JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Lignans from the Root of *Rhodiola crenulata*

Ya-nan Yang, Zhao-zhen Liu, Zi-ming Feng, Jian-shuang Jiang, and Pei-cheng Zhang*

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Supporting Information

ABSTRACT: *Rhodiola crenulata* L. is an important species in genus *Rhodiola* widely used as a health food to reinforce immunity, improve memory and learning, scavenge active-oxygen species, and relieve altitude sickness. Eleven new lignans and a new benzonitrile compound, crenulatanoside A, were isolated from the roots of *R. crenulata* L. along with 25 known compounds, including 12 lignans. The structures of these compounds were elucidated by spectroscopic data and chemical evidence. Among them, compounds **1**−**4** and **5**−**7** were determined to be optical isomers of two 8-*O*-4′ neolignan glycosides. Compounds **8**−**11** were aryl tetralin type lignans, and compounds **12** and **13** were dihydrobenzofuran neolignans. All of the isolated compounds were evaluated for their inhibitory activity against α-glucosidase. From the data obtained, compound **37** showed strong inhibitory activity against α-glucosidase with an IC_{50} value of 96.8 μM.

KEYWORDS: *Rhodiola crenulata, lignan, isolation, health food, α-glucosidase*

INTRODUCTION

The plants of the genus *Rhodiola* are widely distributed in the Himalayan, western, and northern regions of Asia. There are approximately 90 species recorded in the world, and more than 70 species are found in China, mainly in plateau areas, such as Yunnan, Sichuan, and Tibet. Among them, *Rhodiola crenulata* L. is an important species found mostly in the northwest region of China. For a long time, its roots (Golden Root) have been used as a health food, antidepressive, and antifatigue and to reinforce immunity, improve memory and learning, scavenge active-oxygen species, and relieve altitude sickness.[1−8] Recently, some pharmacological studies have demonstrated that the genus *Rhodiola* might have the function of lowering blood glucose.[9,10]

Previous phytochemical studies have reported the isolation of almost 100 compounds from this genus, including phenols and their corresponding glycosides, cyanophoric glycosides, terpenoids, and flavonoids.[11−15] Among them, phenols and their corresponding glycosides, monoterpenoid glycosides, and cyanophoric glycosides were considered as characteristic constituents of *Rhodiola*. To date, only three lignans have been isolated from this genus, one of which was isolated from *R. crenulata*.[16] In our search for constituents from *R. crenulata*, 11 new lignans, **1**, **2**, and **5**−**13**, and a new benzonitrile compound, crenulatanoside A **36**, were isolated from the roots of *R. crenulata*. These new compounds along with the 25 already known constituents, which include 12 lignans, were identified by comparing the spectroscopic data obtained with those previously reported in the literature. Compounds **1**−**4** and **5**−**7** were identified as optical isomers of two 8-*O*-4′ neolignan glycosides. Compounds **8**−**11** were identified as aryl tetralin type lignans, and compounds **12** and **13** were identified as dihydrobenzofuran neolignans. Compound **36** was identified as a rare benzonitrile natural product. All of these compounds were evaluated for their inhibitory activity against α-glucosidase, and compound **37** was observed to exhibit strong inhibitory activity against α-glucosidase.

MATERIALS AND METHODS

Plant Material. The roots of *R. crenulata* were purchased in July 2007 from Pushenglin Corp. The plant material was identified as *R. crenulata* growth in Tibet by Professor Lin Ma. A voucher specimen was deposited in the Herbarium of the Department of Medicinal plants, Institute of Materia Media, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China.

General Apparatus and Chemicals. The optical rotations were measured on a Jasco P-2000 polarimeter. UV spectra were recorded on a Jasco V650 spectrophotometer. IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and 2D NMR spectra were run on INOVA 500 MHz spectrometer with tetramethylsilane (TMS) as an internal standard, and values were given in ppm (δ). HRESIMS was performed on Finnigan LTQ FTMS. Column chromatography was performed with Macroporous resin (Diaion HP-20, Mitsubishi Chemical Corp. Tokyo, Japan), Rp-18 (50 μm, YMC, Kyoto, Japan), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-20A detector, using a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm). HPLC-DAD analysis was performed using an Agilent 1200 series system (Agilent Technologies, Waldbronn, Germany) with an Apollo C18 column (250 mm × 4.6 mm, 5 μm; Grace Davison).

Extraction and Isolation. Air-dried roots (20 kg) of *R. crenulata* were extracted with 80% EtOH. After the solvent was evaporated under reduced pressure, the residue was resuspended in H_2O (5000 mL) and extracted with EtOAc (3 × 5000 mL). The aqueous layer (3600 g) was applied to a HDP100 macroporous adsorbent resin (4000 g, dried weight) column. Successive elution from the column with H_2O, 15% EtOH, 30% EtOH, 50% EtOH, 70% EtOH, and finally 95% EtOH (15 L each) yielded five corresponding fractions after removing solvents. The fraction eluted with 30% EtOH (240 g) was separated on a Sephadex LH-20 column with 0−100% MeOH (using a 10% stepwise increase in MeOH concentration) to yield fractions 1−9

Received: November 14, 2011
Revised: January 5, 2012
Accepted: January 6, 2012
Published: January 6, 2012

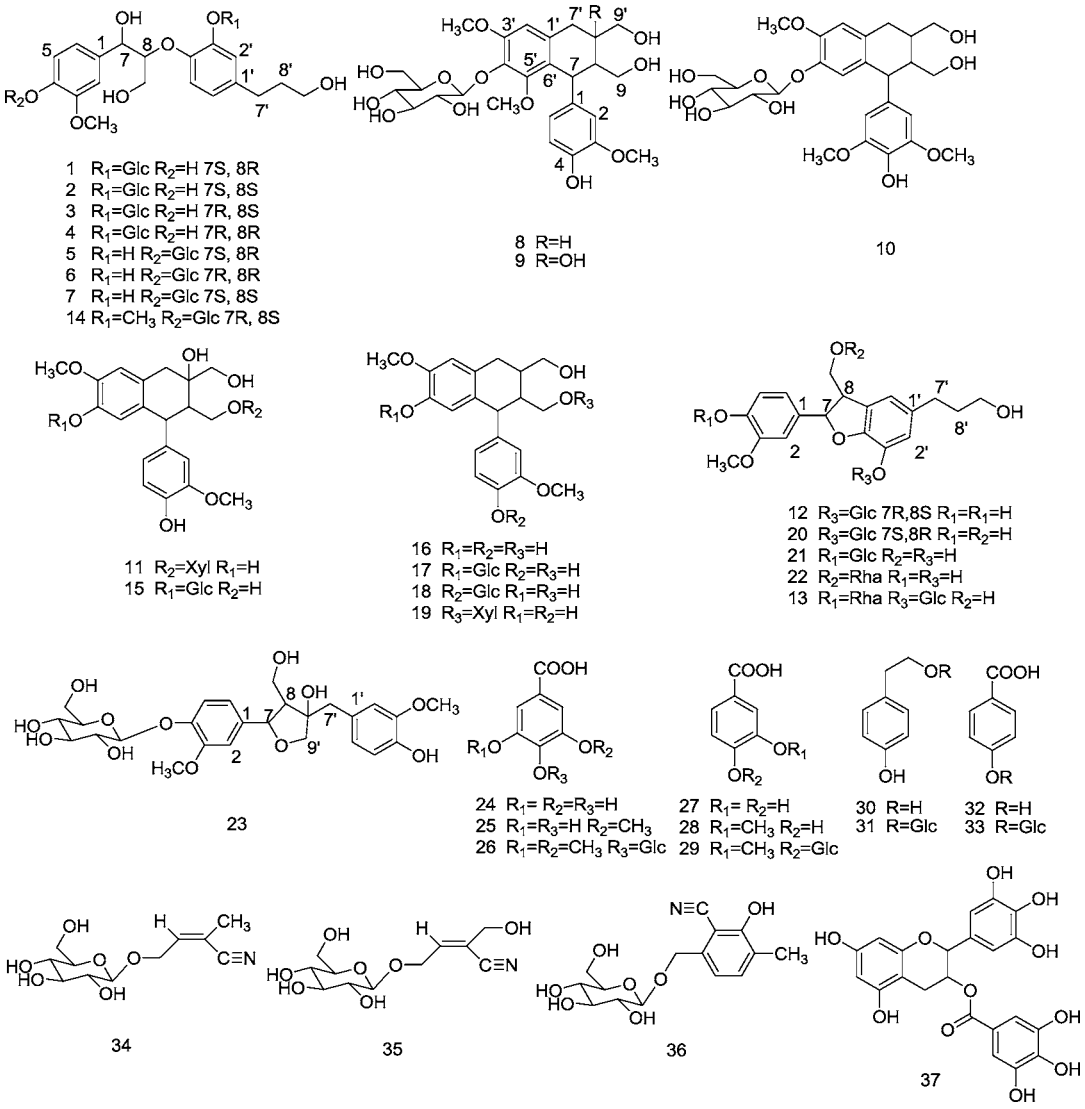

Figure 1. Structures of compounds **1**−**37**.

