Quantitative Determination of Salidroside and Tyrosol from the Underground Part of *Rhodiola rosea* by High Performance Liquid Chromatography

Pham Tuan Linh, Young Ho Kim, Seon Pyo Hong[1], Jiang Ji Jian[2], and Jong Seong Kang

College of Pharmacy, Chungnam National University, Taejon 305-764, Korea, [1]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea, and [2]Yanbian University, Longjing City, Jilin Province, China

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A reversed-phase high performance liquid chromatographic method was developed to determine salidroside and tyrosol simultaneously in the *Rhodiola rosea*. The optimum condition was Nova-Pak C_{18} as stationary phase, 6.5% methanol in water as mobile phase and detection at UV 225 nm. The identification was carried out by comparing the retention time and LC/MS spectrum of the relevant peaks with those of isolated standards. The contents of salidroside and tyrosol in the samples gathered from various area in China were ranged over 1.3-11.1 mg/g and 0.3-2.2 mg/g, respectively.

Key words: *Rhodiola rosea*, Salidroside, Tyrosol

INTRODUCTION

Chinese natural medicines originating in several alpine plants belonging to *Rhodiola* species (Crassulaceae) are given the generic name Rhodiolae Radix. Rhodiolae Radix has been used as "source of adaptation to environment" in Chinese traditional medicine and can effectively enhance the body's ability to resist fatigue and extend human life (Ssaratikov *et al.*, 1968). Its effects on prolyl endopeptidase inhibition (Fan *et al.*, 1999), learning and memory (Petkov *et al.*, 1986), antiallergic activity (Yoshikawa *et al.*, 1996) and liver regeneration (Udintsev *et al.*, 1991a,b) are also reported. Several bioactive glycosides such as rhodiocyanosides (Yoshikawa *et al.*, 1995) and sacranosides (Yoshikawa *et al.*, 1997), and phenolic components (Zong *et al.*, 1991) have been isolated from the Rhodiolae Radix.

Salidroside(SAL, *p*-hydroxyphenethyl-β-$_D$-glucoside), known as rhodioloside, is one of the major phenolic glycoside of *Rhodiola rosea* and the content of salidroside is often used as one of criteria to evaluate the quality of the crude drug. The quantitative determination of salidroside in the fruit of *Ligustrum lucidum* (Shi *et al.*, 1998) and in suspension culture of compact callus aggregate of *Rhodiola sachalinensis* (Xu *et al.*, 1998) was reported. Tyrosol(TYR, *p*-hydroxyphenethyl alcohol), the aglycone of salidroside, seems to be important for production of salidroside. An accurate method for the simultaneous determination of SAL and TYR was developed and applied to the quality evaluation of the *Rhodiola rosea*.

Correspondence to: Jong Seong Kang, College of Pharmacy, Chungnam National University, Taejon 305-764, Korea
E-mail: kangjss@hanbat.chungnam.ac.kr

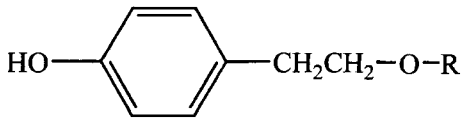

Fig. 1. Chemical structures of SAL(R=glc) and TYR(R=H).

MATERIALS AND METHODS

Materials and instruments

The dried underground parts of *Rhodiola rosea* were obtained from Protection and Development Research Institute of Chang Bai Mountain Natural Resources in Yanbian University. The voucher specimen are deposited at the herbarium in College of Pharmacy, Chungnam National University. SAL and TYR were isolated from *Rhodiola rosea* and used as standard. TYR was also obtained from Aldrich Chemical Co. (WI, U.S.A.). The spectral data of purchased TYR were compared with those of the isolated compound. Methanol as a HPLC grade from Tedia Co. (OH, U.S.A.) and other reagents as A analytical grade were used. The chromatographic system

for quantitative analysis consisted of SCL-10A system controller, LC-10AD pump, SPD-10A UV/VIS detector with variable wavelength (Shimadzu Co., Kyoto, Japan), column temperature controller (Waters, MA, U.S.A.) and Rheodyne 7725 injector with a 20 μl sample loop. For qualitative analysis the QP8000 LC/MS system (Shimadzu Co, Kyoto, Japan) with APCI interface, LC-10AD pump and SPD-10A UV/VIS detector were used.

