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Bioactive Constituents of Chinese Natural Medicines. II.[1] Rhodiolae Radix. (1). Chemical Structures and Antiallergic Activity of Rhodiocyanosides A and B from the Underground Part of *Rhodiola quadrifida* (PALL.) FISCH. *et* MEY. (Crassulaceae)

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Two bioactive cyanoglycosides, rhodiocyanosides A and B, and two oligoglycosides, rhodioflavonoside [gossypetin 7-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranoside] and rhodiooctanoside [octyl α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside), 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 four known compounds: rhodioloside, *n*-hexyl β-D-glucopyranoside, gossypetin 7-*O*-α-L-rhamnopyranoside, and tricetin. The chemical structures of new glycosides 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-2,4-dinitrophenyl IgE. Additionally, rhodiocyanoside A, the major constituent of this natural medicine, was also found to show antiallergic activity in a passive cutaneous anaphylaxis test in rat.

Key words rhodiocyanoside; cyanoglycoside; *Rhodiola quadrifida*; antiallergic activity; passive cutaneous anaphylaxis test; histamine release inhibitor

Chinese natural medicines originating in several alpine plants belonging to *Rhodiola* species (Crassulaceae) are given the generic name Rhodiolae Radix (紅景天). One of these, "Si Lie Hong Jing Tian (四裂紅景天)," which is prepared from the underground part of *Rhodiola* (*R.*) *quadrifida* (PALL.) FISCH. *et* MEY., has been prescribed for hemostatic, antibechic and tonic purposes in Chinese traditional preparations and used as an endermic liniment for burns and contusions. As chemical constituents of *R. quadrifida*, two flavonols (quercetin and kaempferol), two coumarins (scopoletin and umbelliferone), *p*-tyrosol, and rhodioloside were previously reported.[2]

As part of our characterization studies on the bioactive principles of natural medicines,[3] we have investigated antiallergic constituents using the *in vitro* peritoneal exudate cells bioassay testing inhibitory effect on histamine release and the *in vivo* passive cutaneous anaphylaxis (PCA) test. We recently reported several antiallergic constituents: isocoumarins (thunberginols A, B),[4] benzylidenephthalide (thunberginol F),[4] dihydroisocoumarins (thunberginols C, D, E, G),[5] phthalide (hydramacrophyllols A, B),[6] secoiridoid glucoside complexes (hydramacrosides A, B),[7] *ent*-isopimarane type diterpene ketones (trifoliones A, B, C, D),[8] pungent principles (6-gingerol, 6-shogaol),[9] and methyl-migrated secodammarane triterpene glycosides (hovenidulciosides A_1, A_2, B_1, B_2).[10] In the course of continuing survey for antiallergic compounds from natural medicines, we have found that the methanolic extract of the underground part of *R. quadrifida* inhibits histamine release from rat peritoneal exudate cells induced by antigen-antibody reaction. From the active methanolic extract, we have isolated two cyanoglycosides called rhodiocyanosides A (**1**) and B (**2**) and two oligoglycosides, rhodioflavonoside (**3**) and rhodiooctanoside (**4**), together with four known compounds: rhodioloside (**5**),[11] *n*-hexyl β-D-glucopyranoside (**6**),[12] gossypetin 7-*O*-α-L-rhamnoside (**7**),[13] and tricetin (**8**).[14] In this paper, we give a full account of the structure elucidations of **1**, **2**, **3**, and **4** and the antiallergic activity of **1** and **2**.[15]

The dried underground part of *R. quadrifida* was extracted with methanol under reflux. Since the methanol extract was found to exhibit the inhibitory activity on histamine release from rat peritoneal exudate cells induced by antigen-antibody reaction, it was subjected to further separation procedure. Thus the methanolic extract was separated by XAD-2 column chromatography in order to remove carbohydrate (the water-eluted fraction). The methanol-eluted fraction was subjected to repeated ordinary-phase silica-gel and reversed-phase silica-gel column chromatography and, finally, HPLC separation to afford **1** (0.113% from the natural medicine), **2** (0.009%), **3** (0.009%), **4** (0.096%),[16] **5** (0.016%), **6** (0.003%), **7** (0.001%), and **8** (0.001%).

