Bioactive Constituents of Chinese Natural Medicines. IV.[1] Rhodiolae Radix. (2).: On the Histamine Release Inhibitors from the Underground Part of *Rhodiola sacra* (PRAIN ex HAMET) S. H. Fu (Crassulaceae): Chemical Structures of Rhodiocyanoside D and Sacranosides A and B

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The methanolic extract of the underground part of *Rhodiola sacra* (PRAIN ex HAMET) S. H. Fu was found to show inhibitory activity on the histamine release from rat peritoneal exudate cells induced by an antigen–antibody reaction. From the methanolic extract with the inhibitory activity on histamine release, a new cyanoglycoside called rhodiocyanoside D and two new monoterpene glycosides called sacranosides A and B were isolated, together with eight known compounds, rhodiocyanoside A, heterodendrin, lotaustralin, rhodioloside, 2-phenylethyl α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside, 2-methyl-3-buten-2-yl β-D-glucopyranoside, kenposide A, and rhodiooctanoside. The structures of new compounds were determined on the basis of chemical and physicochemical evidence, which included the synthesis of sacranoside A from (−)-myrtenol. All major chemical constituents from *R. sacra* inhibited the histamine release and, among them, lotaustralin and rhodiooctanoside were found to show potent inhibitory activity.

Key words rhodiocyanoside D; sacranoside; *Rhodiola sacra*; histamine release inhibitor; cyanoglycoside; Rhodiolae Radix

Rhodiolae Radix (Chinese name "紅景天"), which originates in alpine plants belonging to the genus *Rhodiola* (Crassulaceae), has been used as a hemostatic, antibechic, tonic, and endemic-liniment for burns and contusions. Recently, we have characterized the structures of two cyanoglycosides, rhodiocyanosides A and B, and oligoglycosides, rhodioflavonoside and rhodiooctanoside, from the underground part of *Rhodiola* (*R.*) *quadrifida* (PALL.) FISCH. et MAY (四裂紅景天), and reported the antiallergic effect of rhodiocyanosides A and B.[2] Furthermore, we have isolated rhodiocyanoside C and crassusoside-a from another Rhodiola Radix originating in Sichuan Province, together with lotaustralin, eugenyl glucoside, naringenin, aromadendrin, pallasiin, and 5,7,3′,4′,5′-pentahydroxyflavonone.[3] As part of our characterization studies on the antiallergic constituents of Chinese natural medicines[4] and medicinal foodstuffs,[5] we have investigated the underground part of *R. sacra* (PRAIN ex HAMET) S. H. Fu (全弁紅景天),[6] which is listed in Dictionary of Chinese Materia Medica (中薬大辞典) as an important and representative Rhodiolae Radix. As chemical constituents of *R. sacra*, umbelliferone, tyrosol, kaempferol, rhodioloside, β-sitosterol, and organic acids were previously reported.[7] The methanolic extract of this natural medicine was found to show inhibitory activity on the histamine release from rat peritoneal exudate cells induced by an antigen–antibody reaction. From the active methanolic extract we have isolated a new cyanoglycoside called rhodiocyanoside D (**1**) and two new monoterpene glycosides called sacranosides A (**2**) and B (**3**), together with a cyanoglycoside, rhodiocyanoside A (**4**),[2] two cyanogenic glycosides, heterodendrin (**5**)[8] and lotaustralin (**6**),[9] two phenethyl glycosides, rhodioloside (**7**)[10] and 2-phenylethyl α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**8**),[11] and three aliphatic glycosides, 2-methyl-3-buten-2-yl β-D-glucopyranoside (**9**),[12] kenposide A (**10**),[13] and rhodiooctanoside (**11**).[2] In this paper, we describe the structural determination of rhodiocyanoside D (**1**) and sacranosides A (**2**) and B (**3**), and the inhibitory activity of the histamine release of the constituents from the underground part of *R. sacra*.[14]

The air-dried underground part of *R. sacra*[6] was extracted with methanol under reflux. As is apparent from Table 1, the methanolic extract was found to exhibit the inhibitory activity on the histamine release from rat peritoneal exudate cells induced by an antigen–antibody reaction. The methanol extract was separated by XAD-2 column chromatography in order to remove carbohydrates (the water-eluted fraction). The methanol-eluted fraction was subjected to normal-phase silica-gel column chromatography to provide eight fractions (fraction 1—8). Fractions 3—6 were separated by normal and reversed-phase silica-gel column chromatography and then HPLC to give **1** (0.0029%), **2** (0.0046%), and **3** (0.0037%), together with **4** (0.029%), **5** (0.0038%), **6** (0.0012%), **7** (0.00067%), **8** (0.10%), **9** (0.0029%), **10** (0.029%), and **11** (0.0056%).

