Variability in the Essential-Oil Composition of *Sideritis scardica* Griseb. from Native Bulgarian Populations

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The essential-oil composition of six native populations of *Sideritis scardica* from Bulgaria was studied by GC-FID and GC/MS analyses. Altogether, 37 components, representing 73.1 to 79.2% of the total oil content were identified. Among them, α-pinene (4.4–25.1%), β-pinene (2.8–18.0%), oct-1-en-3-ol (2.3–8.0%), phenylacetaldehyde (0.5–9.5%), β-bisabolene (1.3–11.0%), benzyl benzoate (1.1–14.3%), and *m*-camphorene (**1**; 0.3–12.4%) were the main compounds. All samples were characterized by low contents of oxygenated mono- and sesquiterpenes ($\leq$1.6 and 2.3%, resp.). Principal component analysis (PCA) and cluster analysis (CA) showed a significant variability in the chemical composition of the studied samples as well as a correlation between the oil profiles and the ecological conditions of the natural habitats of *S. scardica*.

Introduction. – *Sideritis scardica* Griseb. (Lamiaceae; mountain tea) is a Balkan endemic plant encountered in Southwest Albania, Macedonia, Bulgaria, and Greece [1] as well as in Turkey (*cf.* [2] and refs. cit. therein). The species is distributed in the mountainous regions of Southern Bulgaria from 1000 up to 2200 m.a.s.l. (Slavyanka, Pirin, and Rhodope Mts). The plant is used in traditional medicine as herbal tea for the treatment of bronchitis, lung emphysema, *etc.* [3], and for this reason it is intensively collected from the natural habitats for years. Nowadays, the distribution of *S. scardica* is quite limited, and the species has been included in the Red Book of Bulgaria as an endangered plant [4]. It is under governmental protection, and its collection from the native habitats is prohibited.

A literature survey revealed few reports on the *S. scardica* essential-oil composition from native Bulgarian, Macedonian, Greek, and Turkish populations. *Kokkalou* [5] reported that monoterpene hydrocarbons (>80%) were the principal components in the essential oil of Greek origin with α-pinene (52%) as the major compound. β-Pinene (17.9%), carvacrol (14.8%), and α-pinene (7.3%) were found as main constituents in a sample of Turkish origin [2]. In contrast, Macedonian and Bulgarian *S. scardica* essential oils [6] were reported to be poor in monoterpenes (<7%). α-Cadinol (20.0%), a sesquiterpene alcohol, was the most abundant compound in the Macedonian sample, whereas in the oils of Bulgarian origin, the main components were diterpenoids (>21%) and octadecenol (>20%) [6]. Sesquiterpenoids as β-

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caryophyllene (18.8%) and nerolidol (12.1%) dominated in another Bulgarian sample [7]. Recently, it has been published that an essential oil from Shara Mt (Macedonia) contained relatively low concentrations of monoterpene hydrocarbons (1.8%), but high percentages of oxygenated mono- and sesquiterpenes (30.0 and 25.5%, resp.) and substantial amounts of fatty acids with their esters (15.9%) [8].

Therefore, to contribute to a better understanding of the chemical diversity of this Balkan endemic species, the essential oils obtained from six native Bulgarian populations were characterized by GC-FID and GC/MS analyses. In addition, chemometric methods such as principal component and cluster analyses were applied to evaluate the variations of *S. scardica* at the intraspecific level.

