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Uruguayan Essential Oils. Part VI.* Composition of Lemon Oil

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The composition of 31 genuine Uruguayan lemon oils obtained by industrial processing (FMC on line) during the 1995 season is reported. The samples were representative of all production areas situated in the North and South of Uruguay. The volatile fraction was analysed by HRGC–FID and GC–MS; the enantiomeric distribution of β-pinene, sabinene, limonene, linalol, terpinen-4-ol and α-terpineol was studied by multidimensional HRGC–HRGC; coumarins and psoralens present in the non-volatile residue were analysed by normal phase HPLC. The results relative to the volatile fraction were compared with those obtained for Uruguayan oils produced in the 1992 and 1993 seasons and for Italian FMC oils. Coumarins and psoralens have been analysed for the first time in Uruguayan oils; their content is compared with that of Italian oils. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

In recent years, Uruguayan production of lemon oil has been increasing and at the same time the extraction technology has been improved. The composition of the volatile fraction of lemon oil has been widely studied,[2–9] but so far little has been reported about the enantiomeric distribution of its components[10–12] or about the heterocyclic oxygenated compounds present in the non-volatile residue.[13–15]

In previous papers we analysed the composition of the volatile fraction of Uruguayan lemon oils, both laboratory prepared and industrially produced during the 1989, 1992 and 1993 seasons.[16–18] Here we report the results relative to Uruguayan lemon oils industrially produced during the 1995 season. We wished to confirm the data previously obtained; to detect possible differences in the oil composition due to the extraction technology or to the production period; to identify new components in the volatile fraction; to analyse the enantiomeric distribution of some components present in the volatile fraction and to study coumarins and psoralens present in the non-volatile residue. Volatile fraction results were compared with those relative to Italian FMC oils.[19–20]

EXPERIMENTAL

The research was carried out on 31 industrial FMC lemon oils produced in 1995. The oils were divided into north (9 samples) and south (22 samples) oils according to the production area.

GC Analysis

Volatile fraction was analysed by HRGC–FID as described: gas chromatograph Fisons Mega Series 5160 equipped with a Shimadzu data processor C-R3A; fused silica column SE-52, 30 m × 0.32 mm i.d., film thickness 0.40–0.45 μm; column temperature, 45°C (6 min) to 180°C at 3°C/min; injector and detector temperatures, 280°C; carrier gas He 95 kPa; injection mode, split; injected volume, 1 μl of neat oil.

* For Part V, see Ref. 1.

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GC–MS Analysis

Some samples were analysed by GC–MS(EI) on a Fisons MD 800 (Milan, Italy) system coupled with Adams library[21] and FFC banks;[22] GC conditions were: fused silica SE-52 column, 60 m × 0.32 mm i.d.; film thickness 0.40–0.45 μm (Mega, Legnano (MI) Italy); column temperature 45°C (6 min) to 111°C at 3°C/min, then to 160°C at 2°C/min and at 3°/min to 300°C and held for 15 min; carrier gas He was delivered at constant pressure of 70 kPa (40.5 cm/sec). 1 μl of solution (0.33% v/v essential oil/pentane) were injected on a cold on-column system fitted with an automated actuator. The MS scan conditions were: source temperature, 200°C; interface temperature, 260°C; E energy, 70 eV; mass scan range, 39.00–350.00 amu.

Chiral Analysis

Enantiomeric ratios of some monoterpene hydrocarbons (β-pinene, sabinene, limonene) and of some monoterpene alcohols (linalol, terpinen-4-ol, α-terpineol) were obtained by multidimensional gas chromatography, using a developmental model GC–GC[23] set up with: two GC ovens, the first one equipped with a column coated with SE-52 and the second one with a chiral column coated with a derivatized β-cyclodextrin; a hot interface; a rotary switching valve; and a system to maintain a constant flow during the transfer. With this system a heart-cut of the relevant fractions can be made and these fractions transferred from the non-chiral column to the chiral one in the following experimental conditions: precolumn, fused silica SE-52 column, 30 m × 0.32 mm i.d., film thickness 0.40–0.45 μm (Mega, Legnano (MI) Italy); column temperature 45°C (6 min) to 220°C at 2°C/min. Analytical column, fused silica capillary column, 25 m × 0.25 mm i.d.; coated with a diethyl *tert*-butylsilyl-β-cyclodextrin (Mega, Legnano, (MI) Italy); column temperature, 40–180°C, at 2°C/min; interface temperature, 200°C; detector FID, 250°C (for both chromatographs).

