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Volatiles of *Pleurospermum austriacum* (L.) Hoffm. (Apiaceae)

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Abstract: This work presents the first results of detailed GC and GC–MS analyses of the volatile constituents from the fresh leaves, fruits and stalks of an endangered plant species *Pleurospermum austriacum* (L.) Hoffm. (Apiaceae). Sesquiterpene hydrocarbons made up ca. 4/5 of the oils obtained in low yield (0.003–0.086 %, w/w). The major volatiles detected were germacrene D (66.5, 62.4 and 49.0 % in fruits, leaves and stalks, respectively), β -caryophyllene (3.1–5.7 %), δ -cadinene (3.6–5.0 %) and (*E*)- β -farnesene (1.0–1.5 %).

Keywords: *Pleurospermum austriacum*; Apiaceae; essential oil; germacrene D.

INTRODUCTION

Pleurospermum Hoffm. (Apiaceae) is a widespread, heterogeneous genus of complex and controversial taxonomy.^{1–3} Russian authors³ delimit *Pleurospermum sensu stricto* by only two species, *P. austriacum* (that is according to the *Flora Europaea* a sub-boreal Euroasian floral element^{1,2}), and *P. uralense*, referring the other species to *Aulacospermum*, *Hymenidium*, *Hymenolaena*, *Physospermopsis* and *Pterocyclus*. Other morphologically similar genera, where generic boundaries become indistinct, include *Trachydium* and *Pseudotrachydium* Pimenov and Kljuykov.³ As yet, this rather radical classification has not gained widespread acceptance, and the proponents admit that this is a taxonomic hypothesis, and (particularly for some groups) a more natural classification will only be possible following critical revision in the field, herbarium³ and possibly a more general approach, including the use of chemical markers as *principium divisionis*.

Phytochemically speaking, *Pleurospermum* taxa were investigated on a few occasions. The chemistry regarding the volatile compounds of these taxa has been neglected (essential oil investigations), and only *P. lindleyanum* was studied

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in this sense.⁴ Most of the phytochemical work realized in this field resulted in the isolation of a number of coumarins (mono-, di-, and trimers) and related phenylpropanoids and concerned only the non-volatile compounds. Some of these molecules were found for the first time as naturally occurring metabolites.^{5–14} Surprisingly, the previously mentioned analyses⁴ of the oil of *P. lindleyanum* resulted in the identification of certain anthropogenic compounds as oil constituents.

The aim of this work was to provide the very first and detailed GC and GC–MS analyses of the volatile constituents from the leaves, fruits and stalks of the endangered plant species *Pleurospermum austriacum* (L.) Hoffm.¹⁵

EXPERIMENTAL

Plant material

Fresh plants (fruits, leaves and stalks) were collected in July, 2007 at the mountain Stara planina (Babin zub), at an altitude of 1707 m above sea level. The plant was identified by Niko Radulović. A voucher specimen (number 200705) is deposited at the Herbarium of the Faculty of Science and Mathematics, Niš.

Extraction of essential oils

Fresh plant material (three batches of about 250 g of each sample) was subjected to hydrodistillation with *ca.* 2 L of distilled water for 2.5 h using an original Clevenger-type apparatus. Due to the small sample size of the isolated essential oils, which were not completely liquid, the volume of the oils was not measured, and hence the yields are expressed as weight of essential oils per weight of plant material. The obtained oils were separated by extraction with freshly distilled diethyl ether (Merck, Germany) and dried over anhydrous magnesium sulfate (Aldrich, USA). The solvent was evaporated under a gentle stream of nitrogen at room temperature, in order to exclude any loss of the essential oils, and stored at 4 °C until analyzed. When the oil yields were determined, after the bulk of the ether had been removed under a stream of N₂, the residue was exposed to vacuum at room temperature for a short period to eliminate the solvent completely. The pure oil was then measured on an analytical balance and multiple gravimetric measurements were taken during 24 h to ensure that all of the solvent had evaporated.

