5 (~2%) partially characterized. The majority of the identified compounds are esters (nearly 80% of the sample) with the major constituents being methyl hexanoate (~31%) and methyl hex-2-enoate (~27%). The esters comprise an interesting series of chemically related compounds in that the methyl and ethyl esters of the C_4, C_6, and C_8 saturated straight chain carboxylic acids were all present in the sample, together with the six corresponding 2-enoates. A similar, but slightly less complete, series was also obtained with another tropical fruit, wood apple, although in that case the corresponding 3-hydroxy esters were also generally detected (MacLeod and Pieris, 1981). These hydroxy derivatives were definitely not present in soursop essences, since specific searches were made for these compounds. The aforementioned previous publication (MacLeod and Pieris, 1981) includes summaries of the mass spectra of the less common esters of wood apple, since these are not widely published, if at all. Here, Table II provides similar summaries of the spectra of the additional less common esters detected in soursop.

The previous paper (MacLeod and Pieris, 1981) discussed briefly the biosynthetic relationships of these esters and also summarized previous reports of their detection as aroma components. This will not be reiterated here except to emphasize that the 2-enoates are relatively rare aroma constituents and generally they have only been located in tropical or subtropical fruits and products such as passion fruit (Murray et al., 1972; Winter and Kloti, 1972), grapes (Stern et al., 1967), and wood apple (MacLeod and Pieris, 1981). The detection of these esters in soursop further supports the contention that they might be a characteristic of tropical fruits.

Generally, the determined odor qualities (Table I) were undistinguished, and no GC peak seemed to represent any specific element of the characteristic soursop flavor. The obvious, if facile, deduction must be drawn that soursop flavor is basically a blend of the 15 esters, together with at least β-farnesene, in the correct proportions. It would certainly be important in any processed product to retain as far as possible all these components to maintain the characteristic fresh fruit flavor.

LITERATURE CITED

Alves, S.; Jennings, W. G. Food Chem. 1979, 4, 149–159.
Likens, S. T.; Nickerson, G. B. Proc. Am. Soc. Brew. Chem. 1964, 5–13.
MacLeod, A. J.; Cave, S. J. J. Sci. Food Agric. 1975, 26, 351–360.
MacLeod, A. J.; Pieris, N. M. J. Agric. Food Chem. 1981, 29, 49–53.
Murray, K. E.; Shipton, J.; Whitfield, F. B. Aust. J. Chem. 1972, 25, 1921–1933.
Stern, D. J.; Lee, A.; MacFadden, W. H.; Stevens, K. L. J. Agric. Food Chem. 1967, 15, 1100–1103.
Winter, M.; Kloti, R. Helv. Chim. Acta 1972, 55, 1916–1921.

Composition of Rough Lemon Leaf Oil

Eric D. Lund,* Philip E. Shaw, and Cora L. Kirkland

Twenty-five of ninety-two components isolated by gas chromatography from steam-distilled rough lemon (Citrus jambhiri Lush.) leaf oil and aqueous distillate were identified. They were identified by gas chromatographic retention times, infrared spectroscopy, and mass spectroscopy. Quantities of the major identified components were as follows (peak area percent): limonene, 33.7; sabinene, 7.8; γ-terpinene, 7.4; β-ocimene, 7.3; linalool, 5.3; isopulegol, 4.6; geranial, 3.9; neral, 3.6; p-cymene, 3.3; geranyl acetate, 1.1; neryl acetate, 0.8; terpinen-4-ol, 1.0. A number of compounds not previously identified in rough lemon leaf oil were isolated. Some of these may be associated with the host preference of citrus blackfly (Aleurocanthus woglumi) for this citrus species. The data could be useful in taxonomic studies, for identification of new aroma compounds, or in evaluation of compounds affecting citrus blackfly.

The composition of citrus leaf oils has been investigated for determination of taxonomic relationships and identification of unique fragrance components. Recently, lemon leaves from several varieties, especially rough lemon (Citrus jambhiri Lush.), were found to be preferred hosts for citrus blackfly, Aleurocanthus woglumi (Howard, 1979; Dowell et al., 1978). Because of the potential damaging effects of this insect, compounds with attractant or repellant properties or other characteristics that affect its behavior are of interest. For these reasons, we decided to investigate in detail the leaf oil composition of rough lemon.

