The Compositions of the Essential Root Oils from *Pimpinella saxifraga* s.l. and Chemotaxonomic Implications

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The essential root oils of the *Pimpinella saxifraga* subspecies *eusaxifraga, alpestris* and *nigra* were investigated by means of capillary GC and spectroscopic methods. A total of 48 components was identified.

Characteristic constituents of all oils are pseudoisoeugenol derivatives, sesquiterpenoids and trinor-sesquiterpene hydrocarbons, but the qualitative and quantitative patterns of the individual oils exhibit significant differences.

The results obtained were discussed in regard to a chemotaxonomic reliable delimitation of the 3 described subspecies of *Pimpinella saxifraga* L.

Introduction

Pimpinella saxifraga L., a perennial Apiaceae, occurs at grassy and rocky places in temperate Europe extending to Asia [1, 2]. The species is highly polymorphic and taxonomic delimitation has therefore been treated differently. Thellung [1] and Wolff [3] distinguished three subspecies: subsp. *eusaxifraga* Thellung, subsp. *alpestris* (Sprengel) Vollmann and subsp. *nigra* (Miller) Gaudin. This grouping is maintained in most of the recent floras of Central European regions. The status of the individual taxa, however, varies according to the author. In contrast, Tutin in "Flora Europaea" [4] does not subdivide this species, because of the inadequate and conflicting evidence of the status of the various taxa.

The infrageneric splitting of *Pimpinella saxifraga* is mainly based on morphological characters, such as size, pubescence and leaf dissection, which show a high degree of variability, even within the infrageneric taxa. The same is true for the phytochemical feature used for the distinction of subsp. *nigra:* the blue colour of the essential root oil, which is clearly visible after cutting the root.

Recent investigations of diverse Pimpinella species [5, 6] have shown that the essential root oils of *Pimpinella saxifraga* and *Pimpinella major* differ in qualitative and quantitative composition. These results induced us to perform a more detailed investigation of the essential root oils of the three subspecies of *Pimpinella saxifraga* described by Thellung [1]. The aim was to find out whether a more reliable delimitation and characterization of the three infrageneric taxa of *Pimpinella saxifraga* can be achieved by chemical characters than by morphological features.

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Experimental

Plant material and essential oil recovery

Pimpinella saxifraga subsp. *eusaxifraga* Thellung was collected near Würzburg (F.R.G.).

Pimpinella saxifraga subsp. *alpestris* (Sprengel) Vollmann was collected at the Monte Baldo (Italy).

Pimpinella saxifraga subsp. *nigra* (Miller) Gaudin was obtained from the Botanical Garden of Würzburg and from habitats near Riva del Garda (Italy).

In order to obtain the essential oils, the roots were cut into small pieces, filled into a 2 l round-bottom flask, containing 1 l of water, and subjected to hydrodistillation for 6 h. The distillation apparatus used was as described by Sprecher [7]. The essential oils were received in *n*-pentane as solvent and stored at +4 °C in small tightly stoppered amber glass containers.

Isolation of 3,10-dihydro-1,4-dimethylazulene

The blue coloured essential root oil of *Pimpinella saxifraga* subsp. *nigra* was subjected to repetitive dry column chromatography on silica gel (63–200 μm; Woelm, F.R.G.) in the cold (−18 °C); *n*-pentane was used as eluent.

Spectral data of 3,10-dihydro-1,4-dimethylazulene

MS: *m/e* 143 (100%), 128 (91)*, 158 (29), 115 (29), 142 (25), 141 (23), 129 (17), 127 (17).

^{13}C NMR measurements were conducted on a Bruker Spectrometer (62.9 MHz), solvent was $CDCl_3$.

^{13}C NMR: 147.27 (s), 139.22 (s), 136.44 (d), 130.11 (s), 129.11 (d), 127.90 (d), 122.00 (d), 112.06 (d), 43.80 (d), 32.68 (t), 20.88 (q), 12.69 (q).

Gas chromatography (GC)

The essential oils were analyzed on a Fractovap 2900 gas chromatograph (Carlo Erba Strumentazione, Milano) equipped with a 50 m × 0.25 mm i.d. WCOT WG 11 glass capillary (Werner Günther Analysentechnik, F.R.G.). Temperature of the injection port (split injection, split 1:20) and the detector (FID) was 200 °C. Temperature program: 7 min at 70 °C, 70–200 °C (3 °C/min). Carrier gas: 1.5 ml/min N_2. Quantification was performed with a Merck/Hitachi Chromato-Integrator D-2000; percentages are based on calculated peak areas.

