Gas Chromatographic Quality Control for Oil of *Melaleuca* Terpinen-4-ol Type (Australian Tea Tree)

Joseph J. Brophy, Noel W. Davies, Ian A. Southwell,* Ian A. Stiff, and Lyall R. Williams

Detailed GC and GC–MS analyses of oil of *Melaleuca* have identified several constituents not previously reported from *Melaleuca alternifolia* and clarified some earlier assignments. The range, mean, and coefficient of variation for the principle constituents in 800 typical samples are presented along with the compositions of several substandard oils. Isolation and storage procedures affecting the chemical composition of the oil are reported. Ethanolic extraction of mature leaves gave solutions suitable for direct injection into a gas chromatograph for the qualitative determination of tea tree oil. Comparison with conventional steam distillation showed that this technique was suitable for preliminary analysis of tea tree oil yield and quality.

The essential oil of *Melaleuca alternifolia* Cheel (family Myrtaceae) was first reported early this century (Penfold, 1925) from the leaves of a paperbark tea tree growing in the central coastal region of eastern Australia. This taxon, previously known as *Melaleuca linariifolia* var. alternifolia, had been raised to species rank the previous year (Cheel, 1924), and the entire genus is at present undergoing taxonomic revision (Barlow, 1987, personal communication).

Possible economic uses of the oil were immediately investigated as affinities with nutmeg oil were noted (Penfold, 1925; Penfold and Morrison, 1946) and high bactericidal activity was recorded (Penfold and Grant, 1925). Early reports list many medical conditions responding to treatment with oil of *M. alternifolia* (Penfold and Morrison, 1946; Guenther, 1950; Lassak and McCarthy, 1983). The oil was also used in machine "cutting" oils, as a perfumery toner and blender, and as a flavoring and antiseptic agent in denture and mouth washes (Guenther, 1950; Pickering, 1956). Consequently, the oil has maintained a place in the essential oil trade and has subsequently been investigated for use as a nutmeg flavor substitute (Pickering, 1956), in the treatment of furunculosis (Feinblatt, 1960), in combatting wood-destroying fungi (Maruzzella et al., 1960), in treatment of vaginitis (Pena, 1962), as an antiseptic in a "Water-Jel" fire blanket (Water-Jel, 1973), in veterinary products (Lassak and McCarthy, 1983), and as an additive to podophyllotoxin in oral contraceptives (Davis, 1984). An issue of *Phytotherapy* was dedicated principally to the medicinal uses of *M. alternifolia* oil (Belaiche, 1985; Garnero, 1985). Numerous studies have established the bactericidal and fungicidal efficacy of the oil against *Bacillus typhosus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Proteus vulgaris*, and others (Penfold and Grant, 1925; Feinblatt, 1960; Beylier, 1979; Belaiche, 1985b; Altman, 1988). The active component is thought to be terpinen-4-ol (1) acting alone or synergistically with one or more minor components (Penfold and Grant, 1925; Lassak and McCarthy, 1983; Belaiche, 1985a).

As an agricultural enterprise, tea tree oil production, up until this decade, has been a small industry with annual outputs of 5–10 tons, mostly for the export market (Small, 1981; Lassak and McCarthy, 1983; Lawrence, 1985). Very recent years, however, have seen a 3-fold increase in production resulting from more effective promotion and marketing, a shift from synthetic to natural medicines, flavors, and fragrances, a greater international appreciation of the product, and the decreasing viability of some of the region's traditional agricultural enterprises (e.g., sugar and dairy). Whereas previous supplies have been adequately obtained from natural *M. alternifolia* stands growing in the New South Wales northern rivers region, increased production now becomes dependent on either plantation establishment along the lines of earlier experimental plots (Penfold and Morrison, 1946; Guenther, 1950; Small, 1981) or the harvesting of natural stands growing further afield. The former approach is being realized with the recent establishment of several *M. alternifolia* plantations, some of which have the capacity to produce in excess of 15 tons of oil/year. These plantations, which should produce oil of consistent quality and quantity, are of realistic size with more readily attainable goals than the massive operation described by Garnero (1985), which has since failed. In the meantime, the latter approach of harvesting natural stands, a greater distance from what was previously considered good tea tree country, is increasing the chances of collecting substandard oils. Both approaches present quality control problems.

Three chemical varieties ("physiological forms") of *M. alternifolia* have been recognized (Penfold et al., 1948, 1949; Guenther, 1950) and grouped as low (type), intermediate (Var. A), and high (Var. B) cineole (2) forms, with cineole determined by the *o*-cresol method (Cocking, 1920, 1927). The exclusive use of the low-cineole form as a medicinal oil has been often stressed (Penfold and Morrison, 1946; Guenther, 1950; Lassak and McCarthy, 1983) because it is thought that high-cineole oils irritate mucous membranes and skin. These facts were then used as a basis for standardization as by the *British Pharmaceutical Codex* (1949) and the Standards Association of Australia (1967, 1985) where physical constant and later gas chromatographic limits were used to exclude all but low-cineole oils. As other *Melaleuca* species, namely *M. linariifolia* (Davenport et al., 1949; Guenther, 1950) and *Melaleuca dissitiflora* (Brophy and Lassak, 1983), have chemical varieties giving oils with similar constants, the latest standard (Standards Association of Australia, 1985) was broadened to include all *Melaleuca* oils of the terpinen-4-ol type. Consequently, a rapid and accurate microdetermination for yield and quality of *Melaleuca* oils was then required in order to (i) advise cutters on the quality of natural stands, (ii) define the characteristics of seed source

School of Chemistry, University of New South Wales, Kensington, New South Wales 2033, Australia (J.J.B.), Central Science Laboratory, University of Tasmania, Hobart, Tasmania 7001, Australia (N.W.D.), North Coast Agricultural Institute, Wollongbar, New South Wales 2480, Australia (I.A.S., I.A.S.), and School of Chemistry, Macquarie University, North Ryde, New South Wales 2113, Australia (L.R.W.).