HPLC Analysis

Coumarins and psoralens have been analysed by normal phase HPLC, using a Waters Associates instrument and a photo diode array detector (PDA) model 996 under the same experimental conditions as previously reported[24] using a μ-Porasil column (15 cm × 3.9 mm i.d., particle size 10 μm). Mobile phase, eluent A: hexane:ethyl acetate, 92:8; eluent B: hexane:ethyl alcohol, 9:1. Eluent program: 0–15 min, A 100%; 15–20 min, from A 100% to B 100%, with a linear gradient; 20–30 min, B 100%; flow rate: 1.25 ml/min. Peak integration and quantitative calculations were performed with the Millenium 2010 system (Water Associates). The injection volume was 20 μl of a solution obtained by diluting about 90 mg of lemon oil and 0.1 ml of a coumarin solution of known concentration to 1 ml of hexane:ethyl acetate (75:25). Detection was by UV absorbance at 315 nm. The UV spectra of eluting peaks were monitored in the region 200–400 nm.

RESULTS AND DISCUSSION

Volatile Fraction

Figure 1 shows the chromatogram of a lemon oil obtained using an SE-52 column; 67 components have been identified. The quantitative data were divided according to the production areas into north and south oils. For each group the average ($\bar{X}$) and the standard deviation (s) of all components were calculated. These data are reported in Table 1. Moreover, for each sample, the total amount of hydrocarbons, oxygenated compounds, monoterpenes, sesquiterpenes, carbonyl compounds, alcohols and esters were also calculated. The optical rotation, the refraction index and the CD values are also reported in Table 1. In the same table the results obtained for these oils are compared with those for 337 Italian lemon oils obtained by FMC during the period 1984–92.[19,20] The data reported refer to the volatile part of the oil and do not include the non-volatile residue which generally constitutes 1.5–3.0% of the oil.

With respect to previous research on Uruguayan lemon oils,[16–18] the following new components have been identified: tricyclene, 6-methylhept-5-en-2-one, *cis*-limonene oxide, *trans*-limonene oxide, borneol, perilla aldehyde, nonyl acetate, methyl geranate, dodecanal, decyl acetate, tetradecanal, γ-muurolene, γ-elemene and nootkatone.

The quantitative composition of 1995 north oils is similar to that of 1992 and 1993 north oils, previously analysed.[17] Hydrocarbon, monoterpene and oxygenated compound content is the same for 1993 and 1995 oils; ester and alcohol content is slightly higher while the content of carbonyl compounds is slightly lower in 1995 oils. In comparison with 1992 oils, 1995 oils show a