A detailed study of true lemon leaf oil [Citrus limon (L.) Burm. f.] has been reported by Kamiyama (1967). In the Kamiyama study, gas chromatography (GC), thin-layer chromatography (TLC), and infrared spectroscopy (IR) were used to identify 25 compounds; 7 unidentified compounds were also isolated.

Attaway et al. (1966) positively identified a number of rough lemon leaf components. Kesterson et al. (1964) and Scora et al. (1969) identified rough lemon leaf components by retention times only.

Scora et al. (1969) examined nine varieties of rough lemon, two true lemons, and a hybrid and reported differences and similarities between rough and true lemon leaf oils. Rough lemon is a common rootstock in Florida commercial groves. Taxonomically, it is considered to be in a distinctly different group from the true lemon.

In our study, the oil was prepared by steam distillation. The compositions of both aqueous and oil layers were

---

U.S. Citrus and Subtropical Products Laboratory, Southern Region, U.S. Department of Agriculture, Science and Education Administration, Winter Haven, Florida 33880.

Composition of Rough Lemon Leaf Oil

J. Agric. Food Chem., Vol. 29, No. 3, 1981 **491**

examined. Our goal was to isolate and identify components, including minor ones that may have been overlooked.

MATERIALS AND METHODS

Preparation of Oil. Mature rough lemon leaves collected from a commercial grove in Winter Haven, FL, were washed with water and air-dried overnight. The dry leaves (5630 g) were placed in perforated trays in a large covered vessel, and steam was passed through the trays at 100 °C (atmospheric pressure). The volatile product was condensed with a chilled water condenser (9 °C). The oil phase was continuously removed with an oil separator (condensate rate 6000 mL/h). A 3-h period was required for complete volatilization of the oil. The total aqueous condensate volume was 18 L. The oil layer weighed 9.8 g (density 0.86 g/mL).

Extraction of Aqueous Condensate. The aqueous condensate was extracted 3 times with methylene chloride in a separatory funnel (total combined volume 18 L), and the combined extracts were concentrated in a rotary evaporator to ~50 mL. The extract was then distilled through a Vigreux column to give 2.5 g of an oil (concentrated aqueous extract density 0.99 g/mL).

Fractionation of Oil and Concentrated Aqueous Extract. The oil and concentrated aqueous extract were fractionated on a Hewlett-Packard Model 7620A gas chromatograph with a flame ionization detector. They were first separated on a diethylene glycol succinate (DEGS) column; then, fractions from the DEGS were separated on a Carbowax column. The carrier gas for both columns was helium at a flow rate of 200 mL/min. The columns were programmed from 80 to 220 °C at 2 °C/min, and the injector, detector, and collector temperatures were 220 °C. The columns were Teflon-lined stainless steel, 6.3 mm o.d. × 3 m. They were packed with 18% stabilized DEGS (Analabs) or Carbowax HP (Chrompack) on 60/80 Gas Pack FS (CRS). The injection port was glass lined. The stainless steel effluent splitter and collector were coated with ThetaKote (Theta Corp.) for deactivation of the surfaces.

Identification and Quantitation of Components. Components isolated from the gas chromatograph in sufficient quantity (1–5 mg) were analyzed on a Perkin-Elmer Model 727B infrared spectrophotometer. 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 values are expressed as percentage of total peak area.

Identification of Isoisopulegol. The alcohol was dissolved in ethanol and reduced with H_2 and PtO_2 at 60 lb/in^2 for 3 h. The major product (isomenthol) was isolated by gas chromatography on Carbowax HP (see above for conditions).

An authentic sample of isomenthol was prepared by sodium–2-propanol reduction of menthone (Solodar, 1976), followed by gas chromatographic separation on Carbowax HP (see above). Infrared spectra and GC retention times on Carbowax for the isomenthol obtained by reduction of the unidentified alcohol and for the authentic sample were identical.

RESULTS AND DISCUSSION

Of the 92 isolated components, 25 were identified. The major components were limonene, sabinene, γ-terpinene, β-ocimene, linalool, isopulegol, geranial, neral, *p*-cymene, geranyl acetate, neryl acetate, and terpinen-4-ol. Isopulegol has never been positively identified as a component of rough lemon leaf oil. The other major components have

all been previously identified as major constituents of rough lemon leaf oil.