using HPLC-DAD analysis. Fraction 1 (3 g) was further separated via reversed-phase preparative HPLC, using a MeOH:H_2O ratio of 1:4, to yield **1** (21 mg), **3** (17 mg), **4** (22 mg), **6** (18 mg), and **23** (19 mg). Fraction 2 (14 g) was separated on a Sephadex LH-20 column and eluted with a gradient of increasing MeOH (0−20%) in H_2O to yield six subfractions (Fr2-1−Fr2-6). Subfraction Fr2-2 was further separated by reverse-phase preparative HPLC, using a MeOH:H_2O ratio of 1:5, to afford **2** (18 mg), **5** (16 mg), **7** (26 mg), **8** (14 mg), and **10** (8 mg). Subfraction Fr2-3 was further separated by reverse-phase preparative HPLC, using a MeOH:H_2O ratio of 3:7, to afford **11** (17 mg), **15** (11 mg), **20** (17 mg), and **21** (19 mg). Subfraction Fr2-5 was separated on a Sephadex LH-20 column and eluted with a gradient of increasing MeOH (0−30%) in H_2O to yield four subfractions (Fr2-5-1−Fr2-5-4). Subfraction Fr2-5-2 was subjected to reverse-phase preparative HPLC, using a MeOH:H_2O ratio of 1:4 as the mobile phase, to yield **9** (10 mg), **12** (12 mg), **13** (23 mg), and **17** (19 mg). Subfraction Fr2-5-3 was further separated by reverse-phase preparative HPLC, using a MeOH:H_2O ratio of 3:7, to afford **14** (16 mg), **18** (11 mg), **19** (23 mg), and **22** (17 mg). Fraction 6 was further purified by reverse-phase preparative HPLC, using a MeOH:H_2O ratio of 2:3, to yield **16** (25 mg) and **37** (37 mg). The fraction eluted with 15% EtOH (130 g) was separated on a Sephadex LH-20 column with 0−100% MeOH (5% stepwise increase in MeOH concentration) to yield fractions 1−12 using HPLC-DAD analysis. Fraction 3 (3 g) was further separated via reversed-phase preparative HPLC, using a MeOH:H_2O ratio of 1:9, to yield **24** (60 mg) and **34** (22 mg). Fraction 5 (18 g) was separated over a Sephadex LH-20 column and eluted with a gradient of increasing MeOH (0−30%) in H_2O to yield 12 subfractions (Fr5-1−Fr5-12). Subfraction Fr5-6 was further separated by reverse-phase preparative HPLC, using a MeOH:H_2O ratio of 1:8, to afford **26** (19 mg), **29** (26 mg), and **35** (28 mg). Using a MeOH:H_2O ratio of 1:9, subfraction Fr5-9 was further separated by reverse-phase preparative HPLC to afford **31** (18 mg), **32** (29 mg), **33** (31 mg), and **36** (24 mg). Using reverse-phase preparative HPLC, fraction 9 (4 g) was further separated with a MeOH:H_2O ratio of 1:8 to afford **25** (17 mg), **27** (19 mg), **28** (21 mg), and **30** (27 mg). Figure 1 shows the structure of compounds **1**−**37**.

(7*S*,8*R*)-4,7,9,3′,9′-Pentahydroxy-3-methoxyl-8-4′-oxyneolignan-3′-*O*-*β*-D-glucopyranoside (1). White amorphous powder, $[\alpha]_D^{20}$ +19.3 (*c* 0.027, MeOH). CD (MeOH) nm: 233 (−15.0), 278 (−1.7). UV (MeOH) λ_{max} nm: 278, 233. IR (KBr) ν_{max}: 3365, 2925, 1605, 1506, 1453, 1427, 1261, 1217, 1121, 1060, 1020. HRESIMS *m*/*z* 549.1943 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

(7*S*,8*S*)-4,7,9,3′,9′-Pentahydroxy-3-methoxyl-8-4′-oxyneolignan-3′-*O*-*β*-D-glucopyranoside (2). White amorphous powder, $[\alpha]_D^{20}$ +12.3 (*c* 0.031, MeOH). CD (MeOH) nm: 238 (+5.9), 290 (+0.5). UV (MeOH) λ_{max} nm: 275, 225. IR (KBr) ν_{max}: 3361, 2928, 1600, 1508, 1448, 1426, 1267, 1217, 1119, 1067. HRESIMS *m*/*z*

dx.doi.org/10.1021/jf204660c | J. Agric.Food Chem. 2012, 60, 964−972

Table 1. ^{1}H NMR Spectroscopic Data (δ) of Compounds 1, 2, and 5−7[a]

position	1	2	5	6	7
2	7.06 s	6.99 d (1.5)	7.11 s	7.05 s	7.04 s
5	6.91 d (8.0)	6.87 d (8.0)	7.13 d (8.5)	7.09 d (8.0)	7.09 d (8.5)
6	6.97 d (8.0)	6.99 d (8.0)	7.02 d (8.5)	7.01 d (8.0)	7.02 d (8.5)
7	4.99 d (5.5)	4.89 d (7.5)	5.05 d (4.0)	4.87 d (7.0)	4.87 d (7.5)
8	4.62 m	4.71 m	4.57 m	4.53 m	4.58 m
9	3.65 m	3.58 m	3.72 m	3.97 m	4.04 m
			3.91 m		
2′	7.06 s	6.98 d (1.5)	6.77 s	6.65 s	6.66 s
5′	6.92 d (8.0)	6.91 d (8.0)	6.78 d (8.5)	6.76 d (8.0)	6.81 d (8.5)
6′	6.87 d (8.0)	6.87 dd (8.0, 1.5)	6.63 d (8.5)	6.62 d (8.0)	6.65 d (8.5)
7′	2.63 m	2.59 m	2.54 m	2.52 m	2.53 m
8′	1.83 m	1.80 m	1.78 m	1.78 m	1.78 m
9′	3.60 m	3.47 m	3.61 m	3.58 m	3.58 m
1″	5.15 d (7.5)	4.76 d (7.5)	5.05 d (7.5)	5.03 d (7.5)	5.04 d (6.5)
2″	3.67 m	3.58 m	3.61 m	3.61 m	3.64 m
3″	3.60 m	3.37 m	3.55 m	3.57 m	3.57 m
4″	3.54 m	3.49 m	3.53 m	3.53 m	3.52 m
5″	3.65 m	3.55 m	3.58 m	3.60 m	3.62 m
6″	3.94 m	4.03 m	3.91 m	3.91 m	3.81 m
3-CH_3O	3.83 s	3.75 s	3.82 s	3.79 s	3.79 s

[a] ^{1}H NMR data (δ) were measured in D_2O at 500 MHz. Coupling constants (J) in Hz are given in parentheses.

Table 2. ^{13}C NMR Spectroscopic Data (δ) of Compounds 1, 2, 5−13, and 36

position	1[a]	2[a]	5[a]	6[a]	7[a]	8[b]	9[a]	10[b]	11[a]	12[a]	13[a]	36[a]
1	135.2	135.6	138.5	138.3	138.2	138.6	142.4	135.7	140.4	136.0	139.7	141.0
2	113.9	114.2	113.9	114.5	114.2	112.9	115.8	106.6	116.4	122.1	122.0	102.6
3	150.2	150.1	151.4	151.1	151.2	144.1	150.1	147.8	150.4	150.5	152.9	162.0
4	147.7	147.7	147.9	148.0	148.1	147.0	145.9	133.7	146.4	148.1	147.2	130.2
5	118.2	118.2	118.9	118.7	118.6	115.0	118.1	147.8	118.2	119.1	121.4	138.5
6	122.8	123.9	122.3	123.1	123.4	120.0	124.3	106.6	125.4	121.9	121.7	124.0
7	75.6	74.8	75.2	75.0	75.1	40.0	43.5	46.6	46.5	91.0	90.6	72.2
8	87.2	85.4	86.6	86.5	86.4	47.1	51.4	45.3	48.6	55.3	55.5	119.9
9	63.5	63.4	63.7	64.0	64.0	61.4	62.3	60.1	71.1	65.7	65.8	18.4
1′	139.7	139.6	139.7	139.9	139.8	134.0	134.9	130.0	128.6	139.5	139.6	104.3
2′	120.1	120.4	118.8	118.8	118.5	108.2	112.3	112.1	115.6	118.5	119.2	75.9
3′	149.3	148.8	148.5	149.1	148.7	151.1	154.6	146.6	148.9	143.1	143.1	78.7
4″	148.8	148.5	146.9	146.6	146.5	136.7	139.1	144.4	145.9	148.4	148.5	72.4
5′	120.3	121.4	118.8	119.9	119.8	151.2	153.8	116.2	118.9	132.2	132.1	78.6
6′	126.3	126.6	123.0	122.7	122.9	125.8	128.5	132.6	134.7	113.2	113.5	63.5
7′	33.5	33.5	33.4	33.4	33.4	32.6	41.6	32.1	40.9	33.9	33.9	
8′	36.0	36.1	36.0	36.0	36.0	38.8	76.7	38.0	76.2	36.3	36.3	
9′	63.8	63.9	63.9	63.9	63.9	64.2	70.2	63.5	70.1	63.9	63.9	
1″	103.6	103.6	103.4	103.4	103.5	102.4	105.1	100.7	106.7	103.4	103.5	
2″	76.0	76.0	75.7	75.7	75.7	74.0	76.3	73.0	76.3	75.8	75.8	
3″	79.0	79.0	78.9	78.9	78.9	76.9	79.0	77.0	78.3	79.0	79.0	
4″	72.4	72.4	72.2	72.2	72.2	70.0	72.1	69.1	72.0	72.3	72.3	
5″	78.5	78.5	78.4	78.4	78.4	76.4	78.5	76.9	67.8	78.4	78.4	
6″	63.5	63.7	63.3	63.4	63.3	59.8	63.1	60.1		63.4	63.4	
1‴											102.4	
2‴											72.8	
3‴											73.0	
4‴											74.8	
5‴											72.6	
6‴											19.4	
3′-CH_3O						55.7	59.1	55.6	58.8			
5′-CH_3O						61.0	63.2					
3-CH_3O	58.8	58.8	58.6	58.6	58.6	56.2	58.9	56.0	58.8	58.8	58.8	
5-CH_3O								56.0				