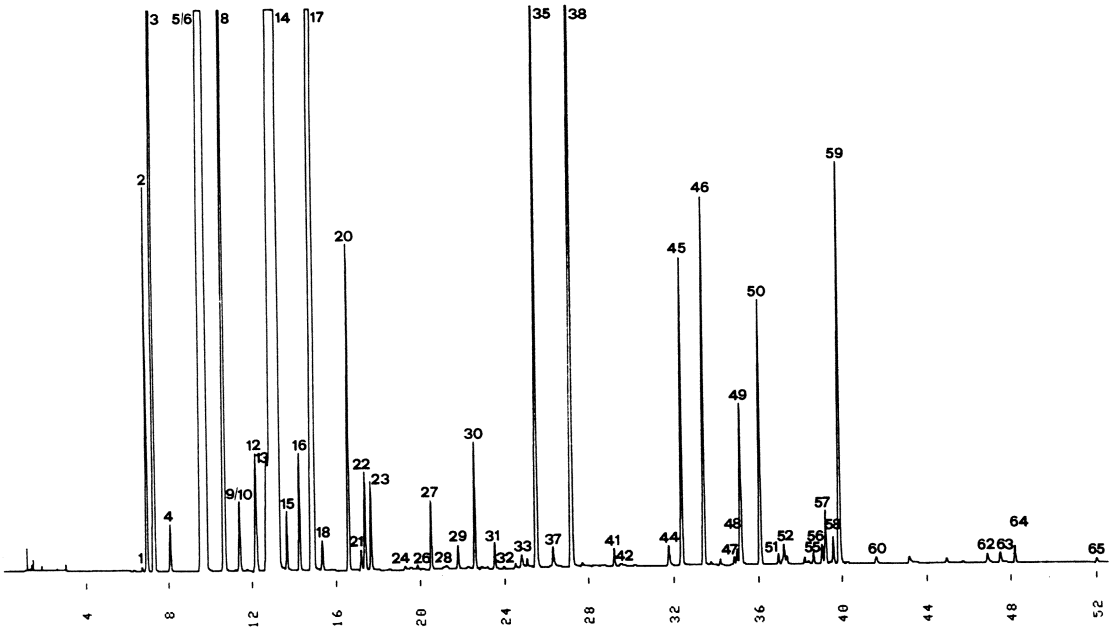

Fig. 1. Gas chromatogram of the volatile fraction of a Uruguayan lemon oil. For peak identification see Table 1.

slightly lower content of hydrocarbons and monoterpenes and a slightly higher content of oxygenated compounds. The amount of each class of substances for north oils of the three different production years are compared in Figure 2.

The quantitative composition of 1995 south oils is very similar to that of 1992 south oils. 1993 south oils have a higher content of oxygenated compounds (mainly carbonyl compounds) and a lower content of hydrocarbons and monoterpenes than 1995 south oils. The amount of each class of substances for south oils of the three different production years is compared in Figure 3.

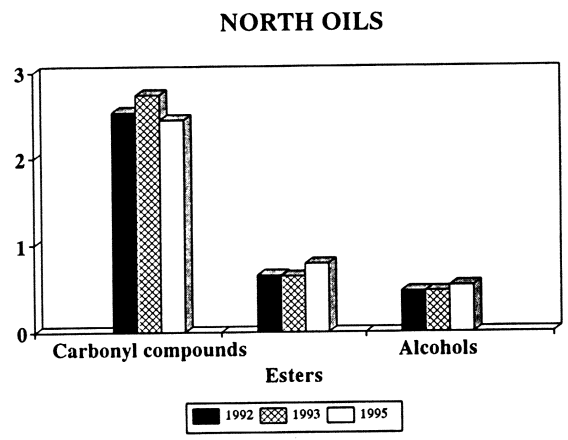

Fig. 2. Carbonyl compound, ester and alcohol average content for north Uruguayan oils produced in 1992, 1993 and 1995 seasons.

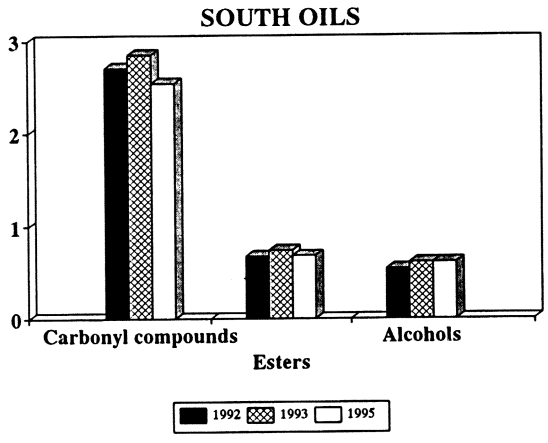

Fig. 3. Carbonyl compound, ester and alcohol average content for south Uruguayan oils produced in 1992, 1993 and 1995 seasons.

