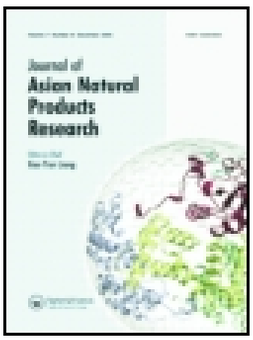

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Aromatic glycosides from the whole plants of *Iris japonica*

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ABSTRACT

Phytochemical investigation on the whole plants of *Iris japonica* led to the isolation of four new aromatic glycosides. Their structures including the absolute configurations were determined by spectroscopic and chemical methods as (–)-4-hydroxy-3-methoxy acetophenone 4-*O*-β-D-{6-*O*-[4-*O*-(7*R*,8*S*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (**1**), (–)-4-hydroxy-3-methoxy acetophenone 4-*O*-β-D-{6-*O*-[4-*O*-(7*S*,8*R*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (**2**), (–)-4-hydroxy-3-methoxy acetophenone 4-*O*-*β*-D-{6-*O*-[4-*O*-(7*R*,8*R*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (**3**), (–)-4-hydroxy-3-methoxy acetophenone 4-*O*-*β*-D-{6-*O*-[4-*O*-(7*S*,8*S*)-(4-hydroxy-3-methoxy-phenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (**4**), respectively.

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Iris japonica; aromatic glycosides; chiral resolution; erythro-threo isomer; CD difference spectrum

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1. Introduction

The genus *Iris* belongs to the Iridaceae family, which consists of approximately 800 species worldwide. In China, there are 71 species and 7 varieties, mostly distributed in the northwest, the southwest, and the northeast of China [1]. *Iris tectorum* is a representative plant of the Iridaceae family, the rhizomes of which are called "chuan she gan" in China. It has been commonly used for treatment of sore throats [2]. *Iris japonica* mainly distributes in the southwest of China, and has been used as a folk medicine for the treatment of traumatic injury and relieving constipation by purgation [3]. Previous studies of *I. japonica* led to the identification of nine isoflavones and four iridals from this plant [4,5]. In our present investigation on the components of this plant material, four new aromatic glycosides have been characterized (Figure 1). The isolation and structure determination of the new isolates are reported in this article.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, and its molecular formula $C_{33}H_{38}O_{15}$ was deduced by HRESIMS (*m/z* 697.2108 $[M + Na]^+$). The IR spectrum showed

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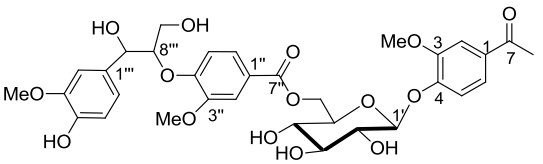

1 7'''*R*,8'''*S*
2 7'''*S*,7'''*R*
3 7'''*R*,7'''*R*
4 7'''*S*,7'''*S*

Figure 1. Structures of compounds **1**–**4**.

absorption bands for hydroxyl (3239 cm^{-1}), carbonyl (1673 cm^{-1}), and aromatic (1402, 1452, and 1595 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** (Table 1) showed signals attributed to three 1, 3, 4-trisubstitued aromatic rings at δ_H 7.44 (H-2), 7.15 (H-5), and 7.32 (H-6); δ_H 7.35 (H-2″), 7.14 (H-5″), and 7.50 (H-6″); δ_H 7.00 (H − 2‴), 6.66 (H − 5‴), and 6.79 (H − 6‴), a methyl singlet, three methoxys, two oxygenated methines, and an oxygenated methylene. Acid hydrolysis of **1** gave a D-glucose, which was clarified by GC analysis. The anomeric proton at δ 5.13 (H-1′) with a large coupling constant ($^3J_{H1',H2'}$ = 7.5 Hz) suggested the presence of a β-glucopyranosyl moiety. The ^{13}C NMR spectrum of **1** showed 33 carbon resonances comprising 18 aromatic, a ketone carbonyl, an ester carbonyl, a methyl, three methoxy, two oxygenated methine, an oxygenated methylene, and six glucopyranosyl carbon signals. The spectral data of **1** were in good agreement with those of scroneoside B [6] except for the presence of an additional phenylglycerol unit [7,8].