GC–MS

The chemical composition of the oils was investigated by GC and GC–MS. The GC–MS analyses (three repetitions) were realized using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5 % phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 µm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface operated at 250 °C and 300 °C, respectively. The oven temperature was increased from 70–290 °C at a heating rate of 5.0 °C min⁻¹ and then isothermally held for 10 min. Helium at a flow rate of 1.0 ml min⁻¹ was used as the carrier gas. The sample, 1 µl of the oil solution in diethyl ether (1:100), was injected in a pulsed split mode (the flow was 1.5 ml min⁻¹ for the first 0.5 min and then set to 1.0 ml min⁻¹ throughout the remainder of the analysis; split ratio 40:1). The MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35–500, scan time 0.32 s.

Gas chromatographic analyses were performed using an HP 5890 gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector (Agilent Technologies, USA). The separation was achieved using a HP-5 (5 % diphenyl- and 95 % dimethylpolysiloxane) fused silica capillary column, 30 m×0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 70 to 290 °C at a rate of 5.0 °C/min. Hydrogen was used as the carrier gas at a flow rate of 1.6 mL min⁻¹ at 45 °C. The injector temperature was 250 °C and the detector temperature: 280 °C; the injection mode was splitless. The percentage composition of the oils was computed from the GC (FID) peak areas without any corrections. The relative standard deviation (*RSD*) of repeated measurements (independent sample preparation and GC–FID) was for all substances below 1 %. The only exceptions which had higher *RSD* were minor components such as methyl salicylate, α -cubebene, 1-hexyl isovalerate, bicycloelemene and germacrene A, for the *RSD* was 2, 3, 5, 9 and 10 %, respectively.

Oil constituents were identified by comparison of their linear retention indices (relative to C₇–C₂₉ alkanes¹⁶ on the HP-5MS column) with literature values¹⁷ and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known oils, and wherever possible, by co-injection with an authentic sample.

RESULTS AND DISCUSSION

Hydrodistillation of fresh plant material yielded 0.003 % (w/w, based on weight of fresh plant material) of essential oil in the stalks, 0.012 % (w/w) in the leaves and 0.086 % (w/w) in the fruits. The chemical compositions of the essential oils from different parts of *P. austriacum* are reported in Table I, together with a summation of the compounds according to their respective compound class (percentage and number of detected constituents). Two hundred and five different constituents were identified in the volatile fraction of *P. austriacum* (156 in fruits, 116 in leaves, 117 in stalks) amounting to 94.5–98.6 % of the total oils. In all samples, the sesquiterpenoid fractions were the most abundant (85.7–90.5 %). About one half of all the identified components belonged to this class, with hydrocarbons in a greater number compared to the oxygenated derivatives. Within this class, the germacrane and related sesquiterpenoids (SGE) were the major ones (71.9 % in the fruits, 63.2 % in the leaves and 52.2 % in the stalks). The major components detected were germacrene D (66.5 % in the fruits, 62.4 % in the leaves and 49.0 % in the stalks), β -caryophyllene (3.1–5.7 %), δ -cadinene (3.6–5.0 %) and (*E*)- β -farnesene (1.0–1.5 %). Additional worth mentioning constituents were: (*Z*)-3-hexen-1-ol (8.8 %), 1-hexanol (1.3 %) and *epi*-cubebol (2.4 %) in the leaf oil, β -phellandrene (2.3 %), bicyclogermacrene (3.3 %) in the fruit oil and humulene (1.4 %), α -cadinol (3.0 %) and hexadecanoic acid (5.2 %) in the stalk oil. Shyobunol (0.3 %), α -phellandrene (0.3 %), *cis*-muurola-3,5-diene (0.1 %), aristolone (0.1 %) and cinnamyl valerate (0.1 %) were found only in the fruit oil, while 1-phenylethyl 3-methylbutanoate (0.2 %) was only detected in the leaf oil. γ -Muurolene (0.8 %), gossonorol (0.3 %), *cis*-calamene-10-ol (0.5 %), α -bisabolol (0.9 %), tetradecanoic acid (0.5 %) and 14-hydroxy- δ -cadinene (0.4 %) were present only in the stalk oil and were completely absent from the other

two oils. An additional dissimilarity between these oils was the content of “green leaf”¹⁸ (G) volatiles (volatile aliphatic aldehydes, alcohols, and their esters formed through the hydroperoxide lyase pathway of oxylipin metabolism, plants start to form Gs after disruption of their tissues and after suffering biotic or abiotic stresses), being predominant in the leaf oil (10.9 %), as expected.