0021-8561/89/1437-1330$01.50/0

trees for plantation establishment, and (iii) investigate subtle changes in oil yield and quality under varying influences of season, temperature, light, moisture, maturity, etc.

The detailed gas chromatography–mass spectrometry (GC–MS) analysis of Australian tea tree oil published by Swords and Hunter (1978) identified many constituents that had not been found by previous investigators (Penfold, 1925; Laakso, 1966; Guenther, 1968). Lawrence (1978) subsequently reviewed this analysis and highlighted some deficiencies with their list of constituents albeit with trace components only. Our involvement with quality control enables us to clarify this list, add several constituents not yet reported from *M. alternifolia*, and comment on some of the factors affecting oil composition.

An extraction method has been developed for the rapid GLC analysis of three components of *Eucalyptus* leaf (Ammon et al., 1985). This investigation also describes the application of a similar extraction technique to the qualitative and quantitative analysis of much smaller quantities of *Melaleuca* leaf using the superior separation of high-resolution capillary columns similar to that used by Swords and Hunter (1978).

EXPERIMENTAL SECTION

Materials. All commercial oils were obtained from steam or hydrodistillation and were used as submitted for quality control by the industry. Although source species could not be established in each case, most samples would have been derived from *M. alternifolia* as only a little *M. linariifolia* and no *M. dissitiflora* are being distilled commercially. Plant material for glass laboratory distillation was from *M. alternifolia* growing in plantation at the North Coast Agricultural Institute, Wollongbar, N.S.W.

Laboratory Distillations. Leaf and terminal branchlets were distilled by hydrodistillation with cohobation in an all-glass apparatus until oil ceased condensing (approximately 4 h) or for shorter periods where specified.

Leaf Extractions. One to three intact leaves were weighed (fresh or air-dried) in a GC injection vial and immersed in absolute ethanol for a minimum of 30 h. Trials showed that substantially all extractable oil had been removed by this time. The vial contents were then automatically sampled and injected into the GC.

GC Conditions. Routine gas chromatograms with percentages expressed in FID areas were obtained in the Wollongbar (615 samples), Hobart (190 samples), and North Ryde (25 samples) laboratories on the following instruments: (a) Perkin-Elmer Sigma 2B and Sigma 10B chromatograph data station; (b) Hewlett-Packhard 5890 chromatograph and 7673 A controller and 3393 A integrator; (c) Shimadzu GC6-AMP chromatograph and Milton Roy CI 10 integrator; (d) Hewlett-Packhard 5790 A chromatograph and Perkin-Elmer 3600 computer with Sigma 15 Data Station; (e) Hewlett-Packhard 5880 A chromatograph with level IV integrator.

N_2, He, or H_2 was used as carrier gas with the following columns: (a) 2 m × 3 mm (i.d.) stainless steel packed FFAP; (b) 50 m × 0.2 mm (i.d.) FSOT FFAP; (c) 50 m and (d) 10 m × 0.2 mm (i.d.) FSOT BP21; (e) 50 m, (f) 40 m, (g) 30 m, and (h) 10 m × 0.2 mm (i.d.) FSOT BP1; (i) 50 m × 0.3 mm (i.d.) FSOT BP 20.

Typical conditions were temperature programming from 50 °C (1 min) to 180 °C at 10 °C/min for polar columns and from 40 °C (1 min) to 250 °C at 10 °C/min for nonpolar columns. Retention indices were calculated against straight-chain hydrocarbon standards.

GC–MS Conditions. The following variations at the Kensington and Hobart Laboratories were used: (a) AEI MS 12 mass spectrometer with a Shimadzu GC6-AMP gas chromatograph with all-glass straight split coupling and SCOT SP 1000 (85 m × 0.5 mm) column programmed from 60 to 225 °C at 3 °C/min or SCOT OV1 (30 m × 0.5 mm) column programmed from 60 to 250 °C at 3 °C/min with ion source at 200 °C, EI ionizing voltage 70 eV, accelerating voltage 8000 V, spectra obtained every 6 s on V.G. Display Digispec data system; (b) HP 5970 mass-selective detector coupled via an open split interface to a HP 5890 chromatograph with FSOT column i above and scanning each second.

General Instrumentation. Melting points were determined on a Reichert hot-stage apparatus, optical rotations in ethanol on an Atago Polax polarimeter, infrared spectra on a Perkin-Elmer 681 spectrophotometer, NMR spectra on a Varian XLFT-100 instrument with $CDCl_3$ as solvent and tetramethylsilane as internal standard, EIMS on an AE1 MS 12 spectrometer at 70 eV, and CIMS (negative ion) on an AEI MS 902.

Trihydroxymenthane. *M. alternifolia* oil, which had been allowed to stand in a clear, glass-stoppered bottle on a bench exposed to light, was filtered and the crystalline residue recrystallized from methanol to give (+)-1(*S*),2(*S*),4(*S*)-trihydroxy-*p*-menthane (**11**): 10.2 g; mp 171 °C (lit. mp 172 °C (Thappa et al., 1976)); $[\alpha]_D$ 52° (*c* 1.2, EtOH) (lit. $[\alpha]_D$ −21.7° (*c* 0.5, EtOH) (Pailer et al., 1981)); IR (Nujol) ν_{max} 3280, 1320, 1205, 1140, 1083, 1065, 1028, 1000, 913, 842 cm^{-1}; EIMS *m/z* (relative intensity) 170 (M − 18, 1) 153 (3), 145 (55), 127 (83), 109 (52), 58 (51), 43 (100); CIMS *m/z* 187 (M − 1); NMR δ 0.92 (6 H, d, J = 6.6 Hz), $(CH_3)_2CH$), 1.33 (3 H, s, CH_3), 3.54 (1 H, br m, $W_{h/2}$ = 13.2 Hz, *CH*OH), 3.82 (1 H, br d, J = 7.6 Hz, CHOH).

RESULTS AND DISCUSSION

Composition. The constituents of *M. alternifolia*, Australian tea tree oil, terpinen-4-ol type, as determined on numerous samples with gas chromatography (GC) and gas chromatography–mass spectrometry techniques are as shown in Table I.