In the HMBC spectrum of **1**, correlations of H-2/C-4, C-6, and C-7; H-6/C-1, C-4, C-5, and C-7; OCH_3-3/C-3; CH_3/C-1 and C-7, suggested the existence of 4-oxy-3-methoxy acetophenone moisty, which was supported by a correlation between H-2 and OCH_3-3 in the ROESY spectrum. The HMBC spectrum also showed correlations of H-2″/C-1″, C-3″, C-4″, C-6″, and C-7″; H-5″/C-1″, C-3″, C-4″, and C-6″; H-6″/C-1″, C-2″, C-4″, C-5″, and C-7″; and OCH_3-3″/C-3″, in combination with chemical shifts of these proton and coupling patterns, indicated that there was a 4″-hydroxy-3″-methoxybenzoyloxy moiety. In addition, correlations of H − 7‴/H − 1‴, C − 2‴, C − 6‴, C − 8‴, and C − 9‴; H − 2‴/C − 1‴, C − 3‴, C − 4‴, C − 6‴, and C − 7‴; H − 5‴/C − 1‴, C − 3‴, C − 4‴, and C − 6‴; C − 6‴/C − 1‴, C − 2‴, C − 3‴ and C − 7‴; and OCH_3 − 1‴/C − 3‴, together with their shifts, demonstrated the presence of a 4‴-hydroxyl-3‴- methoxyphenylglycerol moiety. This was supported by ^{1}H–^{1}H COSY correlations of H − 7‴/H − 8‴/H_2 − 9‴; H − 5‴/H − 6‴. Furthermore, HMBC correlations of H-1′/C-4 and H_2-6′/C-7″ indicated that the 4-oxy-3-methoxy acetophenone and 4″-hydroxyl-3″-methoxybenzoyloxy units were connected at C-1′ and C-6′ of the β-glucopyranosyl moiety, respectively. Meanwhile, HMBC correlation of H − 8‴/C-4″ suggested a linkage between C − 8‴ and C-4″.

The stereochemistry of **1** was elucidated by a comprehensive analysis of the NMR and CD difference data of **1** (Figure 2). The $\Delta\ \delta_{C8'''-C7'''}$ value (11.8 ppm) suggested an 7‴, 8‴-*erythro* configuration of **1** [9–12]. In the CD difference spectrum of **1**, a positive Cotton effect at 238 nm indicated the 8‴ *S* configuration [12]. Therefore, compound **1** was determined as (−)-4-hydroxy-3-methoxyacetophenone

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Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **1**–**4**[a].

