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Short communication

Analysis by gas chromatography–mass spectrometry of the volatiles from the fruits of *Ammodaucus leucotrichus* subsp. *leucotrichus* and subsp. *nanocarpus* grown in North Africa and the Canary Islands, respectively

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 volatiles from the fruits of *Ammodaucus leucotrichus* subsp. *leucotrichus* and subsp. *nanocarpus* (two endemic species, the first from North Africa and the second from the Canary Islands, Spain) were studied by gas chromatography and gas chromatography–mass spectrometry. The major components of the volatiles of subsp. *nanocarpus* were found to be, β-pinene (22.2–33.6%), bornyl angelate (20.6–21.8%) and camphor (8.3–11.7%) whereas in the fruits of subsp. *leucotrichus*, the main constituents were perillaldehyde (63.6%) and limonene (26.8%). We also suggest that subsp. *nanocarpus* should have the status of species and should be named *Ammodaucus nanocarpus*.

Keywords: *Ammodaucus leucotrichus* subsp. *leucotrichus*; *Ammodaucus leucotrichus* subsp. *nanocarpus*; Apiaceae; Volatile oil composition; Bornyl angelate; Camphor; Perillaldehyde; β-Pinene; Limonene

1. Introduction

Ammodaucus Cosson & Durieu belongs to the plant family *Apiaceae* (Umbelliferae), tribe *Caucalideae* subfamily *Apioideae*, and comprises two endemic subspcies *A. leucotrichus* Cosson & Durieu subsp. *leucotrichus* inhabiting the maritime sands in the Saharan and sub-Saharan countries of North Africa, Morocco, Algeria and Tunisia, extending to Egypt and tropical Africa. [1]. The genus is present in the Macaronesian Archipelago comprising the second subspecies [3] *A. leucotrichus* Cosson & Durieu subsp. *nanocarpus* E. Beltrán. This last subspecies was described by Beltrán [2] for Lanzarote and Fuerteventura of the Canary Islands, Spain. This research is part of a project on the volatile oil composition of *Apiaceae* species endemic to the Canary Islands [4,5]. In this work, attempts have been made to achieve two goals: first, to know the oil composition of the fruits of *A. leucotrichus* subsp. *leucotrichus* and subsp. *nanocarpus* by gas chromatography and gas chromatography–mass spectrometry, and second, to establish that the knowledge of the volatiles may be useful to improve the systematic status of genus *Ammodaucus*. Muckensturm et al. [6] studied the ethereal extract of the fruits of *A. leucotrichus* subsp. *leucotrichus* bought in a local market at Casablanca (Morocco), and found a guaianolide lactone, ammolactone, together with limonene, perillaldehyde, 3-hydroxyperillaldehyde, methyl perillate, bornyl angelae and γ-decalactone. This last taxon, locally known as “Kammûn es-Sôfi”, “Hairy Cumin”, is used in the North African countries as a condiment or spice and in traditional medicine for cold, fever, and digestive complaints particularly in children [6]. As far as we know, there is no other previous report on the chemical analysis of subsp. *leucotricus*, and our research is the first report on the essential oil analysis of subsp. *nanocarpus*.

* Corresponding author. Tel.: +34 91 3945058; fax: +34 91 3945037.
E-mail address: AVN44@bio.ucm.es (A. Velasco-Negueruela).

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2. Experimental

2.1. Plant material

Two samples of subsp. *nanocarpus* (ALN1,ALN2) were gathered:

(1) The fruits of the first sample (ALN1) were collected from Trasera, Tarajalejo Beach, Tuineje, Fuerteventura, Canary Islands, Spain on 11 April 2005, N/311873-E/58593, at an altitude of 10 m (above sea level). A voucher specimen, TFC 45930 was deposited at the Herbarium of La Laguna University, La Laguna, Tenerife, Canary Islands, Spain.
(2) The fruits of the second sample (ALN2) were collected from the slope N-NW of Chayofita Mountain, Los Cristianos, Tenerife, Canary Islandas, Spain on 17 April 2005 at an altitude of 25 m (above sea level). A voucher specimen, TFC 45931 was deposited at the Herbarium of La Laguna University, La Laguna, Tenerife, Canary Islands, Spain.
(3) One sample of subsp. *leucotrichus* (ALL) was collected from in Dakhla (Villacisneros), Western Saharan, North Africa, in Spring 2004 at an altitude of 25 m (above sea level). A voucher specimen, TFC 45944 was deposited at the Herbarium of La Laguna University, La Laguna, Tenerife, Canary Islands, Spain.

