Enantiomeric Composition of the Principal Components of the Oil of *Melaleuca alternifolia*

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The concentrations and enantiomeric purity of the major monoterpene constituents in a number of *Melaleuca alternifolia* (tea tree) oils have been determined by enantioselective gas chromatography. Consistent enantiomeric ratios of 65:35 (+:−) for terpinen-4-ol and 76:24 (+:−) for α-terpineol were observed for the range of oils analyzed. Attempts to validate these ratios in the intact oil by ^{1}H NMR together with chiral lanthanide shift reagents have not yet been successful. ^{1}H NMR was, however, successful in confirming the enantiomeric purity determined by gas chromatography of standard terpinen-4-ol [79:21 (+:−)] and α-terpineol [35:65 (+:−)] samples. Under the correct conditions, a single analysis on a β-cyclodextrin column enables the determination of the quality of the oil as set forth by the Australian standard and provides additional enantiomeric evidence of the authenticity of the oil.

INTRODUCTION

The detailed chemical composition of the various types of *Melaleuca alternifolia* oil, commonly but ambiguously referred to as tea tree oil, are now well established (Brophy *et al.*, 1989; Southwell and Stiff, 1989; Kawakami *et al.*, 1990). Little, however, appears to be known about the enantiomeric composition of the constituents of the oil, despite the fact that optical rotation is used as a quality measurement (Australian Standard, 1985). The relationship between the enantiomeric composition of the chiral constituents of essential oils and their origins, processing, and physiological properties has been pointed out (Konig *et al.*, 1992). Thus, information from the enantioselective analysis of the major chiral monoterpenes in *M. alternifolia* oil will enable a more rigorous description of the characteristics of the oil. It would also permit an investigation of the relationships between the bioactivity of the components and their stereochemistry and possible biogenetic pathways. Two instrumental techniques that have the potential to provide rapid chiral analysis on a routine basis are enantioselective chromatography and nuclear magnetic resonance (NMR). Recent advances in capillary gas chromatography column technology have seen the advent of a number of stable chiral stationary phases that enable the direct stereochemical analysis of chiral compounds without derivatization. These have been used to determine the enantiomeric status of a range of natural products including terpinen-4-ol (Schurig *et al.*, 1985, 1990; Takeoka *et al.*, 1990; Bicchi *et al.*, 1990; Dietrich *et al.*, 1992).

While under normal conditions NMR spectroscopy cannot distinguish signals from different enantiomers, the addition of chiral lanthanide shift reagents (LSRs) may, in some cases, cause the nuclei of the different enantiomers to resonate at different frequencies and permit their separation. This change in chemical shift depends upon the substrate, the specific LSR, and the substrate:LSR ratio. The application of this method to the constituents of essential oils including the determination of the enantiomeric purity of terpinen-4-ol in marjoram has been demonstrated previously (Ravid *et al.*, 1988a,b).

This paper describes the application of these methods to the analysis of *M. alternifolia* oil and the implications for quality criteria based on the data obtained.

EXPERIMENTAL PROCEDURES

Reagents. Oil samples were purchased from local retail outlets. Other oils including the *Eucalyptus dives* oil were kindly donated by Mr. G. R. Davis, Davis Essential Oils Pty. Ltd., Sydney, Australia. Tris[3-(heptafluoropropylhydroxymethylene-(+)-camphorato] derivatives of europium(III) (98% pure) and praseodymium(III) (98% pure), praseodymium(III) tris[3-(heptafluoropropylhydroxymethylene)-(−)-camphoratol, terpinen-4-ol ($[\alpha]^{22} = +29°$), α-terpineol ($[\alpha]^{20}_D = -35°$), myrcene, α-terpinene, γ-terpinene, 1,8-cineole, *p*-cymene, and $CDCl_3$ (99.9% pure) were obtained from Aldrich Chemicals. α-Pinene and limonene were donated by Haarman and Reimer, Sydney, Australia.

Instrumental Methods. *NMR Spectroscopy.* All NMR spectra were recorded on a Varian Gemini 200 spectrometer operating at a magnetic field strength of 4.7 T in a 5-mm ^{1}H/multinuclear probe. Oil standards were prepared by dissolution in $CDCl_3$; 200 mM for the ^{13}C spectra, 50 mM for the ^{1}H spectra, and 5 mM for the LSR experiment. All ^{1}H chemical shifts were referenced to residual $CHCl_3$ at 7.24 ppm with respect to tetramethylsilane (TMS), and all ^{13}C chemical shifts were referenced to $CDCl_3$ at 77.0 ppm with respect to TMS.

^{13}C NMR Experiments. All ^{13}C NMR data were collected at 50.0 MHz at a transmitter offset of −1525.9 Hz, as 16 000 data points over a spectral width of 7304.6 Hz. All 1D data sets were zero-filled to 32 768 points and weighted with an exponential apodization function of 2.0 Hz prior to Fourier transformation. The ^{13}C 1D single-pulse spectrum was recorded using a 45° flip angle with a relaxation delay of 1.0 s. Broadband Waltz decoupling (Shaka *et al.*, 1983) was used throughout to collapse any ^{1}H–^{13}C coupling.