Table I shows the complete composition of the oil and aqueous extract. All compounds listed in Table I were obtained in sufficient quantity for infrared spectra. Also included in the table are data from previous studies of rough and true lemon leaf oil composition (Scora et al., 1969; Kamiyama, 1967). Comparison of our data with the values for Florida rough lemon (*C. jambhiri*) in Scora et al. (1969) shows that only the values for γ-terpinene, methylheptenone, and neryl and geranyl acetate were similar. Our data and those reported by Kamiyama for an unspecified true lemon cultivar (*C. limon*) are similar with respect to concentrations of sabinene, limonene, myrcene, γ-terpinene, linalool, and β-caryophyllene. Three of the compounds we identified, isopulegol, isoisopulegol, and linalool oxide, have not been previously reported as present in either *C. limon* or *C. jambhiri* leaf oils.

Isopulegol, a major component, was accompanied by a relatively small amount of its optical isomer, isoisopulegol. Since isopulegol, along with small amounts of isoisopulegol and the other possible optical isomers, can be formed by thermal decomposition of citronellal (Hefendehl, 1970), it is possible that these two alcohols may have been produced by a similar process from citronellal present in leaf oils.

Storing oil in the refrigerator at 9 °C for 7 months greatly increased its caryophyllene oxide content and correspondingly decreased its caryophyllene content. Autooxidation of caryophyllene in Cannabis oil has been previously observed (Paris, 1975). Apparently, even under these mild conditions, particularly sensitive oil components can autooxidize. A major reason for this unexpected instability must be the absence of the nonvolatile natural antioxidants which are present in most cold-pressed citrus oils but not in distilled oils.

Artifacts could also have been formed during the steam distillation, as indicated by the data in Table II. Shown in the table are the relative amount of each component in the oil (or water) phase and the apparent oil–water distribution coefficients. The latter are only approximations of the relative water solubilities, since the phases were not equilibrated. Only four compounds (neral, geranial, linalool, and methylheptenone) had distribution coefficients similar to those previously reported for typical lemon oil components [100–700 (Lund and Bryan, 1976)]. Neral and geranial (532; 626) had coefficients reasonably close to the previously reported values (454; 640). Linalool (513) and methylheptenone (263) also had coefficients close to the expected values, based on relative water solubility. Hydrocarbons had very high coefficients, showing that they were almost insoluble in the aqueous phase.

Those components with abnormally low coefficients included *cis*-3-hexen-1-ol, terpinen-4-ol, nerol, geraniol, and citronellol. Possibly all of these were initially produced from unidentified precursors in the aqueous phase and then did not have time to diffuse into the oil phase.

Unusually high coefficients indicative of decomposition in the aqueous phase were shown by citronellol, isopulegol, neryl acetate, and geranyl acetate. The decomposition reactions must have been more rapid than diffusion from oil to aqueous layer. The hydrolysis of neryl and geranyl acetates could explain both the high distribution coefficients and the exclusive presence of nerol and geraniol in the aqueous layer. We have no explanation for the high coefficients of the other two components.

Several of the unidentified compounds were of interest because of their relatively high concentrations or because they have been isolated from two other citrus leaf oils, i.e.,

