RHODIOCYANOSIDES A AND B, NEW ANTIALLERGIC CYANOGLYCOSIDES FROM CHINESE NATURAL MEDICINE "SI LIE HONG JING TIAN", THE UNDERGROUND PART OF *RHODIOLA QUADRIFIDA* (PALL.) FISCH. ET MEY.

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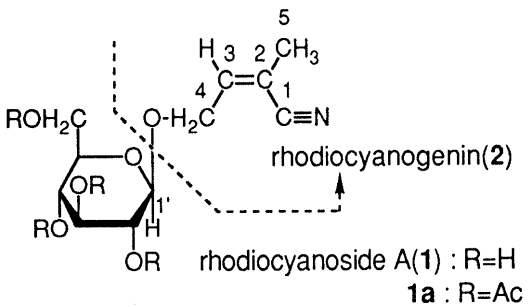
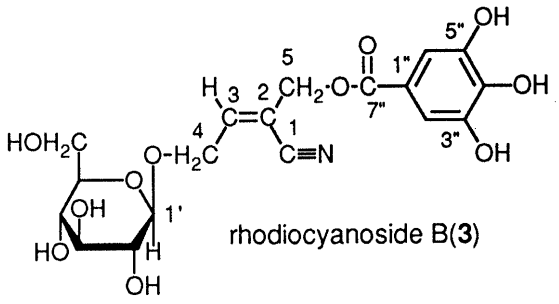
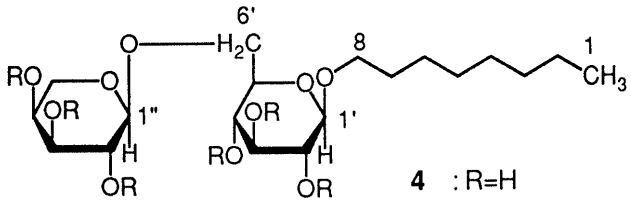
Two new antiallergic cyanoglycosides named rhodiocyanosides A and B were isolated from the Chinese natural medicine "Si Lie Hong Jing Tian" (Shiretsukoukeiten in Japanese), the underground part of *Rhodiola quadrifida* (Pall.) Fisch. et Mey., together with two new glycosides, octyl α-L-arabinopyranosyl(1-6)-β-D-glucopyranoside and gossypetin 7-*O*-β-D-glucopyranosyl(1-3)-α-L-rhamnopyranoside. Their chemical structures were determined on the basis of chemical and physicochemical evidence. Rhodiocyanosides A and B exhibited inhibitory activity on the histamine release from rat peritoneal exudate cells sensitized with anti-DNP IgE. In addition, rhodiocyanoside A was found to inhibit the PCA reaction in rats.

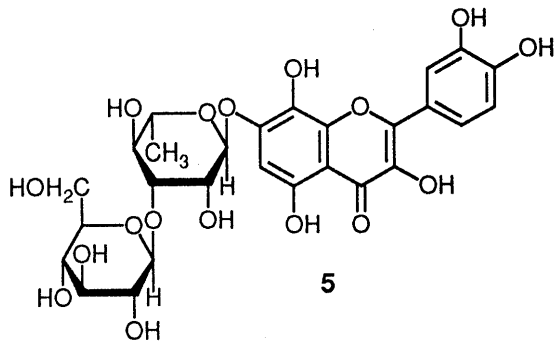
KEY WORDS rhodiocyanoside; cyanoglycoside; *Rhodiola quadrifida*; antiallergic activity; histamine release inhibitor; passive cutaneous anaphylaxis reaction