TABLE I. Percentage composition of *Pleurospermum austriacum* oils^a

<i>R^b</i>	Component	Fruit oil	Leaf oil	Stalk oil	Class	Method ^c
861	(<i>Z</i>)-3-Hexen-1-ol	0.2	8.8	1.4	G	a, b
863	1-Hexanol	tr ^d	1.3	0.4	G	a, b, c
1034	β -Phellandrene	2.3	tr	–	M	a, b
1383	α -Copaene	1.0	1.0	0.7	SCO	a, b
1428	β -Caryophyllene	3.1	5.7	4.9	SC	a, b, c
1460	(<i>E</i>)- β -Farnesene	1.0	1.2	1.5	SA	a, b
1462	Humulene	0.4	0.6	1.4	SC	a, b, c
1498	Germacrene D	66.5	62.4	49.0	SGE	a, b, c
1504	<i>epi</i> -Cubebol	tr	2.4	tr	SCA	a, b
1507	Bicyclogermacrene	3.3	tr	2.3	SGE	a, b
1511	(<i>E,E</i>)- α -Farnesene	0.6	1.3	1.7	SA	a, b
1514	β -Bisabolene	–	0.5	1.0	SBI	a, b, c
1532	δ -Cadinene	3.9	3.6	5.0	SCA	a, b
1650	τ -Muurolol (syn. ^e <i>epi</i> - α -muurolol)	0.5	0.3	1.0	SCA	a, b
1664	α -Cadinol	1.4	0.9	3.0	SCA	a, b
1962	Hexadecanoic acid	tr	–	5.2	O	a, b, c
2027	Isopropyl hexadecanoate	0.3	–	1.0	O	a, b, c
Total identified		95.1	98.6	94.5		
Number of identified compounds		156	116	117		
Monoterpenoids		2.8 (27) ^f	tr (8)	tr (5)		
Hydrocarbons		2.8 (15)	tr (6)	tr (3)		
Oxygenated derivatives		tr (12)	tr (2)	tr (2)		
Sesquiterpenoids		90.5 (66)	85.7 (58)	85.7 (67)		
Hydrocarbons		85.8 (40)	79.5 (37)	72.6 (36)		
Oxygenates derivatives		4.7 (26)	6.2 (21)	13.1 (31)		
Acyclic sesquiterpene (SA)		1.8 (7)	2.6 (7)	4.1 (8)		
Bisabolanes (SBI)		0.5 (5)	2.0 (7)	4.3 (10)		
Caryophyllanes and related (SC)		3.8 (3)	6.8 (3)	7.2 (3)		
Cadinanes and related (SCA)		8.1 (25)	8.6 (19)	14.6 (24)		
Copaanes and related (SCO)		1.3 (3)	1.3 (3)	1.1 (3)		
Germacrane and related (SGE)		71.9 (5)	63.2 (5)	52.2 (3)		
Other unclassified sesquiterpenes (S)		3.1 (18)	1.2 (14)	2.2 (16)		
Diterpenes (DT)		0.1 (2)	0.9 (5)	tr (2)		
“Green leaf” volatiles (G)		0.7 (27)	10.9 (24)	1.8 (16)		
Others (O)		1.0 (34)	1.1 (21)	7.0 (27)		

^aThe remainder of the identified constituents (minor, that have not reached 1 % in any of the samples) of the investigated essential oils are summarized in the following format below: *RI*, Component name, Relative percentage of the component in the fruit oil, Leaf oil and stalk oil, Class of the constituent, Method of identification; - 743, pyridine, -, tr^d, -, O, a, b, c; 744, (*E*)-2-pentenal, -, tr, -, G, a, b; 762, 1-pentanol, -, -, tr, G, a, b, c; 765, (*Z*-