No	**1**		**2**		**3**		**4**	
1		130.8		130.9		130.8		131.0
2	7.44 d (1.9)	110.9	7.43d (1.9)	111.0	7.44 d (2.0)	110.0	7.44 d (2.0)	110.0
3		148.6		148.7		148.6		148.8
4		150.3		150.4		150.3		150.4
5	7.15 d (8.5)	114.0	7.14 d (8.5)	114.2	7.16 d (8.5)	114.1	7.16 d (8.5)	114.2
6	7.32 dd (8.5, 1.9)	122.3	7.32 dd (8.5, 1.9)	122.4	7.32 dd (8.5, 2.0)	122.3	7.32 dd (8.5, 2.0)	122.5
7		196.4		196.7		196.5		196.7
OMe-3	3.80 s	55.4	3.80 s	55.5	3.80 s	55.6	3.80 s	55.7
Me	2.46 s	26.3	2.46 s	26.4	2.47 s	26.3	2.47 s	26.4
1′	5.13 d (7.5)	99.0	5.13 d (7.3)	99.2	5.13 d (7.5)	99.1	5.13 d (7.5)	99.2
2′	3.25–3.82 m	73.0	3.25–3.82 m	73.1	3.23–3.80 m	73.0	3.25–3.82 m	73.1
3′	3.25–3.82 m	76.6	3.25–3.82 m	76.7	3.23–3.80 m	76.7	3.25–3.82 m	76.7
4′	3.25–3.82 m	70.1	3.25–3.82 m	70.2	3.23–3.80 m	70.1	3.25–3.82 m	70.2
5′	3.25–3.82 m	73.9	3.25–3.82 m	74.0	3.23–3.80 m	74.0	3.25–3.82 m	74.1
6′a	4.55 d (12.0)	63.9	4.55 d (12.0)	64.0	4.57 d (11.8)	64.0	4.57 d (11.8)	64.0
6′b	4.20 dd (12.0, 7.5)		4.20 dd (12.0,7.5)		4.20 dd (11.8, 7.5)		4.20 dd (11.8, 7.5)	
1″		121.4		121.5		121.5		121.6
2″	7.35 d (1.9)	112.7	7.35 d (1.9)	112.8	7.40 d (2.0)	112.7	7.40 d (2.0)	112.8
3″		148.9		149.1		148.9		149.1
4″		152.7		152.8		153.1		153.2
5″	7.14 d (8.5)	113.6	7.13 d (8.5)	113.8	7.17 d (8.5)	113.6	7.17 d (8.5)	113.7
6″	7.50 dd (8.5, 1.9)	123.1	7.48 dd (8.5, 1.9)	123.2	7.52 dd (8.5, 2.0)	123.1	7.52 dd (8.5, 2.0)	123.3
7″		165.2		165.4		165.2		165.4
OMe-3″	3.71 s	55.6	3.71 s	55.7	3.77 s	55.8	3.78 s	55.9
1‴		132.9		132.9		132.9		132.9
2‴	7.00 d (1.7)	111.5	6.99 d (1.6)	111.6	6.98 d (1.7)	111.0	6.97 d (1.6)	111.1
3‴		147.0		147.1		147.1		147.2
4‴		145.5		145.6		145.6		145.7
5‴	6.66 d (8.0)	114.6	6.65 d (8.0)	114.7	6.68 d (8.0)	114.7	6.68 d (8.0)	114.9
6‴	6.79 dd (8.0, 1.7)	119.6	6.78 dd (8.0, 1.6)	119.7	6.77 dd (8.0, 1.6)	119.1	6.77 dd (8.0, 1.6)	119.2
7‴	4.71 d (5.0)	71.5	4.71 d (4.5)	71.5	4.72 d (4.7)	71.2	4.72 d (4.7)	71.3
8‴	4.52–4.53 m	83.3	4.52–4.53 m	83.3	4.49 –4.50 m	83.6	4.49–4.50 m	83.7
9‴	3.62–3.64 m	60.1	3.61–3.63 m	60.3	3.59–3.60 m	60.2	3.58–3.60 m	60.3
	3.25–3.27 m		3.25–3.27 m		3.25–3.26 m		3.25–3.26 m	
OMe-3‴	3.70 s	55.7	3.70 s	55.9	3.72 s	55.4	3.72 s	55.6

[a]^{1}H NMR data (δ) were measured in DMSO-d_6 at 600 MHz and ^{13}C NMR data (δ) were measured in DMSO-d_6 at 150 MHz for **1**–**4**. Coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

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4-*O*-β-D-{6-*O*-[4-*O*-(7*R*,8*S*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside.Figure 2. CD difference spectra of compounds **1** (—)and **2** (---).

IR, HRESIMS, and NMR spectroscopic data of compound **2** are almost identical to those of **1**. However, **1** and **2** were separable by chiral HPLC with retention times of 15.8 and 26.3 min (Experimental Section), respectively. The $\Delta\,\delta_{C8'''-C7'''}$ value (11.8 ppm) suggested that **2** was another *erythro*-isomer of **1** [9–12]. The CD difference spectrum (Figure 2) of **2** showed negative Cotton effect at 238 nm indicated the 8‴ *R* configuration [12]. Thus, **2** was determined to be (−)-4-hydroxy-3-methoxy acetophenone 4-*O*-β-D-{6-*O*-[4-*O*-(7*S*,8*R*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside.

