HETEROCYCLES, Vol. 81, No. 4, 2010, pp. 909 - 916. © The Japan Institute of Heterocyclic Chemistry
Received, 14th December, 2009, Accepted, 8th February, 2010, Published online, 9th February 2010
DOI: 10.3987/COM-09-11886

NEW CYANOGLYCOSIDES, HYDRACYANOSIDES D, E, AND F, FROM THE LEAVES OF *HYDRANGEA MACROPHYLLA*

Zhibin Wang,[a,b] Seikou Nakamura,[a] Hisashi Matsuda,[a] Lijun Wu,[b] and Masayuki Yoshikawa[a,*]

[a]Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan
[b]School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract —Three new cyanoglycosides named hydracyanosides D (**1**), E (**2**), and F (**3**) were isolated from the leaves of *Hydrangea macrophylla* cultivated in China together with 17 known constituents including hydracyanoside A (**4**). Their structures were elucidated on the basis of chemical and physicochemical evidence.

In the course of our characterization studies on bioactive constituents from *Hydrangea* species,[1-6] we have reported the isolation and absolute stereostructure elucidation of three cyanogenic glycosides, hydracyanosides A (**4**), B, and C from *H. macrophylla* (Thunb.) Ser. (Saxifragaceae).[1] As a continuing study on the leaves of *H. macrophylla*, we have isolated three new cyanoglycosides named hydracyanosides D (**1**), E (**2**), and F (**3**) together with 17 known constituents. In this paper, we describe the isolation and structure elucidation of these three new constituents.

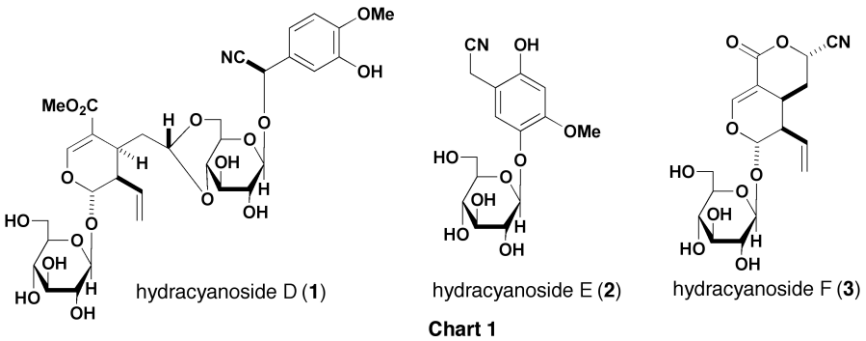

Chart 1

The leaves of *H. macrophylla* were finely cut and treated with MeOH to furnish a MeOH extract (26.0%). The MeOH extract was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (8.4%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH- and a H_2O-soluble fraction (6.5% and 11.1%, respectively).[1] The *n*-BuOH-soluble fraction was separated by normal- and reversed-phase column chromatography, and finally HPLC to give hydracyanosides D (**1**, 0.00071%), E (**2**, 0.00020%), F (**3**, 0.00023%), together with hydracyanoside A (**4**, 0.09%),[1] (2*R*)-taxiphyllin (**5**, 0.00028%),[7,8] tachioside (**6**, 0.00061%),[9] isotachioside (**7**, 0.00072%),[9] 3-(*β*-D-glucopyranosyloxy)-4-methoxybenzaldehyde (**8**, 0.00093%),[10] 2-phenylethyl-*β*-D-glucopyranoside (**9**, 0.00032%),[11] salidroside (**10**, 0.00014%),[12] icariside

F_2 (**11**, 0.00022%),[13] loganin (**12**, 0.0015%),[14] 8-*epi*-loganin (**13**, 0.0011%),[15] secoxyloganin (**14**, 0.00070%),[16] vogeloside (**15**, 0.0023%),[17] *epi*-vogeloside (**16**, 0.00038%),[17] thymidine (**17**, 0.00037%),[18,19] adenosine (**18**, 0.00055%),[20] (–)-methyl shikimate (**19**, 0.00023%),[21] *Z*-hex-3-en-1-ol *β*-D-xylopyranosyl(1-6)-*β*-D-glucopyranoside (**20**, 0.00051%)[22] (Chart 1, 2).