2-penten-1-ol, tr, 0.2, tr, G, a, b; 780, (\pm)-2,3-butandiol, -, -, tr, O, a, b; 785, *meso*-2,3-butandiol, -, -, tr, O, a, b; 801, hexanal, tr, tr, G, a, b; 834, furfural, tr, tr, O, a, b, c; 852, (*E*)-3-hexen-1-ol, -, 0.1, tr, G, a, b; 885, 2-butylfuran, tr, tr, G, a, b; 895, (*2E,4Z*)-2,4-hexadienal, tr, -, -, G, a; 900, nonane, tr, -, tr, O, a, b, c; 903, cyclohexanone, -, tr, -, O, a, b, c; 913, (*2E,4E*)-2,4-hexadienal, -, tr, -, G, a, b; 930, α -thujene, tr, -, -, M, a, b; 939, α -pinene, 0.1, -, tr, M, a, b, c; 955, camphene, tr, -, -, M, a, b, c; 957, (*E*)-2-heptenal, tr, tr, -, G, a, b; 967, benzaldehyde, tr, tr, O, a, b, c; 978, sabinene, 0.1, tr, tr, M, a, b; 984, β -pinene, tr, -, -, M, a, b, c; 993, myrcene, tr, -, -, M, a, b, c; 995, 2-pentylfuran, tr, tr, G, a, b; 999, 2,4,6-trimethylpyridine, -, tr, -, O, a, b, c; 1001, mesitylene, tr, -, -, O, a, b, c; 1005, octanal, tr, tr, G, a, b; 1008, (*E*)-3-hexenyl acetate, 0.1, 0.2, tr, G, a, b; 1010, α -phellandrene, 0.3, -, -, M, a, b; 1013, 1-hexyl acetate, -, tr, -, G, a, b, c; 1014, (*2E,4E*)-2,4-heptadienal, -, tr, tr, G, a, b; 1021, α -terpinene, tr, -, -, M, a, b, c; 1029, *p*-cymene, tr, tr, tr, M, a, b, c; 1033, limonene, tr, tr, -, M, a, b, c; 1044, β -isophorone, tr, -, -, O, a, b, c; 1048, phenylacetaldehyde, tr, 0.2, tr, O, a, b, c; 1048, (*E*)- β -ocymene, tr, -, -, M, a, b; 1059, (*Z*)-2-octenal, tr, -, -, G, a, b; 1062, γ -terpinene, tr, tr, -, M, a, b; 1062, artemisia ketone, tr, -, -, M, a, b, c; 1070, 1-octanol, -, tr, -, G, a, b, c; 1072, acetophenone, -, -, tr, O, a, b, c; 1077, *cis*-linalool oxide (furanoid), tr, -, -, M, a, b, c; 1094, terpinolene, tr, -, -, M, a, b; 1094, *trans*-linalool oxide (furanoid), tr, -, -, M, a, b, c; 1098, 3-isopropyl-2-methoxypyrazine, tr, -, -, O, a, b; 1100, undecane, tr, tr, tr, O, a, b, c; 1103, linalool, tr, tr, tr, M, a, b, c; 1107, nonanal, 0.1, 0.1, -, G, a, b; 1107, hotrienol, tr, -, -, M, a, b; 1109, 2-methylbutyl isovalerate, tr, -, -, O, a, b; 1118, 3-methyl-3-butenyl 3-methylbutanoate, tr, -, -, O, a, b; 1120, *endo*-fenchol, tr, -, -, M, a, b; 1127, dehydrosabina ketone, tr, -, -, M, a, b; 1127, α -isophorone, tr, -, -, O, a, b; 1162, (*E*)-2-nonenal, tr, -, -, G, a, b; 1184, terpinen-4-ol, tr, -, -, M, a, b, c; 1191, naphthalene, tr, -, -, O, a, b, c; 1194, cryptone, tr, -, -, M, a, b; 1199, (*Z*)-4-decenal, tr, -, -, G, a, b; 1201, methyl salicylate, tr, 0.2, tr, O, a, b, c; 1208, decanal, tr, tr, -, G, a, b, c; 1225, *endo*-fenchyl acetate, tr, tr, -, M, a, b; 1227, β -cyclocitral, -, tr, -, O, a, b, c; 1227, (*2E,4E*)-2,4-nonadien-1-ol, tr, -, -, G, a, b; 1234, (*Z*)-3-hexenyl 2-methylbutanoate, tr, tr, -, G, a, b; 1238, (*Z*)-3-hexenyl 3-methylbutanoate, 0.1, 0.2, tr, G, a, b; 1238, 1-hexyl 2-methylbutanoate, tr, -, -, G, a, b; 1238, methyl thymol, tr, -, -, M, a, b, c; 1243, 1-hexyl isovalerate, 0.2, tr, tr, G, a, b; 1248, methyl carvacrol, tr, -, -, M, a, b, c; 1265, (*E*)-2-decenal, tr, -, -, G, a, b; 1294, 1-tridecene, tr, -, -, O, a, b; 1297, (*2E,4Z*)-2,4-decadienal, tr, -, -, G, a, b; 1300, tridecane, tr, tr, -, O, a, b, c; 1300, 2-methylnaphthalene, tr, -, -, O, a, b; 1309, undecanal, tr, -, -, G, a, b; 1317, (*E*)-3-hexenyl tiglate, tr, tr, -, G, a, b; 1319, *p*-vinylguaiaicol, -, tr, -, O, a, b; 