Preparation of SAL and TYR standards

SAL and TYR were isolated as usual method. Dried roots of *Rhodiola rosea* (3 kg) were chopped coarsely and extracted twice with methanol at room temperature. The methanol extract (300 g), concentrated under vacuum, was fractionated with dichloromethane, ethylacete and water, successively. The ethylacetate extract was subjected to silicagel column chromatograpy with dichloromethane-methanol (10:1) as eluents to give 6 fractions. Fraction 2 was further separated by preparative HPLC (Prep Nova-Pak, HR C_{18}, 25 × 100 mm, Waters, MA, USA) with 30% methanol to give TYR. SAL was obtained from fraction 5 by preparative HPLC with 20% methanol.

Chromatography

The HPLC separation of SAL and TYR for the qualitative and quantitative analysis was performed using a reverse phase system. The chromatographic column used was Nova-Pak C_{18} (3.9 × 150 mm, Waters, MA, USA). The mobile phases were mixed solution of methanol and water with various composition at a flow rate 1.0 to 1.2 ml/min. Detection was carried out at UV 225 nm. The qualitative analysis of SAL and TYR in sample was carried out by monitoring the LC/MS spectrum of HPLC effluent. The conditions for LC/MS system were following: Probe voltage -4.5 kV, APCI temp. 400°C, nebulizer gas flow rate 2.5 L/min, CDL voltage 45 V, CDL temp. 230°C, deflector voltage -40 V.

Extraction of TYR and SAL from specimen

The specimens for extraction were prepared from the dried underground part of *Rhodiola rosea* by means of coarsely cut, moderately cut, finely cut, coarsely powder or moderately fine powder. Among chloroform, methanol and methanol:water (1:1) mixture, methanol was selected as a extraction solvent of SAL and TYR from specimen, because it showed the highest extraction ability for SAL and TYR (data not shown). To determine the effect of fineness of the specimen on extraction, the specimen in different fineness (3 g) was extracted with methanol under reflux for 2 h. The extract was then moved into volumetric flask and diluted to 50 ml with methanol. 5 ml of filtered solution was concentrated to 1 ml under vacuum and centrifuged at 13,000 rpm for 10 min. The 10 μl supernatant were injected to HPLC. The extraction efficiency of each sampling method was compared by measuring the concentrations of SAL and TYR in the solution.

RESULTS AND DISCUSSION

Isolation of SAL and TYR from *Rhodiola rosea* for standard materials

SAL and TYR were isolated from the ethylacetate fraction of *Rhodiola rosea*. Repeated silicagel column chromatography and preparative HPLC gave SAL and TYR as pure compounds. Their structures were identified using UV, ^1H NMR, ^{13}C NMR and MS spectral data (data not shown).

Resolution and identification of SAL and TYR from samples

To obtain the optimum conditions for the resolution of SAL and TYR the capacity factors were examined when they were eluted with mobile phase in various composition of methanol. As shown in Fig. 2 the capacity factor of SAL is larger than that of TYR if the methanol content is lower than 12%, while the methanol content is higher than 12% the effects are reversed. The optimum resolution of SAL and TYR from samples was acquired at 6.5% methanol. Fig. 3 shows the chromatograms of standard and sample with the mobile phase of 6.5% methanol in water. The peaks of SAL and TYR from standard mixture and sample were identified by MS single ion chromatogram and MS spectrum shown in Fig. 4. From the MS spectrum the peaks about 10 min and 13.5 min were identified as TYR (m/z 137, $(M-1)^-$) and SAL (m/z 299, $(M-1)^-$), respectively.