The use of nonpolar stationary phases facilitated separations not possible on the more polar columns commonly used [e.g., Swords and Hunter (1978)]. For example, a 30-m BP1 FSOT column consistently separated α-thujene and α-pinene and occasionally separated β-phellandrene from both limonene and 1,8-cineole. This latter separation was at a maximum when the cineole percentage was low and close to that of limonene and β-phellandrene. Often nonpolar columns fail to separate limonene from cineole and polar columns fail to separate β-phellandrene from cineole. This separation is important commercially as low 1,8-cineole oils are preferred by the industry. [After the completion of this investigation, it was found that a 60-m column with intermediate polarity stationary phase (RSL 300) gave excellent base-line separation of the α-thujene–α-pinene, β-pinene–sabinene, α-terpinene–*p*-cymene, and limonene–1,8-cineole–β-phellandrene clusters.] In a similar way, an erroneous figure for α-terpineol can be obtained from some polar columns where the α-terpineol peak is not separated from viridiflorene. The use of two-dimensional GC may be a way of resolving the separation problems. Despite these deficiencies, even the poorer resolving power of a 2-m packed FFAP column gave percentage estimates for cineole and terpinen-4-ol suitable for quality control.

In some laboratory-distilled oils, quantities of *trans*-**3** and *cis*-**4** sabinene hydrates were detected. These arise from the presence of growing tips in the leaf sample and incomplete conversion these thujane precursors to terpinenes and terpinenols (Southwell and Stiff, 1989). When only growing tips were distilled in this manner, the level of *cis*-sabinene hydrate was as high as 5.7%. These laboratory-distilled oils and some commercially distilled oils also contained small amounts of alcohol pairs *trans*-**5** and *cis*-**6** (menth-2-en-1-ol) and *trans*-**7** and *cis*-**8** (piperitol). Swords and Hunter (1978) reported a trace of ambiguously named 1-terpineol that we define as *trans*-menth-2-en-1-ol (**5**). The peak equivalent to their β-terpineol is assigned by us to stereoisomer *cis*-menth-2-en-1-ol (**6**). The unassigned piperitol isomer is defined by us as the cis isomer **8**, and *trans*-piperitol (**7**) was also identified. The assignment of each of these six alcohols was confirmed by cochromatography using authentic products. It is also of interest that these alcohol pairs, along with terpinen-4-ol and α-terpineol, are found greatly enhanced in concen-

Table I. Constituents of the Volatile Oil of *M. alternifolia*, Australian Tea Tree

peak no.	assignment	%[f]	lit.[g] peak no.	RI (pol)[h]	RI (nonpol)[i]	ident	peak no.	assignment	%[f]	lit.[g] peak no.	RI (pol)[h]	RI (nonpol)[i]	ident
1	α-pinene	2.6	1	1034	933	a–c	50	γ-muurolene	sl tr	37	1710		a
2	α-thujene	0.9		1039	926	a, b	51	viridiflorene	1.0	39	1713	1497	a, b
3	camphene	sl tr	2	1075	943	a, b	52	$C_{15}H_{24}$	sl tr				a, b
4	β-pinene	0.3	3	1114	973	a, b	53	α-terpineol	2.4	38	1722	1177	a–c
5	sabinene	0.2[k]	4	1128	969	a–c	54	$C_{15}H_{24}$	tr		1734		a, b
6	myrcene	0.5	5	1172	984	a–c	55	$C_{15}H_{24}$	tr		1739		a, b
7	α-phellandrene	0.3	6	1172	998	a–c	56	piperitone[j]		40			
8	1,4-cineole	sl tr	7		1006	a, b	57	α-muurolene	0.1	41	1743		a, b
9	α-terpinene	10.4	8	1187	1010	a–c	58	α-amorphene	tr		1744		a, b
10	limonene	1.0	9	1206	1021	a–c	59	bicyclogermacrene	0.1		1754		a, b
11	β-phellandrene	0.9		1216	1022	a, b	60	*cis*-piperitol	tr[k]	42	1771	1195	a–c
12	1,8-cineole	5.1	10	1216	1022	a–c	61	unknown		43			
13	γ-terpinene	23.0	11	1257	1050	a–c	62	δ-cadinene	1.3	44	1777	1520	a, b
14	*trans*-β-ocimene	sl tr			1040	a	63	unknown	sl tr			1523	a
15	*p*-cymene	2.9	12	1285	1013	a–c	64	cadina-1,4-diene	0.1	45	1802	1530	a, b
16	terpinolene	3.1	13	1298	1081	a, b	65	nerol	sl tr	46	1807		a
17	hexanol[j]		14				66	$C_{15}H_{24}$	sl tr		1812		a, b
18	allyl hexanoate[j]		15				67	*p*-cymen-8-ol	sl tr	47	1852		a
19	*p*,α-dimethylstyrene	tr	16	1468	1075	a, b	68	calamenene	0.1	48	1857	1515	a, b
20	$C_{15}H_{24}$	sl tr	17	1469		a	69	unknown	sl tr		1880		a
21	α-cubebene	tr	18	1479		a, b	70	$C_{15}H_{24}$	sl tr		1944		a, b
22	*trans*-sabinene hydrate	tr[k]		1484	1058	a–d	71	unknown	sl tr		1956		a
23	α-ylangene	sl tr		1485		a, b	72	palustrol	tr		1980		a, b
24	unknown	sl tr		1490		a	73	$C_{15}H_{26}O$	tr		2027		a, b
25	$C_{15}H_{24}$	tr	19	1496		a, b	74	unknown	tr		2033		a
26	α-copaene	tr	20	1504	1379	a, b	75	$C_{15}H_{26}O$	tr		2038	1550	a, b
27	unknown	tr		1506	1381	a	76	methyl eugenol	tr		2042	1377	a–c
28	camphor[j]		21				77	ledol	sl tr		2057	1604	a, c
29	unknown	tr		1541		a	78	cubenol	0.1		2080		a, b
30	α-gurjunene	0.2	22	1544	1414	a, b	79	unknown	tr		2084		a
31	$C_{15}H_{24}$	sl tr	24	1558		a, b	80	$C_{15}H_{26}O$	0.1		2098		a, b
32	unknown	sl tr	25	1560		a	81	globulol	0.2		2103	1585	a–c
33	linalool	tr	23	1570	1084	a–c	82	viridiflorol	0.1		2113	1593	a–c
34	*cis*-sabinene hydrate	tr[k]		1571	1089	a–e	83	unknown	tr		2123		a
35	*trans*-menth-2-en-1-ol	0.2[k]	26	1584	1112	a–c	84	rosifoliol	tr		2133	1599	a–c
36	β-elemene	0.1	28			a	85	unknown	tr		2142	1625	a
37	β-caryophyllene	0.1	29	1619	1423	a–c	86	spathulenol	tr		2152	1573	a–c
38	β-gurjunene	0.1	30			a, b	87	unknown	sl tr		2175		a
39	aromadendrene	1.5	31	1623	1444	a–c	88	unknown	tr		2193		a
40	terpinen-4-ol	40.1	27	1626	1166	a–e	89	unknown	tr		2200		a
41	α-bulnesene	sl tr				a, b	90	$C_{15}H_{26}O$	tr		2210		a, b
42	*cis*-menth-2-en-1-ol	0.1[k]	32	1652	1129	a–c	91	unknown	sl tr		2217		a
43	unknown	tr		1654		a	92	unknown	sl tr		2222		a
44	$C_{15}H_{24}$	tr		1658		a, b	93	$C_{15}H_{24}O$	tr		2228		a, b
45	*allo*-aromadendrene	0.3	33	1663	1465	a, b	94	unknown	sl tr		2258		a
46	$C_{15}H_{24}$	tr		1673		a, b	95	unknown	sl tr		2292		a
47	$C_{15}H_{24}$	0.2	34	1677	1474	a, b	96	unknown	sl tr		2309		a
48	humulene	tr	35	1688	1457	a–c	97	1,2,4-trihydroxy-menthane	tr			1470	a–e
49	*trans*-piperitol	tr[k]	36?	1705	1185	a–c							