Results and Discussion. – In the present work, the essential-oil composition of six *S. scardica* populations originated from Pirin (*SC1*), Slavyanka (*SC2*), and Rodopi (*SC3*–*SC6*) Mts (*Table 1* and *Fig. 1*) were investigated. The essential-oil yield of the examined populations ranged from 0.04 up to 0.10% (*w*/*w*), based on the dry weight of the plant material (*Table 2*). The lowest yield was recorded in plants from Mugla Village (*SC4*) and the highest in plants from Marciganiza (*SC6*). Altogether, 37 components (*Table 2* and *Fig. 2*), representing 73.1 to 79.2% of the total oil content, were registered as chromatographic peaks and were taken into consideration for the comparison of the studied oils. The major class of compounds was that of the monoterpene hydrocarbons (11.8–48.6%), represented mainly by α-pinene (4.4–25.1%) and β-pinene (2.8–18.0%). Sesquiterpene hydrocarbons (8.8–22.2%), diterpenoids (1.1–19.6%), and aromatic compounds (2.5–25.7%) were detected in quite variable amounts. Oxygenated mono- and sesquiterpenes were minor components and did not exceed 3.3% in total.

Table 1. *Collection-Site Details of the Six Studied* Sideritis scardica *Populations*

Population	Locality	Floristic region	Voucher	Altitude [m]	Climatic zone[a])
SC1	Peak Orelek	Pirin	So-1298	2000	CM
SC2	Peak Tzarev	Slavyanka	So-1299	2086	CM
SC3	Chaeva chuka	Central Rhodopes	So-1297	1822	CM
SC4	Mugla village	Central Rhodopes	So-1302	1505	CM
SC5	Trigrad village	Central Rhodopes	So-1300	1240	CM
SC6	Marciganiza	Western Rhodopes	So-1301	1412	TC

[a]) CM, Continental Mediterranean; TC, transitional continental [9].

To investigate the variations in the essential oils of different populations of *S. scardica*, the composition data were analyzed by principal component analysis (PCA) and cluster analysis (CA). The PCA performed on the content of all constituents for each sample showed that the first two principal axes accounted for 70.79% of the total variation (*Fig. 3*, *a*). Considering the contributions to the variances, Axis 1 (42.77% of the total variation) accounted for positive contributions of α-pinene, β-pinene, myrcene, terpinolene, δ-car-3-ene, limonene, γ-terpinene, terpinolene, α-copaene, β-caryophyllene, germacrene D, and δ-cadinene and negative contributions of oct-1-en-3-ol, benzyl alcohol, eugenol, *trans*-β-farnesene, caryophyllene oxide, and *m*-camphorene

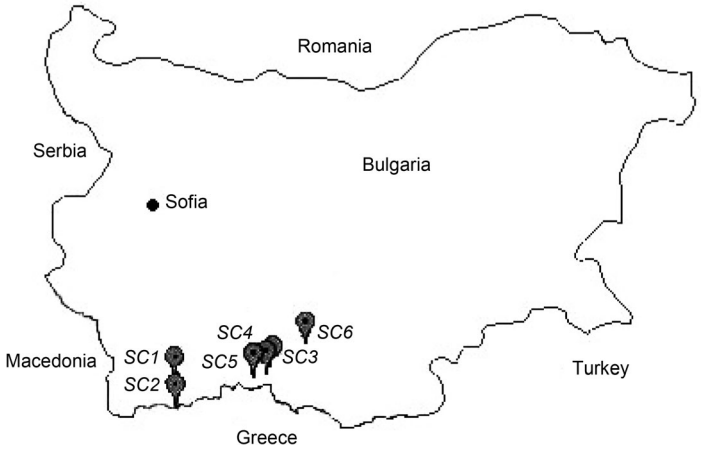


Fig. 1. *Geographical distribution of the six native Bulgarian populations of* Sideritis scardica. For the population details, *cf. Table 1.*

