

Table II. Apparent Distribution Coefficients

compound	apparent dist coefficient, $C_o/C_w$
limonene	11 700
methylheptenone	11.5
linalool	320
isopulegol	970
neral	1 410
geranial	1 380
neryl acetate	24 900

Amaha, 1972), we found it to be a major component in our study. Both our study and theirs, on the other hand, show high concentrations of limonene, geranial, and neral. The high geranial and neral concentrations were also characteristic of Key lime peel oil (Shaw, 1979). We are also reporting methylheptenone, isoisopulegol, and citronellol as components of Key lime leaf oil for the first time.

Table II shows apparent oil-water distribution coefficients for some of the identified components. The coefficient is not necessarily an equilibrium value, since the phases were not equilibrated. These relative values, however, should indicate relative water solubilities.

The coefficient for neryl acetate was much higher and that for methylheptenone lower than we predicted based on previously reported values for equilibrium coefficients [100-700 (Lund and Bryan, 1976)]. This may have been because the phases were not equilibrated. If hydrolysis of neryl acetate in the aqueous phase occurred faster than diffusion from oil to aqueous layer, this could have caused the observed high coefficient for the neryl acetate. Nerol, a product of such hydrolysis, however, was not identified, in contrast to the previous study (Lund et al., 1981). Thus, it was not possible to verify the occurrence of this hydrolytic degradation reaction. Also, the lack of identifiable decomposition products makes it difficult to rationalize the unusually low coefficient for methylheptenone.

The values (1400) for neral and geranial were relatively high compared with previous values [400-600 (Lund and Bryan, 1976)], probably a result of the unusually high polarity of the oil phase. The latter contained only 31% hydrocarbons; the remainder was a mixture of polar compounds, principally geranial and neral (30% of total). In contrast, rough lemon leaf oil contained 78% hydro-

carbons, and the solubility of polar components in it (particularly geranial and neral) would have been much lower.

Thirty components were isolated but not identified. On the basis of GC and other data, most of them have never been reported as constituents of citrus leaf or peel oils. Some of them had potent aromas resembling that of lemon or lime. We previously found a number of them in rough lemon leaf oil (Lund et al., 1981) and in Meyer lemon leaf oils. This unidentified group included two alcohols, eight unsaturated hydrocarbons, one ester, and two uncharacterized compounds. Seven of the isolated unidentified hydrocarbons seem to be related as evidenced by their similar infrared spectra. We also isolated three acids which had spectra similar to each other.

Individual compounds have not been tested as blackfly attractants because a suitable bioassay has not been developed. However, these data on new Key lime leaf oil components may be useful in other areas of research, such as taxonomy or identification of new fragrance components.

#### ACKNOWLEDGMENT

We appreciate the assistance of Bryan Steinberg of the Agricultural Research Center, University of Florida, Ft. Lauderdale, FL, in identification and collection of leaves. Samples were analyzed by GC-MS at the University of Alabama in Birmingham, Comprehensive GC-MS Center (Dr. R. L. Settine).

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Received for review March 24, 1981. Revised manuscript received October 19, 1981. Accepted October 19, 1981. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of others which may also be suitable.

## Components of Meyer Lemon Leaf Oil

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Twenty-two compounds were isolated by gas chromatography from steam-distilled leaf oil of Meyer lemon (*Citrus limon* X *Citrus sinensis*) and identified by infrared spectroscopy, gas chromatographic retention times, and, in some instances, mass spectroscopy. The identified components and relative amounts were as follows (peak area percent): limonene, 73; 1,8-cineole, 7; isopulegol, 4; linalool, 1.7; myrcene, 1.3; citronellal, 1.2;  $\alpha$ -terpineol, 1.1; terpinen-4-ol, 1.0; ocimene, 1.0; geranial, 0.9;  $\gamma$ -terpinene, 0.7; neral, 0.7; methylheptenone, 0.6; geranyl acetate, 0.3; sabinene, 0.27;  $\alpha$ -pinene, 0.2; isoisopulegol, 0.16; thymol, 0.04;  $\beta$ -caryophyllene, 0.010; neryl acetate, 0.004; citronellol, 0.002; caryophyllene oxide,  $8 \times 10^{-5}$ . A number of additional compounds were isolated but not positively identified.

Recently, several citrus species have been observed to be preferential hosts for citrus blackfly [*Aleurocanthus*

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*woglumi* (Howard, 1979)]. Because of the potential serious damage to the citrus crop from this insect, compounds that affect its behavior are of interest. The long-range attractant is probably leaf color (Dowell, 1979); however, volatile leaf components could contribute to the attractancy or affect the behavior of the insect in other ways.

