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Essential oil of *Aloysia virgata* var. *platyphylla* (Briquet) Moldenke from Corrientes (Argentina)

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ABSTRACT: The qualitative and quantitative composition of the essential oil from aerial parts of *Aloysia virgata* var. *plathyphylla* (Verbenaceae) has been investigated for the first time. This species grows naturally in northeast Argentina, where is employed in folk medicine. The essential oils were obtained by steam distillation of leaves and young stems at different stages and analyzed by GC-MS. The oil composition showed quantitative variation during the period of sampling (spring–summer–autumn): sabinene, 1.8–2.4%; δ-elemene, 4.6–8.4%; β-caryophyllene, 16.3–18.1%; germacrene D, 16.0–27.1%; and bicyclogermacrene, 17.6–29.3%. The characterization of *Aloysia virgata* var. *plathyphylla* by enantioselective gas chromatography was performed by evaluation of the enantiomeric ratio of sabinene and germacrene D. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Aloysia virgata* var. *platyphylla*; essential oil; δ-elemene; germacrene D; bicyclogermacrene; enantioselective gas chromatography

Introduction

The family Verbenaceae comprises more than 98 genera, mainly tropical and sub-tropical herbs, shrubs and trees. *Aloysia* is a large genus of this family, represented in the northeastern region of Argentina by 12 species, three of them endemic: *Aloysia citriodora* Palau, *Aloysia virgata* (Ruiz & Pav.) Juss. var. *virgata* and *Aloysia virgata* var. *platyphylla* (Briquet) Moldenke.[1] They are used as medicinal and aromatic plants in this region, where a large proportion of the population use medicinal plants as their unique source of medicines.[2]

Aloysia virgata var. *platyphylla* (Briquet) Moldenke [botanical synonym *Aloysia virgata* var. *elliptica* (Briq.) Moldenke[3]] is known by Guaranians as 'niño rupá guazú'[4] or 'pa'ira yvoty'.[5,6] This species can be found in Bolivia, Brazil, Paraguay and northwest and northeast Argentina,[3,7] and it is commonly undifferentiable by common people from *A. virgata* var. *virgata* because of their morphologic similarities. The latter is, however, the more common species found in Corrientes Province. Despite its importance as medicinal plant, there is little available information on the chemical composition of *Aloysia virgata* var. *platyphylla*.[8,9]

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As part of our continuing search of plants for new sources of valuable oils/perfumery or pharmaceutical products, and owing to the importance of *Aloysia virgata* for medicinal purposes, we report here the relative composition of the oil obtained from aerial parts of this species growing wild in the Corrientes Province (Argentina), and its characterization by enantiomeric analysis using multidimensional gas chromatography.

Experimental

Plant Material and Isolation of the Essential Oil

To evaluate the species behavior in response to seasonal factors in different collections over the year, samples of fresh leaves and young stems, representing an entire population of *A. virgata*, were collected randomly near Río Empedrado (55 km at the South of Corrientes), 27.51°S, 58.44°W, in three different seasons: spring (oil **I**); autumn (oil **II**); and summer (oil **III**). Plant material was identified by Professor Sara G. Tressens (IBONE/UNNE) and voucher specimens were deposited at the Herbarium of the Facultad de Agroindustrias (UNNE), in Sáenz Peña (Chaco, Argentina; FAI 122 and 123).

The essential oils were obtained from the leaves and young stems (1.588 kg of sample **I**; 0.708 kg of sample

II; and 0.690 kg of sample **III**), previously dried for 3 days at controlled temperature, by steam distillation using a macro distillation apparatus with a 12 l flask. The oils were dried with anhydrous sodium sulfate and kept in a sealed flask at −15 °C until analysis.

Physicochemical Indices

The physicochemical indices of the oils were determined following the ISO norms, ISO 279:1981 for the specific gravity (using a 0.5 ml picnometer), ISO 592:1981 for the optical rotation and ISO 280:1976 for the refractive index.

GC Analysis

Capillary gas chromatography was carried out using a Shimadzu 14 B gas chromatograph equipped with a flame ionization detector (FID) and Shimadzu data processing software, EZ-Chrom, using two capillary columns. The first was a SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m × 0.32 mm i.d.), coated with 5% phenylpolymethylsiloxane (0.40–0.45 μm phase thickness); column temperature, 60 °C (8 min) to 180 °C at 3 °C/min, 180–250 °C at 20 °C/min, then 250 °C for 10 min; injector temperature, 250 °C; detector temperature, 280 °C; injection mode, split; split ratio, 1:30; volume injected, 0.2 μl of oil; carrier gas, hydrogen, 55 kPa.

The second column was a Carbowax 20M (Ohio Valley, OW, USA) bonded fused-silica capillary column (25 m × 0.32 mm i.d.), coated with polyethylene glycol (0.25 μm phase thickness); column temperature, 40 °C (8 min) to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min; injector temperature, 250 °C; detector temperature, 250 °C; injection mode, split; split ratio, 1:30; volume injected, 0.2 μl of oil; carrier gas, hydrogen, 30 kPa.

GC-MS Analysis

GC-MS analysis was conducted using a Shimadzu QP 5050 equipped with reference libraries,[10,11] using two capillary columns. The first was an SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m × 0.25 mm i.d.), coated with 5% phenylpolymethylsiloxane (0.25 μm phase thickness); column temperature, 60 °C (8 min) to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min; injector temperature, 250 °C; injection mode, split; split ratio, 1:40; volume injected, 0.2 μl of oil; helium was used as the carrier gas, 122.2 kPa (51.6 cm/s); interface temperature, 250 °C; acquisition mass range, 40–400.

The second column was a BP 20 (SGE, Australia) bonded fused-silica capillary column (25 m × 0.25 mm i.d.), coated with polyethylene glycol 20 000 (0.25 μm phase thickness); column temperature, 40 °C (8 min) to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min; injector temperature, 250 °C; injection mode, split; split ratio, 1:40; volume injected, 0.2 μl of oil. The carrier gas was He, 92.6 kPa (55.9 cm/s); interface temperature, 250 °C; acquisition mass range, 40–400.

Identification and Quantification

The components of the oil were identified by comparison of their linear retention indices (LRIs) on the two columns, determined in relation to a homologous series of *n*-alkanes (C_9–C_{26}), with those from pure standards or reported in the literature.[10,12] Comparison of fragmentation patterns in the mass spectra with those stored on the GC-MS database was also performed.[10,11] Quantification of each compound was performed on the basis of their GC peak areas. Relative proportions of the essential oil constituents were expressed as percentages obtained by peak-area normalization, all relative response factors being taken as 1. Percentages were obtained on SE-52 except for those of limonene and *p*-cymene, which were obtained on Carbowax 20M.

Chiral Analysis

Enantiomeric ratios of sabinene were obtained by multidimensional GC, using a developmental model[13] set up with two GC ovens. The first oven was equipped with a column coated with SE-52 and the second 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 transferred from the non-chiral column to the chiral one in the following experimental conditions: precolumn, SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (30 m × 0.32 mm i.d.), coated with 5% phenylpolymethylsiloxane (0.40–0.45 μm phase thickness); column temperature, 45 °C for 6 min, rising to 280 °C at 2 °C/min, then 280 °C for 15 min; analytical columns, fused-silica capillary column (25 m × 0.25 mm i.d., 0.25 μm phase thickness), coated with, for column (a), 2,3-di-*O*-ethyl-6-*O*-*t*-butyldimethylsilyl-β-cyclodextrin in PS 086 (13% phenylmethyl-polysiloxane; Mega, Legnano, Italy) and, for column (b), 2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl-β-cyclodextrin in OV 1701 (50%); injection temperature, 250 °C; column temperature, for column (a) 50 °C for 6 min, rising to 90 °C at 2 °C/min, 90 °C for 20 min, 90–180 °C at 2 °C/min, then 180 °C for 10 min and, for column (b), as described by Schmidt

et al.;[14] interface temperature, 200 °C; detector FID, 280 °C (for both chromatographs). Volume injected, 1 µl of an oil diluted 1:10 in *n*-hexane; injection mode, split; split ratio, 1:15; carrier gas, helium, 90 kPa (precolumn) and 110 kPa (analytical column). Under the experimental conditions, sabinene resolved best on 2,3-di-*O*-ethyl-6-*O*-*t*-butyldimethylsilyl-β-cyclodextrin, while germacrene D resolved best on 2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl-β-cyclodextrin. The enantiomers of sabinene were assigned by injection of enantiomerically pure standards purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St Louis, MO, USA). Germacrene D has been isolated before in an optically impure state from the leaves of *Hedyosmum scabrum* and then the optical rotation (25 °C) determined, considering that optically pure (−)-germacrene-D was estimated to be 305°.[15] The order of elution of the enantiomers was checked in the same chromatographic conditions as described previously.[14]

Results and Discussion

The yields of essential oil in different harvesting periods in *A. virgata* var. *plathyphylla* aerial parts, their physical constants and chemical composition of oils collected at three different stages of the year are shown in Tables 1 and 2. The three samples gave similar chromatographic profiles, but with important variations in the proportions of the main constituents.

The utilization of the chemical diversity of the plant kingdom is one of main directions of investigation. Search for inter- or intraspecific distribution of well-defined active compounds is one of the most important goals of analysis, resulting in large numbers of cultivars of practical importance. It has been reported that the formation of active principles occurs predominantly during the periods of vigorous growth or during a time of intensive metabolic processes such as when a plant is flowering or fruiting.[16] Over different stages of growth, including full flowering, the oil composition from leaves and young stems of *A. virgata* showed considerable variation within the range of analytical variations for the different samples analyzed. These quantitative variations in the essential oils might be a result of different metabolic stages.

The percentage composition of the essential oil at different growing phases is presented in Table 2. Thirty-five components, representing 89.2–96.8% of the total composition, were identified. All the oil samples were dominated by their sesquiterpene fraction (79.5–90.9%), δ-elemene (4.6–8.4%), β-caryophyllene (16.3–18.1%), germacrene D (16.0–27.1%) and bicyclogermacrene (17.6–29.3%) being the main components. The oxygen-containing sesquiterpenes represented 4.1–8.2%. The monoterpene fraction occurred in smaller amounts (1.8–2.6%), sabinene (1.8–2.4%) being the only monoterpene to attain relative amounts larger than 1%.

Table 1. Physicochemical indices of the *Aloysia virgata* var. *platyphylla* oil

Oil	Yield (%)	$d_4^{20°}$ (g/ml)	$n_D^{20°}$	$\alpha_D^{t°C}$
I (spring)	0.4	0.9097	1.5096	$-48.58^{\circ 25°}$
II (autumn)	0.3	0.9142	1.5083	$-45.46^{\circ 27°}$
III (summer)	0.5	0.9227	1.5094	$-40.62^{\circ 25°}$

A literature search provided only one recent reference on the composition of the essential oil from the leaves and inflorescences of *A. virgata*[9] from Cuba. This oil was characterized by the presence of germacrene D (15.6%), β-caryophyllene (15.4%), bicyclogermacrene (13.8%) and α-humulene (11.7%) as major components. Even though the chemical composition seems to be similar to our report, there are some noteworthy quantitative differences regarding the main components. Furthermore, no detailed information related to the taxonomy of the Cuban material studied is given, making comparison between the results difficult.

These data are based on single samples at each collection site and do not take into account within-site variation. However, the chemical composition of the analysed essential oils appears to show quantitative variation due to the influence of local environmental conditions and seasonal collections period.

Because *A. virgata* is a traditionally used medicinal plant,[5,17–21] more work must be performed in order to establish the probable relationship between the composition of essential oils and its therapeutic properties.

Chirality evaluation of essential oil compounds has been introduced as a new and substantial indicator of genuineness, presupposing high stereoselectivity during biosynthesis of the precursors and enzyimatically induced liberation of the aroma-active chiral volatiles to be analyzed.[22] Therefore, in order to characterize the oil of *A. virgata* growing wild in Corrientes, we also report here the enantiomeric distribution of sabinene and germacrene D (Table 3), the most characteristic monoterpene and sesquiterpene present in the oil, respectively.