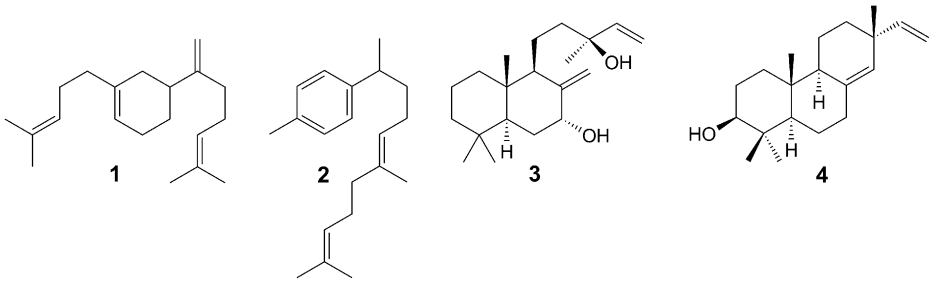


Fig. 2. *Rare essential-oil components identified in* Sideritis scardica

(**1**). Axis 2 (28.02% of the total variation) was positively related to α-pinene, oct-1-en-3-ol, benzyl alcohol, (*E*)-oct-2-en-1-ol, and eugenol and negatively related to sabinene, β-pinene, nonanal, α-terpineol, bicycloelemene, β-bisabolene, 7α-hydroxymanool (**3**), sandaracopimaradien-3β-ol (**4**), and two unidentified diterpenoids (*Entries 36* and *37*, *Table 2*).

As depicted in the score plot (*Fig. 3*, *b*), the investigated populations were highly dispersed and could be grouped as follows:

Populations SC3, *SC4*, and *SC5* constituted the first group and were characterized by oils with the highest contents of oct-1-en-3-ol (7.7–8.0%), compound **1** (6.1–12.4%), and aromatic components (>20%). Among the aromatic compounds, phenylacetaldehyde (9.5%) and benzyl benzoate (7.9%) dominated in *Population SC3*, benzyl benzoate (14.3%) in *Population SC4*, and eugenol (6.8%) in *Population SC5*. β-Caryophyllene and β-bisabolene were the prevalent sesquiterpenes, but differing in their content. These oils contained the lowest amount of β-pinene and moderate concentrations of α-pinene, in comparison with the oils of the other populations.

Table 2. *Chemical Composition of the Essential Oils of the* Sideritis scardica Populations SC1–SC6

