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Note

Phenolic glycosides from *Ficus tikoua* and their cytotoxic activities

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ABSTRACT

Four new phenolic glycosides, named 2-ethylene-3,5,6-trimethyl-4-phenol-1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**1**), 3-methoxy-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranosylpropiophenone (**2**), 3-hydroxy-1-(4-*O*-β-D-glucopyranosyl-3-methoxyphenyl)propan-1-one (**3**) and 4-hydroxy-3,5-bis(3′-methyl-2-butenyl)benzoic acid-*O*-β-D-glucopyranoside (**4**), were isolated from the ethanol extract of *Ficus tikoua*, together with six known compounds: 3,4,5-trimethoxyphenol-1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**5**), 3,4,5-trimethoxyphenol-1-*O*-β-D-glucopyranoside (**6**), 3-methoxy-4-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosylpropiophenone (**7**), baihuaqianhuoside (**8**), 3,5-dimethoxy-4-hydroxybenzoic acid-*O*-β-D-glucopyranoside (**9**) and 2-methoxy-4-allylphenyl-1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**10**). The structures of the four new compounds were elucidated by chemical methods and MS and IR, as well as 1D and 2D NMR analyses. The cytotoxicities of the 10 compounds against HeLa, K562, HL60 and HepG2 cell lines were assessed.

Ficus tikoua Bur., belonging to the Moraceae family, is a Miao ethnomedicine in China and has long been used for the treatment of injuries, rheumatism, diarrhoea and cough caused by lung heat. A recent pharmacological investigation of this plant showed that *Ficus tikoua* possessed anti-tumour, anti-diabetes and anti-bacterial activity.[1] Only a few phytochemical studies on the stems and leaves of *F. tikoua* are documented,[1,2] and there has been no chemical investigation on the rhizomes to the best of our knowledge. In our previous cytotoxic experiments on natural medicines, 90% ethanol extract of the rhizome of *F. tikoua* was found to possess significant cytotoxic activity. To elucidate the anti-tumour bio-active constituents from this ethnomedicine, the rhizome of *F. tikoua* was phytochemically investigated to afford four new phenolic glycosides, 2-ethylene-3,5,6-trimethyl-4-phenol-1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**1**), 3-methoxy-4-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosylpropiophenone (**2**), 3-hydroxy-1-(4-*O*-β-D-glucopyranosyl-3-methoxyphenyl)propan-1-one (**3**) and 4-hydroxy-3,5-bis(3′-methyl-2-butenyl)benzoic acid *O*-β-D-glucopyranoside (**4**) (Fig. 1), as well as six known compounds, 3,4,5-trimethoxyphenol-1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**5**),[3] 3,4,5-trimethoxyphenol-1-*O*-β-D-glucopyranoside (**6**),[4] 3-methoxy-4-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosylpropiophenone (**7**),[5] baihuaqianhuoside (**8**),[6–9] 3,5-dimethoxy-4-hydroxybenzoic acid *O*-β-D-glucopyranoside (**9**)[10] and 2-methoxy-4-allylphenyl-1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**10**).[11] The cytotoxicity of the 10 isolates was tested by the MTT method with HeLa, K562, HL60 and HepG2 cell lines. Herein, we describe the isolation, structural elucidation and cytotoxic activity of the 10 isolates.

Compound **1** was obtained as a white amorphous powder. The positive ESIMS gave a quasi-molecular ion peak at *m/z* 495 ([M+Na]$^+$), in agreement with the molecular formula $C_{22}H_{32}O_{11}$ revealed by the HRESIMS. The IR suggested the existence of hydroxyl (3447 cm^{-1}), olefin (1646 cm^{-1}) and aromatic groups (1601, 1505 and 1457 cm^{-1}) in compound **1**. After the hydrolysis of **1** with 10% HCl in MeOH, D-glucose and D-xylose were identified by a comparison with the authentic sugar samples on TLC (developed by *n*-BuOH/AcOH/H_2O 4:1:5, upper layer; and PhOH/H_2O 4:1). In the ^{1}H NMR spectrum, three olefinic proton signals due to a mono-substituted double bond at δ 6.94 (1H, dd, J = 11.6, 18.4 Hz, H-7), 5.48 (1H, dd, J = 2.4, 11.6 Hz, H-8a) and 5.32 (1H, dd, J = 2.4, 18.4 Hz, H-8b) were observed, as well as three singlet methyl signals at δ 2.23 (3H, s), 2.20, (3H, s) and 2.16 (3H, s). The anomeric proton signals at δ 4.68 (1H, d, J = 7.6 Hz, H-1′) and 4.12 (1H, d, J = 7.6 Hz, H-1″) suggested the presence of two β-linked sugar moieties. The ^{13}C NMR spectrum displayed 22 carbon signals: three methyls at δ 14.3, 14.2 and 13.2; one mono-substituted double bond at δ 134.7 (C-7, d) and 120.2 (C-8, t); a fully substituted