1321, (*2E,4E*)-2,4-decadienal, tr, tr, G, a, b; 1323, 1-hexyl senecioate (hexyl 2-methyl-2-butenolate), tr, tr, -, G, a, b; 1325, (*E*)-9-undecenal, -, -, tr, G, a, b; 1343, bicycloelemene, 0.1, tr, -, S, a, b; 1355, α -cubebene, 0.1, tr, tr, S, a, b; 1374, cyclosativene, tr, -, -, S, a, b; 1378, α -ylangene, tr, tr, tr, S, a, b; 1390, geranyl acetate, tr, tr, tr, M, a, b, c; 1392, β -bourbonene, tr, tr, tr, S, a, b; 1397, β -cubebene, 0.6, 0.8, 0.6, S, a, b; 1398, β -elemene, 0.5, tr, tr, S, a, b; 1411, dodecanal, tr, tr, tr, G, a, b; 1419, α -gurjunene, 0.2, tr, -, S, a, b; 1421, *cis*- α -bergamotene, -, -, tr, SBI, a, b; 1437, β -copaene, 0.3, 0.3, 0.4, SCO, a, b; 1441, *trans*- α -bergamotene, -, tr, tr, SBI, a, b; 1443, β -gurjunene, 0.8, tr, tr, S, a, b; 1448, aromadendrene, tr, tr, -, S, a, b; 1454, *cis*-muurola-3,5-diene, 0.1, -, -, SCA, a, b; 1472, *cis*-muurola-4(14),5-diene, 0.3, 0.2, 0.3, SCA, a, b; 1484, γ -muurolene, -, -, 0.8, SCA, a, b; 1485, γ -curcumene, -, -, tr, SBI, a, b; 1488, herbertene, -, -, tr, SBI, a, b; 1496, 1-phenylethyl 3-methylbutanoate, -, 0.2, -, O, a, b; 1498, 10,11-epoxy-calamenene, -, -, tr, SCA, a, b; 1500, β -selinene, tr, -, 0.2, S, a, b; 1502, α -zingiberene, tr, 0.4, -, SBI, a, b; 1503, *trans*-muurola-4(14),5-diene, 0.4, tr, 0.6, SCA, a, b; 1507, α -muurolene, tr, tr, tr, SCA, a, b; 1511, α -cuprenene, tr, tr, tr, SBI, a, b; 1515, germacrene A, 0.6, tr, -, SGE, a, b; 1515, δ -amorphene, tr, -, -, SCA, a, b; 1517, β -curcumene, 0.2, 0.4, 0.8, SBI, a, b; 1523, γ -cadinene, 0.5, 0.3, 0.5, SCA, a, b; 1524, cubebol, tr, tr, -, SCA, a, b; 1538, (*E*)- γ -bisabolene, 0.2, 0.4, 0.7, SBI, a, b; 1540, *trans*-cadin-1,4-diene, 0.1, tr, tr, SCA, a, b; 1546, α -cadinene, 0.1, tr, tr, SCA, a, b; 1552, α -calacorene, tr, tr, 0.2, SCA, a, b; 1557, *cis*-muurola-5-en-4 β -ol, tr, -, -, SCA, a, b; 1557, dodecanoic acid, -, -, tr, O, a, b, c; 1564, *cis*-cadinene ether, tr, -, -, SCA, a, b; 1565, *cis*-muurola-5-en-4 α -ol, tr, -, -, SCA, a, b; 1568, (*E*)-nerolidol, 0.1, 0.1, 0.5, SA, a, b; 1571, β -calacorene, tr, tr, tr, SCA, a, b; 1574, γ -undecalactone, tr, -, -, O, a, b; 1576, mint oxide, 0.1, 0.1, 0.3, S, a, b; 1582, (*E*)-dendrolasin, tr, tr, tr, SA, a, b; 1584, germacrene D-4-ol, 0.9, 0.3, -, SGE, a, b; 1587, spathulenol, tr, 0.1, 0.2, S, a, b, c; 1590, 10-*epi*-junenol, -, -, tr, S, a, b; 1593, caryophyllene oxide, 0.3, 0.5, 0.9, SC, a, b, c; 1595, β -copaen-4 α -ol, tr, -, -, SCO, a, b; 1597, *cis*- β -elemenone, -, -, tr, S, a, b; 1603, salvial-4(14)-en-1-one, 0.1, tr, tr, S, a, b; 1610, 3-phenylpropyl 2-methylbutanoate, tr, -, -, O, a, b; 1611, rosifoliol, -, -, tr, S, a, b; 1613, 3-phenylpropyl 3-methylbutanoate, tr, -, -, O, a, b; 1615, tetradecanal, 0.1, 0.3, 0.3, O, a, b, c; 1623, 1,10-di-*epi*-cubenol, tr, tr, 0.5, SCA, a, b; 1629, junenol, 0.2, 0.1, 0.6, S, a, b; 1630, α -corocalene, -, -, tr, SCA, a, b; 1632, *nor*-copaenone, -, tr, tr, SCO, a, b; 1637, 1-*epi*-cubenol, 0.1, 0.2, 0.2, SCA, a, b; 1645, gossonorol, -, -, 0.3, S, a, b; 1650, τ -cadinol (syn. ^e *epi*- α -cadinol), 0.5, 0.3, 0.9, SCA, a, b; 1650, τ -muurolol (syn. *epi*- α -muurolol), 0.5, 0.3, 1.0, SCA, a, b; 1655, α -muurolol, 0.2, 0.2, 0.7, SCA, a, b; 1667, *cis*-calamenen-10-ol, -, -, 0.5, SCA, a, b;

