

Figure 1. Chromatogram of an apple sample (1) with 1.0 mg/kg of both ziram (a) and zineb (b) added and (2) without. Full scale corresponds to 0.2 absorbance unit.

given in Table I. They are calculated with regard to the contents of active ingredients in the analytical standards used.

Water solutions of disodium *N,N'*-ethylenebis(dithiocarbamate) of pH 7.0 with and without EDTA added were

analyzed in order to examine if the presence of EDTA influenced the recovery of zineb in a way other than by sequestering zinc cations. No such effect was found. The recoveries of zineb, ziram, and thiram from fortified food samples were linear within the concentration ranges studied, 0.01–1.5 mg/kg for ziram and thiram and 0.02–1.5 mg/kg for zineb when L-cysteine or sodium *N-tert*-butyldithiocarbamate was added. Figure 1 shows chromatograms of an apple sample free from dithiocarbamates and an apple sample fortified with ziram and zineb before extraction with the EDTA solution. The limits of detection for zineb, ziram, and thiram are below 0.02, 0.01, and 0.01 mg/kg, respectively. The limits of detection were not optimized.

Registry No. Thiram, 137-26-8; L-cysteine, 52-90-4; zineb, 12122-67-7; ziram, 137-30-4; EDTA, 60-00-4.

LITERATURE CITED

- Engst, R.; Schnaak, W. *Residue Rev.* 1974, 52, 45–67.
 Gustafsson, K. H.; Thompson, R. A. *J. Agric. Food Chem.* 1981, 29, 729–732.
 Thier, H.-P., et al. *Lebensmittelchem. Gerichtl. Chem.* 1977, 31, 25–27.

K. Håkan Gustafsson*¹
 Christer H. Fahlgren²

¹Department of Natural Sciences with Technology
 University College of Kalmar
 S-391 29 Kalmar, Sweden
²Food Research Department
 National Food Administration
 S-751 26 Uppsala, Sweden

Received for review November 16, 1981. Revised manuscript received October 6, 1982. Accepted November 8, 1982.

The Volatile Constituents of *Schinus molle* L.

The volatile constituents of the fruit of *Schinus molle* L. have been investigated by gas chromatography-mass spectrometry. Forty-six compounds were identified or partially identified in the oil which was obtained from this fruit by a distillation-extraction method (Likens-Nickerson apparatus). The compounds reported here were 9 monoterpene hydrocarbons, 1 aromatic compound, 1 aliphatic acid ester, 2 monoterpene esters, 16 sesquiterpene hydrocarbons, and 17 other sesquiterpenoids. Major components of this oil were myrcene, α -phellandrene, δ -cadinene, limonene, α -cadinol, and β -phellandrene.

The California pepper tree (*Schinus molle* L.) is grown extensively as an ornamental plant in many areas of the Americas. Fruit from this tree yields a volatile oil that has been used as a substitute for black pepper, in flavor compositions, and in pharmaceutical products (Gonzales and Lombardo, 1946; Ottolino, 1948). In Greece the fruit serves for the preparation of certain beverages (Guenther, 1952).

A number of investigators have examined the physico-chemical properties and chemical composition of the oil, but relatively few components have been identified and only tentative identification of some compounds has been accomplished (Brückner van der Lingen, 1930; Gonzales, 1931; Ottolino, 1948; Bernhard and Wrolstad, 1963). Cremonini (1928, 1930) indicated the presence of *trans*-

terpin, ferrojone, and a sugar with properties similar to those of glucose. Bernhard and Wrolstad (1963) reported the presence of α -pinene, β -pinene, α -phellandrene, β -phellandrene, myrcene, D-limonene, camphene, *p*-cymene, and three unidentified constituents. More recently Jennings and Bernhard (1975) found, in addition to the above, sabinene, α -terpinene, γ -terpinene, terpinolene, methyl *n*-octanoate, bourbonene, α -*trans*-bergamotene, caryophyllene, α -terpineol, germacrene D, and δ -cadinene. Terhune et al. (1974) reported isolating and characterizing a new sesquiterpene, β -spatulene. Quite recently Pozzo-Balbi et al. (1976, 1978) have isolated several triterpenoid acids from an acidic fraction of an oleoresin obtained from the fruit.