Rhodiocyanoside D (1) Rhodiocyanoside D (**1**) was isolated as a white powder of negative optical rotation ($[\alpha]_D^{25}$ −14.1°). The negative-ion and positive-ion FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 258 $(M-H)^-$, 260 $(M+H)^+$, and 282 $(M+Na)^+$, and high-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ revealed the molecular formula of **1** to be $C_{11}H_{17}NO_6$. The IR spectrum of **1** showed absorption bands assignable to hydroxy, nitrile, and olefin functions at 3401, 2224, 1655, and 1076 cm^{-1}, while an absorption maximum was observed at 211 nm (log ε 4.8) in the UV spectrum. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 2) spectra of **1**, which was assigned by various NMR

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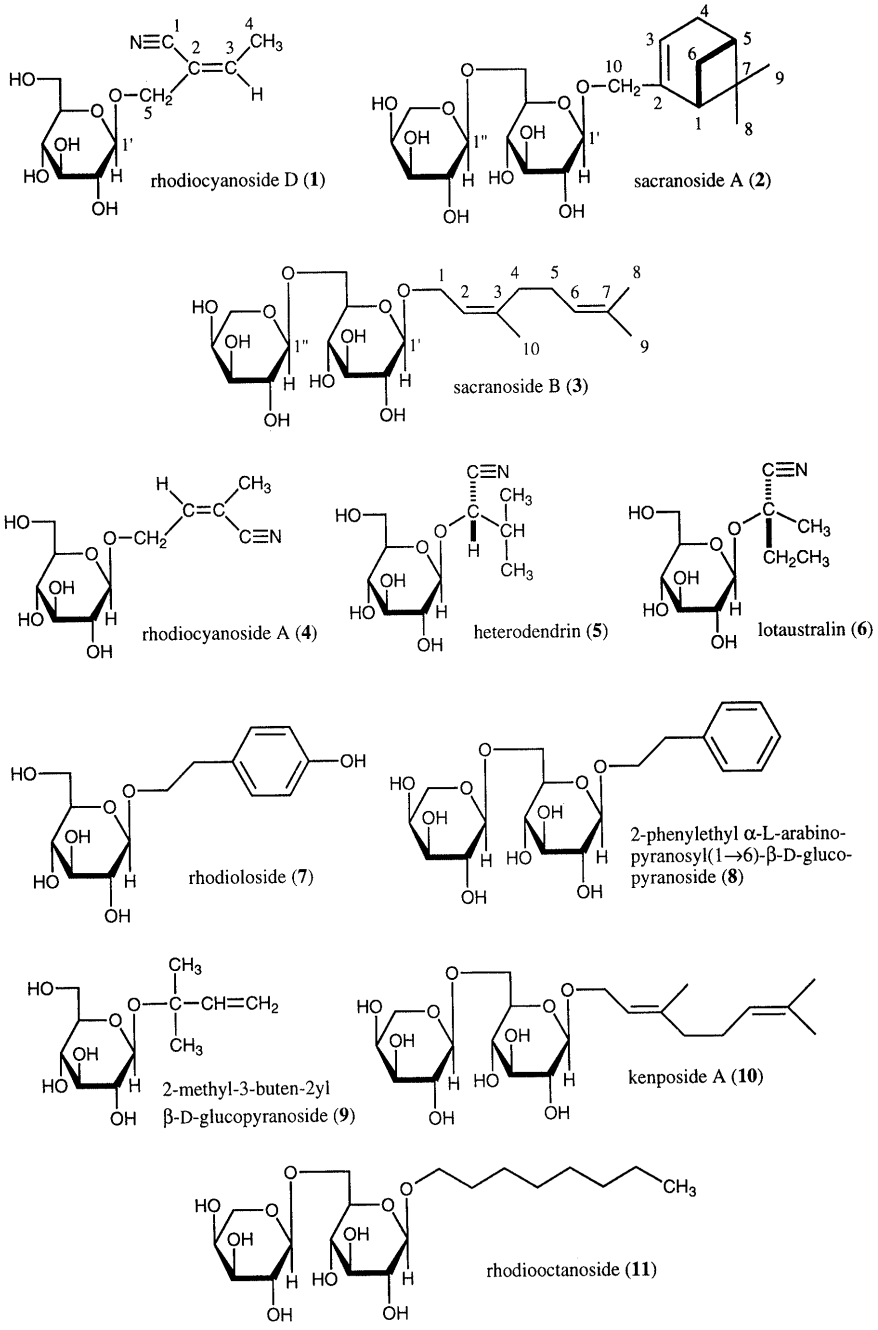

