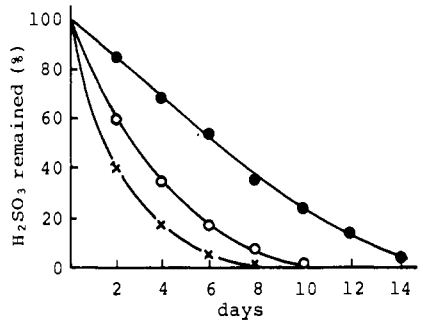

Figure 3. Oxidative decomposition of sulfites in phosphate buffer. A 500-mL flask containing 250 mL of 0.1 M sulfites in 0.2 M phosphate buffer (pH 7.0) was kept at 24 ± 1 °C. Sulfurous acid content was determined every other day. Solid circles represent lysine sulfite, open circles represent sodium bisulfite, and crosses represent sodium sulfite.

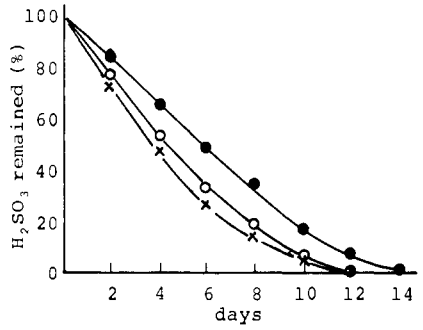

Figure 4. Oxidative decomposition of sulfites in acetate buffer. A 500-mL flask containing 250 mL of 0.1 M sulfites in 0.2 M acetate buffer (pH 4.0) was kept at 24 ± 1 °C. Sulfurous acid content was determined every other day. Solid circles represent lysine sulfite, open circles represent sodium bisulfite, and crosses represent sodium sulfite.

shown in Figures 2, 3, and 4. As shown in Figure 2, lysine sulfite is extremely stable in aqueous solution. After 14 days, remaining sulfurous acid content of lysine sulfite solution was more than 80% of the initial amount, while the contents in sodium bisulfite and in sodium sulfite were only 20% and almost 0%, respectively. In the buffered solutions, sulfites were oxidized more rapidly than in aqueous solution (see Figures 3 and 4). Also in this case, lysine sulfite was most stable, followed by sodium bisulfite and then sodium sulfite. However, the difference of stabilities of these salts became smaller at the lower pH. It may be speculated that lysine sulfite is liable to liberate sulfurous acid moiety at the lower pH.

In conclusion, lysine sulfite is much more stable than lysine hydrochloride in the browning reaction. Also the sulfite of this form is more stable than inorganic sulfites in the oxidative decomposition. Although further extensive studies should be done for the practical applications of lysine sulfite, the salt seems to be very versatile and promising. The application of lysine sulfite for food processing, especially for lysine fortified biscuit, seems advantageous. In the pharmaceutical field, sulfites are added in parenteral amino acid infusion to prevent oxidative deterioration. Further, amino acid infusion containing no sodium ion is recommended for some patients with renal disease. Lysine sulfite seems suitable as sulfurous acid source for such a sodium free parenteral infusion. Of course, lysine sulfite should be subject to the same regulatory considerations as other sulfite derivatives.

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Composition of Australian Tea Tree Oil (*Melaleuca alternifolia*)

Greg Swords and G. L. K. Hunter*

Australian tea tree oil (*Melaleuca alternifolia*) was fractionated by column chromatography and analyzed by combined gas chromatography–mass spectrometry. Preparative GLC of selected fractions yielded pure compounds for analysis by infrared and nuclear magnetic resonance spectroscopy. Forty compounds were identified, including viridiflorene which has not been previously reported as occurring in nature.

Tea tree oil is obtained by the steam distillation of *Melaleuca alternifolia*, a small paper-barked tree which grows in natural stands on swampy land along the north coast of New South Wales and the south coast of Queensland, Australia. Tea tree oil has been used in soap perfumes and as a bactericide. It is a good natural source of terpinen-4-ol.

The chemical composition of tea tree oil has been previously investigated by the Instrumental Laboratories of Fritzsche Brothers, Inc.; New York, and the following components were reported (Guenther, 1968): α-pinene, 2.2%; α-terpinene, 7.5%; limonene, 1.0%; 1,8-cineole, 5.6%; γ-terpinene, 17.5%; *p*-cymene, 3.0%; terpinolene, 3.1%; 1-terpinen-4-ol, 44.9%; α-terpineol, 5.2%; aroma-

Corporate Research & Development Department, The Coca-Cola Company, Atlanta, Georgia 30301.

