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## Phytochemical and analytical studies of extracts from *Rhodiola rosea* and *Rhodiola quadrifida*

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Column chromatography of hydrophilic extracts from *Rhodiola rosea* and *Rodiola quadrifida* led to the isolation of cinnamic alcohol, chlorogenic acid, rhodiocetoside, rosiridin, rosavin and the phenolic compounds salidroside, rhodiolin and a novel compound consisting of viridoside with an attached arabinose unit (mongroside). HPLC analysis of plant material from different sources and from different collection periods showed a great variability in the composition and in the amount of pharmacologically active compounds contained.

### 1. Introduction

*Rhodiola rosea* L. (Crassulaceae), also known as “golden root”, grows primarily in dry areas at high altitudes in the arctic regions of Asia and Europe. For centuries *Rh. rosea* has been used in the traditional medicine of Russia, Scandinavia and some Asian countries. Since 1960 (when “golden root” was identified as *Rh. rosea* by a botanic expedition in the Altai mountains) more than 180 phytochemical, pharmacological and clinical studies have been published. In 1969 the plant has been included in the official Russian materia medica and since 1975 a registered preparation under the name Rhodiola Extract Liquid has been existing. In Sweden it was recognized as a herbal medicinal product in 1985 (Sandberg and Bohlin 1993). Medical and pharmacological texts describe the use of *Rh. rosea* as a stimulant against fatigue, for the treatment of somatic and infectious diseases, for psychiatric and neurological problems, and in healthy individuals to relieve fatigue and to increase attention span, memory and work productivity. Many studies identified the plant as an adaptogen (a non-specific increase of the resistance of an organism without disturbing normal biological parameters). Trials in cell cultures, animals and humans have revealed antifatigue, anti-stress, antihypoxic, anticancer, antioxidant, immune enhancing and sexually stimulating properties (Saratikov 1974; Saratikov and Krasnov 1987; Furmanowa et al. 1995; Darbinyan et al. 2000; Spasov et al. 2000a, b). Phytochemical investigations of *Rh. rosea* have revealed six distinct groups of chemical compounds: phenylpropanoids, phenylethanol derivatives, flavonoids, monoterpenes, triterpenes, phenolic acids. Initially, the phenylethanol derivative salidroside was believed to be responsible for the pharmacological effect. Later it was found that salidroside is present in all investigated *Rh.* species and that it is not the only active ingredient. Nowadays, the most important compounds (from a pharmacological standpoint) are sali-

droside and the phenylpropanoids rosin, rosavin, rosarin and rosiridin. Comparative studies with those components showed CNS activity (Sokolev et al. 1985), adaptogenic activity (Barnaulov et al. 1986) as well as immunostimulating properties (Sokolev et al. 1990). Nevertheless, *Rh.* extracts are superior to the single components which indicates that the glycosides mentioned are not the only compounds responsible for the medicinal effect but can be used as diagnostic markers. Consequently the main marker compound (also used for the standardization of *Rh.* root extracts) is rosavin, which was demonstrated to be specific for this species (Dubichev et al. 1991; Ganzera et al. 2001). Besides this, rosiridin is contained in *Rh. rosea* in an amount of about 3% and should also be used as a diagnostic sign (Kurkin et al. 1985).

In the traditional medicine of Mongolia and Tibet *Rh. quadrifida* (Pall.) Fisch. et May. is used, too. Under the names “Ere-gombo” (Mongolia) and “Tsan” (Tibet) the plant is applied for the treatment of fatigue, blood-pressure, dysentery, genital diseases of women and as a stimulator of the nervous system (Saratikov et al. 1967; Yoshikawa et al. 1995; Yoshikawa et al. 1996). *Rh. quadrifida* is a perennial grassy plant occurring predominantly in some highland regions of the former USSR (Altai, Sayan), in East Siberia, in some mountainous regions of China (Sichuan) and in high mountain regions of Mongolia (Hentii, Hangai, Hovsgol, Hovd and Mongol Altai).

The phytochemical composition of the ingredients (without cinnamic alcohol and rosiridin) is similar to that of *Rh. rosea*.