1678, *trans*-calamene-10-ol, tr, 0.2, tr, SCA, a, b; 1688, cinnamyl valerate (correct isomer not determined), 0.1, -, -, O, a; 1690, *epi-α*-bisabolol, 0.1, 0.3, 0.9, SBI, a, b; 1692, *α*-bisabolol, -, -, 0.9, SBI, a, b, c; 1696, germacra-4(15),5,10(14)-trien-1 α -ol, 0.6, 0.5, 0.9, SGE, a, b; 1701, shyobunol, 0.3, -, -, S, a, b; 1701, 3-phenylpropyl hexanoate (correct isomer not determined), tr, -, -, O, a; 1705, (2*Z*,6*Z*)-farnesol, -, -, tr, SA, a, b; 1713, amorpho-4,9-dien-2 α -ol, tr, -, tr, SCA, a, b; 1717, pentadecanal, tr, -, tr, O, a, b; 1720, (2*E*,6*Z*)-farnesal, tr, tr, SA, a, b; 1727, (2*Z*,6*E*)-farnesol, 0.1, tr, 0.4, SA, a, b; 1747, (2*E*,6*E*)-farnesal, tr, tr, SA, a, b; 1749, mint sulfide, tr, 0.1, tr, S, a, b; 1763, tetradecanoic acid, -, -, 0.5, O, a, b, c; 1772, aristolone, 0.1, -, -, S, a, b; 1782, 14-hydroxy- α -muurolene, tr, tr, SCA, a, b; 1811, 14-hydroxy- δ -cadinene, -, -, 0.4, SCA, a, b; 1818, hexadecanal, tr, tr, O, a, b, c; 1841, neophytadiene, isomer II, 0.1, 0.8, tr, DT, a, b; 1862, pentadecanoic acid, -, -, tr, O, a, b; 1900, nonadecane, tr, -, -, O, a, b, c; 1928, methyl hexadecanoate, -, -, tr, O, a, b, c; 1944, (Z)-9-hexadecenoic acid (palmitoleic acid), -, -, tr, O, a, b, c; 1950, isophytol, -, -, DT, a, b; 1971, (Z,Z)-geranyl linalool, -, -, DT, a, b; 1996, ethyl hexadecanoate, -, -, tr, O, a, b, c; 2000, eicosane, tr, -, -, O, a, b, c; 2034, (E,E)-geranyl linalool, tr, 0.1, tr, DT, a, b; 2083, 1-octadecanol, -, -, tr, O, a, b, c; 2100, heneicosane, 0.1, tr, -, O, a, b, c; 2117, (E)-phytol, -, -, DT, a, b; 2200, docosane, tr, tr, -, O, a, b, c; 2281, 1-eicosanol, tr, -, -, O, a, b, c; 2300, tricosane, 0.2, 0.1, tr, O, a, b, c; 2396, 1-tetracosene, -, tr, tr, O, a, b; 2400, tetracosane, tr, tr, tr, O, a, b, c; 2500, pentacosane, 0.2, 0.1, tr, O, a, b, c; 2600, hexacosane, -, -, tr, O, a, b, c; 2700, heptacosane, -, tr, -, O, a, b, c; 2833, (all E)-squalene, -, -, tr, O, a, b; ^bexperimentally determined retention indices by co-injection of a homologous series of *n*-alkanes (C₇–C₂₉); ^ca – constituent identified by mass spectra comparison, b – constituent identified by retention index matching, c – constituent identity confirmed by co-injection of an authentic sample; ^dtrace (<0.05 %); ^esynonym; ^fthe value in brackets represents the number of identified compounds belonging to this specific class; M – monoterpenoids; SA – acyclic sesquiterpenes; SBI – bisabolanes; SC – caryophyllanes and related sesquiterpenoids; SCA – cadinanes and related sesquiterpenoids; SCO – copaanes and related sesquiterpenoids; SGE – germacrane and related sesquiterpenoids; S – other unclassified sesquiterpenes; DT – diterpenes; G – “green leaf” volatiles (aliphatic aldehydes, alcohols, and their esters formed as the plants response after suffering biotic or abiotic stresses); O – other unclassified constituents

