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Short Communication

Analysis by gas chromatography–mass spectrometry of the essential oils from the aerial parts of *Pimpinella anagodendron* Bolle and *Pimpinella rupicola* Svent., two endemic species to the Canary Islands, Spain

A. Velasco-Negueruela [a,*], M.J. Pérez-Alonso [a], P.L. Pérez de Paz [b], J. Palá-Paúl [a], J. Sanz [c]

[a] *Departamento de Biología Vegetal I (Botánica), Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain*
[b] *Departamento de Biología Vegetal, Facultad de Farmacia, Universidad de la Laguna, 3807 La Laguna, Santa Cruz de Tenerife, Islas Canarias, Spain*
[c] *Instituto de Química Orgánica (CSIC), C/Juan de la Cierva 3, 28006 Madrid, Spain*

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Abstract

The essential oils from the aerial parts of *Pimpinella anagodendron* Bolle and *Pimpinella rupicola* Svent., two endemic species growing in Tenerife, Canary Islands, Spain, were studied by gas chromatography and gas chromatography–mass spectrometry. The major components of the flowering tops (flowers + unripe fruits) of *P. rupicola* (PRFT) were found to be β-bisabolene (34.8%), limonene (10.9%) and α-zingiberene (10.5%), whereas in the flowering tops of *P. anagodendron* (PAFT), the main constituents were α-zingiberene (32.9%), β-bisabolene (17.9%), β-pinene (15.8%) and *ar*-curcumene (11.5%). The major compounds found in the stems + leaves of *P. rupicola* (PRSL) were β-bisabolene (31.6%), α-zingiberene (11.4%) and limonene (10.8%), whereas those of *P. anagodendron* (PASL) were α-zingiberene (32.3%), β-bisabolene (14.0%) and *ar*-curcumene (12.6%). In all the oils were found the characteristic constituents of genus *Pimpinella*, the pseudoisoeugenol esters. In accordance with the morphological, chorological and chemical differences between both species, we suggest that *P. rupicola* Svent. is a good taxon and not a synonym of *P. anagodendron*.

Keywords: *Pimpinella anagodendron*; *Pimpinella rupicola*; Apiaceae; Essential oil composition; α-Zingiberene; β-Pinene; *ar*-curcumene; β-Bisabolene; Limonene; Pseudoisoeugenol esters

1. Introduction

Pimpinella L. belongs to the plant family Apiaceae (Umbelliferae), subfamily Apioideae, and comprises about 150 species which occur largely in Europe and Asia extending to China [1]. The genus is present in the Macaronesian Archipelago comprising five endemic species: *Pimpinella cumbrae* Link. *Pimpinella dendrotragium* Webb and Berth., *Pimpinella anagodendron* Bolle, *Pimpinella junoniae* Ceb. and Ort., and *Pimpinella rupicola* Svent. This last species was described by Sventenius [2] for the Tena mountains, Tenerife Island. This taxon was included in most of the Canary Islands Floras [3–5] as a synonym of *P. anagodendron*. Bramwell and Bramwell [6] proposed that the populations of *P. anagodendron* of Teno-Masca (NE Tenerife) could be treated as a variety of *P. anagodendsron*. Both taxa are endemic to the Macaronesian Archipelago and inhabit only in Tenerife Island but in different chorological habitats. As a part of a project on the volatile composition of *Pimpinella* species endemic to the Canary Islands [7,8], we have studied in this work the oil composition of the aerial parts of *P. anagodendron* and *P. rupicola* by gas chromatography and gas chromatography–mass spectrometry. The knowledge of the essential oils may be useful to clarify the systematic status of *P. rupicola*. Kubeczka and Ullmann [9] surveyed the presence of geijerene + pregeijerene in the root oil of *P. anagodendron* and they found 2.9% of these components. Martinez et al. [10] found a new coumarin, masquine, in the roots of *P. rupicola* and as far as we know there is no other previous report on the chemical analysis of *P. rupicola* and *P. anagodendron*.

