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COMPOSITION OF *ALOYSIA GRATISSIMA* LEAF ESSENTIAL OIL*

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Key Word Index—*Aloysia gratissima*; Verbenaceae; volatile leaf oil; sabinene; β-pinene; copaenol; copaenone.

Abstract—Sabinene, β-pinene, copaenol and copaenone have been isolated and identified in *A. gratissima* leaf essential oil from plants growing in Uruguay.

INTRODUCTION

Verbenaceae, and particularly genera *Lippia* and *Aloysia*, grow in tropical and sub-tropical regions [1]. These genera are well known for their aromatic character [2]. Their essential oils have been extensively studied in Brazil and Argentina. Some species, like *L. citriodora* HBK, are grown commercially for their use in perfumery [1], while others are used in folk medicine [3–5]. One interesting aspect of their chemistry is the great diversity of compositions found. Such differences can exist in samples which are morphologically identical, and the existence of chemical races or the influence of climatic and geographical conditions have been proposed as possible explanations [6, 7]. These differences are usually limited to the substitution of a major component by another having an obvious chemical relationship [8], but in some cases there seem to be completely different compositions within the same morphological species [2, 9].

Several species grow in Uruguay, five of which are woody. *L. citriodora* is cultivated as an ornamental plant. The other four, *A. gratissima* (Gill. et Hook.) Tronc., *A. sellowi* Briq., *A. chamaedryfolia* Cham. and *A. virgata* Ruiz et Pav. [10] have not been previously analysed in spite of their interesting aromas. These species are native and usually grow bordering rivers and streams. In this contribution we study the composition of the leaf essential oil from *A. gratissima* (= *A. lycioides* = *L. lycioides* = *A. ligustrina* = *L. ligustrina* [10]) growing in Minas, Uruguay.

RESULTS

Taking into account the possibility of finding dramatic year to year variations due to climatic influences, samples from plants of the same population were collected in two successive years at flowering time (October). Flowers were not included in the distillation.

The essential oils were analysed by capillary GLC and the results are shown in Table 1. The identification of constituents was done by co-injection against pure standards, IR, NMR and GC/MS. As can be observed in Table 1, little chemical variation was observed in *A. gratissima* leaf essential oils. More than 70% of the samples consisted of hydrocarbons (half of which were sesquiterpenes). The main constituent was sabinene (30%), with minor amounts of β-pinene (8%). The oxygenated constituents were mainly sesquiterpenic alcohols and ketones. Oxygenated monoterpenes represented less than 6% of the total. Pinocamphone and *iso*-pinocamphone were identified, this being the first time that these compounds have been found in the group. Given the percentage of sabinene, it is interesting to note the very slight optical activity of the samples.

DISCUSSION

In Table 2 the average composition for *A. gratissima* is compared with the published data for other species of these genera. We can observe that several groups can be established (for references, see notes in Table 2).

(i) The most important, regarding the number of analysed species, is that of plants in which ketones are the main constituents. Most of the species are from Argentina and none grows in Brazil.

(ii) A second group is that of species where phenolics are present, all of them being found in Brazil.

(iii) There is a third group formed by two species with high aldehyde contents, including *L. citriodora*.

(iv) The final group is that of species where hydrocarbons are important (including mono- and sesquiterpenes). *A. gratissima* (Minas) falls within this group, which includes all the other species described for Brazil. There is a clear relationship between *A. gratissima* (Minas) (30% sabinene) and *L. aristata* (21% sabinene, described for N.E. Brazil). The composition found for *A. gratissima* growing in Minas is different from that of the species with the same morphological description growing in Argentina [19].

EXPERIMENTAL

General. Fractional distillations were carried out in a SIBATA High Temperature Spinning Band Column. Preparative GCs were completed with a Pye UNICAM 104 GC with programmed temperature. Capillary GCs were carried out in a Shimadzu GC6-

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Table 1. Composition of *A. gratissima* leaf essential oils

	Compound	%(82)*	%(83)	Identification†				Ref.
1	α-Pinene	2.67	3.01	1	2	3	4	
2	β-Pinene	9.66	7.78	1	2	3	4	
3	Sabinene	29.95	35.29	1	2	3	4	
4	α-Terpinene	1.38	1.19	1	4			
5	Limonene	1.31	1.31	1	4			
6	1,8-Cineole	1.39	1.52	1	4			
7	p-Cimene	0.54	0.58	1	4			
8	Terpinolene	—	0.49	4				
9	α-Elemene	1.69	1.65	4				
10	Pinocamphone	1.71	2.06	2	3	4		[11]
11	$C_{15}H_{24}$ (M^+ 204)	—	0.71	4				
12	*iso*-Pinocamphone	2.14	1.50	2	3	4		[11]
13	*cis*-Sabinene hyidrate	—	0.8	4				
14	α-Cedrene	3.35	2.88	2	3	4		[12]
15	*iso*-Caryophyllene	—	0.69	4				
16	β-Caryophyllene	2.08	1.73	1	4			
17	Hydrocarbon	7.38	7.94					
18	Unknown	0.95	1.00					
19	Unknown	—	0.36					
20	Unknown	—	0.36					
21	Unknown	—	0.77					
22	β-Cadinene	2.08	1.08	4				
23	β-Bisabolene	5.81	2.08	4				
24	$C_{15}H_{24}$ (M^+ 204)	0.85	1.23	4				
25	$C_{15}H_{24}$ (M^+ 204)	0.97	0.96	4				
26	Unknown	—	0.37					
27	α-Curcumene	2.57	1.81	4				
28	Calamenene	1.28	1.22	4				
29	$C_{15}H_{24}$ (M^+ 204)	0.50	1.34	4				
30	Caryophyllene epoxide	0.50	0.89	4				
31	Unknown	1.93	1.54					
32	Copaenone	3.59	4.14	2	3	4		
33	Globulol or ledol	3.48	3.08	4				[13]
34	Spathulenol	1.10	0.81	4				[14]
35	Copaenol	4.29	4.28	2	3	4		[15]
36	C_{15} alcohol	3.87	1.54	4				

* Percentages are w/w as determinated by GC.