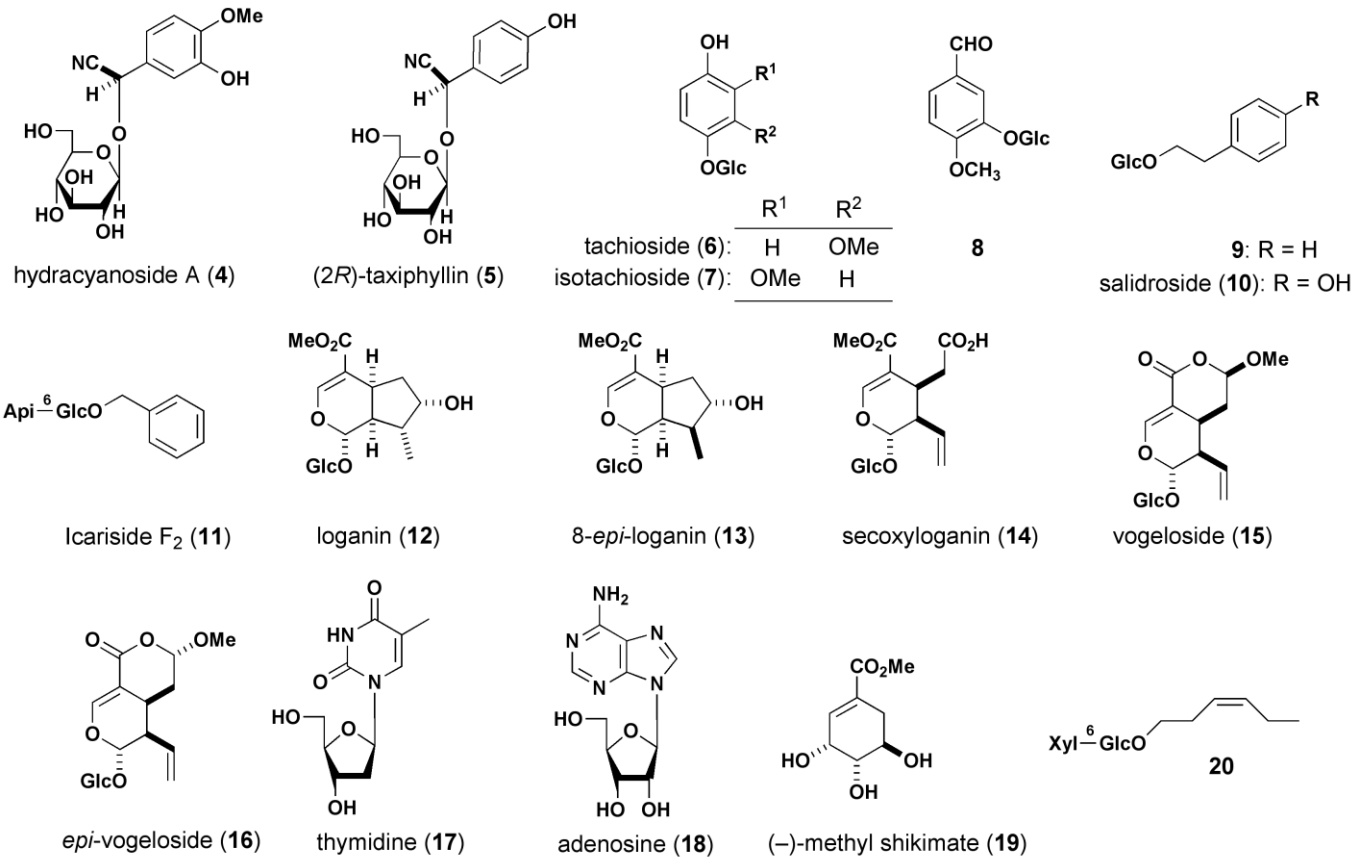

Chart 2

Hydracyanoside D (**1**) was isolated as a white powder with negative optical rotation ($[a]_D^{23}$ –111.6° in MeOH). The molecular formula $C_{32}H_{41}NO_{17}$ of **1** was clarified from the positive-ion FABMS [*m/z* 734 $(M+Na)^+$] and by HRFABMS measurement. The IR spectrum showed the presence of cyano group (2365 cm^{-1}), ester (1701 cm^{-1}), olefin (1655 cm^{-1}), and an oligoglycoside structure (3420 and 1078 cm^{-1}). Acid hydrolysis of **1** with 1 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[23,24] The ^{1}H-(CD_3OD) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[25] showed signals assignable to a methoxy group [δ 3.87 (3H, s)], a carbomethoxy group [δ 3.70 (3H, s)], a methine bearing a cyano functional group [δ 5.60 (1H, s, 2-H)], four olefinic protons [δ 5.24 (1H, br d, *J* = 11.0 Hz, 10'''a-H), 5.30 (1H, br d, *J* =

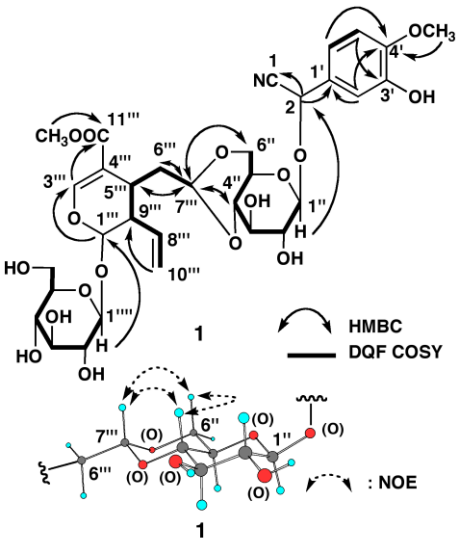

Figure 1

17.8 Hz, 10'''b-H), 5.73 (1H, ddd, *J* = 8.2, 11.0, 17.8 Hz, 8'''a-H), 7.45 (1H, s, 3'''-H)], an aromatic ring [δ6.95 (1H, dd, *J* = 2.1, 8.3 Hz, 6'-H), 6.96 (1H, d, *J* = 8.3 Hz, 5'-H), 7.00 (1H, d, *J* = 