The only previously published investigated essential oil from a *Pleuro-spermum* species was the one from *P. lindleyanum* (according to Pimenov and Kljuykov,³ this taxon is transferred to the genus *Hymenidium* (*H. stellatum*)).⁴ The authors state that 73 oil constituents were identified, and among them were: 1-propoxy-2-propanol, myristicin, 1,2,3-trimethoxy-5-(2-propenyl)-benzene, *cis*-asarone, *n*-hexane, apiol, dimethyl ether, 1,2-dimethoxy-4-(2-propenyl)benzene, ethyl acetate, spathulenol, α,α' -4-trimethyl-benzenemethanol, *trans*-methyl *iso*-eugenol, and β -phellandrene. Apparently a number of the listed compounds represent either misidentifications or identification of a solvent contaminant introduced during the work-up of the essential oil sample (1-propoxy-2-propanol, dimethyl ether, *n*-hexane, ethyl acetate). Apart from this, the authors refer to the content (20.77 %) of cycloserine “as the highest” in conjunction with the essential oil.⁴ The compounds in common with the present *P. austriacum* oil were spathulenol and β -phellandrene.

The sesquiterpenoid fraction of the oils of *P. austriacum* was mostly comprised of germacrane and highly related types of compounds (Table I) that are the first steps in the biosynthesis pathway of sesquiterpenes from farnesyl diphosphate. Sesquiterpene biosynthesis seems to be complex since the formation *via* either pathway (mevalonic or methylerythritol) or a combination of both has been reported.¹⁹ However, these appear to be omnipresent in plant taxa and some insects, and is related to the cytosol–mitochondria. A previous investigation showed