Rhodiocyanosides A (1) and B (2) Rhodiocyanoside A (**1**) was isolated as a white powder. The IR spectrum of **1** showed absorption bands assignable to hydroxyl, nitrile, and olefin groups at 3410, 2222, 1655, and 1076 cm^{-1}, while an absorption maximum was observed at 208 nm ($\log \varepsilon$ 4.0) in the UV spectrum. The negative-mode and positive-mode FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 258 $(M-H)^-$ and *m/z* 260 $(M+H)^+$ and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{11}H_{17}NO_6$. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra of **1**, which were completely assigned by various NMR analytical experiments,[17] indicated the presence of a trisubstituted olefin [δ 6.46 (qdd, J=1.7, 6.3, 6.9 Hz, 3-H)] bonding with a

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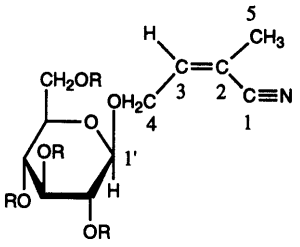
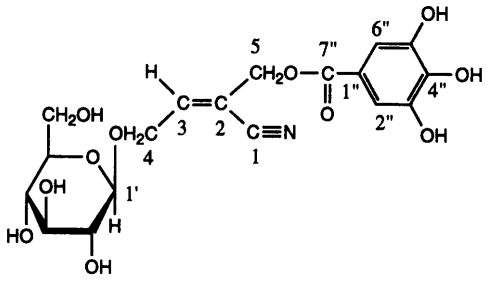


rhodiocyanoside A (**1**) : R=H
1a : R=Ac



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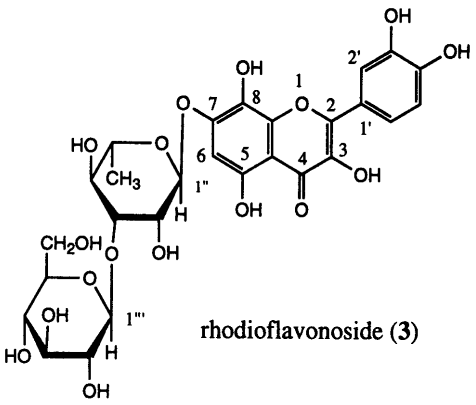
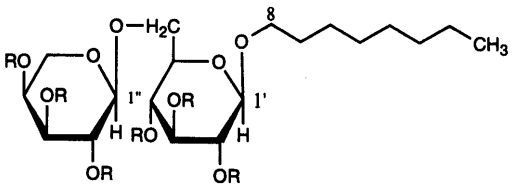
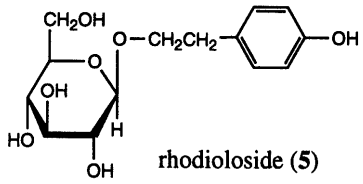


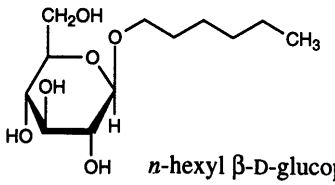
rhodioflavonoside (**3**)



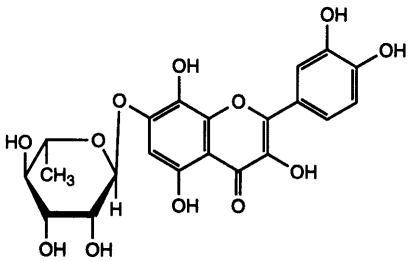
rhodiooctanoside (**4**) : R=H
4a : R=Ac



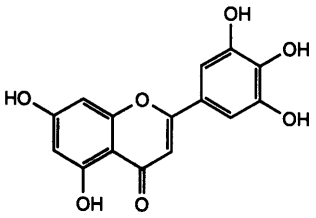
rhodioloside (**5**)



n-hexyl β-D-glucopyranoside (**6**)



gossypetin 7-*O*-α-L-rhamnopyranoside (**7**)



tricetin (**8**)

Chart 1

methyl [δ 1.93 (ddd, J=1.3, 1.3, 1.7 Hz, 5-H_3)], oxymethylene [δ 4.43 (qdd, J=1.3, 6.9, 13.5 Hz), 4.54 (qdd, J=1.3, 6.3, 13.5 Hz) (4-H_2)], and nitrile group together with a β-D-glucopyranosyl moiety [δ 4.30 (d, J=7.9 Hz, 1′-H)].

Acetylation of **1** with acetic anhydride in pyridine furnished the tetraacetate (**1a**). On enzymatic hydrolysis of **1** with naringinase, the aglycone, rhodiocyanogenin (**9**) was obtained, while methanolysis of **1** with 9% hydrogen chloride–dry methanol liberated methyl glucoside. Comparison of the ^{1}H-NMR and ^{13}C-NMR spectra for **1**, **1a**, and **9** with those for various known cyanoglycosides such as osmaronin (**10**)[18] led us to formulate the structure of **1** as 4-β-D-glucopyranosyloxy-2-methylbut-2-enenitrile. The geometric structure of the trisubstituted olefin in **1** was confirmed by the nuclear Overhauser effect spectros-

copy (NOESY) experiment of **1** and **9**. Namely, NOE correlations were observed between the 2-methyl group (5-H_3) and the 3-proton and between the 3-proton and the 4-methylene. Based on those findings, the structure of rhodiocyanoside A was determined to be 4-β-D-glucopyranosyloxy-2-methyl-2*Z*-butenenitrile (**1**).