The spectroscopic data of **3** were similar to those of **1**. Comparison of the NMR data of **1** and **3**, indicated that $\Delta\,\delta_{C8'''-C7'''}$ value was 12.4 ppm in **3**, while it was 11.8 ppm in **1** [9–12]. This suggested that **3** was a *threo*-isomer of **1**, which was further confirmed by 2D NMR data. In the CD difference spectrum (Figure 3) of **3**, a negative Cotton effect at 234 nm indicated

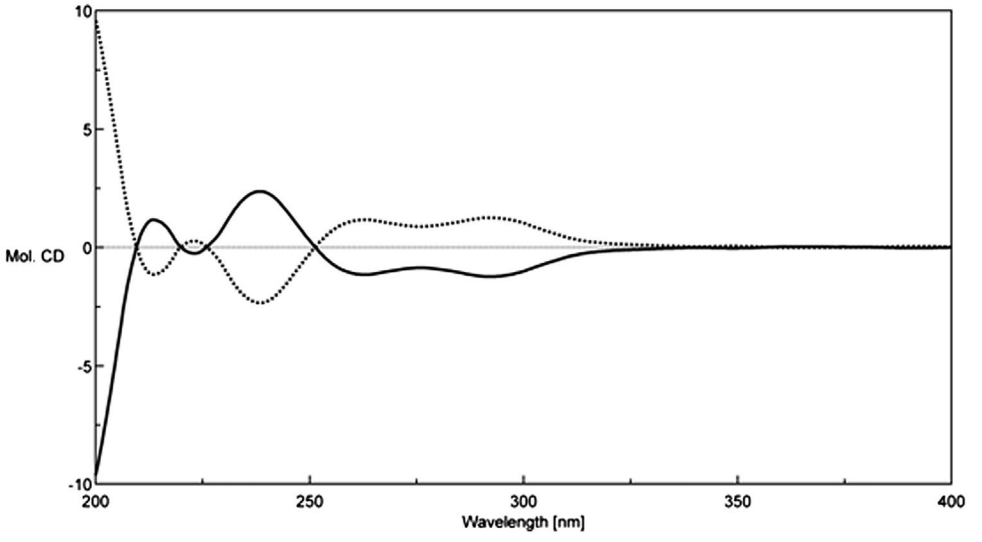

Figure 2. CD difference spectra of compounds **1** (—)and **2** (---).

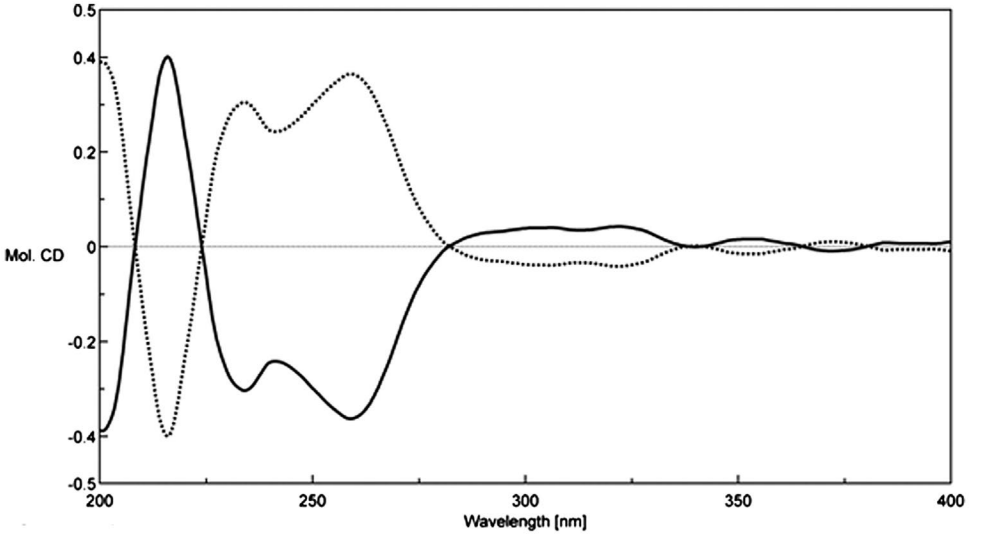

Figure 3. CD difference spectra of compounds **3** (—) and **4** (---).

the 8‴*R* configuration [12]. Hence, compound **3** was characterized as (−)-4-hydroxy-3-methoxy acetophenone 4-*O*-β-D-{6-*O*-[4-*O*-(7*R*,8*R*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside.

