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Volatiles from rhizomes of *Rhodiola rosea* L.

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Abstract

Terpenes and aroma volatiles from rhizomes of *Rhodiola rosea* L. from Norway have been isolated by both steam distillation and headspace solid-phase micro-extraction coupled with gas chromatography and mass spectrometry analysis. The dried rhizomes contained 0.05% essential oil with the main chemical classes: monoterpene hydrocarbons (25.40%), monoterpene alcohols (23.61%) and straight chain aliphatic alcohols (37.54%). *n*-Decanol (30.38%), geraniol (12.49%) and 1,4-*p*-menthadien-7-ol (5.10%) were the most abundant volatiles detected in the essential oil, and a total of 86 compounds were identified in both the SD and HS-SPME samples. Geraniol was identified as the most important rose-like odour compound besides geranyl formate, geranyl acetate, benzyl alcohol and phenylethyl alcohol. Floral notes such as linalool and its oxides, nonanal, decanal, nerol and cinnamyl alcohol highlight the flowery scent of rose root rhizomes.

Keywords: *Rhodiola rosea*; Crassulaceae; GC–MS; Headspace SPME; Terpenes; Aroma volatiles

1. Introduction

Rhodiola rosea L. or rose root, also commonly known as golden root and arctic root, is a perennial herbaceous plant of the family Crassulaceae. In all, 14 homonyms and infraspecific taxa have been described for *Rhodiola rosea*. The yellow-flowered taxon of rose root is spread widely and can be found in the mountain regions of Central and Northern Europe as well as in Russia and in the east coastal regions of North America at altitudes between 1000 and 5000 m above sea level. Rose root has an approximate plant height of 75 cm, with the characteristic flower scent of roses which impart its name.

Rose root is a multipurpose medicinal plant with adaptogenic properties by increasing the body's non-specific resistance and normalizing functions, and it has traditionally been grown and used in Russia and Mongolia for the treatment of long-term illness and weakness due to infection. Due to changing consumer demands towards natural health products and the growing interest for unknown plant secondary metabolites and their applications in biotechnology and therapy in the last decades, a great deal of focus has been put on rose root and its medical properties with regard to memory and learning (Petkov et al., 1986), immune response (Lishmanov et al., 1987; Furmanowa et al., 1999b), organ function, CNS and stress (Sokolov et al., 1985; Maslova et al., 1994; Afanas'ev et al., 1996; Lishmanov et al., 1999) and cancer therapy (Bocharova et al., 1994, 1995; Udintsev and Shakhov, 1991; Razina et al., 2000).

The chemical composition of rhizomes of *Rhodiola rosea* has been exhaustively studied by East European research groups (Khnykina and Zotova, 1966; Saratikov et al., 1967; Dubichev et al., 1991; Revina et al., 1976; Komar et al., 1980; Kurkin et al., 1986; Furmanowa et al., 1999b). Active metabolites within the chemical groups of phenols such as salidroside and its aglycon tyrosol (Peshekova et al., 1973; Kurkin et al., 1989; Kur'yanov et al., 1991; Satsyperova et al., 1993; Antipenko and Kuznetsov, 1998; Linh et al., 2000) and cinnamic glycosides such as rosin, rosavin, and rosarin (Zapesochnaya and Kurkin, 1982; Kurkin et al., 1985b, 1986; Satsyperova et al., 1993; Furmanowa et al., 1999a) have been identified. Other important constituents from rose root are flavonoids (Kurkin et al., 1982; Zapesochnaya and Kurkin, 1983; Zapesochnaya et al., 1985), tannins (Revina et al., 1976; Nekratova et al., 1992), gallic acid and its esters (Dubichev et al., 1991; Satsyperova et al., 1993) and essential oil (Kurkin et al., 1985a; Shirokov et al., 1980; Belov et al., 1994).