Gas chromatography-mass spectrometry (GC-MS)

Hewlett-Packard GC 5890A, MSD 5970B, Data system 59970A; cross-linked 30 m × 0.25 mm DB-WAX 0.25 µm film fused silica capillary (J & W Scientific); directly coupled to the MS (transfer line 200 °C); flow: 1 ml/min He; temperature program: 7 min at 60 °C, 60–200 °C (3 °C/min). Injection port: 200 °C, split 1:16. The 70 eV EI-spectra were recorded with 2.5 scans/sec for the first 20 min and afterwards with 1.7 scans/sec.

Results and Discussion

In former examinations pseudoisoeugenol-derivatives and some C_{12}-hydrocarbons derived from sesquiterpenes were found to be characteristic constituents of most Pimpinella root oils [6]. In addition to these investigations we have analyzed the essential root oils from *Pimpinella saxifraga* subsp. *eusaxifraga* and *P. s.* subsp. *nigra* in more detail, including *P. s.* subsp. *alpestris*. The results of our capillary GC and GC/MS measurements of all these root oils are summarized in Table I, the corresponding gas chromatograms are depicted in Fig. 1.

Pimpinella saxifraga subsp. eusaxifraga

Because of the wide range of morphological variability and the unsatisfactory systematic division it seemed necessary to investigate the essential root oils from plants with simple segmented leaves and dissected leaves separately. Main constituent (about 60%) of the root oils from both forms is *trans*-epoxy-pseudoisoeugenyl-2-methylbutyrate. Furthermore, it is characteristic for these two oils that 1,4-dimethylazulene and its postulated precursors (Fig. 1a, b; peak No. 13, 18, 20, 44 and 55) are only present in relatively low concentrations (totally about 5%). In a very few cases we could not at all establish the occurrence of 1,4-dimethylazulene in the volatile oils of roots from *Pimpinella saxifraga* subsp. *eusaxifraga*.

Interestingly, the root oils of plants with simple segmented and dissected leaves also exhibit significant differences, mainly in the sesquiterpenes.

The root oil obtained from plants with simple segmented leaves contains 7.5% of an unknown sesquiterpene hydrocarbon $C_{15}H_{22}$ (Fig. 1, peak No. 36), besides germacrene-B (2.7%) and germacrone. The latter can be recognized by a broad peak in the gas chromatogram (Fig. 1; peak No. 53), due to cope-rearrangement, representing 17.2% together with its rearrangement product β-elemenone. In contrast, these three compounds usually do not occur, or could only be detected in traces, in the root oil from plants with a dissected leaf-form. The major sesquiterpene in this oil is *trans*-β-farnesene (10.9%).

Pimpinella saxifraga subsp. alpestris

This subspecies comprises small plants, growing in mountainous regions and cannot be distinguished reliably from special forms of the subsp. *eusaxifraga* by morphological features.

The essential root oil of *Pimpinella saxifraga* subsp. *alpestris* (Fig. 1c) is dominated by the C_{12}-hydrocarbons pregeijerene and its thermal rearrangement product geijerene (together amounting to 31.5%). The phenylpropanoid *trans*-epoxy-pseudoisoeugenyl-2-methylbutyrate shows a lower proportion (23.9%) in comparison with the subsp. *eusaxifraga*. Besides this, the large amount of β-bisabolene (17.5%), being distinctively higher than in the other root oils, seems remarkable. As already

* A significantly lower value (65%) is given for the abundance of this ion species in [11].

Table I. Composition of the essential root oils from different subspecies of *Pimpinella saxifraga* L.: (a), (b), (c), (d): see Fig. 1; (e): subsp. *nigra* (from surroundings of Riva del Garda, Italy).