Chart 1

Table 1. Inhibitory Effect of the MeOH Ext. from *R. sacra* on Histamine Release from Rat Exudate Cells Induced by an Antigen-Antibody Reaction

	Conc. (μg/ml)	*n*	Inhibition of histamine release (%)
R. sacra MeOH ext.	10	4	55.8± 2.9
	50	4	107.7± 6.2
	100	4	105.2±10.9

analytical methods,[15] showed a tri-substituted olefin [δ 6.72 (qdd-like, 3-H)] bonding with a methyl [δ 2.02 (ddd, J=1.0, 1.0, 6.9 Hz, 4-H_3)], oxymethylene [δ 4.24, 4.41 (both m, 5-H_2)], and nitrile groups and a β-D-glucopyranosyl moiety [δ 4.31 (d, J=7.9 Hz, 1′-H)]. In the heteronuclear multiple bond correlation (HMBC) experiment of **1**, a long-range correlation was observed between the anomeric proton and the 5-oxymethylene carbon. The geometric structure of **1** was characterized to be a *Z* form by the ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) experiment of **1**, in which NOE correlations were observed between the following protons: 3-H and 4-H_3, 3-H and 5-H_2. On enzymatic hydrolysis of **1** with β-glucosidase, 2-hydroxymethyl-2*Z*-butenenitrile[16] was obtained as the aglycone, while acid hydrolysis of **1** with 5%H_2SO_4–dioxane (1 : 1) liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolidine derivative.[17] Consequently, rhodiocyanoside D was determined to be 2-(β-D-glucopyranosyloxymethyl)-2*Z*-butenenitrile (**1**).

Sacranosides A (2) and B (3) Sacranoside A (**2**) was obtained as a white powder. The IR spectrum of **2** showed

absorption bands due to hydroxyl and olefin functions at 3422 and 1655 cm^{-1}. In the negative-ion and positive-ion FAB-MS of **2**, quasimolecular ion peaks were observed at m/z 445 $(M-H)^-$ and 469 $(M+Na)^+$, respectively, and the molecular formula $C_{21}H_{34}O_{10}$ of **2** was determined by high-resolution MS measurement. The acid hydrolysis of **2** yielded L-arabinose and D-glucose, which were also identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[17] The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 2) spectra of **2**, which were completely assigned by means of various NMR analytical methods,[15] showed the presence of a monoterpene moiety [δ 0.87, 1.30 (both s, 8, 9-H_3), 4.00 (dd, J=1.7, 12.5 Hz), 4.20 (dd, J=1.3, 12.5 Hz)(10-H_2), 5.58 (tt-like, 3-H)], a β-D-glucopyranosyl moiety [δ 4.28 (d, J=7.6 Hz, 1′-H), 3.73 (dd, J=5.1, 11.6 Hz), 4.08 (dd, J=2.0, 11.6 Hz) (6′-H_2)], and an α-L-arabinopyranosyl moiety [δ 4.32 (d, J=6.6 Hz, 1″-H)]. The carbon signals of the disaccharide moiety in the ^{13}C-NMR (Table 2) of **2** were superimposable on those of **8**, kenposide A (**10**), and rhodiooctanoside (**11**), whereas the carbon signals of the aglycone part were very similar to those of (−)-myrtenol (**12**)[18] except for the signals due to the 3 and the 10-carbons, which showed a glycosylation shift.[19]

Table 2. ^{13}C-NMR Data of Rhodiocyanoside D (**1**) and Sacranosides A (**2**) and B (**3**)

	1	2	3
C-1	118.3	44.6	69.3
C-2	114.8	146.3	122.5
C-3	148.9	121.0	141.9
C-4	17.9	32.3	33.1
C-5	70.4	42.2	27.8
C-6		32.6	125.0
C-7		39.0	132.9
C-8		21.6	17.9
C-9		26.7	26.0
C-10		72.9	23.7
Glc-1′	104.0	103.5	103.1
2′	75.7	75.1	75.1
3′	78.8	78.0	78.0
4′	72.4	71.7	71.5
5′	78.9	76.9	76.8
6′	63.6	69.5	69.3
Ara-1″		105.2	105.1
2″		72.4	72.4
3″		74.2	74.2
4″		69.5	69.5
5″		66.7	66.7

The spectra were taken in CD_3OD (68 MHz).