[a] GC retention index (RI). [b] GC–MS. [c] CO–GC. [d] ^1H NMR. [e] IR. [f] From a typical commercial steam-distilled oil. Key: sl tr = slight trace; tr = trace. [g] Swords and Hunter (1978). [h] Column c. [i] Column g. [j] Not confirmed by this investigation. [k] Higher percentages from flush growth.

tration in the solvent extracts of the noncohobated still waters obtained from *M. alternifolia* steam distillations.

Viridiflorene (**9**), assigned structure **10** by Swords and Hunter (1978) should have the (*R*)-methyl configuration at C3 as established by Buchi et al. (1969) for the 9-hydroxy epimers viridiflorol and ledol. As dehydration products viridiflorene and ledene are identical, this was not the first reported natural occurrence as ledene had already been isolated from the essential oil of sweet marjoram (Taskinen, 1974). The monoterpene similarities between *M. alternifolia* and marjoram (*Majorana hortensis*) have already been noted (Southwell and Stiff, 1989). It is noted here that the presence of β-caryophyllene, humulene, alloaromadendrene, α-copaene, ledene, and bicyclogermacrene in both species indicate sesquiterpene similarities as well. These could well be related to the presence of a single precursor such as bicyclogermacrene as suggested by Taskinen (1974) for majoram.

This investigation records for the first time *M. alternifolia* as a source of the sesquiterpenoids α-ylangene, bicyclogermacrene, palustrol, globulol, rosifoliol, and spathulenol in addition to monoterpenoids β-phellandrene, *trans*- and *cis*-sabinene hydrate, and *trans*-piperitol.

Variation among Commercial Oils. The *M. alternifolia* oil sample analyzed by Swords and Hunter (1978) was not typical of tea tree oils on the commercial market when compared with hundreds of samples analyzed by us in recent years (Table II). Typical commercial oils contain significantly less *p*-cymene and 1,8-cineole and more α-terpinene, γ-terpinene, and terpinolene than reported by Swords and Hunter. In an attempt to determine whether this oxidation was brought about by aging or by extrinsic factors such as moisture, light, or oxygen, several samples of different ages and storage conditions were analyzed and compositions compared (Table III). Deterioration rates were variable, with occasional samples oxidizing rapidly and failing to meet the requirements of the Standards Association of Australia (1967, 1985). After periods as brief as 21 months, some samples contain 20–40% *p*-cymene and are almost devoid of α-terpinene, γ-terpinene, and terpinolene, all of which oxidize to *p*-cymene. Small amounts of crystals settling out from these oxidized oils were found by optical rotation, melting point, infrared, NMR, and mass spectral comparison to be 1(*S*),2(*S*),4-

Chart I

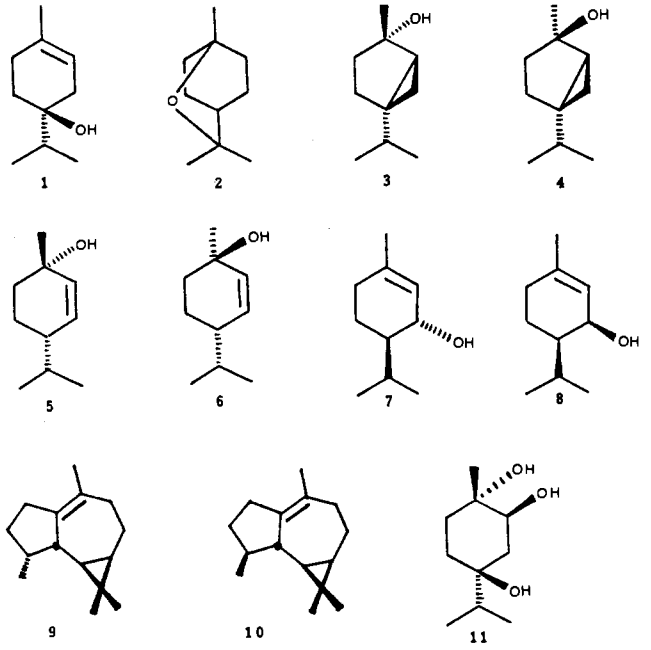

Table II. Mean Percentage Composition, Minimum and Maximum Percentages, and Coefficient of Variation of the Major Components of Oil of *Melaleuca*, Terpinen-4-ol Type