Peak No.	Compound	Area [%]				
		(a)	(b)	(c)	(d)	(e)
1	α-pinene	trace	trace	trace	–	trace
2	camphene	–	–	0.37	–	–
3	undecane	0.13	trace	trace	1.19	0.72
4	β-pinene	trace	trace	trace	trace	–
5	myrcene	–	trace	trace	trace	–
6	limonene	–	–	–	trace	–
7	*cis*-ocimene	trace	–	–	0.28	trace
8	γ-terpinene	–	–	0.15	trace	–
9	*trans*-ocimene	–	–	–	0.83	–
10	*p*-cymene	trace	–	0.26	trace	–
11	*n*-octanal	0.50	1.20	0.32	trace	0.13
12	geijerene isomer	trace	trace	0.87	0.15	0.82
13	geijerene	0.41	0.74	12.48	3.60	11.96
14	6-methyl-hept-5-en-2-one	–	–	–	–	0.57
15	unknown	–	–	0.24	–	trace
16	nonanone-2	trace	trace	trace	trace	trace
17	nonanal	trace	trace	trace	trace	trace
18	trinoranastreptene (= clavukerin B)	0.48	1.29	2.48	17.71	5.23
19	longicyclene	0.22	2.08	–	trace	–
20	pregeijerene (+ trace thymolmethylether)	1.52	2.90	19.05	6.01	13.95
21	bornyl acetate	0.29	trace	0.31	–	–
22	α-bergamotene	trace	0.30	trace	0.16	0.10
23	β-elemene	0.11	–	–	0.29	trace
24	carvacrolmethylether	0.14	0.68	3.03	trace	trace
25	β-caryophyllene	trace	trace	0.41	2.18	0.76
26	unknown	trace	–	–	0.31	–
27	thymolhydroquinonedimethylether	trace	0.35	–	trace	–
28	trinoranastreptene isomer	–	–	0.18	trace	0.67
29	γ-elemene	0.11	–	–	–	–
30	$C_{15}H_{24}$	0.38	1.10	trace	trace	trace
31	$C_{15}H_{24}$	0.53	2.50	trace	trace	0.52
32	*trans*-β-farnesene	0.57	10.91	0.65	0.12	1.54
33	unknown	–	–	–	0.38	–
34	$C_{15}H_{24}$	–	–	0.45	–	trace
35	humulene (?)	–	–	0.69	0.30	0.13
36	$C_{15}H_{22}$	7.58	–	–	–	–
37	unknown	–	–	–	0.85	–
38	isocaryophyllene (?)	trace	–	trace	8.61	0.19
39	germacrene D + β-himachalene	0.49	0.14	trace	1.53	1.48
40	β-bergamotene	0.17	0.72	2.41	1.56	1.37
41	β-bisabolene	0.40	1.41	17.53	3.17	1.37
42	β-sesquiphellandrene	0.78	4.28	0.24	1.58	1.10
43	$C_{12}H_{14}$	–	–	trace	1.48	0.70
44	3,10-dihydro-1,4-dimethylazulene	0.15	1.12	2.28	15.10	43.56
45	germacrene B	2.73	–	–	–	–
46	MW: 194	–	trace	0.20	1.42	trace
47	*trans*-anethole	–	trace	0.61	–	–
48	unknown	–	–	1.67	trace	trace
49	MW: 194	trace	0.59	–	trace	trace
50	unknown	–	–	trace	0.98	trace
51	β-elemenone	0.26	–	–	–	–
52	unknown	–	–	–	1.20	–
53	germacrone	16.92	–	–	–	–
54	anyl-2-methylbutyrate	0.22	1.03	2.34	0.23	0.11
55	1,4-dimethylazulene	trace	0.40	1.98	16.10	8.25
56	epoxy-anyl 2-methylbutyrate	0.45	0.46	2.90	0.45	trace
57	pseudoisoeugenyl-2-methylbutyrate	0.80	4.03	0.69	0.20	0.12
58	*cis*-epoxy-pseudoisoeugenyl-2-methylbutyrate	0.18	trace	trace	trace	trace
59	*trans*-epoxy-pseudoisoeugenyl-2-methylbutyrate	61.39	56.43	23.97	6.81	1.92

Further trace constituents, detected in the root oil of *Pimpinella saxifraga* subsp. *eusaxifraga:* α-ylangene, germacrene A, germacrene C, β-copaene (also detected in subsp. *alpestris* and *nigra*), δ-elemene (also detected in subsp. *nigra*), fenchyl-alcohol (also detected in subsp. *alpestris*), 2-methyl-5-methoxybenzofuran, epoxy-anyl-tiglate, pseudoisoeugenyl-tiglate, epoxy-pseudoisoeugenyl-tiglate (also detected in subsp. *alpestris*).

