

On the Genuineness of Citrus Essential Oils. Part LVII.† The Composition of Distilled Lime Oil

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Received 22 March 1997

Accepted 22 May 1997

ABSTRACT: The composition of distilled lime oil has been investigated. The analytical techniques used to fractionate the oils and identify each component were: open column chromatography, gas chromatography, gas chromatography–mass spectrometry. Among the 113 compounds identified, 28 are reported to be present in distilled lime oil for the first time. The main differences between the cold-pressed and the distilled lime oil are also discussed. © 1998 John Wiley & Sons, Ltd.

Flavour Fragr. J., **13**, 93–97 (1998)

KEY WORDS: *Citrus aurantifolia* Swingle; lime; distilled lime oil composition; column chromatography; HRGC–MS

Introduction

Distilled lime oil is obtained from the distillation of the oil/juice emulsion produced by pressing whole lime, *Citrus aurantifolia* Swingle, fruits^{2,3} through a screw-press which crushes the fruits. The complexity of its composition^{4–20} is due to the reactions, catalysed by the acidic medium, that occur during the distillation.

The chemistry involved during these transformations has been the subject of different studies,^{11,19,20} mainly regarding the monoterpene bicyclic hydrocarbons α -thujene, α -pinene, β -pinene and sabinene and the isomerization and hydration reactions of limonene forming alcohols, aldehydes and oxides.¹⁹ Moreover, under the conditions used for the distillation, neral and geranial undergo cyclization/oxidation and polymerization reactions.¹⁹ The complexity of the distilled lime oil makes it difficult to resolve and identify all of the components in a single gas chromatographic analysis. Therefore, we first separated the oil into simpler mixtures by column chromatography before gas chromatographic analysis, avoiding the inconvenience represented by too many overlapping peaks.^{17,20}

This research note gives the composition of several distilled lime oils of different geographical origins, obtained by open column chromatography, gas

chromatography and gas chromatography–mass spectrometry.

Experimental

The research was carried out on six distilled lime oil samples, four of which were obtained from Mexico, one from Peru and one from the Ivory Coast. All of the samples were analysed by GC and GC–MS. One of the Mexican samples and the samples from Peru and Ivory Coast were also fractionated on a column packed with neutral Al₂O₃. The fractions so obtained were analysed by GC–MS in the same conditions used for the whole oils.

Column Chromatography

The essential oil (100 μ l) was introduced on to a column (1.6 cm i.d.) containing neutral alumina, grade II activity (20 g) in light petroleum (b.p. 30–50°C). Elution with light petroleum (150 ml) gave fraction 1 (hydrocarbons); elution with light petroleum:diethyl ether (90:10 v:v, 100 ml) gave fraction 2 (ethers, ketones, oxides, esters); elution with light petroleum:diethyl ether (80:20, 100 ml) gave fraction 3 (aldehydes); elution with light petroleum:diethyl ether (50:50, 100 ml) gave fraction 4 (alcohols); elution with diethyl ether (100 ml) gave fraction 5 (alcohols).

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† For Part LVI, see Ref. 1

Contract grant sponsor: Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Italy.

GC Analysis

A 5160 Mega Series gas chromatograph (Fisons Instruments, Milan, Italy) equipped with a data processor DP 700 was used with a SE-52 fused silica column (25 m × 0.32 mm, 0.40–0.45 μm film thickness, Mega, Legnano, Italy); column temperature, 45°C (6 min) to 240°C at 3°C/min; injector temperature, 250°C; detector temperature, 280°C; injection mode, split; split ratio 1:50; volume injected, 0.2 μl of the oil; carrier gas, He, 100 kPa.

GC-MS Analysis

A gas chromatograph mass spectrometer (quadrupole) system MD 800 (Fisons Instruments, Milan, Italy) equipped with commercial libraries (NIST, Adams,²¹ Parfume²²) and a home-made bank FFC (flavour and fragrance components)²³ was used with a DB-5 fused silica column (30 m × 0.25 mm, 0.25 μm film thickness, J & W, Folson, California, USA); column temperature, 60–240°C at 3.0°C/min, to 300°C at 30.0°C/min; injector temperature, 250°C; injection mode, split; split

ratio, 1:20; volume injected, 1 μl of a solution 1/20 in pentane of the oil; carrier gas He, 83 kPa; linear velocity 35 cm/sec at 60°C; interface temperature 250°C; source temperature 200°C; EI⁺ acquisition mass range of 41–300 amu.