peak no.	assignment	mean	min	max	CV[a]	n[b]
1	α-pinene	2.46	0.8	3.6	13.5	523
2	α-thujene	0.83	0.1	2.1	54.2	527
4	β-pinene	0.66	0.1	1.6	24.7	642
5	sabinene	0.45	0.0	3.2	103.1	626
6	myrcene	0.86	0.1	1.8	40.1	831
7	α-phellandrene	0.44	0.1	1.9	40.2	798
9	α-terpinene	9.56	4.6	12.8	15.3	801
10	limonene	1.01	0.4	2.7	30.0	498
11	β-phellandrene	0.94	0.4	1.6	27.3	82
12	1,8-cineole	3.87	0.5	17.7	74.5	126
11, 12	unresolved	3.98	1.0	14.7	46.7	421
13	γ-terpinene	20.20	9.5	28.3	11.4	822
15	p-cymene	2.80	0.4	12.4	54.3	796
16	terpinolene	3.45	1.6	5.4	11.0	831
39	aromadendrene	1.68	0.1	6.6	45.7	815
40	terpinen-4-ol	37.93	28.6	57.9	12.4	825
51	viridiflorene	1.68	0.3	6.1	42.8	723
53	α-terpineol	3.01	1.5	7.6	20.9	737
62	δ-cadinene	1.49	0.1	7.5	44.1	758
81	globulol	0.86	0.1	3.0	11.6	356
82	viridiflorol	0.33	0.1	1.4	52.9	414

[a] CV = 100 × standard deviation/mean. [b] n = number of samples analyzed with adequate GC resolution.

(*S*)-trihydroxy-*p*-menthane (11). These crystals have previously been reported from *M. linariifolia* oil and from a terpinen-4-ol fraction from the oil that had been standing in contact with air and moisture for 6 months (Jones and Oakes, 1940). Other sources of this triol include *Zanthoxylum budrunga* fruit volatile oil (Thappa et al., 1976) and *Boswellia carteri* Birdw. incense pyrolysis product (Pailer et al., 1981). The triol is however not easy to detect by GC as it is present in small quantities that do not elute readily from polar columns and precipitate readily from the oil because of their insolubility. The most obvious evidence for their formation is the observation of crystalline plates precipitating from a rapidly yellowing oil. The variability of the composition of aged oils suggests that an oil will retain original quality for 10 years or more if stored under cool, dark, and dry conditions. The extrinsic factors that accelerate oxidation are undergoing further investigation, which has already shown that, in the presence of air and sunlight for 7 months, *p*-cymene concentration can increase 2-fold while α-terpinene, γ-terpinene, and terpinolene concentrations are halved. Although on this occasion 1,2,4-trihydroxy-*p*-menthane was not formed, the terpinenes may provide the hydrogen atoms necessary for the production of the triol from terpinen-4-ol peroxide (Figure 1).

Duration of distillation is another factor affecting oil quality. Incomplete distillation gives enhanced terpinen-4-ol levels and smaller amounts of sesquiterpenoids (Table

Table III. Monoterpenoid Composition Comparison of Aged Oils of *M. alternifolia*

sample no.	1	2	3	4	5
age, years	10	10	5	2	1
rel deter rate	slow	rapid	rapid	rapid	moderate
composition, %					
α-thujene	0.6			0.2	0.8
α-pinene	2.2	3.2	tr	2.0	2.5
sabinene		0.1		tr	tr
β-pinene	0.6	0.3	tr	0.4	0.7
myrcene	0.5	0.2	tr	0.1	0.7
α-phellandrene	0.2	tr		tr	0.4
α-terpinene	5.8	0.2		0.1	6.6
p-cymene	4.3	32.0	21.7	35.3	8.0
limonene / β-phellandrene / 1,8-cineole	0.4	7.3	2.1	3.1	4.0
γ-terpinene	15.0	tr	tr	tr	17.6
terpinolene	2.7	tr	tr	tr	3.1
terpinen-4-ol	41.6	31.5	45.9	23.8	37.3
α-terpineol	3.7	6.4	9.6	8.2	2.9
1,2,4-trihydroxymenthane	tr	4.6	2.5	3.6	tr
Australian std status	pass	fail	fail	fail	fail

Table IV. Percentage Composition of *M. alternifolia* Oils Collected at Different Times during the Distillation

peak no.	assignment	0–30 min (4.5 mL)	30–90 min (2.6 mL)
1	α-pinene	1.4	3.5
2	α-thujene	0.6	1.1
4	β-pinene	0.5	0.9
5	sabinene	0.2	0.1
6	myrcene	0.7	1.3
7	α-phellandrene	0.2	0.4
9	α-terpinene	7.8	14.0
10	limonene		
11	β-phellandrene	5.7	4.1
12	1,8-cineole		
13	γ-terpinene	15.6	29.1
15	p-cymene	1.3	1.4
16	terpinolene	2.6	4.8
39	aromadendrene	0.3	1.2
40	terpinen-4-ol	55.9	25.1
51	viridiflorene	0.5	1.5
53	α-terpineol	3.8	2.1
62	δ-cadinene	0.3	1.2

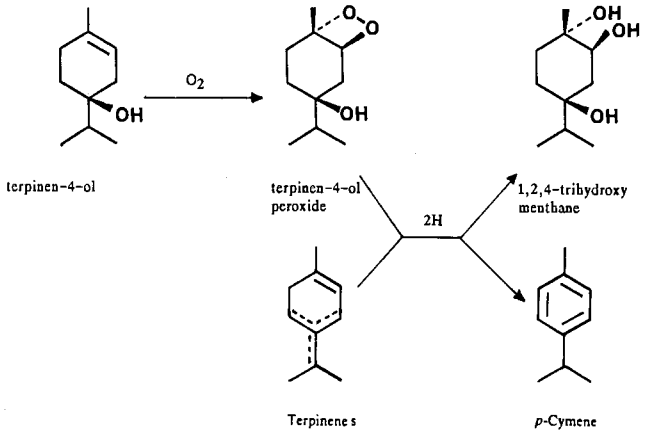

Figure 1. Possible pathway for the deterioration of *M. alternifolia* oil components.