Table 1. Average composition as single components and as classes of substances of the North and South Uruguayan and Italian FMC lemon oils

	1995 North oils		1995 South oils		Italian FMC oils	
	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s
1 Tricyclene	0.01	<0.001	0.01	<0.001	0.01	0.001
2 α-Thujene	0.40	0.022	0.04	0.018	0.43	0.019
3 α-Pinene	1.92	0.089	1.89	0.072	1.95	0.067
4 Camphene	0.06	0.003	0.06	0.003	0.06	0.004
5 Sabinene	13.88	0.655	14.31	0.758	2.02	0.157
6 β-Pinene					13.01	1.308
7 6-Methylhept-5-en-2-one	t	–	t	–	t	–
8 Myrcene	1.58	0.081	1.49	0.046	1.44	0.077
9 Octanal	0.04	0.002	0.04	0.002	0.06	0.016
10 α-Phellandrene	0.07	0.004	0.07	0.005	0.05	0.023
11 δ-3-Carene	0.01	0.001	0.01	0.002	0.01	0.001
12 α-Terpinene	0.18	0.009	0.19	0.010	0.19	0.025
13 *p*-Cymene			0.15	0.022	0.13	0.096
14 Limonene	67.15	1.372	67.02	1.334	65.23	1.570
15 (*Z*)-β-Ocimene	0.05	0.005	0.07	0.012	0.07	0.021
16 (*E*)-β-Ocimene	0.10	0.008	0.12	0.017	0.13	0.024
17 γ-Terpinene	8.88	0.440	8.81	0.394	9.54	0.423
18 *cis*-Sabinene hydrate	0.04	0.004	0.05	0.012	0.04	0.007
19 Octanol	0.01	0.002	0.01	0.010	t	–
20 Terpinolene	0.36	0.019	0.35	0.018	0.38	0.024
21 *trans*-Sabinene hydrate	0.03	0.005	0.03	0.007	0.03	0.009
22 Linalol	0.12	0.012	0.12	0.008	0.11	0.017
23 Nonanal	0.10	0.005	0.12	0.016	0.11	0.021
24 *cis*-Limonene oxide	0.01	0.001	0.01	0.003	0.01	0.004
25 *trans*-Limonene oxide	0.01	0.001	0.01	0.003	0.01	0.003
26 Camphor	0.01	0.001	0.01	0.003	0.01	0.002
27 Citronellal	0.09	0.005	0.08	0.010	0.09	0.021
28 Borneol	0.02	0.002	0.02	0.006	0.01	0.004
29 Terpinen-4-ol	0.04	0.005	0.06	0.010	0.04	0.011
30 α-Terpineol	0.16	0.014	0.19	0.018	0.17	0.034
31 Decanal	0.05	0.004	0.04	0.006	0.04	0.013
32 Octyl acetate	t	–	0.01	0.002	t	–
33 Nerol + Citronellol	0.03	0.003	0.02	0.004	0.04	0.019
34 Carbonyl compound	0.02	0.006	0.02	0.006	0.01	0.005
35 Neral	0.76	0.058	0.81	0.086	0.83	0.131
36 Piperitone	t	–	t	–	t	–
37 Geraniol	0.03	0.004	0.04	0.012	0.02	0.006
38 Geranial	1.32	0.094	1.38	0.153	1.39	0.216
39 Perilla aldehyde						
40 Bornyl acetate	0.01	0.005	0.01	0.007	t	–
41 Undecanal	0.03	0.005	0.02	0.005	0.02	0.006
42 Nonyl acetate	0.01	0.002	0.01	0.002	0.01	0.003
43 Methyl geranate	0.01	0.001	t	–	t	–
44 Citronellyl acetate	0.03	0.002	0.02	0.004	0.03	0:009
45 Neryl acetate	0.42	0.033	0.33	0.033	0.40	0.079
46 Geranyl acetate	0.31	0.019	0.31	0.042	0.42	0.123
47 Dodecanal	0.01	<0.001	0.01	<0.001	t	–
48 Decyl acetate	0.01	0.001	0.01	0.001	t	–
49 (*E*)-Caryophyllene	0.26	0.020	0.24	0.023	0.23	0.026
50 α-*trans*-Bergamotene	0.34	0.019	0.30	0.023	0.34	0.031
51 α-Humulene	0.02	0.006	0.02	0.008	0.02	0.003
52 (*E*)-β-Farnesene + β-Santalene	0.05	0.007	0.05	0.011	0.04	0.009
53 γ-Muurolene	t	–	t	–	0.01	0.003
54 Germacrene-D	0.02	0.001	0.01	0.004	0.01	0.004
55 Sesquiterpene	0.03	0.009	0.02	0.004	0.02	0.003
56 Valencene	0.04	0.017	0.02	0.014	0.03	0.018
57 Bicyclogermacrene	0.06	0.006	0.07	0.014	0.07	0.016