Results and discussion

Figure 1 shows the total ion chromatograms (TIC) obtained for a Mexican distilled lime oil and the fractions obtained therefrom by column chromatography on an open column packed with neutral alumina.

Table 1 reports the composition of the distilled lime oil samples analysed. As can be inferred from the Figure and from the Table, 113 components were identified, corresponding to 99% of the whole oil. The GC-MS analyses and the fractionation allowed the identification of several compounds whose peaks were, otherwise, co-eluted. From the GC analyses of the single fractions it was possible to determine the amount of some of the co-eluted peaks. Twenty-eight components were identified for the first time in the

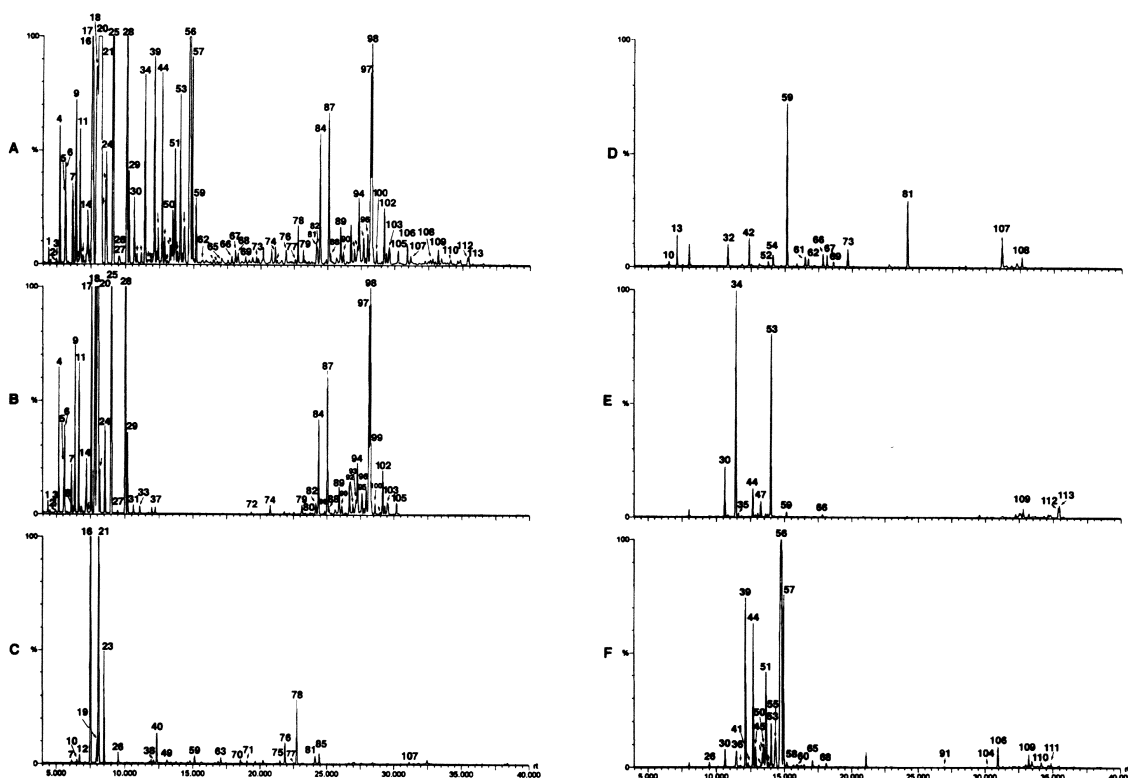


Figure 1. Total ion chromatograms of a distilled lime oil (A) and of the fractions obtained by column chromatography: (B) hydrocarbons; (C) ethers, ketones, oxides, esters; (D) aldehydes; (E and F) alcohols. For peak identification, see Table 1.