Table I. Compounds Identified in the Oil of *S. molle* L. Fruit and Their Kovats Indices

peak	compound	peak area, %	Kovats indices		reference essential oil
			I_u	I_k	
1	α -pinene ^j	2.66	1031	1031	lemon oil ^b
3	camphene ^j		1071	1072	lemon oil ⁱ
4	β -pinene ^j	0.17	1108	1107	lemon oil ^b
5	sabinene ^j		1109	1109	lemon oil ^b
6	myrcene ^j	20.41	1150	1149	lemon oil ^b
7	α -phellandrene ^j	17.34	1164	1161	grapefruit oil ^a
9	limonene ^j	7.23	1188	1190	lemon oil ^b
10	β -phellandrene ^j	6.21	1198	1197	grapefruit oil ^a
15	<i>p</i> -cymene ^j	2.93	1245	1245	lemon oil ^a
16	terpinolene ^j	0.10	1262	1263	lemon oil ⁱ
19	methyl octanoate ^j	0.95	1352	1355	
20	unknown	0.11	1365		
25	unknown		1415		
28	α -cubebene	0.13	1442	1442	cubeb oil ^f
30	α -copaene	0.12	1476	1477	hop oil ^e
36	unknown	0.15	1487		
39	α -gurjunene (tentative)	0.71	1511		
43	β -elemene	0.52	1556	1556	grapefruit oil ^h
45	β -caryophyllene ^j	2.02	1570	1570	lemon oil ^e
47	sesquiterpene hydrocarbon M = 204	0.44	1607		
51	α -humulene	0.58	1632	1635	hop oil ^e
55	γ -muurolene	0.48	1655	1658	hop oil ^e
58	sesquiterpene hydrocarbon M = 204	0.24	1670		
61	α -muurolene	1.48	1684	1685	cubeb oil ^f
63	δ -cadinene ^j	9.11	1721	1722	hop oil ^e
64	sesquiterpene hydrocarbon M = 204	0.67	1732		
67	β -guainene (tentative)	0.22	1745		
68	sesquiterpene M = 220	0.16	1762		
70	calamenene	0.28	1778	1779	hop oil ^e
71	sesquiterpene M = 222	0.16	1806		
72	sesquiterpene M = 220		1816		
73	geranyl butyrate	0.34	1833	1836	
74	γ -calacorene	0.15	1847	1850	hop oil ^e
75	unknown	0.28	1852		
78	α -calacorene	0.26	1904	1903	hop oil ^e
79	unknown	0.72	1909		
80	sesquiterpene M = 220	0.28	1950		
83	sesquiterpene M = 220	0.25	1976		
84	sesquiterpene M = 222	0.22	1981		
85	elemol	1.32	1987	1991	elemi oil ^g
86	sesquiterpene M = 222	0.34	1994		
88	neryl hexanoate	0.28	2031	2033	
92	γ -eudesmol	1.04	2074	2079	eucalyptus oil ^d
93	<i>T</i> -cadinol	1.39	2078	2081	basil oil ^c
94	<i>T</i> -muurolol	2.32	2092	2091	hop oil ^e
95	δ -cadinol	0.69	2100	2100	hop oil ^e
96	α -eudesmol	0.90	2122	2128	eucalyptus oil ^d
97	β -eudesmol	1.01	2131	2135	eucalyptus oil ^d
98	α -cadinol	6.64	2139	2138	hop oil ^d
99	sesquiterpene M = 222	0.26	2150		
106	sesquiterpene M = 222	0.41	2210		

^a Ashoor and Bernhard (1967). ^b Ikeda et al. (1962). ^c Lawrence et al. (1972). ^d McQuillin and Parrack (1956). ^e Naya and Kotake (1971). ^f Ohta (1966). ^g Summa (1960). ^h Wilson and Shaw (1978). ⁱ Ziegler (1971). ^j Previously reported in the literature.