Rhodiocyanoside B (**2**), obtained as a white powder, gave the quasimolecular ion peak at *m/z* 450 $(M+Na)^+$ in the positive-mode FAB-MS and the molecular composition was defined as $C_{18}H_{21}NO_{11}$ from the high-resolution MS analysis. In the IR spectrum of **2**, it showed absorption bands at 3410, 2230, 1714, 1620, 1529, and 1075 cm^{-1} ascribable to hydroxyl, nitrile, ester, and aromatic ring. The UV spectrum of **2** showed absorption maxima at 217 nm ($\log\varepsilon$ 4.5) and 279 nm ($\log\varepsilon$ 4.0), which suggested the presence of a galloyl group. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (CD_3OD)[17] spectra of **2** showed signals due to the 2-oxymethyl-4-oxy-2-butenenitrile moiety [δ 6.86 (dd, J=5.9, 6.2 Hz, 3-H), 4.53 (1H, dd, J=6.2, 14.5 Hz), 4.69 (1H, dd, J=5.9, 14.5 Hz) (4-H_2), 4.88 (s, 5-H_2)], β-D-glucopyranosyl part [δ 4.43 (d, J=7.6 Hz, 1′-H)], and galloyl part [δ 7.09 (s, 2″, 6″-H)]. The positions of a β-D-glucopyanosyl and galloyl group in **2** were clarified by the COLOC experiment of **2**, which showed long-range correlations between the carbonyl carbon (7″-C) of galloyl group and the 5-methylene protons and between the aromatic carbon (1′-C) of β-D-glucopyranosyl group and the 4-methylene protons. Finally, the geometric structure of the 2-butenenitrile moiety of **2** was determined by the NOESY experiment, which showed NOE correlations between the 5-H_2 and the 3-H and between the 3-H and the 4-H_2. On the basis of the above evidence and comparison of the ^{1}H-NMR and ^{13}C-NMR data for **2** with those for the known cyanoglycosides sarmentosin (**11**) and sutherlandin (**12**),[19] the structure of rhodiocyanoside B was characterized as 4-β-D-glucopyranosyloxy-2-galloyloxymethyl-2*Z*-butenenitrile (**2**).

Rhodioflavonoside (3) and Rhodiooctanoside (4) Rhodioflavonoside (**3**) was isolated as a yellow powder and

Table 1. Inhibitory Effect of *Rhodiola quadrifida* MeOH Extract on Histamine Release from Rat Peritoneal Exudate Cells Sensitized with Anti-DNP IgE

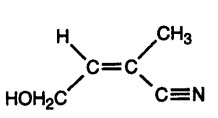
	Conc. (mg/ml)	*n*	Inhibition of histamine release (%)
R. quadrifida MeOH ext.	10	4	−2.34±1.41
	50	4	70.12±3.72
	100	4	97.95±1.54

Each value represents the mean±S.E. Peritoneal exudate cells were incubated with test samples for 15 min.

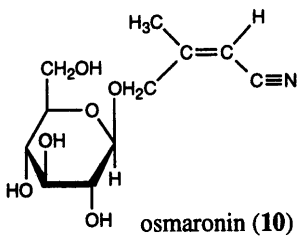
Table 2. ^{13}C-NMR Data for **1**, **1a**, **2**, and **9**[a]

	1	**1a**	**2**	**9**
C-1	118.2	118.0	116.2	118.2
C-2	112.6	113.0	113.0	111.1
C-3	145.0	143.7	148.9	147.8
C-4	68.4	68.2	68.2	61.5
C-5	20.3	20.3	64.6	20.3
C-1′	104.0	101.1	104.3	
C-2′	74.9	72.7	74.9	
C-3′	78.0	74.2	77.8	
C-4′	71.4	69.8	71.8	
C-5′	78.0	73.0	78.0	
C-6′	62.6	63.0	62.6	
C-1″			120.6	
C-2″			110.3	
C-3″			146.6	
C-4″			140.3	
C-5″			146.6	
C-6″			110.3	
C-7″			167.3	
OAc		20.6		
		20.6		
		20.6		
		20.7		
		171.1		
		171.2		
		171.6		
		172.3		

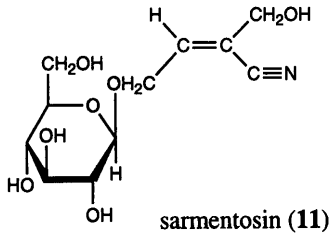
a) Compounds **1** and **9** were measured in CD_3OD, compound **1a** in $CDCl_3$, and compound **2** in DMSO-d_6.