The Chinese natural medicine "Si Lie Hong Jing Tian" (Shiretsukoukeiten in Japanese), which is produced from *Rhodiola quadrifida* (Pall.) Fisch. et Mey.(Crassulaceae), has been used as a hemostatic, an antibechic, and an endermic liniment for burns. In the chemical study of this natural medicine, several components such as rhodioloside(salidroside) and flavonol glycosides were isolated.[1] As part of continuing studies on the bioactive constituents of natural medicine,[2] we have isolated new antiallergic cyanoglycosides, rhodiocyanosides A(**1**) and B(**3**) together with two new glycosides, octyl α-L-arabinopyranosyl(1-6)-β-D-glucopyranoside(**4**) and gossypetin 7-*O*-β-D-glucopyranosyl(1-3)-α-L-rhamnopyranoside(**5**), and four known compounds, rhodioloside,[1,3] n-hexyl β-D-glucopyranoside,[4] tricetin,[5] and gossypetin 7-*O*-α-L-rhamnopyranoside,[6] from the underground part of *Rhodiola quadrifida* (Pall.) Fisch. et Mey. This paper communicates the structure elucidation of **1**, **3**~**5**, and the antiallergic activity of **1** and **3**.[7]









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The MeOH extract of the underground part was subjected to XAD-2 column chromatography, and then the MeOH-eluate was purified by repeated ordinary SiO_2($CHCl_3$-MeOH-H_2O) and reversed phase SiO_2(chromatorex ODS DM 1020T, H_2O-MeOH) column chromatography and finally HPLC (YMC-Pack R&D, H_2O-MeOH) to furnish **1**(0.113% from the crude drug), **3**(0.009%), **4**(0.009%), **5**(0.004%), rhodioloside(0.016%), n-hexyl β-D-glucopyranoside(0.003%), tricetin(0.001%), and gossypetin 7-*O*-α-L-rhamnopyranoside(0.001%).

Rhodiocyanoside A(**1**), a white powder, $[\alpha]_D$ -16.1°(MeOH), $C_{11}H_{17}NO_6$, UV(log ε, MeOH) : 208(4.03)nm, IR(KBr) : 3410, 2222, 1655, 1076cm^{-1}, positive mode FAB-MS : m/z 260$(M+H)^+$, negative mode FAB-MS : m/z 258$(M-H)^-$, furnished the tetraacetate(**1a**),[8] colorless oil, $[\alpha]_D$ -10.4°(MeOH), $C_{19}H_{25}NO_{10}$, IR(KBr) : 2235, 1757, 1231, 1042cm^{-1}, negative mode FAB-MS : m/z 426$(M-H)^-$ by acetylation with Ac_2O and pyridine. Enzymatic hydrolysis of **1** with β-glucosidase or naringinase gave the aglycone, rhodiocyanogenin(**2**), colorless oil, C_5H_7NO, IR(film) : 3300, 2222, 1655cm^{-1}, EI-MS : m/z 97(M^+), while methyl D-glucoside was identified by methanolysis of **1** with 9% HCl-dry MeOH. The ^1H NMR(CD_3OD) and ^{13}C NMR(Table 1) spectra of **1** and **2** which were assigned by DEPT, COSY(^1H-^1H, ^1H-^{13}C), HMBC, COLOC, and HOHAHA(^1H-^1H, ^1H-^{13}C), indicated the presence of trisubstituted olefin [**1** : δ 6.46(qdd, J=1.7, 6.3, 6.9); **2** : δ 6.36(tq, J=1.3, 6.6)(3-H)] which was bonded with methyl [**1** : δ 1.93(ddd, J=1.3, 1.3, 1.7); **2** : δ 1.96(tq, J=1.0, 1.3)(5-H_3)], nitrile group, and oxymethylene [**1** : δ 4.43(qdd, J=1.3, 6.9, 13.5), 4.54(qdd, J=1.3, 6.3, 13.5); **2** : δ 4.26(dq, J=1.0, 6.6)(4-H_2)] bearing a β-D-glucopyranoside moiety in the case of **1**[δ 4.30(d, J=7.9, 1'-H)]. The geometric structure of the trisubstituted olefin in **1** was characterized by the NOESY experiment of **1** and **2** ; namely, the NOE correlations were observed between 5-H_3 and 3-H and between 3-H and 4-H_2. Based on the above given evidence, the structure of rhodiocyanoside A(**1**) was determined.