In this paper we report the results of our phytochemical investigations of hydrophilic extracts from roots of *Rh. rosea* and *Rh. quadrifida*. We included in our study samples from different sources as well as from different collecting times. It could be shown that not only the amount but also the content of pharmacologically active ingredients can vary in a large scale.

## 2. Investigations, results and discussion

The extraction procedure (3.3.) of the plant materials (3.2.) led to different amounts of resulting BE-extracts as listed in Table 1.

The data show that the highest DEV resulted from *Rh. quadrifida*, 2001, *Rh. rosea* 2001 from Poland and from Sweden (4–8:1). On the other hand, we found much smaller amounts of extracts in the other drug samples which indicates that great variations in the quality of the drugs occur.

The extracts were analysed by HPLC. The quantification of the measured compounds is given in Table 2.

These results show that – besides the amount of extracts – also the compound composition shows a broad range so that clear guidelines for the standardization are urgently needed.

When referring to the proposals for using salidroside, rosavin, rosiridin (Ganzera et al. 2001) and cinnamic alcohol as the marker compounds for the identification of *Rh. rosea* the plant material collected in 2002 in Mongolia did not undergo this qualification standard.

According to a proposal by Russian scientists and their Pharmacopeia (Bykov et al. 1999), rosavin (including rosin and rosarin) as well as salidroside should be used for the standardization of water/alcohol tinctures which are prepared from roots. Taking into consideration that those components are not the only ones responsible for the pharmacological activity as mentioned before, it can be accepted that they are used as an indicator for the desired efficacy described in clinical studies. Therefore *Rh. rosea* extracts used for medicinal purposes were standardized to a minimum of 3% rosavin and 0.8–1% salidroside (Brown et al. 2002).

The results of our analytical studies show that it is possible to receive plant material of good quality by wild collecting and it can be assumed that this material will produce health benefits (*Rh. rosea* from Mongolia as well as from Poland collected in 2001). But on the other hand, surprising results were found: although the drug from a Swedish health market showed the highest amount of ro-

savin and rosiridin, the important marker compound salidroside was missing. According to the described guidelines for characterizing of the efficacy, this drug should not be used medicinally. Another remarkable result was found in the *Ph. rosea* sample collected in Mongolia in 2002: it showed only a low content of salidroside and furthermore, the marker compounds rosavin and cinnamic alcohol were not contained. Similarly to the Swedish drug, it should not be used medicinally. As an explanation for the last findings we assume that the bad climatic conditions may be responsible: a hard and snowy winter season was followed by a dry and short summerperiod in 2002. The plants had a very short vegetation period and did not develop their normal habitats which could be seen in a small shape. From this it can be deduced that the biochemical synthesis of the interesting secondary metabolites was decreased, too.

The data concerning *Rh. quadrifida* (Table 2) show that its medicinal use similar to *Rh. rosea* (in Tibet and Mongolia) is justified because the pharmacological active markers are contained, too.

## 3. Experimental

### 3.1. General procedure

NMR spectra (Bruker AC-400) were measured in DMSO- $D_6$ . Chemical shifts ( $\delta$  = ppm) were referenced to DMSO (2.50 and 39.43 ppm, respectively). Coupling constants in Hz. Flash liquid chromatography (FLC): 150 × 2 cm column, packed with Polygoprep  $C_{18}$ , 60–30 (Macherey-Nagel, Germany). HPLC: Dionex system (pump 480, Gina 50 autosampler, DAD 320 s) with a CC 250/4 Nucleodur  $C_{18}$  Pyramid, 5  $\mu$ , column (Macherey-Nagel, Germany); data acquisition: Chromeleon V. 6.40, build 800.

### 3.2. Plant material

Underground parts of *Rh. rosea* were collected in the Mongol Altain mountain at the territory of Hovd aimag Dariv sum (altitude 3200–3400 m) in August 2001 (40 g) and 2002 (47 g). The plant material was identified by Ch. Sanchir and M. Urgamal, voucher specimen were deposited at the herbarium of the Institute of Botany of Mongolian Academy of Sciences (MAS) in Ulaanbaatar. Underground parts of *Rh. rosea* from Poland were collected in the Garden of Medicinal Plants of the Research Institute of Medicinal Plants in Poznan, Poland, in 2001 (41 g). Underground parts of *Rh. rosea* from Sweden were received from an official supermarket store for medicinal plants (45 g). Underground parts of *Rh. quadrifida* were collected in the Jargalant mountain in the western part of Mongolia, at the territory Hovd aimag Dariv sum (altitude 3400 m) in July–August 2001 (23 g) and 2002 (790 g). The plant material was identified by Ch. Sanchir and M. Urgamal, voucher specimen were deposited at the herbarium of the Institute of Botany of Mongolian Academy of Sciences (MAS) in Ulaanbaatar. The roots were air-dried and powdered.

