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Chemical composition of the essential oils from the roots, fruits, leaves and stems of *Pimpinella cumbrae* Link growing in the Canary Islands (Spain)

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ABSTRACT: The oil constituents from the roots, fruits, leaves and stems of *Pimpinella cumbrae* Link gathered in the Canary Islands, Spain, have been studied by GC and GC–MS in combination with retention indices. The major constituents in the root oil were found to be isokessane (17%), β-dihydroagarofuran (15%), 2-methylbutyric acid (10%), geijerene (10%) and pregeijerene (7%). In the fruit oil the main components found were α-bisabolol (39%), δ-3-carene (16%) and limonene (8%). In the leaf oil, α-bisabolol (53%) and δ-3-carene (11%) were the predominant constituents. The most important compounds from the stem oil were α-bisabolol (39%), isokessane (10%) and β-dihydroagarofuran (9%). Pseudoisoeugenol esters were also detected in the oils from the roots, fruits and stems. These components were partially hydrolysed in the course of prolonged hydrodistillation to 2-methyl-5-methoxybenzofuran. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: *Pimpinella cumbrae*; Apiaceae; essential oil composition; isokessane; β-dihydroagarofuran; δ-3-carene; 2-methyl-5-methoxybenzofuran; α-bisabolol

Introduction

Pimpinella L. belongs to the Family Apiaceae, subfamily Apioideae and comprises about 150 species, which occur largely in Europe and Asia extending to China.[1] As part of our research on the constituents of the essential oils of Macaronesian endemic taxa[2–4] we have studied the essential oils of the roots, fruits, leaves and stems of *Pimpinella cumbrae* Link. This species is endemic to the Canary Islands, Spain and is present in large numbers at the top of the Tenerife mountains (Teide National Park) but less abundant at the summit of the La Palma mountains.[5]

Kubeczka and Ullmann[6] surveyed the presence of geijerene and pregeijerene in the root oils of three Macaronesian species of *Pimpinella*, viz. *P. cumbrae* Link, *P. anagodendron* Bolle and *P. junionae* Ceb. & Ort. and they found geijerene plus pregeijerene (35%, 3%, 46%) in the three species, respectively.

As far as we know there is no other previous report on the chemical analysis of oils from Macaronesian species of *Pimpinella*.

Experimental

Plant Material

P. cumbrae Link was gathered in Degollada de Guajara, Las Cañadas, Tenerife, Canary Islands (Spain), at an altitude of 2300 m on 29 August 2000. A voucher specimen, TFC 42811, has been deposited at the Herbarium of the Faculty of Pharmacy, La Laguna University, La Laguna, Tenerife, Canary Islands, Spain.

Analysis

Dried plant material was hydrodistilled for 3 h in a Clevenger-type apparatus according to the method recommended in the Spanish Pharmacopoeia.[7] The oils were dried over anhydrous sodium sulphate and stored at 4 °C in the dark.

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GC

GC was carried out on a Varian 3300 gas chromatograph fitted with a fused methyl silicone DB-1 column (50 m × 0.25 mm), film thickness 0.25 μm. The temperature was programmed at 95–240 °C at 4 °C/min. Injection was performed at 250 °C in the split mode. A flow of 1.5 ml/min carrier gas (N_2) was used. Detection was performed by FID at 250 °C. Injection volume for all samples was 0.1 μl.

GC–MS Analyses

These were carried out on a Hewlet-Packard 5890 gas chromatograph fitted with a phase-bonded poly(5% diphenyl 95% dimethylsiloxane) silicone PTE-5 capillary column (30 m × 0.25 mm), film thickness 0.25 μm. Helium was used as carrier gas at a flow rate of 1.5 ml/min. The temperature programme was 70 °C for 2 min, then programmed to 250 °C at 2 °C/min; injector temperature, 250 °C. The chromatograph was coupled to a HP 5971A mass selective detector at 70 eV.

Qualitative Analyses

Most constituents were tentatively identified by GC, by comparison of their retention indices with those of authentic standards available in the author's laboratory or with retention indices in close agreement with references.[8–12] Further identification was achieved by GC–MS. Other constituents were either synthesized, purchased or identified in oils of known composition. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer data base using the NBS54K.L and Wiley.L built-in libraries and with those published in the literature.[8–12] Mass spectra of pseudoisoeugenol esters were identical to those of references.[13,14] The mass spectra of sesquiterpene ethers were also identical to those of references[15–17] and *trans-p*-(1-butenyl)-anisole was tentatively identified according to its mass spectrum and comparing it with that of reference.[18] C_{12} compounds, including azulenes, were identified according to references.[14,19–21]

Results and Discussion

Table 1 summarizes the identified compounds, their retention indices and their percentage composition. The constituents are arranged in order to their elution on DB-1 column. The yields based on dry weight of the samples were as follows: roots, 0.34%; fruits, 1.60%; leaves, 0.56%; and stems, 0.16%.