* Corresponding author. Fax: +34 91 3945034.
E-mail address: AVN44@bio.ucm.es (A. Velasco-Negueruela).

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2. Experimental

2.1. Plant material

The air-dried aerial parts of *P. rupicola* Svent. were gathered at flowering in Cruz de Gala, Cumbre del Bolico, Buenavista del Norte, Teno-Masca, Tenerife, Canary Islands, Spain (31/05/2004, at an altitude of 1250 m above sea level, E/32117-N/3132892). A voucher specimen, TFC 45137 was deposited at the Herbarium of La Laguna University, La Laguna, Tenerife, Canary Islands, Spain. *P. anagodendron* was collected between Montaña Tafada and Roque Anambro, Anaga, Tenerife, Canary Islands, Spain (25/06/2004, at an altitude of 500 m above sea level, E/386942-N/3162045). A voucher specimen, TFC 45143 was deposited at the Herbarium of La Laguna University, La Laguna, Tenerife, Canary Islands, Spain. Two collective samples of *P. rupicola* were gathered, stems and leaves (PRSL) and flowering tops (PRFT), and two samples of *P. anagodendron* stems and leaves (PASL) and flowering tops (PAFT).

2.2. Isolation procedure

The aerial parts of *P. anagodendron* and *P. rupicola* were left to dry at room temperature and 274 g of the plant material (PRFT), 314.8 g (PAFT), 887 g (PRSL) and 1462 g (PASL) were coarsely minced and placed in flasks containing 1 L of water, respectively, and hydrodistilled in a Clevenger-type apparatus according to the method recommended in the Spanish Pharmacopoeia [11] for 8 h. The essential oils were dried over anhydrous magnesium sulphate and stored at 4 °C in the dark. Essential oil yields were 1.00% PRFT, 0.92% PAFT, 1.88% PRSL and 0.36% PASL, based on dried weight of samples.

2.3. Gas chromatography (GC)

A Varian GC 3300 fitted with a fused silica capillary column coated with dimethylpolysiloxane (DB-1) as stationary phase (50 m × 0.25 mm I.D., 0.25 μm film thickness) was used for GC analysis. Oven temperature was programmed from 90 to 240 °C at 4 °C/min. Injection was performed at 250 °C using a 1:100 split ratio. A flow of 1.5 mL/min carrier gas (N_2) was used. Detection was performed by flame ionization detector (FID) at 300 °C.

2.4. Gas chromatography–mass spectrometry (GC–MS)

Analyses were carried out on a Hewlett Packard 5890 gas chromatograph fitted with a fused silica capillary column coated with crosslinked dimethylpolysiloxane (DB-1) as stationary phase (Agilent Technologies, 25 m × 0.20 mm I.D., 0.33 mm film thickness). Carrier gas, He; flow rate, 1 mL/min. Temperature was programmed from 70 to 250 °C at 4 °C/min. Samples were injected at 250 °C, using a 1:20 split ratio. Spectra were recorded in the scan mode at 70 eV.

2.5. Qualitative and quantitative analyses

Most constituents were identified by gas chromatography by comparison of their GC retention indices (KI) with those of literature [12–18] or with those of standards purchased, synthesized or identified in *Pimpinella* oils of known composition [7,8]. Further identification was confirmed when possible by comparison of their mass spectra with those stored in the MS databases (NIST and WILEY libraries) or with mass spectra from literature [7,8,12–18]. Mass spectra of pseudoisoeugenol esters were identical to those of references [7,8,19–21]. C_{12} compounds were identified according to reference [20]. The mass spectra of sesquiterpene ethers were also identical to those of references [8,13,22–24]. *Trans-p*-(1-butenyl)anisole was tentatively identified according to its mass spectrum comparing it with that of reference [25]. Relative component concentrations were obtained directly from GC peak areas.

3. Results and discussion

The components of the oils from the aerial parts of *P. anagodendron* and *P. rupicola*, their retention indices, their percentage composition and identification methods are given in Table 1 where the components are listed in order of elution on the DB-1 column.

