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The absolute stereostructures of cyanogenic glycosides, hydracyanosides A, B, and C, from the leaves and stems of *Hydrangea macrophylla*

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ABSTRACT

Three new cyanogenic glycosides named hydracyanosides A (**1**), B (**2**), and C (**3**) were isolated from the leaves and/or stems of *Hydrangea macrophylla* in China. The absolute stereostructures of hydracyanosides were characterized on the basis of chemical and physiochemical evidence including single crystal X-ray crystallographic analysis. To the best of our knowledge, this is the first scientific report of cyanogenic glycosides from *Hydrangea* plants.

1. Introduction

A Saxifragaceae plant, *Hydrangea macrophylla* (Thunb.) Ser., is native to Japan and widely cultivated in many countries including Japan and China. The blossoms are mainly used for ornamental purposes. In June 2008, the cases of food poisoning that present symptoms of vomiting, etc. after eating the leaves of *H. macrophylla* were generated in Osaka and Ibaraki prefectures of Japan. At first, the Ministry of Health, Labor, and Welfare of Japan advised the possibility that the food poisoning was caused by cyanogenic glycosides in the leaves of *H. macrophylla*. However, since the scientific information for the cyanogenic glycoside constituents in the leaves of *H. macrophylla* are insufficient, the notice was corrected. Actually, to the best of our knowledge, there is no scientific evidence concerning cyanogenic glycoside constituents of *Hydrangea* plants. Thus, the findings of cyanogenic glycosides from *H. macrophylla* were ambiguous, so that the cause of the food poisoning remained to be not clarified. In the course of our chemical and pharmacological studies on *Hydrangea* plants,[1] we tried to examine the chemical constituents from the leaves and stems of *H. macrophylla* and isolated three new cyanogenic glycosides named hydracyanosides A (**1**), B (**2**), and C (**3**) together with a known cyanogenic glycoside, taxiphyllin (**5**).[2,3] This Letter deals with the absolute stereostructure elucidation of these three new constituents (**1**–**3**).

2. Results and discussion

The fresh leaves (2.2 kg) and stems (3.0 kg) of *H. macrophylla* cultivated in Sichuan province of China[4] were finely cut and extracted with MeOH under reflux to provide a MeOH extract [573 g (26.0%) from leaves, 361 g (12.0%) from stems]. The MeOH extract was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (8.4% from leaves, 2.6% from stems) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (6.5% from leaves, 3.3% from stems) and a H_2O-soluble fraction (11.1% from leaves, 6.1% from stems). The *n*-BuOH-soluble fractions from the leaves and stems were subjected to normal- and reversed-phase silica gel column chromatographies, and finally HPLC to give hydracyanosides A [**1**, 1.92 g (0.089%) from leaves, 3.89 g (0.13%) from stems], B [**2**, 11 mg (0.00037%) from stems], and C [**3**, 12 mg (0.00041%) from stems] together with taxiphyllin [**5**, 6 mg (0.00027%) from leaves]. In a similar method, hydracyanoside A (**1**, 201 mg, 0.0079%) was obtained from the EtOAc-soluble fraction of the stems (Fig. 1).

Hydracyanoside A (**1**),[5] $[\alpha]_D^{25}$ −67.4 (MeOH), was isolated as a white powder. The IR spectrum of **1** showed an absorption band at 2365 cm^{-1} ascribable to cyano group and broad bands at 3380 and 1080 cm^{-1} suggestive of an oligoglycoside structure. In the positive-ion FABMS of **1**, a quasimolecular ion peak was observed at *m/z* 364 $(M+Na)^+$. The HRFABMS analysis revealed the molecular formula of **1** to be $C_{15}H_{19}NO_8$. Acid hydrolysis of **1** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[6] The ^1H (CD_3OD) and ^{13}C NMR (Table 1) spectra[7] of **1**