Table I. Composition of Meyer Lemon Leaf Oil and Aqueous Phase

no.	compound <sup>a</sup>	composition, peak area %		
		oil	aqueous	combined
1	unidentified, hydrocarbon or ether		0.020	0.020
2	$\alpha$ -pinene	0.21		0.21
3	sabinene <sup>b</sup>	0.27		0.27
4	1,8-cineole		7.0	7.0
5	myrcene <sup>b</sup>	1.3		1.3
6	limonene	73		73
7	ocimene	0.99		0.99
8	$\gamma$ -terpinene <sup>b</sup>	0.70		0.70
9	methylheptenone <sup>b</sup>		0.60	0.60
10	citronellal <sup>b</sup>	1.0	0.18	1.2
11	linalool <sup>b</sup>	0.23	1.5	1.7
12	isopulegol <sup>b</sup>	3.0	1.0	4.0
13	isopulegol <sup>b</sup>	0.16		0.16
14	terpinen-4-ol <sup>b</sup>		1.0	1.0
15	$\beta$ -caryophyllene	0.010		0.010
16	$\alpha$ -terpineol <sup>b</sup>		1.1	1.1
17	neral <sup>b</sup>	0.07	0.60	0.67
18	geranial		0.90	0.90
19	geranyl acetate	0.3		0.3
20	neryl acetate <sup>b</sup>		0.004	
21	citronello <sup>b</sup>	0.002		0.002
22	caryophyllene oxide <sup>b</sup>	0.00008		0.00008
23	thymol	0.007	0.03	0.04
	unidentified, total <sup>c</sup>	2.3	2.5	4.8

<sup>a</sup> In order of elution from diethylene glycol succinate. <sup>b</sup> Identified by mass spectral comparison, in addition to IR spectra. <sup>c</sup> Total peak area of unidentified compounds including two hydrocarbons, five alcohols, nine esters, five aldehydes, three ketones, and three carboxylic acids.

Among the citrus species, lemon leaves from several varieties are preferred hosts (Dowell et al., 1978). Meyer lemon, one of the more common lemon species grown in Florida, was selected for a detailed study of leaf oil composition.

No published data could be found on Meyer lemon leaf oil composition; however, oil from the flowers (Goryaev et al., 1979) and peel (Moshonas et al., 1972) has been studied. In addition, leaf oil from other lemon varieties has been analyzed (Lund et al., 1981; Scora et al., 1969; Kamiyama, 1967; Attaway et al., 1966; Kesterson et al., 1964).

We prepared steam-distilled oil from Meyer lemon leaves and analyzed the oil and aqueous fractions separately. Our purpose was to identify components isolated from both fractions.

#### MATERIALS AND METHODS

**Preparation of Oil and Concentrated Extract of Aqueous Codistillate.** Mature Meyer lemon leaves (10900 g) were obtained from a budwood test grove maintained by the State of Florida. The Meyer lemon trees were grown on sour orange rootstock. The leaves were steam distilled and extracted as described previously (Lund et al., 1981). The oil layer weighed 9.0 g (density at 25 °C was 0.85 g/mL). A 2.0-g sample of concentrated extract (density at 25 °C was 0.95 g/mL) was obtained by methylene chloride extraction from a total aqueous codistillate volume of 6.6 L.

**Fractionation of Oil and Concentrated Extract.** Methods for fractionation were described previously (Lund et al., 1981). The oil and extract were first fractionated separately by gas chromatography on a stabilized diethylene glycol succinate (DEGS; Analabs, Inc.) column. Individual fractions from the DEGS column were then separated on a column packed with a high molecular weight Carbowax liquid phase, Carbowax HP (Chrom-pack). Columns were Teflon-lined stainless steel 6.3 mm (o.d.)  $\times$  3 m, packed with 18% of the liquid phase on 60/80 Gas Pack FS (CRS). The carrier gas was helium and the flow rate was 200 mL/min. The instrument was a Hewlett-Packard Model 7620A gas chromatograph. A flame

ionization detector was used. The injection port was glass lined, and the effluent splitter and collector were coated internally with Thetakote (a glasslike inert surface; Theta Corp.). The oven temperature was programmed from 80 to 220 °C at 2 °C/min. The injector, detector, and collector were held at 220 °C.