The root oil from *P. cumbrae* was of a deep blue colour, but blue oils are commonly detected in the genus *Pimpinella*, as in the root oils of *P. saxifraga* L., *P. nigra* Mill. and *P. aromatica* Bieb.[14,22] The major components of this oil were found to be isokessane (17%), β-dihydroagarofuran (15%), 2-methyl-butyric acid (10%), geijerene (10%), pregeijerene (7%) and trinoranastreptene (6%). The azulene constituents found were dihydro-1,4-dimethyl-azulene (4%), 1,4-dimethyl-azulene (1%) and 3,10-dihydro-1,4-dimethyl-azulene (0.3%). The seldom-found sesquiterpene ether, isokessane, has previously only been found in the root oils of *Valeriana officinalis* L. (Valerianaceae) and *Pimpinella villosa* Schousboe (Apiaceae)[15–22] and has also been cited[15] in *Bothriochloa intermedia* (R. Br.) A. Camus and *B. bladhii* (Retz.) S.T. Blake (Poaceae), *Heracleum dissectum* Ledeb. (Apiaceae) and *Senecio* sp. (Asteraceae). It is important to mention that both sesquiterpene ethers, dihydroagarofuran and isokessane were also found together in the roots of *Rubus rosifolius* Sm. (Rosaceae).[16]

The yellow fruit oil was characterized by high amounts of α-bisabolol (39%), δ-3-carene (16%), limonene (8%) and β-pinene (7%). The main constituents of the blue-green stem oil were shown to be α-bisabolol (39%), isokessane (10%) and dihydroagarofuran (9%) whereas the light yellow leaf oil contained α-bisabolol (53%) and δ-3-carene (11%) as major compounds.

It is important to note the high content of α-bisabolol in the oils of the stems, leaves and fruits of *P. cumbrae* and, as far as we know, α-bisabolol has been previously detected, but in lower amounts, in *P. villosa* herb and fruit oils (0.2–2%).[22] C_{12} sesquiterpenes are not exclusive to *Pimpinella* spp. or to the Family Apiaceae and they have been found in both *in vivo* plants and *in vitro* cultures.[23] Geijerenes have been found previously in 15 species of the genus *Pimpinella*[6,22] (also in this paper) and in: Asteraceae (*Chromolaena odorata* (L.) R. King & H. Robinson and *Eupatorium odoratum* L.); Lamiaceae (*Nepeta govaniana* Benth.); Rosaceae (*Rubus rosifolius* Sm.); and Rutaceae (*Geijera parviflora* Lindley, *Ruta graveolens* L., *Boenninghausenia albiflora* Reichb. and *Vepris heterophylla* (Engl.) W. Mzirag.).[23]

Together with C_{12} compounds, pseudoisoeugenol esters (Figure 1) are important chemosystematic characters in the genus *Pimpinella*.[14,22,24–26] They have been found so far in *P. cumbrae* (this paper), *P. anisum* L.[14,22] *P. peregrina* L.[14,22] *P. saxifraga* L.[14,22] *P. nigra* Mill.,[14,22] *P. major* (L.) Hudson,[14,22] *P. diversifolia* DC,[24] *P. aromatica* Bieb.[22] *P. villosa* Schousboe,[22] *P. tripartita* Kalenicz. = *Albovia tripartita* (Kalenicz.) Schischk.,[22] *P. isaurica* Mathews[22] and only in an other species of Apiaceae, namely *Ligusticum mucronatum* (Shrenk) Leute.[27]

Table 1. Essential oil composition (%) of different parts of *Pimpinella cumbrae* Link