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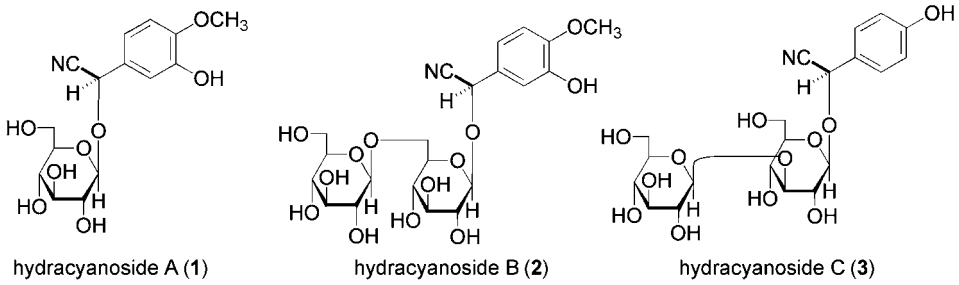

Figure 1. Structures of hydracyanosides A (**1**), B (**2**), and C (**3**).

Table 1
^{13}C NMR data for **1**–**3**

Position	1	2	3
1	119.6	119.7	119.6
2	68.1	68.7	68.1
1′	126.8	127.4	116.8
2′	115.7	115.8	130.9
3′	147.9	148.2	125.1
4′	150.4	150.4	160.4
5′	112.6	112.6	125.1
6′	121.0	120.8	130.9
4′-*O*-Me	56.5	56.4	—
1″	101.2	102.2	100.7
2″	74.5	74.7	74.1
3″	77.6	77.6	87.4
4″	71.3	71.5	70.0
5″	78.0	77.9	78.0
6″	62.6	69.9	62.7
1‴	—	104.9	105.2
2‴	—	75.3	75.7
3‴	—	77.8	77.8
4‴	—	71.6	71.6
5‴	—	77.9	78.1
6‴	—	62.7	62.6

Measured in CD_3OD at 150 MHz.

indicated the presence of a methoxy group [δ 3.85 (3H, s)], a methine bearing an oxygen function [δ 5.77 (1H, s, H-2)], an ABX-type aromatic ring [δ 6.94 (1H, d, J = 8.2 Hz, H-5′), 7.02 (1H, dd, J = 2.0, 8.2 Hz, H-6′), 7.05 (1H, d, J = 2.0 Hz, H-2′)], a β-D-glucopyranosyl moiety [δ 4.27 (1H, d, J = 7.6 Hz, H-1″)], and a cyano group (δ_c 119.6, C-1). As shown in Figure 2, the DQF COSY experiment on **1** indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-2 and C-1, 1′, 2′, 6′; H-2′ and C-4′, 6′; H-5 and C-1′, 3′; H-1″ and C-2. In addition, the NOE correlation between methoxy proton and H-5′ was observed in the difference NOESY experiment on **1**. Thus, **1** was characterized to be a cyanogenic glycoside with a 3-hydroxy-4-methoxyphenyl moiety. Next, the absolute configuration at the C-2 position of **1** was characterized by comparison of the 2- and 1″-proton signals of **1** with those of known cyanogenic glycosides on the ^{1}H NMR (CD_3OD) spectrum (Fig. 3, Table 2). Namely, Seigler et al. reported that the 2- and 1″-proton signals in the ^{1}H NMR (CD_3OD) spectrum of prunasin [**4**, δ 5.89 (H-2), 4.25 (H-1″)][8] and taxiphyllin [**5**, δ 5.78 (H-2), 4.17 (H-1″)][3] having

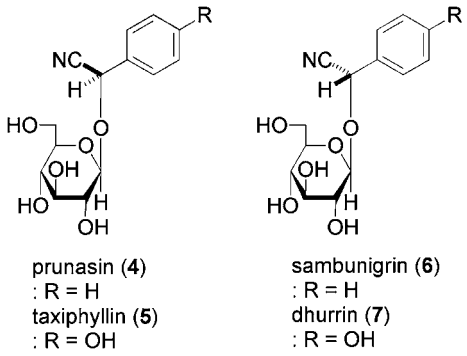

Figure 3. Known cyanogenic glycosides (**4**–**7**).