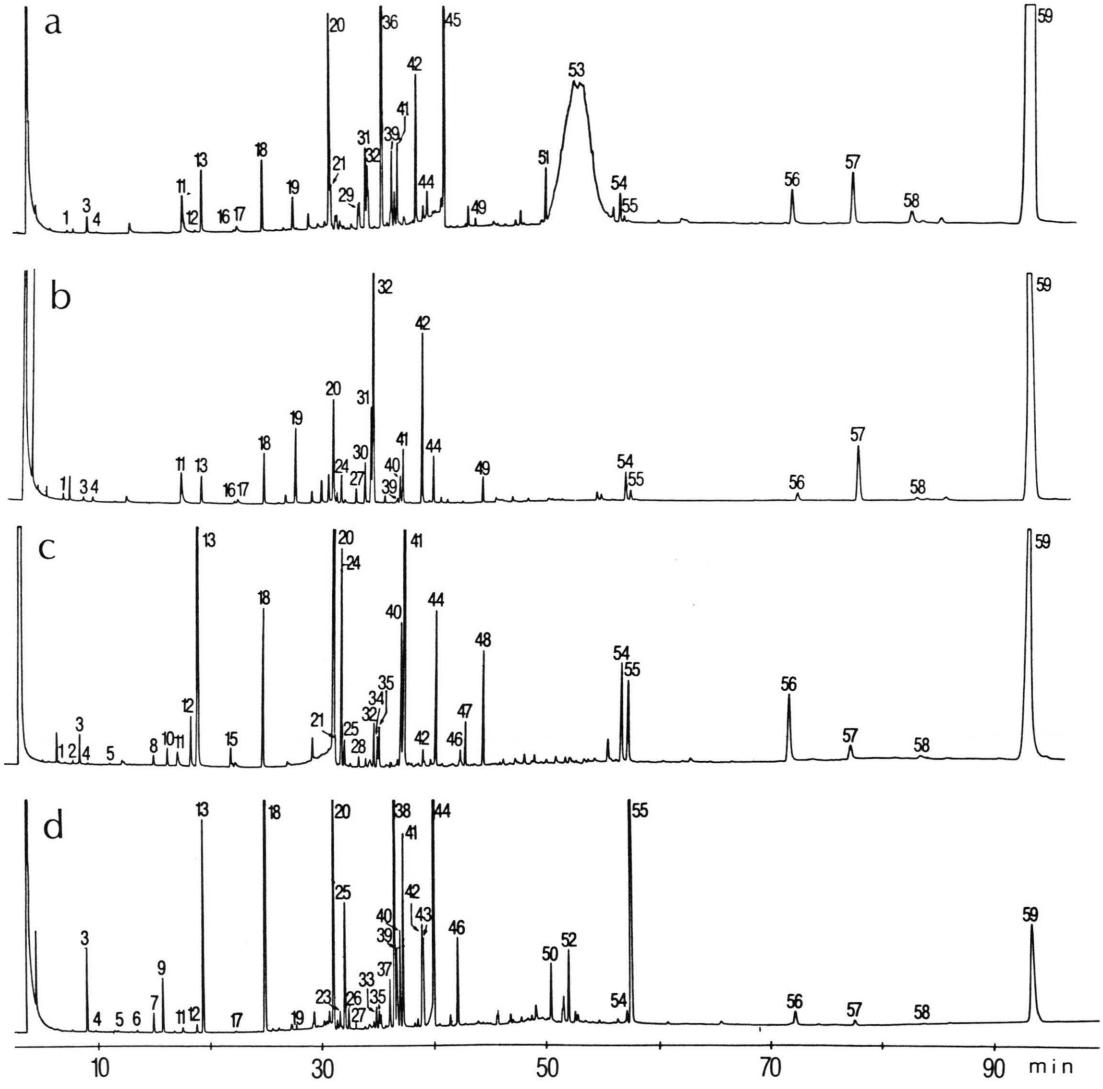


Fig. 1. Capillary gas chromatograms of the essential root oils from different subspecies of *Pimpinella saxifraga* (peak numbers refer to Table I). a) Subsp. *eusaxifraga* (plants with simple segmented leaves); b) subsp. *eusaxifraga* (plants with dissected leaves); c) subsp. *alpestris*; d) subsp. *nigra* (from the Botanical Garden of Würzburg).

observed in case of the root oils of the other Pimpinella subspecies (Table I) monoterpene hydrocarbons can only be detected in traces.

Pimpinella saxifraga subsp. nigra

This subspecies can be delimited from the others chiefly by cutting the fresh root from which a blue oil emerges, indicating the formation of an azulene derivative (1,4-dimethylazulene). In order to examine the chemotaxonomic value of this phytochemical property we studied the composition of the volatile root oil of *Pimpinella saxifraga* subsp. *nigra* in more detail and investigated plant material of different origins (Botanical Garden of Würzburg and surroundings of Riva del Garda). The results of the capillary GC and GC/MS analyses of the root hydrodistillates are summarized in Fig. 1d and Table Id, e.

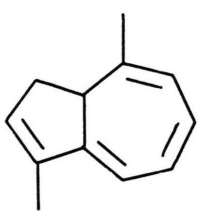
It is noteworthy that in contrast to the previously mentioned oils, the root oils of *Pimpinella saxifraga*

subsp. *nigra* are dominated by different C_{12}-compounds (Fig. 1d, peak No. 13, 18, 20, 43, 44, 55), which represent approximately 60% and 80%, respectively, of these oils (Table I).