Table 1. ^{13}C NMR Data for **1**, **1a**, **2**, and **3**(68MHz, CD_3OD)

	1	1a	2	3
C-1	118.2	116.9	118.2	116.2
2	112.6	112.6	111.1	113.0
3	145.0	142.4	147.8	148.9
4	68.4	68.3	61.5	68.2
5	20.3	20.2	20.3	64.6
1'	104.0	100.2		104.3
2'	74.9	71.2		74.9
3'	78.0	72.8*		77.8
4'	71.4	67.4		71.4
5'	78.0	72.1*		78.0
6'	62.6	61.8		62.6
1"				120.6
2"				110.3
3"				146.6
4"				140.3
5"				146.6
6"				110.3
7"				167.3

*Assignments may be interchangeable.

Rhodiocyanoside B(**3**), a white powder, $[\alpha]_D$-12.2°(MeOH), $C_{18}H_{21}NO_{11}$, UV(log ε, MeOH) : 279(3.99), 217(4.48)nm, IR(KBr) : 3410, 2230, 1714, 1620, 1529, 1075cm^{-1}, positive mode FAB-MS : m/z 449$(M+Na)^+$, showed the signals due to butenenitrile moiety [δ 6.86(dd, J=5.9, 6.2, 3-H), 4.53(dd, J=6.2, 14.5), 4.69(dd, J=5.9, 14.5)(4-H_2), 4.88(s, 5-H_2)], β-D-glucopyranosyl moiety [δ 4.43(d, J=7.6, 1'-H)], and galloyl group [δ 7.09(s, 2",6"-H)] in the ^1H NMR(CD_3OD) spectrum of **3**. In the NOESY data of **3**, the NOE correlations were observed in the following pairs of protons [5-H_2 & 3-H ; 3-H & 4-H_2]. Detailed comparison of the ^1H NMR and ^{13}C NMR data(Table 1) for **3** with those for **1** led us to formulate the structure of rhodiocyanoside B(**3**).

Octyl α-L-arabinopyranosyl(1-6)-β-D-glucopyranoside(**4**), a white powder, $[\alpha]_D$ -29.2°(MeOH), $C_{19}H_{36}O_{10}$, IR(KBr) : 3410, 1074cm^{-1}, ^1H NMR(CD_3OD) : δ 4.26(d, J=7.6, 1'-H), 4.32(d, J=6.6, 1"-H), 0.90(t, J=6.4, 1-H_3), negative mode FAB-MS : m/z 423$(M-H)^-$, liberated 1-octanol, methyl L-arabinoside, and methyl D-glucoside by the methanolysis. Ordinary acetylation of **4** furnished the hexaacetate(**4a**).[9] Finally, examination of the ^{13}C NMR of **4**[10] and **4a** led us to characterize the structure of **4**. Gossypetin 7-*O*-β-D-glucopyranosyl(1-3)-α-L-rhamnopyranoside(**5**), a yellow powder, $[\alpha]_D$ -50.8°(MeOH), $C_{27}H_{30}O_{17}$,

Table 2. Inhibitory Effect of Rhodiocyanosides A(**1**) and B(**3**) on Histamine Release from Rat Peritoneal Exudate Cells Sensitized with anti-DNP IgE

		Inhibition of histamine release (%)	
Concentration(M)	n	1	3
10^{-5}	4	9.8±5.8	-3.1±4.9
3×10^{-5}	4	42.1±6.3	28.2±5.9
10^{-4}	4	60.7±3.4	19.3±3.8

Each value represents the mean±S.E. sensitized peritoneal exudate cells were preincubated with **1** or **2** 20 min prior to the antigen-challenge with DNP-BSA.

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Table 3. Effect of Rhodiocyanoside A(1) and DSCG on the Passive Cutaneous Anaphylaxis Reaction in Rats

	Dose (mg/kg)	Time (min)	Area of bluing spots(cm^2)	Inhibition (%)
Control		–	1.74±0.08	–
1	100	20	1.28±0.14*	26.9
		60	1.11±0.16**	36.5
DSCG	100	20	0.94±0.13**	46.2
		60	0.84±0.25**	51.8

Test samples were injected intravenously at each time prior to the challenge. Each value represents the mean±S.E. of 5-8 experiments. Asterisks denote the significant differences from the control at *p<0.01, **p<0.05, respectively.