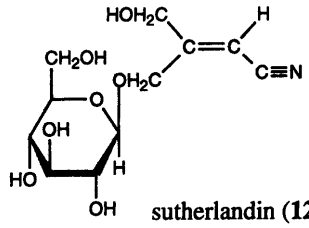
rhodiocyanogenin (**9**)



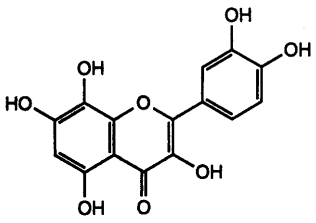
osmaronin (**10**)



sarmentosin (**11**)



sutherlandin (**12**)



gossypetin (**13**)

Chart 2

its IR and UV spectra indicated the flavonoid glycoside structure of **3**. The molecular formula $C_{27}H_{30}O_{17}$ of **3** was clarified from the quasimolecular ion peak [*m/z* 625 $(M-H)^-$] in the negative-mode FAB-MS and by high-resolution MS measurement. Methanolysis of **3** with 9% hydrogen chloride (HCl)–dry methanol (MeOH) liberated methyl rhamnoside and methyl glucoside, while enzymatic hydrolysis of **3** with naringinase furnished gossypetin (**13**).[20] The ^{1}H-NMR (dimethyl sulfoxide (DMSO)-d_6) and ^{13}C-NMR (DMSO-d_6)[17] of **3** showed signals due to a gossypetin moiety [δ 6.62 (s, 6-H), 6.93 (d, J=8.5 Hz, 5′-H), 7.68 (dd, J=2.0, 8.5 Hz, 6′-H), 7.83 (d, J=2.0 Hz, 2′-H); δ_C 149.4 (7-C)], α-L-rhamnopyranosyl part [δ 5.56 (br s, 1″-H); δ_C 80.8 (3′-C)], and β-D-glucopyranosyl part [δ 4.53 (d, J=7.3 Hz, 1‴-H)]. In the heteronuclear multiple bond correlation (HMBC) experiment of **3**, long-range correlations were observed between the 1‴-proton of β-D-glucopyranosyl part and the 3″-carbon of α-L-rhamnopyranosyl part and between the 1″-proton of α-L-rhamnopyranosyl part and the 7-carbon of gossypetin moiety. Finally, comparison of the physical data for **3** with those for gossypetin (**13**) and gossypetin 7-*O*-α-L-rhamnopyranoside (**7**) led us to characterize the structure of rhodioflavonoside as gossypetin 7-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranoside (**3**).

Rhodiooctanoside (**4**), obtained as a white powder, liberated 1-octanol, methyl glucoside, and methyl arabinoside upon methanolysis. Here again, the molecular formula $C_{19}H_{36}O_{19}$ of **4** was determined by the negative-mode FAB-MS [*m/z* 423 $(M-H)^-$] and high-resolution MS measurement. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (CD_3OD)[17] of **4** showed signals assignable to an 1-octanol moiety [δ 0.90 (t, J=6.4 Hz, 1-H_3), 1.61 (tdd, J=6.9, 6.9, 6.9 Hz, 7-H_2), 3.55, 3.83 (both m, 8-H_2)], β-D-glucopyranosyl part [δ 4.26 (d, J=7.6 Hz, 1′-H), 3.74 (dd, J=5.0, 11.4 Hz), 4.09 (br d, J=11.4 Hz) (6′-H_2)], and α-L-arabinopyranosyl part [δ 4.32 (d, J=6.6 Hz, 1″-H), δ_C 105.1 (1″-C)]. In the correlation spectroscopy *via* long-range coupling (COLOC) experiment of **4**, a C–H long-range correlation was observed between the anomeric carbon (1″-C) of α-L-arabinopyranosyl part and the 6′-methylene proton of the β-D-glucopyranosyl part. Ordinary acetylation of **4** yielded the hexaacetate (**4a**). On the basis of this evidence and examination of the physical data of **4a**, the structure of rhodiooctanoside was determined to be octyl α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**4**).

Inhibitory Effect of Rhodiocyanoside A (1) and B (2) on Histamine Release from Rat Peritoneal Exudate Cells Since the underground part of *Rhodiola quadrifida* has been used as an antibechic and endermic liniment for burns and contusions in Chinese traditional medicine, the components of this natural medicine were expected to show antiallergic activity. Inhibitory effect of rhodiocyanosides A (**1**) and B (**2**) on histamine release from rat peritoneal exudate cells is shown in Table 3. A major component of this Chinese natural medicine, **1** was found to inhibit the histamine release by 10^{-5} to 10^{-4} in a concentration-dependent manner. On the other hand, **2** exhibited only slight inhibitory effect, even at 10^{-4} M.