[a] ^{13}C NMR data (δ) were measured in D_2O at 125 MHz. [b] ^{13}C NMR data (δ) were measured in DMSO at 125 MHz.

dx.doi.org/10.1021/jf204660c | J. Agric.Food Chem. 2012, 60, 964−972

Table 3. ^{1}H NMR Spectroscopic Data (δ) of Compounds 8−13 and 36

position	8[b]	9[a]	10[b]	11[a]	12[a]	13[a]	36[a]
2	6.66 s	6.84 s	6.36 s	6.86 s	7.04 s	7.12 s	
5	6.61 d (8.0)	6.87 d (8.5)		6.90 d (7.5)	6.92 d (7.0)	7.21 d (7.5)	7.45 d (7.5)
6	6.35 d (8.0)	6.73 d (8.5)	6.36 s	6.78 d (7.5)	6.91[c]	6.99 d (7.5)	7.05 d (7.5)
7	4.19 d (5.5)	4.06 d (8.5)	3.78 d (5.5)	3.98 m	5.61 d (6.0)	5.67 d (5.0)	4.84 dd (12.0,3.5)
							4.96 dd (12.0,3.5)
8	1.76 m	2.07 m	1.70 m	2.27 m	3.61 m	3.61 m	
9	3.32 m	3.82 m	3.46 m	3.75 d (11.5)	3.77 m	3.74 m	2.27 s
				3.96 d (11.5)	3.93 m	4.02 m	
1′							4.54 d (8.0)
2′	6.62 s	6.83 s	6.69 s	6.86 s	6.98 s	6.99 s	3.35 m
3′							3.46 m
4′							3.48 m
5′			6.38 s	6.33 s			3.49 m
6′					6.93 s	6.94 s	3.78 dd (5.5,12.0)
							3.93 dd (5.5,12.0)
7′	2.45 d (14.0)	2.81 d (16.5)	2.72 m	2.81 d (17.0)	2.64 m	2.65 m	
	2.65 dd (15.5,4.0)	3.21 d (16.5)		3.18 d (17.0)			
8′	1.49 m		1.89 m		1.84 m	1.84 m	
9′	3.39 m	3.74 m	3.57 dd (10.5, 1.5)	3.74 m	3.63 m	3.62 m	
			3.43 m	3.62 m			
1″	4.83 d (7.5)	4.94 d (7.5)	4.33 d (7.5)	4.15 d (7.5)	5.17 d (7.0)	5.19 d (6.5)	
2″	3.17[c]	3.37 m	3.13 m	3.28 m	3.58 m	3.56 m	
3″	3.03 m	3.37 m	2.80 m	3.42 m	3.54 m	3.54 m	
4″	3.11	3.66 m	3.18 m	3.60 m	3.59 m	3.61 m	
5″	3.17[c]	3.53 m	3.11 m	3.82 m	3.61 m	3.58 m	
6″	3.56 m	3.89[c]	3.46 m		3.63 m	3.63 m	
1‴						5.48 brs	
2‴						3.63 m	
3‴						3.91 m	
4‴						3.54 m	
5‴						3.89 m	
6‴						1.24 d (5.5)	
3-CH_3O	3.75 s	3.82 s	3.69 s	3.86 s	3.84 s	3.73 s	
5-CH_3O			3.69 s				
3′-CH_3O	3.68 s	3.89 s	3.72 s	3.82 s			
5′-CH_3O	3.17 s	3.27 s					

[a] ^{1}H NMR data (δ) were measured in D_2O at 500 MHz. [b] ^{1}H NMR data (δ) were measured in DMSO-d_6 at 500 MHz. Coupling constants (J) in Hz are given in parentheses. [c] Overlapping signals.

549.1950 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

(7*S*,8*R*)-4,7,9,3′,9′-Pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-*β*-D-glucopyranoside (5). White amorphous powder, $[\alpha]_D^{20}$ +9.2 (*c* 0.026, MeOH). CD (MeOH) nm: 233 (−15.9), 277 (−2.4). UV (MeOH) λ_{max} nm: 277, 235. IR (KBr) ν_{max}: 3347, 2930, 1594, 1509, 1453, 1377, 1268, 1222, 1128, 1073, 1041. HRESIMS *m*/*z* 549.1949 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

(7*R*,8*R*)-4,7,9,3′,9′-Pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-*β*-D-glucopyranoside (6). White amorphous powder, $[\alpha]_D^{20}$ −35.3 (*c* 0.015, MeOH). CD (MeOH) nm: 237 (−4.1), 276 (−1.4). UV (MeOH) λ_{max} nm: 276, 237. IR (KBr) ν_{max}: 3316, 2932, 1592, 1505, 1454, 1419, 1265, 1221, 1124, 1072, 1024. HRESIMS *m*/*z* 549.1935 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

(7*S*,8*S*)-4,7,9,3′,9′-Pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-*β*-D-glucopyranoside (7). White amorphous powder, $[\alpha]_D^{20}$ −26.5 (*c* 0.023, MeOH). CD (MeOH) nm: 235 (+4.6), 272 (−0.8). UV (MeOH) λ_{max} nm: 277, 220. IR (KBr) ν_{max}: 3363, 2933, 1598, 1511, 1453, 1413, 1268, 1221, 1105, 1053. HRESIMS *m*/*z* 549.1939 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

5′-Methoxy-(+)-isolariciresinol-4′-*O*-*β*-D-glucopyranoside (8). White amorphous powder, $[\alpha]_D^{20}$ −21.0 (*c* 0.021, MeOH). CD (MeOH) nm: 238 (+5.9), 273 (+1.2), 287 (−0.3). UV (MeOH) λ_{max} nm: 282, 238. IR (KBr) ν_{max}: 3341, 2938, 1601, 1511, 1489, 1453, 1411, 1265, 1223, 1117, 1071. HRESIMS *m*/*z* 575.2106 [M + Na]$^+$ (calcd for $C_{27}H_{36}O_{12}Na$, 575.2099). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

5′-Methoxy-8′-hydroxyl-(+)-isolariciresinol-4′-*O*-*β*-D-glucopyranoside (9). White amorphous powder, $[\alpha]_D^{20}$ −5.4 (*c* 0.026, MeOH). CD (MeOH) nm: 239 (+10.1), 273 (+3.6), 288 (−0.9). UV (MeOH) λ_{max} nm: 282, 237. IR (KBr) ν_{max}: 3351, 2930, 1600, 1512, 1492, 1453, 1412, 1270, 1227, 1111, 1072, 1031. HRESIMS *m*/*z* 591.2056 [M + Na]$^+$ (calcd for $C_{27}H_{36}O_{13}Na$, 591.2048). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

5-Methoxy-(+)-isolariciresinol-4′-*O*-*β*-D-glucopyranoside (10). White amorphous powder, $[\alpha]_D^{20}$ −49.5 (*c* 0.012, MeOH). CD (MeOH) nm: 236 (+1.7), 270 (+0.9), 287 (−2.3). UV (MeOH) λ_{max} nm: 282, 234. IR (KBr) ν_{max}: 3368, 2914, 1610, 1511, 1458, 1425, 1263, 1221, 1115, 1060. HRESIMS *m*/*z* 575.2095 [M + Na]$^+$ (calcd for $C_{27}H_{36}O_{12}Na$, 575.2099). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

8′-Hydroxy-(+)-isolariciresinol-9-*O*-*β*-D-xylopyranoside (11). White amorphous powder, $[\alpha]_D^{20}$ +43.5 (*c* 0.023, MeOH). CD (MeOH) nm: 238 (+8.6), 274 (+7.1), 292 (−9.4). UV (MeOH) λ_{max}

dx.doi.org/10.1021/jf204660c | J. Agric.Food Chem. 2012, 60, 964−972

nm: 285, 233. IR (KBr) ν_{max}: 3382, 2935, 1600, 1512, 1462, 1430, 1368, 1255, 1218, 1124, 1072, 1035. HRESIMS at m/z 531.1837 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_{11}Na$, 531.1842). For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