The INEPT (insensitive nuclei enhancement polarization transfer) spectrum was obtained using the standard pulse sequence (Morris and Freeman, 1978) with a delay, Δ, of 2 ms and a relaxation delay of 2 s.

The DEPT (distortionless enhancement by polarization transfer) subspectra were obtained using the standard pulse sequence (Doddrell *et al.*, 1982; Pegg *et al.*, 1982). Experimental conditions were optimized for a ^{13}C–^{1}H coupling of 140 Hz with a relaxation delay of 2 s. Four separate DEPT subspectra were acquired using θ pulse values of 45°, 90°, 90°, and 135°, which were then manipulated using a standard Varian macroprogram to produce the following spectra: (1) methyl only; (2) methylene only; (3) methine only; and (4) all protonated carbon spectrum.

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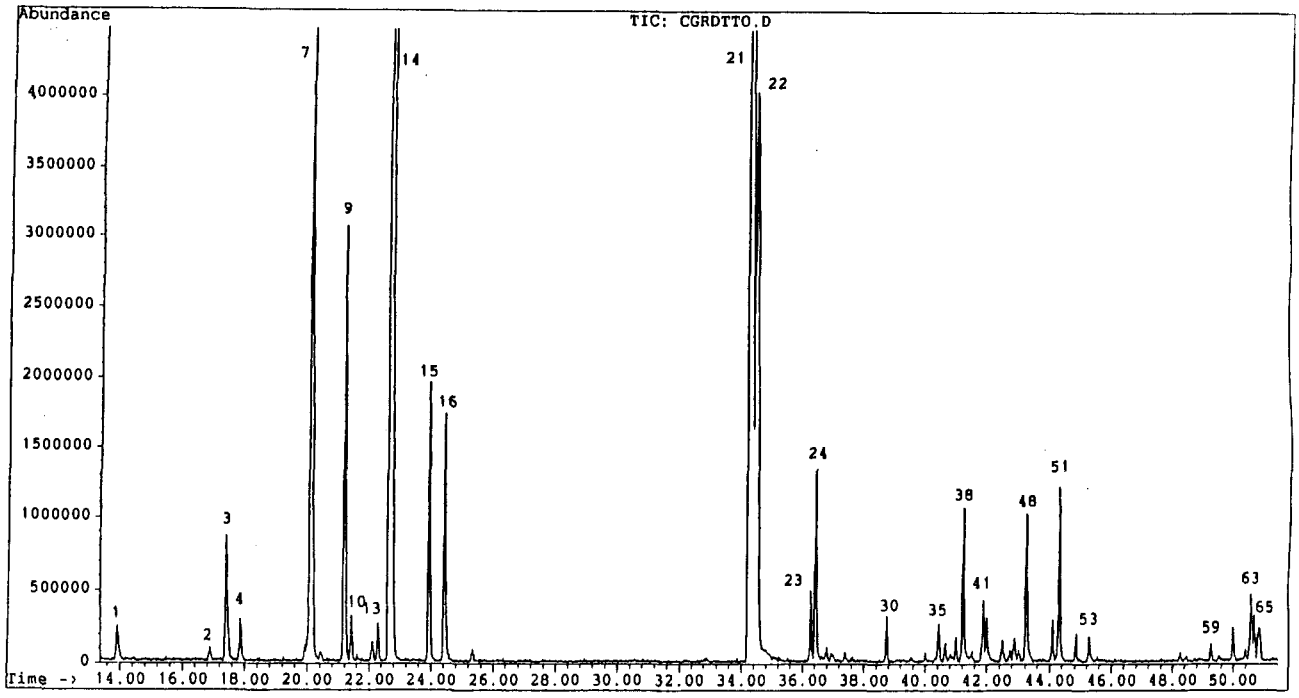

Figure 1. Chiral gas chromatogram of *M. alternifolia* oil.

The 2D heteronuclear ^{13}C–^{1}H shift correlation spectrum (HETCOR) (Bax and Morris, 1981; Bax, 1983) was obtained using a standard pulse sequence in which ^{1}H decoupling was achieved using the Waltz method (Shaka *et al.*, 1983) during the acquisition period only. Experimental conditions were optimized for a ^{13}C–^{1}H coupling of 140 Hz; 2048 data points were collected in t_2 using a spectral width of 7304.6 Hz, and 128 t_1 increments were collected over a ^{1}H spectral width of 1499.9 Hz and a decoupler offset of −355.0 Hz. The data were zero-filled to 4096 points in t_2 and 256 in t_1 prior to Fourier transformation. An exponential apodization function was used to weigh the data in t_2, while a pseudo-echo apodization function was used to weigh the data in t_1.