Table continues on next page

Table 1. Continued

	1995 North oils		1995 South oils		Italian FMC oils	
	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s
58 (*Z*)-α-Bisabolene	0.04	0.005	0.04	0.006	0.04	0.007
59 β-Bisabolene	0.51	0.031	0.44	0.040	0.51	0.057
60 γ-Elemene	0.01	0.001	0.01	0.002	0.01	0.002
61 Tetadecanal	0.01	0.001	0.01	0.002	0.01	0.002
62 2,3-Dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.02	0.001	0.02	0.003	0.02	0.004
63 Campherenol	0.02	0.002	0.02	0.003	0.02	0.004
64 β-Bisabolol	0.02	0.003	0.02	0.002	0.02	0.004
65 Nootkatone	0.01	0.001	t	–	t	–
Hydrocarbons	96.01	0.229	96.02	0.375	95.83	0.421
Monoterpenes	94.64	0.273	94.81	0.476	94.52	0.470
Sesquiterpenes	1.37	0.068	1.21	0.115	1.31	0.127
Oxygenated compounds	3.78	0.213	3.82	0.331	3.91	0.339
Carbonyl compounds	2.44	0.161	2.54	0.259	2.56	0.335
Esters	0.80	0.040	0.68	0.070	0.86	0.178
Alcohols	0.53	0.030	0.60	0.072	0.49	0.082
CD	0.46	0.020	0.55	0.040		
Optical rotation	63.1	1.25	62.4	1.55		
Refraction index	1.475	<0.001	1.475	<0.001		

Table 2. Enantiomeric ratios for β-pinene, sabinene, limonene, linalol, terpinen-4-ol and α-terpineol in Uruguayan lemon oils

	North			South		
	1	2	3	4	5	6
β-Pinene (+/−)	6.4/93.6	6.3/93.7	5.9/94.1	5.9/94.1	5.9/94.1	5.8/94.2
Sabinene (+/−)	15.5/84.5	15.1/84.9	15.5/84.5	14.4/85.6	14.4/85.6	14.2/85.2
Limonene (+/−)	3.5/96.5	3.5/96.5	3.5/96.5	3.6/96.4	3.6/96.4	3.6/96.4
Linalol (−/+)	51.0/49.0	51.8/48.2	49.5/50.5	56.4/43.6	55.8/44.2	56.8/43.2
Terpinen-4-ol (+/−)	32.5/67.5	30.1/69.9	31.2/68.8	29.3/70.7	29.5/70.5	28.4/71.6
α-Terpineol (−/+)	75.9/24.1	77.1/22.9	74.5/25.5	78.8/21.2	78.7/21.3	79.0/21.0

A comparison of 1995 north and south oils shows that the oils of these different areas have a similar average composition (Table 1) and the quantitative differences for classes of substances and for single components are minor.

The differences found between the composition of the oils produced in the 1992, 1993 and 1995 seasons are probably due to metereological conditions of these years and to improvements in the extraction technology. As regards the meteorological conditions, the winter of 1995 was rather rainy (like those of 1987–92), while the winter of 1993 was mild. In addition, from 1994 the north Uruguayan industries began to use a more efficient water recycling process.

Table 1 also shows that the composition of Uruguayan lemon oils, both north and south, is similar to that of Italian FMC lemon oils. The major differences concern the ester and alcohol content. Italian oils have a higher ester content while Uruguayan 1995 south oils have a higher alcohol content.