(7*R*,8*S*)-Dihydrodehydrodiconiferyl Alcohol-3′-*O*-*β*-D-glucopyranoside (12). White amorphous powder, $[\alpha]_D^{20}$ +46.9 (*c* 0.029, MeOH). CD (MeOH) nm: 239 (−11.4), 281 (−1.7). UV (MeOH) λ_{max} nm: 282, 232. IR (KBr) ν_{max}: 3347, 2927, 1604, 1517, 1493, 1434, 1275, 1209, 1157, 1126, 1074, 1031. HRESIMS at m/z 531.1852 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_{11}Na$, 531.1842). For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

(7*R*,8*S*)-Dihydrodehydrodiconiferyl Alcohol-3′-*O*-*α*-L-rhamnopyranosyl-4-*O*-*β*-D-glucopyranoside (13). White amorphous powder, $[\alpha]_D^{20}$ −31.5 (*c* 0.014, MeOH). CD (MeOH) nm: 238 (−3.2), 279 (−1.0). UV (MeOH) λ_{max} nm: 280, 238. IR (KBr) ν_{max}: 3262, 2930, 1598, 1495, 1404, 1262, 1210, 1062, 1018. HRESIMS m/z 677.2407 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{15}Na$, 677.2421). For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

Crenulatanoside A (36). Colorless oil. UV (MeOH) λ_{max} nm: 302, 238. IR (KBr) ν_{max}: 3370, 2929, 2224, 1585, 1508, 1492, 1424, 1275, 1235, 1160, 1076, 1044. HRESIMS at m/z 348.1048 $[M + Na]^+$ (calcd for $C_{15}H_{19}NO_7Na$, 348.1059). For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

Inhibitory Activity of *α*-Glucosidase. The inhibitory activity of compounds **1**−**37** on *α*-glucosidase was determined spectrophotometrically on 96-well microplate reader. Twenty microliters of 0.2 U/mL *α*-glucosidase was premixed with 10 μL of compounds at various concentrations in 50 μL of 100 mM phosphate buffer (pH 7.0) at 37 °C for 5 min. Then, 20 μL of 2.5 mM substrate *p*-nitrophenyl-*α*-D-glucopyranoside was added to the mixture to initiate the reaction. The reaction was incubated at 37 °C for 15 min and stopped by the addition of 50 μL of 0.4 M Na_2CO_3. *α*-Glucosidase activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-*α*-D-glucopyranoside at 400 nm. The control was the mixture of the test sample with solvent instead. The sample and control blanks were the mixtures of sample and control except *α*-glucosidase was instead with phosphate buffer, respectively. The inhibition (%) of sample on *α*-glucosidase could be calculated by the following formula:

$$\text{inhibition (\%)} = [(A_{\text{sample}} - A_{\text{sample blank}}) / (A_{\text{control}} - A_{\text{control blank}})] \times 100$$

Acid Hydrolysis of 1, 2, 5−13, and 36. A solution of each compound in H_2O (3 mL) was individually hydrolyzed with 1 N HCl (0.5 mL) at 80 °C for 4 h. Each reaction mixture was extracted with EtOAc (3 × 3 mL) to yield an EtOAc extract and H_2O phase after removing the solvents. The aqueous phases of the hydrolysates were purified by chromatograph column over silica gel and eluted with a mixture of MeCN−H_2O (8:1) to yield glucose with positive optical rotations.

RESULTS AND DISCUSSION

Phytochemical Investigation. Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was determined as $C_{25}H_{34}O_{12}$ from an HRESIMS ion at m/z 549.1950 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). The ^1H NMR spectrum of **1** (see Table 1) showed six aromatic proton signals at *δ* 7.06 (s, 2, H-2, 2′), 6.91 (d, 1, *J* = 8.0 Hz, H-5), 6.97 (d, 1, *J* = 8.0 Hz, H-6), 6.92 (d, 1, *J* = 8.0 Hz, H-5′), and 6.87 (d, 1, *J* = 8.0 Hz, H-6′), revealing the presence of two ABX system aromatic rings. Two methylene protons at *δ* 1.83 (m, 2, H-8′) and 2.63 (m, 2, H-7′), two oxymethine protons at *δ* 4.99 (d, 1, *J* = 5.5 Hz, H-7) and 4.62 (m, 1, H-8), and two oxymethylene protons at *δ* 3.65 (m, 2, H-9) and 3.60 (m, 2, H-9′) established the presence of a 1,2,3-propanetriol moiety and a 1-propanol moiety. Additionally, a methoxy group attached to the aromatic ring at *δ* 3.83 (s, 3, OCH_3) and a glucopyranosyl anomeric proton at *δ* 5.15 (d, 1, *J*=7.5 Hz, H-1″) were observed in the ^1H NMR spectrum. The ^{13}C NMR spectrum of **1** (see Table 2) showed 25 carbon signals. Aside from the six carbon signals from the *O*-glucose unit and a methoxy group, the remaining 18 carbon signals, including 12 aromatic and six aliphatic carbons, and the HMBC correlations (see Figure 2) of

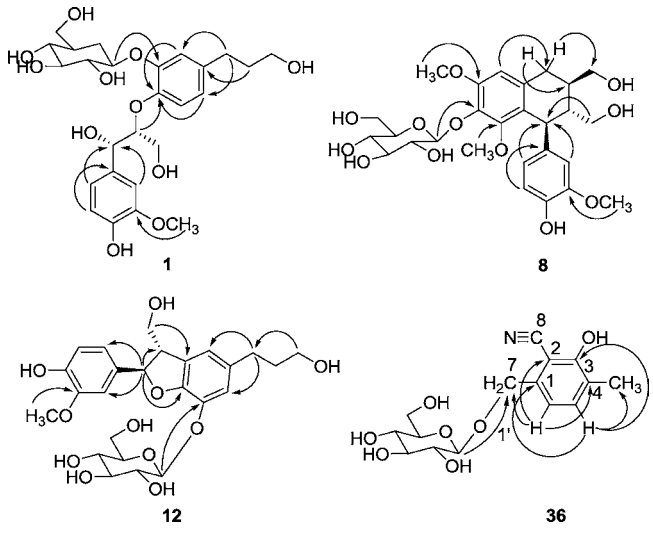

Figure 2. Selected HMBC correlations of **1**, **8**, **12**, and **36**.

H-7 at *δ* 4.99 with C-1, C-2, C-6, C-8, and C-9 and of H-7′ at *δ* 2.63 with C-1′, C-2′, C-6′, C-8′, and C-9′ confirmed the presence of two phenyl propanoid units. In the HMBC spectrum, the correlation of H-8 at *δ* 4.62 with C-4′ at *δ* 148.8 suggested that **1** was an 8-*O*-4′ system neolignan. The methoxy group was determined to be at C-3 based on the HMBC correlation of methoxy group at *δ* 3.83 with C-3 at *δ* 150.2, while the HMBC correlation of the anomeric proton H-1″ of sugar moiety at *δ* 5.15 correlated with C-3′ at 149.3, indicating that the glucose unit tethered to C-3′. Acid hydrolysis of **1** yielded D-glucose, which was identified by the positive optical rotation ($[\alpha]_D^{20}$ + 39.4), and the *β* form was determined by the presence of an anomeric proton at *δ* 5.15 (d, 1, *J* = 7.5 Hz, H-1″). The *erythro* configuration between two chiral centers at C-7 and C-8 positions was determined by its small coupling constant ($J_{7,8}$ = 5.5 Hz). The 8*R* absolute configuration was determined by its CD spectrum, which displays a negative Cotton effect at 233 nm.[17] Thus, the structure of **1** was determined to be (7*S*,8*R*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8−4′-oxyneolignan-3′-*O*-*β*-D-glucopyranoside (**1**).