Table I.   Percentage Compositions[a] of Rough Lemon Leaf Oils

		% composition				
		rough lemon				true lemon, Kamiyama (1967)
		present study			Scora et al. (1969)	
no.	compound[b]	oil	aqueous extract	combined		
1	unidentified, hydrocarbon	3.3		3.3		
2	unidentified, hydrocarbon	2.4		2.4		
3	α-pinene				0.59	3.4[c]
	α-thujene					3.4[c]
	β-pinene					12.4[c]
	camphene					0.1
4	sabinene	7.4	0.4	7.8		12.4[c]
5	limonene	32.4	1.3	33.7	22.9	23.1
6	γ-terpinene	7.4		7.4	5.1	3.3
	trans-2-hexenal					2.0
7	p-cymene	3.3		3.3		0.5
8	unidentified, alcohol	1.1		1.1		
9	myrcene	1.2		1.2	20.6	1.7
10	β-ocimene	7.3		7.3		
11	α-terpinene	0.2		0.2		trace
12	terpinolene	0.2		0.2		trace
13	unidentified, hydrocarbon	0.06		0.06		
14	methylheptenone	0.10	0.6	0.7	1.08	
15	citronellal	0.8	0.14	0.94	11.3	
16	linalool	1.3	4	5.3	9.4	3.1
	nonanol				2.02	
17	isoisopulegol	0.6		0.6		
18	isopulegol	4.4	0.23	4.6		
19	unidentified, ketone	0.05		0.05		
20	unidentified, hydrocarbon	0.017		0.017		
21	unidentified, hydrocarbon	0.10		0.10		
22	unidentified, alcohol	0.017		0.017		
23	caryophyllene oxide	0.2		0.2		
24	unidentified, carbonyl	0.02		0.02		
25	unidentified, carbonyl	0.008		0.008		
26	unidentified, alcohol	0.09		0.09		
27	unidentified, aldehyde	0.018		0.018		
	cis-3-hexen-1-ol					trace
28	unidentified, ketone	0.005		0.005		
29	unidentified, alcohol		0.00020	0.00020		
30	cis- or trans-linalool oxide	0.03		0.03		
31	β-caryophyllene	0.3		0.3		0.2
32	unidentified, ketone	0.025	0.011	0.04		
33	unidentified, alcohol		0.0016	0.0016		
34	unidentified, alcohol	0.0025		0.0025		
35	terpinen-4-ol	0.022	0.93	0.95		0.4[c]
36	α-terpineol		0.13	0.13		
37	unidentified, alcohol		0.003	0.003		
38	unidentified, alcohol		0.003	0.003		
39	unidentified, carbonyl	0.008		0.008		
40	unidentified, carbonyl	0.003		0.003		
41	unidentified, carbonyl	0.05		0.05		
42	unidentified, alcohol		0.020	0.020		
43	unidentified, alcohol plus ester	0.10	0.25	0.35		
44	unidentified, ester	0.05		0.05		
45	unidentified, ester	0.005		0.005		
46	unidentified, ester	0.05		0.05		
	neryl formate				1.59	
47	neral	0.91	2.7	3.6	10.6	16.4
	terpenyl acetate				1.28	
	geranyl formate				0.83	
48	unidentified, ester	0.012		0.012		
49	geranyl acetate	1.1		1.1	1.43	
50	geranial	1.11	2.8	3.9	6.2	24.3
51	neryl acetate	0.8		0.8	0.93	
52	unidentified, ester	0.017		0.017		
53	unidentified, ester	0.10		0.10		
54	unidentified, ester	0.0017		0.0017		
55	unidentified, alcohol		0.016	0.016		
56	unidentified, ketone	0.005		0.005		
57	unidentified, ketone	0.005		0.005		
58	unidentified, ketone	0.008		0.008		
59	nerol		0.15	0.15	1.81	2.0
60	unidentified, alcohol		0.19	0.19		
61	geraniol		0.09	0.09		2.8
62	citronellol		0.07	0.07		

Composition of Rough Lemon Leaf Oil

J. Agric. Food Chem., Vol. 29, No. 3, 1981 **493**

Table I (*Continued*)

no.	compound[b]	oil	aqueous extract	combined	Scora et al. (1969)	true lemon, Kamiyama (1967)
		% composition				
		rough lemon				
		present study				
	β-elemene					0.4[c]
63	unidentified, hydrocarbon	0.20		0.20		
64	unidentified	0.004		0.004		
65	unidentified	0.002		0.002		
66	unidentified	0.0015		0.0015		
67	unidentified, alcohol	0.05		0.05		
68	unidentified, hydrocarbon	0.004		0.004		
69	unidentified, hydrocarbon	0.002		0.002		
70	unidentified, hydrocarbon	0.0015		0.0015		
	β-selinene					1.9
71	unidentified, alcohol		0.04	0.04		
72	unidentified, hydrocarbon	0.10		0.10		
73	unidentified, hydrocarbon	0.15		0.15		
74	unidentified	0.010		0.010		
75	unidentified, acid	0.03		0.03		
76	unidentified	0.012		0.012		
77	unidentified	0.02		0.02		
78	unidentified	0.03		0.03		
79	unidentified	0.2		0.2		
80	unidentified	0.10		0.10		
81	unidentified, aromatic alcohol		0.5	0.5		
82	unidentified, acid		0.06	0.06		
83	unidentified, ether, M_r 306		0.33	0.33		
84	unidentified, ether, M_r 237		0.5	0.5		
85	unidentified, alcohol		0.04	0.04		
86	unidentified, acid		0.015	0.015		
87	unidentified, acid		0.014	0.014		
88	unidentified, ether		0.022	0.022		
89	unidentified, ether		0.06	0.06		
90	unidentified, ether		0.06	0.06		
91	unidentified, alcohol		0.011	0.011		
92	unidentified, alcohol		0.09	0.09		
	unidentified, total small peaks[d]	5		5		