2.1 Hz, 2'-H)], two β-D-glucopyranosyl moieties [δ4.40 (1H, d, *J* = 8.0 Hz, 1''-H), 4.67 (1H, d, *J* = 7.6 Hz, 1''-H)], and a cyano group (δc 119.4, 1-C). The double quantum filter correlation spectroscopy (DQF COSY) experiment on **1** indicated the presence of partial structures written in bold lines, and in the heteronuclear multiple bond connectivity spectroscopy (HMBC) experiment, long-range correlations were observed between the following protons and carbons: 2-H and 1, 1'-C; 2'-H and 1', 4'-C; 5'-H and 3'-C; 6'-H and 4'-C; 1''-H and 2-C; 4''-H and 7'''-C; 6''-H and 7'''-C; 1'''-H and 3'''-C; 3'''-H and 11'''-C; 5'''-H and 7'''-C; 6'''-H and 7'''-C; 7'''-H and 4'', 6''-C; -OC*H*$_3$ and 4'-C; -COOC*H*$_3$ and 11'''-C (Figure 1). Furthermore, the NOESY spectrum showed NOE correlations between the following proton pairs (4''-H and 6''β, 7'''-H; 6''β -H and 7'''-H), so that the stereostructure of **1** was characterized. Finally, methanolysis of **1** with mild conditions was carried out to give hydracyanoside A (**4**)[1] and secoxyloganin dimethylacetal.[17] Consequently, the structure of hydracyanoside D (**1**) was determined as shown.