The present investigation was conducted to reevaluate results of previous studies and to determine if additional identification of volatile components could be carried out by using more modern chromatographic techniques.

EXPERIMENTAL SECTION

Taxonomic identification of this plant was made by Andrew Sanders, Museum Scientist, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

Fully mature *S. molle* fruit was collected from a single tree on the Riverside campus of the University of California in November of 1980. The fruit was separated from twigs, stems, and leaves by hand, weighed (362.9 g), and rinsed rapidly with deionized water. The fruit was mixed

with 300 mL of deionized water and comminuted in a blender for 10 min. This slurry was subjected to simultaneous distillation and extraction (Schultz et al., 1977), a modification of the method of Likens and Nickerson (1964). The extracting solvent was methylene chloride, and steam distillation-extraction (CH₂Cl₂/H₂O = 250 mL/1500 mL) was continued for 18 h. The extract was dried over anhydrous sodium sulfate for 12 h, and the solvent was removed by using a rotary flash evaporator. Approximately 10.3 g (2.84% relative to the quantity of fruit used) of a pale yellow volatile oil with a pungent aroma was obtained. The oil had a specific rotation of $[\alpha]_D^{25} +71.97^\circ$ (Union Giken Model PM-101 automatic digital polarimeter).

The oil was subjected to gas chromatographic analyses

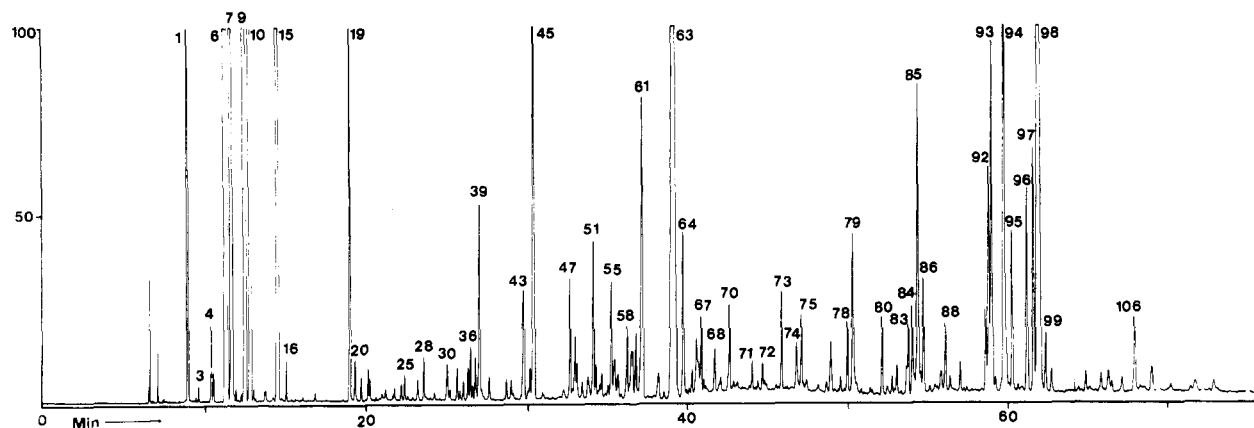


Figure 1. Chromatogram of 1 μ L of the oil of *S. molle* L. fruit, split ca. 1:100, on a 50 m \times 0.28 mm WCOT glass capillary column coated with PEG Carbowax 20M. The column temperature was programmed from 80 to 220 $^{\circ}$ C at 2 $^{\circ}$ C/min. The injector and detector temperatures were 250 $^{\circ}$ C. For peak identification, see Table I.

