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New compounds from *Rhodiola kirilowii*

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Abstract

Five compounds were isolated from root extracts of *Rhodiola kirilowii*: arbutin, epigallocatechin gallate, rhodiocyanoside A, fructopyrano-(1-4)-glucopyranose and lotaustralin. The first four compounds were found in this plant for the first time. Lotaustralin, salidroside, daucosterol and tyrosol were already described to be contained. Especially salidroside (as a *Rhodiola* marker compound) could not be detected in our samples. The structures were established by NMR studies.

Keywords

Rhodiola kirilowii; arbutin; epigallocatechin gallate; rhodiocyanoside A; lotaustralin; fructopyrano-(1-4)-glucopyranose.

Introduction

Rhodiola kirilowii (Reg.) Reg. (Crassulaceae) is used in the Chinese traditional medicine for the enhancement of the ability of anti-anoxia, it shows anticoagulative properties and decreases the level of blood sugar. *Rh. kirilowii* is also said to protect people efficiently against cardiopulmonary function problems when moving to high altitude (4500 m) [1]. The plant was reported to contain salidroside, tyrosol, daucosterol, lotaustralin, sucrose, beta-sitosterol [2,3,4,5]. Our reinvestigation of

cultivated *Rh. krilowii* led to the isolation of 5 compounds which were identified by NMR-studies as arbutin (**1**), epigallo-catechin gallate (**2**), rhodiocyanoside A (**3**), lotaustralin (**4**), fructopyrano-(1-4)-glucopyranose (**5**).

Results and Discussion

The genus *Rhodiola* contains 15 species; among them *Rh. rosea, Rh. quadrifida* and *Rh. kirilowii* are used medinically. Especially the species *rosea* is investigated intensively [2,3]. *Rh. kirilowii* is traditionally used in Northern China and in southern parts of the former USSR. It is mainly applied to protect and to reduce cardiopulmonary pathological damages occurring in high altitudes [1]. As ingredients, salidroside, tyrosol, daucosterol, lotaustralin, sucrose, beta-sitosterol are mentioned [4,5,6,7]. Our reinvestigation of cultivated plant material led to the isolation of arbutin, rhodiocyanoside A, epigallogatechin gallate, fructopyrano-(1-4)-glucopyranose and lotaustralin.

Only lotaustralin is already mentioned as being contained in the plant [8]. Especially the absence of the marker compound salidroside seems to be remarkable. Arbutin, rhodiocyanoside A and epigallocatechin gallate are reported to have antibacterial, histamine release inhibiting and antioxidative as well as antiallergic properties, respectively [9,10,11,12].

Experimental

Plant material

Roots and rhizomes of *Rh. kirilowii* were collected in September 2003 and identified in the Research Institute of Medicinal Plants, Poznań. The plant growth was controlled: The reaction and mineral components of the soil, the air temperature, the average sum of humidity and rain as well as the sun periods were monitored permanently.

Instruments

HPLC: Dionex system (pump 480, Gina 50 autosampler, DAD 320s) with a CC 250/4 Nucleodur C_{18} Pyramid, 5 µ, column (Macherey-Nagel, Germany); data

acquisition: Chromeleon V. 6.40, build 800. NMR: Bruker AC-400, DMSO-D_6, chemical shifts (δ=ppm) were referenced to DMSO (2.50 and 39.43 ppm, respectively), coupling constants in Hz. Flash liquid chromatography (FLC): 150 x 2 cm column, Polygoprep C_{18}, 60-30 (Macherey-Nagel, Germany).

Extraction and isolation

Air-dried underground parts of *Rh. kirilowii* were extracted with n-hexane for 24 hours, using a Soxhlet apparatus, followed by methanolic extraction for 48 hours. The alcoholic solution was evaporated to dryness under reduced pressure. The residue was partitioned between 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) leading to compounds **1** - **5**.

Arbutin (1)

NMR (δ=ppm; *J*[Hz]): C-1: 152.36; C-2/6: 115.64; 6.85 (2H,d,*J*=8.7); C-3/5: 117.81; 6.65 (2H,d,*J*= 8.7); C-4: 150.54; C-1´: 101.90; 4.62 (1H,d,*J*=7.0); C-2´: 73.46; 2.90-3.60 (m); C-3´: 76.80; 2.90-3.60 (m); C-4´: 69.93; 2.90-3.60 (m); C-5´: 77.13; 2.90-3.60 (m); C-6´: 60.93; 2.90-3.60 (m).