Entry	*RI*[a])	Compound name and class	Content [%]					
			SC1	*SC2*	*SC3*	*SC4*	*SC5*	*SC6*
1	932	α-Pinene	13.7	4.4	12.0	7.4	6.8	25.1
2	970	Sabinene	1.8	1.4	tr[b])	tr	tr	0.8
3	976	β-Pinene	18.0	8.6	5.3	3.5	2.8	11.6
4	980	Oct-1-en-3-ol	3.2	2.6	8.0	7.8	7.7	2.3
5	990	Myrcene	0.6	tr	0.6	tr	tr	1.2
6	1002	α-Phellandrene	n.d.[c])	n.d.	n.d.	n.d.	n.d.	2.1
7	1008	δ-Car-3-ene	n.d.	n.d.	n.d.	n.d.	n.d.	2.8
8	1026	Limonene	2.4	2.4	0.7	0.4	0.4	3.7
9	1028	Benzyl alcohol	tr	tr	1.1	0.5	2.4	0.4
10	1040	Phenylacetaldehyde	1.9	2.2	9.5	1.4	1.7	0.5
11	1059	γ-Terpinene	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
12	1061	(*E*)-Oct-2-en-1-ol	0.7	tr	1.2	0.5	tr	1.0
13	1088	Terpinolene	n.d.	n.d.	n.d.	n.d.	n.d.	0.4
14	1095	Linalool	0.8	1.6	0.7	0.5	1.8	0.4
15	1101	Nonanal	0.8	0.8	tr	0.4	0.6	0.4
16	1110	2-Phenylethyl alcohol	1.4	2.8	3.6	1.9	5.1	0.2
17	1174	Terpinen-4-ol	tr	tr	tr	tr	1.0	0.3
18	1188	α-Terpineol	tr	1.6	tr	0.5	tr	0.3
19	1339	Bicycloelemene	0.6	1.0	n.d.	n.d.	n.d.	0.2
20	1358	Eugenol	0.7	tr	3.6	3.3	6.8	0.3
21	1376	α-Copaene	0.6	tr	tr	tr	tr	2.2
22	1390	β-Elemene	tr	tr	tr	tr	0.6	tr
23	1419	β-Caryophyllene	5.5	4.0	2.8	4.6	2.8	5.2
24	1456	*trans*-β-Farnesene	1.1	0.8	1.7	1.4	1.7	tr
25	1482	Germacrene D	0.5	2.2	0.6	0.8	1.2	4.7
26	1500	Bicyclogermacrene	1.7	2.0	0.6	1.1	2.6	3.4
27	1508	β-Bisabolene	4.2	11.0	2.5	2.3	7.1	1.3
28	1525	δ-Cadinene	1.0	1.2	0.6	tr	0.8	3.9
29	1578	Spathulenol	0.6	0.6	0.5	0.9	1.3	0.7
30	1585	Caryophyllene oxide	0.6	0.6	0.7	0.9	1.0	0.6
31	1762	Benzyl benzoate	1.3	2.6	7.9	14.3	4.2	1.1
32	1953	*m*-Camphorene (**1**)	2.4	1.2	7.4	12.4	6.1	0.3
33	1958	2,6-Dimethyl-10-(*p*-tolyl)undeca-2,6-diene (**2**)	0.4	0.6	3.5	0.4	1.9	0.5
34	2237	7α-Hydroxymanool (**3**)	2.4	3.4	0.8	1.4	1.6	tr
35	2269	Sandaracopimaradien-3β-ol (**4**)	1.0	3.6	1.1	1.5	1.1	0.1
36	2325	Diterpene ($M^+ = 288$, $C_{20}H_{32}O$)	3.5	6.2	0.8	1.3	1.1	0.1
37	2349	Diterpene ($M^+ = 288$, $C_{20}H_{32}O$)	0.8	4.6	1.4	2.1	0.9	0.1
		Monoterpene hydrocarbons	37.3	18.4	19.3	11.8	11.8	48.6
		Oxygenated monoterpenes	tr	1.6	tr	0.5	1.0	0.6
		Sesquiterpene hydrocarbons	15.2	22.2	8.8	10.2	16.8	20.9
		Oxygenated sesquiterpenes	1.2	1.2	1.2	1.8	2.3	1.3
		Diterpene hydrocarbons	2.8	1.8	10.9	12.8	8.0	0.8
		Oxygenated diterpenes	7.7	17.8	4.1	6.3	4.7	0.3
		Aromatic compounds	5.3	7.6	25.7	21.4	20.2	2.5
		Aliphatic compounds	4.7	3.4	9.2	8.7	8.3	3.7
		Total	74.2	74.0	79.2	73.5	73.1	78.7
		Oil yield [% (*w*/*w*)]	0.07	0.08	0.08	0.04	0.06	0.10

[a]) *RI:* Retention index determined relative to a homologous series of *n*-alkanes (C_8–C_{25}) on a *HP5-MS* column. [b]) tr: Trace (< 0.1%). [c]) n.d.: Not detected.