Table 2
Comparison of the chemical shifts of **1**–**3** and known cyanogenic glycosides on the ^{1}H NMR (CD_3OD) spectrum

H	1	2	3	4	5	6	7
2	5.77	5.73	5.79	5.89	5.78	6.03	5.90
1″	4.27	4.29	4.23	4.25	4.17	4.67	4.67

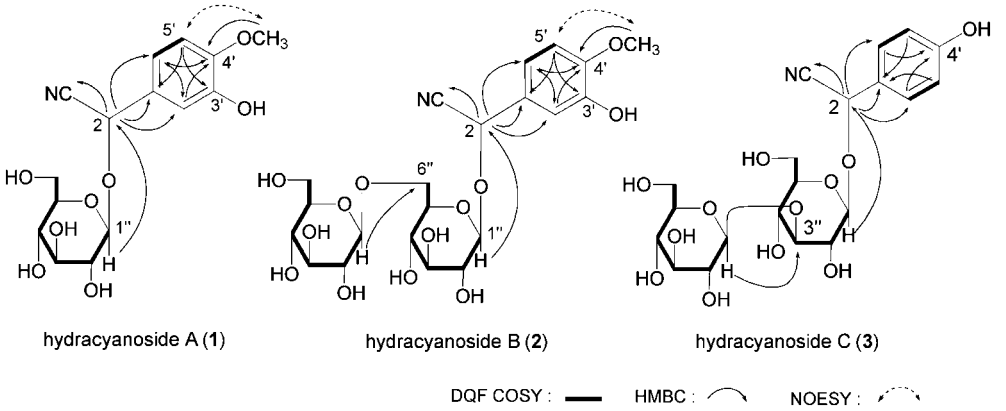

Figure 2. DQF COSY, HMBC, and NOE correlations of hydracyanosides A (**1**), B (**2**), and C (**3**).

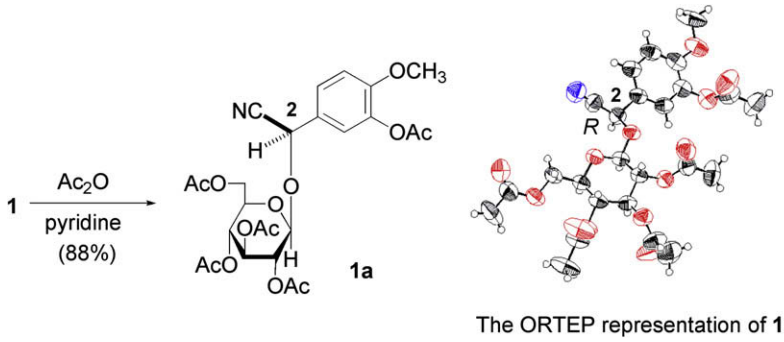

Figure 4. Absolute stereostructure of hydracyanoside A (**1**).

R orientation were shifted upfield relative to those of sambunigrin [**6**, δ 6.03 (H-2), 4.67 (H-1″)][8] and dhurrin [**7**, δ 5.90 (H-2), 4.67 (H-1″)][3] having *S* orientation. The 2- and 1″-proton signals of **1** were observed at δ 5.77 and 4.27, respectively, so that the C-2 orientation of **1** was characterized to be *R*. Finally, the absolute stereostructure at the 2-position in **1** was confirmed to be *R* orientation by the single crystal X-ray crystallographic analysis of the penta-*O*-acetyl derivative (**1a**) from **1** upon acetylation reaction with acetic anhydride in pyridine. The ORTEP representation of the X-ray structure on **1** is presented in Figure 4.[9] Consequently, the total structure of hydracyanoside A (**1**) was determined as shown.