2.2. Isolation procedure

The fruits of both species were left to dry at room temperature and 291 g of the plant material (ALN1) and 384 g (ALN2) were coarsely minced and placed in individual flasks containing 2 L of water each, and 51 g (ALL) was mixed with 1 L of water and hydrodistilled in a Clevenger-type apparatus according to the method recommended in the Spanish Pharmacopoeia [7] for 8 h. The volatiles were dried over anhydrous magnesium sulphate and stored at 4 °C in the dark. Volatile oil yields were 1.33% ALN1, 1.22% ALN2 and 2.76% ALL, based on dried weight of samples.

2.3. Gas chromatography (GC)

Analyses were carried out on a Varian 3300 gas chromatograph fitted with a fused silica capillary column coated with dimethypolysiloxane(DB-1) as stationary phase (50 m × 0.25 mm I.D., 0.25 μm film thickness). Carrier gas was N_2 at a flow rate of 1.5 mL/min. Oven temperature was programmed from 90 to 240 °C at 4 °C/min. Injection was performed at 250 °C using 1:100 split ratio. Detection was performed by flame ionization detection (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 dimethylpolysiloxane (DB-1) as stationary phase (25 m × 0.20 mm I.D., 0.33 mm film thickness). Carrier gas was He at a flow rate of 1 mL/min. Temperature was programmed from 70 to 250 °C at 4 °C/min. Samples were injected at 250 °C using 1:20 split ratio. Spectra were recorded in 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 (I) with those reported in literature [9,11,13,14] or with those of standards purchased, synthesized or identified in oils of known composition. Further identification was confirmed when possible by comparison of their mass spectra with those stored in MS databases (NIST and Wiley libraries) or with mass spectra reported in literature [8–13]. Relative component concentrations were obtained directly from GC peak areas.

3. Results and discussion

The components of the oils from the fruits of *A. leucotrichus* subsp. *nanocarpus* and *A. leucotrichus* subsp. *leucotrichus* their

Table 1
Percentage composition of the volatiles from the fruits of *Ammodaucus leucotrichus* subsp. *nanocarpus* and subsp. *leucotrichus*

Component	I (DB1)	ALN1	ALN2	ALL	IM
α-Thujene	916	0.3	0.2	t	MS.I_2
α-Pinene	924	5.5	5.2	4.7	MS, I_2
Camphene	938	3.8	3.3	0.3	MS, I_2
Sabinene	961	7.0	3.7	t	MS, I_2
β-Pinene	964	22.2	33.6	1.4	MS, I_2
Myrcene	975	5.4	1.8	0.4	MS, I_2
α-Phellandrene	989	0.1	0.1	1.1	MS, I_2
δ-3-Carene	998	0.3	0.7	t	MS, I_2
α-Terpinene	1003	0.3	0.3	t	MS.I_2
p-Cymene	1005	0.3	1.1	t	MS, I_2
Limonene	1012	4.0	3.5	26.8	MS, I_2
β-Phellandrene	1016	–	–	0.1	MS.I_1
γ-Terpinene	1040	5.6	4.6	t	MS, I_2
cis-Sabinene hydrate	1053	t	t	–	MS.I_1
Terpinolene	1068	0.3	0.2	t	MS, I_2
Linalool	1070	0.3	0.3	–	MS, I_2
Camphor	1128	11.7	8.3	–	MS.I_2
Borneol	1150	t	t	–	MS.I_2
Terpinen-4-ol	1157	0.2	0.2	–	MS, I_2
p-Cymen-8-ol	1163	t	t	–	MS, I_2
α-Terpineol	1169	0.1	0.1	0.1	MS, I_2
Myrtenal	1178	t	t	–	MS.I_2
Thymol methyl ether	1199	0.1	0.1	–	MS, I_1
Carvacrol methyl ether	1205	0.1	0.1	–	MS, I_1
Perillaldehyde	1272	–	–	63.6	MS.I_1
Bornyl acetate	1279	5.0	4.7	–	MS.I_2
Perilla alcohol	1285	–	–	0.2	MS.I_1
Thymol	1290	t	t	–	MS.I_2
α-Cubebene	1341	t	t	–	MS.I_1
Methyl perillate	1350	–	–	0.5	MS.I_1
3-Hydroxyperillaldehyde	1352	–	–	0.4	MS.I_1
α-Copaene	1360	0.1	0.1	–	MS, I_1
β-Cubebene	1372	t	t	–	MS.I_1
Bornyl isobutanoate	1390	0.4	0.4	–	MS.I_1
β-Caryophyllene	1414	0.2	0.2	–	MS, I_2
γ-Decalactone	1448	0.3	0.5	0.2	MS.I_1
ar-Curcumene	1453	0.2	0.1	t	MS, I_1
α-Zingiberene	1469	0.2	0.1	–	MS, I_1