Enantiomeric Ratios

Enantiomeric ratios of six components were determined by four subsequent transfers during the same analysis. Figure 4 shows the chromatogram, obtained by SE-52; the zones which were transferred to chiral column are indicated. Figure 5 shows the chiral chromatogram.

Enantiomeric ratios of the components analysed (Table 2) are similar for north and south oils. The most noticeable difference was observed for linalol, which showed a (−)/(+) enantiomeric ratio of 51 : 49 in north oils and of 56 : 44 in south oils. Italian oils, previously analysed,[10] showed a (−)/(+)-linalol enantiomeric ratio which varies from 51:49 to 58:42.

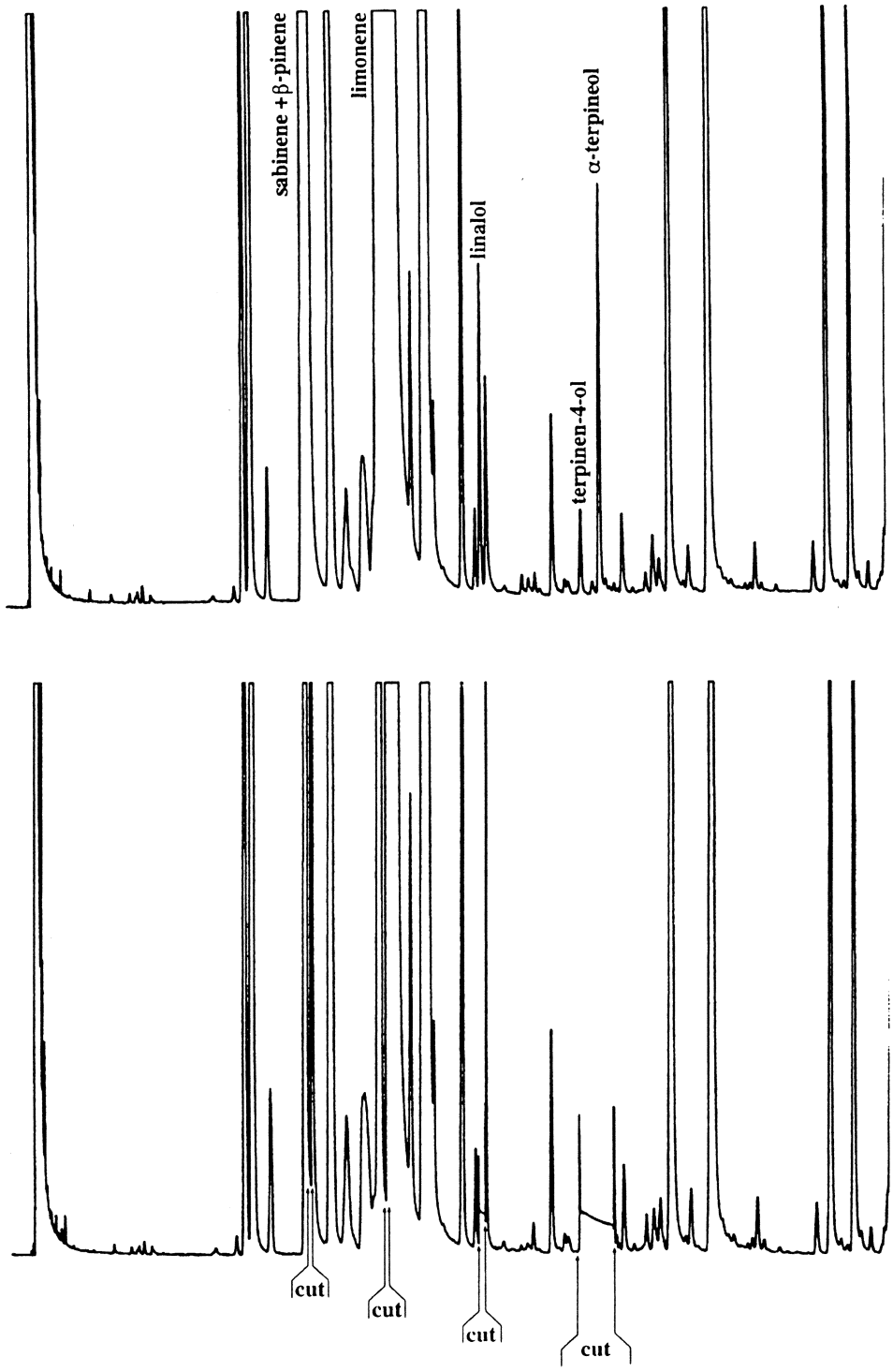