Inhibitory Effect of Rhodiocyanoside A (1) on Rat 48 h PCA Reaction Seeing that rhodiocyanoside A (**1**) showed inhibitory activity on histamine release from rat peritoneal cells, **1** was examined by *in vivo* PCA test. Intravenous injections of **1** significantly inhibited the PCA reaction as shown in Table 4. Injection of **1** at an interval of 60 min after the challenge exhibited greater inhibitory effect than 20 min afterward. Disodium cromoglycate (DSCG) was used as a reference drug.

Antiallergic activity of **1**, the major component of the underground part of *R. quadrifida*, may be preliminary evidence to substantiate the traditional effect of this natural medicine such as the antibechic effect.

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

Con. (M)	*n*	Inhibition of histamine release (%)	
		1	**2**
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.

Table 4. 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.

Experimental

The following instruments were used to obtain physical data : specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high resolution FAB-MS, JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC, Shimadzu LC-10AS chromatograph; GLC, Shimadzu GC-14A chromatograph.

The following experimental conditions were used for chromatography: ordinary-phase column chromatography; Silica-gel BW-200 (Fuji Silysia Chemical Ltd., 150—350 mesh), reversed-phase column chromatography; Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 100—200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary-phase) and Silica gel RP-18 60 F_{254} (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 60 WF_{254S} (Merck, 0.25 mm) (reversed-phase). Detection was done by spraying 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Bioassay Reagents : Mouse monoclonal anti-2,4-dinitrophenyl immunoglobulin E (anti-DNP IgE, PCA titer 1000000, Seikagaku Corporation), 2,4-dinitrophenylated bovine serum albumin (DNP-BSA, Cosmo Bio Co., Ltd.,Tokyo). Other reagents were purchased from Wako Pure Chemical Industries.

Extraction and Isolation The underground part (3.5 kg, purchased from Honzo Seiyaku Co., Ltd., Nagoya) was finaly minced and extracted three times with MeOH under reflux. Evaporation of the solvent from

the extract solution under reduced pressure gave the MeOH extract (227 g). This extract (217 g) was subjected to XAD-2 (Organo Co., Ltd., 2 kg, H_2O, MeOH) column chromatography to give the MeOH-eluted fraction (94.8 g), which was separated by silica gel column chromatography (2 kg, $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O) to afford six fractions [fraction 1 (4.3 g), fraction 2 (44.5 g), fraction 3 (2.4 g), fraction 4 (2.1 g), fraction 5 (26.3 g) and fraction 6 (19.1 g)].

Silica gel column chromatography (170 g, $CHCl_3$–MeOH) of fraction 2 (8.5 g) followed by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 15 g), H_2O–MeOH] yielded tricetin (**8**, 35.2 mg, 0.001%). Fraction 4 (2.1 g) was subjected to reversed-phase silica gel column chromatography (Chromatorex DM1020T, 40 g, H_2O–MeOH) and HPLC [YMC Pack R&D (YMC Co., Ltd.), 70% aqueous MeOH] to furnish *n*-hexyl β-D-glucopyranoside (**6**, 100.8 mg, 0.003%). Fraction 5 (22.2 g) was separated by silica gel column chromatography (1 kg, $CHCl_3$–MeOH–H_2O) to give five fractions [fraction 5-1 (2.4 g), fraction 5-2 (12.7 g), fraction 5-3 (2.4 g), fraction 5-4 (1.8 g), and fraction 5-5 (3.1 g)]. Fraction 5-2 (12.7 g) was further separated by reversed-phase silica gel (Chromatorex DM1020T, 250 g, H_2O–MeOH) and silica gel column chromatography (75 g, $CHCl_3$–MeOH–H_2O) and finally HPLC (YMC Pack R&D, H_2O–MeOH) to furnish rhodiocyanoside A (**1**, 3.9 g, 0.113%), rhodiooctanoside (**4**, 3.2 g, 0.096%), and rhodioloside (**5**, 569.6 mg, 0.016%). Silica gel (90 g, $CHCl_3$–MeOH–H_2O) and reversed-phase silica gel column chromatography (Chromatorex DM1020T, 20 g, H_2O–MeOH) of fraction 5-4 (1.8 g) yielded gossypetin 7-*O*-α-L-rhamnopyranoside (**7**, 35.2 mg, 0.001%). Fraction 5-5 (3.1 g) was subjected to silica gel (150 g, $CHCl_3$–MeOH–H_2O) and reversed-phase silica gel column chromatography (Chromatorex DM1020T, 30 g, H_2O–MeOH) and HPLC (YMC Pack R&D, H_2O–MeOH) to give rhodiocyanoside B (**2**, 329.2 mg, 0.009%) and rhodioflavonoside (**3**, 129.5 mg, 0.004%). The physical data for the known compounds (**5**—**8**) were identified by reported values.[11-14]