Table V. Percentage Composition of Some Substandard Oils from *Melaleuca* (a–c) or of Unknown Origin (d–f)

peak no.	assignment	a	b	c	d	e	f
1	α-pinene	1.3	1.2	2.3	2.2	1.5	10.4
2	α-thujene	0.2		0.2	1.3	1.6	0.2
4	β-pinene	0.5	0.7	0.3	0.9	0.5	2.9
5	sabinene	0.2	0.2		0.2		1.0
6	mycrene	0.9	1.5	0.5	1.9	1.7	3.2
7	α-phellandrene	0.1	0.2	1.1	1.9	19.0	0.8
9	α-terpinene	1.8	0.3	7.5	2.9	4.1	0.5
10	limonene	47.6	3.0	4.4	5.4	8.1	11.8
11	β-phellandrene						
12	1,8-cineole		82.2		25.7		30.6
13	γ-terpinene	3.3	0.5	13.1	6.3	6.8	0.6
15	p-cymene	0.3	0.1	1.9	0.5	6.7	2.3
16	terpinolene	0.6	0.2	3.1	tr	1.4	0.7
33	linalool	0.4	0.2		36.1	7.1	0.3
39	aromandendrene			7.3			
40	terpinen-4-ol	17.3	1.3	19.4	4.3	32.7	0.3
51	virdiflorene			6.5			
53	α-terpineol	4.2	8.8	3.8	2.6	1.2	16.0
62	δ-cadinene			7.0			

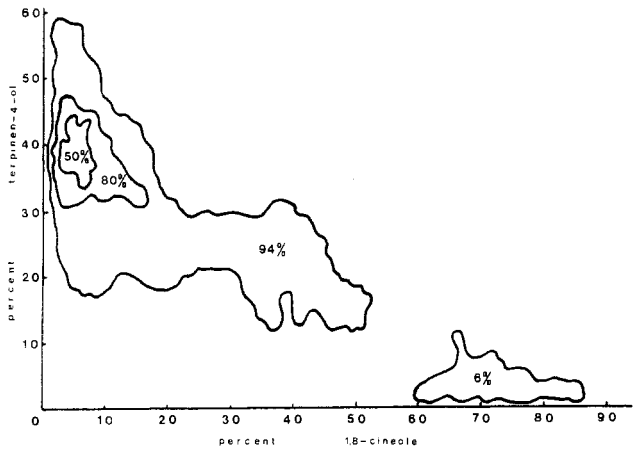

Figure 2. Contour diagram indicating the proportion of analyzed samples containing the indicated percentages of 1,8-cineole and terpinen-4-ol.

IV). Hence, this distillation of the higher boiling alcohol constituents before the hydrocarbons in *M. alternifolia* supports the hydrodiffusion principles proposed by Koedam et al. (1980) for *Abies*.

The presence or absence of recirculation of condensed waters (cohobation) also affects oil composition and yield in laboratory distillations (Murtagh, personal communication). The solubility of terpinen-4-ol in the still waters causes terpinen-4-ol levels to fall when steam/leaf ratios are high. When the ratio is smaller, as in commercial distillations, this lack of cohobation has a smaller but still significant affect on oil quality.

Substandard Oils. The existence of chemical forms of *M. alternifolia* and *M. linariifolia* increases the chances of substandard oils being produced and reaching the commercial market. The composition of a typical acceptable low-cineole oil is summarized in Table I and the usual range presented in Table II. The compositions of typical intermediate and high-cineole forms are summarized in Table V. The bulk of the commercial samples analyzed contained 30–45% terpinen-4-ol and less than 10% cineole (Figure 2). Substandard oil samples showed gradually decreasing terpinen-4-ol levels with concomitant increases in cineole levels. A secondary concentration of samples occurred with cineole at 60–80% and terpinen-4-ol at less than 10%. This grouping clearly represents the high-cineole Var. B chemical form established for *M. alternifolia* (Penfold et al., 1948) and suggested to be the

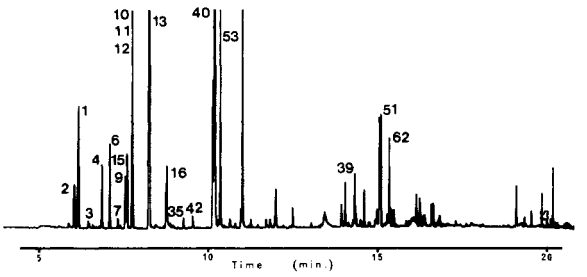

Figure 3. Gas chromatographic trace of *M. alternifolia* single leaf extract on a 30-m BP1 FSOT column. Peak numbers are consistent with Table I.

case for *M. linariifolia* as well (Guenther, 1950). This form is unsatisfactory for commercial tea tree oil production (Penfold and Morrison, 1946). On the other hand, Figure 2 indicates that the intermediate cineole Var. A chemical form with 31–45% cineole is less obvious due to a gradual mergence of Var. A with the commercial low-cineole type form.

On occasions, other oils of unknown origin, not of the terpinen-4-ol variety typical of *M. alternifolia*, reach the market. Some of these are rich in linalool, α-pinene, or α-terpineol and are almost certainly either distilled from different species or blended. The compositions of some such oils are also shown in Table V. As mentioned earlier, oils that are poorly dried and stored will undergo accelerated oxidation, accumulate *p*-cymene, and hence also fit into the substandard oil category. A good example of this is the sample analyzed by Swords and Hunter (1978). Similarly, oils distilled from leaf material dried at temperatures exceeding 60 °C will show elevated *p*-cymene levels (Curtis and Murtagh, personal communication).