Fig. 4. SE-52 gas chromatogram of a Uruguayan lemon oil: zones transferred are shown.

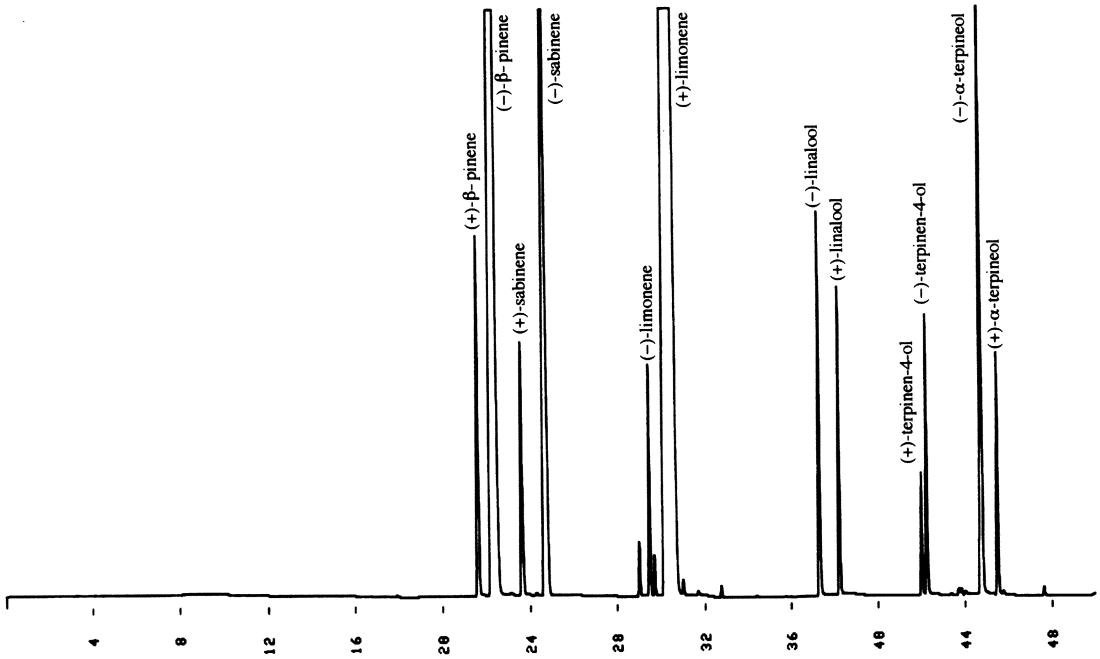

Fig. 5. Chiral gas chromatogram of components of a Uruguayan lemon oil.

Coumarins and Psoralens

Figure 6 shows the HPLC chromatogram of a lemon oil. Three coumarins: citropten, 5-geranyloxy-7-methoxycoumarin and 5-isopentenyloxy-7-methoxycoumarin; ten psoralens: bergamottin, 8-geranyloxypsoralen, byakangelicol, oxypeucedanin, isoimperatorin, imperatorin, phellopterin, 5-isopent-2′-enyloxy-8-(2′,3′epoxyisopentyloxy)-psoralen, oxypeucedanin hydrate and byakangelicin were identified in lemon oil. Moreover two unknown psoralens were detected. Quantitative

Table 3. Average content (g/100 g of oil) of coumarins in Uruguayan and Italian lemon oils