Table 1. Composition of the samples of distilled lime oils (*Citrus aurantifolia* Swingle) analysed

Peak No.	Compound	Peru	Ivory Coast	Mexico			
				1	2	3	4
1	Nonane	0.03	0.02	0.03	0.04	0.02	0.02
2	Tricyclene	0.03	0.02	0.02	0.02	0.02	0.02
3	α -Thujene	0.01	tr	0.01	0.02	0.01	0.01
4	α -Pinene	0.99	1.26	0.94	1.17	1.01	0.99
5	α -Fenchene	0.79	0.68	0.69	0.69	0.73	0.73
6	Camphene ^d						
7	2,6,6-Trimethyl-2-ethenyl-tetrahydropyran	0.24	0.18	0.23	0.21	0.23	0.24
8	Sabinene	tr	tr	tr	tr	tr	tr
9	β -Pinene	0.77	1.90	1.27	1.85	1.23	1.19
10	6-Methyl-hept-5-en-2-one	0.01	tr	tr	tr	0.01	0.01
11	Myrcene ^c	1.00	1.17	0.90	0.85	1.10	1.08
12	2,3-Dehydro-1,8-cineole ^c	0.21	0.27	0.24	0.19	0.26	0.29
13	Octanal	0.05	0.03	0.04	0.02	0.05	0.04
14	α -Phellendrene	0.44	0.48	0.44	0.34	0.44	0.45
15	δ -3-Carene	0.03	tr	tr	0.02	0.01	0.03
16	1,4-Cineole ^c	3.52	3.72	2.43	2.48	3.22	3.22
17	α -Terpinene ^c	2.35	1.47	1.62	1.66	2.14	2.15
18	<i>p</i> -Cymene	2.30	2.17	4.28	3.71	1.81	2.06
19	2,2-Dimethyl-5-(1-methyl-1-propenyl)-tetrahydrofuran ^b	0.02	0.03	0.06	0.04	0.03	0.05
20	Limonene ^c	39.19	38.95	41.27	42.24	41.98	40.49
21	1,8-Cineole ^c	4.35	9.47	7.25	7.45	7.23	7.15
22	(<i>Z</i>)- β -Ocimene	0.20	0.18	0.19	0.15	0.20	0.20
23	2,2-Dimethyl-5-(1-methyl-1-propenyl)-tetrahydrofuran ^b	0.25	0.14	0.24	0.18	0.24	0.23
24	(<i>E</i>)- β -Ocimene	0.45	0.45	0.43	0.20	0.48	0.50
25	γ -Terpinene	13.35	11.71	8.51	10.55	10.94	10.89
26	Octanol	0.02	0.02	0.02	0.01	0.01	0.01
27	<i>p</i> -Mentha-3,8-diene ^d	0.02	0.01	0.02	0.01	0.01	0.02
28	Terpinolene	9.70	7.28	6.91	7.96	8.59	8.58
29	<i>p</i> -Cymenene	0.16	0.08	0.13	0.11	0.14	0.15
30	Linalol ^c	0.16	0.14	0.22	0.11	0.14	0.14
31	Undecane ^c	0.02	0.02	0.03	0.01	0.02	0.02
32	Nonanal ^c	0.01	0.03	0.04	0.01	0.02	0.02
33	<i>p</i> -Mentha-1,3,8-triene ^{d,c}	tr	tr	0.01	tr	tr	tr
34	α -Fenchol	0.82	0.71	0.83	0.66	0.73	0.69
35	β -Fenchol	0.01	tr	0.02	tr	tr	0.01
36	Myrcenol	0.01	tr	0.08	0.01	0.01	0.07
37	<i>allo</i> -Ocimene ^d	tr	tr				
38	<i>cis</i> -Limonene oxide ^d	tr	tr	0.03	0.01	0.01	0.01
39	Terpinen-1-ol	1.27	0.97	1.11	0.74	1.11	1.11
40	<i>trans</i> -Limonene oxide ^d	tr	tr	0.04	0.02	0.02	0.02
41	<i>trans</i> -Pinene hydrate ^d	0.03	0.02	0.01	tr	tr	0.01
42	4,8-Epoxy- <i>p</i> -menth-1-ene ^d	0.03	tr	0.03	0.02	0.02	0.02
43	<i>neo-allo</i> -Ocimene ^d	tr	tr	tr	tr	tr	tr
44	<i>cis</i> - β -Terpineol	0.80	0.72	0.91	0.60	0.74	0.74
45	(<i>Z</i>)-Ocimenol ^a						
46	Isopulegol ^d	0.08	tr				
47	Isoborneol	0.06	0.05	0.15	0.10	0.15	0.11
48	(<i>E</i>)-Ocimenol ^a						
49	<i>trans</i> -Pinocamphone ^d	0.05	0.02	0.06	0.04	0.06	0.05
50	<i>trans</i> - β -Terpineol		0.20	0.10	0.10	0.15	0.10
51	Borneol	0.82	0.64	0.69	0.59	0.70	0.59
52	<i>cis</i> -Pinocamphone ^d	0.05		0.01	0.04	0.02	0.05
53	Terpinen-4-ol	0.71	0.88	0.81	0.56	0.82	0.79
54	<i>p</i> -Methylacetophenone			0.02	tr	tr	0.01
55	<i>p</i> -Cymen-8-ol	0.11	0.18	0.28	0.21	0.13	0.12
56	α -Terpineol	7.00	7.36	7.59	8.04	7.04	7.03
57	γ -Terpineol	1.24	1.07	1.11	1.31	1.11	1.11
58	Dihydrocarveol ^{a,d}						
59	Decanal	0.11	0.27	0.20	0.02	0.13	0.14
60	Nerol	0.02	0.02	0.01	tr	tr	0.01
61	Neral	0.03	0.01	0.01	0.01	tr	0.01
62	Carvone	tr	tr	0.01	tr	0.01	0.02
63	<i>p</i> -Menth-1-en-8-yl vinyl ether ^d			tr	0.01	tr	0.01
64	Piperitone	tr					tr
65	Geraniol	0.01	tr	0.04	0.04	0.01	0.02