a direct correlation between the amount and chemical composition of the essential oils.¹⁸ The results of these statistical analyses strongly suggest that the main volatiles of essential oil-poor species (yields less than 0.1 %) are fatty acid- and carotenoid-derived compounds, while the essential oil rich taxa (essential oil yields much higher than 0.1 %) are generally characterized by the specific production of monoterpene and/or phenylpropanoids.¹⁸ Two facts, the monoterpenes that accounted only for 0.0–2.8 % and that the yield of each oil was quite low (0.003–0.086 %, w/w), corroborate the previously introduced hypothesis concerning a possible link between the oil yield and the corresponding oil composition. It is, then, directly possible to conclude that if some plant is rich in oil, it will probably have a big portion of monoterpenes, or the phenylpropanoids are predominant. A related hypothesis concerning a possible link between oil yield and composition was already proposed.²⁰ Lawrence suggested that the oil-poor species of the family Lamiaceae produce essential oils rich in hydrocarbons, with germacrene D often being one of the predominant components.²⁰ This does not seem to stand for the Lamiaceae alone. For example, in the oil-poor (0.18–0.57 %) aerial parts of *Tamarix boveana* (Tamaricaceae), germacrene D (7.69–31.43 %) with its congeners represented the predominant class of volatile compounds.²¹ In some previous investigations of *Lippia alba* (Verbenaceae), possible connections between the morphological characteristics and chemotypes were discussed, and a large content of germacrene D was a feature of one of the chemotypes.²² More strikingly, a study on *Artemisia annua* showed that the glandless leaves were estimated to contain more than twice as much total sesquiterpenes per unit of fresh weight as the glanded leaves. In this way, the absence of monoterpenes in the steam-distilled oil of the glandless biotype is a good indication that the monoterpenes accumulate exclusively in specialized tissue structures, the glandular trichomes.²³ Thus, one may expect to find germacrene D and/or its hydrocarbon congeners as major constituents of essential oil in plant species not possessing an elaborate biosynthetic apparatus for the production of volatile monoterpenes or phenylpropanoids, as *P. austriacum*.

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ИЗВОД

ИСПАРЉИВИ СASTOЈЦИ БИЉНЕ ВРСТЕ *Pleurospermum austriacum* (L.) Hoffm.
(APIACEAE)

НИКО С. РАДУЛОВИЋ, НЕВЕНКА Д. ЂОРЂЕВИЋ И РАДОСАВ М. ПАЛИЋ

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Резултати детаљних GC и GC–MS анализа испарљивих састојака свежих листова, стабљика и плодова угрожене биљне врсте *Pleurospermum austriacum* (L.) Hoffm. (Apiaceae) приказани су у овом раду по први пут. Око 4/5 уља, добијених у ниском приносу (0,003–0,086

mas. %), је било сачињено од сесквитерпенских угљоводоника. Главне компоненте, идентификоване у уљима, су гермакрен Д (66,5 % у плоду, 62,4 % у лишћу и 49,0 % у стабљници), β -кариофилен (3,1–5,7 %), δ -кадинен (3,6–5,0 %) и (*E*)- β -фарнезен (1,0–1,5 %).

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