### 3.3. Extraction and isolation

As the traditional use of the plant takes place in form of alcohol-water extracts we focussed our study on hydrophilic extraction: the air-dried underground parts of *Rh. rosea* and *Rh. quadrifida* were extracted with n-hexane for 24 h in a Soxhlet apparatus, followed by methanolic extraction for 48 h. The alcoholic solution was evaporated under reduced pressure to dryness. The residue was partitioned in  $CCl_4$ : $CH_3OH$ : $H_2O$ , (5:4:1). The  $MeOH/H_2O$  extract was again evaporated to dryness. The residue was dissolved in  $BuOH-H_2O$  leading to a butanolic (BE) as well as a water extract, respectively. The BE was used for the FLC. Elution was done by  $H_2O/MeOH/AcCN$  80:10:10, 4 ml/min. The resulting fractions (10 ml) were monitored by HPLC (1.3 ml/min; 0.04 M  $H_3PO_4/CH_3CN/MeOH$ : 0–7 min: 75/12/13, 7–20 min: 60/20/20, 20–22 min: 60/20/20). Prep. HPLC (SP 250/10 Nucleosil 120–7  $C_{18}$ , Macherey & Nagel) was used for final purification yielding the compounds.

### 3.4. Characterization of the compounds

#### 3.4.1. Cinnamic alcohol (1)

NMR:  $^1H$ : 7.36, H-2/6; 7.29, H-3/5; 7.23, H-4; 6.69, H-7, J = 15.2 (E-config.); 6.34, H-8, J = 15.2 (E-config.); 4.35, H<sub>2</sub>-9;  $^{13}C$ : 136.4, C-1; 130.0, C-7; 128.3, C-4; 128.1, C-3/5; 127.1, C-8; 126.0, C-2/6; 62.7, C-9. The data are in accordance with those reported in literature (Zapesochayna & Kurkin, 1982).