Table continued over page

Table 1. Continued

Peak No.	Compound	Peru	Ivory Coast	Mexico			
				1	2	3	4
66	Geranial	0.02	0.04	0.04	0.03	0.04	0.03
67	Perilla aldehyde	0.05	0.02	0.04	0.03	0.01	0.04
68	Decanol	tr	tr	0.03	0.04	0.01	0.01
69	α -Terpinen-7-ol ^d	0.02	0.01	0.01	0.01	tr	0.01
70	Bornyl acetate ^d	0.02	0.01	0.02	0.03	tr	0.01
71	<i>trans</i> -Pinocarvyl acetate ^d	0.01	0.01	0.04	0.01	tr	0.01
72	Tridecane	tr	0.01	0.02	tr	tr	tr
73	Undecanal	0.01	0.02	0.06	0.04	0.01	0.02
74	δ -Elemene	0.05	0.04	0.08	0.06	0.05	0.05
75	Citronellyl acetate ^d	tr	0.01	0.01	tr	tr	tr
76	Neryl acetate	0.06	0.04	0.07	0.02	0.02	0.05
77	Undecanol	0.01	tr	tr	tr	tr	tr
78	Geranyl acetate	0.07	0.10	0.13	0.06	0.07	0.08
79	β -Elemene	0.06	0.04	0.08	0.05	0.05	0.05
80	Isocaryophyllene ^d	tr	0.09	0.01	tr	0.06	0.07
81	Dodecanal	0.06	tr	0.10	0.01	tr	0.01
82	<i>cis</i> - α -Bergamotene	0.06	0.04	0.09	0.04	0.05	0.05
83	Decyl acetate ^d	tr	tr	tr	tr	tr	tr
84	β -Caryophyllene	0.42	0.43	0.57	0.52	0.53	0.57
85	Dihydrocaryophyllene oxide ^d	tr	tr	0.01	0.01	tr	tr
86	γ -Elemene ^d	tr	tr	tr	tr	tr	tr
87	<i>trans</i> - α -Bergamotene	0.72	0.62	0.80	0.45	0.65	0.71
88	(<i>Z</i>)- β -Farnesene	0.09	0.08	0.06	tr	0.01	0.01
89	α -Humulene	0.08	0.07	0.15	0.12	0.11	0.13
90	β -Santalene	0.04	0.02	0.08	0.03	0.08	0.08
91	Dodecanol	tr	tr	0.01	0.04	0.01	0.01
92	γ -Curcumene ^d	0.11	0.09	0.09	0.08	0.11	0.07
93	<i>cis</i> - β -Guaiene	0.09	0.08	0.16	0.08	0.10	0.10
94	1,1,3a,7-Tetramethyl-1 <i>H</i> -1a,2,3,3a,4,5,6,7b-octahydro-cyclopropa [a] naphthalene ^{b,d}	0.18	0.15	0.23	0.13	0.22	0.24
95	α -Selinene	0.09	0.07	0.15	0.06	0.09	0.10
96	(<i>Z</i>)- α -Bisabolene	0.11	0.08	0.14	0.06	tr	0.12
97	(<i>E</i>)- α -Farnesene	2.07	1.99	2.47	1.09	1.74	1.93
98	β -Bisabolene ^a	tr	tr	tr	tr	tr	tr
99	(<i>Z</i>)- γ -Bisabolene	0.01	tr	tr	tr	tr	tr
100	δ -Cadinene	0.08	0.04	0.08	0.03	0.06	0.06
101	(<i>E</i>)- γ -Bisabolene	0.04	0.02	0.01	0.02	0.04	0.04
102	1,1,3a,7-Tetramethyl-1 <i>H</i> -1a,2,3,3a,4,5,6,7b-octahydro-cyclopropa [a] naphthalene ^{b,d}	0.19	0.15	0.25	0.13	0.16	0.18
103	Selina-3,7(11)-diene	0.10	0.07	0.11	0.03	0.17	0.05
104	Elemol ^d	tr	tr	tr	tr	0.04	0.04
105	Germacrene-B	0.05	0.01	0.08	0.04	0.06	0.06
106	Caryophyllene alcohol	0.06	0.05	0.09	0.02	0.07	0.07
107	Caryophyllene oxide ^d	0.02	tr	0.05	0.03	0.01	0.01
108	Tetradecanal ^d	0.04	0.03	0.04	0.01	0.03	0.03
109	γ -Eudesmol	0.06	0.05	0.10	0.03	0.07	0.07
110	α -Cadinol	0.03	0.02	0.03	0.01	0.03	0.03
111	Campherenol	0.03	0.01	0.05	0.01	0.04	0.03
112	α -Bisabolol	0.06	0.02	0.02	0.02	0.06	0.03
113	<i>epi</i> - α -Bisabolol ^d	0.06	0.02	0.03	0.01	0.01	0.03
Totals							
	Monoterpene hydrocarbons	71.62	67.86	67.62	71.78	69.03	69.54
	Sesquiterpene hydrocarbons	4.64	4.18	5.55	3.06	4.36	4.67
	Total hydrocarbons	76.52	72.04	73.21	74.68	74.30	74.35
	Alcohols	13.48	13.20	14.33	13.26	13.21	12.97
	Aldehydes	0.40	0.46	0.58	0.19	0.29	0.35
	Esters	0.16	0.17	0.27	0.12	0.09	0.15
	Ethers and oxides	8.64	13.81	10.61	10.96	11.27	11.25
	Ketones	0.11	0.02	0.09	0.06	0.09	0.14
	Total oxygenated components	22.80	27.66	25.89	24.59	24.95	24.87
	Total identified	99.35	99.69	99.15	99.27	99.25	99.20