† Identification key: 1, Retention time on PEG 20 M and OV 17; 2, ^{1}H NMR spectra; 3, IR; 4, GC/MS.

AMPrF GC. Capillary columns were drawn with a Shimadzu GDM-1 and coated with a Shimadzu MCT-1A machine following ref. [24]. Refractive indexes were determined with an ATAGO M Refractometer. Optical rotations were measured using 50 mm cells and an ATAGO Polax polarimeter. IR spectra were run neat on NaCl discs, on a Perkin–Elmer 177. NMRs on a Varian T-60 using CCl_4 as solvent. GC/MS were measured on a Hitachi M-80 GC/MS with an ionization potential of 20 eV. All solvents were distilled in glass prior to their use. All other reagents used were of analytical grade.

Plant material. Young twigs of *A. gratissima* were collected from wild plants growing 20 km SW of Minas, Uruguay. Collections were carried out during the flowering periods on 11/10/82 and 13/10/83. The leaves were picked by hand after 3 days at room temp.

Distillation. The dry leaves were steam-distilled. The condensed H_2O was separated from the supernatant oil and extracted with Et_2O. The ethereal extract was added to the oily layer, dried (Na_2SO_4) and evaporated to yield a greenish oil. For 1982 the yield was 1.6% (v/w; 10.5 ml from 650 g leaves; $d_{20°}$ = 0.9002; $[\alpha]_D^{20°} = 0°$; RI = 1.4876), and for 1983, 1.9% (v/w; 14.1 ml from 750 leaves; $d_{20°} = 0.8997$; $\alpha_D^{20°} = -1°$; RI = 1.4874).

Fractional distillation. The essential oil (35 ml) was distilled under vacuum. The system was first refluxed closed for 30 min. The reflux ratio was then set at 40, with a distillation-rate of 5 ml/hr and a band-speed of 2000 rpm.

Four fractions were distilled at 8 mm Hg within a range of 40–50°; three at 5 mm Hg and a range of 45–65°; six at 2.5 mm Hg and a range of 60–80°. All fractions were then analysed by capillary GC and examined against the original oil to detect the presence of artifacts.

Column chromatography. Column chromatography was carried out with silica gel (Merck 10181), using a 50/1 ratio of silica to sample. Hydrocarbons were eluted with petrol (bp 60–70°), and the oxygenated fractions eluted with increasing percentages of Me_2CO.

Argentation column chromatography. Hydrocarbon fractions were resolved on silica gel with 10% $AgNO_3$ added.

Table 2. Comparison of *Lippia* and *Aloysia* essential oils by groups of components

Species	O_{10} compounds				O_{15} compounds			Ref.
	H	O1	Ket	Al	H	Ox	Phen	
L. alba B.	5.3	—	—	22.5	33.3	—	—	[2]
L. citriodora	1.4	11.6	—	59.4	—	—	—	[16]
L. grata	48.5	0.6	—	—	3.5	—	43.1	[2]
L. sidoides	44.4	—	0.6	—	9.7	—	48.6	[2]
L. alnifolia	3.8	—	—	—	41.0	—	34.8	[2]
L. fissicalis	36.3	1.6	60.4	—	—	—	—	[17]
L. alba A.	22.0	1.8	61.0	—	1.8	—	—	[18]
A. lycioides	26.0	7.2	52.0	—	6.2	—	—	[19]†
A. polystachia	30.0	—	70.0	—	—	—	—	[20]
L. adoensis	15.0	4.1	69.5	—	—	—	—	[21]
L. grisebaquiana	16.5	47.0	22.0	0.3	5.3	—	—	[22]
L. ukambensis	9.5	35.1	36.5	—	7.3	—	—	[23]
L. aff. aristata	24.8	0.8	—	—	65.8	4.5	—	[2]
L. aristata	42.3	1.2	—	—	46.1	—	—	[2]
A. gratissima	47.0	—	4.0	—	14.7	15.8	†	

*Key: H, hydrocarbons; O1, alcohols; Ket, ketones; Ald, aldehydes; Ox, oxygenated compounds; Phen, phenols.

† See comments in Discussion.

Gas chromatography. Analytical GLC was carried out with WCOTs: (a) stainless steel, FFAP, i.d. 0.25 mm, 45 m; (b) glass, PEG 20 M, i.d. 0.3 mm, 30 m; (c) OV-17, i.d. 0.5 mm, 60 m. Injection and detector temps were 250°; column oven temp. from 60 to 200° at 6°/min; carrier: N_2 at 30 ml/min; splitter ratio 32. Preparative GLC was carried out using glass columns (OV-101 5% on Diatomite C-AW DMCS 80–90 mesh), of 4.6 m length, i.d. 8 mm; carrier: N_2 at 100 ml/min, using a 1/100 splitting ratio, with CO_2/Me_2CO cold-traps.

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