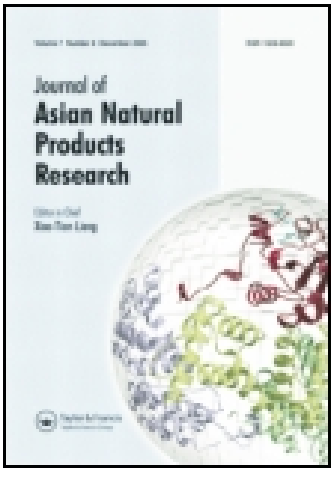

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Five new stilbene glycosides from the roots of *Polygonum multiflorum*

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Five new stilbene glycosides (**1**–**5**), together with six known ones, were isolated from the roots of *Polygonum multiflorum*. Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence.

Keywords: *Polygonum multiflorum*; Polygonaceae; stilbene glycoside

1. Introduction

The root of *Polygonum multiflorum* Thunb (Polygonaceae), He-Shou-Wu in Chinese, is a popular traditional Chinese medicine, which has been widely used in China and other Asian countries as a tonic, anti-oxidative, and anti-aging agent for centuries [1]. Stilbene glycosides, one of the main active constituents of this plant, had been proved to possess anti-inflammatory, anti-oxidant, anti-HIV, anti-tumor, and liver protection activities [2–7]. However, to date, only 13 stilbene glycosides had been isolated from the plant [8–13]. In our previous study, we had reported the isolation of a new flavonostilbene glycoside and several known stilbene glycosides from the roots of *P. multiflorum* [14]. Our further investigation of this plant has led to the finding of five new stilbene glycosides, (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(4″-*O*-α-D-glucopyranosyl)-β-D-glucopyranoside (**1**), (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(6″-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside (**2**), (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranosyl-4′-*O*-α-D-glucopyranoside (**3**), (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranosyl-5-*O*-α-D-glucopyranoside (**4**), and (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(2″-*O*-β-D-fructofuranosyl)-β-D-glucopyranoside (**5**) (Figure 1), together with six known ones (**6**–**11**). In this paper, we describe the isolation and structural elucidation of these new stilbene glycosides.

2. Results and discussion

The molecular formula of **1** was determined to be $C_{26}H_{32}O_{14}$ by its HR-ESI-MS at *m/z* 591.1683 $[M + Na]^+$. The UV spectrum showed the absorption maxima at 206 and 314 nm. The IR spectrum implied the presence of hydroxyl group (3407 cm^{-1}) and aromatic ring (1605 and 1508 cm^{-1}). The ^1H NMR spectrum of **1** revealed proton signals for a *para*-disubstituted benzene ring [δ_H 7.45 (2H, d, $J = 8.4$ Hz) and 6.77 (2H, d, $J = 8.4$ Hz)], a tetrasubstituted benzene ring [δ_H 6.63 (1H, d, $J = 2.4$ Hz) and 6.26 (1H, d, $J = 2.4$ Hz)], and a *trans*-disubstitued double bond [δ_H 7.70 (1H, d, $J = 16.4$ Hz) and 6.92 (1H, d, $J = 16.4$ Hz)]. In addition, the ^1H NMR spectrum of **1** displayed the signals of two anomeric protons [δ_H 5.22 (1H, d,