Table 1. ^{13}C-NMR data for **1**

Position		Position	
1	119.4	1'''	97.7
2	69.1	3'''	153.7
1'	127.3	4'''	111.5
2'	115.7	5'''	29.6
3'	148.3	6'''	35.1
4'	150.5	7'''	102.7
5'	112.6	8'''	135.8
6'	120.7	9'''	45.4
4'-O*C*H$_3$	56.5	10'''	119.9
1''	103.1	11'''	169.3
2''	75.6	COO*C*H$_3$	51.9
3''	74.5	1''	100.0
4''	81.6	2''	74.7
5''	67.9	3''	78.0
6''	69.2	4''	71.6
	-	5''	78.4
	-	6''	62.8

Measured in CD$_3$OD at 150 MHz

Hydracyanoside E (**2**), $[\alpha]_D^{24}$ –29.4° (MeOH), was isolated as a white powder. The IR spectrum of **2** showed absorption bands at 3570, 2257, and 1074 cm^{-1} ascribable to hydroxyl, cyano, and ether functional groups. The molecular formula $C_{15}H_{19}NO_8$ was determined from the positive-ion FABMS at *m/z* 364 $(M+Na)^+$ and by HRFABMS measurement. Acid hydrolysis of **2** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[23,24] The ^{1}H- (CD$_3$OD) and ^{13}C-NMR (Table 2) spectra[25] of **2** indicated the presence of a methoxy group [δ 3.81 (3H, s)], a methylene [δ 3.65 (1H, s, 2-H$_2$)], an aromatic ring [δ6.54 (1H, s, 3'-H), 7.13 (1H, s, 6'-H)], a β-D-glucopyranosyl moiety [δ4.72 (1H, d, *J* = 7.6 Hz, 1''-H)], and a cyano group (δc 119.8, 1-C). Next, long-range correlations in the HMBC experiment were observed between the following proton and carbon: 2-H$_2$ and 1, 1', 2', 6'-C; 3'-H and 1', 2', 5'-C; 6'-H and 2, 2', 4'-C; 1''-H and 5'-C; -OCH$_3$ and 4'-C (Figure 2). Furthermore, the NOESY spectrum showed NOE correlations between the following proton pairs (1''-H and 6'-H; -OCH$_3$ and 3'-H). On the basis of this evidence, the structure of **2** was elucidated as shown.

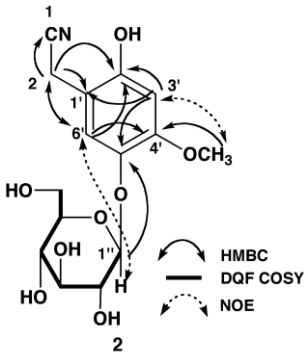

Figure 2

Table 2. ^{13}C-NMR data for **2**

Position		Position	
1	119.8	1''	104.3
2	18.1	2''	75.1
1'	109.9	3''	77.9
2'	152.5	4''	71.4
3'	101.7	5''	78.2
4'	152.0	6''	62.5
5'	140.9		
6'	121.1		
4'-*O*-*C*H$_3$	56.7		

Measured in CD$_3$OD at 150 MHz

Hydracyanoside F (**3**), $[\alpha]_D^{25}$ –106.2° (MeOH), was isolated as a white powder. The IR spectrum showed the presence of cyano group (2361 cm^{-1}), ester (1719 cm^{-1}), olefin (1655 cm^{-1}), and a glycoside structure (3420 and 1074 cm^{-1}). The molecular formula $C_{17}H_{21}NO_9$ was determined from the positive-ion FABMS at *m/z* 406 $(M+Na)^+$ and by HRFABMS measurement. Acid hydrolysis of **3** liberated D-glucose.[23,24] The ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 3) spectra[25] of **3** indicated the presence of a methine bearing a cyano functional group [δ5.59 (1H, dd, *J* = 2.0, 5.5 Hz, 7-H)], four olefinic protons [δ5.31 (1H, dd, *J* = 2.1, 10.3 Hz, 10a-H), 5.36 (1H, dd, *J* = 2.1, 17.3 Hz, 10b-H), 5.54 (1H, ddd, *J* = 9.7, 10.3, 17.3 Hz, 8-H), 7.70 (1H, br s, 3-H)], a β-D-glucopyranosyl moiety [δ4.75 (1H, d, *J* = 7.6 Hz, 1'-H)], and a cyano group (δc 118.0). The proton and carbon signals in the ^{1}H- and ^{13}C-NMR of **3** were similar to those of *epi*-vogeloside (**16**)[17] except for the signals around the 7-position. The connectivity of a sugar part in **3** was characterized by HMBC experiment, which showed long-range correlations between the 1'-proton and the 1-carbon (Figure 3). Furthermore, the NOESY spectrum showed NOE correlations between the following proton pairs (1β-H and 8-H; 5-H and 6α, 9-H; 6α-H and 7-H; 6β-H and 7-H). On the basis of this evidence, the structure of **3** was elucidated as shown.