In the HMBC experiment of **2**, long-range correlations were observed between the 1′-proton of the β-D-glucopyranosyl part and the 10-carbon and between the 1″-proton of α-L-arabinopyranosyl part and 6′-carbon of the β-D-glucopyranosyl part. Finally, the absolute stereostructure of **2** was confirmed by synthesis from (−)-myrtenol (**12**) (Chart 2). Thus, glycosidation of **12** with *O*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)trichloroacetimidate[20] in the presence of boron trifluoride etherate ($BF_3 \cdot Et_2O$) in chloroform furnished 2′,3′,4′,6′-*O*-acetyl-β-D-glucopyranosyl (−)-myrtenol, which was subjected to a deacetylation reaction with 10% aqueous potassium carbonate to give β-D-glucopyranosyl (−)-myrtenol (**13**). Monomethoxytritylation (MMTr) of **13**, followed by acetylation and detritylation, yielded 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyl (−)-myrtenol (**14**), which was subjected to glycosidation with *O*-(2,3,4-tri-*O*-acetyl-L-arabinopyranosyl)trichloroacetimidate[20] followed by deacetylation to provide sacranoside A (**2**). On the basis of the above evidence, the structure of sacranoside A was determined to be (−)-myrtenol α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**2**).

Sacranoside B (**3**), also isolated as a white powder, gave quasimolecular ion peaks at m/z 447 $(M-H)^-$ and 471 $(M+Na)^+$ in the negative-ion and positive-ion FAB-MS, and the molecular composition was defined as $C_{21}H_{36}O_{10}$ from the high-resolution FAB-MS analysis. The acid hydrolysis of **3** liberated L-arabinose and D-glucose, which were identified by GLC analysis of the trimethylsilyl thiazolidine derivatives.[17] The ^{1}H-NMR and ^{13}C-NMR (Table 2) spectra[15] showed signals due to a nerol moiety [δ 1.61, 1.67 (both s, 8, 9-H_3), 1.75 (d, J=0.7 Hz, 10-H_3),

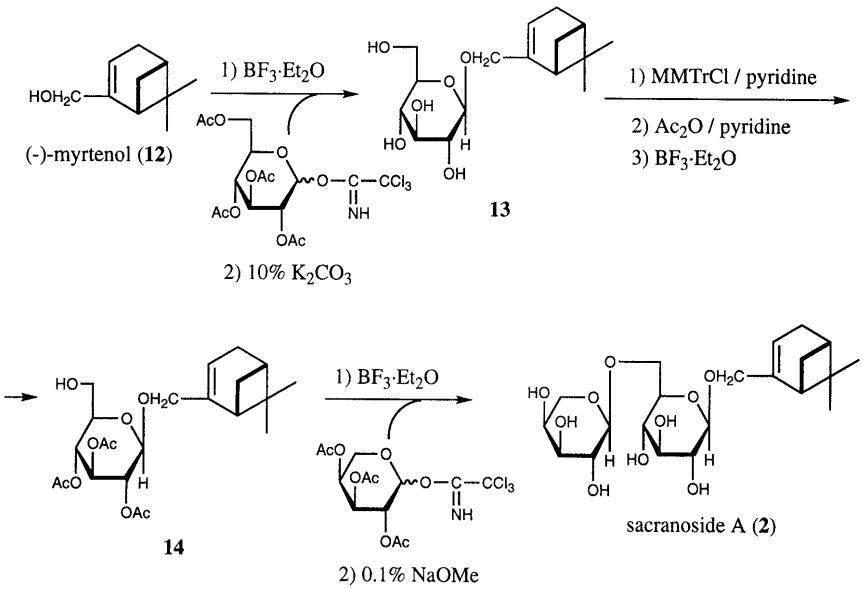

Chart 2

Table 3. Inhibitory Effects of Major Constituents (**1**—**3**, **5**, **6**, **8**, **10**, **11**) from *R. sacra* on Histamine Release from Rat Peritoneal Exudate Cells Induced by an Antigen–Antibody Reaction

	n	Inhibition of histamine release (%)	
		10^{-5} M	10^{-4} M
Rhodiocyanoside C (**1**)	4	20.6±10.4	47.4±4.6
Sacranoside A (**2**)	4	14.2± 3.9	30.5±6.0
Sacranoside B (**3**)	4	8.2±16.5	43.2±6.7
Heterodendrin (**5**)	4	27.9± 4.3	35.3±5.4
Lotaustralin (**6**)	4	75.5± 7.4	65.9±8.5
2-Phenylethyl glycoside (**8**)	4	7.7± 2.2	32.8±1.2
Kenposide A (**10**)	4	11.2± 5.2	35.6±7.1
Rhodiooctanoside (**11**)	4	44.9± 5.7	71.4±6.1
Amlexanox	4	11.8± 3.4	61.2±3.1

Means±S.E.