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The aim of the present study was to characterize the composition of terpenes and aroma volatiles from rhizomes of *Rhodiola rosea* as a potential source for large-scale production of essential oils and flavour essences. The investigation was partly perfomed within the framework of the project "Norwegian Herb Production (NUP) " in the period 1995–1998, which focused on the cultivation, improvement and marketing of medicinal plants and herbs in Norway. Samples from both steam distillation (SD) and headspace solid-phase microextraction (HS-SPME) have been analysed by gas chromatography/mass spectrometry (GC–MS). HS-SPME, introduced by Zhang and Pawliszyn (1993), has been widely applied in environmental research for the detection of air pollutants, water impurities and pesticides (Chai and Pawliszyn, 1995; Santos et al., 1996; Magdic et al., 1996). A considerable number of applications in the field of food chemistry and quality control was established which focussed on aroma volatiles (orange juice: Steffen and Pawliszyn, 1996; wine: Garcia et al., 1998; coffee: Roberts et al., 2000) and terpenes of essential oils from different sources (Schafer et al., 1995; Czerwinski et al., 1996; Fuchs et al., 1999; Paniandy et al., 2000). The HS-SPME technique has to a great extent been successfully introduced in the author's laboratory for biochemical and plant physiological-related applications (Rohloff, 1999; Rohloff et al., 2000a,b, 2002).

In the present study, HS-SPME has been applied to gain detailed information about the terpenic and aroma volatile composition of *Rhodiola rosea* rhizomes from Norway, and to identify and quantify the specific compounds responsible for the characteristic rose scent. To the best of the author's knowledge, a detailed investigation of the aroma volatiles from rose root has not yet been undertaken.

2. Results and discussion

The steam distillation of 100 g of raw plant material yielded a clear and colourless essential oil (0.05% of dry wt.) in contrast to results from Shirokov and co-workers (1980) who found an average content of 1%. With regard to other well-known terpene-containing root drugs from different plant families such as Compositae, Umbelliferae, Zingiberaceae and Araceae with oil contents from 0.5 up to 10% (Wichtl, 1997), the yield from rose root was rather low. However, the characteristic site of terpene synthesis and accumulation in higher plants is often restricted to aerial plant organs rather than to roots and rhizomes (Hay and Svoboda, 1993). An analysis task on cultivated rose root plants from Norway carried out at the Plant Biocentre in 2001 showed that the average oil content was below 0.05% (Dragland, 2001), and thus, underscores the presented data.

In total, 75 compounds were identified in the essential oil from the SD samples on the basis of a mass spectrum database search and retention indices (see Table 1), while 59 compounds were detected by peak area measurement (94.74% identified). In contrast, only 69 peaks were identified by the use of HS-SPME, and a total of 36 compounds was detected. The main chemical classes of volatile compounds were monoterpene hydrocarbons, monoterpene alcohols and straight chain aliphatic alcohols (see Table 2) which accounted for more than 86% of the essential oil. The most abundant compound was decanol (30.38%), which also was the main peak detected by HS-SPME. Other important constituents were geraniol (12.49%), 1,4-*p*-menthadien-7-ol (5.10%), limonene (4.90%), α-pinene (4.69%) and dodecanol (3.67%). In an investigation carried out at the author's laboratory on cultivated plant material from Norway (Dragland, 2001), monoterpenes (geraniol, myrtenol, pinocarveol) as well as alipathic (heptanol) and aromatic alcohols (cinnamyl alcohol and cuminyl alcohol) were found to be the main compounds in *Rhodiola* essential oil and showed similarities to the present study. In contrast to results reported by a Russian research group (Belov et al., 1994), the proposed main constituents such as octadecadienoic acid, heptanol derivates and hexadecanoic acid were only detected in insignificant amounts in the Norwegian samples.

