_{15}H_{20}$	—	t
34.	[3] β-phenylethylacetate	0.2	—
	[3] $C_{15}H_{20}$	—	0.3
35.	$C_{15}H_{22}$	t	t
36.	$C_{15}H_{20}$	t	t
37.	$C_{10}H_{14}O$	0.2	a
38.	$C_{15}H_{22}$	a	t
39.	$C_{10}H_{18}O$	0.2	a
40.	u	t	a
41.	$C_{15}H_{20}$	a	t
42.	$C_{15}H_{20}$	a	t
43.	$C_{15}H_{24}O$	t	0.1
44.	$C_{15}H_{26}O$	t	0.1
45.	u	t	0.1
Region III			
1.	$C_{15}H_{24}O$	0.1	1.0

TABLE 1 (Cont.)

Peak[1]	Assignment	*E. youmanii*[2]	*E. macrorhyncha*[2]
2.	u	0.1	t
3.	u	t	t
4.	u	t	t
5.	$C_{15}H_{24}$	0.1	0.3
6.	$C_{15}H_{24}$	t	0.2
7.	u	0.1	0.2
8.	$C_{15}H_{26}O$	0.2	0.4
9.	globulol?	1.4	1.6
10.	u	0.3	0.6
11.	viridiflorol	0.3	0.3
12.	u	0.1	2.3
13.	u[4]	t	t
14.	γ-eudesmol	13.4	5.0
15.	u	1.4	0.8
16.	u	1.0	0.5
17.	u	0.2	0.2
18.	α-eudesmol	12.5	7.3
19.	β-eudesmol	15.4	9.3
20.	u[4]	0.3	0.2
21.	u	0.2	t
22.	u	0.1	0.1
23.	u	0.1	0.1
24.	u	t	t
25.	u	0.2	0.2
26.	u	t	0.1

Explanation 1 = Peak numbers refer to Figure 1; FFAP column
2 = % of total
3 = different compounds, same retention times
4 = group of peaks
a = absent, u = unknown, t = trace

It is also worthwhile noting that errant structural assignments may result if based on G.L.C. comparisons alone; even if co-injection of samples on more than one column type shows identical retention times. In the present work, differences in several very minor components between the two oils (Table 1), notably in the hydroxylated monoterpene/sesquiterpene hydrocarbon region, could only be realised by concomitant mass spectral analysis.

The occurrence of the azulene derivatives, globulol and viridiflorol, was of interest and in an attempt to ascertain the range of terpenoid skeletal types present, a sample of the oil from *E. youmanii* was partially dehydrogenated. G.L.C.-M.S. analyses of the brilliant turquoise product showed the anticipated azulenes as well as a large increase in *p*-cymene and the presence of substituted naphthalenes.

Whilst the volatile leaf oil of *E. youmanii* had not been previously investigated, some early work on *E. macrorhyncha* showed the presence of 1,8-cineole[8], (*ca* 30%), eudesmol[8], phellandrene[8] and *l*-pinene[9]. Of the remaining two species of the subseries *Macrorhynchinae, E. laevopinea* R.T. Baker and *E. muelleriana* Howitt (syn. *E. dextropinea* R.T. Baker), early investigations reported the presence of *l*-pinene (major component)[12], 1,8-cineole[13] and possibly geranyl acetate[13] in the former and α-pinene[12] (major component), 1,8-cineole[14], geranyl acetate[13] and phellandrene[15] in the latter.

Because of the small number of compounds identifiable by the then available analytical techniques, and the lack of precise information as to which specific isomers of pinene, phellandrene and eudesmol were present, these early results are of limited chemotaxonomic value. However, it does appear that the leaf oils of the subseries *Macrorhynchinae* may be basically similar and thus not particularly useful for differentiating between these closely related species. A similar conclusion was reached during an investigation of the *Eucalyptus* subseries *Strictinae*[16].

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บทคัดย่อ

ได้ตรวจส่วนประกอบของน้ำมันที่ระเหยด้วยไอน้ำจาก Eucalyptus สองสปีชีส์ โดยวิธี G.L.C. และ G.L.C.-M.S. ได้พบความแตกต่างน้อยมาก ซึ่งเป็นการสนับสนุนทางเคมีอนุกรมวิธาน ว่าสปีชีส์ทั้งสองมีความสัมพันธ์กันใกล้ชิดทางพฤกษศาสตร์ ในจำนวนสารประกอบประมาณ 30 ตัว ซึ่งมีอยู่มากกว่า 1% ได้สามารถกำหนดได้ว่า 23 ตัว ในนั้นเป็นสารอะไร ในทั้งสองกรณีได้พบว่า 1,8-cineole และ isomeric eudesmols สามตัวเป็น mono- และ sesquiterpenoids ที่สำคัญตามลำดับ