4.13 (m, 1-H_2), 5.11 (t-like, 6-H), 5.37 (br t, J=6.4 Hz, 2-H)], a β-D-glucopyranosyl part [δ 4.27 (d, J=7.6 Hz, 1′-H)], and an α-L-arabionopyranosyl part [δ 4.30 (d, J=6.6 Hz, 1″-H)]. The carbon signals of the disaccharide moiety in the ^{13}C-NMR (Table 2) of **3** were superimposable on those of **2**, whereas the carbon signals of the aglycone part were similar to those of nerol. Furthermore, HMBC long-range correlations were observed between the 1′-proton and the 1-carbon and between the 1″-proton and the 6′-carbon. Comparisons of the ^{1}H-NMR and ^{13}C-NMR data for **3** with those for kenposide A (**10**) led us to formulate the structure of sacranoside B as nerol α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**3**).

Inhibitory Effect of Chemical Constituents of *R. sacra* on the Histamine Release from Rat Peritoneal Exudate Cells As part of our characterization studies of the antiallergic constituents of medicinal foodstuffs[5] and natural medicines,[4] we recently isolated rhodiocyanosides A and B from the underground part of *R. quadrifida*, and those cyanoglycosides were found to show inhibitory activity on the histamine release from rat exudate cells.[2] As an extension of this work, we have continued to examine the antiallergic constituent of *R. sacra*. Since the methanolic extract of *R. sacra* showed potent inhibitory activity on the histamine release from rat exudate cells induced by an antigen–antibody reaction (Table 1), the chemical constituents of *R. sacra* were expected to exhibit antiallergic activity. As shown in Table 3, all major constituents (**1**—**3**, **5**, **6**, **8**, **10**, **11**) were found to inhibit histamine release. Particularly, lotaustralin (**6**) and rhodiooctanoside (**11**) showed potent inhibitory activity.

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, MS, and high-resolution 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: normal-phase column chromatography, Silica gel BW-200 (Fuji Silysia Chemical Ltd., 150—350 mesh), Cosmosil 75 C_{18}-OPN (Nakalai Tesque Co., Ltd., 75 μm); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 60F_{254} (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 60WF_{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 100000, Seikagaku Corporation) and 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) of *R. sacra*[6] was minced and extracted three times with MeOH under reflux. Evaporation of the solvent from the extract solution under reduced pressure gave the MeOH extract (1050 g). This extract (312 g) was separated by XAD-2 (Organo Co., Ltd., 2 kg, H_2O, MeOH) column chromatography to give a MeOH-eluted fraction (45 g), which was separated by normal-phase silica-gel column chromatography (1 kg, $CHCl_3$–MeOH) to afford eight fractions [fr 1 (6.4 g), fr 2 (2.5 g), fr 3 (3.3 g), fr 4 (2.5 g), fr 5 (8.6 g), fr 6 (2.0 g), fr 7 (0.4 g), fr 8 (7.8 g)]. Fraction 3 (3.3 g) was further subjected to normal-phase silica-gel (160 g, $CHCl_3$–MeOH–H_2O) and reversed-phase silica-gel column chromatography (5 g, MeOH–H_2O) and then HPLC [YMC-pack ODS-A, MeOH–H_2O (15 : 85, v/v)] to give rhodiocyanosides D (**1**, 30 mg), A (**4**, 306 mg), heterodendrin (**5**, 39 mg), lotaustralin (**6**, 12 mg), and 2-methyl-3-buten-2-yl β-D-glucopyranoside (**9**, 30 mg). Fraction 4 (2.5 g) was separated by reversed-phase silica-gel (49 g, MeOH–H_2O) and HPLC [YMC-pack ODS-A, MeOH–H_2O (60 : 40, v/v)] to furnish sacranosides A (**2**, 16 mg), B (**3**, 18 mg), rhodioloside (**7**, 7 mg), and kenposide A (**10**, 143 mg). Reversed-phase silica-gel column chromatography (40 g, MeOH–H_2O) of fraction 5 (8.6 g) yielded 2-phenylethyl α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**8**, 641 mg) and the glycoside mixture, which was subjected to HPLC separation [YMC-pack ODS-A, MeOH–H_2O (60 : 40, v/v)] to furnish **2** (32 mg), **3** (20 mg), **10** (164 mg), and rhodiooctanoside (**11**, 58 mg). Fraction 6 (2.0 g) was subjected to normal-phase silica-gel column chromatography (100 g, $CHCl_3$–MeOH–H_2O) to give **8** (424 mg). Three known compounds (**4**, **7**, **11**) were identified by comparison of TLC behavior, ^{1}H-NMR, and ^{13}C-NMR spectra with those of authentic samples,[2] while other known compounds (**5**, **6**, **8**—**10**) were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-NMR, and ^{13}C-NMR spectra) with reported values.[8-13]