Spectroscopic data of compound **4** are almost identical to those of **3**. However, **3** and **4** were separable by chiral HPLC with retention times of 15.4 and 25.6 min (Experimental Section), respectively. In addition, the $\Delta\,\delta_{C8'''-C7'''}$ (12.4 ppm) suggested that **4** was another *threo*-isomer of **3** [9–12]. The CD difference spectrum of **4** (Figure 3) showed positive Cotton effect at 234 nm indicating the 8‴ *S* configuration [12]. Consequently, **4** was determined

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to be (−)-4-hydroxy-3-methoxy acetophenone 4-*O*-β-D-{6-*O*-[4-*O*-(7*S*,8*S*)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a JASCO P-2000 polarimeter (Tokyo, Japan), and UV spectra with a JASCO V-650 spectrophotometer (JASCO). IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission; Thermo Electron Corporation, Madison, WI, USA). CD spectra were recorded on a JASCO J-815 CD spectrometer (JASCO, Tokyo, Japan). NMR measurements were performed on Bruker AV600 IIIHD spectrometers (Bruker Corp., Karlsruhe, Germany) in DMSO-d_6. HRESIMS were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent Technologies Ltd., Santa Clara, CA, USA). Preparative HPLC was conducted using a Shimadzu LC-6AD instrument with an SPD-20A detector and YMC-Pack ODS-A column (250 × 10 mm, 5 μm). Chirality resolutions were performed on a DAICEL AD-H column (250 × 4.6 mm, 5 μm; Daicel, Tokyo, Japan). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), Diaion HP 20 (Mitsubishi, Japan) and ODS (50 μm, YMC, Kyoto, Japan) were used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out with GF254 plates (Qingdao Marine Chemical Factory). Spots were visualized by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

3.2. Plant material

Whole plants of *Iris japonica* were collected in Yunnan Province, China, in May 2014, and identified by associate Professor Lin Ma (Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College). A voucher specimen (ID-S-2618) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing.

3.3. Extraction and isolation

The air-dried whole plants (50 kg) of *I. japonica* were extracted with 95% EtOH (3 × 100 L) under reflux. The extracts were combined and concentrated under a vacuum to give a residue (2.1 kg), which was suspended in water and partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH soluble fraction (520 g) was subjected to a Diaion HP20 column eluted with a stepwise gradient of EtOH/H_2O (10:90, 30:70, 50:50, 70:30, and 95:5, v/v) to afford five fractions. After removing the solvent, the EtOH/H_2O (50:50) eluate (178 g) was subsequently separated by silica gel chromatography eluting with $CHCl_3$/MeOH (50:1–0:1, v/v) to afford six fractions (A–F) based on TLC analysis. The F_D (35 g) that eluted with 10:1 $CHCl_3$/MeOH was further chromatographed on a reversed-phase C_{18} silica gel column (40 × 6 cm), eluted with 10, 30, 50, 70, and 100% MeOH in H_2O, to afford F_{D1} to F_{D8}. F_{D2} (5 g) was purified using Sephadex LH-20 (MeOH) to give $F_{D2\text{-}1}$ to $F_{D2\text{-}5}$. $F_{D2\text{-}2}$ (67 mg) was isolated by a silica gel ($CHCl_3$/MeOH 20:1) to yield $F_{D2\text{-}2\text{-}1}$ and $F_{D2\text{-}2\text{-}2}$. $F_{D2\text{-}2\text{-}2}$ (10.5 mg) was

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isolated by preparative HPLC separation (20% MeCN in H_2O, 3 ml/min) to afford fractions $F_{D2\text{-}2\text{-}2\text{-}1}$ (3.6 mg, t_R 46 min) and $F_{D2\text{-}2\text{-}2\text{-}2}$ (3.8 mg, t_R 42 min). Finally, chiral resolution on $F_{D2\text{-}2\text{-}2\text{-}1}$ and $F_{D2\text{-}2\text{-}2\text{-}2}$ were performed by a DAICEL AD-H column (50% isopropanol in hexane, 0.6 ml/min) to yield **1** (1.2 mg) and **2** (1.5 mg), **3** (1.2 mg) and **4** (1.6 mg), respectively.