	North Uruguay		South Uruguay		Italian	
	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s
1. Unknown psoralen	+		+		+	
2. Unknown psoralen	+		+		+	
3. Bergamottin	0.218	0.015	0.235	0.021	0.229	0.036
4. Isoimperatorin	+		+		+	
5. 5-Geranyloxy-7-methoxycoumarin	0.174	0.008	0.174	0.017	0.213	0.020
6. 5-Isopentenyloxy-7-methoxycoumarin	+		+		+	
7. Citropten	0.127	0.008	0.169	0.021	0.091	0.026
8. 8-Geranyloxypsoralen	0.033	0.001	0.030	0.004	0.028	0.005
9. Imperatorin*	+		+		+	
10. Phellopterin**	+		+		+	
11. 5-Isopent-2′-enyloxy-8-(2′-3′-epoxyisopentyloxy) psoralen*	+		+		+	
12. Oxypeucedanin	0.107	0.012	0.142	0.014	0.129	0.020
13. Byakangelicol	0.136	0.015	0.152	0.015	0.129	0.024
14. Oxypeucedanin hydrate	+		+		+	
15. Byakangelicin	+		+		+	

* Tentative.
** Tentative, identified according to McHale and Sheridan.[13]

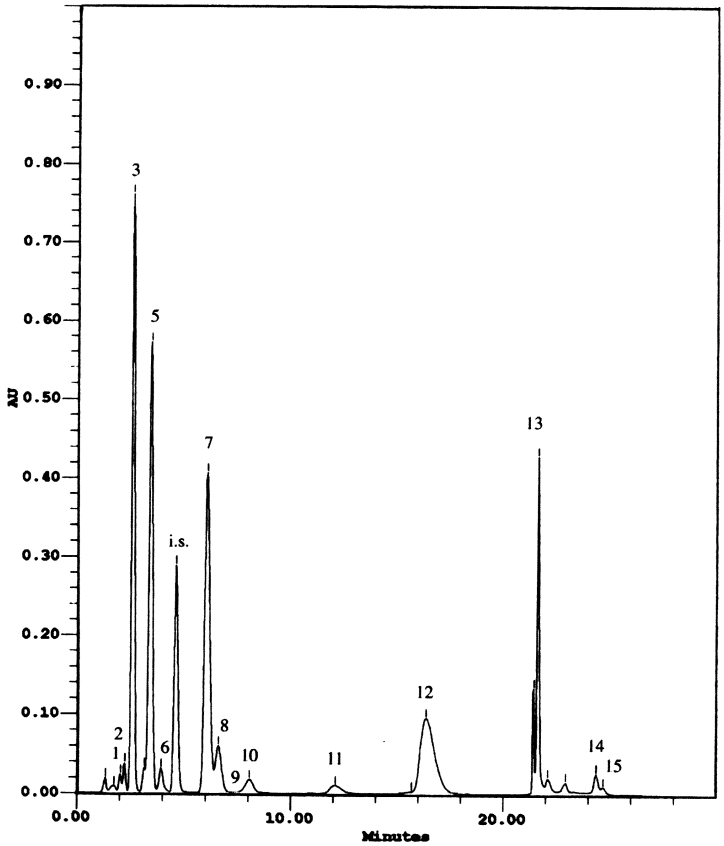

Fig. 6. HPLC chromatogram of a Uruguayan lemon oil. For peak identification see Table 3.

results were only obtained for bergamottin, 5-geranyloxy-7-methoxycoumarin, citropten, 8-geranyloxypsoralen, oxypeucedanin and byakangelicol. For the other components, a sufficient amount at the required degree of purity was not isolated to build a calibration curve and calculate the correction factor, so the quantitative data are not reported.[24] These results are reported in Table 3. The same table reports coumarin and psoralen content for 37 Italian lemon oils produced during the 1994–95 season.[24] The same components were identified in Uruguayan and Italian oils. Oils from the south showed a higher average content of bergamottin, citropten, oxypeucedanin and byakangelicol than those from north.

The most evident difference between Uruguayan and Italian lemon oils concerns the relative content of 5-geranyloxy-7-methoxycoumarin and of citropten. 5-Geranyloxy-7-methoxycoumarin is higher in Italian oils while citropten is higher in Uruguayan ones.

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