Microanalysis. The recently described (Southwell and Stiff, 1989) 30-h room-temperature ethanolic extraction of a single mature *Melaleuca* leaf (approximately 6 mg) in a 0.1-mL insert in a GC autosampler vial gave a solution suitable for direct injection into the chromatograph. The monoterpene region of the resultant trace accurately reflected the quality of the oil obtained by steam distillation of the same leaf material, as long as a mature leaf was extracted (Figure 3). When a lighter green growing tip was extracted, γ-terpinene and terpinen-4-ol were found to be present in their precursor sabinene hydrate forms. These thujane precursors convert to their stable products either during steam distillation by artifact formation or as the leaf matures. In addition, some of the oil components, e.g., α-terpinene and terpinolene, are enhanced during steam distillation because of artifact formation even when mature leaf is distilled (Southwell and Stiff, 1989). This microextraction method provides an excellent way of determining whether the tree under investigation has acceptable levels of terpinen-4-ol and cineole. The use of tridecane as an internal standard enabled estimates of oil yield to be calculated.

Registry No. α-Pinene, 80-56-8; α-thujene, 2867-05-2; camphene, 79-92-5; β-pinene, 127-91-3; sabinene, 3387-41-5; myrcene, 123-35-3; α-phellandrene, 99-83-2; 1,4-cineole, 470-67-7; α-terpinene, 99-86-5; limonene, 138-86-3; β-phellandrene, 555-10-2; 1,8-cineole, 470-82-6; γ-terpinene, 99-85-4; *trans*-β-ocimene, 3779-61-1; *p*-cymene, 99-87-6; terpinolene, 586-62-9; hexanol, 25917-35-5; allyl hexanoate, 123-68-2; *p*-α-dimethylstyrene, 1195-32-0; α-cubebene, 17699-14-8; *trans*-sabinene hydrate, 17699-16-0; α-ylangene, 14912-44-8; α-copaene, 3856-25-5; camphor, 76-22-2; α-gurjunene, 489-40-7; linalool, 78-70-6; *cis*-sabinene hydrate, 15537-55-0; *trans*-menth-2-en-1-ol, 29803-81-4; β-elemene, 33880-83-0; β-caryophyllene, 87-44-5; β-gurjunene, 73464-47-8; aromadendrene, 72747-25-2; terpinen-4-ol, 562-74-3; α-bulnesene, 3691-11-0; *cis*-menth-2-en-1-ol, 29803-82-5; *allo*-aromadendrene,

25246-27-9; humulene, 6753-98-6; *trans*-piperitol, 16721-39-4; γ-muurolene, 10208-80-7; viridiflorene, 21747-46-6; α-terpineol, 98-55-5; piperitone, 89-81-6; α-muurolene, 10208-80-7; α-amorphene, 20085-19-2; bicyclogermacrene, 24703-35-3; *cis*-piperitol, 16721-38-3; δ-cadinene, 483-76-1; γ-cadina-1,4-diene, 29837-12-5; nerol, 106-25-2; *p*-cymen-8-ol, 1197-01-9; calamenene, 483-77-2; palustrol, 5986-49-2; methyl eugenol, 93-15-2; ledol, 577-27-5; cubenol, 21284-22-0; globulol, 489-41-8; viridiflorol, 552-02-3; rosifoliol, 63891-61-2; spathulenol, 6750-60-3; 1,2,4-trihydroxymenthane, 98109-59-2.

LITERATURE CITED

Altman, P. M. Australian Tea Tree Oil. *Aust. J. Pharm.* **1988**, *69*, 276–278.

Ammon, D. G.; Barton, A. F. M.; Clarke, D. A.; Tjandra, J. Rapid and Accurate Determination of Terpenes in the Leaves of *Eucalyptus* species. *Analyst* **1985**, *110*, 921–924.

Balaiche, P. Editorial. *Phytotherapy* **1985a**, *15*, 3.

Belaiche, P. L'huile essentielle de *Melaleuca alternifolia* (Cheel) dans les infections urinaires colibacillaires chroniques idiopathiques. *Phytotherapy* **1985b**, *15*, 9.

Belaiche, P. L'huile essentielle de *Melaleuca alternifolia* (Cheel) dans les infections vaginales a *Candida albicans*. *Phytotherapy* **1985c**, *15*, 13.

Belaiche, P. L'huile essentielle de *Melaleuca alternifolia* (Cheel) dans les infections cutanees. *Phytotherapy* **1985d**, *15*, 15.

Beylier, M. F. Bateriostatic activity of some Australian essential oils. *Perfum. Flavor.* **1979**, *4*(2), 23–25.

Oleum Melaleucae. *British Pharmaceutical Codex*; General Medical Council: London, 1949; pp 597–598.

Brophy, J. J.; Lassak, E. V. The Volatile Oils of *Melaleuca armillaris*, *M. dissitiflora* and *M. trichostachya*. *J. Proc. Roy. Soc. N.S.W.* **1983**, *116*, 7–10.

Buchi, G.; Hofheinz, W.; Paukstelis, J. V. The Synthesis of (−)-Aromadendrene and Related Sesquiterpenes. *J. Am. Chem. Soc.* **1969**, *91*, 6473–6478.

Cheel, E. Notes on Melaleuca, with Descriptions of Two New Species and a New Variety. *J. Proc. Roy. Soc. N.S.W.* **1924**, *58*, 189–197.

Cocking, T. A New Method for the Estimation of Cineole in Eucalyptus Oil. *Pharm. J.* **1920**, *105*, 81–83.

Cocking, T. Estimation of Cineole. *Perfum. Essent. Oil Rec.* **1927**, *18*, 254–257.