Rhodiocyanoside D (**1**): A white powder, $[\alpha]_D^{25}$ −14.1° (c=0.4, MeOH), High-resolution positive-ion FAB-MS: Calcd for $C_{11}H_{17}NNaO_6$ $(M+Na)^+$: 282.0953; Found: 282.0970. UV λ_{max}^{MeOH} nm: 211 (log ε 4.8). IR (KBr) cm^{-1}: 3401, 2924, 2884, 2224, 1655, 1076. ^{1}H-NMR (CD_3OD) δ: 2.02 (3H, ddd, J=1.0, 1.0, 6.9 Hz, 4-H_3), 3.67 (1H, dd, J=5.0, 11.9 Hz), 3.87 (1H, br d, J=11.9 Hz) (6′-H_2), 4.24, 4.41 (1H each, both m, 5-H_2), 4.31 (1H, d, J=7.9 Hz, 1′-H), 6.72 (1H, qdd-like, 3-H). ^{13}C-NMR: given in Table 2. Negative-ion FAB-MS (m/z): 258 $(M-H)^-$. Positive-ion FAB-MS (m/z): 260 $(M+H)^+$, 282 $(M+Na)^+$.

Sacranoside A (**2**): A white powder, $[\alpha]_D^{25}$ −33.9° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{34}NaO_{10}$ $(M+Na)^+$: 469.2050; Found: 469.2043. IR (KBr) cm^{-1}: 3422, 2921, 1655, 1076, 1046. ^{1}H-NMR (CD_3OD) δ: 0.87, 1.30 (3H each, both s, 8, 9-H_3), 1.18, 2.42 (1H each, both m, 6-H_2), 2.08 (1H, m, 5-H), 2.24 (1H, t, J=5.6 Hz, 1-H), 2.28 (2H, m, 4-H_2), 3.73 (1H, dd, J=5.1, 11.6 Hz), 4.08 (1H, dd, J=2.0, 11.6 Hz) (6′-H_2), 4.00 (1H, dd, J=1.7, 12.5 Hz), 4.20 (dd, J=1.3, 12.5 Hz) (10-H_2), 4.28 (1H, d, J=7.6 Hz, 1′-H), 4.32 (1H, d, J=6.6 Hz, 1″-H), 5.58 (1H, tt-like, 3-H). ^{13}C-NMR: given in Table 2. Negative-ion FAB-MS (m/z): 445 $(M-H)^-$. Positive-ion FAB-MS (m/z): 469 $(M+Na)^+$.

Sacranoside B (**3**): A white powder, $[\alpha]_D^{25}$ +11.6° (c=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{36}NaO_{10}$ $(M+Na)^+$: 471.2206; Found: 471.2216. IR (KBr) cm^{-1}: 3407, 2926, 1655, 1649, 1086. ^{1}H-NMR (CD_3OD) δ: 1.61 (3H, s, 8-H_3), 1.67 (3H, s, 9-H_3), 1.75 (3H, d, J=0.7 Hz, 10-H_3), 2.09 (4H, m, 4, 5-H_2), 3.53 (1H, dd, J=1.7, 12.5 Hz), 3.86 (1H, dd, J=3.3, 12.5 Hz) (5″-H_2), 3.73 (1H, dd, J=4.5, 11.9 Hz), 4.07 (1H, br d, J=11.9 Hz) (6′-H_2), 4.13 (1H, m, 1-H_2), 4.16 (1H, dd, J=7.6, 11.9 Hz), 4.32, (1H, d, J=11.9 Hz) (1-H_2), 4.27 (1H, d, J=7.6 Hz, 1′-H), 4.30 (1H, d, J=6.6 Hz, 1″-H), 5.11 (1H, t-like, 6-H), 5.37 (1H, br t, J=6.4 Hz, 2-H). ^{13}C-NMR: given in Table 2. Negative-ion FAB-MS (m/z): 447 $(M-H)^-$. Positive-ion FAB-MS (m/z): 471 $(M+Na)^+$.