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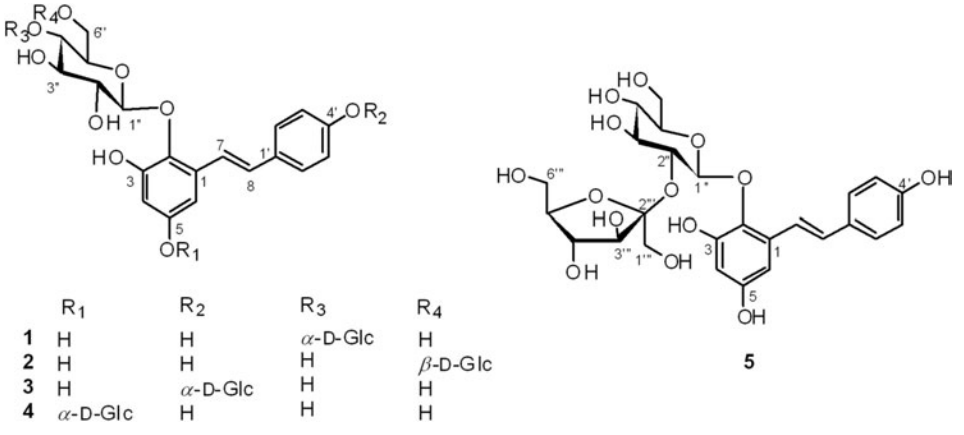

	R_1	R_2	R_3	R_4
1	H	H	α-D-Glc	H
2	H	H	H	β-D-Glc
3	H	α-D-Glc	H	H
4	α-D-Glc	H	H	H

Figure 1. Chemical structures of compounds **1**–**5**.

$J = 4.0$ Hz) and 4.53 (1H, d, $J = 8.0$ Hz)], suggesting the presence of two sugar moieties. The above spectral data suggested that **1** was a stilbene glycoside with an (*E*)-2,3,5,4′-tetrahydroxystilbene aglycone and two sugar moieties [3]. Acid hydrolysis of **1** afforded D-glucose, which was identified by high-performance liquid chromatography (HPLC) analysis [15]. The β-configuration of a D-glucose unit ($J = 8.0$ Hz) and α-configuration of another D-glucose unit ($J = 4.0$ Hz) were, respectively, determined based on the $^3J_{H1,H2}$ coupling constants of anomeric protons. The ^{13}C NMR and DEPT spectra of **1** revealed the presence of 26 carbon signals including 12 aromatic carbons, 2 olefinic carbons, and 2 glucose moieties. With the aid of ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY experiments, all the ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in Table 1. In the HMBC spectrum of **1**, the long-range correlations between H-1‴ (δ_H 5.22) of the α-D-glucose and C-4″ (δ_C 80.1) of the β-D-glucose, as well as between H-1″ (δ_H 4.53) of the β-D-glucose and C-2 (δ_C 137.8) of the stilbene aglycone were observed. Based on the above results, the structure of **1** was established as (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(4″-*O*-α-D-glucopyranosyl)-β-D-glucopyranoside.

Compound **2** displayed the same molecular formula $C_{26}H_{32}O_{14}$ as **1** by its HR-ESI-MS data (*m/z* 591.1686 $[M + Na]^+$, calcd for $C_{26}H_{32}O_{14}Na$, 591.1684). Similar to **1**, compound **2** showed the characteristic UV (λ_{max} 206 and 312 nm) and IR (ν_{max} 3402, 1609, 1508 cm^{-1}) absorptions for a stilbene glycoside. The ^{1}H NMR spectrum of **2** displayed aromatic and olefinic proton signals at δ_H 7.69 (1H, d, $J = 16.4$ Hz), 7.44 (2H, d, $J = 8.8$ Hz), 6.93 (1H, d, $J = 16.4$ Hz), 6.77 (2H, d, $J = 8.8$ Hz), 6.63 (1H, d, $J = 2.4$ Hz), and 6.25 (1H, d, $J = 2.4$ Hz), as well as two anomeric protons at δ_H 4.54 (1H, d, $J = 8.0$ Hz) and 4.32 (1H, d, $J = 7.6$ Hz), suggesting that **2** was also a stilbene glycoside with two sugar units. The ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY spectra of **2** allowed the full assignments of all proton and carbon signals (Table 1). Comparison of the NMR data of the aglycone part of **2** with those of **1** revealed that they were very similar, indicating that **2** possessed the same (*E*)-2,3,5,4′-tetrahydroxystilbene aglycone as **1**. Acid hydrolysis of **2** also afforded D-glucose. The relative anomeric configurations of the two D-glucose moieties were determined to be β on the basis of the large $^3J_{H1,H2}$ coupling constants of the anomeric protons. The sequence and linkage position of the glucose moieties were deduced by the HMBC experiment. In the HMBC spectrum, the correlations between H-1‴ (δ_H 4.32) of

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1–5** (CD_3OD, J in Hz).