Compound **2** was obtained as a white amorphous powder. Positive HRESIMS data of **2** indicated an ion at m/z 549.1943 $[M + Na]^+$, corresponding to a molecular formula of $C_{25}H_{34}O_{12}$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). The ^1H NMR spectrum of **2** (see Table 1) exhibited six aromatic proton signals at *δ* 6.99 (d, 1, *J* = 1.5 Hz, H-2), 6.87 (d, 1, *J* = 8.0 Hz, H-5), 6.99 (d, 1, *J* = 8.0 Hz, H-6), 6.98 (d, 1, *J* = 1.5 Hz, H-2′), 6.91 (d, 1, *J* = 8.0 Hz, H-5′), and 6.87 (dd, 1, *J* = 8.0, 1.5 Hz, H-6′), revealing the presence of two ABX system aromatic rings. A methoxy group at *δ* 3.75 (s, 3, OCH_3) and a glucopyranosyl anomeric proton at *δ* 4.76 (d, 1, *J* = 7.5 Hz, H-1″) were also observed in the ^1H NMR spectrum. The ^{13}C NMR spectrum of **2** (see Table 2) showed 25 carbon signals, including two C6−C3 units, six carbons in an *O*-glucose unit, and a methoxy carbon at *δ* 58.8.

dx.doi.org/10.1021/jf204660c | J. Agric.Food Chem. 2012, 60, 964−972

The key correlation of H-8 at δ 4.71 with C-4′ at δ 148.5 in the HMBC spectrum indicated that **2** was also an 8-*O*-4′ neolignan. The HMBC correlations of the methoxy proton at δ 3.75 with C-3 at δ 150.1 and the anomeric proton H-1″ at δ 4.76 with C-4′ at δ 148.5 showed that **2** was an optical isomer of **1**. The sugar unit was determined to be β-D-glucose by acid hydrolysis and the anomeric proton doublet at δ4.76 (d, 1, J = 7.5 Hz, H-1″) in the ^{1}H NMR spectrum. Comparison of the ^{1}H NMR data (see Table 1) of **2** and **1** showed that the 5.5 Hz coupling constant between H-7 and H-8 in 1 was 7.5 Hz in **2**. This difference suggested that the configuration between C-7 and C-8 was *threo* instead of the *erythro* form in **1**. The 8*S* absolute configuration in **2** was determined by the positive Cotton effect at 238 nm in the CD spectrum of **2**. On the basis of these results, the structure of **2** was determined to be (7*S*, 8*S*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-4′-oxyneolignan-3′-*O*-β-D-glucopyranoside (**2**).

Compound **5** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{25}H_{34}O_{12}$ by the positive HRESIMS ion observed at m/z observed 549.1949 ([M + Na]$^+$. The ^{1}H NMR spectrum of **5** also showed six aromatic proton signals at δ7.11 (s, 1, H-2), 7.13 (d, 1, J = 8.5 Hz, H-5), 7.02 (d, 1, J = 8.5 Hz, H-6), 6.77 (s, 1, H-2′), 6.78 (d, 1, J = 8.5 Hz, H-5′), and 6.63 (d, 1, J = 8.5 Hz, H-6′) (see Table 1). Furthermore, the ^{13}C NMR spectrum of **5** (see Table 2) showed 25 carbon signals, including six carbons of an *O*-glucose unit and a methoxy carbon at δ 58.6. All of these spectroscopic data were similar to those of **1**, suggesting that **5** was also an 8-*O*-4′ system neolignan. Careful analysis of the HMBC spectrum of **5** revealed that the anomeric proton H-1″ at δ 5.05 was correlated with C-4 at δ 147.9, indicating the linkage position of glucose unit was at C-4 instead of C-3′ in 1 and 2. The sugar unit was determined to be in the β-D-glucose form by the same methods used to characterize the sugar units of **1** and **2**. The *erythro* configuration of C-7 and C-8 was confirmed by the coupling constant ($J_{7,8}$ = 4.0 Hz). The 8*R* absolute configuration of **5** was determined by the negative Cotton effect at 233 nm displayed in the CD spectrum. All of these data indicated that the structure of **5** was (7*S*, 8*R*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-β-D-glucopyranoside (**5**).

Compound **6** was obtained as a white amorphous powder. The same molecular formula as **5**, $C_{25}H_{34}O_{12}$, was determined from the positive HRESIMS ion at m/z 549.1935 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). The ^{1}H NMR spectrum (see Table 1) of **6** also showed six aromatic proton signals at δ7.05 (s, 1, H-2), 7.09 (d, 1, J = 8.0 Hz, H-5), 7.01 (d, 1, J = 8.0 Hz, H-6), 6.65 (s, 1, H-2′), 6.76 (d, 1, J = 8.0 Hz, H-5′), and 6.62 (d, 1, J = 8.0 Hz, H-6′), revealing the presence of two ABX system aromatic rings. The ^{13}C NMR spectrum of **6** (see Table 2) showed 25 carbon signals, including six carbons of an *O*-glucose unit and a methoxy carbon at δ 58.6. The HMBC correlations of the methoxy proton at δ 3.79 with C-3 at δ 151.1 and the anomeric proton H-1″ at δ 5.03 with C-4 at δ 148.0 indicated that the methoxy group and the sugar unit were located at C-3 and C-4, respectively. The anomeric proton at δ 5.03 (J = 7.5 Hz) revealed that the glucose moiety was in the β-form. The NMR data of **6** were similar to those of **5** with the exception of the coupling constant between H-7 and H-8. A bigger coupling constant ($J_{7,8}$ = 7.0 Hz) confirmed the *threo* configuration of **6**, and its 8*R* absolute configuration was determined by the CD spectrum with a negative Cotton effect at 237 nm. Thus, the structure of **6** was identified as (7*R*,8*R*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-β-D-glucopyranoside (**6**).

Compound **7** was obtained as a white amorphous powder. The molecular formula of **7** was determined as $C_{25}H_{34}O_{12}$ from the HRESIMS at m/z 549.1939 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{12}Na$, 549.1948). The UV, IR, and NMR spectroscopic data of **7** indicated that this compound was also an 8-*O*-4′ system neolignan and an optical isomer of **5** and **6**. The *threo* configuration of **6** was confirmed by its coupling constant ($J_{7,8}$ = 7.5 Hz). However, the CD spectrum of **7** showed a positive Cotton effect at 235 nm, which differed from that of **6**, indicating that **7** had an 8*S* absolute configuration. The structure of **7** was determined to be (7*S*, 8*S*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-β-D-glucopyranoside (**7**).

Compound **8** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{27}H_{36}O_{12}$ from the positive HRESIMS ion at m/z 575.2106 [M + Na]$^+$ (calcd for $C_{27}H_{36}O_{12}Na$, 575.2099). The ^{1}H NMR spectrum of **8** (see Table 3) showed four aromatic proton signals at δ 6.66 (s, 1, H-2), 6.61 (d, 1, J = 8.0 Hz, H-5), 6.35 (d, 1, J = 8.0 Hz, H-6), and 6.62 (s, 1, H-2′), revealing the presence of an ABX system of an aromatic ring and a 1,3,4,5,6-pentasubstituted benzene ring. Additionally, three methoxy groups at δ 3.75 (s, 3, OCH_3), 3.68 (s, 3, OCH_3), and 3.17 (s, 3, OCH_3) and a glucopyranosyl anomeric proton at δ 4.83 (d, 1, J = 7.5 Hz, H-1″) were also observed in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum of **8** (see Table 2), the 18 skeleton carbon signals suggested that the aglycone of **8** was also a lignan with the exception of three methoxyl carbons and six carbons of an *O*-glucose unit. In the HMBC spectrum (see Figure 2), the following correlation peaks confirmed that **8** was an aryl tetralin type lignan: H-7 at δ 4.19 with C-1, C-2, C-6, C-8, and C-9; H-7′ at δ 2.45 with C-1′, C-2′, C-6′, C-8′, and C-9′; and H-8 at δ 1.76 with C-6′, C-7′, and C-9′. The positions of three methoxy groups were determined by the HMBC correlations of the methoxy protons at δ 3.75 (s, 3, OCH_3), 3.68 (s, 3, OCH_3), and 3.17 (s, 3, OCH_3) with C-3, C-3′, and C-5′, respectively. Additionally, the HMBC correlation of the anomeric proton H-1″ at δ 4.83 with C-4′ at δ 136.7 indicated that the linkage position of the glucose unit was at C-4′. The anomeric proton at δ 4.83 (d, 1, J = 7.5 Hz, H-1″) confirmed that the glucose unit was in the β-form. Acid hydrolysis of **8** afforded D-glucose, which was identified by the positive optical rotation, $[\alpha]_D^{20}$ +29.6. The absolute configuration of C-7 could be determined from the CD spectrum of **8**. The negative Cotton effect at 287 nm indicated that the absolute configuration of C-7 in **8** was *S*.[18] Consequently, **8** was identified as 5′-methoxy-(+)-isolariciresinol-4′-*O*-β-D-glucopyranoside (**8**).

Compound **9** was obtained as a white amorphous powder. The molecular formula of **9** was determined as $C_{27}H_{36}O_{13}$ from an HRESIMS ion at m/z 591.2056 [M + Na]$^+$ (calcd for $C_{27}H_{36}O_{13}Na$, 591.2048). The ^{1}H NMR spectrum of **9** (see Table 3) also revealed the presence of four aromatic proton signals at δ 6.84 (s, 1, H-2), 6.87 (d, 1, J = 8.5 Hz, H-5), 6.73 (d, 1, J = 8.5 Hz, H-6), and 6.83 (s, 1, H-2′); three methoxy proton signals at δ 3.89 (s, 3, OCH_3), 3.82 (s, 3, OCH_3), and 3.27 (s, 3, OCH_3); and a glucopyranosyl anomeric proton signal at δ 4.94 (d, 1, J = 7.5 Hz, H-1″). The ^{13}C NMR spectrum of **9** (see Table 2) displayed 27 carbon signals corresponding to three methoxy carbons, 18 carbons of two phenyl propanoid units, and six carbons of an *O*-glucose unit. The similar ^{1}H and ^{13}C NMR data to those of **8** suggested that

dx.doi.org/10.1021/jf204660c | J. Agric.Food Chem. 2012, 60, 964–972

9 was also an aryl tetralin type lignan. Considering the 36 ppm downfield shift of C-8′ in the ^{13}C NMR spectrum and the difference in the mass spectrum, a hydroxy group was proposed to be attached to C-8′ in **9**. The presence of this hydroxy group was further confirmed by the HMBC correlation of H-7 and H-9 with C-8′. The anomeric proton doublet at δ 4.94 (d, 1, J = 7.5 Hz, H-1″) indicated that the sugar moiety was in the β-configuration. The absolute configuration of C-7 in **9** was determined by its CD spectrum. A negative Cotton effect at 288 nm indicated that the absolute configuration of C-7 in **9** was *S*. Thus, **9** was identified as 5′-methoxy-8′-hydroxyl-(+)-isolariciresinol-4′-*O*-β-D-glucopyranoside (**9**).