Regarding the different analytical methods for the isolation of the volatile compounds, SD followed by GC–MS revealed a higher number of identified and detected peaks compared to HS-SPME coupled with GC–MS. With regard to the chosen extraction methods (conventional SD and HS-SPME), neither the monoterpene alcohol glycoside rosiridin nor its aglycon rosiridol found in rose root by Kurkin and co-workers (1985a), could be isolated. As already pointed out by Field et al. (1996), Coleman and Lawrence (1997), and Rohloff (1999), the applicability of SPME for terpene extraction is intimately linked to the parameters of sampling time and temperature conditions. In addition, it is influenced by the chosen fibre type and its diameter (Schafer et al., 1995). Depending on the applied method, the affinity of the non-polar and semi-polar terpenes and volatiles to the fibre type differs greatly, i.e. analysis of the headspace gas by SPME will under no circumstances render results from essential oil analysis of solvent-based samples (Rohloff, 1999). Although the number of detected compounds in distillates might be higher than those reported for sesqiterpenes for example, the soft and non-invasive SPME represents an analytical tool for the identification of additional volatiles not being isolated by SD (Rohloff, 1999; Paniandy et al., 2000; Beaulieu and Grimm, 2001). On the background of the number of identified compounds from essential oil and HS-SPME samples (75 versus 69, respectively), the presented results underscore the excel-

Table 1
Terpenes and headspace volatiles from rhizomes of *Rhodiola rosea* ordered by retention time from SD samples

No.	Compound	RA[a] (SD)	RT[b]	RA (HS-SPME)	RT
1	Santene	0.84	1.92	–	–
2	Tricyclene	0.36	2.07	–	–
3	α-Pinene	4.69	2.15	t	3.65
4	Camphene	0.91	2.43	t	4.85
5	Hexanal	0.05	2.57	1,54	5.53
6	β-Pinene	1.47	2.83	t	7.20
7	Sabinene	1.45	2.97	t	7.23
8	3-Carene	2.04	3.30	t	7.77
9	β-Myrcene	2.25	3.48	t	8.78
10	α-Terpinene	0.81	3.73	t	8.98
11	2-Heptanone	–	–	t	9.30
12	Heptanal	t[c]	3.82	t	9.47
13	Limonene	4.91	4.03	0,38	9.77
14	β-Phellandrene	2.31	4.18	t	9.95
15	2-Pentyl furan	0.08	4.65	0,54	11.65
16	*cis*-Ocimene	0.21	4.72	t	11.93
17	γ-Terpinene	1.83	4.92	–	–
18	*trans*-Ocimene	0.24	5.08	–	–
19	Styrene	0.06	5.18	1,73	12.43
20	*n*-Pentanol	t	5.35	t	12.70
21	*p*-Cymene	2.97	5.47	0,85	12.95
22	Terpinolene	0.42	5.77	t	13.53
23	*n*-Octanal	–	–	t	14.05
24	6-Methyl-5-hepten-2-one	0.03	7.30	0,54	16.27
25	*n*-Hexanol	0.03	7.90	t	17.33
26	*n*-Nonanal	t	9.17	0,77	18.70
27	Ocimenone	–	–	1,24	19.32
28	*trans*-2-Octenal	–	–	t	20.02
29	β-Thujone	t	10.62	–	–
30	β,*p*-Dimethyl styrene	t	10.72	0,52	20.40
31	*cis*-Linalool oxide	0.15	10.93	0,77	20.70
32	Menthone	0.44	11.45	1,28	21.03
33	*trans*-Linalool oxide	0.05	12.12	0,46	21.95
34	Isomenthone	0.12	12.57	t	22.18
35	*n*-Decanal	0.13	13.43	1,17	23.23
36	Benzaldehyde	0.12	14.18	2,07	23.82
37	Isopinocamphone	0.10	14.77	t	24.22
38	*trans*-2-Nonenal	0.03	14.90	t	24.43
39	Pinocarvone	0.05	15.87	t	25.08
40	Linalool	2.31	16.18	1,37	25.42
41	*n*-Octanol	2.77	16.60	3,66	25.67
42	Bornyl acetate	t	16.80	–	–
43	β-Caryophyllene	t	17.28	t	26.07
44	Neomenthol	–	–	0,29	26.58
45	Myrtenal	–	–	0,91	26.90
46	Menthol	0.08	20.28	t	27.68
47	*trans*-Pinocarveol	0.54	20.65	1,61	27.90
48	Neral	t	20.92	0,27	28.50
49	Estragol	0.10	21.43	–	–
50	*n*-Nonanol	0.72	22.52	2,09	28.68
51	α-terpineol	0.40	23.15	0,25	29.00
52	Geranyl formate	–	–	2,90	29.25
53	Carvone	0.15	24.03	t	29.50
54	Geranial	0.07	24.63	0,62	29.65
55	Geranyl acetate	0.11	26.38	t	30.25
56	Cuminyl aldehyde	0.35	26.50	–	–
57	Perilla aldehyde	0.09	26.70	t	30.62
58	*n*-Decanol	30.38	27.12	10,53	30.47
59	Myrtenol	1.73	27.88	1,45	30.92