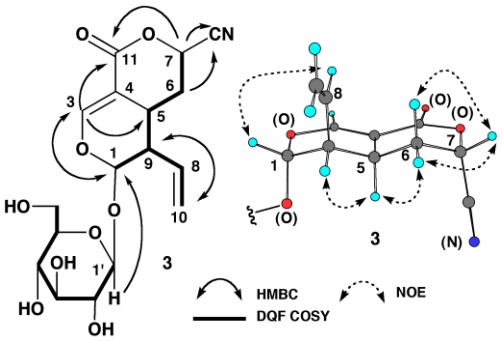

Figure 3

Table 3. ^{13}C-NMR data for **3**

Position		Position	
1	98.7	1'	100.6
3	156.0	2'	74.6
4	103.7	3'	78.1
5	25.6	4'	71.5
6	28.4	5'	78.5
7	66.8	6'	62.7
8	132.8		
9	43.1		
10	121.8		
11	165.0		
*C*N	118.0		

Measured in CD_3OD at 150 MHz

EXPERIMENTAL

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (*l* = 5 cm); UV spectra, Shimadzu UV-1600; IR spectra, Shimadzu FTIR-8100 spectrophotometer; FABMS and high-resolution FABMS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz), JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors; and HPLC column, Cosmosil 5C_{18}-MS-II (250 x 4.6 mm i.d.) and (250 x 20 mm i.d.) columns were used for analytical and preparative purposes, respectively. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC

plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material

The fresh leaves of *H. macrophylla*, which were cultivated in Sichuan province of China, were collected in 2008. A voucher of the plant is on file in our laboratory (Phamacognosy-2008-HM).