	1		**2**		**3**		**4**		**5**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	133.7		133.8		133.6		133.9		133.8	
2	137.8		137.7		138.1		139.8		137.9	
3	151.9		151.6		152.1		152.1		151.6	
4	103.6	6.26 (d, $J = 2.4$)	103.7	6.25 (d, $J = 2.4$)	103.9	6.27 (d, $J = 2.5$)	105.7	6.63 (d, $J = 3.0$)	103.9	6.28 (d, $J = 2.8$)
5	155.9		156.1		156.0		156.2		156.0	
6	102.7	6.63 (d, $J = 2.4$)	102.9	6.63 (d, $J = 2.4$)	102.8	6.63 (d, $J = 2.5$)	105.2	7.03 (d, $J = 3.0$)	103.0	6.63 (d, $J = 2.8$)
7	121.7	7.70 (d, $J = 16.4$)	121.4	7.69 (d, $J = 16.4$)	123.4	7.79 (d, $J = 16.5$)	121.4	7.71 (d, $J = 16.5$)	121.3	7.68 (d, $J = 16.4$)
8	130.1	6.92 (d, $J = 16.4$)	130.2	6.93 (d, $J = 16.4$)	129.5	6.96 (d, $J = 16.5$)	130.7	7.00 (d, $J = 16.5$)	130.3	6.93 (d, $J = 16.4$)
1′	130.8		130.8		133.4		130.8		130.8	
2′, 6′	129.2	7.45 (d, $J = 8.4$)	129.2	7.44 (d, $J = 8.8$)	129.0	7.55 (d, $J = 8.5$)	129.3	7.46 (d, $J = 8.5$)	129.3	7.45 (d, $J = 8.6$)
3′, 5′	116.4	6.77 (d, $J = 8.4$)	116.5	6.77 (d, $J = 8.8$)	118.3	7.14 (d, $J = 8.5$)	116.4	6.77 (d, $J = 8.5$)	116.5	6.78 (d, $J = 8.6$)
4′	158.2		158.4		158.2		158.4		158.3	
1″	108.0	4.53 (d, $J = 8.0$)	108.0	4.54 (d, $J = 8.0$)	108.2	4.51 (d, $J = 8.0$)	108.0	4.54 (d, $J = 8.0$)	108.0	4.52 (d, $J = 7.9$)
2″	75.0	3.62	75.5	3.59 (t, 8.0)	75.5	3.57	75.4	3.58 (t, 8.0)	75.3	3.56
3″	76.9	3.38 (dt, $J = 9.2, 2.0$)	77.8	3.46	77.9	3.44	77.9	3.45	77.8	3.44
4″	80.1	3.77	70.9	3.57 (t, 8.0)	70.8	3.54	70.8	3.54 (t, 9.5)	71.8	3.45
5″	77.6	3.71	77.3	3.46	78.2	3.28 m	78.2	3.27 m	77.2	3.46
6″	61.6	3.88 (dd, $J = 12.0, 3.2$)	69.4	4.15 (dd, $J = 11.2, 2.0$)	62.4	3.77	62.3	3.77	62.6	4.06 (d, $J = 9.4$)
		3.81 (t, $J = 12.0$)		3.82 (t, $J = 11.2$)		3.72		3.73		3.69
1‴	102.7	5.22 (d, $J = 4.0$)	104.6	4.32 (d, $J = 7.6$)	99.3	5.50 (d, $J = 4.0$)	99.7	5.45 (d, $J = 3.5$)	62.0	3.66
										3.57
2‴	74.1	3.46 (dd, $J = 9.6, 4.0$)	75.3	3.25	73.3	3.59 (dd, $J = 9.6, 4.0$)	73.4	3.57	105.2	
3‴	75.0	3.62	77.9	3.36 (t, 8.4)	75.0	3.87 (t, 9.6)	75.0	3.86 (t, 9.5)	76.6	3.99 (d, 8.2)
4‴	71.5	3.27 (t, $J = 9.2$)	71.6	3.31	71.5	3.45	71.5	3.44	79.1	4.10 (dd, $J = 8.2, 3.5$)
5‴	74.7	3.67	77.9	3.26	74.4	3.67	74.4	3.72	83.4	3.74
6‴	62.7	3.83 (t, $J = 8.0$)	62.7	3.85 (dd, $J = 12.4, 2.4$)	62.1	3.81 (dd, $J = 11.6, 2.4$)	62.1	3.80 (dd, $J = 12.0, 2.5$)	64.0	3.72
		3.66		3.65 (t, $J = 12.4$)		3.75		3.76		3.56