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	RA[a] (SD)	RT[b]	RA (HS-SPME)	RT
60	Nerol	0.07	28.77	–	–
61	*trans*-Carveol	t	29.85	–	–
62	*cis*-Carveol	0.10	30.32	–	–
63	Geraniol	12.49	31.37	t	31.87
64	Hexanoic acid	–	–	0,55	32.17
65	Benzyl alcohol	–	–	4,88	32.50
66	Octadecyl acetate	0.06	33.07	0,17	32.62
67	Phenylethyl alcohol	t	33.88	2,29	33.05
68	Cuminyl acetate	0.11	36.43	0,31	33.87
69	Dodecanol	3.67	37.42	0,66	33.98
70	Perilla alcohol	0.45	38.47	–	–
71	Cinnamaldehyde	0.15	39.33	3,67	34.98
72	Benzene propanol	0.09	40.47	0,47	35.17
73	1,4-*p*-Menthadien-7-ol	5.10	40.98	–	–
74	Octanoic acid	t	42.32	t	35.53
75	Cumin alcohol	2.66	43.17	1,44	35.98
76	Cinnamyl acetate	0.12	45.10	t	36.65
77	Thymol	t	47.90	–	–
78	Carvacrol	t	48.95	–	–
79	Cinnamyl alcohol	0.28	51.35	3,70	38.53
80	Decanoic acid	0.32	52.00	–	–
81	Dodecanoic acid	0.12	60.95	t	41.22
82	Acetovanillone	–	–	t	42.33
83	Benzyl benzoate	t	64.55	–	–
84	Tetradecanoic acid	t	69.25	t	44.85
85	Pentadecanoic acid	–	–	t	47.42
86	Hexadecanoic acid	t	77.13	t	50.80

[a] Relative area in % (peak area relative to total peak area).
[b] Retention time in minutes.
[c] Trace compound.

Table 2
Composition of the essential oil from SD samples of *Rhodiola rosea* rhizomes ordered by chemical groups (summarized peak area in %)

Compound group	
Monoterpene hydrocarbons	25.40
Oxygenated monoterpenes	
Alcohols	23.61
Aldehydes	0.29
Ketones	0.86
Straight chain alipathic compounds	
Alcohols	37.54
Aldehydes	0.08
Ketones	0.03
Acids + esters	0.50
Aromatic compounds	3.94
Phenols	0.10
O-Heterocyclic compounds	2.39
Total	94.74

lent suitability of solid-phase micro-extraction for qualitative analyses.

With regard to the characteristic rose fragrance of the rhizomes of *Rhodiola rosea*, several compounds with rose odour and other floral notes have been identified (see Table 1). Geraniol was identified as the main rose-like odour compound, which is one of the most abundant monoterpene alcohols in the essential oil from *Rosa* sp. (Lawless, 1996). Minor important constituents such as geranyl formate and geranyl acetate, benzyl alcohol and phenylethyl alcohol were also detected. Hegnauer (1964, 1989) relates also rose scent to the aglycon tyrosol, but from the presented data it can not be concluded in which way this phenolic compound contributes to the appearance of rose root fragrance in relation to geraniol.