Isolation of Constituents from the Leaves of *H. macrophylla*

The fresh leaves (2.2 kg) of *H. macrophylla* cultivated in Sichuan province of China were finely cut and extracted with MeOH under reflux to provide a MeOH extract (573 g, 26.0%). The MeOH extract (563 g) was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (182 g, 8.4%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (140 g, 6.5%) and a H_2O-soluble fraction (240 g, 11.1%). The *n*-BuOH-soluble fraction (140 g) was subjected to ordinary-phase silica gel column chromatography {3.0 kg, $CHCl_3$ → $CHCl_3$–MeOH–H_2O [(30:3:1, v/v/v, lower layer) → (10:3:1, v/v/v, lower layer) → (7:3:1, v/v/v, lower layer) → (6:4:1, v/v/v)] → MeOH} to give five fractions [Fr. 1 (20.0 g), Fr. 2 (10.9 g), Fr. 3 (60.3 g), Fr. 4 (30.7 g), Fr. 5 (12.7 g)]. Fraction 1 (20.0 g) was subjected to reversed-phase silica gel column chromatography [500 g, MeOH–H_2O (15:85 → 25:75 → 45:55 → 65:35 → 85:15, v/v) → MeOH → $CHCl_3$] to afford six fractions [Fr. 1-1, Fr. 1-2 (1.0 g), Fr. 1-3 (4.2 g), Fr. 1-4 (1.2 g), Fr. 1-5, Fr. 1-6]. Fraction 1-2 (1.0 g) was subjected to HPLC [MeOH–H_2O (15:85, v/v)] to afford thymidine (**17**, 8.1 mg, 0.00037%). Fraction 1-3 (4.2 g) was subjected to HPLC [MeCN–H_2O (7:93, v/v)] to afford nine fractions [Fr. 1-3-1, Fr. 1-3-2, Fr. 1-3-3 [= 3-(*β*-D-glucopyranosyloxy)-4-methoxybenzaldehyde (**8**, 20 mg, 0.00093%)], Fr. 1-3-4, Fr. 1-3-5, Fr. 1-3-6, Fr. 1-3-7 (23 mg), Fr. 1-3-8, Fr. 1-3-9 [= 2-phenylethyl-*β*-D-glucopyranoside (**9**, 7.0 mg, 0.00032%)]. Fraction 1-3-7 (23 mg) was further purified by HPLC [MeOH–H_2O (28:72, v/v)] to give hydracyanoside F (**3**, 5.0 mg, 0.00023%). Fraction 1-4 (1.2 g) was subjected to HPLC [MeOH–H_2O (32:68, v/v)] to afford four fractions [Fr. 1-4-1, Fr. 1-4-2 (100 mg), Fr. 1-4-3, Fr. 1-4-4]. Fraction 1-4-2 (100 mg) was further purified by HPLC [MeCN–H_2O (10:90, v/v)] to give vogeloside (**15**, 50 mg, 0.0023%), *epi*-vogeloside (**16**, 8.3 mg, 0.00038%). Fraction 3 (60 g) was subjected to reversed-phase silica gel column chromatography [1.2 kg, MeOH–H_2O (15:85 → 25:75 → 45:55 → 65:35 → 85:15, v/v) → MeOH → $CHCl_3$] to afford ten fractions [Fr. 3-1, Fr. 3-2, Fr. 3-3, Fr. 3-4 (4.9 g), Fr. 3-5 (1.5 g), Fr. 3-6, Fr. 3-7 (1.3 g), Fr. 3-8 (1.8 g), Fr. 3-9, Fr. 3-10 (580 mg)]. Fraction 3-4 (4.9 g) was subjected to HPLC [MeOH–H_2O (15:85, v/v)] to afford hydracyanoside A (**4**, 1.92 g, 0.09%), hydracyanoside E (**2**, 4.3 mg, 0.00020%), (2*R*)-taxiphyllin (**5**, 6.0 mg, 0.00028%), tachioside (**6**, 13 mg, 0.00061%), isotachioside (**7**, 16 mg, 0.00072%), salidroside (**10**, 3.1 mg, 0.00014%), adenosine (**18**, 12 mg, 0.00055%), (–)-methyl shikimate (**19**, 5.0 mg, 0.00023%). Fraction 3-5 (1.5 g) was subjected to HPLC [MeCN–H_2O (10:90, v/v)] to afford icariside F_2 (**11**, 4.8 mg, 0.00022%). Fraction 3-7 (1.3 g) was subjected to HPLC [MeOH–H_2O (37:63, v/v)] to afford 8-*epi*-loganin (**13**, 23 mg, 0.0011%), *Z*-hex-3-en-1-ol *β*-D-xylopyranosyl(1-6)-*β*-D-glucopyranoside (**20**, 11 mg, 0.00051%). Fraction 3-8 (1.8 g) was subjected to HPLC [MeOH–H_2O (55:45, v/v)] to afford loganin (**12**, 33 mg, 0.0015%), secoxyloganin (**14**, 15 mg, 0.00070%).

Fraction 3-10 (580 mg) was subjected to HPLC [MeOH–H_2O (75:25, v/v)] to afford hydracyanoside D (**1**, 15 mg, 0.00071%).