Table 1 (*Continued*)

Component	I (DB1)	ALN1	ALN2	ALL	IM
Valencene	1481	0.1	0.1	–	MS, I_1
β-Bisabolene	1485	0.1	0.1	–	MS, I_1
α-Muurolene	1489	0.3	0.3	–	MS, I_1
γ-Cadinene	1490	0.1	0.1	–	MS, I_1
δ-Cadinene	1509	2.1	1.9	–	MS.I_1
Germacrene B	1541	t	t	–	MS.I_1
β-Calacorene	1548	t	t	–	MS.I_1
Bornyl angelate	1550	20.6	21.8	–	MS.I_1
Germacrene D-4-ol	1559	0.1	0.1	–	MS.I_1
Isobornyl angelate	1592	1.5	0.6	–	MS.I_1
1-*epi*-Cubenol	1612	0.2	0.3	–	MS.I_1
T-Muurolol (*epi*-α-*m*uurolol)	1638	0.2	0.3	–	MS, I_1
α-Muurolol	1642	t	t	–	MS.I_1
α-Cadinol	1650	0.8	0.7	–	MS.I_1
(Z,E)-Farnesol	1687	t	t	–	MS.I_2
10-nor-Calamenen-10-one	1700	0.3	0.2	–	MS.I_1
Chamazulene	1715	–	–	0.2	MS.I_1
Total monoterpenes		94.7	94.9	99.6	
Total sesquiterpenes		5.0	4.6	0.2	
Total various compounds		0.3	0.5	0.2	

I = programmed temperature retention indices relative to the homologous series of *n*-alkanes (C_5–C_{25}); MS = mass spectra data; I_1 = retention data according to literature values; I_2 = retention data according to authentic standards; IM = identification method; t = traces <0.1%; DB1 = column phase; ALN1 = fruits of *Ammodaucus leucotrichus* subsp. *nanocarpus* sample 1; ALN2 = fruits of *Ammodaucus leucotrichus* subsp. *nanocarpus* sample 2; ALL = fruits of *Ammodaucus leucotrichus* subsp. *leucotrichus*.

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 main results of this work can be summarized as follows. The major constituents of the essential oil from the fruits of subsp. *nanocarpus* were found to be β-pinene (22.2–33.6%), bornyl angelate (20.6–21.8%) and camphor (8.3–11.7%). Other representative components of the oil were identified as α-pinene (5.2–5.5%), camphene (3.3–3.8%), sabinene (3.7–7.0%), myrcene (1.8–5.4%), limonene (3.5–4.0%), γ-terpinene (4.6–5.6%), bornyl acetate (4.7–5.0%) and δ-cadinene (2.1–1.9%). Total amount of monoterpenes was 94.7–94.9%, that of sesquiterpenes 4.6–5.0% and that of various components (γ-decalactone) 0.3–0.5%. The major components of the oil from the fruits of subsp. *leucotrichus* were perillaldehyde (63.6%) and limonene (26.8%). Other characteristic components of the oil were α-pinene (4.7%), β-pinene (1.4%), 3-hydroxyperillaldehyde (0.4%), methyl perillate (0.5%) and perilla alcohol (0.2%). The amount of monoterpenes was 99.6%, that of sesquiterpenes 0.2% and that of various copmponents (γ-decalactone) 0.2%.

From the above results, it is coherent to think that subsp. *nanocarpus* drives the biosynthesis of major terpenic compounds, camphor and bornyl angelate, through the bornanic pathway [15,16], namely geranyl pyrophosphate (GPP)-linalyl pyrophosphate (LPP)-α-terpinyl cation-bornyl pyrophosphate-camphor whereas in subsp. *leucotrichus p*-memthanic constituents were formed through the biosynthetic pathway geranyl pyrophosphate (GPP)-linalyl pyrophopsphate (LPP)-α-terpinyl cation-limonene-perillaldehyde. As biosynthetic pathways [17] are the only chemical characters valid in chemosystematics and these are so different in both subspecies, and according to Ref. [2] there are significant morphological differences particularly in the lower size of the fruits and much higher length of the fruit bristles in subsp. *nanocarpus*, we propose a new combination and status novo of this subspecies as: *Ammodaucus nanocarpus* (E. Beltrán) P. Pérez & A. Velasco *comb. et status novo* (Basionym: *Ammodaucus leucotrichus* Cosson & Durieu subsp. *nanocarpus* E. Beltrán.).

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