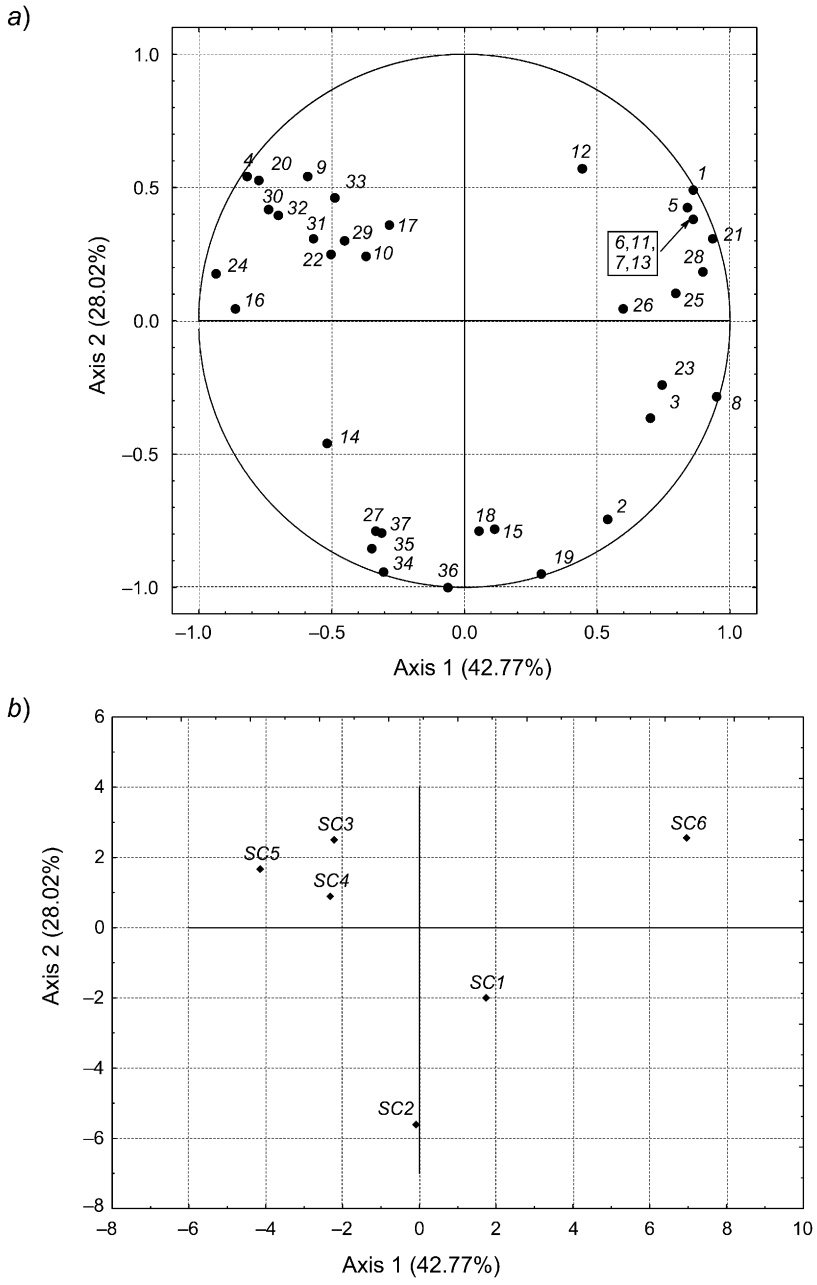


Fig. 3. *Principal component analysis,* a) *loading plot and* b) *score plot, of the volatile components of* Sideritis scardica Populations SC1–SC6*, based on the content of all components for each population.* For the numbering of the compounds (*Entry*) in *a*), *cf. Table 2*, and for the population details in *b*), *cf. Table 1.*

Population SC2 was situated far below the origin of Axis 2 and slightly below the origin of Axis 1 (negative coordinates on Axis 1 and 2) on the score plot. It could be associated with none of the other populations studied, because of quantitative differences in the chemical oil composition. The oil of *Population SC2* was characterized by the lowest content of α-pinene (4.4%) and the highest amounts of β-bisabolene (11.0%), compounds **3** and **4** (3.4 and 3.6%, resp.), and the two unidentified diterpenoids (*Entries 36* and *37*; 6.2 and 4.6%, resp.; *Table 2*).

Populations SC1 and *SC6* were situated at the positive side of Axis 1 on the score plot. The high percentage of monoterpene hydrocarbons (sum of α- and β-pinene $>$ 30%) was characteristic for the oils of these populations. However, β-pinene was the principal component in the oil of *Population SC1*, while α-pinene was the major compound in that of *Population SC6*. The two populations also differed in the oil contents of diterpenoids (10.5 *vs.* 1.1%) and some sesquiterpenes as α-copaene, trans-β-farnesene, germacrene D, and δ-cadinene. In addition, α-phellandrene, δ-car-3-ene, γ-terpinene, and terpinolene were detected only in the oil of *Population SC6*. The observed quantitative and qualitative differences in the chemical oil compositions explained the placement of *Populations SC1* and *SC6* at the positive and negative side of Axis 2, respectively.