Extraction efficiency of SAL and TYR

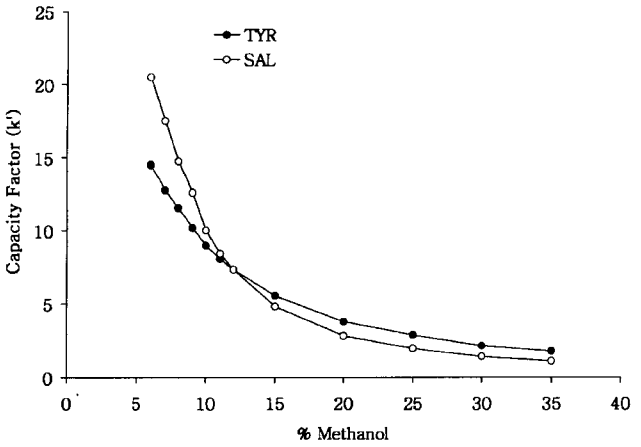

Fig. 2. The effect of methanol concentration on the retention of SAL(–○–) and TYR(–●–). Stationary phase; Nova-Pak C_{18} (3.9 × 150 mm), mobile phase; methanol in water, column temperature; 30°C. The capacity factor, k', was calculated by $k'=(t_R-t_o)/t_o$ where t_R is the retention time of the compound of interest and t_o the retention time of an unretarded component.

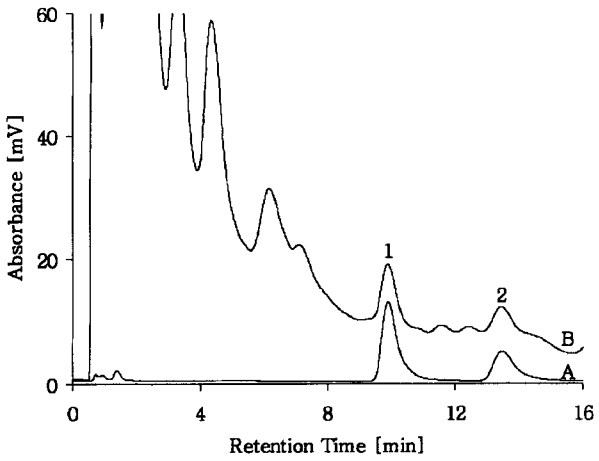

Fig. 3. Chromatograms of a mixture of SAL and TYR standard (A) and extract of Rhodiola Root (B). Stationary phase; Nova-Pak C_{18}(3.9×150 mm), mobile phase; 6.5% methanol in water, column temperature; 30°C, flow rate; 1.1 ml/min, detection; UV 225 nm. Peaks: 1. TYR, 2. SAL.

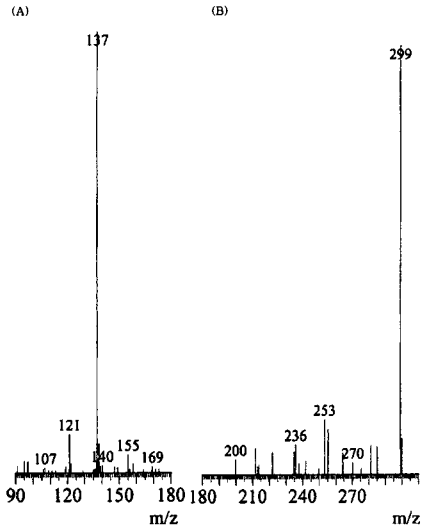

Fig. 4. LC/MS spectrum of an extract of Rhodiola Root for TYR(A) and SAL(B). Measuring conditions: Probe voltage -4.5 kV, APCI temp. 400°C, nebulizer gas flow rate 2.5 L/min, CDL voltage 45 V, CDL temp. 230°C, deflector voltage -40 V. Identification; TYR m/z 137 $(M-1)^-$, SAL m/z 299 $(M-1)^-$

The coarsely cut, moderately cut, finely cut, coarse powdered or moderately fine powdered specimen was extracted and the extraction efficiency depended on the fineness of specimen was examined. As shown in Table I the content of SAL and TYR from coarsely cut specimen was significantly lower than that from moderately cut specimen, but there were not any significant differences in content of SAL and TYR between moderately cut, finely cut and powdered specimen. This result shows that the components cannot be completely extracted from coarsely cut specimen but the fineness of the specimen is not an important factor for extraction if it is smaller than moderately cut specimen.