Compound **10** was obtained as a white amorphous powder and its positive HRESIMS data ([M + Na]$^+$, m/z found 575.2095) indicated the molecular formula of **10** to be $C_{27}H_{36}O_{12}$. The similar ^1H and ^{13}C NMR data to those of **8** (see Table 3) confirmed that **10** was an aryl tetralin type lignan. Additionally, four aromatic proton signals at δ 6.36 (s, 2, H-2, 6), 6.69 (s, 1, H-2′), and 6.38 (s, 1, H-5′), found in the ^1H NMR spectrum, indicated that the lignan skeleton has the structures of 1,3,4,6-tetrasubstituted and 1,3,4,5-tetrasubstituted aromatic rings. This finding was further confirmed by an HMBC experiment, which showed the following correlation peaks: H-7 at δ 3.78 with C-1, C-2, C-6, C-8, and C-9; H-7′ at δ 2.72 with C-1′, C-2′, C-6′, C-8′, and C-9′; and H-8 at δ 1.70 with C-6′, C-7′, and C-9′. The positions of three methoxy groups at δ 3.72 (s, 3, OCH_3), 3.69 (s, 3, OCH_3), and 3.69 (s, 3, OCH_3) were determined to be at C-3′, C-3, and C-5 by HMBC correlations. The β-configuration of the sugar moiety was determined based on the coupling constant (J = 7.5 Hz). A negative Cotton effect at 287 nm in the CD spectrum of **10** indicated that the absolute configuration of C-7 was *S*. These spectroscopic data identified **10** as 5-methoxy-(+)-isolariciresinol-4′-*O*-β-D-glucopyranoside (**10**).

Compound **11** was obtained as a white amorphous powder. The molecular formula of **11** was determined as $C_{25}H_{32}O_{11}$ from an HRESIMS ion at m/z 531.1851 [M + Na]$^+$ (calcd for $C_{25}H_{32}O_{11}Na$, 531.1842). Five aromatic proton signals at δ6.86 (s, 2, H-2, 2′), 6.90 (d, 1, J = 7.5 Hz, H-5), 6.78 (d, 1, J = 7.5 Hz, H-6), and 6.33 (s, 1, H-5′); two methoxyl proton signals at δ 3.86 (s, 3, OCH_3) and 3.82 (s, 3, OCH_3); and an anomeric proton signal at δ 4.03 (d, 1, J = 7.5 Hz, H-1″) were detected by ^1H NMR (See Table 3). In the ^{13}C NMR spectrum (see Table 2), 25 carbon signals were detected and assigned as two methoxy carbons, 18 carbons of two C6−C3 units, and five carbons of an *O*-xylose unit. The NMR spectroscopic data of **11** were very similar to those of the known compound **15**, with the exception of a set of signals assigned to a xylose moiety in **11**. In the HMBC spectrum, the correlation of the anomeric proton H-1″ at δ 4.15 with C-9 at δ 71.1 confirmed that the linkage position with the xylose unit is at C-9. The xylose unit was determined to be in a β-form by the anomeric proton at δ 4.15 (J = 7.5 Hz). In addition, the absolute configuration of C-7 of **11** is *S* based on the negative Cotton effect at 292 nm in the CD spectrum. Hence, **11** was identified as 8′-hydroxy-(+)-isolariciresinol-9-*O*-β-D-xylopyranoside (**11**).

Compound **12** was obtained as a white amorphous powder. The molecular formula of **12** was determined as $C_{25}H_{32}O_{11}$ from the HRESIMS ion at m/z 531.1852 [M + Na]$^+$ (calcd for $C_{25}H_{32}O_{11}Na$, 531.1842). The ^1H NMR spectra of **12** exhibited a 1,3,4-trisubstituted aromatic ring [δ 7.04 (s, 1, H-2), 6.92 (d, 1, J = 7.0 Hz, H-5), and 6.91 (overlap, 1, H-6)] and a 1′,3′,4′,5′-tetrasubstituted aromatic ring [δ 6.98 (s, 1, H-2′) and 6.93 (s, 1, H-6′)]. Additionally, a methoxy proton [δ 3.84 (s, 3, OCH_3)], two methylene protons [δ2.64 (m, 2, H-7′), 1.84 (m, 2, H-8′)], two oxymethylene protons [δ 3.77 (m, 1, H-9a), 3.93 (m, 1, H-9b), and 3.63 (m, 2, H-9′)], and two methine protons [δ 5.61 (d, 1, J = 6.0 Hz, H-7), 3.61 (m, 1, H-8)] were also observed. The ^{13}C NMR spectra of **12** displayed six carbon signals in the high-field region attributed to a methine carbon at δ 55.3 (C-8), an oxymethine carbon at δ 91.0 (C-7), two oxymethylene carbons at δ 65.7 (C-9) and 63.9 (C-9′), two aliphatic methylene carbons at δ 33.9 (C-7′) and 36.3 (C-8′), and **12** aromatic carbons in the downfield region. These spectroscopic data suggested that **12** was a dihydrobenzofuran neolignan glycoside. In the HMBC experiment of **12** (see Figure 2), the correlation peaks of H-7 with C-2, C-6, C-8, C-4′, and H-1″ with C-3′ determined that the planar structure of **12** was the same as the known compound **20**. The anomeric proton at δ 5.17 (d, 1, J = 7.0 Hz, H-1″) in the ^1H NMR spectrum of **12** indicated that the glucose unit is in the β-form. CD data of **20** showed a positive Cotton effect at 282 nm, indicating that the absolute configuration of C-7 is *S*. Consequently, the 7*R* configuration of **12** was determined by the negative Cotton effect at 281 nm in the CD spectrum of this compound. On the basis of these results, the structure of **12** was concluded to be (7*R*, 8*S*)-dihydrodehydrodiconiferyl alcohol-3′-*O*-β-D-glucopyranoside (**12**).

Compound **13** was obtained as a white amorphous powder. The molecular formula of compound **13** was determined as $C_{31}H_{42}O_{15}$ from the HRESIMS ion at m/z 677.2407 [M + Na]$^+$ (calcd for $C_{31}H_{42}O_{15}Na$, 677.2421). Five aromatic proton signals at δ7.12 (s, 1, H-2), 7.21 (d, 1, J = 7.5 Hz, H-5), 6.99 (d, 1, J = 7.5 Hz, H-6), 6.99 (s, 1, H-2′), and 6.94 (s, 1, H-6′) were detected in the ^1H NMR spectrum of **13** (see Table 3). The ^{13}C NMR spectrum (see Table 2) showed 31 carbon signals, 25 of which were similar to the ^{13}C NMR data of **12** with the exception of a set of additional signals assigned to the α-L-rhamnose moiety. In the HMBC spectrum, the correlation of H-1″ (δ 5.19) with C-3′ (δ 143.1) indicated that the linkage point of the glucose unit was at C-3′. Additionally, another correlation of H-1‴ (δ 5.48) with C-4 (δ 147.2) established that the rhamnose unit was attached to C-4. The absolute configuration of C-7 in **13** was determined by investigating its CD spectrum. The negative Cotton effects at 238 and 279 nm in the CD spectrum of **13** indicated that the absolute configuration of C-7 is *R*.[19] Thus, the structure of **13** was determined to be (7*R*, 8*S*)- dihydrodehydrodiconiferyl alcohol-3′-*O*-α-L-rhamnopyranosyl-4-*O*-β-D-glucopyranoside (**13**).