The major constituents of the essential oil from the stems + leaves of *P. rupicola* (PRSL) were found to be β-bisabolene (31.6%), α-zingiberene (11.4%) and limonene (10.8%). Other representative components of the oil were identified as α-pinene (6.3%), δ-3-carene (7.6%), β-caryophyllene (2.8%), *ar*-curcumene (3.8%), germacrene-D (2.3%) and pseudoisoeugenyl 2-methyllbutyrate II (1.8%). The total amount of monoterpenes was 26.9%, that of sesquiterpenes 62.3%, that of arylpropanoids 4.7% and that of various non terpenoid components (2-methyl-5-methoxybenzofuran + 2-methylbutyric acic + isomers of *p*-(1-butenyl)anisole) 6.1%.

The major constituents of the oil from the flowering tops of *P. rupicola* (PRFT) were shown to be β-bisabolene (34.8%), limonene (10.9%) and α-zingiberene (10.5%). Other characteristic components of the oil were α-pinene (7.4%), δ-3-carene (7.8%), β-pinene (3.3%), *ar*-curcumene (4.1%), β-caryophyllene (1.8%), dihydroagarofuran isomer (2.7%), epoxypseudoisoeugenyl 2-methylbutyrate (3.9%) and epoxypseudoisoeugenyl tigale II (3.0%). The amount of monoterpenes was 30.0%, that of sesquiterpenes 59.7%, that of various non terpenoid components (5-methoxy-2-methylbenzofuran + 2-methylbutyric acic) 1.2% and that of aryl propanoids 9.1%.

The oil of the stems + leaves of *P. anagodendron* (PASL) was characterized by high amounts of α-zingiberene (32.3%), β-bisabolene (14.0%), *ar*-curcumene (12.6%) and smaller amounts of geijerene (3.5%), (*E*)-β-farnesene (4.9%), β-caryophyllene (4.0%), β-sesquiphellandrene (3.1%), pseudoisoeugenyl 2-methylbutyrate II (2.9%), psedoisoeugenyl tiglate II (2.1%), epoxypseudoisoeugenyl 2-methylbutyrate (5.5%) and epoxypseudoisoeugenyl tiglate I (6.0%). The total amount of monoterpenes in this oil was 0.2%, that of sesquiter-

Table 1
Percentage composition of the essential oils from the aerial parts of *P. anagodendron* Bolle and *P. rupicola* Svent