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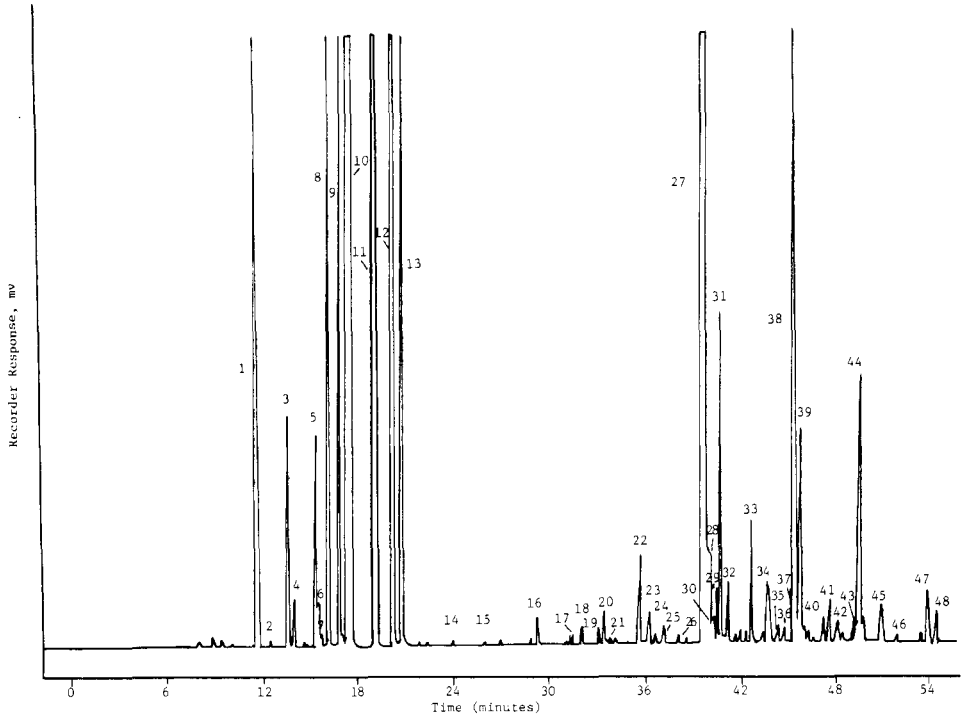


Figure 1. Australian Tea Tree Oil gas chromatogram.

dendrene, 2.7%; two unknown sesquiterpenes, 1.6% each. The purpose of this work was to characterize more fully the components of Australian Tea Tree Oil.

EXPERIMENTAL SECTION

Two commercial oils, one supplied by Citrus and Allied Essences Ltd. and the other by J. Manheimer, Inc., were compared by capillary GLC using a Perkin-Elmer 910 equipped with a 300 ft × 0.01 in. i.d. glass capillary column coated with Carbowax 20M and programmed from 60–180 °C at 2 °C/min with a helium flow rate of 2 mL/min. Relative peak areas were measured and calculated using a P.E.P.-2 data system. Chromatograms of the two samples were virtually identical. The Manheimer sample was selected for further investigation.

Ten grams of the oil was separated into ten fractions through a 44 × 1.5 cm column containing 40 g of Woelm activity 1 silica gel. The hydrocarbons were essentially removed from the oil in three 30-mL fractions using *n*-hexane as the eluent. Following this, the nonalcoholic oxygenated components were removed in three 30-mL fractions using 100 mL of ethyl acetate. The remaining components, consisting essentially of alcohols, were removed from the column in four separate fractions using a total of 100 mL of methanol. Each of the ten fractions was concentrated to 2 mL by distillation through a short (10 cm) fractionating column. Subsequent capillary gas chromatograms of each fraction showed no artifact formation when compared to one of the whole oil. Fractions 1 and 10 contained solvents only. Mass spectral and retention time data were sufficient for the identification of most of the components of fractions 3–7. Fraction 2 contained a number of sesquiterpenes which were identified by their infrared and NMR spectra following component separation and purification. Infrared and NMR spectra of some components of fractions 8 and 9 were also obtained, but most of those compounds remain unidentified.