^a Co-eluted with the former compound.^b *Cis* or *trans* isomer.^c Pairs of compounds co-eluted. The percentage of each component was determined from the chromatograms of the relative fractions obtained by open column chromatography.^d Compounds reported in distilled lime oil for the first time.

Table 2. Average composition in classes of substances of Key lime cold-pressed oil, type A, and distilled lime oil

	Cold pressed lime oil type A ^a (%)	Distilled lime oil ^b (%)
Monoterpene hydrocarbons	86.33	69.58
Bicyclic monoterpene hydrocarbons	26.22	3.17
Sesquiterpene hydrocarbons	7.61	4.41
Monoterpene aldehydes	2.77	0.09
Aliphatic aldehydes	0.55	0.29
Alcohols	1.43	13.41
Esters	0.33	0.16
Ethers and oxides	tr	11.09
Ketones	0.03	0.08

^a See reference 24. ^b See Table 1.

distilled lime oil. From the data reported it is seen that the samples analysed differ little in their qualitative composition. Their quantitative composition, with regard to the major components, is also very similar.

Monoterpene hydrocarbons represent 68–72% of the whole oil, while the sesquiterpenes vary between 3.1% and 5.5%. Aldehydes and esters only account for 0.2–0.6% and 0.1–0.3% respectively. Alcohols and ethers are the most abundant oxygenated components; alcohols were around 13% of the whole oil; ethers and oxides were between 9% and 14%, respectively.

The composition of distilled lime oil differs greatly from the volatile composition of cold-pressed lime oils. Such differences are seen in Table 2, where the average compositions in classes of substances of the two oils are compared. Our results agree with those of Clark and Chamblee.^{19,20}

The mean percentage of monoterpene hydrocarbons in the distilled lime oils analysed is about 70% while in cold-pressed Key lime oils, type A, previously analysed,²⁴ it is about 86%. These differences are mainly due to monoterpene bicyclic hydrocarbons such as sabinene, β -pinene, α -thujene and α -pinene; in fact these compounds, in heated aqueous medium, very easily undergo isomerization and hydration reactions^{19,25–28} and therefore tend to disappear in distilled oils. The most abundant monoterpene hydrocarbon, limonene, even if involved in transformation processes that occur during the distillation, does not change very drastically in content.