Enzymatic Hydrolysis of Rhodiocyanoside D (1) with β-Glucosidase A solution of **1** (6.3 mg) in acetate buffer (pH 4.4, 1.4 ml) was treated with

β-glucosidase (Sigma Chemical Co., 6.3 mg) and the whole solution was left standing at 38 °C for 18 h. The reaction solution was poured into water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine and dried over $MgSO_4$ powder, then filtered. After removal of the solvent under reduced pressure, the residue was separated by normal-phase silica-gel column chromatography [1 g, *n*-hexane–AcOEt (20 : 1)] to give 2-hydroxymethyl-2*Z*-butenenitrile (2.0 mg, 84.8%), which was identified by comparison of its physical data with the reported values.[16]

Acid Hydrolysis of Rhodiocyanoside D (1), Sacranosides A (2) and B (3) i) A solution of **1** (2.0 mg) in 5% H_2SO_4–dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was extracted with AcOEt. The water layer was neutralized with Amberlite IRA-400 and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was dissolved in pyridine (0.02 ml) and L-cysteine methyl ester hydrochloride (1.0 mg) and the whole mixture was stirred at 60 °C for 1 h. The reaction was then treated with *N,O*-bis(trimethylsiliy)trifluoroacetamide (BSTFA, 0.01 ml) and the whole was stirred at 60 °C for 1 h. The reaction mixture was subjected to GLC analysis to identify the trimethylsilyl (TMS) thiazolidine derivative of D-glucose (**a**). GLC conditions: column, Supelco STB™-1 [0.25 mm(i.d.) × 30 m]; injector temp., 230 °C; detector temp., 230 °C; column temp., 230 °C. He flow rate; 15 ml/min. [t_R: **a**, 24.2 min].

i) A solution of **2** (2.0 mg) or **3** (2.0 mg) in 5% H_2SO_4–dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 2.5 h. Work-up of the reaction mixture as described above gave a product which was then subjected to GLC analysis (the same conditions as described above) to identify the TMS thiazolidine derivatives of D-glucose (**a**) and L-arabinose (**b**) [t_R: **a**, 24.2 min, **b**, 15.0 min].

Glycosidation of (−)-Myrtenol (12) A solution of **12** (Aldrich Chemical Company, Inc., 300 m g) in dry $CHCl_3$ (5 ml) in the presence of molecular sieves-4A (1 g) was treated with 1-imidate-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (1.95 g), and the mixture was stirred at room temperature under an N_2 atmosphere for 10 min. It was treated with $BF_3 \cdot Et_2O$ (510 μl), and stirring was continued for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine then dried over $MgSO_4$. After work-up of the AcOEt extract in the usual manner, the crude product was separated by normal-phase silica-gel column chromatography [100 g, *n*-hexane–acetone (4 : 1)] to give 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl (−)-myrtenol (156 mg). A solution of 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl (−)-myrtenol (156 mg) in MeOH (2 ml) was treated with 10% aqueous K_2CO_3 (3 ml) and the mixture was stirred at 20 °C for 15 min. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and then filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by reversed-phase (2 g, $H_2O \rightarrow$MeOH) and normal-phase [3 g, $CHCl_3$–MeOH (10 : 1)] silica-gel column chromatography and HPLC [MeOH–H_2O (55 : 45), v/v] to furnish **13** (6.4 mg); a white powder, ^{1}H-NMR ($CDCl_3$) δ: 0.83, 1.28 (3H each, both s, 8,9-H_3), 1.18 (1H, d, J=8.6 Hz, 6-H), 1.98—2.43 (5H, m, 1,4,5,6-H), 3.27—3.63 (4H, m, 2′,3′,4′,5′-H), 3.96 (2H, br s, 6′-H_2), 3.98, 4.21 (1H each, both dd-like, 10-H_2), 4.33 (1H, d, J=7.6 Hz, 1′-H), 5.56 (1H, br s, 3-H).