Rhodiocynoside A (**1**): A white powder, $[\alpha]_D^{25}$ −16.1° (c=0.4, MeOH). High-resolution negative-mode FAB-MS : Calcd for $C_{11}H_{16}NO_6$ $(M-H)^-$: 258.0978. Found: 258.0977. UV λ_{max}^{MeOH} nm (log ε): 208 (4.0). IR (KBr): 3410, 2222, 1655, 1076 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.93 (3H, ddd, J=1.3, 1.3, 1.7 Hz, 5-H_3), 3.19 (1H, dd, J=7.9, 8.9 Hz, 2′-H), 3.68 (1H, dd, J=5.0, 11.9 Hz), 3.87 (1H, dd, J=2.0, 11.9 Hz, 6′-H_2), 4.30 (1H, d, J=7.9 Hz, 1′-H), 4.43 (1H, qdd, J=1.3, 6.9, 13.5 Hz),4.54 (1H, qdd, J=1.3, 6.3, 13.5 Hz, 4-H_2), 6.46 (1H, qdd, J=1.7, 6.3, 6.9 Hz, 3-H). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 260 $(M+H)^+$. Negative-mode FAB-MS m/z: 258 $(M-H)^-$.

Rhodiocyanoside B (**2**): A white powder, $[\alpha]_D^{25}$ −12.2° (c=0.5, MeOH). High-resolution positive-mode FAB-MS : Calcd for $C_{18}H_{21}NaNO_{11}$ $(M+Na)^+$: 450.1032. Found: 450.0984. UV λ_{max}^{MeOH} nm (log ε): 279 (4.0), 217 (4.5). IR (KBr): 3410, 2230, 1714, 1620, 1529, 1075 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 4.43 (1H, d, J=7.6 Hz, 1′-H), 4.53 (1H, dd, J=6.2, 14.5 Hz), 4.69 (1H, dd, J=5.9, 14.5 Hz, 4-H_2), 4.88 (2H, s, 5-H_2), 6.86 (1H, dd, J=5.9, 6.2 Hz, 3-H), 7.09 (2H, s, 2′, 6′-H). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 450 $(M+Na)^+$.

Rhodioflavonoside (Gossypetin 7-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranoside, **3**): A yellow powder, $[\alpha]_D^{27}$ −50.8° (c=0.4, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{27}H_{29}O_{17}$ $(M-H)^-$: 625.1405. Found: 625.1375. UV λ_{max}^{MeOH} nm (log ε): 340 (3.9), 280 (4.1), 260 (4.2). IR (KBr): 3389, 1655, 1076 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.15 (3H, d, J=6.0 Hz, 6″-H_3), 3.15 (1H, m, 2‴-H), 3.16 (1H, m, 4‴-H), 3.23 (1H, m, 3‴-H), 3.25 (1H, m, 5‴-H), 3.50 (1H, m), 3.72 (1H, m, 6‴-H), 3.53 (1H, m, 4″-H), 3.65 (1H, m, 5″-H), 4.08 (1H, br d, J=8.9 Hz, 3″-H), 4.22, (1H, br s, 2″-H), 4.53 (1H, d, J=7.3, 1‴-H), 5.56 (1H, br s, 1″-H), 6.62 (1H, s, 6-H), 6.93 (1H, d, J=8.5 Hz, 5′-H), 7.68 (1H, dd, J=2.0, 8.5 Hz, 6′-H), 7.83 (1H, d, J=2.0 Hz, 2′-H), 11.92 (1H, br s, 5-OH). ^{13}C-NMR (DMSO-d_6) δ_C: 17.9 (6″-C), 60.9 (6‴-C), 68.9 (2″-C), 69.3 (5″-C), 69.7 (4‴-C), 70.4 (4″-C), 73.9 (2‴-C), 76.1 (5‴-C), 76.7 (3‴-C), 80.8 (3″-C), 99.1 (1″-C), 98.2 (6-C), 104.4 (10-C), 104.7 (1‴-C), 115.4 (2′-C), 115.5 (5′-C), 120.2 (6′-C), 122.1 (1′-C), 127.2 (8-C), 135.8 (3-C), 144.3 (9-C), 145.0 (3′-C), 147.3 (2-C), 147.8 (4′-C), 149.4 (7-C), 151.4 (5-C), 176.2 (4-C). Negative-mode FAB-MS m/z: 625 $(M-H)^-$.