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aromatic ring at δ 150.4 (C-4, s), 147.0 (C-1, s), 130.8 (C-2, s), 129.6 (C-5, s), 125.3 (C-6, s) and 122.4 (C-3, s); one β-D-glucopyranosyl moiety at δ 104.8 (C-1′, d), 75.9 (C-2′, d), 77.8 (C-3′, d), 71.6 (C-4′, d), 77.7 (C-5′, d) and 69.5 (C-6′, t);[12,13] and one β-D-xylopyranosyl group at δ 104.6 (C-1″, d), 74.7 (C-2″, d), 77.3 (C-3″, d), 71.1 (C-4″, d) and 66.5 (C-5″, t).[12,14] The absolute configurations of the D-glucose and D-xylose were verified by GC analysis of their trimethylsilyl L-cysteine derivatives. The long-range HMBC correlations (Fig. 2) of H-7 (δ 6.94) with δ 147.0 (C-1), 130.8 (C-2) and 122.4 (C-3); H-9 (δ 2.23) with δ 130.8 (C-2), 122.4 (C-3) and 150.4 (C-4); H-10 (δ 2.16) with δ 150.4 (C-4), 129.6 (C-5), and 125.3 (C-6); and H-11 (δ 2.20) with δ 147.0 (C-1), 129.6 (C-5) and 125.3 (C-6) suggested that the mono-substituted double bond is located at C-2, and the three methyls are attached to C-3, C-5 and C-6. Simultaneously, HMBC correlations (Fig. 2) of H-1′ (δ 4.68) with δ 147.0 (C-1) and H-1″ (δ 4.12) with δ 69.5 (C-6′) were observed, demonstrating that the β-D-xylopyranose was located at the C-6′ of the inner glucopyranose, and the inner glucopyranose was linked at C-1 in the aromatic ring. The carbon signal in the low field at δ 150.4 (C-4, s) suggested the existence of the hydroxyl at C-4. These deductions were supported by the HRESIMS and ^{1}H–^{1}H COSY, ROESY and HMBC (Fig. 2) correlations. Consequently, the structure of compound **1** was assigned as 2-ethylene-3,5,6-trimethyl-4-phenol-1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**1**) (Fig. 1).

Compound **2** was obtained as a white amorphous powder and had the molecular formula $C_{21}H_{30}O_{12}$, deduced by the HRESI peak at *m*/*z* 497.1643 ($C_{21}H_{30}O_{12}Na^+$, calcd 497.1634). The IR spectrum showed absorption bands at 3444, 1679, 1595, 1511 and 1461 cm^{-1}, corresponding to hydroxyl, carbonyl and aromatic ring groups, respectively. The hydrolysis of **2** with 10% HCl in MeOH liberated D-glucose and D-apiose, identified by a comparison with the authentic sugar samples on TLC (*n*-BuOH/AcOH/H_2O 4:1:5, upper layer; PhOH/H_2O 4:1). The absolute configurations of the D-glucose and D-apiose were also confirmed by GC analysis. The ^{1}H NMR spectrum showed three aromatic proton signals at δ 7.63 (1H, dd, *J* = 1.6, 8.8 Hz), 7.56 (1H, d, *J* = 1.6 Hz) and 7.18 (1H, d, *J* = 8.8 Hz); one methoxy at δ 3.89 (3H, s); one methylene at δ 3.02 (2H, q, *J* = 7.2 Hz); one methyl at δ 1.17 (3H, t, *J* = 7.2 Hz); together with two anomeric proton signals at δ 5.11 (1H, d, *J* = 7.6 Hz, H-1′) and 5.54 (1H, d, *J* = 1.2 Hz, H-1″).[15–17] The coupling constant of the anomeric proton ascribable to the glucose suggested that the glucose was β-linked, and the β-linkage of apiose was deduced by comparing the *J* value of anomeric proton with those of methyl-α-D-apiose (*J* = 4.6 Hz) and methyl-β-D-apiose (*J* = 2.4 Hz) in the previous report.[15] The ^{13}C NMR also displayed two sugar moieties corresponding to a β-D-glucopyranose with signals at δ 100.2 (d), 77.3 (d), 78.7 (d), 71.3 (d), 78.2 (d) and 62.4 (t);[12,13] and a β-D-apiofuranose with signals at δ 110.2 (d), 77.9 (d), 80.8 (s), 75.4 (t) and 66.1 (t);[15–17] as well as one carbonyl at δ 202.2; an aromatic ring with signals at δ 152.2 (s), 150.6 (s), 132.4 (s), 123.6 (d), 115.6 (d) and 112.1 (d); and a methoxy at δ 56.4 (q). Comparing the NMR data with those of the known compound baihuaqianhuoside (**8**)[6–9] suggested that compound **2** possessed the same aglycone as that of baihuaqianhuoside (**8**) and contained one more apiose sugar moiety. The additional β-D-apiofuranosyl unit was assigned to be attached at the C-2′ of the inner β-D-glucopyranose based on the HMBC correlation between H-1″ (δ 5.54) and δ 77.3 (C-2′). The other HMBC and ^{1}H–^{1}H COSY (Fig. 2) data further confirmed this deduction. Accordingly, the structure of compound **2** was determined to be 3-methoxyl-4-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosylpropiophenone (**2**) (Fig. 1).