Preparation of 2′,3′,4′-Tri-*O*-Acetyl β-D-Glucopyranosyl (−)-Myrtenol (14) A solution of **13** (6.4 mg) in dry pyridine (1.0 ml) was treated with *p*-methoxyphenyldiphenylmethyl chloride (MMTrCl) (30 mg), and the mixture was stirred at room temperature under an N_2 atmosphere for 1 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with diluted aqueous HCl, aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. After removal of the solvent from the AcOEt extract under reduced pressure, the product was purified by normal-phase silica-gel column chromatography [7 g, *n*-hexane–acetone (1 : 1)→$CHCl_3$–MeOH (6 : 1)] to give the 6′-*O*-monomethoxytritylate (8.5 mg): a white powder, ^{1}H-NMR ($CDCl_3$) δ: 0.85, 1.28 (3H each, both s, 8, 9-H_3), 1.18 (1H, d, J=8.6 Hz, 6-H), 2.03—2.43 (5H, m, 1,4,5,6-H), 3.30—3.56 (4H, m, 2′,3′,4′,5′-H), 3.80 (3H, s, OMe), 3.96, 4.31 (1H each, both dd-like, 10-H_2), 4.12 (2H, m, 6′-H_2), 4.31 (1H, d, J=7.6 Hz, 1′-H), 5.55 (1H, br s, 3-H), 6.82—7.46 (14H, m, triphenyl).

A solution of the 6′-*O*-monomethoxytritylate (8.5 mg) in pyridine (0.3 ml) was treated with Ac_2O (0.2 ml), and the whole was stirred at room temperature under an N_2 atmosphere for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract, the residue was purified by normal-phase silica-gel column chromatography [200 mg, *n*-hexane–AcOEt (2 : 1)] to give the 2′,3′,4′-tri-*O*-acetyl-6′-*O*-monomethoxytritylate (12.4 mg): a white powder, ^{1}H-NMR ($CDCl_3$) δ: 0.87, 1.29 (3H each, both s, 8, 9-H_3), 1.19 (1H, d, J=8.6 Hz, 6-H), 1.76, 1.99, 2.05 (3H each, all s, OAc), 2.00—2.43 (5H, m, 1,4,5,6-H), 3.10, 3.23 (1H each, both dd-like, 6′-H_2), 3.55 (1H, m, 5′-H), 3.79 (3H, s, OMe), 4.08, 4.24 (1H each, both dd-like, 10-H_2), 5.05—5.15 (3H, m, 2′,3′,4′-H), 5.55 (1H, br s, 3-H), 6.80—7.47 (10H, m, triphenyl). A solution of the 2′,3′,4′-tri-*O*-acetyl-6′-*O*-monomethoxytritylate (12.4 mg) in dry tetrahydrofuran (THF)–Et_2O (1 : 2, 1.0 ml) was treated with $BF_3 \cdot Et_2O$ (0.1 ml) and the mixture was stirred at room temperature under an N_2 atmosphere for 15 min. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and brine. After work-up of the AcOEt extract, the residue was purified by normal-phase silica-gel column chromatography [5 g, *n*-hexane–acetone (5 : 1)] to give 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyl (−)-myrtenol (**14**, 4.0 mg): a white powder, ^{1}H-NMR ($CDCl_3$) δ: 0.84, 1.28 (3H each, both s, 8, 9-H_3), 1.16 (1H, d, J=8.6 Hz, 6-H), 2.01 (3H, s), 2.05 (6H, s) (OAc), 2.00—2.42 (5H, m, 1,4,5,6-H), 3.60 (1H, m, 5′-H), 3.80 (2H, m, 6′-H_2), 3.96, 4.19 (1H each, both dd-like, 10-H_2), 4.56 (1H, d, J=7.9 Hz, 1′-H), 4.97—5.30 (3H, m, 2′,3′,4′-H), 5.50 (1H, br s, 3-H).

Glycosidation of 14 In the presence of molecular sieves-4A (0.1 g), a solution of **14** (4.0 mg) in dry CH_2Cl_2 (0.5 ml) was treated with 2,3,4-tri-*O*-acetyl-L-arabinopyranosyl-1-*O*-trichloroacetamidate (11.4 mg) and $BF_3 \cdot Et_2O$ (2.3 μl). The mixture was stirred at room temperature for 30 min, then poured into ice-water. The whole was extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with brine. After work-up of the CH_2Cl_2 extract, the residue was treated with 0.1% NaOMe (0.5 ml) at room temperature for 10 min. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and then filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by HPLC [YMC-pack ODS-A, MeOH–H_2O (60 : 40, v/v)] to give sacranoside A (**2**, 0.6 mg), which was identified on the basis of TLC, HPLC, $[\alpha]_D$, and ^{1}H-NMR (CD_3OD) comparisons with the natural product.

References and Notes

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