3.3.1. (–)-4-Hydroxy-3-methoxyacetophenone 4-O-β-D-{6-O-[4-O-(7R,8S)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (1)

White amorphous powder, $[\alpha]_D^{20}$ –36.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 203 (5.11), 222 (4.86), 265 (4.62) nm; CD (MeOH) $\Delta\varepsilon_{225\ nm}$ –2.92, $\Delta\varepsilon_{257\ nm}$ –1.50, $\Delta\varepsilon_{290\ nm}$ –0.65; IR ν_{max} 3239, 1718, 1673, 1595, 1516 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectral data see Table 1; HRESIMS: *m/z* 697.2108 $[M + Na]^+$ (calcd for $C_{33}H_{38}NaO_{15}$, 697.2103).

3.3.2. (–)-4-Hydroxy-3-methoxyacetophenone 4-O-β-D-{6-O-[4-O-(7S,8R)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (2)

White amorphous powder, $[\alpha]_D^{20}$ –27.9 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 203 (5.10), 222 (4.81), 265 (4.54) nm; CD (MeOH) $\Delta\varepsilon_{234\ nm}$ –5.33, $\Delta\varepsilon_{299\ nm}$ +1.10; IR ν_{max} 3258, 1716, 1674, 1595, 1515 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectral data see Table 1; HRESIMS: *m/z* 697.2106 $[M + Na]^+$ (calcd for $C_{33}H_{38}NaO_{15}$, 697.2103).

3.3.3. (–)-4-Hydroxy-3-methoxyacetophenone 4-O-β-D-{6-O-[4-O-(7R,8R)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (3)

White amorphous powder, $[\alpha]_D^{20}$ –9.4 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 203 (5.11), 222 (4.81), 265 (4.54) nm; CD (MeOH) $\Delta\varepsilon_{222\ nm}$ –1.52, $\Delta\varepsilon_{256\ nm}$ –0.97; IR ν_{max} 3363, 1716, 1689, 1612, 1592 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectral data see Table 1; HRESIMS: *m/z* 697.2103 $[M + Na]^+$ (calcd for $C_{33}H_{38}NaO_{15}$, 697.2103).

3.3.4. (–)-4-Hydroxy-3-methoxyacetophenone 4-O-β-D-{6-O-[4-O-(7S,8S)-(4-hydroxy-3-methoxyphenylglycerol-8-yl)-3-methoxybenzoyl]}-glucopyranoside (4)

White amorphous powder, $[\alpha]_D^{20}$ –6.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 203 (5.07), 222 (4.77), 265 (4.46) nm; CD (MeOH) $\Delta\varepsilon_{223\ nm}$ –1.39, $\Delta\varepsilon_{273\ nm}$ +0.21; IR ν_{max} 3379, 1717, 1688, 1611, 1594 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectral data see Table 1; HRESIMS: *m/z* 697.2104 $[M + Na]^+$ (calcd for $C_{33}H_{38}NaO_{15}$, 697.2103).

3.4. Acid hydrolysis of 1–4

Each compound (1 mg) was individually refluxed in 2 M HCl (2.0 ml) at 80 °C for 15 h. The reaction mixture was extracted with EtOAc, and the H_2O phase was evaporated under vacuum, diluted repeatedly with H_2O, and evaporated *in vacuo* to furnish a neutral residue. The residue was dissolved in anhydrous pyridine (1.0 ml), to which 1 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h, and after evaporation *in vacuo* to dryness, 0.1 ml of N-trimethylsilylimidazole was added; the mixture was kept at 60 °C for another 2 h. The reaction mixture was partitioned between *n*-hexane

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and H_2O (1.0 ml each), and then the *n*-hexane extract was analyzed by GC under the following conditions: capillary column, HP-5 (30 m × 0.25 mm, with a 0.25 μm film; Dikma); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature, 160 °C, then raised to 280 °C at 5 °C/min, final temperature maintained for 10 min; carrier gas, N_2. From the acid hydrolysate of each compound, D-glucose was confirmed by comparison of the retention time of its derivative with that of authentic sugar derivatized in a similar way, which showed a retention time of 18.91 min, respectively.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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