^{1}H 2D Experiments. The ^{1}H NMR spectra were collected at 200.0 MHz with a spectral width of 1499.9 Hz and a transmitter offset of −355.0 Hz in both dimensions. A relaxation delay of 2.0 s was incorporated into the sequence. Homonuclear ^{1}H DQF-COSY (double quantum filtered correlated spectroscopy) was performed in the phase sensitive mode using the hypercomplex method of data collection (Muller and Ernst, 1979; Keeler and Neuhaus, 1985). Data were collected as 1024 points in t_2 for each of the 256 t_1 values with quadrature detection in both dimensions. Both data sets were zero-filled to 2048 and 1024 points, respectively, prior to Fourier transformation, and a pseudo-echo apodization function was used to weigh the data to ensure that the interferogram decayed to zero by the end of the acquisition.

LSR Experiments. All shift reagents were freeze-dried and stored under N_2 in a freezer until used. Aliquots of a binary solution of 20 mM LSR and 5 mM oil standard in $CDCl_3$ were added to a 5 mM oil standard dissolved in $CDCl_3$ (0.5 mL). After the addition of each aliquot, the solution was mixed and allowed to equilibrate at 20 °C in the NMR spectrometer for 10 min prior to data acquisition. Using this technique, the oil standards were always kept at a constant 5 mM concentration through the entire experiment. A series of 1D ^{1}H NMR spectra were acquired at different LSR:oil standard ratios. Data were collected at 200.0 Hz at a transmitter offset of −355.0 Hz, as 16 000 data points over a spectral width of 1499.9 Hz and a relaxation delay of 1.0 s. Data sets were zero-filled to 32 768 points and weighted with an exponential apodization function of 0.1 Hz prior to Fourier transformation.

Gas Chromatography. High-resolution chiral phase gas chromatograms were recorded on a Hewlett-Packard 5890 Series II chromatograph fitted with a J&W Cyclodex-B, 0.25-μm film thickness, 30 m × 0.25 mm capillary column. Data were acquired under the following conditions: initial temperature, 50 °C, 10 min; program rate; 3 °C/min; final temperature, 180 °C; final time, 2 min; total time, 55 min; injector temperature, 240 °C; transfer line temperature, 280 °C; carrier gas, He at 22 cm/s; split ratio, 1:40. The column was terminated at a Hewlett-Packard mass selective detector (MSD) (HP 5971A). The ion source was run in the EI mode at 190 °C using an ionization energy of 70 eV. The scan rate was 0.9 scans/s. Data from the MSD were stored and processed using a Hewlett-Packard Vectra QS20 computer installed with Mustang software and the Wiley Mass Spectral Library. Kovats indices were calculated against external hydrocarbon standards.

RESULTS AND DISCUSSION

A chromatogram of a typical *M. alternifolia* oil sample obtained using a β-cyclodextrin column is shown in Figure 1. The components together with their retention data are shown in Table I. Identification was confirmed by GCMS and by comparison with known standards and with literature retention values using the same or similar columns (Takeoka *et al.*, 1990; Konig *et al.*, 1990). The conditions for the analysis need to be carefully controlled to achieve sufficient resolution over the whole of the chromatogram. An initial temperature of 50 °C, held for 10 min followed by a temperature ramp to 180 °C at 3 °C/min, provided the separation required. Assignment of the (+) and (−) forms to the various enantiomers was based on published data. Detection and identification of many of the chiral trace monoterpenes and sesquiterpenes were achieved only by increasing the amount of sample injected. This, however, resulted in a marked decrease in the resolution obtained for the major components. The β-cyclodextrin column clearly resolves 1,8-cineole from the other monoterpenes, a separation that is not easily achieved on other commonly used columns. This is of particular significance in relation to the *M. alternifolia* oil quality standard criteria in which 1,8-cineole concentration is one of the key characteristics.

Separation of the enantiomers of several of the chiral monoterpene hydrocarbons, namely α-pinene and β-phellandrene, was also achieved, and enantiomeric ratios of 8:92 and 35:65, respectively, were observed. Although the enantiomers of limonene and α-phellandrene were ob-

Table I. Chiral Composition of *M. alternifolia* Oil Determined on β-Cyclodextrin