Component	RI	*P. rupicola*		*P. anagodendron*		IM
		S + L	FT	S + L	FT	
2-Methylbutyric acid	818	2.1	0.1	t	t	MS, RI_1
Tiglic acid	865	0.1	t	t	t	MS, RI_1
α-Pinene	924	6.3	7.4	0.1	0.9	MS, RI_2
Camphene	938	0.1	0.1	–	0.1	MS, RI_2
Sabinene	961	0.1	0.2	–	0.2	MS, RI_2
β-Pinene	964	0.1	3.3	0.1	15.8	MS, RI_2
Myrcene	975	0.3	t	–	0.6	MS, RI_2
α-Phellandrene	989	0.1	0.1	–	–	MS, RI_2
δ-3-Carene	998	7.6	7.8	t	0.2	MS, RI_2
p-Cymene	1005	0.2	t	t	–	MS, RI_2
Limonene	1012	10.8	10.9	t	0.3	MS, RI_2
γ-Terpinene	1040	0.7	0.2	t	0.3	MS, RI_2
p-Cresol	1052	–	t	–	–	MS, RI_2
m-Cresol	1055	–	t	–	–	MS, RI_2
Terpinolene	1068	0.3	t	t	t	MS, RI_2
Linalool	1070	0.2	t	t	t	MS, RI_2
Geijerene isomer $C_{12}H_{18}$[a]	1130	0.2	–	0.1	–	MS
Geijerene	1136	4.1	t	3.5	t	MS.RI_1
p-Cymen-8-ol	1163	t	–	–	–	MS, RI_2
α-Terpineol	1169	t	t	t	t	MS, RI_2
Trinoranastreptene	1189	t	–	t	–	MS
Thymol,methyl ether	1199	t	–	–	–	MS, RI_1
Carvacrol,methyl ether	1205	0.1	–	–	–	MS, RI_1
p-(1-Butenyl)anisole isomer[a]	1259	0.2	–	–	–	MS
Trinoranastreptene isomer[a]	1269	t	–	–	–	MS
trans-*p*-(1-Butenyl)-anisole	1278	0.8	–	0.5	–	MS
Pregeigerene	1284	2.1	–	0.8	–	MS, RI_1
2-methyl-5-Methoxybenzofuran	1336	1.0	1.1	2.4	0.8	MS, RI_2
α-Cubebene	1341	0.2	–	–	t	MS, RI_1
α-Copaene	1360	t	–	t	t	MS, RI_1
β-Bourbonene	1366	–	–	t	–	MS, RI_1
β-Elemene	1372	t	–	–	–	MS, RI_1
β-Caryophyllene	1414	2.8	1.8	4.0	5.5	MS, RI_2
γ-Elemene	1423	–	–	–	t	MS,RI_1
β-Gurjunene	1425	0.1	–	–	–	MS, RI_1
trans-α-Bergamotene	1427	0.3	0.2	1.0	0.7	MS, RI_1
α-Guaiene	1430	0.1	t	–	–	MS, RI_1
(*E*)-β-Farnesene	1438	0.2	0.4	4.9	3.4	MS, RI_1
α-Humulene	1445	0.2	0.8	0.3	0.4	MS, RI_2
(*Z*)-methylisoeugenol	1450	0.3	0.4	0.2	0.1	MS, RI_1
ar-Curcumene	1453	3.8	4.1	12.6	1.5	MS, RI_1
Germacrene D	1460	2.3	0.7	1.6	0.4	MS, RI_1
α-Zingiberene	1469	11.4	10.5	32.3	32.9	MS, RI_1
β-Dihydroagarofuran	1475	t	t	t	t	MS, RI_1
α-Muurolene	1479	0.3	0.7	0.4	0.1	MS, RI_1
β-Bisabolene	1485	31.6	34.8	14.0	17.9	MS, RI_1
γ-Cadinene	1490	0.1	t	0.1	t	MS, RI_1
β-Sesquiphellandrene	1501	1.5	1.5	3.1	2.1	MS, RI_1
Isokessane	1509	0.2	1.5	0.3	0.9	MS, RI_1
Cadina-1,4-diene	1523	t	–	t	–	MS, RI_1
α-Cadinene	1529	t	–	t	–	MS, RI_1
Dihydroagarofuran isomer[a]	1551	1.4	2.7	0.1	t	MS
ar-Turmerol	**1568**	t	–	t	–	MS, RI_1
Anyl-2-methyl butyrate	1574	t	–	t	–	**MS**
Caryopyllene oxide	1579	0.3	–	t	–	MS, RI_2
γ-Eudesmol	1612	t	t	t	t	MS, RI_1
T-Muurolol (*epi*-α-Muurolol)	1638	0.3	t	t	t	MS, RI_1
T-Cadinol (*epi*-α-Cadinol)	1640	0.2	–	0.3	t	MS, RI_1
α-Cadinol	1648	0.5	–	0.3	0.3	MS, RI_1
epi-α-Bisabolol	1660	–	t	–	t	MS, RI_1
(*E*,*E*)-Farnesol	1700	t	t	0.2	t	MS, RI_2
Xanthorrhizol	1730	t	t	t	t	MS, RI_1

Table 1 (*Continued*)

