· Letters ·

A New Cyano-compound from *Rhodiola kirilowii*

YANG Lian-mei[1, 2], HU Rong[2], FU Hong-zheng[1*]

1. State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China

2. Department of Pharmaceutics, School of Medical Academy, Yangzhou University, Yangzhou 225009, China

Abstract: **Objective** To study the chemical constituents of *Rhodiola kirilowii*. **Methods** The compounds were separated and purified by various chromatographic techniques and their structures were elucidated on the basis of physicochemical properties and spectroscopic methods. **Results** Five compounds were purified and their structures were identified as 4-(β-*D*-glucopyranosyloxy)-3-hydroxy-2-(hydroxymethyl)-butanenitrile (**1**), epicatechin (**2**), arbutin (**3**), rutin (**4**), and β-*D*-glucose (**5**). **Conclusion** Compound **1** is a new cyano-compound and other compounds are isolated from the plant for the first time.

Key words: arbutin; cyano-compound; 4-(β-*D*-glucopyranosyloxy)-3-hydroxy-2-(hydroxymethyl)-butanenitrile; β-*D*-glucose; rutin
DOI: 10.3969/j.issn.1674-6384.2011.04.001

Introduction

Many plants produce cyanogenic glucosides for their chemical defense. They are α-hydroxynitrile glucosides which release toxic hydrogen cyanide (HCN) upon cleavage by endogenous plant β-glucosidases. In addition to cyanogenic glucosides, several plant species produce β- and γ-hydroxynitrile glucosides. These do not release HCN upon hydrolysis by β-glucosidases, and β- and γ-hydroxynitrile glucosides are produced by diversification of the cyanogenic glucoside biosynthetic pathway at the level of nitrile intermediate (Akgul *et al*, 2004, Bjarnholt *et al*, 2008). *Rhodiola kirilowii* (Regel) Maxim is a traditional Tibetan medicine in China with adaptogenic properties. It has a pronounced antifatigue effect reflected in an antifatigue index (Yousef *et al*, 2006). Here we report the isolation and characterization of a new cyano-compound and four compounds isolated from the ethanol extract of the roots of *R. kirilowii*. In order to make full use of the roots of *R. kirilowii*, it is necessary to further investigate its chemical constituents. Moreover, we present the biological activities of the new cyano-compound.

Materials and methods

Equipments

IR spectra were recorded on a Nicolet Impact 410 Spectrometer using KBr disk. Melting points were measured on an XT_{4A} Micromelting Point Apparatus. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker—400 Spectrometer with tetramethylsilane (TMS) as the internal standard. HR-ESI-MS were measured using a Finnigan LCQ-DECA Instrument, and HR-ESI-MS data were obtained on a Mariner Spectrometer. A lab alliance HPLC system consisted of a binary pump and a Model 201 Detector was used. Silica gel (100－200 and 200－300 mesh) was purchased from Qingdao Marine Chemical Factory. Solvents of analytical grade were purchased from Beijing Chemical Factory.

Plant materials

The roots of *Rhodiola kirilowii* (Rege1) Maxim. and ethanol extract were collected and identified by Prof. FU Hong-zheng of Peking University. A voucher specimen (No. H090707) was deposited at State Key Laboratory of Natural and Biomimetic Drugs in Peking University.

* Corresponding author: Fu HZ Address: State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China
Tel: +86-10-8280 5212 Fax: +86-10-8280 2724 E-mail: drhzfu@yahoo.com.cn
Received: March 25, 2011; Revised: June 16, 2011; Accepted: August 23, 2011

Extraction and isolation

The roots (50.0 kg) of *R. kirilowii* were air dried and powdered, and then extracted with 95% ethanol (10 times volume) for three times to afford ethanol extract (5.0 kg). The ethanol extract (5.0 kg) was suspended inwater and extracted with *n*-BuOH. The *n*-BuOH soluble portion was concentrated under reduced pressure. The *n*-BuOH extract (3.0 kg) was subjected to silica gel column chromatography eluting successively with $CHCl_3$-CH_3OH (1:0→0:1) to yield five parts (I—V). Part IV of $CHCl_3$-CH_3OH (20:1) was subjected to silica gel column chromatography eluting successively with $CHCl_3$-CH_3OH (1:0→0:1) to yield fractions 1 and 2. Fraction 1 (7 g) was separated by column chromatography on silica gel (EtOAc-CH_3OH = 10:1) to yield compounds **1** (70 mg) and **5** (17 mg). Part I of $CHCl_3$-CH_3OH (50:1) was separated by column chromatography on silica gel (petroleum ether-EtOAc 30:1→5:1) and Sephadex LH-20 column (petroleum ether-$CHCl_3$-CH_3OH = 4:5:1) to afford compounds **2** (10 mg), **3** (25 mg), and **4** (25 mg).