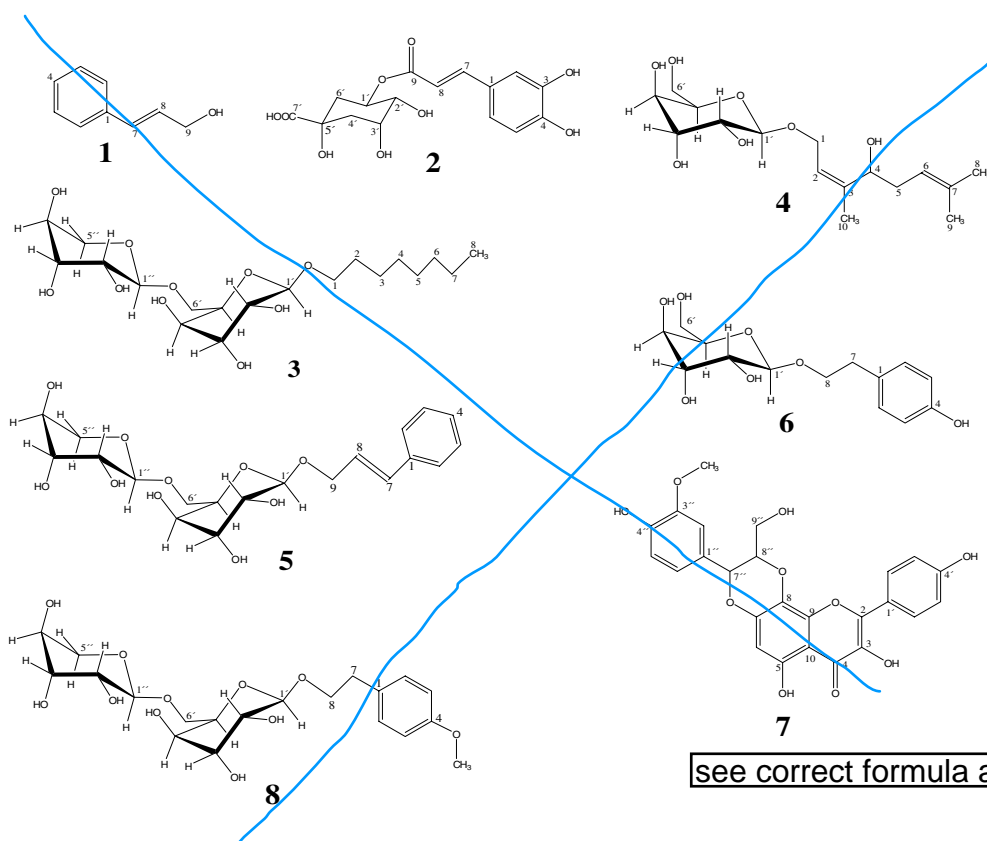
**Table 1: Extraction results**

	Plant material (g)	BE (g)	DEV <sub>native</sub>
<i>Rh. rosea</i> , Mongolia, 2001	40.0	3.0	13.3 : 1
<i>Rh. rosea</i> , Mongolia, 2002	47.0	3.74	12.6 : 1
<i>Rh. quadrifida</i> , Mongolia, 2001	23.0	5.35	4.3 : 1
<i>Rh. quadrifida</i> , Mongolia, 2002	790.0	50.0	15.8 : 1
<i>Rh. rosea</i> , Poland, 2001	41.0	6.34	6.5 : 1
<i>Rh. rosea</i> , Sweden, 2002	45.0	5.35	8.4 : 1

DEV<sub>native</sub>: according to Gaedtke and Steinhoff 2000

**Table 2: Quantification of compounds 1 to 8 ( $\mu$ g/mg BE)**

Plant source	6	2	5	4	3	7	1	8
<i>Rh. rosea</i> , Mongolia, 2001	13.1		18.7	6.0		3.9	18.9	
<i>Rh. rosea</i> , Mongolia, 2002	4.6					1.4		
<i>Rh. quadrifida</i> , Mongolia, 2001	2.5		1.9		1.2	2.2		1.0
<i>Rh. quadrifida</i> , Mongolia, 2002	4.5	0.9	0.9			2.3		2.9
<i>Rh. rosea</i> , Poland, 2001	4.0	4.6	27.9			3.9	10.5	
<i>Rh. rosea</i> , Sweden, 2002			50.7	24.2			15.6	



### 3.4.2. Chlorogenic acid (2)

NMR: caffeic acid part:  $^1\text{H}$ : 7.04, H-2; 6.76, H-5; 6.94, H-6; 7.55, H-7; 6.25, H-8;  $^{13}\text{C}$ : 168.7, C-9; 149.6, C-4; 147.1, C-8; 146.8, C-3; 127.8, C-1; 123.0, C-7; 116.5, C-5; 115.2, C-6; 115.2, C-2) and those for the quinic acid part ( $^1\text{H}$ : 5.32, H-3'; 4.16, H-1'; 3.72, H-2'; 2.22/2.09, H<sub>2</sub>-4'; 2.17/2.03, H<sub>2</sub>-6';  $^{13}\text{C}$ : 177.1, C-7'; 76.2, C-5'; 73.5, C-2'; 72.0, C-3'; 71.3, C-1'; 38.7, C-6'; 38.2, C-4'). Data were corresponding to those reported by Haslam and Turner (1971); Kelley et al. (1976).

### 3.4.3. Rhodiocyanoside (3)

NMR octyl part: six methylene signals at 1.6 to 1.2 ( $^1\text{H}$ ) and 22 to 32 ( $^{13}\text{C}$ ), methylfunction 1.1 ( $^1\text{H}$ ) and 68.7 ( $^{13}\text{C}$ ), methylen-1 4.10 ( $^1\text{H}$ ) and 68.7 ( $^{13}\text{C}$ ). The sugar moiety consisted of a  $\beta$ -D-arabino(1-6) $\beta$ -D-glucopyranose like in 5 and 8. Data are in accordance with those reported by Yoshikawa et al. (1996).