Component	RI	*P. rupicola*		*P. anagodendron*		IM
		S + L	FT	S + L	FT	
Pseudoisoeugenyl 2-ethylbutyrate I[a]	**1736**	**t**	**t**	**t**	**t**	**MS**
Pseudoisoeugenyl tiglate I[a]	**1785**	**t**	**t**	**t**	**t**	**MS**
Pseudoisoeugenyl 2-methylbutyrate II[a]	**1792**	**1.8**	**1.1**	**2.9**	**0.1**	**MS, RI_1**
Pseudoisoeugenyl tiglate I[a]	**1817**	**0.5**	**0.4**	**2.1**	**1.1**	**MS, RI_1**
Epoxypseudoisoeugenyl 2-methylbutyrate[a]	**1844**	**1.6**	**3.9**	**5.5**	**0.8**	**MS, RI_1**
Epoxypseudoisoeugenyl tiglate I[a]	**1848**	**0.5**	**0.3**	**6.0**	**0.3**	**MS, RI_1**
Epoxypseudoisoeugenyl tiglate II[a]	**1873**	**–**	**3.0**	**0.3**	**2.3**	**MS**
Hexadecanoic acid	2151	t	t	t	t	MS
Phytol	2272	t	t	t	t	MS
Total monoterpenes		26.9	30.0	0.2	18.4	
Total various components		6.1	1.2	2.9	0.8	
Total sesquiterpenes		62.3	59.7	79.9	76.1	
Total aryl-propanoids		4.7	9.1	17.0	4.7	

RI = programmed temperature retention indices relative to the homologous series of *n*-alkanes (C_5–C_{25}); MS = mass spectra data; RI_1 = retention data according to literature values; RI_2 = retention data according to authentic standards; IM = identification method; *t* = traces >0.1%; In bold-face characteristic chemosystematic constituents of genus *Pimpinella*, underlined = components that separate the species; S + L = stems + leaves; FT = flowering tops (flowers + unripe fruits).

[a] Correct isomer not determined.

penes 79.9%, that of various non terpenoid components (2-methyl-5-methoxybenzofuran + *p*-(1-butenyl)anisole) 2.9%.

The oil from the flowering tops of *P. anagodendron* (PAFT) had as major components, α-zingiberene (32.9%), β-bisabolene (17.9%), β-pinene (15.8%) and *ar*-curcumene (11.5%). Also were detected smaller amounts of β-caryophyllene (5.5%), (*E*)-β-farnesene (3.4%), β-sesquiphellandrene (2.1%) and epoxypseudoisoeugenyl tiglate II (2.3%). The total amount of monoterpenes in this oil was 18.4%, that of sesquiterpenes 76.1%, that of various non terpenoid components (2-methyl-5-methoxybenzofuran) 0.8% and finally that of arylpropanoids 4.7%. More detailed results are shown in Table 1 in which the complete list of the identified compounds is given.

The arylpropanoids found in these oils are all derivatives of pseudoisoeugenol (4-methoxy-2-propenyl-phenol). Together with C_{12} compounds as geijerene and pregeijerene, pseudoisoeugenyl esters are important chemosystematic characters in the genus *Pimpinella* [5,6,18]. They have been found so far in *P. anagodendron* and *P. rupicola* (this paper), *P. junoniae* Ceb and Ort., *P. cumbrae* Link, *P. anisum* L., *P. peregrina* L., *P. saxifraga* L., *P. nigra* Mill., *P. major* (L.) Hudson, *P. diversifolia* DC., *P. aromatica* Bieb., *P. villosa* Schousboe, *P. tripartrita* Kalenicz. (=*Albovia tripartita* (Kalenicz.) Schischk., *P. isaurica* Mathews [7,8,20,26]. The occurrence of 2-methyl-5-methoxybenzofuran is also interesting as this component is a decomposition product formed from epoxypseudoisoeugenyl esters during hydrodistillation and gas chromatography [7,8,19,26]. Psedoisoeugenyl esters have only been found in other genus of *Apiaceae* namely *Ligusticum mucronatum* (Shrenk) Leute [27] and also but only in *Apiaceae*, in in vitro systems [28,29]. Finally, we have found significant quantitative differences between the oils of *P. rupicola* and *P. anagodendron* therefore as also were described [2] differences in morphology and chorology of both species, it seems to be useful to give *P. rupicola* the systematic status of species as Sventenius [2] proposed.

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