Epigallocatechin gallate (2)

NMR (δ=ppm; *J*[Hz]): C-2: 77.10; 4.95 (1H,d,*J*=3.0); C-3: 68.19; 5,36 (1H, dd, *J*= 3.8); C-4: 24.99; 1,95 (2H, dd, *J* =8.60Hz, 7.1); C-5: 156.67; C-6: 95.64; 5.93 (1H, d, *J* =1.3); C-7: 156.70; C-8: 94.48; 5.82 (1H, d, *J* =1.3); C-9: 155.81; C-10: 97.52; C-1´: 128.77; C-2´/6´: 105.62; 6.39 (2H, d, *J* =1.5); C-3´/5´: 145.80; C-4´: 132.52; C-1´´: 120.81; C-2´´/6´´: 108.81; 6.81 (2H, d, *J* =1.5); C-3´´/5´´: 145.56; C-4´´: 138.71; C-7´´: 165.38.

Rhodiocyanosid A (3)

NMR (δ=ppm; *J*[Hz]): C-1: 66.79; 4.41 (1H,d,*J*=6.3)/ 4.31 1H,d,*J*=6.3); C-2: 144.54; 6.50 (1H,t,*J*=6.3); C-3: 110.75; C-4: 19.68; 1.96 (3H,s); C-5: 117.62; C-1´:

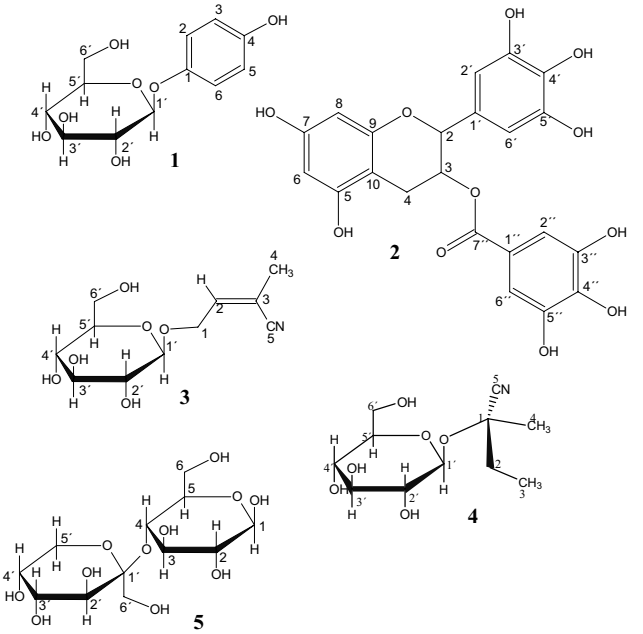
102.82; 4.19 (1H,d,*J*=7.7); C-2`: 73.51; 2,94 (1H,dd,*J* =7.90 Hz); C-3´: 77.15; 2.96 (1H, dd, *J*=11.0); C-4´: 70.08; 3.06 (1H, m); C-5´: 76.83; 3.12 (1H, m); C-6´: 61.11; 3.67 (1H_A, d, *J*=11.50), 3.47 (1 H_B, d, *J* =11.5).

Lotaustralin (4)

NMR (δ=ppm; *J*[Hz]): C-1: 74.78; C-2: 32.96; 1.82 (2H;q;*J*=7.3); C-3: 8.62; 0.98 (3H;t,*J*=7.2); C-4: 24.11; 2.49 (3H,s); C-5: 119.40; C-1´: 99.49; 4.45 (1H,d,*J*=7.7); C-2´: 73.33: 2.94 (1H,dd,*J*=7.9); C-3´: 76.83; 2.96 (1H,dd,*J*=11.0); C-4´: 69.92; 3.06 (1H,m); C-5´: 76.65; 3.12 (1H,m); C-6´: 61.03; 3.67 (1H_A,d,*J*=11.5)/ 3.43 (1H_B,d,*J*=11.5).

Fructopyrano-(1-4)-glucopyranose (5)

NMR (δ=ppm; *J*[Hz]): C-1: 91.91; 5,03 (1H, d, *J* =2,5); C-2: 74.37; 3,0 – 4,3 (1H, m); C-3: 73.00; 3,0 – 4,3 (1H, m); C-4: 82.68; 3,0 – 4,3 (1H, m); C-5: 77.08; 3,0 – 4,3 (1H, m); C-6: 60.60; 3,0 – 4,3 (2H, m); C-1´: 104.16; 3,0 – 4,3 (1H, m); C-2´: 73.00; 3,0 – 4,3 (1H, m); C-3´: 71.77; 3,0 – 4,3 (1H, m); C-4´: 69.93; 3,0 – 4,3 (1H, m); C-5´: 62.29; 3,0 – 4,3 (2H, m); C-6´: 62.16; 3,0 – 4,3 (2H, m).



Figures of compounds **1** – **5**:

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