peak	compound	Kovat index	%	I.D.
1	α-thujene	976	0.44	MS[a]
2	(−)-α-pinene	1007	0.18	MS, RT
3	(+)-α-pinene	1017	1.68	MS, RT
4	β-myrcene	1025	0.46	MS, RT
5	(−)-α-phellandrene	1058	c	MS[a]
6	(+)-α-phellandrene	1058	c	MS[a]
7	α-terpinene	1062	9.90	MS, RT
8	(−)-limonene	1079	c	MS, RT
9	p-cymene	1079	4.96	MS, RT
10	(+)-limonene	1082	0.51	MS, RT
11	isocineole	1085	tr	MS[a]
12	(±)-β-phellandrene	1092	0.22	MS[a]
13	(±)-β-phellandrene	1095	0.36	MS[a]
14	γ-terpinene	1100	21.15	MS, RT
15	α-terpinolene	1126	3.18	MS, RT
16	1,8-cineole	1135	3.04	MS, RT
17	dimethylstyrene	1153	0.17	MS[a]
18	(±)-linalool	1254	tr	MS[a]
19	(±)-linalool	1257	tr	MS[a]
20	sabinene hydrate	1301	tr	MS[a]
21	(+)-terpinen-4-ol	1334	24.73	MS, RT
22	(−)-terpinen-4-ol	1340	13.13	MS, RT
23	(−)-α-terpineol	1381	0.69	MS, RT
24	(+)-α-terpineol	1385	2.03	MS, RT
25	α-copaene	1389	0.17	MS[a]
26	α-cubebene	1394	0.09	MS[a]
27	p-cymen-8-ol	1397	tr	MS[a]
28	MW 204	1408	0.14	MS
29	MW 204	1415	tr	MS
30	α-gurjunene	1442	0.45	MS[a]
31	β-gurjunene	1452	tr	MS[a]
32	β-maaliene	1456	tr	MS[b]
33	MW 204	1462	tr	MS
34	MW 204	1473	0.07	MS
35	β-caryophyllene	1483	0.44	MS[a]
36	α-bulnescene	1489	0.19	MS[a]
37	MW 204	1492	tr	MS
38	MW 204	1496	0.24	MS
39	aromadendrene	1501	1.61	MS[a]
40	γ-gurjunene	1510	0.15	MS[a]
41	alloaromadendrene	1519	0.69	MS[a]
42	epibicyclosesquiphellandrene	1522	0.51	MS[b]
43	α-muurolene	1536	0.26	MS[a]
44	methyleugenol + MW 204	1538	tr	MS[a]
45	MW 204	1542	0.12	MS
46	α-amorphene	1546	0.29	MS[a]
47	bicyclogermacrene	1550	0.13	MS[a]
48	viridiflorene	1555	1.75	MS[a]
49	MW 150 + MW 204	1559	tr	MS
50	MW 204	1577	0.44	MS
51	δ-cadinene	1582	1.86	MS[a]
52	calamenene	1596	0.27	MS[a]
53	cadina-1,4-diene	1607	0.30	MS
54	MW 204	1610	tr	MS
55	MW 150 + MW 204	1615	tr	MS
56	MW 220	1631	tr	MS
57	MW 220	1673	tr	MS
58	palustrol	1689	0.06	MS[a]
59	epiglobulol	1717	0.18	MS[b]
60	MW 222	1725	tr	MS
61	MW 222	1738	0.38	MS
62	spathulenol	1750	0.15	MS[a]
63	globulol	1755	0.80	MS[a]
64	cubenol	1759	0.52	MS[a]
65	viridiflorol	1764	0.58	MS[a]

[a] Previously reported by Brophy *et al.* (1989). [b] Tentative. [c] Insufficient resolution to allow accurate quantitation.

served, overlap occurred with *p*-cymene and α-terpinene, respectively, making accurate quantitation difficult. Separation of these common terpenes will be therefore somewhat dependent on their relative concentrations.

Further optimization of chromatographic conditions may provide the additional resolution needed to fully resolve these compounds. In addition, the sesquiterpenes, which constitute only a small fraction of *M. alternifolia* oil, are also separated, although no attempt was made to investigate or substantiate any chiral separations of this group. This gas chromatographic technique therefore provides a powerful analytical tool for the determination of *M. alternifolia* oil quality, including optical purity, in one injection.

A comparison of the composition figures obtained for the same oil using both β-cyclodextrin and a standard nonpolar column (BP1) is shown in Table II. In almost all cases the summed percentages of the enantiomeric pairs is in good agreement with the data obtained on BP1.

Although the enantiomeric purity of the major component of the commercial samples of terpinen-4-ol and α-terpineol used as standards in this work could be inferred from their optical rotations, another means of validating the enantiomeric purity is essential. NMR using specific lanthanide shift reagents (LSRs) has been shown to be capable of distinguishing between some of the types of enantiomers encountered in essential oils (Ravid *et al.*, 1988b). This technique was therefore explored both to confirm the enantiomeric data generated by gas chromatography and to ascertain whether the technique could be used on a whole oil sample to give both compositional and enantiomeric purity data.