Results and discussion

Compound **1** was isolated as white amorphous power and its molecular formula was established as $C_{11}H_{19}NO_8$ by analyses of HR-ESI-MS at *m/z*: 294.136 93 (calcd. 294.136 98 $[M + H]^+$). The IR spectra indicated the presence of hydroxyl group (3395 cm^{-1}) and cyano group (2245 cm^{-1}). In particular, the broad bands at around 3455 and 1050 cm^{-1} in the IR spectra suggested the existence of glycosidic moieties in the structure. The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum (Table 1) showed the signals of two methylene groups at δ_H 3.97 (2H, dd, J = 2.3, 11.6 Hz) and 3.65 (2H, d, J = 7.6 Hz), two methine groups at δ_H 3.03 (1H, m) and 3.75 (1H, m), and a sugar moiety at δ_H 4.21, 3.97, 3.52, 3.31, 3.24, 3.14, and 2.89. The value of the proton coupling constant of the anomeric proton resonance (J = 7.7 Hz) showed that the sugar moiety adopted a β-configuration. The ^{13}C-NMR spectrum (100 MHz, DMSO-d_6) including the HSQC and DEPT spectra, exhibited 11 carbon signals, consisting of three methylene groups, seven methine groups, and one cyano group, and six of them were attributed to a sugar unit. By means of HSQC spectrum, all of the direct connections between proton and carbon signals were assigned. Analysis of the ^{1}H-^{1}H COSY spectrum of compound **1** showed the connection between H-2, 3, 4 and sugar fragment in Fig. 1. From the anomeric proton [δ_H 4.21 (1H, d, J = 7.7 Hz)], the anomeric carbon (δ_C 97.7), and other characteristic NMR resonances (δ_C 79.5, 72.9, 70.3, 78.5, and 60.7), the sugar unit was identified as β-glucopyranose. The sugar sequences and their linkage sites were derived from the HMBC signals at H-2′ (δ_H 2.89) and C-4 (δ_C 67.7) in its HMBC spectrum, indicating that the β-glucopyranosyl group was attached to the -CH_2 (C-4). In addition, the HMBC spectrum showed that δ_H 2.89 (1H, m) was correlated with δ_C 67.7, 70.5, 97.7, and 72.9, respectively and δ_H 3.97 (2H, dd, J = 2.3, 11.6 Hz) was correlated with δ_C 70.5 and 97.7. From the above evidences, compound **1** was deduced to be 4-(β-*D*-glucopyranosyloxy)-3-hydroxy-2-(hydroxymethyl)-butanenitrile.

Table 1 ^{13}C-NMR and ^{1}H-NMR data of compound 1 (in DMSO-d_6)

Position	δ_C	δ_H
1	118.9	—
2	35.9	3.03 (1H, m)
3	70.5	3.75 (1H, m)
4	67.7	3.97 (2H, dd, J = 2.3, 11.6 Hz)
1′	97.7	4.21(1H, d, J = 7.7 Hz)
2′	79.5	2.89 (1H, m)
3′	72.9	3.31 (1H, m)
4′	70.3	3.14 (1H, m)
5′	78.5	3.24 (1H, m)
6′	60.7	3.97 (1H, m)
		3.52 (1H, m)
CH_2OH	58.6	3.65 (2H, d, J = 7.6 Hz)

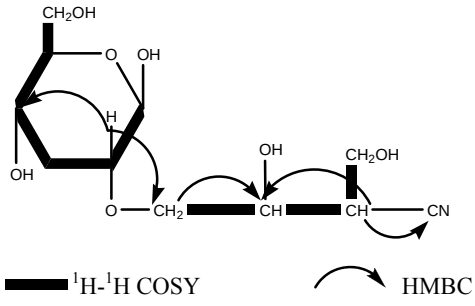

Fig. 1 Key HMBC and ^{1}H-^{1}H COSY correlations of compound 1

Acid hydrolysis

Compound **1** (20 mg) was hydrolyzed with 2 g/L

aqueous HCl (10 mL) at 100 ℃ for 12 h in a sealed tube. The reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The aqueous layer was repeatedly evaporated with MeOH under vacuum until the solvent was completely removed and analyzed by TLC. The TLC behavior of glucose was similar to glucose standard in $CHCl_3$-MeOH (2:1) (Li *et al*, 2009).

References

Akgul Y, Ferreira D, Abourashed EA, Khan IA, 2004. Lotaustralin from *Rhodiola rosea* roots. *Fitoterapia* 75(6): 612-614.

Bjarnholt N, Rook F, Motawia MS, Cornett C, Jørgensen C, Olsen CE, Jaroszewski JW, Bak S, Møller BL, 2008. Diversification of an ancient theme: Hydroxynitrile glucosides. *Phytochemistry* 69(7): 1507-1516.

Li X, Li JF, Wang D, Wang WN, Cui Z, 2009. Isoflavone glycosides from the bark of *Maackia amurensis*. *Acta Pharm Sin* 44(1): 63-68.

Yousef GG, Grace MH, Cheng DM, Belolipov IV, Raskin I, Lila MA, 2006. Comparative phytochemical characterization of three *Rhodiola* species. *Phytochemistry* 67(21): 2380-2391.