Floral notes such as linalool and its oxides, nonanal, decanal, nerol and cinnamyl alcohol emphasize the flowery scent of rose root rhizomes, while alipathic alcohols such as decanol and dodecanol may be responsible for the fat- or wax-like background scent. Regarding the low oil yield and the diverging composition of terpenes and volatiles in rose root rhizomes, the applicability of essential oil or extracts from rose root in cosmetic and perfume industries will be rather restricted to the exclusive production for the national market. On the other hand, the present study reports only results from a local variety originating from Mid-Norway, and in fact, further investigation of this wide-spread mountain plant

might supplement and broaden the knowledge of the biochemistry of *Rhodiola rosea*.

3. Experimental

3.1. Plant material

R. rosea roots (5 kg fr. wt) were collected from wild growing plants at Alvdal, Norway (10°37/62°07) in September 1998. The roots were cut into small segments (1–2 cm), dried at 35 °C in a drying cabinet with fan (Termaks TS 5410) for 48 h and stored at room temperature prior to analysis in December 1998. Five samples each were used for extraction by steam distillation and SPME, respectively.

3.2. Steam distillation (SD)

The distillation apparatus consisted of a heating cap, a 3 l extraction flask, a 3 ml graduated receiver (Dean and Stark) and a condenser (jacketed coil). One-hundred grams of dried plant material and 1.5 l H_2O were used and the distillation was carried out for 3 h after reaching the boiling point. The collected oil was directly used for sample preparation by diluting 10 μl oil in 1 ml ethanol in brown autosampler flasks.

3.3. Headspace solid-phase micro-extraction (HS-SPME)

A PDMS coated fiber (100 μm) and a manual SPME holder (SUPELCO Inc.) were used for sample extraction. In a blank run, the fibre was exposed to the GC inlet for 3 min for thermal desorption at 250 °C before headspace sampling. One gram of each sample was sealed in a 10 ml screw top vial with phenolic cap and PTFE/ silicone septa (SUPELCO Inc.) and stored in a drying cabinet at 50 °C for 15 min. The SPME fibre was exposed to each sample for 1 min by manually penetrating the septum (0.25 cm depth).

3.4. Gas chromatography–mass spectrometry (GC–MS)

Analyses were carried out by using a Varian Star 3400CX gas chromatograph coupled with a Saturn 3 mass spectrometer. The capillary column was a Chrompack CP-Wax 52CB (30 m × 0.32 mm ID, 0.25 μm film thickness), and the carrier gas was He (5 psi). *Oil samples*—the initial oven temperature was 60 °C, rising at 2 °C min^{-1} to 210 °C and then held isothermal for 5 min. The injector, transfer line and detector temperatures were 220 °C, 210 and 175 °C, respectively. Samples were injected by splitless injection. *SPME samples*—the SPME fibre was inserted into the injection port of the GC for 2 min for sample desorption. The oven temperature was held isothermal at 35 °C for 5 min, programmed from 35 to 90 °C at 3 °C min^{-1}, subsequently at 7 °C min^{-1} up to 210 °C and then held isothermal for 15 min. The injector, transfer line and detector temperatures were 240, 210 and 175 °C, respectively. Samples were injected by using the split-sampling technique with a ratio of 1:27.5. All mass spectra were acquired in EI mode (scan range $m/z = 40$–300, 5 μscans s^{-1}, multiplier voltage 1700 V, ionization energy 70 eV). Chromatogram peaks were identified by mass spectra database search (VARIAN NIST MS Database 1992 and IMS Terpene Library 1992) and on the basis of relative retention indices (ESO 00, Database of Essential Oils, BACIS 1999). Quantitative analysis (in%) was performed by peak area measurement (TIC).

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