Hydracyanoside D (1): a white powder, $[a]_D^{23}$ –111.6° (*c* 0.07, MeOH). High-resolution positive-ion FABMS: Calcd for $C_{32}H_{41}NO_{17}Na$ $(M+Na)^+$: 734.2272. Found: 734.2269. UV [MeOH, nm, (logε)]: 282 (3.55), 236 (4.24). IR (KBr): 3420, 2923, 2365, 1701, 1655, 1078 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ: 3.54 (1H, dd, *J* = 9.6, 10.3 Hz, 6"β-H), 4.16 (1H, dd, *J* = 4.4, 10.3 Hz, 6"α-H), 3.70 (3H, s, COOCH_3), 3.87 (3H, s, OCH_3), 4.40 (1H, d, *J* = 8.0 Hz, 1"-H), 4.67 (1H, d, *J* = 7.6 Hz, 1""-H), 4.69 (1H, dd, *J* = 5.5, 7.0 Hz, 7"'-H), 5.24 (1H, br d, *J* = 11.0 Hz, 10"'a-H), 5.30 (1H, br d, *J* = 17.8 Hz, 10"'b-H), 5.54 (1H, d, *J* = 6.2 Hz, 1"'-H), 5.60 (1H, s, 2-H), 5.73 (1H, ddd, *J* = 8.2, 11.0, 17.8 Hz, 8"'a-H), 6.95 (1H, dd, *J* = 2.1, 8.3 Hz, 6'-H), 6.96 (1H, d, *J* = 8.3 Hz, 5'-H), 7.00 (1H, d, *J* = 2.1 Hz, 2'-H), 7.45 (1H, s, 3"'-H). ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 1. Positive-ion FABMS: *m/z* 734 $(M+Na)^+$.

Hydracyanoside E (2): a white powder, $[a]_D^{24}$ –29.4° (*c* 0.02, MeOH). High-resolution positive-ion FABMS: Calcd for $C_{15}H_{19}NO_8Na$ $(M+Na)^+$: 364.1009. Found: 364.1012. UV [MeOH, nm, (logε)]: 289 (3.55), 224 (3.84). IR (KBr): 3570, 2926, 2257, 1074 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ: 3.81 (3H, s), 3.70 (1H, dd, *J* = 5.2, 11.7 Hz, 6"a-H), 3.88 (1H, dd, *J* = 2.0, 11.7 Hz, 6"b-H), 3.65 (1H, s, 2-H_2), 4.72 (1H, d, *J* = 7.6 Hz, 1"-H), 6.54 (1H, s, 3'-H), 7.13 (1H, s, 6'-H). ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FABMS: *m/z* 364 $(M+Na)^+$.

Hydracyanoside F (3): a white powder, $[a]_D^{25}$ –106.2° (*c* 0.02, MeOH). High-resolution positive-ion FABMS: Calcd for $C_{17}H_{21}NO_9Na$ $(M+Na)^+$: 406.1114. Found: 406.1119. UV [MeOH, nm, (logε)]: 242 (3.73). IR (KBr): 3420, 2924, 2361, 1719, 1655, 1074 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ: 2.01 (1H, ddd, *J* = 5.5, 13.7, 14.5 Hz,, 6β-H), 2.11 (1H, ddd, *J* = 2.0, 4.8, 14.5 Hz,, 6α-H), 4.75 (1H, d, *J* = 7.6 Hz, 1'-H), 5.31 (1H, dd, *J* = 2.1, 10.3 Hz, 10a-H), 5.36 (1H, dd, *J* = 2.1, 17.3 Hz, 10b-H), 5.54 (1H, ddd, *J* = 9.7, 10.3, 17.3 Hz, 8-H), 5.59 (1H, dd, *J* = 2.0, 5.5 Hz, 7-H), 5.60 (1H, d, *J* = 5.5 Hz, 1-H), 7.70 (1H, br s, 3-H). ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 3. Positive-ion FABMS: *m/z* 406 $(M+Na)^+$.

Acid Hydrolysis of 1–3. A solution of **1**, **2**, and **3** (each 1.0 mg) in 1.0 M aqueous HCl (2.0 mL) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was partitioned with EtOAc. The aqueous layer was subjected to HPLC analysis to identify the D-glucose under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, MeCN–H_2O (85:15, v/v); flow rate 0.8 mL/min; column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with that of authentic sample. t_R: D-glucose, 13.5 min (positive optical rotation).

Methanolysis of 1

A solution of hydracyanoside D (**1**, 5 mg, 0.007 mmol) in MeOH containing 0.1M HCl (2mL) was stirred

at 70 °C for 2 h. The solution was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue which was purified by HPLC [Cosmosil 5C_{18}-MS-II, MeOH–H_2O (30:70, v/v)] to afford hydracyanoside A (**4**, 0.4 mg) and secoxyloganin dimethylacetal (0.3 mg).