Rhodiooctanoside (Octyl α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside, **4**): An amorphous powder, $[\alpha]_D^{24}$ −29.2° (c=0.8, MeOH). High-resolution negative-mode FAB-MS : Calcd for $C_{19}H_{35}O_{10}$ $(M-H)^-$: 423.2230. Found: 423.2224. IR (KBr): 3410, 1074 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.90 (3H, t, J=6.4 Hz, 1-H_3), 1.22—1.47 (10H, m, 2, 3, 4, 5, 6-H_2), 1.61 (2H, tdd, J=6.9, 6.9, 6.9 Hz, 7-H_2), 3.19 (1H, m, 2′-H), 3.32 (2H, m, 3′, 4′-H), 3.40 (1H, m, 5′-H), 3.53 (3H, m, 8, 3″, 5″-H), 3.62 (1H, m, 2″-H), 3.83 (3H, m, 8, 4″, 5″-H), 3.74 (1H, dd, J=5.0, 11.4 Hz), 4.09 (1H, br d, J=11.4 Hz) (6′-H_2), 4.26 (1H, d, J=7.6 Hz, 1′-H), 4.32 (1H, d, J=6.6 Hz, 1″-H). ^{13}C-NMR (CD_3OD) δ_C: 14.4 (1-C), 23.7 (2-C), 27.1, 30.4, 30.5, 33.0 (3, 4, 5, 6-C), 30.8 (7-C), 66.7 (5″-C), 69.4 (6′, 4″-C), 71.5 (4′-C), 71.8 (8-C), 72.3 (2″-C), 74.1 (3″-C), 75.0 (2′-C), 76.7 (5′-C), 77.9 (3′-C), 104.3 (1′-C), 105.1 (1″-C). Positive-mode FAB-MS m/z: 447 $(M+Na)^+$. Negative-mode FAB-MS m/z: 423 $(M-H)^-$.

Acetylation of Rhodiocyanoside A (1) A solution of **1** (82.0 mg) in pyridine (5.0 ml) was treated with Ac_2O (2.5 ml) and the reaction mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4, and filtered. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography (650 mg, *n*-hexane–AcOEt = 1 : 1) to yield rhodiocyanoside A tetraacetate (**1a**, 126.9 mg, 94%).

Rhodiocyanoside A Tetraacetate (**1a**): Colorless oil, $[\alpha]_D^{25}$ −10.4° (c=0.1, MeOH). IR (KBr): 2235, 1757, 1231, 1042 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.01 (6H, s), 2.03, 2.06, 2.10 (3H each, all s, Ac×4, 5-H_3), 4.17 (1H, dd, J=2.4, 12.3 Hz), 4.27 (1H, dd, J=4.8, 12.3 Hz, 6′-H_2), 4.42 (1H, qdd, J=1.4, 6.7, 12.2 Hz), 4.52 (1H, qdd, J=1.4, 6.7, 12.2 Hz, 4-H_2), 4.55 (1H, d, J=7.6 Hz, 1′-H), 6.24 (1H, qdd, J=1.4, 6.7, 6.7 Hz, 3-H). ^{1}H-NMR (CD_3OD) δ: 1.96 (3H, br s), 2.01, 2.02, 2.02, 2.06 (3H each, all s, 5-H_3, Ac×4), 4.17 (1H, dd, J=2.3, 12.2 Hz), 4.27 (1H, dd, J=4.6, 12.2 Hz, 6′-H_2), 4.44 (2H, qd-like, 4-H_2), 4.73 (1H, d, J=7.9 Hz, 1′-H), 6.37 (1H, qt-like, 3-H). ^{13}C-NMR: given in Table 2. Negative-mode FAB-MS m/z: 426 $(M-H)^-$.

Enzymatic Hydrolysis of Rhodiocyanoside A (1) with Naringinase A solution of **1** (21.0 mg) in acetate buffer (pH 3.8, 5 ml) was treated with naringinase (SIGMA Chemical Co., 10 mg) and the entire solution was left standing at 38 °C for 12 h. The reaction solution was separated by silica gel column chromatography (2 g, $CHCl_3$–MeOH = 10 : 1) to give rhodiocyanogenin (**9**, 4.0 mg).

Rhodiocyanogenin (**2**): Colorless oil. High-resolution EI-MS: Calcd for C_5H_7NO (M^+): 97.0531. Found: 97.0527. UV λ_{max}^{MeOH} nm (log ε): 207 (3.8). IR (film): 3300, 2222, 1655 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.96 (3H, tq, J=1.0, 1.3 Hz, 5-H_3), 4.26 (2H, dq, J=1.0, 6.6 Hz, 4-H_2), 6.36 (1H, tq, J=1.3, 6.6 Hz, 3-H). ^{13}C-NMR: given in Table 2. EI-MS m/z: 97 (M^+).

Methanolysis of Rhodiocyanoside A (1) with 9% HCl–Dry MeOH A solution of **1** (1.0 mg) in 9% HCl–dry MeOH (0.1 ml) was heated under reflux for 4 h. After cooling, the reaction solution was neutralized with Ag_2CO_3 powder and was subjected to TLC [$CHCl_3$–MeOH–H_2O = 65 : 35 : 10, 10 : 3 : 1 (lower layer)] analysis to identify methyl glucoside.