Compound **3** was also isolated as a white amorphous powder and assigned the molecular formula $C_{16}H_{22}O_9$ based on the HRESIMS peak at *m*/*z* 381.1169 ([M+Na]$^+$, $C_{16}H_{22}O_9Na^+$, calcd 381.1162). A comparison of the NMR data with those of baihuaqianhuoside (**8**)[6–9] showed a high similarity, with the exception that there was one more oxygenated methylene and one less methyl in compound **3**. Acid hydrolysis of **3** resulted in D-glucose, which was determined by a TLC comparison with the authentic sample and GC analysis. Considering that compound **3** had 16 mass units more than baihuaqianhuoside (**8**),[6–9] it could be concluded that compound **3** was a derivative of baihuaqianhuoside (**8**), with a hydroxyl added at C-9. This was further verified by the HMBC correlation (Fig. 2) between δ 3.95 (2H, t, *J* = 6.0 Hz, H-9) and C-7 (δ 199.7) and the ^{1}H–^{1}H COSY cross peak (Fig. 2) between δ 3.95 (2H, t, *J* = 6.0 Hz, H-9) and δ 3.20 (2H, t, *J* = 6.0 Hz, H-8). The other HSQC, HMBC and ^{1}H–^{1}H COSY correlations (Fig. 2) were used for the other proton and carbon assignments. Thus, the structure of compound **3** was elucidated to be 3-hydroxy-1-(4-*O*-β-D-glucopyranosyl-3-methoxyphenyl)propan-1-one (**3**) (Fig. 1).

Compound **4** was isolated as a white amorphous powder, with the molecular formula $C_{23}H_{32}O_8$, revealed by the HRESIMS peak at *m*/*z* 459.1986 ([M+Na]$^+$). A comparison of the NMR data with those of 4-methoxy-3,5-bis(3′-methyl-2-butenyl)benzoic acid[18] suggested that compound **4** had a similar 4-hydroxy-3,5-bis(3′-methyl-2-butenyl)benzoic acid nucleus. However, compound **4** contained one more β-D-glucopyranose unit than 4-methoxy-3,5-bis(3′-methyl-2-butenyl)benzoic acid,[18] as shown by the TLC and GC analysis of the alkaline methanolysis product of compound **4**. According to the previous literatures,[10,13,19,20] the anomeric carbon signal should appear at the relatively higher field (with a chemical shifts smaller than 96.0 ppm) when a D-glupyranose was linked at a carboxyl function. In our experiments, the anomeric carbon signal at δ 95.9 (s, C-1′) at a relatively higher field indicated that the β-D-glucopyranose might be linked at the carboxyl group. This idea was substantiated by the HMBC correlation between H-1′ (δ 5.69 1H, d, *J* = 7.8 Hz) and C-7 (167.2, s). The other 2D NMR correlations (Fig. 2) further confirmed the above deduction. Lastly, the structure of compound **4** was characterised as 4-hydroxy-3,5-bis(3′-methyl-2-butenyl)benzoic acid *O*-β-D-glucopyranoside (**4**) (Fig. 1).

The known compounds **5**–**10** were identified by comparing the NMR data with those reported in the literature.[3–11]

All 10 isolates were tested for their cytotoxic activities by the MTT method[21] against HeLa, K562, HL60 and HepG2 cell lines. As summarised in Table 3, compounds **1**, **2**, **4**, **7**, **8** and **10** showed superior cytotoxicity against the HeLa cell line, with IC_{50} values less than 25 μM. Compounds **3**, **5**, **6**, **8** and **9** showed good inhibitory activity against K562 cells. For the HL60 cell line, compounds **4**, **6** and **7** exhibited a similar potency as the positive control, and compounds **1** and **3** showed noticeable activity against HepG2 cells, with IC_{50} values smaller than 20 μM. Cisplatin, an approved agent for clinical anti-tumour treatment, was employed as a positive control in our cytotoxicity bioassay.