[a] Based on the peak area. [b] In order of elution from the DEGS column. [c] Single peaks containing two components. [d] Total peak area of compounds too dilute for IR identification.

Table II. Apparent Distribution Coefficients

compound	% of total in oil (water) phase	apparent dist. coeff, C_o/C_w
appreciably soluble in oil phase		
citronellal	85	9023
linalool	25	513
isopulegol	96	30200
neral	25	532
geranial	28	626
neryl acetate	>99.9	>10⁵
geranyl acetate	>99.9	>10⁵
hydrocarbons	>99.9	>10⁵
largely in water phase		
methylheptenone	86	263
cis-3-hexen-1-ol	>99.9	<1
terpinen-4-ol	98	37
nerol	>99.9	<1
geraniol	>99.9	<1
citronellol	>99.9	<1

Key lime and Meyer lemon (Lund et al., 1980). Compound 1, a volatile hydrocarbon, and the aromatic alcohol 81 were among the more abundant components. Both of these have also been isolated from the two other citrus leaf oils. Unidentified compounds 2 and 8 were also abundant. Other less concentrated components that have also been isolated from the two other citrus leaf oils were alcohols 33, 38, 67, and 71, hydrocarbons 63, 72, and 73 (sesquiterpenes), a series of related unsaturated hydrocarbons (68,

69, 70, 76, 78, 79, and 80), and an acid (82). A series of related ethers (83, 84, 88, 89, and 90) were isolated from rough lemon but have not been isolated from any other citrus leaf oil. The concentrations of two of these ethers (83 and 84) were relatively high (0.3 and 0.5%, respectively). The mass spectra of these ethers were similar, and both ethers showed infrared bands characteristic of unsaturated compounds at 6.0, 8.7, 9.5 and 10.0 μm. None of these unidentified compounds have been isolated from citrus peel oils.

Relatively high concentrations of geranial, neral, and geranyl and neryl acetates are characteristic of true and rough lemon leaf oils (Scora et al., 1969) as well as the peel oils (Shaw, 1979). The high concentrations of oxygenated compounds can explain the powerful and characteristic aroma of this leaf oil. In addition, many of the minor components had pronounced odors related to lemon or lime, and they probably contribute prominently to the overall aroma.

We were not able to obtain data on the effect of individual components on blackflies because of difficulties with the field evaluations. However, the predominance of terpenoids, which are known to be active attractants, shows that rough lemon oil contains many likely candidate compounds.

Many of the 92 compounds we isolated have never been identified in citrus leaf oils or peel oils. In addition, significant quantitative differences were found between our data and previous data on lemon leaf oils. Although a number of possible artifacts were identified, most com-

ponents were probably present as such in the leaves. The data may therefore be useful in taxonomic studies, as well as in identification of compounds in leaf oils obtained commercially by steam distillation.

ACKNOWLEDGMENT

LITERATURE CITED

Attaway, J. A.; Pieringer, A. P.; Barabas, L. J. Phytochemistry 1966, 5, 141-151.
Dowell, R. V.; Reinert, J. A.; Fitzpatrick, G. E. Environ. Entomol. 1978, 7, 524-525.
Hefendehl, F. W. Arch. Pharm. (Weinheim, Ger.) 1970, 303, 345-357.
Howard, F. W. Folia Entomol. Mexicana 1979, 41, 33-40.
Kamiyama, S. Agric. Biol. Chem. 1967, 31, 1091-1096.
Kesterson, J. W.; Pieringer, A. P.; Edwards, G. J.; Hendrickson, R. Proc. Am. Soc. Hortic. Sci. 1964, 84, 199-203.
Lund, E. D.; Bryan, W. L. J. Food Sci. 1976, 41, 1194-1197.
Lund, E. D.; Shaw, P. E.; Kirkland, C. L., U.S. Department of Agriculture, unpublished data, 1980.
Paris, M. Riv. Ital. Essenze, Profumi, Piante Off., Aromi, Saponi, Cosmet., Aerosol 1975, 57, 83.
Scora, R. W.; England, A. B.; Chang, D. Proc. Int. Citrus Symp., 1st, 1968 1969, 1, 441-450.
Shaw, P. E. J. Agric. Food Chem. 1979, 27, 246-257.
Solodar, J. J. Org. Chem. 1976, 41, 3461-3464.