Davenport, J. B.; Jones, T. G. H.; Sutherland, M. D. The Essential Oils of the Queensland Flora. Part XXIII. A Re-examination of the Essential Oil of *Melaleuca linariifolia*. *Univ. Queensl. Pap., Dep. Chem.* **1949**, *1*(36), 1–12.

Davis, G. L. R. Podophyllotoxin and Oil of *Melaleuca alternifolium*, Pharmaceutical composition. Pat. Specif. (Aust) AU 534, 571, 1980 (C1 A61K31/565); *Chem. Abstr.* **1984**, *101*, P43630b.

Feinblatt, H. M. Cajeput-type oil (Tea Tree Oil) for the Treatment of Furunculosis. *J. Natl. Med. Assoc.* **1960**, *52*, 32–34.

Garnero, M. J. L'huile essentielle de 'Tea Tree' d'Australie. *Phytotherapy* **1985**, 15, 5–8.

Guenther, E. *The Essential Oils*; Van Nostrand: New York, 1950; Vol. 4, p 526.

Guenther, E. Australian Tea Tree Oils. Report of a Field Survey. *Perfum. Essent. Oil Rec.* **1968**, *59*, 642–44.

Jones, T. G. H.; Oakes, H. C. The Crystalline Solid Formed in the Oil of *Melaleuca linariifolia*. *Univ. Queensl. Pap., Dep. Chem.* **1940**, *1*(18), 1–3.

Koedam, A.; Scheffer, J. J. C.; Svendsen, A. B. Monoterpenes in the Volatile Leaf of *Abies* × *arnoldiana* Nitz. *J. Agric. Food Chem.* **1980**, *28*, 862–866.

Laakso, P. V. Fractionation of Tea Tree Oil (*Melaleuca alternifolia*). *Proceedings of the 25th Congress of Pharmaceutical Sciences*, Prague, 1965; Hanc, O., Hubik, J., Eds.; Czechoslovakian Medical Press: Prague, 1966; Vol. 1, pp 485–492.

Lassak, E. V.; McCarthy, T. *Australian Medicinal Plants*; Methuen: Sydney, Australia, 1983.

Lawrence, B. M. Tea Tree Oil. *Perfum. Flavor.* **1978**, *3*(5), 40.

Lawrence, B. M. A Review of the World Production of Essential Oils (1984). *Perfum. Flavor.* **1985**, *10*(5), 1–16.

Maruzzella, J. C.; Scrandis, D.; Scrandis, J. B.; Grabon, G. Action of Odoriferous Organic Chemicals and Essential Oils on Wood Destroying Fungi. *Plant Dis. Rep.* **1960**, *44*, 789–792.

Pailer, M.; Scheidl, O.; Gutwillinger, H.; Klein, E.; Obermann, H. Uber die Zusammensetzung des Pyrolysates von Weihrauch "Aden", dem Gummiharz von *Boswellia carteri* Birdw., 3 Mitt. *Monatsh. Chem.* **1981**, *112*, 987–1006.

Pena, E. F. *Melaleuca alternifolia* Oil – Its Use for Trichomonal Vaginitis and Other Vaginal Infections. *Obstet. Gynecol.* **1962**, *19*, 793–795.

Penfold, A. R. The Essential Oils of *Melaleuca linariifolia* (Smith), and *M. alternifolia* (Cheel). *J. Proc. Roy. Soc. N.S.W.* **1925**, *59*, 306–325.

Penfold, A. R.; Grant, R. The Germicidal Values of Some Australian Essential Oils and their Pure Constituents. *J. Proc. Roy. Soc. N.S.W.* **1925**, *59*, 346–350.

Penfold, A. R.; Morrison, F. R. Australian Tea Trees of Economic Value. Part 1. Bulletin Tech. Museum, Sydney; Government Printer: Sydney, 1946; No. 14, pp 5–16.

Penfold, A. R.; Morrison, F. R.; McKern, H. H. G. Studies in the Physiological Forms of the Myrtaceae. Part II. The Occurrence of Physiological Forms in *Melaleuca alternifolia* Cheel. *Researches on Essential Oils of the Australian Flora*; Museum of Technology and Applied Science: Sydney, 1948; Vol. 1, pp 18–19.

Penfold, A. R.; Morrison, F. R.; McKern, H. H. G. *Perfum. Essent. Oil Rec.* **1949**, *40*, 149.

Pickering, G. B. Cedarwood-oil compounds, silica gel separation and tea tree oil as nutmeg substitute. *Manuf. Chem.* **1956**, *27*, 105–106.

Small, B. E. J. Effects of Plant Spacing and Season on Growth of *Melaleuca alternifolia* and Yield of Tea Tree Oil. *Aust. J. Exp. Agric. Anim. Husb.* **1981**, *21*, 439–442.

Southwell, I. A.; Stiff, I. A. Ontogenetical Changes in Monoterpenoids of *Melaleuca alternifolia* Leaf. *Phytochemistry* **1989**, *28*, 1047–1051.

Standards Association of Australia. *Oil of Melaleuca alternifolia*; Standards Australia: Sydney, 1967; K175.

Standards Association of Australia. *Essential Oils – Oil of Melaleuca, Terpinen-4-ol type*; Standards Australia: Sydney, 1985; 2782.

Swords, G.; Hunter, G. L. K. Composition of Australian Tea Tree Oil (*Melaleuca alternifolia*). *J. Agric. Food Chem.* **1978**, *26*, 734–737.

Taskinen, J. Composition of the Essential Oil of Sweet Marjoram Obtained by Distillation with Steam and by Extraction and Distillation with Alcohol-Water mixture. *Acta Chem. Scand.* **1974**, *B28*, 1121.

Thappa, R. K.; Dhar, K. L.; Atal, C. K. A New Monoterpene Triol from *Zanthoxylum budrunga*. *Phytochemistry* **1976**, *15*, 1568–1569.

Water Jel International Pty. Ltd. Neth. Appl. 7309, 130, 1973 (OA 62d); *Chem. Abstr.* **1973**, *83*, p120849u.

Received for review October 17, 1988. Accepted March 21, 1989.