using a wall-coated open-tubular glass capillary column (WCOT) coupled to a glass splitter of our own design. Identifications were based on mass spectra obtained on a gas chromatograph-mass spectrometer interfaced with a glass capillary column (Carbowax 20M, 100 m \times 0.28 mm i.d.; programmed from 80 to 200 $^{\circ}$ C at 3 $^{\circ}$ C/min) and confirmed by retention data (Kovats, 1958; Ettre, 1964) on another high-resolution system. The other WCOT glass capillary column used in these investigations was 50 m \times 0.28 mm i.d. that was also coated with Carbowax 20M. The columns were prepared in our own laboratory by using the heated inlet tube modification of the Golay technique (Jennings et al., 1974). Both columns possessed over 4000 theoretical plates per m at $K = 3$. A Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector modified for capillary use was used for all retention determinations. Column temperature was programmed from 80 to 220 $^{\circ}$ C at 2 $^{\circ}$ C/min. Columns were operated with nitrogen carrier gas at an average linear velocity of 13 cm/s based on a methane peak at 120 $^{\circ}$ C. This was equivalent to a flow rate of ca. 0.68 cm³/min.

A Hewlett-Packard Model 3385-A reporting integrator was used to determine the peak areas reported in Table I.

A Hitachi Model M-80 mass spectrometer equipped with a Hitachi Model 00-3B data system was used for mass spectral identification of the gas chromatographic components under the following conditions: ion source temperature, 180 $^{\circ}$ C; ionization voltage, 20 eV.

RESULTS AND DISCUSSION

Table I shows the compounds identified in the oil of *S. molle* L. fruit. Peak numbers on the left side show the elution order on the 50-m Carbowax 20M column (Figure 1). Peak areas which had a value of less than 0.1% are not listed. I_u designates retention indices of unknowns. I_s represents the retention indices of authentic samples. For some compounds, formulas were deduced from mass spectral data, but known compounds were not available for comparison. We listed those compounds as "tentatively" identified. Known samples were isolated from the sources (essential oils) listed in the column to the right together with pertinent literature references.

The largest fraction of the oil consists of monoterpene hydrocarbons (57.55 area %) of which myrcene and α -phellandrene are present in the greatest quantities. There appears to be small amounts of an aliphatic acid ester and two terpenoid esters. There are at least 16 sesquiterpene

hydrocarbons present (17.41 area %) of which δ -cadinene is present in the largest amount and 17 sesquiterpenoids (17.39 area %) of which α -cadinol is present in the largest quantity. We were unable to detect the presence of β -spathulene as reported by Terhune et al. (1974).

The oil has a rather pungent, pepper-like aroma and some of this pungency is quite likely due to the presence of the many terpenoid hydrocarbons. Many of the monoterpene hydrocarbons have been described as having light citrus-like aromas, while the sesquiterpenoids have woody-spicy and tarry odors. The aromatic compound *p*-cymene is a well-characterized oxidation product of γ -terpinene (Ikeda et al., 1961). It is interesting to note the large number of sesquiterpenes found in this oil and the relative absence of esters, aldehydes, ketones, etc.

Registry No. α -Pinene, 80-56-8; camphene, 79-92-5; β -pinene, 127-91-3; sabinene, 3387-41-5; myrcene, 123-35-3; α -phellandrene, 99-83-2; limonene, 138-86-3; β -phellandrene, 555-10-2; *p*-cymene, 99-87-6; terpinolene, 586-62-9; methyl octanoate, 111-11-5; α -cubebene, 17699-14-8; α -copaene, 3856-25-5; α -gurjunene, 489-40-7; β -elemene, 515-13-9; β -caryophyllene, 87-44-5; α -humulene, 6753-98-6; γ -muurolene, 30021-74-0; α -muurolene, 10208-80-7; δ -cadinene, 483-76-1; β -guaiene, 88-84-6; calamenene, 483-77-2; geranyl butyrate, 106-29-6; γ -calacorene, 24048-45-1; α -calacorene, 21391-99-1; elemol, 639-99-6; neryl hexanoate, 68310-59-8; δ -eudesmol, 1209-71-8; *T*-cadinol, 5937-11-1; *T*-muurolol, 19912-62-0; δ -cadinol, 36564-42-8; α -eudesmol, 473-16-5; β -eudesmol, 473-15-4; α -cadinol, 481-34-5.