The addition of an LSR to a substrate may result in a shift of resonances due to the different enantiomers to higher or lower frequencies; the size of the chemical shift is determined primarily by the distance of a given lanthanide nucleus from a donor group on the substrate molecule. For the analysis of terpenes electron-donating groups such as alcohols and ketones are of particular importance (Parker, 1991; Sullivan, 1979). The complexes that are formed for each of the two enantiomers can have different averaged chemical shifts (Sullivan, 1979). Europium shift reagents are known to shift resonances to higher field (Parker, 1991), while praseodymium shift reagents induce shifts to lower frequency (Sullivan, 1979). For this study the chiral tris[3-(heptafluoropropylhydroxymethylene-(+)-camphorato] (hf(+)c) derivatives of europium and praseodymium [$Eu(hf(+)c)_3$, $Pr(hf(+)c)_3$] and praseodymium(III) tris[3-(heptafluoropropylhydroxymethylene)-(−)-camphorato] [$Pr(hf(-)c)_3$] were used.

Prior to analysis with LSR, commercial samples of (+)-terpinen-4-ol and (−)-α-terpineol were subjected to detailed ^{13}C and ^{1}H NMR using DEPT, INEPT, and ^{13}C–^{1}H HETCOR analyses using standard techniques to confirm NMR spectral assignments (data not shown). A preliminary study with LSR showed that the 9-CH_3 and 10-CH_3 methyl resonances in the ^{1}H NMR spectrum were the most suitable indicators of the enantiomeric purity of these alcohols. Figures 2–4 show the effects of various LSRs and [LSR]:[substrate] ratios on the ^{1}H NMR spectra of terpinen-4-ol and α-terpineol. Addition of $Eu(hf(+)c)_3$ to terpinen-4-ol caused the 9-CH_3 and 10-CH_3 methyl resonances previously centered at 0.93 and 0.89 ppm, respectively, to move to higher field, and the chemical shift differences between the two resonances increased (Figure 2). The 9-CH_3 resonances resolved into two sets of doublets. The larger signal was assigned to the (+)-isomer and the smaller signal to the (−)-enantiomer on the basis of the reported optical rotation, $[\alpha] = +29°$, of the sample. The 10-CH_3 ^{1}H resonance at lower field was not resolved. Addition of further $Eu(hf(+)c)_3$ caused partial overlap of the (+)- and (−)-enantiomeric resonances. The best separation was obtained at a ratio of $Eu(hf(+)c)_3$:terpinen-4-ol of 0.1. An enantiomeric purity of 75:25 was obtained

Table II. Monoterpene Composition of Some Commercial *M. alternifolia* Oils

compound	oil A,[a] %	oil A,[b] %	oil B,[b] %	oil C,[b] %	oil D,[b] %	oil E,[b] %	oil F,[d] %
α-thujene	0.75	0.44	0.74	0.73	0.65		2.00
(−)-α-pinene	2.65	0.18	0.26	tr	0.15	0.13	0.13
(+)-α-pinene		1.68	1.83	1.99	1.62	0.72	0.30
β-myrcene	0.86	0.46	0.45	0.55	0.50	0.17	0.55
(+)-sabinene				0.54			
(−)-sabinene				0.25			
(−)-α-phellandrene	0.50	0.14	0.10	0.25	0.15	0.99	9.66
(+)-α-phellandrene		*e*	*e*	*e*	*e*		
α-terpinene	9.71	9.90	11.00	13.05	10.39	0.83	0.65
(+)-β-pinene	0.61	0.15	0.23	0.21	0.11		
(−)-limonene	*c*					1.37	
p-cymene	3.89	4.96	3.93	3.45	2.80	3.53	10.90
(+)-limonene	*c*	0.51	0.63	0.64	0.62	11.03	0.59
(−)-β-phellandrene	*c*	0.22	0.24	0.36	0.25	0.69	0.89
(+)-β-phellandrene	*c*	0.36	0.38	0.55	0.46	0.21	
γ-terpinene	20.88	21.15	22.58	22.77	20.01	7.05	0.38
terpinolene	3.45	3.18	3.31	3.82	3.16	4.17	0.97
1,8-cineole	5.67[c]	3.04	2.62	3.47	2.65	8.15	0.23
(±)-linalool				tr		2.08	0.20
(±)-linalool						4.40	0.36
(+)-terpinen-4-ol	37.21	24.73	26.58	25.97	23.69	13.46	0.51
(−)-terpinen-4-ol		13.13	13.89	12.14	13.79	27.00	2.39
(−)-α-terpineol	2.86	0.69	0.65	0.57	0.56	1.48	0.75
(+)-α-terpineol		2.03	2.11	1.89	1.82	1.42	0.15
(+)-piperitone						3.64	60.45
(−)-piperitone						0.98	3.40

[a] Determined on BP1. [b] Determined on β-cyclodextrin. [c] Coelutes. [d] *E. dives*. [e] Detected but not quantified.