Hydracyanoside B (**2**),[10] $[\alpha]_D^{26}$ −26.0 (MeOH), was isolated as a white powder. The IR spectrum of **2** showed absorption bands at 3400, 2365, and 1080 cm^{-1} ascribable to hydroxyl, cyano, and ether functions. The molecular formula $C_{21}H_{29}NO_{13}$ was determined from the positive-ion FABMS at *m/z* 526 $(M+Na)^+$ and by HRFABMS measurement. Acid hydrolysis of **2** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[6] The proton and carbon signals of **2** in the ^1H and ^{13}C NMR spectra were superimposable on those of **1**, except for the signals due to the 6-positon of the 2-*O*-β-D-glucopyranoside moiety on **2**. The planar structure of **2** was characterized by means of DQF COSY and HMBC experiments,[7] which showed long-range correlations between the following protons and carbons: H-1″ and C-2; H-1‴ and C-6″ (Fig. 2). On the basis of the above-mentioned evidence, the structure of **2** was characterized to be a cyanogenic diglycoside with the same aglycon as **1**. Finally, by comparison of the 2- and 1″-proton signals of **2** with those of known cyanogenic glycosides on the ^1H NMR (CD_3OD) spectrum as that used to characterize the configuration at the C-2 position of **1**, the C-2 orientation of **2** was determined to be *R* (Table 2). Consequently, the structure of hydracyanoside B (**2**) was determined as shown.

Hydracyanoside C (**3**),[11] $[\alpha]_D^{25}$ −16.7 (MeOH), was isolated as a white powder. The IR spectrum of **2** showed absorption bands ascribable to hydroxyl, cyano, and ether functions. The molecular formula $C_{20}H_{28}NO_{12}$ was determined from the positive-ion FABMS at *m/z* 496 $(M+Na)^+$ and by HRFABMS measurement. Acid hydrolysis of **3** liberated D-glucose.[6] The ^1H (CD_3OD) and ^{13}C NMR (Table 1) spectra[7] of **3** indicated the presence of a methine bearing an oxygen function [δ 5.79 (1H, s, H-2)], an A_2B_2-type aromatic ring [δ 6.84 (2H, d, *J* = 8.2 Hz, H-3′,5′), 7.39 (2H, d, *J* = 8.2 Hz, H-2′,6′)], two β-D-glucopyranosyl moieties [δ 4.23 (1H, d, *J* = 7.6 Hz, H-1″), 4.51 (1H, d, *J* = 8.2 Hz, H-1‴)], and a cyano group (δ_C 119.6, C-1). The planar structure of **3** was characterized by means of DQF COSY and HMBC experiments, which showed long-range correlations between the following proton and carbon: H-1″ and C-2; H-1‴ and C-3″ (Fig. 2). Finally, by using a similar NMR method as that used to characterize the configuration at the C-2 position of **2**, the C-2 orientation of **3** was determined to be *R* (Table 2). Consequently, the structure of hydracyanoside B (**2**) was determined as shown.

In conclusion, a cyanogenic glycoside, hydracyanoside A (**1**), was isolated as a major constituent from the leaves and stems of *H. macrophylla* cultivated in Sichuan province of China. In addition, two cyanogenic glycosides, hydracyanosides B (**2**) and C (**3**), were also isolated from the stems. To the best of our knowledge, this is the first scientific report of the isolation of cyanogenic glycosides from *Hydrangea* plants. In the cases of food poisoning after eating the leaves of *H. macrophylla*, the possibility of the involvement of cyanogenic glycosides was suggested. Continuously, toxicity assay of these cyanogenic glycosides should be subjected further.