LITERATURE CITED

- Ashoor, S. H. M.; Bernhard, R. A. *J. Agric. Food Chem.* **1967**, *15*, 1044.
 Bernhard, R. A.; Wrolstad, R. *J. Food Sci.* **1963**, *28*, 59.
 Brückner van der Lingen, G. W. *Perfum. Essent. Oil Rec.* **1930**, *21*, 154.
 Cremonini, A. *Ann. Chim. Appl.* **1928**, *18*, 361.
 Cremonini, A. *Ann. Chim. Appl.* **1930**, *20*, 309.
 Ettre, L. S. *Anal. Chem.* **1964**, *36*, 31A.
 Gonzales, M. *An. Fac. Quim. Farm., Univ. Montevideo* **1931**, *1*, 133.
 Gonzales, M.; Lombardo, A. *Rev. Farm. (Buenos Aires)* **1946**, *88*, 297.
 Guenther, E. "The Essential Oils"; Van Nostrand: New York, 1952; Vol. V, p 165.
 Ikeda, R. M.; Stanley, W. L.; Rolle, L. A.; Vannier, S. H. *J. Food Sci.* **1962**, *27*, 593.
 Ikeda, R. M.; Stanley, W. L.; Vannier, S. H.; Rolle, L. A. *Food Technol. (Chicago)* **1961**, *15*, 379.

- Jennings, W. G.; Bernhard, R. A. *Chem., Mikrobiol., Technol. Lebensm.* 1975, 4, 95.
- Jennings, W. G.; Yabumoto, K.; Wohleb, R. H. *J. Chromatogr. Sci.* 1974, 12, 344.
- Kovats, E. *Helv. Chim. Acta* 1958, 41, 1915.
- Lawrence, B. M.; Hogg, J. W.; Terhune, S. J.; Pitchifakul, N. *Flavour Ind.* 1972, 3, 47.
- Likens, S. T.; Nickerson, G. B. *Proc. Am. Soc. Brew. Chem.* 1964, 5.
- McQuillin, F. J.; Parrak, J. D. *J. Chem. Soc.* 1956, 2973.
- Naya, Y.; Kotake, M. *Bull. Chem. Soc. Jpn.* 1971, 44, 3116.
- Ohta, Y. Ph.D. Thesis, Institute of Food Chemistry, Osaka, Japan, 1966.
- Ottolino, G. *Atti Relaz.—Accad. Pugliese Sci.* 1948, 6, 49.
- Pozzo-Balbi, T.; Nobile, L.; Scapini, G.; Cini, M. *Gazz. Chim. Ital.* 1976, 106, 785.
- Pozzo-Balbi, T.; Nobile, L.; Scapini, G.; Cini, M. *Phytochemistry* 1978, 17, 2107.
- Schultz, T. H.; Flath, R. A.; Mon, T. R.; Egging, S. B.; Teranishi, R. *J. Agric. Food Chem.* 1977, 25, 446.
- Summa, A. F. Ph.D. Thesis, University of Connecticut, 1960.
- Terhune, S. J.; Hogg, J. W.; Lawrence, B. M. *Phytochemistry* 1974, 13, 865.
- Wilson, C. W., III; Shaw, P. E. *J. Agric. Food Chem.* 1978, 26, 1432.
- Ziegler, E. *Flavour Ind.* 1971, 2, 647.

Richard A. Bernhard^{*1}
Takayuki Shibamoto²
Kenji Yamaguchi³
Elaine White¹

¹Departments of Food Science and Technology
University of California, Davis
Davis, California 95616

²Department of Environmental Toxicology
University of California, Davis
Davis, California 95616

³Ogawa & Co., Ltd.
6-32-9 Akabanenishi, Kita-Ku, Tokyo, Japan

Received for review May 17, 1982. Accepted December 20, 1982.
The research was performed at Davis. The mass spectral data
were obtained in Japan.