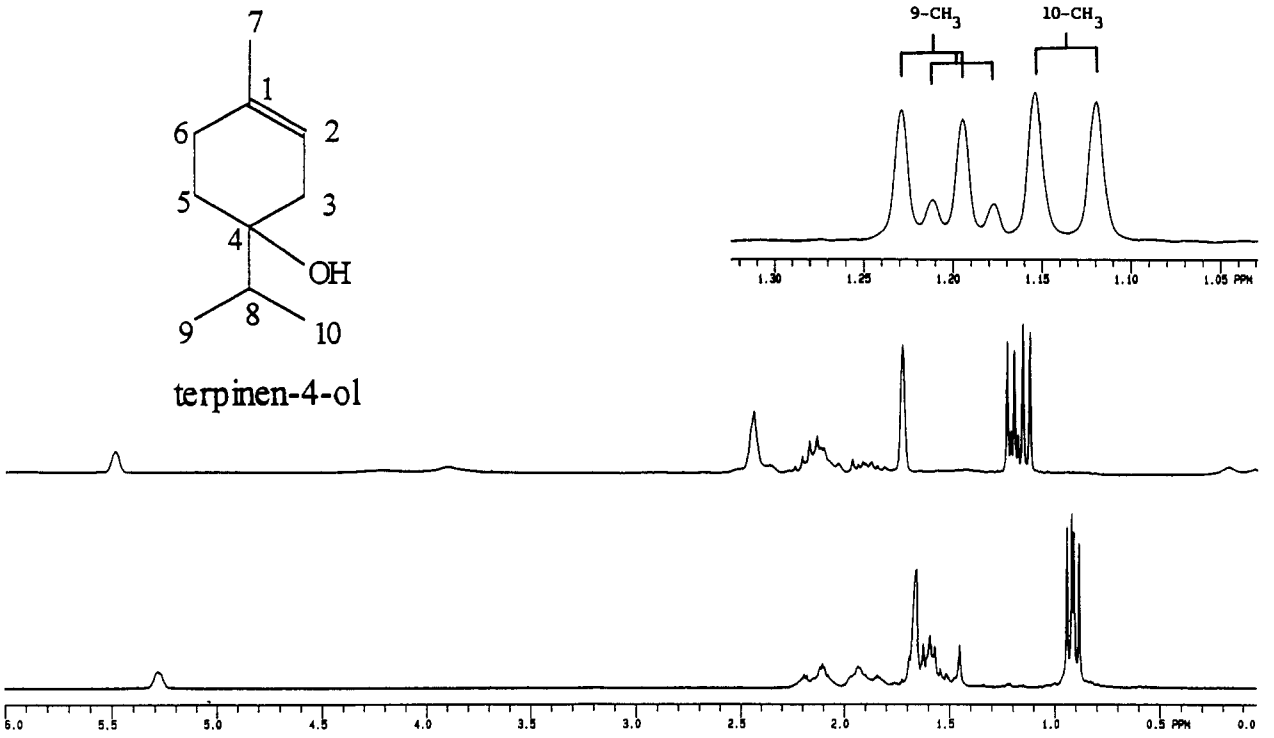

Figure 2. ^{1}H NMR spectra of terpinen-4-ol before (lower trace) and after (upper trace with expansion) the addition of Eu(hf(+)c)$_3$.

for (+)-terpinen-4-ol:(−)-terpinen-4-ol by integration of the 9-CH_3 peaks. Summation of the integral areas for the doublets at 1.19 and 1.21 ppm representing the (+)- and (−)-enantiomers of the 9-CH_3 group was equivalent in area to that of the 10-CH_3 group centered at 1.14 ppm. This confirmed that each group of signals represents three protons, without other overlapping resonances and no loss of signal. A similar study with the LSRs Pr(hf(+)c)$_3$ and Pr(hf(−)c)$_3$ failed to achieve resolution of the enantiomers.

Addition of Pr(hf(−)c)$_3$ to α-terpineol shifted the 9- and 10-CH_3 ^{1}H resonances at 1.17 and 1.15 ppm, respectively, to lower field (Figure 3). The 9-CH_3 ^{1}H resonance was resolved into two peaks representing the two enantiomeric forms, while the 10-CH_3 resonance remained unresolved. Increasing the amount of Pr(hf(−)c)$_3$ increased the separation between the three observed methyl resonances. Integration of the areas of the two 9-CH_3 ^{1}H resonances at Pr(hf(−)c)$_3$:α-terpineol ratios of both 0.77 and 1.54 yielded an enantiomeric purity of 65:35 for (−)-α-terpineol:(+)-α-terpineol. As above, the summation of the areas of the two 9-CH_3 enantiomeric resonances equaled the area of the single 10-CH_3 resonance.

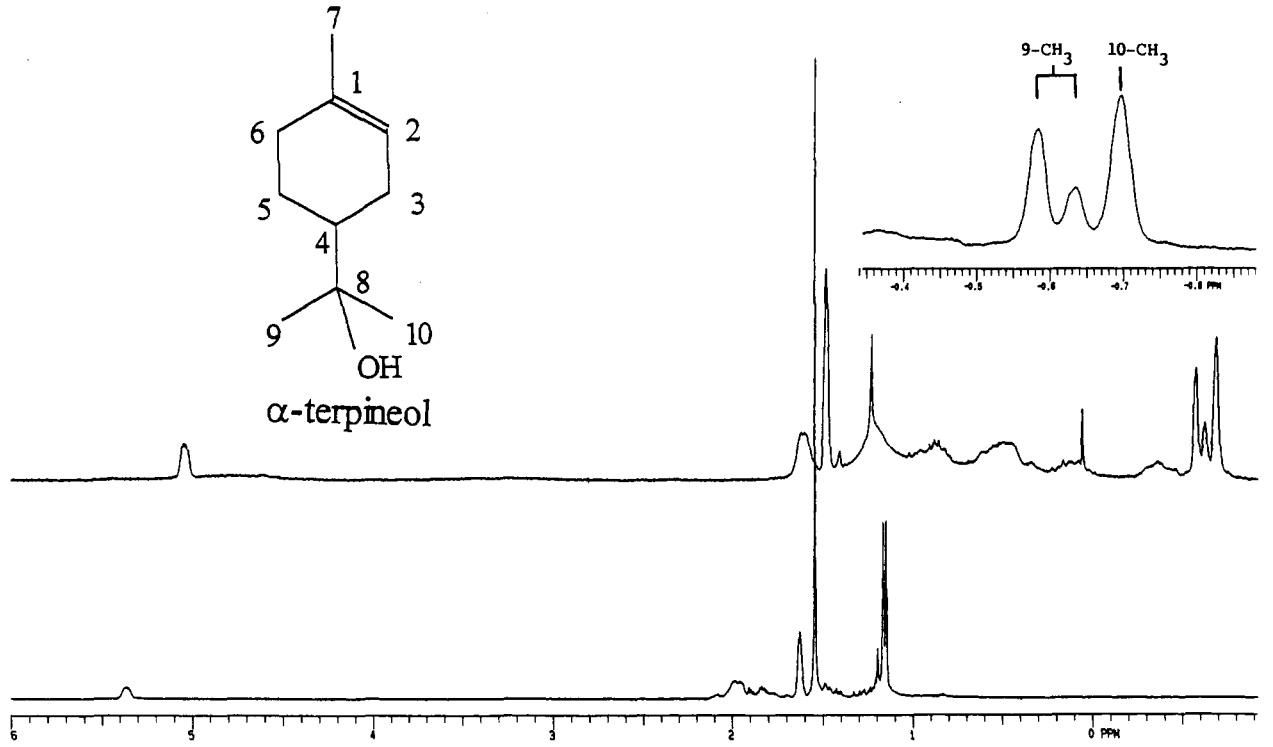

Figure 3. ^{1}H NMR spectra of α-terpineol before (lower trace) and after (upper trace with expansion) the addition of Pr(hf(−)c)$_3$.