Moreover, the CA (*Fig. 4*) on the basis of linkage distances combined with the unweighted pair-group method with arithmetic mean (UPGMA) supported the differentiation of the samples obtained by the PCA analysis.

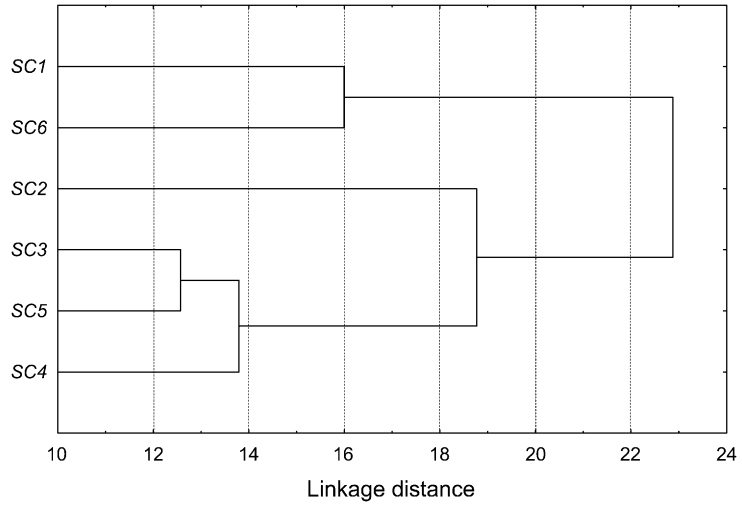


Fig. 4. *Dendrogram obtained by cluster analysis based on linkage distances* (*Euclidean* distances) *and the UPGMA method for the whole set of data of the six native Bulgarian populations of* Sideritis scardica *studied*. For the population details, *cf. Table 1*.

The common and distinguishing characteristics of the six studied *S. scardica* populations correlated partly with the altitude and climate of the natural habitats. Indeed, *Populations SC3*, *SC4*, and *SC5*, originating from closely situated places in the

southern part of the Western Rhodope Mts (*Table 1 and Fig. 1*), exhibited significant similarity. Although *Populations SC2* and *SC1* grow in the same climatic zone and the highest altitude, the strong Mediterranean influence on *Population SC2* might explain its different essential oil composition. Further, *Population SC6* is the only one growing in the transitional continental climatic zone, and its oil was found to differ chemically from the others.

The obtained results were compared with those of samples of Bulgarian origin published previously [6], and a great difference in the chemical composition of the essential oils was observed. Thus, the oil of *Population SC1*, originating from Pirin Mt, was rich in monoterpene hydrocarbons (37.3%), while this type of compounds was detected at only 2.7% in the sample described by *Kostadinova et al.* [6]. In contrast, the former oil was poorer in diterpenoids (10.5%) and aliphatic compounds (4.7%) than the latter. Similarly, the oil of *Population SC5* from Trigrad (Rodopi Mts) also differed from that reported earlier by *Kostadinova et al.* [6] by lower amounts of diterpenoids and aliphatic compounds and by higher contents of aromatic compounds and mono- and sesquiterpenoids. The different plant parts collected (flowering tops *vs.* whole aerial parts) and the method of extraction of the oils (*Likens–Nickerson vs. Clevenger*-type apparatuses) between these samples might be the reasons for such a big qualitative and quantitative disparity.