Notes: Assignments were established by interpretation of the ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY spectra. Overlapped signals are reported without designating multiplicity.

the terminal β-D-glucose and C-6″ (δ_C 69.4) of the inner β-D-glucose, as well as between H-1″ (δ_H 4.54) of the inner β-D-glucose and C-2 (δ_C 137.7) of the stilbene aglycone were observed. Therefore, the structure of **2** was characterized as (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(6″-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside.

The molecular formula of **3** was established as $C_{26}H_{32}O_{14}$ by its HR-ESI-MS at *m/z* 591.1686 $[M + Na]^+$, which was identical to that of **1** and **2**. Similar to **1** and **2**, acid hydrolysis of **3** afforded D-glucose. Comparison of NMR data of **3** with those of **1** suggested they possessed the same (*E*)-2,3,5,4′-tetrahydroxystilbene aglycone, as well as α-D-glucose and β-D-glucose units. The difference between them was the linkage position of the α-D-glucose moiety. In the HMBC spectrum of **3**, the correlations between H-1‴ (δ_H 5.50) of the α-D-glucose and C-4′ (δ_C 158.2) of the aglycone, as well as between H-1″ (δ_H 4.51) of the β-D-glucose and C-2 (δ_C 138.1) of the stilbene aglycone were observed. Thus, the structure of **3** was confirmed as (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranosyl-4′-*O*-α-D-glucopyranoside.

Compound **4** showed the same molecular formula as **1**–**3** by its HR-ESI-MS data. Acid hydrolysis of **4** also afforded D-glucose. The ^1H and ^{13}C NMR data of **4** were similar to those of **3**, suggesting that they possessed the same stilbene aglycone and glucose units. Different from **3**, in the HMBC spectrum of **4**, the correlation between H-1‴ (δ_H 5.45) of α-D-glucose and C-5 (δ_C 156.2) of aglycone was observed, indicating that the α-D-glucose unit in **4** was attached to the C-5 position of aglycone. Hence, the structure of **4** was determined as (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranosyl-5-*O*-α-D-glucopyranoside.

The molecular formula of **5** was also deduced as $C_{26}H_{32}O_{14}$ by its HR-ESI-MS at *m/z* 591.1686 $[M + Na]^+$. The UV and IR spectra of **5** showed the characteristic absorptions corresponding to a stilbene glycoside. The ^1H NMR spectrum of **5** showed proton signals due to a *para*-disubstituted benzene ring [δ_H 7.45 (2H, d, $J = 8.6$ Hz) and 6.78 (2H, d, $J = 8.6$ Hz)], a tetrasubstituted benzene ring [δ_H 6.63 (1H, d, $J = 2.8$ Hz) and 6.28 (1H, d, $J = 2.8$ Hz)], a *trans*-disubstitued double bond [δ_H 7.68 (1H, d, $J = 16.4$ Hz) and 6.93 (1H, d, $J = 16.4$ Hz)], and an anomeric proton signal at δ_H 4.52 (1H, d, $J = 7.9$ Hz). Besides 12 aromatic carbons and 2 olefinic carbons, the ^{13}C NMR spectrum of **5** indicated the presence of two sugar units. All the above information suggested that **5** was also a stilbene glycoside with an (*E*)-2,3,5,4′-tetrahydroxystilbene aglycone and two sugar moieties. Interpretation of ^1H–^1H COSY, HSQC, HMBC, and NOESY spectra of **5** led to the assignment of all proton and carbon signals as shown in Table 1. Different from **1** to **4**, acid hydrolysis of **5** afforded D-glucose and D-fructose, which were identified by the gas chromatography (GC) analysis. The β-configuration of the D-glucose was determined based on the $^3J_{H1,H2}$ coupling constant ($J = 8.0$ Hz) of the anomeric proton. The β-configuration of the D-furanose was deduced by comparison of the NMR data with those reported in the literature [16]. In addition, the HMBC correlation between H-5‴ (δ_H 3.74) of fructose and C-2‴ (δ_C 105.2) of fructose was observed, which confirmed the existence of β-D-fructofuranose. Furthermore, in the HMBC spectrum of **5**, the correlations between H-2″ (δ_H 3.56) of β-D-glucose and C-2‴ (δ_C 105.2) of β-D-fructofuranose, as well as between H-1″ (δ_H 4.52) of β-D-glucose and C-2 (δ_C 137.9) of aglycone were observed. Hence, the structure of **5** was established to be (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(2″-*O*-β-D-fructofuranosyl)-β-D-glucopyranoside.

The six known stilbene glycosides were identified as (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranosyl-4′-*O*-β-D-glucopyranoside (**6**) [17], (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(6″-*O*-α-D-glucopyranosyl)-β-D-glucopyranoside (**7**) [12],

polygonimitin (**8**) [13], (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (**9**) [3], (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-(3″-*O*-galloyl)-β-D-glucopyranoside (**10**) [11], and (*Z*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (**11**) [18], respectively, by comparison of their physical and spectroscopic data with literature values.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter at room temperature (JASCO, Tokyo, Japan). UV spectra were obtained on a JASCO-V-550 UV/VIS spectrophotometer (JASCO). IR spectra were recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer with KBr pellets (JASCO). HR-ESI-MS data were obtained on an Agilent 6210 LC/MSD TOF mass spectrometer (Agilent, Pala Alto, CA, USA). 1D and 2D NMR experiments were performed on a Bruker AV-400 spectrometer (Bruker, Billerica, MA, USA). GC–MS analyses were performed on a Shimadzu GCMS-QP2010 plus gas chromatograph–mass spectrometer (Shimadzu, Kyoto, Japan). Analytical HPLC was performed on an Agilent 1260 chromatography equipped with a G1311C pump, a G1315D photodiode array detector (Agilent) and a Cosmosil $5C_{18}$-MS-II Waters column (4.6 × 250 mm, 5 μm; Nacalai Tesque, Inc., Kyoto, Japan). Preparative HPLC was carried on an Agilent 1260 chromatography equipped with a G1310B pump, a G1365D detector (Agilent), and a Cosmosil $5C_{18}$-MS-II Waters column (20 × 250 mm, 5 μm; Nacalai Tesque, Inc.). Column chromatographies were performed on macroporous resin Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (300–400 mesh; Qingdao Marine Chemical Co. Ltd, Qingdao, China), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC analyses were carried on precoated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute, Yantai, China).

3.2 Plant material

The roots of *P. multiflorum* were collected in Deqing County, Guangdong Province of China, in October of 2010 and authenticated by Prof. Guang-Xiong Zhou (College of Pharmacy, Jinan University). A voucher specimen (No. 20101003) had been deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, China.

3.3 Extraction and isolation

The air-dried and powdered roots of *P. multiflorum* (14.5 kg) were extracted with 95% (v/v) EtOH under reflux for two times (2 × 25 liters, 2 h each). The combined EtOH solution was concentrated under vacuum to yield a residue (1200 g). The crude extract was suspended in water and then partitioned with petroleum ether (b.p. 60–90°C), ethyl acetate, and *n*-butanol, respectively. The *n*-butanol soluble fraction (144 g) was redissolved in H_2O and subjected to macroporous resin HP-20 column eluted with EtOH–H_2O mixtures. The 30% EtOH eluate (14 g) was subjected to Sephadex LH-20 column and eluted with MeOH–H_2O mixtures (70:30 → 100:0, v/v) to obtain nine fractions (1–9). Fraction 5 (2.0 g) was separated by silica gel column with gradient mixtures of $CHCl_3$–MeOH (95:5, 90:10, 85:15, 80:20, 75:25, v/v) as eluents to yield five subfractions (5a–5e). Subfraction 5d (0.5 g) was further separated by preparative HPLC on a reversed-phase C18 column (20 mm × 250 mm, 5 μm) using MeOH–H_2O (25:75, v/v, 6 ml/min, 320 nm) as mobile phase to afford **3** (8.0 mg, t_R = 37.7 min), **4** (12.0 mg, t_R = 25.3 min), **6** (24.4 mg, t_R = 33.8 min), and **8** (25.0 mg, t_R = 51.4 min), respectively. Fraction 7 (1.0 g) was separated by silica gel column ($CHCl_3$–MeOH, 90:10, v/v) and then further purified by preparative

HPLC on a reversed-phase C18 column (20 mm × 250 mm, 5 μm) using MeOH–H_2O (35: 65, v/v, 6 ml/min, 320 nm) as eluent to yield **2** (15.4 mg, t_R = 20.7 min), **5** (12.0 mg, t_R = 29.9 min), and **7** (26.0 mg, t_R = 18.1 min), respectively. Fraction 8 (0.8 g) was separated by silica gel column with gradient mixtures of $CHCl_3$–MeOH–H_2O (85:15:1.5, 80:20:2, 75:25:3, 70:30:5, v/v) as eluents to yield four subfractions (8a–8d). Subfraction 8a (0.25 g) was purified by Sephadex LH-20 column (MeOH) to yield **10** (220 mg). Subfraction 8c (0.2 g) was reseparated by preparative HPLC on a reversed-phase C18 column (20 mm × 250 mm, 5 μm) using MeOH–H_2O (38:62, v/v, 6 ml/min, 320 nm) as eluent to affford **1** (11.0 mg, t_R = 23.5 min), **9** (34.0 mg, t_R = 39.5 min), and **11** (22.0 mg, t_R = 21.9 min), respectively.

3.3.1 *Compound* ***1***

Amorphous powder; $[\alpha]_D^{20}$ + 79.5 (c = 0.28, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.54), 314 (4.26) nm; IR (KBr) ν_{max}: 3407, 1605, 1508, 1463, 1038, 828 cm^{-1}; ^1H and ^{13}C NMR spectral data (see Table 1); ESI-MS *m/z*: 591 $[M + Na]^+$, 567 $[M - H]^-$; HR-ESI-MS *m/z*: 591.1683 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{14}Na$, 591.1684).

3.3.2 *Compound* ***2***

Amorphous powder; $[\alpha]_D^{20}$ − 19.4 (c = 0.35, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.30), 312 (4.11) nm; IR (KBr) ν_{max}: 3402, 1609, 1508, 1455, 1058, 828 cm^{-1}; ^1H and ^{13}C NMR spectral data (see Table 1); ESI-MS *m/z*: 591 $[M + Na]^+$, 567 $[M - H]^-$; HR-ESI-MS *m/z*: 591.1686 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{14}Na$, 591.1684).

3.3.3 *Compound* ***3***

Amorphous powder; $[\alpha]_D^{20}$ + 68.2 (c = 0.43, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.39), 314 (4.26) nm; IR (KBr) ν_{max}: 3407, 1605, 1508, 1459, 1018, 839 cm^{-1}; ^1H and ^{13}C NMR spectral data (see Table 1); ESI-MS *m/z*: 591 $[M + Na]^+$, 567 $[M - H]^-$; HR-ESI-MS *m/z*: 591.1686 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{14}Na$, 591.1684).

3.3.4 *Compound* ***4***

Amorphous powder; $[\alpha]_D^{20}$ + 47.3 (c = 0.60, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.09), 314 (3.94) nm; IR (KBr) ν_{max}: 3387, 1609, 1516, 1456, 1018, 828 cm^{-1}; ^1H and ^{13}C NMR spectral data (see Table 1); ESI-MS *m/z*: 591 $[M + Na]^+$, 567 $[M - H]^-$; HR-ESI-MS *m/z*: 591.1683 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{14}Na$, 591.1684).

3.3.5 *Compound* ***5***

Amorphous powder; $[\alpha]_D^{20}$ − 16.3 (c = 0.45, MeOH); UV (MeOH) λ_{max} (log ε): 216 (4.09), 322 (4.10) nm; IR (KBr) ν_{max}: 3431, 1609, 1512, 1036, 828 cm^{-1}; ^1H and ^{13}C NMR spectral data (see Table 1); ESI-MS *m/z*: 591 $[M + Na]^+$, 567 $[M - H]^-$; HR-ESI-MS *m/z*: 591.1686 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{14}Na$, 591.1684).

3.4 *Acid hydrolysis and HPLC analysis of 1–4*

Each solution of compounds **1**–**4** (each 2 mg) in 2 mol/l HCl (5 ml) was heated in water bath (80°C) for 4 h. The solution was evaporated under reduced pressure. Each residue was dissolved in pyridine (1.0 ml) and stirred with L-cysteine methyl ester hydrochloride (2 mg) for 1 h at 60°C, and then *O*-tolyl isothiocyanate (20 μl) was added to the mixture and heated at 60°C for another 1 h. The reaction mixtures were analyzed by HPLC and detected at 250 nm. Analytical HPLC was performed on a Cosmosil $5C_{18}$-MS-II column (4.6 mm × 250 mm, 5 μm) at 20°C using CH_3CN–0.05% CH_3COOH (25:75,

1.0 ml/min) as the mobile phase. Peaks were detected with a G1315D photodiode array detector. D-glucose ($t_R = 16.36$ min) was identified as sugar moiety of **1**–**4** based on comparisons with authentic samples of D-glucose ($t_R = 16.36$ min) and L-glucose ($t_R = 14.99$ min).

3.5 Acid hydrolysis and GC analysis of 5

Compound **5** (3.7 mg) was heated in an ampoule with 4.0 ml of 2 mol/l HCl at 80°C for 6 h. The solution was evaporated with a steam of N_2 to yield a residue, which was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was evaporated by N_2 and treated with anhydrous pyridine (3 ml) and L-cysteine methyl ester hydrochloride (4 mg), followed by heating at 60°C for 2 h and then concentrated to dryness with N_2. The residue was added to *N*-(trimethylsilyl) imidazole (0.3 ml) and kept at 60°C for 1 h. Subsequently, the solution was diluted with H_2O (1 ml) and extracted with hexane (2 ml). The organic layer was analyzed using GC under the following conditions: column: HT-SE-30 (0.32 mm × 30 mm, 0.5 μm), detector: FID, column temperature: 200–250°C (5°C/min), detector temperature: 280°C, injector temperature: 250°C, and carrier gas: N_2. The standard D-glucose and L-glucose were subjected to the same reaction. As a result, D-glucose ($t_R = 18.86$ min) and D-fructose ($t_R = 16.06$ min) were identified as sugar moiety of **5** based on comparisons with authentic samples of D-glucose ($t_R = 18.86$ min) and D-fructose ($t_R = 16.06$ min).

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