Component	KI	Root	Fruit	Leaf	Stem
Hexanal	808	0.1	—	—	—
2-Methyl-butyric acid	818	10.2	1.1	—	—
Tiglic acid	865	3.4	0.7	—	—
α-Pinene	924	t	0.5	—	—
β-Pinene	964	t	6.5	—	—
Myrcene	975	t	0.6	—	—
α-Phellandrene	989	t	0.2	—	—
δ-3-Carene	998	0.3	15.9	11.4	4.8
p-Cymene	1005	0.4	0.1	0.1	0.2
Limonene	1012	t	8.3	1.0	—
γ-Terpinene	1040	0.4	—	—	—
cis-Linalyl oxide (furanoid)	1050	—	—	1.1	—
p-Cresol	1052	—	0.1	0.4	—
Terpinolene	1068	—	1.5	0.6	3.3
Linalool	1070	—	—	0.6	—
Geijerene	**1131**	**10.2**	—	—	**1.1**
Trinoranastreptene	**1189**	**5.6**	—	—	**0.4**
Thymol–methyl ether	1199	1.8	—	—	—
Carvacrol–methyl ether	1205	1.2	—	—	—
Linalyl acetate	1237	0.1	—	0.6	—
trans-p-(1-Butenyl)-anisole	1270	2.5	—	—	—
Pregeijerene	**1277**	**7.1**	—	—	**0.3**
2,4-Decadienal	1290	0.7	—	—	—
3,10-Dihydro-1,4-dimethyl-azulene	**1322**	**0.3**	—	—	—
Dihydro-1,4-dimethyl-azulene*	**1330**	**3.6**	—	—	—
2-Methyl-5-methoxy-benzofuran	1336	1.5	2.1	1.2	3.2
cis-β-Farnesene	1428	t	0.3	t	0.6
ar-Curcumene	1453	0.4	3.5	4.9	2.3
β-Dihydroagarofuran	1475	15.2	0.5	t	8.5
α-Zingiberene	1469	0.5	3.4	4.4	1.5
β-Bisabolene	1485	0.3	1.0	1.3	t
β-Sesquiphellandrene	1500	t	0.4	0.3	t
Isokessane	1509	17.0	0.7	1.8	10.2
1,4-Dimethyl-azulene	**1523**	**1.1**	—	—	**0.1**
α-Bisabolol	1658	1.1	38.7	53.1	38.8
Pseudoisoeugenyl-2-methyl-butyrate*	**1795**	**1.0**	**0.8**	—	**0.3**
Pseudoisoeugenyl-tiglate*	**1818**	**t**	**0.1**	—	**0.2**
Epoxy-pseudoisoeugenyl-2-methyl-butyrate*	**1844**	**0.3**	—	—	—
Epoxy-pseudoisoeugenyl-2-methyl-butyrate*	**1850**	**3.5**	**1.1**	—	**0.6**
Epoxy-pseudoisoeugenyl-tiglate*	**1873**	**1.4**	**0.5**	—	**2.8**

KI, temperature-programmed Kovàts indices using the homologous series of *n*-hydrocarbons (C_7–C_{22}). t, traces (<0.1%); *, correct isomer not determined; boldtype, characteristic chemosystematic constituents of the genus *Pimpinella*.

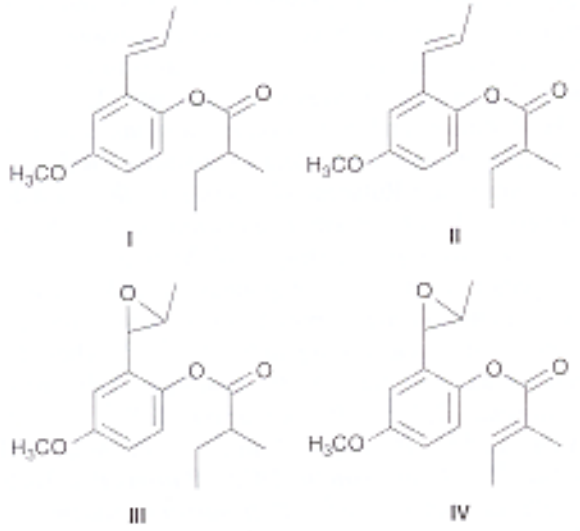

Figure 1. Structure of pseudoisoeugenol and epoxypseudoisoeugenol esters from *P. cumbrae* Link

Pseudoisoeugenol esters have also been found, but only in Apiaceae in *in vitro* systems.[28,29] Our finding in the oil from the roots of *P. cumbrae* of pregeijerene, geijerene, 1,4-dimethyl-azulene, trinoranastreptene, two isomers of dihydro-1,4-dimethylazulene, pseudoisoeugenyl-2-methyl-butyrate (**I**), pseudoisoeugenyl-tiglate (**II**), pseudoisoeugenyl-2-methyl-butyrate epoxides (**III**) and epoxy-pseudoisoeugenyl-tiglate (**IV**) strongly support the proposal of Kubeczka *et al.*[14] and Kubeczka[22] in that the knowledge of the chemical composition of the volatiles from *Pimpinella* spp. hold substantial promise for establishing taxonomic infrageneric relationships.

Interestingly, and according to Martin *et al.*,[13] acid hydrolysis of epoxypseudoisoeugenol esters (**V**) led to the component (**VI**) 2-methyl-5-methoxy-benzofuran (Figure 2). This compound has been found in our root oil (2%), fruit oil (3%), leaf oil (1%) and stem oil (3%). We have also seen in the course of our research that if the time of hydrodistillation was prolonged, the

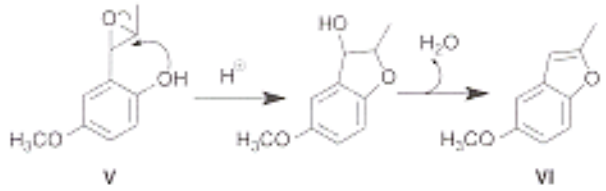

Figure 2. Hydrolysis of epoxypseudoisoeugenol (V) to 2-methyl-5-methoxy-benzofuran (VI)

epoxypseudoisoeugenol esters were partially converted into 2-methyl-5-methoxy-benzofuran.

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