Further, the oil of *Population SC1* was partly similar to an oil of Turkish origin [2], with the presence of α- and β-pinene as dominant compounds, but the oils differed remarkably in the content of aromatic compounds. On the other hand, the oils of *Populations SC3–SC5* were rich in aromatic compounds as the Turkish sample [2], but phenylacetaldehyde, eugenol, and benzyl benzoate were the main aromatic constituents in the oils of *Populations SC3–SC5*, whereas carvacrol and thymol characterized the oil from Turkey. α-Pinene was the major component in the essential oils of a Greek sample [5] and of *Population SC6*, but in diverse percentages (52 *vs.* 25.1%). Sesquiterpenoids were detected in substantial amounts in the essential oils of *Populations SC2* and *SC6* and of a Macedonian sample [6]. However, α-cadinol was the most abundant compound in the Macedonian sample, whereas in the oils of Bulgarian origin, the main components were sesquiterpene hydrocarbons. In fact, the oils of all studied populations (*SC1–SC6*) were scarce of oxygenated mono- and sesquiterpenes, unlike the essential oil from Shara Mt (Macedonia) [8].

Conclusions. – The essential oils obtained from six native populations of *S. scardica* were characterized by GC-FID and GC/MS analyses. Some common features as well as significant variability in the chemical compositions of their oils were observed by PCA and CA. It seems that there is a relation between the oil content and the population locality. This work reiterated the diversity in the essential oil profiles of this Balkan endemic species. Considering the use of the examined species in the countries of the Balkan Peninsula as herbal medicine, a detailed documentation of its chemical diversity is essential for its rational exploitation and further cultivation.

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Experimental Part

Plant Material. Flowering heads at the flowering stage of *Sideritis scardica* GRISEB. were collected in July 2010 from six native populations in Bulgaria (*Fig. 1* and *Table 1*). They were air dried and kept in a dark and cool place until analysis. Voucher specimens have been deposited with the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Isolation of Essential Oils. The essential oils were obtained by micro steam distillation–solvent extraction of the air-dried plant material (5 g) for 3 h in a modified *Lickens–Nickerson* apparatus [10] using Et_2O as a solvent.

GC Analysis. The GC analyses were performed with a *Shimadzu 17A* gas chromatograph equipped with FID and an *HP5-MS* fused-silica cap. column ((5%-phenyl)-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temp. was programmed rising from 50 to 240° at 4°/min and then kept isothermal at 240° for 10 min; injector temp., 260°; detector temp., 260°; carrier gas, N_2 (linear velocity, 33 cm/s); split ratio, 1:100. The relative peak areas (electronic integration) were used for the quantitative estimation of the contents.

GC/MS Analysis. The GC/MS analyses were performed with an *HP 6890* instrument coupled to an *HP MSD 5973* mass selective detector (electron energy, 70 eV). All chromatographic conditions and the column were as described above (*cf. GC Analysis*), with the exception of the carrier gas, which was He.

Compound Identification. The oil components were identified by comparison of their retention indices (*RI*s, determined rel. to the t_R of a series of *n*-alkanes (C_7–C_{30})) and mass spectra with those published in the literature [11][12] and those registered in the *NIST 2008* as well as in a home-made mass spectral library. The EI-MS data in *m*/*z* (%) of the two unidentified diterpenoids (*Entries 36* and *37*, *Table 2*) are given bellow:

Entry 36. 288 (20, M^+), 270 (100), 255 (49), 242 (10), 199 (14), 187 (20), 145 (15), 131 (20), 107 (20), 105 (20), 94 (43), 79 (20), 69 (16).

Entry 37. 288 (29, M^+), 273 (15), 257 (30), 161 (47), 145 (30), 133 (30), 131 (30), 119 (50), 105 (100), 91 (70), 81 (20), 79 (22), 67 (25).

Statistical Analysis. Principal component analysis (PCA) based on the complete data set was conducted to evaluate the chemical variation and relationship between the populations. The UPGMA (unweighted pair-group method with arithmetic means) clustering method based on linkage distances was used to determine similarities between the examined populations. All analyses were performed with the Statistica 6.0 package.

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