Acknowledgments

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4. The fresh leaves and stems 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).
5. *Hydracyanoside A* (**1**): A white powder, $[\alpha]_D^{25}$ −67.4 (*c* 0.02, MeOH). HRFABMS: Calcd for $C_{15}H_{19}NO_8Na$ $(M+Na)^+$: 364.1008. Found: 364.1012. UV (MeOH) λ_{max} (log ε) nm 282 (3.68), 237 (3.96), 209 (4.54). IR (KBr) λ_{max} cm^{-1} 3380, 2943, 2365, 1618, 1516, 1080 cm^{-1}. ^1H NMR (600 MHz, CD_3OD) δ 3.74 (1H, dd, *J* = 2.0, 12.0 Hz, Ha-6″), 3.94 (1H, dd, *J* = 6.2, 12.0 Hz, Hb-6″), 3.85 (3H, s, OCH_3), 4.27 (1H, d, *J* = 7.6 Hz, H-1″), 5.77 (1H, s, H-2), 6.94 (1H, d, *J* = 8.2 Hz, H-5′), 7.02 (1H, dd, *J* = 2.0, 8.2 Hz, H-6′), 7.05 (1H, d, *J* = 2.0 Hz, H-2′). ^{13}C NMR data (150 MHz, CD_3OD) δ_C: given in Table 1. FABMS: *m/z* 364 $[M]^+$.
6. (a) Sugimoto, S.; Nakamura, S.; Yamamoto, S.; Yamashita, C.; Oda, Y.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2009**, *57*, 257–261; (b) Nakamura, S.; Hongo, M.; Sugimoto, S.; Matsuda, H.; Yoshikawa, M. *Phytochemistry* **2008**, *69*, 1565–1572.
7. The ^1H 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 (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.
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9. Crystal data for acyl derivative (**1a**) of hydracyanoside A (**1**). $C_{25}H_{29}NO_{13}$, M_W 551.50, *T* = 294 K, λ = 1.54187 Å, monoclinic, space group $P2_12_12_1$ (#19), *a* = 8.24352(15) Å, *b* = 16.7081(3) Å, *c* = 20.7844(4) Å, *V* = 2862.71(9) $Å^3$, *Z* = 4, D_{calcd} = 1.155 mg/m^3, μ(Cu Kα) = 8.960 cm^{-1}, *F*(0 0 0) = 1160.00, crystal size, 0.35 × 0.08 × 0.07 mm. No. of reflections measured: total, 33343; unique, 5184

(R_{int} = 0.033). Refinement method: full-matrix least-squares on F^2, goodness of fit indicator 1.011, final $R1[I > 2.00\sigma(I)]$ = 0.0509 maximum peak in final Diff. Map and minimum peak 0.49 $e^-/Å^3$ and −0.45 $e^-/Å^3$.

10. *Hydracyanoside B* (**2**): A white powder, $[\alpha]_D^{26}$ −26.0 (*c* 0.30, MeOH). HRFABMS: Calcd for $C_{21}H_{29}NO_{13}Na$ $(M+Na)^+$: 526.1537. Found: 526.1542. UV (MeOH) λ_{max} (log ε) nm 282 (3.62), 236 (3.87), 207 (4.51). IR (KBr) λ_{max} cm^{-1} 3400, 2926, 2365, 1618, 1516, 1080. ^1H NMR (600 MHz, CD_3OD) δ 3.87 (3H, s, OCH_3), 4.29 (1H, d, *J* = 7.6 Hz, H-1″), 4.51 (1H, d, *J* = 7.6 Hz, H-1‴), 5.73 (1H, s, H-2), 6.96 (1H, d, *J* = 8.8 Hz, H-5′), 7.03 (1H, dd, *J* = 2.0, 8.8 Hz, H-6′), 7.03 (1H, d, *J* = 2.0 Hz, H-2′). ^{13}C NMR data (150 MHz, CD_3OD) δ_C: given in Table 1. FABMS: *m/z* 526 $[M]^+$.
11. *Hydracyanoside C* (**3**): A white powder, $[\alpha]_D^{25}$ −16.7 (*c* 0.15, MeOH). HRFABMS: Calcd for $C_{20}H_{28}NO_{12}Na$ $(M+Na)^+$: 496.1431. Found: 496.1435. UV (MeOH) λ_{max} (log ε) nm 275 (3.41), 232 (4.02), 203 (4.23). IR (KBr) λ_{max} cm^{-1} 3400, 2924, 2363, 1616, 1518, 1080. ^1H NMR (600 MHz, CD_3OD) δ 4.23 (1H, d, *J* = 7.6 Hz, H-1″), 4.51 (1H, d, *J* = 8.2 Hz, H-1‴), 5.79 (1H, s, H-2), 6.84 (2H, d, *J* = 8.2 Hz, H-3′,5′), 7.39 (2H, dd, *J* = 8.2 Hz, H-2′,6′). ^{13}C NMR data (150 MHz, CD_3OD) δ_C: given in Table 1. FABMS: *m/z* 496 $[M]^+$.