UV(log ε, MeOH) : 260(4.17), 280(4.06), 340(3.90)nm, IR(KBr) : 3389, 1655cm^{-1}, negative mode FAB-MS : m/z 625$(M-H)^-$, liberated gossypetin[11] by the enzymatic hydrolysis with naringinase. Based on comparison of the ^{13}C NMR data for **5**[12] with those for gossypetin 7-*O*-α-L-rhamnopyranoside and observation of the HMBC correlations between 1"-H and 3'-C and between 1'-H and 7-C, the structure of **5** was determined.

Inhibitory effect of rhodiocyanosides A(**1**) and B(**3**) on the histamine release from rat peritoneal exudate cells sensitized with anti-dinitrophenylated IgE(anti-DNP IgE) by the antigen-challenge with dinitrophenylated bovine serum albumin(DNP-BSA)[13] is summarized in Table 2. Rhodiocyanoside A(**1**), which is a major component of the Chinese natural medicine "Shiretsukoukeiten", has been found to exhibit activity inhibiting the histamine release; rhodiocyanoside B(**3**) shows a little activity. Furthermore, **1** also exhibits inhibitory effect on rat 48h passive cutaneous anaphylaxis(PCA) reaction[13] as shown in Table 3. Those activities of the major component may be preliminary evidence to substantiate the traditional effect of this natural medicine.

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8) ^1H NMR data of **1a** : δ 2.01, 2.03, 2.06, 2.10(3H each, all s), 2.01(6H, br s)(Acx4, 5-H_3), 4.17(dd, J=2.4, 12.3), 4.27(dd, J=4.8, 12.3)(6'-H_2), 4.42(qdd, J=1.4, 6.7, 12.2), 4.52(qdd, J=1.4, 6.7, 12.2)(4-H_2), 4.55(d, J=7.6, 1'-H), 6.24(qdd, J=1.4, 6.7, 6.7, 3-H).

9) **4a**, a white powder, IR(KBr) : 1760, 1055cm^{-1}, ^1H NMR(CD_3OD) : δ 0.90(t, J=6.6, 1-H_3), 1.96, 2.00, 2.01, 2.05, 2.08, 2.12(Acx6).

10) The ^{13}C NMR(CD_3OD) data of **4** : δc 14.4(1-C), 23.7(2-C), 30.8, 30.4, 30.5, 27.1(3, 4, 5, 6-C), 33.0(7-C), 71.0(8-C), 104.3(1'-C), 75.0(2'-C), 77.9(3'-C), 71.5(4'-C), 76.7(5'-C), 69.4(6'-C), 105.1(1"-c), 72.3(2"-C), 74.1(3"-C), 69.4(4"-C), 66.7(5"-C); **4a** : δc 14.4(1-C), 23.7(2-C), 30.6, 30.4, 30.4, 27.0(3, 4, 5, 6-C), 33.0(7-C), 70.9(8-C),* 101.7(1'-C), 72.9(2'-C), 74.6(3'-C), 70.3(4'-C),* 73.9(5'-C), 69.3(6'-C), 101.9(1"-c), 70.5(2"-C),* 71.7(3"-C), 68.3(4"-C), 64.1(5"-C), 20.6, 20.6, 20.6, 20.8, 20.8, 20.9, 171.1, 171.2, 171.4, 171.6, 171.7, 171.9(Acx6). *Assignments may be interchangeable.

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12) The ^{13}C NMR(DMSO-d_6) data of **5** : δc 147.3(2-C), 135.8(3-C), 176.2(4-C), 104.4(10-C), 151.4(5-C), 98.2(6-C), 149.4(7-C), 127.2(8-C), 144.3(9-C), 122.1(1'-C), 115.87(2'-C), 145.0(3'-C), 147.8(4'-C), 115.5(5'-C), 120.2(6'-C).

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