A Varian Aerograph series 1200 FID gas chromatograph, containing a 6 m × 2 mm i.d. glass column packed with 5% Carbowax 20M on 100/120 mesh AW DCMS Chromasorb W, was fitted to the mass spectrometer. The temperature was programmed at 2 °C/min from 70–200 °C with a helium flow of 25 mL/min and the injector and detector temperatures maintained at 200 and 230 °C, respectively. Part of the column effluent was by-passed through a fine metering valve into a glass jet separator coupled to a Hitachi RMU-6L single focusing magnetic sector mass spectrometer. The separator and valve were maintained at 190 °C with a convection circulated air oven. All mass spectra were obtained at 70 eV and 70 μA with an ion source temperature of 190 °C.

Preparative gas chromatography was accomplished on an F/M 720 gas chromatograph using a T.C. detector. This instrument was fitted with an 8 ft × 0.375 in. o.d. stainless steel column packed with 25% Carbowax 20M on AW DCMS Chromasorb W to isolate pure components from fraction 2. An 8 ft × 0.25 in. o.d. stainless steel column packed with 10% SE-30 on AW DCMS Chromasorb W was used for fractions 8 and 9. Both columns were programmed from 75–200 °C at 2 °C/min. A helium flow rate of 150 mL/min was used for the Carbowax column and 70 mL/min for the SE-30 column while maintaining the injector and detector temperatures at 200 °C. The purified fractions were collected in cooled glass capillary tubes.

Infrared spectra were obtained by the addition of 2–5 μL of pentane or methanol to the capillaries containing the purified component. The solution was then slowly transferred via a microliter syringe onto NaCl plates and the solvent allowed to evaporate leaving neat liquids or cast solid films. Spectra were obtained using a Perkin-Elmer 247 or a Perkin-Elmer 281 grating infrared spectrophotometer equipped with beam condensers.

NMR spectra were obtained on a Varian T-60A 60 MHz NMR spectrophotometer using a microcell apparatus.

RESULTS AND DISCUSSION

The preliminary separation by compound type along with the use of a relatively long packed column greatly reduced the problem of resolving compounds in the GC–MS analysis. Peaks corresponding to the mass spectra