### 3.4.4. Rosiridin (4)

Rosiridin consists of a (2Z)-3,7-dimethylocta-2,6-diene-1,4-diol attached with a  $\beta$ -D-glucopyranose at position 1. Base moiety NMR:  $^1\text{H}$ : 6.68, H-2; 6.42, H-6; 4.05, H<sub>2</sub>-1; 3.95, H-4; 2.15, H<sub>2</sub>-5; 1.65, H<sub>3</sub>-8; 1.53, H<sub>3</sub>-9 and H<sub>3</sub>-10;  $^{13}\text{C}$ : 141.7, C-7; 136.7, C-3; 121.5, C-2; 120.9, C-6; 70.5, C-4; 65.1, C-1; 34.1, C-5; 25.9, C-8; 18.0, C-9; 12.1, C-10. The glucose unit showed the expected values. Data are according to Kurkin et al. (1985).

### 3.4.5. Rosavin (5)

Rosavin consists of a phenylpropenol attached with a  $\beta$ -D-arabino(1-6) $\beta$ -D-glucopyranose (identical with that in 8 and 3). NMR values of the base moiety are equal to those in 1. Data are similar to those reported by Zapesochayna and Kurkin (1982).

### 3.4.6. Salidroside (6)

NMR:  $^1\text{H}$ : 7.08, H-2/6; 6.68, H-3/5; 3.5/3.4, H<sub>2</sub>-8; 3.1/2.9, H<sub>2</sub>-7;  $^{13}\text{C}$ : 155.8, C-4; 130.0, C-2/6; 128.8, C-1; 115.2, C-3/5; 70.1, C-8; 35.0, C-7. The data for the  $\beta$ -D-glucopyranosyl moiety were in the expected range. The data are in agreement with those reported earlier (Lu et al. 1980; Shimomura et al. 1987).

### 3.4.7. Rhodiolin (7)

NMR: herbacetin part:  $^1\text{H}$ : 6.92, H-3'/H-5'; 8.15, H-2'/H-6'; 6.37, H-6;  $^{13}\text{C}$ : 176.3, C-4; 159.6, C-4'; 152.5, C-5; 148.9, C-7; 147.2, C-2; 143.8, C-9; 136.3, C-3; 129.8, C-2'/6'; 124.8, C-8; 121.8, C-1'; 115.7, C-3'/5'; 104.4, C-10; 98.3, C-6; 1-(4-hydroxy-3-methoxyphenyl)propane-1,2,3-triol part:

$^1\text{H}$ : 6.86, H-5''; 6.82, H-6''; 7.03, H-2''; 5.09, H-8''; 4.26, H-7''; 3.79, H<sub>2</sub>-9'';  $^{13}\text{C}$ : 147.8, C-3''; 147.4, C-4''; 127.0, C-1''; 120.8, C-6''; 115.6, C-5''; 112.0, C-2''; 77.9, C-8''; 77.3, C-7''; 60.3, C-9''; 55.9, methyl-O-4''. Data agree with those reported by Min-Won Lee et al. (2000).

### 3.4.8. 4-Methylsalidroside-6'-beta-D-arabino(1-6)glucopyranoside (8)

NMR: viridoside (= salidroside-O-methylether) part corresponding to salidroside (6) with a downfield-shift of C-4 (O-methyl) to 158 ppm and an additional attached methyl group ( $^1\text{H}$ : 3.68 ppm;  $^{13}\text{C}$ : 55.1 ppm). Those data are in agreement with those reported for viridoside (Golovina and Nikonov 1988) except the values for CH<sub>2</sub>-6' ( $^1\text{H}$ : 3.85 ppm;  $^{13}\text{C}$ : 68.2 ppm) where an arabinose unit is attached. The data for the sugar moiety ( $\beta$ -D-arabino(1-6) $\beta$ -D-glucopyranose) are the same as in 3 and 5 and were verified by the  $^{13}\text{C}$ -highfield-shifts for C-2'' (70.7 ppm), C-3'' (72.7 ppm) and C-4'' (67.5 ppm) instead of those reported for the xylosyl-glucopyranose unit in cuchiloside (= salidroside-xyloside) (73.9; 76.6; 70.3 ppm, respectively) (Bisset et al. 1989). This new compound is named mongrhoside.

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Corrected formula:

