

Novel Cyanoglucosides from the Leaves of *Hydrangea macrophylla*

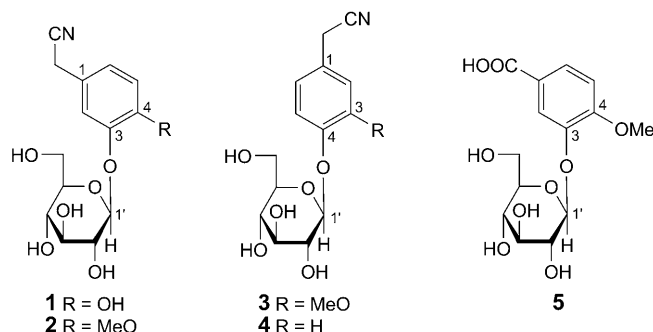
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Four non-cyanogenic cyanoglucosides including hydranitrilosides A₁, A₂, B₁, and B₂ (**1–4**, resp.), together with a new phenolic glucoside, 3-hydroxy-4-methoxybenzoic acid 3-*O*-β-D-glucopyranoside (**5**), were isolated from the leaves of *Hydrangea macrophylla*. Their structures were determined on the basis of chemical and spectral evidence.

Introduction. – *Hydrangea macrophylla* (THUNB.) SER, a member of Saxifragaceae family, is widely cultivated in many countries including China and Japan. *Hydrangea dulcis folium*, which is the fermented and dried leaves of *H. macrophylla*, is listed in the Japanese Pharmacopoeia XV and extensively used in confectionery, drinks, and foods as a sweetener [1]. Dihydroisocoumarins [2][3] and secoiridoid glycosides [4][5] isolated from this plant have showed many pharmacological effects, such as antidiabetic [6], antiallergic, and antimicrobial activities [7].

Here, we report the isolation and structure elucidation of three new non-cyanogenic cyanoglucosides, hydranitrilide A₁ (**1**), hydranitrilide A₂ (**2**), and hydranitrilide B₁ (**3**), a related derivative isolated for the first time as a new natural product, hydranitrilide B₂ (**4**) [8], and a new phenolic glucoside, 3-hydroxy-4-methoxybenzoic acid 3-*O*-β-D-glucopyranoside (**5**) from the leaves of the plant.



Results and Discussion. – Compound **1** was obtained as white amorphous powder and possessed a molecular formula C₁₄H₁₇NO₇, deduced from the HR-ESI-MS analysis ([*M* + Na]⁺ at *m/z* 334.0901; [*M* – H][–] at *m/z* 310.0929). The IR spectrum indicated the presence of OH (3380 cm^{–1}) and CN (2255 cm^{–1}) groups, and an aromatic ring (1608

and 1518 cm⁻¹). The ¹H-NMR (Table 1) spectrum indicated the presence of a CH₂ group ($\delta(\text{H})$ 3.75 (s)) bearing a CN group and an aromatic ring with an *ABX*-coupling system ($\delta(\text{H})$ 7.17 (*d*, *J* = 2.0, 1 H), 6.82 (*d*, *J* = 8.2, 1 H), 6.89 (*dd*, *J* = 8.2, 2.0, 1 H)). The signal at $\delta(\text{H})$ 4.76 (*d*, *J* = 7.6, 1 H) was assigned to an anomeric H-atom signal of the sugar unit. The acid hydrolysis of **1** with aqueous HCl (1M) yielded D-glucose, which was identified by HPLC analysis using an optical rotation detector [9]. The ¹³C-NMR (Table 2) spectrum showed 14 C-atom signals including those of a cyano C-atom at $\delta(\text{C})$ 120.0 and six aromatic C-atoms at $\delta(\text{C})$ 148.2, 147.0, 124.3, 123.6, 118.8, and 117.6. As shown in the Figure, the DQF-COSY experiment on **1** indicated the presence of

Table 1. ¹H-NMR Data (at 600 MHz, δ in ppm, *J* in Hz) of Compounds **1–4**

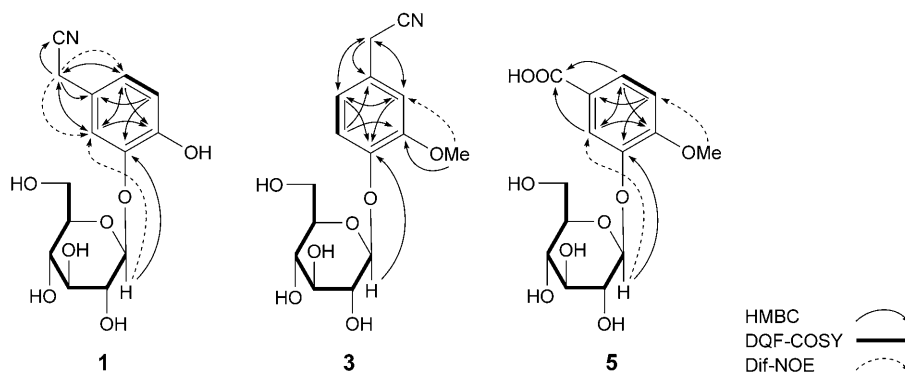
	1 ^{a)}	2 ^{b)}	3 ^{a)}	4 ^{a)}
H–C(2)	7.17 (<i>d</i> , <i>J</i> = 2.0)	7.56 (<i>d</i> , <i>J</i> = 2.0)	6.98 (<i>d</i> , <i>J</i> = 2.1)	7.28 (<i>d</i> , <i>J</i> = 8.5)
H–C(3)				7.11 (<i>d</i> , <i>J</i> = 8.5)
H–C(5)	6.82 (<i>d</i> , <i>J</i> = 8.2)	6.91 (<i>d</i> , <i>J</i> = 8.3)	7.16 (<i>d</i> , <i>J</i> = 8.3)	7.11 (<i>d</i> , <i>J</i> = 8.5)
H–C(6)	6.89 (<i>dd</i> , <i>J</i> = 8.2, 2.0)	7.00 (<i>dd</i> , <i>J</i> = 8.3, 2.0)	6.89 (<i>dd</i> , <i>J</i> = 8.3, 2.1)	7.28 (<i>d</i> , <i>J</i> = 8.5)
CH ₂ –C(1)	3.75 (<i>s</i>)	3.81 (<i>s</i>)	3.83 (<i>s</i>)	3.82 (<i>s</i>)
MeO		3.71 (<i>s</i>)	3.86 (<i>s</i>)	
H–C(1')	4.76 (<i>d</i> , <i>J</i> = 7.6)	5.61 (<i>d</i> , <i>J</i> = 7.6)	4.90 (<i>d</i> , <i>J</i> = 7.6)	4.90 (<i>d</i> , <i>J</i> = 7.5)
H–C(2')	3.48 (<i>dd</i> , <i>J</i> = 8.9, 7.6)	4.27–4.32 (<i>m</i>)	3.49 (<i>dd</i> , <i>J</i> = 8.9, 7.6)	3.46 (<i>dd</i> , <i>J</i> = 8.9, 7.5)
H–C(3')	3.45 (<i>dd</i> , <i>J</i> = 8.9, 8.2)	4.27–4.32 (<i>m</i>)	3.45 (<i>dd</i> , <i>J</i> = 8.9, 9.7)	3.40–3.45 (<i>m</i>)
H–C(4')	3.39–3.43 (<i>m</i>)	4.27–4.32 (<i>m</i>)	3.39–3.43 (<i>m</i>)	3.36–3.42 (<i>m</i>)
H–C(5')	3.37–3.41 (<i>m</i>)	4.01–4.05 (<i>m</i>)	3.37–3.41 (<i>m</i>)	3.36–3.42 (<i>m</i>)
CH ₂ (6')	3.90 (<i>dd</i> , <i>J</i> = 11.7, 2.0), 3.71 (<i>dd</i> , <i>J</i> = 11.7, 4.8)	4.49 (<i>dd</i> , <i>J</i> = 11.7, 2.0), 4.33 (<i>dd</i> , <i>J</i> = 11.7, 4.8)	3.88 (<i>dd</i> , <i>J</i> = 12.4, 1.9), 3.69 (<i>dd</i> , <i>J</i> = 12.4, 4.8)	3.90 (<i>dd</i> , <i>J</i> = 12.2, 2.0), 3.69 (<i>dd</i> , <i>J</i> = 12.2, 5.1)

^{a)} Recorded in CD₃OD. ^{b)} Recorded in C₅D₅N.

Table 2. ¹³C-NMR Data (at 150 MHz, δ in ppm) of Compounds **1–4**

	1 ^{a)}	2 ^{a)}	2 ^{b)}	3 ^{a)}	4 ^{a)}
C(1)	124.3 (<i>s</i>)	125.0 (<i>s</i>)	123.9 (<i>s</i>)	126.9 (<i>s</i>)	126.0 (<i>s</i>)
C(2)	118.8 (<i>d</i>)	118.0 (<i>d</i>)	116.7 (<i>d</i>)	113.6 (<i>d</i>)	130.3 (<i>d</i>)
C(3)	147.0 (<i>s</i>)	148.2 (<i>s</i>)	148.2 (<i>s</i>)	151.2 (<i>s</i>)	118.4 (<i>d</i>)
C(4)	148.2 (<i>s</i>)	150.6 (<i>s</i>)	149.8 (<i>s</i>)	147.6 (<i>s</i>)	158.8 (<i>s</i>)
C(5)	117.6 (<i>d</i>)	114.0 (<i>d</i>)	113.5 (<i>d</i>)	118.3 (<i>d</i>)	118.4 (<i>d</i>)
C(6)	123.6 (<i>d</i>)	123.5 (<i>d</i>)	121.9 (<i>d</i>)	121.1 (<i>d</i>)	130.3 (<i>d</i>)
CH ₂ –C(1)	22.9 (<i>t</i>)	23.0 (<i>t</i>)	22.8 (<i>t</i>)	23.1 (<i>t</i>)	22.8 (<i>t</i>)
C(8)	120.0 (<i>s</i>)	119.9 (<i>s</i>)	119.1 (<i>s</i>)	119.8 (<i>s</i>)	119.9 (<i>s</i>)
MeO		56.8 (<i>q</i>)	56.2 (<i>q</i>)	56.8 (<i>q</i>)	
C(1')	104.3 (<i>d</i>)	102.7 (<i>d</i>)	102.3 (<i>d</i>)	102.7 (<i>d</i>)	102.3 (<i>d</i>)
C(2')	74.9 (<i>d</i>)	74.9 (<i>d</i>)	74.9 (<i>d</i>)	74.9 (<i>d</i>)	74.9 (<i>d</i>)
C(3')	77.6 (<i>d</i>)	77.9 (<i>d</i>)	78.5 (<i>d</i>)	77.7 (<i>d</i>)	78.0 (<i>d</i>)
C(4')	71.3 (<i>d</i>)	71.3 (<i>d</i>)	71.2 (<i>d</i>)	71.3 (<i>d</i>)	71.4 (<i>d</i>)
C(5')	78.4 (<i>d</i>)	78.3 (<i>d</i>)	78.8 (<i>d</i>)	78.2 (<i>d</i>)	78.2 (<i>d</i>)
C(6')	62.4 (<i>t</i>)	62.5 (<i>t</i>)	62.4 (<i>t</i>)	62.5 (<i>t</i>)	62.6 (<i>t</i>)

^{a)} Recorded in CD₃OD. ^{b)} Recorded in C₅D₅N.



partial structures indicated in bold lines, and, in the HMBC experiment, long-range correlations were observed between the following H- and C-atoms: H–C(7) and C(1), C(2), C(6), and C(8); H–C(1') and C(3). In addition, the position of glucose was confirmed by the observation of an NOE effect at $\delta(\text{H})$ 7.17 (H–C(2)) upon irradiation of anomeric H-atom ($\delta(\text{H})$ 4.76 (H–C(1'))). On the basis of the above analysis, the structure of hydranitriloside **A**₁ (**1**) was established as (3,4-dihydroxyphenyl)acetone nitrile 3-*O*- β -D-glucopyranoside.

Compound **2** was obtained as white amorphous powder, and the molecular formula was determined as $\text{C}_{15}\text{H}_{19}\text{NO}_7$ on the basis of the HR-ESI-MS ($[M + \text{Na}]^+$ at m/z 348.1058). The IR spectrum indicated the presence of OH (3380 cm^{-1}) and CN (2255 cm^{-1}) groups, and an aromatic ring (1608 and 1518 cm^{-1}). In the ^1H -NMR spectrum, the signals of aromatic H-atoms overlapped when CD_3OD was used as solvent, and better resolution was achieved in $\text{C}_5\text{D}_5\text{N}$. The NMR data were very similar to those of compound **1**, with the exception of the presence of a MeO signal at $\delta(\text{H})$ 3.71 in the ^1H -NMR (Table 1) spectrum and at $\delta(\text{C})$ 56.2 in the ^{13}C -NMR spectrum (Table 2). In the HMBC spectrum, the cross-peaks between H–C(7) and C(1), C(2), C(6), and C(8); H–C(1') and C(3); and MeO and C(4) were observed. The irradiation of the MeO group ($\delta(\text{H})$ 3.71) resulted in an enhancement of the signal at $\delta(\text{H})$ 6.91 (H–C(5)) in the difference NOE experiment. Therefore, compound hydranitriloside **A**₂ (**2**) was identified as (3-hydroxy-4-methoxyphenyl)acetone nitrile 3-*O*- β -D-glucopyranoside.

Compound **3** was obtained as white amorphous powder with a molecular formula of $\text{C}_{15}\text{H}_{19}\text{NO}_7$, deduced from the HR-ESI-MS ($[M + \text{Na}]^+$ at m/z 348.1061). The presence of OH (3400 cm^{-1}) and CN (2249 cm^{-1}) functions, and of an aromatic ring (1597 and 1516 cm^{-1}) were suggested by its IR spectrum. The ^1H - and ^{13}C -NMR (Tables 1 and 2) spectra of **3** indicated the presence of a CH_2 group ($\delta(\text{H})$ 3.83 (s)) bearing a CN group, an aromatic ring with an *ABX*-coupling system ($\delta(\text{H})$ 7.16 (d, $J = 8.9, 1\text{ H}$), 6.98 (d, $J = 2.1, 1\text{ H}$), 6.89 (dd, $J = 8.9, 2.1, 1\text{ H}$)), a MeO group ($\delta(\text{H})$ 3.86 (s)), an anomeric H-atom ($\delta(\text{H})$ 4.90 (d, $J = 7.6$)), and a CN C-atom ($\delta(\text{C})$ 119.1). The acid hydrolysis of **3** liberated D-glucose, which was identified by HPLC analysis [9]. In the difference-NOE experiment, the irradiation of the MeO group ($\delta(\text{H})$ 3.86) resulted in an enhancement

of the signal at $\delta(\text{H})$ 6.98 (H–C(2)), which suggested that compound **3** was an isomer of compound **2** with respect to the MeO position. The 2D-NMR data, including HMQC, HMBC, and DQF-COSY experiments, indicated that in **3** the respective positions of the MeO and glucopyranosyloxy substituents reversed, compared to **2**. Thus, compound **3**, named hydranitriloside B₁, was elucidated as (4-hydroxy-3-methoxyphenyl)acetonitrile 4-*O*- β -D-glucopyranoside.

Compound **4** was obtained as white amorphous powder with a molecular formula of C₁₄H₁₇NO₆, on the basis of HR-ESI-MS ($[M + \text{Na}]^+$ at m/z 318.0956). The acid hydrolysis of **4** liberated D-glucose [9]. The ¹H- and ¹³C-NMR spectra indicated the presence of a CH₂ group ($\delta(\text{H})$ 3.82 (s)), an AA'BB'-coupling of an aromatic ring ($\delta(\text{H})$ 7.28 (d, $J = 8.5$, 2 H), 7.11 (d, $J = 8.5$, 2 H)), a β -D-glucopyranosyl anomeric H-atom ($\delta(\text{H})$ 4.90 (d, $J = 7.5$)), and a CN group ($\delta(\text{C})$ 119.9 (C(8))). Furthermore, long-range correlations in the HMBC spectra were observed between the following H- and C-atoms: H–C(7) and C(1), C(2), and C(8); and H–C(1') and C(4). On the basis of this evidence, the structure of **4** was determined as (4-hydroxyphenyl)acetonitrile 4-*O*- β -D-glucopyranoside. This compound, for which we propose the name hydranitriloside B₂, was previously synthesized by Helferich *et al.* in 1935 [8], and it is reported here for the first time as a natural product.

Compound **5**, obtained as white amorphous powder, was found to possess a molecular formula of C₁₄H₁₈O₉, as evidenced by the HR-ESI-MS ($[M + \text{Na}]^+$ at m/z 353.0851). The IR spectrum indicated the presence of OH (3380 cm^{–1}) and CO (1698 cm^{–1}) groups, a benzene ring (1605 and 1509 cm^{–1}), and an ether (1074 cm^{–1}) function. The ¹H-NMR spectrum (Table 3) showed signals assignable to a trisubstituted aromatic system at $\delta(\text{H})$ 8.55 (d, $J = 2.0$, 1 H), 7.02 (d, $J = 8.2$, 1 H), and 8.11 (dd, $J = 8.2$, 2.0, 1 H), of a MeO group at $\delta(\text{H})$ 3.73 (s), and of β -D-glucopyranosyl group at $\delta(\text{H})$ 5.75 (d, $J = 7.2$). The acid hydrolysis of **5** gave D-glucose [9]. The ¹³C-NMR spectrum (Table 3) revealed 14 C-atom signals, including those of a CO C-atom ($\delta(\text{C})$

Table 3. ¹H- and ¹³C-NMR Data (at 600 and 150 MHz, resp., δ in ppm, J in Hz) of Compound **5**

	$\delta(\text{H})^{\text{a}}$	$\delta(\text{C})^{\text{a}}$	$\delta(\text{C})^{\text{b}}$
C(1)		123.9 (s)	124.0 (s)
H–C(2)	8.55 (d, $J = 2.0$)	117.5 (d)	119.2 (d)
C(3)		147.3 (s)	147.4 (s)
C(4)		153.7 (s)	155.1 (s)
H–C(5)	7.02 (d, $J = 8.2$)	111.9 (d)	112.6 (d)
H–C(6)	8.11 (dd, $J = 8.2$, 2.0)	125.2 (d)	126.7 (d)
COOH		168.8 (s)	170.0 (s)
MeO–C(4)	3.73 (s)	55.9 (q)	56.6 (q)
H–C(1')	5.75 (d, $J = 7.2$)	102.5 (d)	102.7 (d)
H–C(2')	4.29–4.34 (m)	74.8 (d)	74.9 (d)
H–C(3')	4.29–4.34 (m)	78.3 (d)	77.9 (d)
H–C(4')	4.29–4.34 (m)	70.9 (d)	71.1 (d)
H–C(5')	4.02–4.06 (m)	78.7 (d)	78.1 (d)
CH ₂ (6')	4.46 (dd, $J = 12.4$, 2.4), 4.35 (dd, $J = 12.4$, 4.8)	62.1 (t)	62.3 (t)

^a) Recorded in C₅D₅N. ^b) Recorded in CD₃OD.

168.8), a MeO group ($\delta(\text{C})$ 55.9), and six aromatic C-atoms ($\delta(\text{C})$ 123.9, 117.5, 147.3, 153.7, 111.9, and 125.2). In the HMBC spectrum, the cross-peaks were observed between H–C(2), and C(1) and C(7); H–C(6), and C(1) and C(7); and H–C(1') and C(3). In addition, the irradiation of MeO ($\delta(\text{H})$ 3.73) resulted in an enhancement of the signal at $\delta(\text{H})$ 7.02 (H–C(2)) in the difference NOE spectrum. Based on the above evidence, compound **5** was established as 3-hydroxy-4-methoxybenzoic acid 3-*O*- β -D-glucopyranoside.

Non-cyanogenic glucosides are less common than their cyanogenic counterparts. They represent a small group of compounds comprising *ca.* 30 representatives with a scattered distribution in plants [10]. While their function in plants is not fully understood, they possess some interesting bioactivities. For example, they were found to enhance the antibiotic activity of rifampicin, tetracycline, and ampicillin against the *Gram*-positive and *Gram*-negative bacteria, and also to enhance the absorption of drugs, vitamins, and nutrients through the gastrointestinal membrane and increase their bioavailability [11].

To our knowledge, this is the first report on the presence of non-cyanogenic cyanoglucosides in the genus *Hydrangea*.

Experimental Part

General. Column chromatography (CC): normal-phase CC: silica gel (SiO_2 ; 200–300 mesh; Qingdao Marine Chemical Inc., P. R. China); reversed-phase (RP) silica-gel CC: ODS (100–200 mesh; Fuji Silysia Chemical, Ltd., Japan). HPLC pump and detector: Shimadzu LC10AT and RID-6A (refractive index detector), HPLC column: ODS-A (C-18, 10 mm $\times$ 25 cm, 5 μm , YMC), flow rate: 2.5 ml/min. IR Spectra: Shimadzu FTIR-8100 spectrometer; KBr discs; in cm^{-1} . NMR Spectra: JNM-ECA600 spectrometer; TMS as internal standard, δ in ppm, *J* in Hz. HR-ESI-MS: Bruker micromass Q-Tof mass spectrometer; in *m/z*.

Plant Material. The dried leaves of *H. macrophylla* were collected from Shizhu, Sichuan Province, P. R. China, in May 2009, and identified by associate Prof. Jincai Lu of School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University, P. R. China. A voucher specimen (HM 090505) was deposited with the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, P. R. China.

Extraction and Isolation. The dried leaves of *H. macrophylla* (2.0 kg) were extracted with MeOH (3 $\times$ 8 l) for 3 h at 80° and evaporated *in vacuo*. The extract (452 g, 22.2%) was dissolved in H_2O (5 l) and partitioned into AcOEt/ H_2O 1:1 (*v/v*) for three times, and removal of the solvent *in vacuo* yielded an AcOEt-soluble fraction (103.5 g, 4.3%) and an aq. phase. The aq. phase (5 l) was further extracted with BuOH (3 $\times$ 5 l) to give a BuOH-soluble fraction (125.5 g, 6.5%) and an H_2O -soluble fraction. The BuOH-soluble fraction was subjected to CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$) to give six fractions, *Frs.* 1–6. Compound **1** (7.1 mg, 0.000355%) was obtained from *Fr.* 5 after CC (ODS; MeOH/ H_2O 1:9 $\rightarrow$ 3:7) and HPLC (MeOH/ H_2O 15:85; t_{R} 11.5 min). *Fr.* 3 was separated by HPLC (MeOH/ H_2O 20:80) to give compounds **2** (7.5 mg, 0.000375%; t_{R} 7.4 min) and **3** (26.6 mg, 0.001330%; t_{R} 10.4 min). *Fr.* 2 was separated by CC (ODS; MeOH/ H_2O 1:9 $\rightarrow$ 3:7) and HPLC (MeOH/ H_2O 12:88) to afford compounds **4** (5.1 mg, 0.000255%; t_{R} 14.5 min) and **5** (220.3 mg, 0.011015%; t_{R} 20.5 min).

Acid Hydrolysis of 1–5. A soln. of **1–5** (each 2.0 mg) in 1M HCl (1.0 ml) was heated under reflux for 3 h. After cooling, each mixture was neutralized with Amberlite IRA-400 (OH^- form, Alfa Aesar, USA) and filtered, and the soln. was partitioned with AcOEt to give two layers. The aq. layer was evaporated and then subjected to HPLC analysis using NH_2 column (4.6 mm $\times$ 25 cm, Tokyo Kasei Co., Ltd., Japan) and an optical-rotation detector (Shodex OR-2, Showa Denko Co., Ltd., Japan). D-Glucose were confirmed by comparison of the t_{R} with that of the authentic sample (NICPBP, P. R. China; mobile phase: MeCN/ H_2O 85:15 (*v/v*); flow rate: 0.8 ml/min; t_{R} 12.8 min (D-glucose, positive optical rotation)).

Hydranitriloside A₁ (= [3-(β-D-Glucopyranosyloxy)-4-hydroxyphenyl]acetonitrile; **1**). White amorphous powder (MeOH). $[\alpha]_D^{25} = -66.0$ ($c = 2.3$, MeOH). UV (MeOH): 204 (4.62), 224 (4.10), 279 (3.70). IR (KBr): 3380, 2924, 2255, 1609, 1518, 1439, 1287, 1236, 1075, 897, 804. ¹H-NMR (600 MHz, CD₃OD): *Table 1*. ¹³C-NMR (150 MHz, CD₃OD): *Table 2*. HR-ESI-MS: 334.0901 ($[M + Na]^+$, C₁₄H₁₇NNaO₇⁺; calc. 334.0903); 310.0929 ($[M - H]^-$, C₁₄H₁₆NO₇⁻; calc. 310.0927).

Hydranitriloside A₂ (= [3-(β-D-Glucopyranosyloxy)-4-methoxyphenyl]acetonitrile; **2**). White amorphous powder (MeOH). $[\alpha]_D^{25} = -75.0$ ($c = 2.5$, MeOH). UV (MeOH): 205 (4.65), 227 (4.18), 278 (3.64). IR (KBr): 3380, 2919, 2251, 1595, 1518, 1458, 1267, 1233, 1080, 897, 806. ¹H-NMR (600 MHz, C₅D₅N): *Table 1*. ¹³C-NMR (150 MHz, C₅D₅N): *Table 2*. HR-ESI-MS: 348.1058 ($[M + Na]^+$, C₁₅H₁₉NNaO₇⁺; calc. 348.1059).

Hydranitriloside B₁ (= [4-(β-D-Glucopyranosyloxy)-3-methoxyphenyl]acetonitrile; **3**). White amorphous powder (MeOH). $[\alpha]_D^{25} = -65.1$ ($c = 8.3$, MeOH). UV (MeOH): 205 (4.64), 227 (4.13), 278 (3.66). IR (KBr): 3400, 2923, 2249, 1597, 1516, 1425, 1267, 1229, 1076, 895, 804. ¹H-NMR (600 MHz, CD₃OD): *Table 1*. ¹³C-NMR (150 MHz, CD₃OD): *Table 2*. HR-ESI-MS: 348.1061 ($[M + Na]^+$, C₁₅H₁₉NNaO₇⁺; calc. 348.1059).

Hydranitriloside B₂ (= [4-(β-D-Glucopyranosyloxy)phenyl]acetonitrile; **4**). White amorphous powder (MeOH). ¹H-NMR (600 MHz, CD₃OD): *Table 1*. ¹³C-NMR (150 MHz, CD₃OD): *Table 2*. HR-ESI-MS: 318.0956 ($[M + Na]^+$, C₁₄H₁₇NNaO₆⁺; calc. 318.0954).

3-(β-D-Glucopyranosyloxy)-4-methoxybenzoic Acid (5). White amorphous powder (MeOH). $[\alpha]_D^{25} = -68.0$ ($c = 2.3$, MeOH). UV (MeOH): 217 (4.46), 255 (4.15), 285 (3.77). IR (KBr): 3380, 2930, 1698, 1605, 1522, 1509, 1437, 1281, 1219, 1075, 891, 828. ¹H- and ¹³C-NMR (600 and 150 MHz, resp., C₅D₅N): *Table 3*. HR-ESI-MS: 353.0851 ($[M + Na]^+$, C₁₄H₁₈NaO₇⁺; calc. 353.0849); 329.0873 ($[M - H]^-$, C₁₄H₁₇O₇⁻; calc. 329.0873).

REFERENCES

- [1] M. Kikuchi, R. Kakuda, M. Kikuchi, Y. Yaoita, *Chem. Pharm. Bull.* **2008**, *56*, 610.
- [2] H. Suzuki, T. Ikeda, T. Matsumoto, M. Noguchi, *Agric. Biol. Chem.* **1977**, *41*, 1815.
- [3] T. Hashimoto, M. Tori, Y. Asakawa, *Phytochemistry* **1987**, *26*, 3323.
- [4] H. Inouye, Y. Takeda, S. Uesato, K. Uobe, T. Hashimoto, *Tetrahedron Lett.* **1980**, *21*, 1059.
- [5] S. Uesato, Y. Takeda, T. Hashimoto, K. Uobe, H. Inouye, H. Taguchi, T. Endo, *Helv. Chim. Acta* **1984**, *67*, 2111.
- [6] H. Zhang, H. Matsuda, A. Kumahara, Y. Ito, S. Nakamura, M. Yoshikawa, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4972.
- [7] M. Yoshikawa, E. Harada, Y. Naitoh, K. Inoue, H. Matsuda, H. Shimoda, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.* **1994**, *42*, 2225.
- [8] B. Helferich, H. E. Scheiber, R. Streeck, F. Vorsatz, *Ann. Chem.* **1935**, *518*, 211.
- [9] S. Nakamura, X. Li, H. Matsuda, M. Yoshikawa, *Chem. Pharm. Bull.* **2008**, *56*, 536.
- [10] K. Yamasaki, *Stud. Plant Sci.* **1999**, *6*, 335.
- [11] S. P. S. Khanuja, J. S. Arya, R. S. K. Tiruppadiripuliyur, D. Saikia, H. Kaur, M. Singh, S. C. Gupta, A. K. Shasany, M. P. Darakar, S. K. Srivastava, M. M. Gupta, S. C. Verma, A. Pal, US Pat. US6858588 B2, 2005.

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