Table I. The extraction efficiency of SAL and TYR depends on the fineness of specimen.

Fineness of specimen	Salidroside	Tyrosol
Coarsely cut	0.957 ± 0.036	0.286 ± 0.013
Moderately cut	1.201 ± 0.029*	0.314 ± 0.009*
Finely cut	1.224 ± 0.032*	0.318 ± 0.010*
Coarsely powdered	1.230 ± 0.031*	0.319 ± 0.009*
Moderately fine powdered	1.223 ± 0.031*	0.320 ± 0.009*

Data are given mean ± S.D. (mg/g dried root) of 5 measurements. *$p<0.01$ when compared with coarsely cut specimen. The degree of fineness of crude drug was determinated by sieving with No.4 (4.76 mm) for coarsely cut, No.7 (2.83 mm) for moderately cut, No.10 (2.00 mm) for finely cut, No.20 (0.84 mm) for coarsely powdered and No.50 (0.297 mm) for moderately fine powdered specimen.

Table II. The recovery of SAL and TYR from *Rhodiola* root in the reversed phase HPLC method

Salidroside			Tyrosol		
Added (mg/g)	Found (mg/g)	Recovery (%)	Added (mg/g)	Found (mg/g)	Recovery (%)
0.500	0.488	97.6	0.100	0.088	88.0
1.000	1.005	100.5	0.200	0.196	98.0
1.500	1.416	94.4	0.300	0.293	97.7

Linearity, recovery and limit of detection

The calibration functions of SAL and TYR standard calculated with peak height(y) and concentration(x) in mg/ml were $y=154x+0.602$ ($R=0.9998$) and $y=519x-0.181$ ($R=0.9999$), respectively, over the concentration range 0.001 to 0.5 mg/ml. For the recovery test known amount of SAL and TYR standard was added to the moderately cut specimen and extracted. The result of recovery test is shown as Table II. The linearity and recovery test indicate that this method is suitable and applicable for use as the quality evaluation of the *Rhodiola rosea*. The detection limits of SAL and TYR at a signal to noise ratio of 3 were 100 ng/ml and 50 ng/ml, respectively.

Quantitation of SAL and TYR in the *Rhodiola rosea*

The contents of SAL and TYR in the samples collected from various area of China were calculated from the relevant peak heights by external standard method. As presented in Table III, the variation of SAL and TYR in specimens obtained from various drug stores in Yanbian area was very large, the highest concentration is about 10 times more than that of the lowest. Apparent size and shape of specimen 1, 2 and 3 were all 2-5 cm long and 1-1.5 cm thick. The cross sectional surface of them was

Table III. Concentration of SAL and TYR in Rhodiola Root

Crude drug	Salidroside	Tyrosol
Specimen 1	8.540 ± 0.713	0.252 ± 0.009
Specimen 2	11.064 ± 1.350	0.378 ± 0.052
Specimen 3	5.021 ± 0.697	2.191 ± 0.295
Specimen 4	1.256 ± 0.213	0.320 ± 0.011

Data are given as mean ± S.D. (n=4-6) in mg/g dried sample.

rigid and colored as light pink. On the other hand, specimen 4 was much bigger (4-8 cm long and 2-3.5 cm thick) than others and cultivated more than 5 years. The cross sectional surface was lax and coarse and colored as brownish-red. This seems to be the reason why the content of SAL of specimen 4 was lower than others. In specimen 3, the content of TYR was 6.8-8.7 times higher than others, because it was cultivated some other area in China. Thus it was important to control the gathering time and area of *Rhodiola rosea* to obtain the higher amount of SAL and TYR. TYR would be a precursor or metabolite of SAL, but the close relationship of the content between SAL and TYR was not clear in this experiment.

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