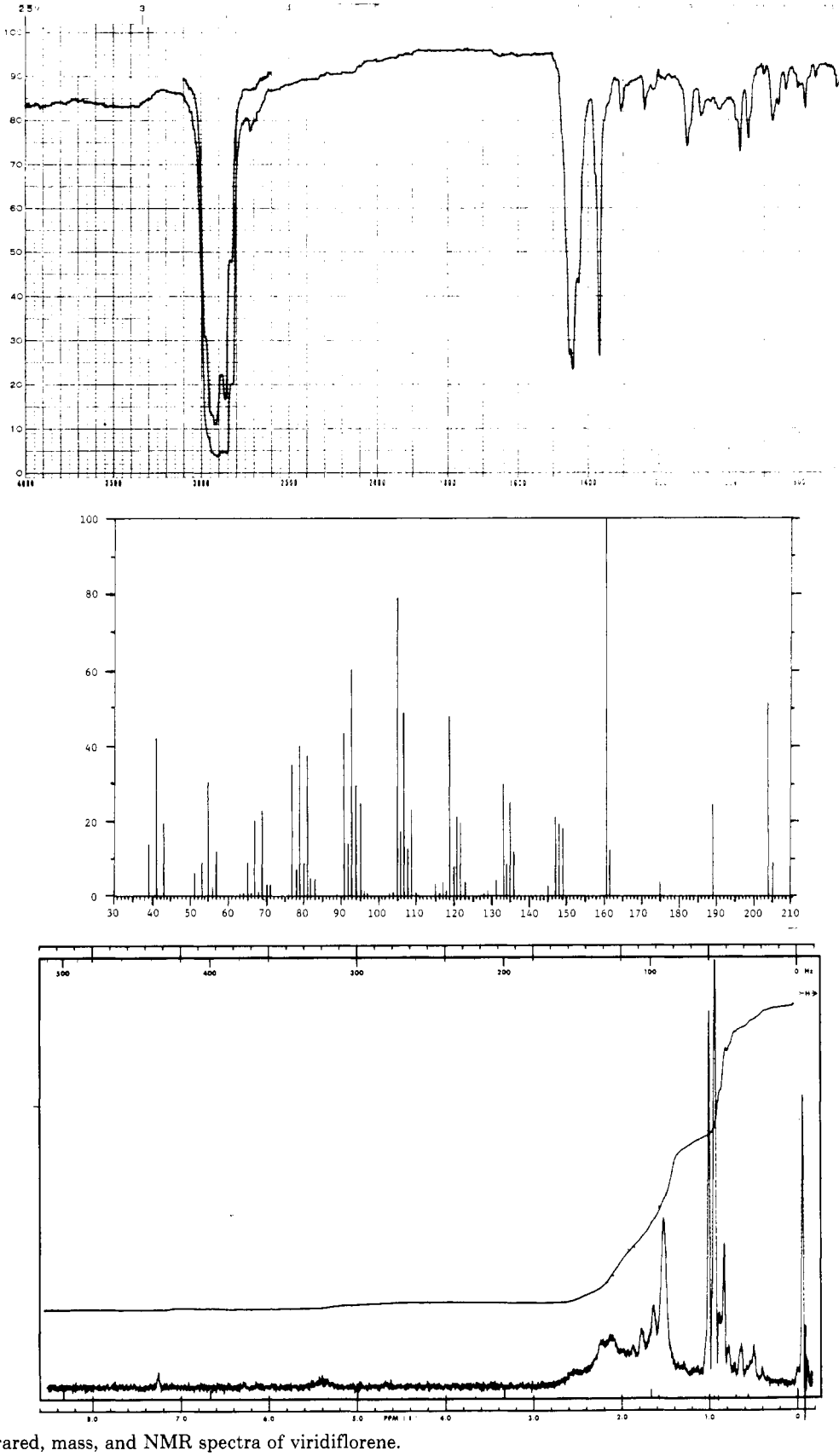


Figure 2. Infrared, mass, and NMR spectra of viridiflorene.

obtained from GC–MS analysis of the fractions were located on a capillary chromatogram of the whole oil by comparing the packed column chromatogram of each fraction with its capillary chromatogram, and then comparing the capillary chromatogram of the fraction with one of the whole oil. The purified components were identified by comparison of their retention times, mass, IR, and NMR spectra with data obtained in this laboratory from authentic compounds or with that published in the literature. A capillary gas chromatogram of the Manheimer sample is shown in Figure 1. Table I lists by peak numbers the compounds reported, the fraction(s) in which they were found, the experimental evidence for their identification, and their relative peak areas obtained from

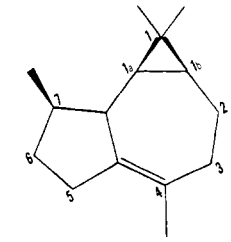
Table I

	Compound	Fraction	Evidence for Assignment[b]	Percent of Integration
1.	α-Pinene	0,[a] 2-5	GC MS	2.8
2.	Camphene	2	GC MS	Tr
3.	β-Pinene	0, 2-4	GC MS	0.59
4.	Sabinene	0	GC MS	0.12
5.	Myrcene	0, 2-5	GC MS	0.52
6.	α-Phellandrene	0, 2-5	GC MS	0.11
7.	1,4-Cineole	5, 6	GC MS	Tr
8.	α-Terpinene	0, 2-5	GC MS	2.74
9.	Limonene	0, 2-5	GC MS	3.09
10.	1,8-Cineole	0, 5-7	GC MS	16.50
11.	γ-Terpinene	0, 2-5	GC MS	11.54
12.	p-Cymene	0, 2-7	GC MS	11.42
13.	Terpinolene	0, 2-6	GC MS	2.36
14.	Hexanol	7	GC MS	Tr
15.	Allyl hexanoate	6, 7	GC MS	Tr
16.	p,α-Dimethylstyrene	0, 5-7	GC MS	0.07
17.	a Sesquiterpene	0, 2, 3	GC MS	Tr
18.	α-Cubebene	2, 3	GC MS[1,2]	0.04
19.	a Sesquiterpene	2-4	GC MS	Tr
20.	α-Copaene	0, 2-4	GC MS	0.10
21.	Camphor	6, 7	GC MS	Tr
22.	α-Gurjunene	0, 2-4	GC[3] MS[2] IR[4]	0.23
23.	Linalool	6, 7	GC MS	0.10
24.	a Sesquiterpene	2, 3	GC MS	Tr
25.	Unidentified	6, 7		0.05
26.	1-Terpineol	6	GC MS	Tr
27.	1-Terpinen-4-ol	0, 6, 7	GC MS	29.41
28.	β-Elemene	3-5	GC MS[1,5]	Tr
29.	Caryophyllene	3-5	GC MS[1]	Tr
30.	a Sesquiterpene	3-5	GC MS	0.06
31.	Aromadendrene	2-5	GC MS[2] IR[6]	2.35
32.	β-Terpineol	6, 7	GC MS	0.24
33.	Alloaromadendrene	2-5	GC MS[2] IR[6]	0.45
34.	Unidentified	6, 7		0.27
35.	Humulene	4-6	GC MS	Tr
36.	Unidentified	6, 7		Tr
37.	γ-Muurolene	2-4	GC[3] MS[2]	Tr
38.	α-Terpineol	0, 5-7	GC MS	3.61
39.	Viridiflorene	0, 2-4	GC MS IR[7] NMR	1.03
40.	Piperitone	7	GC MS	0.08
41.	α-Muurolene	2-4	GC[3] MS[2] IR[6]	0.12
42.	Piperitol	7	GC MS	0.07
43.	Unidentified	7, 8		0.07
44.	σ-Cadinene	0, 2-4	GC[3] MS[2] IR[4]	1.43
45.	4,10-Dimethyl-7-isopropyl bicyclo[4.4.0]-1,4-decadiene	4	MS[2]	0.10
46.	Nerol	7	GC MS	Tr
47.	8-p-Cymenol	6, 7	GC MS	0.13
48.	Calamenene	0, 2-5	GC[8,9] MS[5] IR[4,5]	0.10

[a] 0 refers to the whole oil. [b] 1, Moshanas and Lund (1970); 2, Stenhagen et al. (1970); 3, Andersen and Falcone (1969); 4, Wenninger et al. (1967); 5, Yukawa and Ito (1973); 6, Wenninger et al. (1970); 7, Serkebaeva et al. (1968); 8, Lawrence et al. (1969); 9, Yates and Wenninger (1970).

a capillary chromatogram of the whole oil.

The structure of compound 39, viridiflorene (I), was identified from mass, IR, and NMR spectral data. Subsequently, the infrared spectrum of I was shown to be in good agreement with that reported by Serkebaeva et al. (1968). The infrared, NMR, and mass spectra of viridiflorene are reproduced in Figure 2.



Viridiflorene (I)

The absence of strong absorption around 900 cm^{-1} in the infrared spectrum of viridiflorene suggests that there are no vinylic hydrogens. The lack of splitting of the peak near 1375 cm^{-1} indicates the absence of a normal gem dimethyl group. Typical C–H stretching and bending absorbances are found around 2900 and 1440 cm^{-1}. The mass spectrum of I is similar to that of aromadendrene and alloaromadendrene, exhibiting a base peak at m/e 161, a relatively large molecular ion at m/e 204, and characteristic groups of ions around m/e 105, 93, 119, 79, 133, and 147. The NMR spectrum contains two small (1 H) multiplets at δ 0.57 and 0.70 due to the two cyclopropyl protons, one peak of a doublet (3 H, δ 0.95, J = 6 Hz) from the C-7 methyl group at δ 0.92, a singlet containing the other peak of the doublet and one of the C-1 methyl groups at δ 1.0 ppm, and another singlet (3 H) at δ 1.04 from the other C-1 methyl group. A broad singlet (3 H) at δ 1.58 is due to the C-4 methyl group. A complex array of unresolved multiplets between δ 1.6–2.6 is attributed to the remaining protons on carbon atoms 2, 3, and 5–8. No signals were found in the vinylic region.

Viridiflorene, which has previously been prepared by the dehydration of viridiflorol (Serkebaeva et al., 1968), is now for the first time being reported as occurring in nature.

Compounds 17, 19, 24, and 30 were identified as sesquiterpenes by the molecular ion peak at m/e 204, and characteristic sesquiterpene peaks at 41, 69, 91, 105, 161, and 189. They were not further characterized.

Compound 45 was tentatively identified as 4,10-dimethyl-7-isopropyl-bicyclo[4.4.0]1,4-decadiene by its mass spectrum as reported by Stenhagen et al. (1970).

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