Chiral flavor and fragrance components in natural products are generally characterized by a specific distribution of enantiomers; in other words, the compound is not always present as a pure enantiomer, but rather with a specific enantiomeric ratio, which may differ according to variety and environmental conditions. In this work, the experimental conditions used avoided partial or total racemization of sensitive compounds that could be brought about during the work-up procedure of the analytical method. The high content of germacrene D and bicyclogermacrene found in the oils is proof of this procedure, as the high lability of both sesquiterpenes is known.[23,24] In this way the specific enantiomeric ratio of sabinene and germacrene D was analyzed, which was

Table 2. Percentage composition of the essential oil of *Aloysia virgata* var *platyphylla* (Briquet) Moldenke

Compound[a]	Percentage[b]			
	LRI (SE52)	I (spring)	II (autumn)	III (summer)
α-Thujene	936	tr	0.1	0.1
α-Pinene	941	tr	0.1	tr
Sabinene	976	1.8	2.2	2.4
β-Myrcene	990	tr	—	tr
p-Cymene	1021	tr	0.1	0.1
Limonene	1025	0.1	tr	tr
γ-Terpinene	1059	tr	0.1	tr
δ-Elemene	1338	8.4	4.6	4.9
α-Cubebene	1348	tr	0.1	0.1
α-Copaene	1375	1.1	1.7	4.1
β-Bourbonene	1384	0.7	3.3	2.5
β-Cubebene	1391	—	0.2	tr
β-Elemene	1391	2.7	0.4	3.0
α-Gurjunene	1406	0.1	0.2	0.4
β-Caryophyllene	1424	16.8	16.3	18.1
β-Copaene	1428	0.7	1.7	1.0
γ-Elemene	1431	1.6	0.3	2.4
Aromadendrene	1441	—	1.2	—
α-Humulene	1450	1.0	2.7	1.5
9-*epi*-(*E*)-Caryophyllene	1459	1.9	3.7	2.7
Germacrene D	1483	25.1	27.1	16.0
Bicyclogermacrene	1500	29.3	17.6	20.3
α-Muurolene	1500	—	0.3	—
γ-Cadinene	1502	0.5	0.4	0.8
δ-Amorphene	1504	0.7	0.3	1.6
10-*epi*-Cubebol	1506	0.1	0.2	0.4
Germacrene A	1520	0.1	1.6	0.3
Germacrene B	1555	1.3	1.3	1.9
Germacrene D-4-ol	1575	1.2	—	3.0
Spathulenol	1576	0.9	0.6	1.3
trans-Sesquisabinene hydrate	1596	0.2	tr	0.3
Globulol	1605	0.1	0.5	0.7
Viridiflorol	1610	—	0.4	—
Guaiol	1615	0.2	0.4	0.4
Bulnesol	1677	0.1	tr	0.2
Identified components		96.8	89.2	90.5
Grouped components				
Monoterpene hydrocarbons	1.8	2.4	2.6	
Oxygen-containing monoterpenes	—	—	—	
Sesquiterpene hydrocarbons	90.9	83.4	79.5	
Oxygen-containing sesquiterpenes	4.1	3.4	8.2	
Others	—	0.1	0.1	

[a] The components are reported according their elution order on SE-52.
[b] tr, percentage values less than 0.1%.

Table 3. Enantiomeric ratios for sabinene and germacrene D in *Aloysia virgata* var. *platyphylla* essential oil

	I (spring)		II (autumn)		III (summer)	
Sabinene						
Enantiomer	(1*R*,5*R*)-(+)	(1*S*,5*S*)-(−)	(1*R*,5*R*)-(+)	(1*S*,5*S*)-(−)	(1*R*,5*R*)-(+)	(1*S*,5*S*)-(−)
Ratio (%)	87.7	12.3	87.2	12.8	87.5	12.5
Germacrene D						
Enantiomer	7*R*-(+)	1*S*-(−)	7*R*-(+)	1*S*-(−)	7*R*-(+)	1*S*-(−)
Ratio (%)	0.5	99.5	0.7	99.3	0.7	99.3

characteristic of this species in the selected environmental conditions. There were only minor variations in enantiomeric composition between the samples studied, representing the entire population of *A. virgata*, during the different harvesting periods for the chiral terpenes studied.

In summary, on the basis of this information and according to the evaluation of the species behaviour in

response to seasonal factors in different collections over the year, we report the essential oil composition of aerial parts from *A. virgata* var. *platyphylla*, and contributed to the characterization of this medicinal plant from a metabolic point of view, by the chiral analysis of selected components of the oil.

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