Methanolysis of Rhodioflavonoside (3) and Rhodiooctanoside (4) i) Methanolysis of **3** (1.2 mg) in 9% HCl–dry MeOH (0.1 ml) was heated under reflux for 4 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the insoluble part was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was dissolved in pyridine (0.01 ml) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 ml) and the whole mixture was left standing for 1 h. The reaction mixture was subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (**a**) and methyl rhamnoside (**b**). GLC conditions: column, CBR1-M25-025 [0.25 mm (i.d.) × 25 m]; injector temp., 140 °C; detector temp., 280 °C; column temp., 140—240 °C, 5 °C/min; initial time, 5 min. He flow rate, 15 ml/min. t_R: **a**, 22.3, 23.7 min; **b**, 16.1, 16.5 min.

ii) Methanolysis of **4** (0.8 mg) in 9% HCl–dry MeOH (0.1 ml) was heated under reflux for 2.5 h. Workup of the reaction mixture as described above gave a product which was subjected to GLC analysis (the same conditions as described above) to identify the TMS derivatives of methyl glucoside (**a**) and methyl arabinoside (**c**) [t_R: **a**, 22.3, 23.7 min; **c**, 15.3, 15.4, 15.7 min).

Enzymatic Hydrolysis of Rhodioflavonoside (3) A solution of **3** (18.1 mg) in acetate buffer (pH 3.8, 5 ml) was treated with naringinase (9.0 mg) and the whole solution was left standing at 38 °C for 12 h. The reaction solution was extracted with AcOEt and the AcOEt extract was washed with brine, and then dried over Na_2SO_4, and filtered. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography (1 g, $CHCl_3$–MeOH = 10 : 1) to give

gossypetin (**13**), which was identified by comparison of the physical data with reported values.[20]

Acetylation of Rhodiooctanoside (4) A solution of **4** (7.7 mg) in pyridine (1.0 ml) was treated with Ac_2O (0.5 ml) and the reaction mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4, and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography (1 g, *n*-hexane–AcOEt = 1 : 1) to yield rhodiooctanoside hexaacetate (**4a**, 10.2 mg, 83%).

Rhodiooctanoside Hexaacetate (**4a**): A white powder. IR (KBr): 1760, 1055 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J = 6.6 Hz, 1-H_3), 1.96, 2.00, 2.01, 2.05, 2.08, 2.12 (3H each, all s, Ac × 6). ^{13}C-NMR (CD_3OD) δ: 14.4 (1-C), 20.6, 20.6, 20.6, 20.8, 20.8, 20.9 (Ac × 6), 23.7 (2-C), 27.0, 30.4, 30.4, 30.6 (3, 4, 5, 6-C), 33.0 (7-C), 64.1 (5″-C), 68.3 (4″-C), 69.3 (6′-C), 70.3 (4′-C),* 70.5 (2″-C),* 70.9 (8-C), 71.7 (3″-C), 72.9 (2′-C), 73.9 (5′-C), 74.6 (3′-C), 101.7 (1′-C), 101.9 (1″-C), 171.1, 171.2, 171.4, 171.6, 171.7, 171.9 (Ac × 6). *Assignments may be interchangeable.

Histamine Release from Rat Peritoneal Exudate Cells This experiment was performed according to the method previously reported.[4c,21] Briefly, the peritoneal exudate cells from male Wistar rats weighing 350 to 500 g were sensitized with diluted anti-DNP IgE (× 100) at 37 °C for 1 h. The cell suspension (10^4/1.62 ml) and 180 ml of test compound were preincubated for 15 min; 200 ml of phosphatidylserin (1 mg/ml) and 222 μl of DNP-BSA (1 mg/ml) were added at the same time. The incubation was continued for 20 min. The test tube was dropped into an ice-cold bath to stop the reaction. The supernatant was obtained by centrifugation for 10 min at 100 × *g*, 4 °C, and the histamine concentration was measured by the method of Imada *et al.*[22]

PCA Reaction The method used in the experiment was similar to that reported previously.[21] Briefly, 0.1 ml of diluted anti-DNP IgE (× 6250) was injected intradermally on the dorsal of male Wistar rats weighing about 200 g. After 48 h, the rats were challenged by an intravenous injection of 10% Evans blue containing 0.75 mg of DNP-BSA. Thirty minutes after the challenge, the rats were killed and the skin was removed. The area of bluing spot was measured by a planimeter. Testing compounds were injected intravenously at 20 or 60 min prior to the challenge.

Statistical Analysis Each value represents the mean and standard error. Statistical significance was estimated by analysis of variance (ANOVA) followed by Dunnet's test.

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