

Components of Key Lime Leaf Oil

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Thirteen components isolated from steam-distilled Key lime leaf oil and aqueous distillate were identified by gas chromatography and infrared spectroscopy. The identified components were as follows (peak area percent): limonene, 22.8; geranial, 25.6; neral, 18.1; neryl acetate, 7.1; methylheptenone, 1.32; linalool, 1.2; myrcene, 0.6; geranyl acetate, 0.3; isopulegol, 0.15; ocimene, 0.14; citronellal, 0.03; citronellol, undetermined; isoisopulegol, 0.009. Neryl acetate, methylheptenone, isopulegol, citronellal, and isoisopulegol were positively identified as Key lime leaf oil components for the first time. Six unidentified components were present in relatively high concentration (0.8–1.5% of peak area).

Identification of compounds in citrus leaf oils has been useful in taxonomic studies and for identification of new fragrance components. Recent observations of preferential host behavior of citrus blackfly [*Aleurocanthus woglumi* (Howard, 1979)] for several citrus species has stimulated interest in isolation from citrus leaves of attractants for this serious pest.

Key lime leaves were among the best hosts for citrus blackfly (Dowell and Steinberg, 1979). The only published data on the composition of Key lime leaf oil appeared in 1972 (Kamiyama and Amaha, 1972). Forty compounds were identified by gas chromatographic (GC) retention times only, and the relationship of oil composition to taxonomy was discussed. Limonene and the common essential oil components geranial and neral were the major constituents.

Our purpose in this study was to isolate and identify components from the oil and aqueous fraction of steam distilled leaves. The composition of the oil layer was examined separately from that of the aqueous layer.

MATERIALS AND METHODS

Preparation of Oil and Concentrated Aqueous Extract. Mature Key lime leaves (1700 g) were collected from residences in Ft. Lauderdale, FL. The leaves were steam distilled, and the oil and aqueous phases were analyzed separately, as described previously (Lund et al., 1981). The oil layer weighed 3.2 g (density at 25 °C was 0.87 g/mL). A 1.2-g sample of concentrated extract (density at 25 °C was 1.0 g/mL) was obtained by methylene chloride extraction of a total aqueous condensate volume of 2.4 L.

Fractionation of Oil and Concentrated Extract. Previously described methods were employed for isolation (Lund et al., 1981). The final GC analysis was preceded by fractionation on stabilized diethylene glycol succinate (DEGS; Analabs, Inc.). Individual fractions from the DEGS column were separated on Carbowax HP (Chrompack, a high molecular weight Carbowax liquid phase). The columns were Teflon-lined stainless steel, 6.3 mm × 3 m. They were packed with 18% of the liquid phase on 60/80 Gas Pack FS (CRS). The carrier gas was helium at a flow rate of 200 mL/min. The instrument was a Hewlett-Packard Model 7620A gas chromatograph with a flame ionization detector and a glass-lined injection port. The stainless steel effluent splitter and collector were coated with Thetakote (Theta Corp.). The columns were programmed from 80 to 220 °C at 2 °C/min, and the injector, detector, and collector were held at 220 °C.

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Table I. Composition of Key Lime Leaf Oil and Aqueous Phase

no.	compound ^a	composition, peak area %		
		oil	aqueous	combined
1	unidentified, hydrocarbon or ether	0.03		0.03
2	myrcene	0.6		0.6
3	limonene	21.6	1.2	22.8
4	ocimene	0.14		0.14
5	methylheptenone	0.023	1.3	1.32
6	citronellal	0.03		0.03
7	linalool	0.4	0.8	1.2
8	isopulegol	0.09	0.06	0.15
9	isobornyl acetate	0.009		0.009
10	neral	12.4	5.7	18.1
11	geranyl acetate	^b	0.3	0.3
12	geranial	17.4	8.2	25.6
13	neryl acetate	6.9	0.18	7.1
14	citronellol + unidentified (ester)	0.04		0.04
	unidentified, total ^c	12.6	9.6	22.2

^a In order of elution from diethylene glycol succinate.

^b Geranyl acetate concentration in oil could not be obtained due to interference from the large geranial peak.

^c Total peak area of unidentified compounds including nine hydrocarbons, two alcohols, one ester, one aldehyde, three ketones, and four carboxylic acids.

Identification and Quantitation of Components.

Infrared absorption spectra were obtained on isolated fractions by using a Perkin-Elmer Model 727B infrared spectrophotometer. In addition, several fractions and components were analyzed on a GC-MS instrument (SP-1000 packed column; 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 as described previously (Lund et al., 1981) by reduction to isomenthol and comparison with an authentic sample prepared by reduction of menthone.

RESULTS AND DISCUSSION

Thirteen compounds were identified from the oil and aqueous layers of steam-distilled Key lime leaves (see Table I). Limonene, geranial, neral, and neryl acetate together accounted for 74% of the total product collected.

The data in Table I show that although neryl acetate was not reported by the previous workers (Kamiyama and

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; α -terpineol, 1.1; terpinen-4-ol, 1.0; ocimene, 1.0; geranial, 0.9; γ -terpinene, 0.7; neral, 0.7; methylheptenone, 0.6; geranyl acetate, 0.3; sabinene, 0.27; α -pinene, 0.2; isoisopulegol, 0.16; thymol, 0.04; β -caryophyllene, 0.010; neryl acetate, 0.004; citronellol, 0.002; caryophyllene oxide, 8×10^{-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.