

PII: S0031-9422(97)00400-7

# VOLATILE CONSTITUENTS OF LEAVES, ROOTS AND STEMS FROM ARISTOLOCHIA ELEGANS

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(Received in revised form 5 March 1997)

**Key Word Index**—*Aristolochia elegans*; Aristolochiaceae; essential oil; sesquiterpenes;  $\beta$ -caryophyllene; isocaryophyllene; bicyclogermacrene; E-nerolidol.

Abstract—The volatile oils from roots, stems and leaves of Aristolochia elegans Mast., collected at different stages of development, were obtained by hydrodistillation and analysed by GC and GC-mass spectrometry. All the samples of essential oils were found to be rich in sesquiterpenes. Sesquiterpene hydrocarbons, in particular  $\beta$ -caryophyllene, isocaryophyllene and bicyclogermacrene were the predominated components in the oil from the leaves, whereas their amounts were significantly decreased in the essential oils of the other plant parts. The oxygenated sesquiterpenes, mainly E-nerolidol, were the main constituents of the oils from stems and roots. © 1997 Elsevier Science Ltd

## INTRODUCTION

The genus Aristolochia comprises several species used in traditional medicine e.g. A. radix [1], A. mollisima [2] and A. indica [2, 3]. Among them, A. elegans, a woody vine which grows in the forests of north-eastern Argentina [4], has been reported to contain an alkaloid with uterus contraction stimulating activity [5]. Barnard [6] found that its alcoholic extract showed antimitotic activity. The antiviral activity of this species has been also screened [7].

Previous phytochemical investigations of acetone extracts from A. elegans revealed the presence of lignanes, kaurane diterpenes and the sesquiterpenes nerolidol and caparrapidiol [8]. The present work deals with the composition of the essential oil of the leaves, stems and roots of this species, collected at different seasons of the year.

#### RESULTS AND DISCUSSION

The qualitative and quantitative analytical results are shown in Table 1. In total 58 constituents were identified and collectively these accounted for more than 80% of the total oil in all samples, except in the roots in which they accounted for only 62% of the oil. Sesquiterpenes were shown to be the main group of

constituents in all the essential oils analysed, whereas monoterpenes only constituted a small percentage of all the samples investigated. The essential oils analysed showed different composition according to the plant part from which they were obtained. In the case of the leaves, little seasonal variation was found.

 $\beta$ -Caryophyllene and other sesquiterpene hydrocarbons, like bicyclogermacrene, isocaryophyllene or germacrene D, predominate in the oil from leaves. Their percentages significantly decrease in the oils obtained from stems and roots, in which a tendency to accumulate oxygenated sesquiterpenes, mainly Enerolidol, was found. Previous work on the composition of the essential oil of other *Aristolochia* spp. showed also that sesquiterpene hydrocarbons, particularly  $\beta$ -caryophyllene, tend to accumulate in the leaves but not in the roots or stems of the plants [9–12].

The two essential oil samples coming from leaves L-1 and L-2 showed a similar composition with little differences due to seasonal variation. The major constituents in both samples were the sesquiterpene hydrocarbons, particularly caryophyllane derivatives (L-1 and L-2) and compounds with a germacrane skeleton (L-2). In addition, sample L-1 contained a higher percentage of minor constituents, such as: longifolene, *allo*-aromadendrene, α-copaene, γ-cadinene and spathulenol, than L-2, which could be due to a higher enzymatic activity at the flowering stage. Isocaryophyllene and bicyclogermacrene, which reached per-

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Table 1. Composition of the essential oil from leaves, roots and stems of A. elegans

Components		% total*		
	L-I	L-2	R	S
Monoterpene hydrocarbons	0.6		2.2	
α-Pinene			0.6	
$\beta$ -Pinene			0.4	_
Camphene			1.2	
Limonene	0.6			_
Oxygenated monoterpenes	_		3.5	0.5
1,8-Cineole			0.4	
Bornyl acetate			2.2	
Borneol	_		0.9	_
Linalool	_		_	0.1
Cyclocitral	_	-	_	0.4
Sesquiterpene hydrocarbons	72.8	85.1	15.3	8.0
$\delta$ -Elemene	_	1.1		_
α-Cubebene		0.2	_	tr†
α-Copaene	2.9	1.1	3.8	0.3
$\beta$ -Bourbonene	1.5	1.1	_	0.2
Isocaryophyllene	16.2	0.6	0.2	0.1
β-Elemene	1.1	1.2	0.3	0.2
α-Gurjunene	1.3	0.4		_
β-Gurjunene	0.6		_	0.1
β-Caryophyllene	16.9	27.8	0.7	3.5
Longifolene	7.4			0.2
Isolongifolene	0.9		_	
6,9-Guaiadiene			1.3	_
allo-Aromadendrene	1.3	0.2	1.9	
α-Himachalene	1.6		_	_
α-Humulene	3.0	2.6	0.3	0.1
β-Cubebene	0.5	0.2		
γ-Muurolene	0.6		0.1	0.1
Germacrene B		1.0		
Germacrene D	3.4	12.7		1.8
Bicyclogermacrene	9.2	32.4		0.8
α-Bisabolene		-	0.2	
γ-Cadinene	3.7	2.4	2.9	_
$\delta$ -Cadinene	0.2	0.1	0.6	0.5
α-Curcumene	_	tr		
Calacorene‡	0.1	****	1.4	_
Calamenene	0.4		0.2	_
Guaiazulene			1.4	_
trans-β-Farnesene				0.1