Compound **36** was obtained as colorless oil. The molecular formula of **36** was determined as $C_{15}H_{19}NO_7$ from the HRESIMS ion at m/z 348.1048 [M + Na]$^+$ (calcd for $C_{15}H_{19}NO_7Na$, 348.1059). The ^1H NMR spectra of **36** revealed an AB system, which was attributed to a 1,2,3,4-tetrasubstituted aromatic ring at δ 7.45 (d, 1, J = 7.5 Hz, H-5) and 7.05 (d, 1, J = 7.5 Hz, H-6). In addition, a methyl proton signal at δ 2.27 (s, 3, CH_3), methylene proton signals at δ 4.96 (dd, 1, J = 12.0, 3.5 Hz, H-7a) and 4.84 (dd, 1, J=12.0, 3.5 Hz, H-7b), and an anomeric proton signal at δ 4.54 (d, 1, J = 8.0 Hz, H-1′) were also detected by ^1H NMR (see Table 3). The ^{13}C NMR spectrum of **36** displayed 15 carbon signals attributed to six carbons from an *O*-glucose unit and six carbons assigned to an aromatic ring, and the other three carbon signals were determined to be a methyl carbon, a methylene carbon, and a quaternary carbon. The quaternary carbon signal was identified as the carbon signal of a cyano group. From the

dx.doi.org/10.1021/jf204660c | J. Agric.Food Chem. 2012, 60, 964−972

above data, the skeleton of **36** was determined as benzenemethanol, and the positions of the other substituents were confirmed by the HMBC spectrum (see Figure 2). The correlation peaks of H-1′ (δ 4.54) with C-7 (δ 72.2) were used to determine that the linkage position of glucose was at C-7. The position of the methyl group was confirmed at C-4 based on the correlation peaks of H-6 (δ 7.05) with C-7 (δ 72.2) and H-5 (δ 7.45) with CH_3 (δ 18.4). Upon further analysis of the HMBC spectrum, the correlation peak of H-5 (δ 7.45) with C-3 (δ 162.0) indicated the linkage position of the hydroxyl group was at C-3. The anomeric proton at δ4.54 (d, 1, J = 8.0 Hz, H-1′) in the ^{1}H NMR spectrum indicated that the glucose unit was in the β-form. On the basis of these results, the structure of **36** was concluded to be 2-cyano-3-hydroxy-4-methxyl-benzenemethanol-7-*O*-β-D-glucopyranoside and named crenulatanoside A.

Known compounds **3**, **4**, **14**−**35**, and **37** were identified by NMR and MS analyses, compared with reference data, and identified as the following: (7*R*, 8*S*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4-*O*-β-D-glucopyranoside (**3**),[20] (7*R*, 8*R*)-4,7,9,3′,9′-pentahydroxy-3-methoxyl-8-4′-oxyneolignan-4 -*O*-β-D-glucopyranoside (**4**),[21] (7*R*, 8*R*)-threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**14**),[17] (+)-cycloolivil-4′-*O*-β-D-glucopyranoside (**15**),[22] (+)-isolarisiresinol (**16**),[23] (+)-isolarisiresinol-4′-*O*-β-D-glucopyranoside (**17**),[24] (+)-isolarisiresinol-4-*O*-β-D-glucopyranoside (**18**),[25] (+)-isolarisiresinol-9-*O*-β-D-xylopyranoside (**19**),[26] (7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol-3′-*O*-β-D-glucopyranoside (**20**),[27] (7*R*, 8*S*)-dihydrodehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside (**21**),[28] (7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol-9-*O*-α-L-rhamnopyranoside (**22**),[29] olivil-4-*O*-β-D-glucopyranoside (**23**),[30] gallic acid (**24**),[31] 3-*O*-methyl gallic acid (**25**),[32] 4-*O*-β-D-glucopyranosyloxy-3,5-dimethoxy-benzoic acid (**26**),[33] protocatechuic acid (**27**),[34] vanillic acid (**28**),[35] vanillic acid 4-*O*-β-D-glucopyranoside (**29**),[36] tyrosol (**30**),[37] salidroside(**31**),[38] 4-hydroxybenzoic acid (**32**),[39] 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside (**33**),[40] rhodiocyanoside A (**34**),[41] sarmentosin (**35**),[42] and EGCG (**37**).[43]

Inhibitory Activity of α-Glucosidase. The inhibitory activity of compounds **1**−**37** against α-glucosidase was examined in this study. Compound **37** exhibited strong inhibitory activity against α-glucosidase with an IC_{50} value of 96.8 μM, and its activity was in a dose-dependent manner (see Figure 3). Statistical analysis was done using the Graphpad prism statistical package.

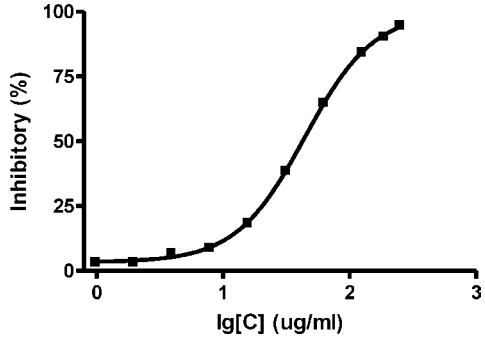

Figure 3. Inhibitory effect of compound **37**.

In conclusion, further investigation of the constituents in the health food *R. crenulata* revealed the presence of 11 new lignans and a new benzonitrile compound isolated from the roots of *R. crenulata* L. Additionally, 25 known compounds, including 12 known lignans, were also isolated. To our knowledge, only three lignans have been isolated previously from the genus *Rhodiola*. All of the isolated compounds were evaluated for their inhibitory activity against α-glucosidase. From the data obtained, compound **37** showed strong inhibitory activity against α-glucosidase with an IC_{50} value of 96.8 μM and may be useful in the prevention and treatment of diabetes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of compounds **1**, **2**, **5**−**13**, and **36**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-10-63165231. Fax: +86-10-63017757. E-mail: pczhang@imm.ac.cn.

Funding

This research was supported by the National Natural Science Foundation of China (No. 30973861) and the Nation Science and Technology Project of China (No. 2009YZH-HXY07).

■ ACKNOWLEDGMENTS

We thank Ying-hong Wang and Hong-yue Liu for NMR measurements.

■ REFERENCES

(1) Abidov, M.; Crendal, F.; Grachev, S.; Seifulla, R.; Ziegenfuss, T. Effect of extracts from *Rhodiola rosea* and *Rhodiola crenulata* (Crassulaceae) roots on ATP content in mitochondria of skeletal muscles. *Bull. Exp. Biol. Med.* **2003**, *136*, 585−587.

(2) Shevtsov, V. A.; Zholus, B. I.; Shervarly, V. I.; Volskij, V. B.; Korovin, Y. P.; Khristich, M. P.; Roslyakova, N. A.; Wikman, G. A randomized trial of two different doses of a SHR-5 *Rhodiola rosea* extract versus placebo and control of capacity for mental work. *Phytomedicine* **2003**, *10*, 95−105.

(3) Ohsugia, M.; Fan, W. Z.; Hasea, K. J.; Xiong, Q. B.; Tezuka, Y.; Komatsu, K.; Namba, T.; Saitoh, T.; Tazawa, K. J.; Kadota, S. Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola sacra*. *J. Ethnopharmacol.* **1999**, *67*, 111−119.

(4) Abidov, M.; Grachev, S.; Seifulla, R. D.; Ziegenfuss, T. N. Extract of *Rhodiola rosea* radix reduces the level of C-reactive protein and creatinine kinase in the blood. *Bull. Exp. Biol. Med.* **2004**, *38*, 63−64.

(5) Mishra, K. P.; Padwad, Y. S.; Jain, M.; Karan, D.; Ganju, L.; Sawhney, R. C. Aqueous Extract of *Rhodiola imbricata* Rhizome Stimulates Proinflammatory Mediators via Phosphorylated IκB and Transcription Factor Nuclear Factor-κB. *Immunopharmacol. Immunotoxicol.* **2006**, *28*, 201−212.

(6) Blomkvist, J.; Taube, A.; Larhammar, D. Perspective on Roseroot (*Rhodiola rosea*) studies. *Planta Med.* **2009**, *75*, 1187−1190.

(7) van Diermen, D.; Marston, A.; Bravo, J.; Reist, M.; Carrupt, P. A.; Hostettmann, K. Monoamine oxidase inhibition by *Rhodiola rosea* L. roots. *J. Ethnopharmacol.* **2009**, *122*, 397−401.

(8) Panossian, A.; Wikman, G.; Sarris, J. Rosenroot (*Rhodiola rosea*): Traditional use, chemicalcomposition, pharmacology and clinical efficacy. *Phytomedicine* **2010**, *17*, 481−493.

(9) Kwon, Y.; Jang, H.; Shetty, K. Evaluation of *Rhodiola crenulata* and *Rhodiola rosea* for management of type II diabetes and hypertension. *Asia Pac. J. Clin. Nutr.* **2006**, *15*, 425−432.

(10) Kim, S. H.; Hyun, S. H.; Choung, S. Y. Antioxidative effects of *Cinnamomi cassiae* and *Rhodiola rosea* extracts in liver of diabetic mice. *Biofactors* **2006**, *26*, 209−219.