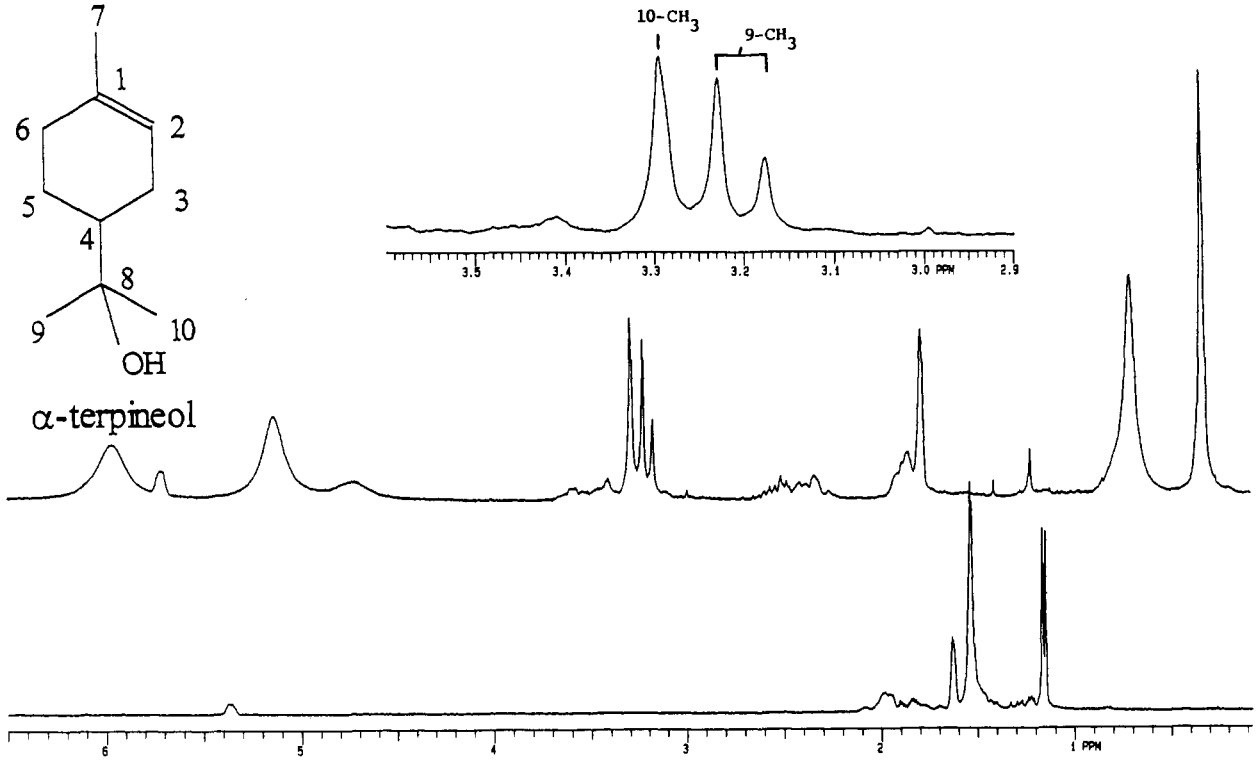

Figure 4. ^{1}H NMR spectra of α-terpineol before (lower trace) and after (upper trace with expansion) the addition of Eu(hf(+)c)$_3$.

Addition of Eu(hf(+)c)$_3$ to α-terpineol caused the 9- and 10-CH_3 ^{1}H resonances to move to higher field instead of lower field as in the case with Pr(hf(−)c)$_3$ (Figure 4). Again, only the 9-CH_3 resonances of the enantiomers were resolved, while the 10-CH_3 resonances remained unresolved. Integration of the resonances at a Eu(hf(+)c)$_3$:α-terpineol ratio of 0.27 indicated a 67:33 ratio of (−)-α-terpineol:(+)-α-terpineol, which was in good agreement with the previous result. Integration of the 9-CH_3 and 10-CH_3 methyl resonances confirmed that each group of signals represented three protons, without any overlapping resonances or loss of signal. A similar study with Pr(hf-(+)c)$_3$ failed to resolve the enantiomers.

The application of this approach to *M. alternifolia* oil is complicated by the complex nature of the mixture, and further investigation is required before this technique can provide the necessary quality information.

Analysis of the commercial (+)-terpinen-4-ol and (−)-α-terpineol samples using the gas chromatographic technique described above also provided excellent separation of the two enantiomeric pairs. Agreement between the NMR and the GC results for terpinen-4-ol (76:24, 72:28)

and α-terpineol (35:65, 33:67) was good and confirmed the enantiomeric purities.

A number of commercial oils were analyzed by the gas chromatographic method (Table II) to establish the variability of their enantiomeric composition. Although these oils showed minor variations in their composition, their enantiomeric composition remained uniform. A consistent 65:35 ratio of (+):(−) terpinen-4-ol confirmed the (+) form as the predominant isomer in *M. alternifolia* oil. This consistency of enantiomeric composition is also apparent for the other monoterpene alcohol, α-terpineol (76:24), with again the (+) form predominating. Other clearly resolved enantiomeric pairs such as α-pinene and β-phellandrene also show consistent enantiomeric composition and could be used as quality indicators.

An example of the application of this analytical method is demonstrated by the comparison of the analysis of high-quality *M. alternifolia* oil (oils A–D, Table II) with that of a tea tree oil of unknown origin (oil E). While the poor quality of the latter oil is apparent from the high levels of limonene, linalool, and piperitone, the chiral analysis is more informative as the enantiomeric ratios are inverted for both the monoterpene alcohols. The resolving power of this chiral column is further highlighted by the excellent enantiomeric separation under these conditions of linalool and piperitone. The tea tree oil may have been blended from a distilled fraction from *E. dives* oil (oil F, Table II), a primary source of piperitone. We have shown (unpublished results) that *E. dives* oil contains a predominance of the (−)-terpinen-4-ol. The comparatively low enantiomeric purity of the terpinen-4-ol found in *M. alternifolia* oil reflects the combination of biochemical and chemical pathways that can lead to the formation of this compound (Southwell and Stiff, 1989). The similarities between the enantiomeric purity of terpinen-4-ol from marjoram and *M. alternifolia* oil suggest comparable pathways for its formation both within the plant and during extraction.

CONCLUSION

The chiral phase gas chromatographic technique described above can be readily accommodated in most essential oils laboratories. The potential for this method to provide a complete and unambiguous quality standard for *M. alternifolia* and other essential oils has been clearly demonstrated.

The use of NMR techniques to determine the enantiomeric purity of single chiral compounds illustrates the capacity of this method to provide this information and to validate chromatographic data, but its application to the complex mixtures usually encountered in essential oils must be further investigated.

ACKNOWLEDGMENT

We thank the Rural Industries Research and Development Corp. and the UWS, Hawkesbury, for financial support of this work.

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Received for review February 3, 1993. Accepted June 3, 1993.