Sesquiterpene hydrocarbons, which in cold-pressed oils accounts for 7.6%, drop to 4.4% in the distilled oils. These differences are mostly due to loss of β -caryophyllene, *trans*- α -bergamotene, germacrene-D and germacrene-B.

Aliphatic aldehydes in distilled oils are about 50% less than in cold-pressed oils. Monoterpene aldehydes undergo cyclization and oxidation reactions,¹⁹ therefore their content in distilled oils is about 30% less than the values observed for cold-pressed oils.²⁴ The main products formed by the acidic catalyzed reaction of hydrocarbons and aldehydes are alcohols and oxides,¹⁹ as is evident from the high content of these components in distilled lime oil.

Acknowledgements — This research was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica of Italy (60% and 40% research funds). The coordinator of the research groups was Professor Giovanni Dugo.

References

1. Part LVI: A. Verzera, A. Trozzi, G. Licandro and S. Scuderi, *Essenz. Deriv. Agrum.*, **66**, 353 (1996).
2. L. Haro-Guzman, *Essenz. Deriv. Agrum.*, **50**, 332 (1980).
3. N. Anand and C. L. Green, *IFEAT/CITRAG International Conference on Citrus Oils*, Taormina, Italy (November 1987).
4. E. Guenter and E. Langenau, *J. Am. Chem. Soc.*, **65**, 959 (1943).
5. C. A. Slater, *Chem. Ind.*, **17**, 883 (1961).
6. C. A. Slater, *J. Sci. Food Agric.*, **12**, 732 (1961).
7. E. Kovats, *Helv. Chim. Acta*, **46**, 2705 (1963).
8. J. Perez Zayas and R. Tapanes, *Rev. CENIC, Cienc. Fis.*, **5**(1), 1 (1974).
9. R. Tapanes, *Rev. CENIC, Cienc. Fis.*, **5**(1), 13 (1974).
10. M. A. Azzouz and G. A. Reineccius, *J. Food. Sci.*, **41**, 324 (1976).
11. D. McHale, *8th International Congress of Essential Oils*, Cannes, France (October 1980).
12. M. G. Moshonas and P. E. Shaw, *J. Agric. Food. Chem.*, **28**, 680 (1980).
13. J. A. Pino and R. Tapanes, *J. Food. Technol.*, **18**, 523 (1983).
14. Analytical Methods Committee, *Analyst*, **109**, 1343 (1984).
15. R. T. Alessandro, J. M. Adams and M. A. Miskiewicz, *J. Assoc. Off. Anal. Chem.*, **68**, 1154 (1985).
16. L. Haro and W. E. Faas, *Perf. Flav.*, **10**(5), 67 (1985).
17. T. S. Chamblee, B. C. Clark, T. Radford and G. A. Iacobucci, *J. Chromatogr.*, **330**, 141 (1985).
18. D. S. Khurdiya and M. L. Maheshwari, *Papai J.*, (2), 25 (1988).
19. B. C. Clark and T. S. Chamblee, in *Off-flavors in Food and Beverages*, Elsevier, Amsterdam (1992).
20. T. S. Chamblee and B. C. Clark, *J. Essent. Oil Res.*, **9**, 267 (1997).
21. R. P. Adams, *Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy*, Allured Publishing, Carol Stream, IL (1995).
22. Flavor and Fragrance Library, C. E. Instruments, Rodano, Milano, Italy (1995).
23. L. Mondello, P. Dugo, A. Basile, G. Dugo and K. D. Bartle, *J. Microcol. Sep.*, **7**, 581 (1995).
24. P. Dugo, L. Mondello, G. Lamonica and G. Dugo, *J. Agric. Food Chem.*, **45**, 3608 (1997).
25. C. M. Williams and D. Whittaker, *J. Chem. Soc. (B)*, 668 (1971).
26. C. M. Williams and D. Whittaker, *J. Chem. Soc. (B)*, 672 (1971).
27. M. A. Cooper, C. M. Holden, P. Loftus and D. Whittaker, *J. Chem. Soc. Perkin Trans., II*, 665 (1971).
28. A. Verzera, I. Stagno d'Alcontres, M. G. Donato, R. Del Duce and C. Gigliotti, *Industria delle Bevande*, **119**, 379 (1992).