(11) Yousef, G. G.; Grace, M. H.; Cheng, D. M.; Belolipov, I. V.; Raskin, I.; Lila, M. A. Comparative phytochemical characterization of three *Rhodiola* species. *Phytochemistry* **2006**, *67*, 2380−2391.
(12) Fan, W.; Tezuka, Y.; Ni, K. M.; Kadota, S. Prolyl endopeptidase inhibitors from the underground part of *Rhodiola sachalinensis*. *Chem. Pharm. Bull.* **2001**, *49*, 396−401.
(13) Yoshikawa, M.; Shimada, H.; Horikawa, S.; Murakami, T.; Shimoda, H.; Yamahara, J.; Matsuda, H. Bioactive constituents of Chinese natural medicines. IV. Rhodiolae Radix. 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. *Chem. Pharm. Bull.* **1997**, *45*, 1498−1503.
(14) Ma, G. Z .; Li, W.; Dou, D. Q.; Chang, X. L.; Bai, H.; Satou, T.; Li, J.; Sun, D. J.; Kang, T. G.; Nikaido, T.; Koike, K. Rhodiolosides A-E, monoterpene glycosides from *Rhodiola rosea*. *Chem. Pharm. Bull.* **2006**, *54*, 1229−1233.
(15) Lee, M. W.; Lee, Y. A.; Park, H. M.; Toh, S. H.; Lee, E. J.; Jang, H. D.; Kim, Y. H. Antioxidative phenolic compounds from the roots of *Rhodiola sachalinensis* A. Bor. *Arch. Pharm. Res.* **2000**, *23*, 455−458.
(16) Nakamura, S.; Li, X. Z.; Matsuda, H.; Yoshikawa, M. Bioactive constituents from Chinese natural medicines. XXVIII.Chemical Structures of acyclic alcohol glycosides from the roots of *Rhodiola crenulata*. *Chem. Pharm. Bull.* **2008**, *56*, 536−540.
(17) Huo, C. H.; Liang, H.; Zhao, Y. Y.; Wang, B.; Zhang, Q. Y. Neolignan glycosides from *Symplocos caudata*. *Phytochemistry* **2008**, *69*, 788−795.
(18) Ohashi, K.; Watanabe, H.; Okumura, Y.; Uji, T.; Kitagawa, I. Comparative estimation of the liposomal content of phosphatidylcholine/triolein emulsions using fluorescence quenching and ^{1}H-NMR. *Chem. Pharm. Bull.* **1994**, *42*, 1924−1926.
(19) Machida, K.; Takano, M.; Kakuda, R.; Yaoita, Y.; Kikuchi, M. A new lignan glycoside from the leaves of *Sambucus sieboldiana* (MIQ.) BLUME *ex*. GRAEBN. *Chem. Pharm. Bull.* **2002**, *50*, 669−671.
(20) Matsuda, N.; Kikuchi, M. Studies on the constituents of *Lonicera* Species. X. neolignan glycosides from the leaves of *Lonicera gracilipes* var. Glandulosa MAXIM. *Chem. Pharm. Bull.* **1996**, *44*, 1676−1679.
(21) Gan, M. L.; Zhang, Y. L.; Lin, S.; Liu, M. T.; Song, W. X.; Zi, J. C.; Yang, Y. C.; Fan, X. N.; Shi, J. G.; Hu, J. F.; Sun, J. D.; Chen, N. H. Glycosides from the root of *Iodes cirrhosa*. *J. Nat. Prod.* **2008**, *71*, 647−654.
(22) Ida, Y.; Satoh, Y.; Ohtsuka, M.; Nagasao, M.; Shoji, J. Phenolic constituents of *Phellodendron Amurense* Bark. *Phytochemistry* **1993**, *35*, 209−215.
(23) Emi, O.; Kuniharu, S.; Mikio, Y. A new lignan glycoside from the leaves of *Sambucus sieboldiana* (MIQ.) BLUME *ex*. GRAEBN. *Chem. Pharm. Bull.* **1995**, *50*, 669−671.
(24) Marinos, V. A.; Tate, M. E.; Williams, P. J. Lignan and phenylpropanoid glycerol glucosides in wine. *Phytochemistry* **1992**, *31*, 4307−4312.
(25) Kazuki, Y.; Hiroshi, K.; Shigenobu, A. Non-basic components of *Coptis rhizoma*. II. Four new hemiterpenoid glucosides, two new phenylpropanoid glucosides and a new flavonoid glycoside from *Coptis japonica* var. dissecta. *Natural Medicines (Tokyo)* **1997**, *51*, 244−248.
(26) Takani, M.; Ohya, K.; Takahashi, K. Studies on constituents of medicinal XXII. constituents of *Schizandra nigra* Max. *Chem. Pharm. Bull.* **1979**, *27*, 1422−1425.
(27) Kizu, H.; Shimana, H.; Tomimori, T. Studies on the constituents of *Clematis* Species. VI. The constituents of *Clematis Stans* SIEB. *et* ZUCC. *Chem. Pharm. Bull.* **1995**, *43*, 2187−2194.
(28) Koichi, M.; Misako, T.; Rie, K.; Yasunori, Y.; Masao, K. A New Lignan Glycoside from the Leaves of *Sambucus sieboldiana* (MIQ.) BLUME ex. GRAEBN. *Chem. Pharm. Bull.* **2002**, *50*, 669−671.
(29) Nakanishi, T.; Iida, N.; Inatomi, Y.; Murata, H.; Inada, A.; Murata, J.; Lang, F. A.; Iinuma, M.; Tanaka, T. Neolignan and flavonoid glycosides in *Juniperus communis* var. *depressa*. *Phytochemistry* **2004**, *65*, 207−213.
(30) Abe, F.; Yamauchi, T.; Wan, A. S. C. Lignans related to olivil from genus *Cerbera* (*Cerbera*. VI). *Chem. Pharm. Bull.* **1988**, *36*, 795−799.
(31) Barakat, H. H.; Hussein, S. A. M.; Marzouk, M. S.; Merfort, I.; Linscheid, M.; Nawwar, M. A. M. Polyphenolic metabolites of *Epilobium Hirsutum*. *Phytochemistry* **1997**, *46*, 935−942.
(32) Wu, W. L.; Chen, S. E.; Chang, W. L.; Chen, C. F.; Lee, A. R. Synthesis and antihepatotoxicity of some Wuweizisu analogues. *Eur. J. Med. Chem.* **1992**, *27*, 353−358.
(33) Sano, K.; Sanada, S.; Ida, Y.; Shoji, J. Studies on the constituents of the Bark of *Kalopanax pictus* NAKAI. *Chem. Pharm. Bull.* **1991**, *39*, 865−870.
(34) Cui, C. B.; Tezuka, Y.; Yamashita, H.; Kikuchi, T.; Nakano, H. Constituents of a fern, *Davallia mariesii* MOORE. V. Isolation and structures of davallin, a new tetrameric proanthocyanidin and two new phenolic glycosides. *Chem. Pharm. Bull.* **1993**, *41*, 1491−1497.
(35) Goda, Y.; Shibuya, M.; Sankawa, U. Inhibitors of prostaglandin biosynthesis from *Mucuna birdwoodiana*. *Chem. Pharm. Bull.* **1987**, *35*, 2675−2677.
(36) Gerothanassis, I. P.; Exarchou, V.; Lagouri, V.; Troganis, A.; Tsimidou, M.; Boskou, D. Methodology for identification of phenolic acids in complex phenolic mixtures by high-resolution two-dimensional nuclear magnetic resonance. Application to methanolic extracts of two *Oregano* species. *J. Agric. Food. Chem.* **1998**, *46*, 4185−4192.
(37) Choi, J. H.; Lee, D. U. A new citryl glycoside from *Gastrodia elata* and its inhibitory activity on GABA transaminase. *Chem. Pharm. Bull.* **2006**, *54*, 1720−1721.
(38) Morikawa, T.; Xie, H. H.; Matsuda, H.; Wang, T.; Yoshikawa, M. Bioactive constituents from Chinese natural medicines. XVII. Constituents with radical scavenging effect and new glucosyloxybenzyl 2-isobutylmalates from *Gymnadenia conopsea*. *Chem. Pharm. Bull.* **2006**, *54*, 506−513.
(39) Machida, K.; Kikuchi, M. Norisoprenoids from *Viburnum Dilatatum*. *Phytochemistry* **1996**, *41*, 1333−1336.
(40) Saito, N.; Toki, K.; Uesato, K.; Shigihara, A.; Honda, T. An acylated cyanidin glycoside from the red-purple flowers of *Dendrobium*. *Phytochemistry* **1994**, *37*, 245−248.
(41) Yoshikawa, M.; Shimada, H.; Shimoda, H.; Matsuda, H.; Yamahara, J.; Murakami, N. Rhodiocyanosides A and B, new antiallergic cyanoglycosides from Chinese natural medicine Si lie Hong Jing Tian, the underground part of *Rhodiola Quanrifida* (PALL.) Fisch. *et* MEY. *Chem. Pharm. Bull.* **1995**, *43*, 1245−1247.
(42) Nishida, R.; Rothschild, M.; Mummery, R. A cyanoglucoside, sarmentosin, from the Magpie Moth, *Abraxas Grossulariata*, geometridae: lepidoptera. *Phytochemistry* **1994**, *36*, 37−38.
(43) Motoaki, K.; Teruaki, A.; Lisa, I.; Keiji, D.; Takashi, Y.; Takuo, O.; Kyoichi, K. Enzymatic sulfation of isoamyl gallate and (−)-epigallocatechin gallate by bacterial arylsulfotransferase. *Chem. Pharm. Bull.* **1992**, *40*, 1864−1867.