Pregeijerene (mol.wt. 162; peak No. 20), 1,5-dimethyltricyclo-(4.4.0.$0^{2,6}$)deca-4,7-diene (mol.wt. 160; peak No. 18) and an unknown constituent (mol.wt. 158; peak No. 44) are supposed to be intermediates, arising by stepwise dehydrogenation and cyclization during the formation of 1,4-dimethylazulene (mol.wt. 156; peak No. 55). The latter component, which was described in *Pimpinella saxifraga* subsp. *nigra* for the first time by Meuche and Huneck [8], is responsible for the characteristic blue colour of the root oil of this plant. It is remarkable that this norsesquiterpene is not a distillation artefact in the root oil of this plant.

This is worth mentioning, because in the volatile oils of *Thapsia garganica* L. and *Ruta graveolens* L. 1,4-dimethylazulene is a decomposition product formed during hydrodistillation [9, 10].

The formula of the unknown compound No. 44, amounting to 43.5% in the root oil of the plants from Italy, was established from ^{1}H NMR, ^{13}C NMR and mass spectral data to be $C_{12}H_{14}$ (mol.wt. 158). Hence, it follows that the molecule must be bicyclic and bears four double bonds. The definite structure (**1**) was finally elucidated by selective proton decoupling ^{1}H NMR experiments and determined as 3,10-dihydro-1,4-dimethylazulene.



The ^{1}H NMR and MS data obtained were in good accordance with those given by Takeda and Katoh [11], who isolated this compound from the liverworth *Calypogeia granulata*. They also found the two above mentioned norsesquiterpene hydrocarbons 1,5-dimethyltricyclo-(4.4.0.$0^{2,6}$)deca-4,7-diene (peak No. 18) called trinoranastreptene and 1,4-dimethylazulene (peak No. 55) [11, 12]; the latter has already previously been described in *Calypogeia trichomanis* [8].

Trinoranastreptene proved to be identical with clavukerin B isolated from the soft coral *Clavularia koellikeri* [13].

This compound and 3,10-dihydro-1,4-dimethylazulene, isolated from roots of *Pimpinella saxifraga* subsp. *nigra* and also detected in the other *Pimpinella* root oils (*cf.* Table I and [6]) have been discovered in a higher plant for the first time, as far as we know. The essential root oils of *Pimpinella saxifraga* subsp. *nigra* plants from two different localities had a closely similar qualitative composition. However, some differences could be observed in the relative amounts of 1,4-dimethylazulene and its supposed precursors (see Table I).

Conclusion

While it is difficult to clearly subdivide the complex aggregate "*Pimpinella saxifraga*" into distinct taxonomic groups by morphological features, this can be done successfully by comparing the chemical composition of the individual essential root oils.

The oil of *Pimpinella saxifraga* subsp. *eusaxifraga* is characterized by a relatively high content of *trans*-epoxy-pseudoisoeugenyl-2-methylbutyrate. Further differentiation within this subspecies was established, which could be correlated to plants with different degrees of leaf dissection. The most distinguishing feature of plants with simple segmented leaves is the high amount of germacrone, being absent in plants with dissected leaves.

The volatile root oil of *Pimpinella saxifraga* subsp. *alpestris* contains about 32% of the two isomers geijerene and pregeijerene and about 5% of further C_{12}-hydrocarbons, which are supposed to be precursors of 1,4-dimethylazulene. Additionally, the occurrence of considerable amounts of β-bisabolene (about 17%) and *trans*-epoxy-pseudoisoeugenyl-2-methylbutyrate (about 24%) is remarkable.

In contrast, the essential root oil of *Pimpinella saxifraga* subsp. *nigra*, which has a significant lower content of *trans*-epoxy-pseudoisoeugenyl-2-methylbutyrate, is characterized by a dark-blue colour due to appreciable quantities of 1,4-dimethylazulene (from 8 to 16%); its presumed C_{12}-precursors amount to a value of not less than 50% of the oil, including geijerene and pregeijerene. These C_{12}-compounds which dominate the essential root oil belong to a dehydrogenation sequence running from pregeijerene to 1,4-dimethylazulene; the structure of

the so far unknown intermediate $C_{12}H_{14}$ of this pathway was elucidated as 3,10-dihydro-1,4-dimethylazulene.

Though the qualitative composition of essential root oils from the individual subspecies of *Pimpinella saxifraga* aggregate is very similar, a reliable separation of this complex taxon can be performed by the characteristic quantitative patterns of essential oil components.

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