Importance of Thymol, Methyl N-Methylanthranilate, and Monoterpene Hydrocarbons to the Aroma and Flavor of Mandarin Cold-Pressed Oils

Charles W. Wilson, III,* and Philip E. Shaw

Various combinations of thymol, methyl N-methylanthranilate, (DMA), γ-terpinene, and β-pinene were added to tangerine cold-pressed oil at levels found in Argentine (Sicilian) mandarin oil. The aromas of the compounded oils and Argentine mandarin oil were compared, and then the flavors of tangerine juice containing the compounded oils and mandarin oil were compared. The presence of both β-pinene and γ-terpinene in addition to thymol and DMA was necessary to give the tangerine oil an acceptable mandarin aroma. However, additional citrus components were necessary to give the tangerine oil an acceptable mandarin flavor in tangerine juice. Flavor thresholds were determined for DMA in water and for thymol and DMA in single-strength tangerine juice.

Mandarin oranges are a large and distinctive group of loose-skinned citrus fruit that show more variation among cultivars than do oranges or grapefruit (Hodgson, 1967). Horticulturists usually restrict the name tangerine to a class of mandarins having a deep red color characteristic of the variety Dancy tangerine (Hodgson, 1967). Kugler and Kovats (1963) attributed the distinctive flavor and aroma of Sicilian mandarin peel oil to the presence of methyl N-methylanthranilate and thymol but gave no evidence to support this claim. In addition, the Sicilian mandarin oil they examined was designated Citrus reticulata Blanco, whereas Hodgson (1967) described the principal mandarin in Sicily (and in Argentina) to be the Mediterranean cultivar Citrus deliciosa Tenore.

Thymol has been identified in Dancy tangerine peel oil and both methyl N-methylanthranilate (DMA) and thymol have been identified in Sicilian mandarin peel oil (Shaw, 1977). The reported quantities of thymol vary from 0.04 to 0.2% of the oil (Shaw, 1979), while the quantity of DMA as quantitated only once in mandarin peel oil was 0.9%. The flavor threshold of thymol in water has been reported to be 1.7 ppm (Moshonas et al., 1972), but the flavor

U.S. Citrus and Subtropical Products Laboratory, Southern Region, Science and Education Administration, U.S. Department of Agriculture, Winter Haven, Florida 33880.

threshold of DMA in water has not been reported. Neither threshold has been determined in mandarin or tangerine juice. Thus, the relative contributions of these two constituents to the flavor and aroma of mandarin peel oil or juice are difficult to assess from the available information.

The current study reports quantities of DMA and thymol present in Argentine mandarin and tangerine peel oils. The relative importance of these two constituents to the flavor of mandarin and tangerine cold-pressed oils and to tangerine juice flavored with these oils was determined.

MATERIALS AND METHODS

Mandarin oil from Argentina was obtained from J. Manheimer, Inc., Long Island City, NY; commercial cold-pressed tangerine oil (a blend of oil from mostly Dancy plus a small percentage of Robinson tangerines) and frozen concentrated tangerine juice wthout added oil or other flavor fractions (evaporator pump out) were obtained from Citrus Central, Inc., Orlando, FL. Thymol (NF, Fisher Scientific Co., Fair Lawn, NJ) was 99.5% pure by gas chromatography (GC). DMA (ICN Pharmaceuticals Inc., Plainview, NY) was purified by thin-layer chromatography (TLC) on Silica gel G in 90:10 hexane–acetone prior to use, and the purified sample was shown to be 99.8% pure by GC.

β-Pinene (Columbia Organic Chemicals, Columbia, SC) and γ-terpinene (SCM Corp., Jacksonville, FL) were purified by GC on a 4 mm i.d. × 5 m glass column containing