**Identification and Quantitation of Components.** Components were isolated from the gas chromatograph and analyzed on a Perkin-Elmer Model 727B infrared spectrophotometer. Several fractions and individual components were analyzed by gas chromatography-mass spectrometry (GC-MS) on a packed column (Supelco SP-1000; see Acknowledgment). Peak areas were determined by planimetry or triangulation. Percentage composition was expressed as percentage of total peak area. Apparent oil-water distribution coefficients were obtained by dividing the concentration [weight/volume percent in the oil phase ( $C_o$ )] by the concentration in the water phase ( $C_w$ ).

**Identification of Isoisopulegol.** Isoisopulegol was identified by correlation with isomenthol as described previously (Lund et al., 1981).

#### RESULTS AND DISCUSSION

Twenty-two compounds from Meyer lemon leaf oil were isolated and identified (see Table I). Limonene was the major constituent, accounting for 73% of the combined product. Two other components were present in fairly high amounts: 1,8-cineole (7%) and isopulegol (4%). The presence of 1,8-cineole,  $\alpha$ -pinene, and thymol and the relatively high  $\alpha$ -terpineol concentration distinguish this oil from the other related leaf oils we have analyzed [rough lemon and Key lime (Lund et al., 1981, 1982)].

Table I shows the complete composition of the oil and aqueous codistillate. All compounds listed in the table were obtained in sufficient quantity for infrared spectra. Identification of most of the compounds was also verified by mass spectral comparison. Comparison with the reported composition of Meyer lemon flower and peel oils shows that, aside from the large amount of limonene, all three oils also contain thymol. Notable differences are the

Table II. Apparent Distribution Coefficients

compound	apparent dist coefficient, $C_o/C_w$
hydrocarbons	$>10^5$
1,8-cineole	$<1$
methylheptenone	$<1$
citronellal	3490
linalool	96
isopulegol	1890
terpinen-4-ol	$<1$
$\alpha$ -terpineol	$<1$
neral	73
geranial	$<1$
geranyl acetate	$>10^5$
neryl acetate	$>10^5$
citronellol	$>10^5$
thymol	147

lack of 1,8-cineole and isopulegol in the flower and peel oils.

The apparent oil-water distribution coefficients shown in Table II give some indication of relative water solubility and reveal possible artifact formation. Only thymol had a coefficient in the range previously reported for typical oxygenated components [100-700 (Lund and Bryan, 1976; Lund, 1981, 1982)]. Four compounds had very low coefficients: 1,8-cineole, linalool, terpinen-4-ol, and  $\alpha$ -terpineol. These compounds may have been produced in part in the aqueous phase and remained substantially in this phase because of the short contact time. In contrast, geranyl acetate and neryl acetate had very high coefficients. These two may have diffused into the aqueous phase and been rapidly destroyed by hydrolysis; reequilibration would not have been possible because the contact time was too short. Coefficients for methylheptenone, neral, and geranial were lower, and those for citronellal and isopulegol were higher, than expected. The hydrocarbons all had very high coefficients, as expected.

Of the 70 compounds isolated in this study, 48 remain unidentified. However, on the basis of GC, IR, and MS data, most of the 48 unidentified components have never been reported as citrus leaf or peel components. Some of them had strong lemon-lime or menthol aromas. A number of the unidentified compounds were considered significant because they were relatively concentrated or because closely analogous compounds (i.e., compounds with similar retention times, IR, or other properties) were also isolated from other citrus leaf oils [rough lemon and Key

lime (Lund et al., 1981, 1982)]. Thus, a compound with physical properties very similar to compound 1 (a volatile hydrocarbon) was found in abundance in both Meyer lemon and rough lemon leaf oils. Likewise, analogous compounds present in Meyer lemon and rough lemon, but at lower concentrations, included five hydrocarbons, three aldehydes, two ketones, and one alcohol; two other hydrocarbons appeared to be present only in Meyer lemon and Key lime.

Individual compounds have not been tested as blackfly attractants because a suitable bioassay has not been developed. Identification of Meyer lemon leaf oil components may, however, be useful for taxonomic studies or in flavor research.

#### ACKNOWLEDGMENT

We appreciate the assistance of Donald A. Sims. Samples were analyzed by GC-MS at the University of Alabama in Birmingham, Comprehensive GC-MS Center (Dr. R. L. Settine). We thank Don Bridges and Burt Nixon of the State of Florida, Division of Plant Industry, for the leaf samples.

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Received for review March 30, 1981. Revised manuscript received October 19, 1981. Accepted October 19, 1981. Mention of a trademark of proprietary product is for identification only and does not recommend its approval over other products which may also be suitable by the U.S. Department of Agriculture.