centages of 16.2% in sample L-1 and 32.4% in L-2, respectively, have never been previously reported in the essential oils of the genus *Aristolochia*. *Allo-*aromadendrene (0.2 to 1.9%) is chemically related with aromadendrene, found in *A. longa* [13], and with ledol, found in *A. asclepiadifolia* [11].

### **EXPERIMENTAL**

Plant material. Leaves, stems and roots from A. elegans were collected from ornamental plants cultivated in San Isidro, province of Buenos Aires (Argentina). Leaves were collected in October 1993 (L-1) and July 1994 (L-2); roots in October 1993 (R)

Table 1—Continued.

Components	% total*				
	L-1	L-2	R	S	
Oxygenated sesquiterpenes	8.4	6.3	41.0	84.2	
Z-Nerolidol		-	0.4	_	
E-Nerolidol	0.6	0.5	35.1	79.5	
Bourbonanol§	-	0.1			
Caryophyllenol§	0.4			_	
β-Caryophyllene oxide		0.9		3.5	
Cubenol	_	0.1	3.8	_	
epi-Cubenol	_	0.2	0.3	-	
Hedycaryol	_	_	0.3		
Guaiol		_	0.5	_	
Globulol	1.5	0.4			
Viridiflorol	0.9	0.3		_	
Spathulenol	1.7	1.9		0.4	
T-Cadinol		0.2	0.2		
T-Muurolol	0.9	0.5		0.3	
α-Cadinol	1.8	1.0		0.5	
Cadinol isomer§	0.7		0.1	_	
Farnesyl ketone		0.2	Tenerity our		
Farnesol§			0.3	_	
Others				0.2	
1-Octen-3-yl acetate	_	_		tr	
1-Octen-3-ol		-		0.2	
3-Octanol	_			tr	
Total identified	81.8	91.4	62.0	92.9	

<sup>\*</sup>Sample L-1: leaves, October 1993; sample L-2: leaves July, 1994; sample R: roots; sample S: stems.

and stems in July 1994 (S). The fresh plant material was subsequently air-dried, with protection from light. A voucher specimen was deposited in the Herbarium BAF of the Museo de Farmacobotánica Juan A. Domínguez (Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina) under the number 8718.

Analysis of the essential oils. All samples of plant material were submitted to hydrodistillation in a Clevenger type apparatus [14] and the essential oils obtained were analysed by GC and GC-MS using two fused-silica CC of different stationary phases. Analytical conditions for GC (Hewlett-Packard 5890A): columns (25 m  $\times$  0.2 mm i.d., 0.25  $\mu$ m film) of Carbowax 20M (CW-20M) and methyl silicone 30 (SE-30), carrier gas He, flow rate 1 ml min<sup>-1</sup>, split 1:60, injection temp. 250°, oven temp. programme from 80° to 250° at 4° min<sup>-1</sup>, detection (FID) temp. 270°. GC-MS [computerized system (GC Hewlett-Packard 5890 coupled to a mass selective detector Hewlett-Packard 5971A)]: Supelcowax<sup>TM</sup> 10 column (30 m  $\times$  0.2 mm i.d., 0.25  $\mu$ m film) and the same SE-30 column as above; the analytical conditions were: carrier gas He, flow rate 1 ml min<sup>-1</sup>, split 1:60, injection temp. 250°, interface temperature 280°, oven temp. programme

<sup>†</sup> tr: Trace ( $\leq 0.05\%$ ).

<sup>‡</sup> Identification based on mass spectrum only.

<sup>§</sup> Exact isomer not identified.

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from 80° to 220° at 6° min<sup>-1</sup>. Identification of components was made on the basis of their retention index, with reference to an homologous series of fatty acid Me esters, and their MS, which were compared with those in our library and with lit. data [15–16]. The quantification of the components was performed on the basis of their GC peak areas, without corrections for response factors.

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