ACKNOWLEDGMENTS

This research was supported by the 21st COE program, Academic Frontier Project, and Grand-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES AND NOTES

1. S. Nakamura, Z. Wang, F. Xu, H. Matsuda, L. Wu, and M. Yoshikawa, *Tetrahedron Lett.*, 2009, **50**, 4639.
2. H. Zhang, H. Matsuda, C. Yamashita, S. Nakamura, and M. Yoshikawa, *Eur. J. Pharmacol.*, 2009, **606**, 255.
3. A. Kurume, Y. Kamata, M. Yamashita, Q. Wang, H. Matsuda, M. Yoshikawa, I. Kawasaki, and S. Ohta, *Chem. Pharm. Bull.*, 2008, **56**, 1264.
4. H. Matsuda, Q. Wang, K. Matsuhira, S. Nakamura, D. Yuan, and M. Yoshikawa, *Phytomedicine*, 2008, **15**, 177.
5. H. Zhang, H. Matsuda, A. Kumahara, Y. Ito, S. Nakamura, and M. Yoshikawa, *Bioorg. Med. Chem., Lett.*, 2007, **17**, 4972.
6. Q. Wang, H. Matsuda, K. Matsuhira, S. Nakamura, D. Yuan, and M. Yoshikawa, *Biol. Pharm. Bull.*, 2007, **30**, 388.
7. G. H. N. Towers, A. G. McInnes, and A. C. Neish, *Tetrahedron*, 1964, **20**, 71.
8. D. S. Seigler, G. F. Pauli, R. Fröhlich, E. Wegelius, A. Nahrstedt, K. E. Glander, and J. E. Ebinger, *Phytochemistry*, 2005, **66**, 1567.
9. X.-N. Zhong, H. Otsuka, T. Ide, E. Hirata, and Y. Takeda, *Phytochemistry*, 1999, **52**, 923.
10. A. E. Pavlov, V. M. Sokolov, and V. I. Zakharov, *Russ. J. Gen. Chem.*, 2001, **71**, 1811.
11. K. Kurashima, M. Fujii, Y. Ida, and H. Akita, *Chem. Pharm. Bull.*, 2004, **52**, 270.
12. H. Nishimura, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, 1984, **32**, 1735.
13. T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, and H. Oguchi, *Chem. Pharm. Bull.*, 1988, **36**, 2475.
14. K. Mitsunaga, K. Koike, H. Fukuda, K. Ishii, and T. Ohmoto, *Chem. Pharm. Bull.*, 1991, **39**, 2737.
15. A. Bianco and P. Passacantilli, *Phytochemistry*, 1981, **20**, 1873.
16. I. Calis and O. Sticher, *Phytochemistry*, 1984, **23**, 2539.
17. H. Kawai, M. Kuroyanagi, and A. Ueno, *Chem. Pharm. Bull.*, 1988, **36**, 3664.
18. This compound was identified by comparison of the physical data with commercially obtained sample.
19. A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *J. Am. Chem. Soc.*, 1970, **92**, 4079.
20. P. Ciuffreda, S. Casati, and A. Manzocchi, *Magn. Reson. Chem.*, 2007, **45**, 781.

21. J. Huang and F.-E. Chen, *Helv. Chim. Acta*, 2007, **90**, 1366.
22. M. Kishida, M. Fujii, Y. Ida, and H. Akita, *Heterocycles*, 2005, **65**, 2127.
23. S. Sugimoto, S. Nakamura, S. Yamamoto, C. Yamashita, Y. Oda, H. Matsuda, and M. Yoshikawa, *Chem. Pharm. Bull*., 2009, **57**, 257.
24. S. Nakamura, M. Hongo, S. Sugimoto, H. Matsuda, and M. Yoshikawa, *Phytochemistry*, 2008, **69**, 1565.
25. The ^{1}H- and ^{13}C-NMR spectra of **1**–**3** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence spectroscopy (HMQC), and heteronuclear multiple bond connectivity spectroscopy (HMBC) experiments.