In conclusion, our research described the isolation of 10 phenolic chemicals from this plant for the first time. Furthermore, the cytotoxic activities of all the isolates were assessed in vitro. Although the cytotoxicities of compounds **1**–**10** were not comparable to the positive control in our tested cell lines, these results revealed the phenolic constituents in *F. tikoua* might be the main anti-tumour bioactive components, which provide a scientific support to the ethnomedicine for the anti-tumour activity.

1. Experimental

1.1. General experimental procedures

Optical rotations were determined on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-210A spectrophotometer. GC experiments were conducted on an Agilent 7890A instrument. IR spectra were obtained on a Bio-Rad FTS-135

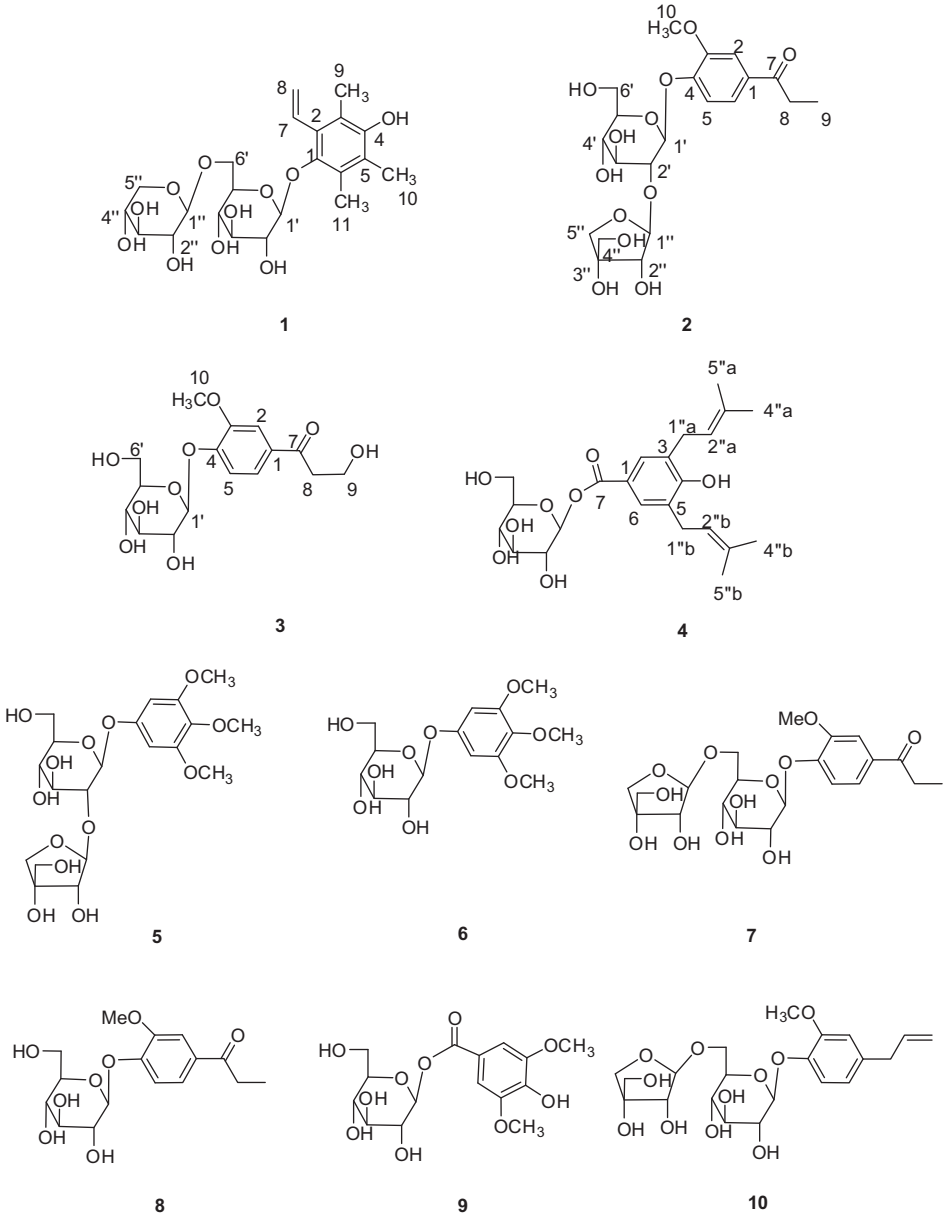

Figure 1. The structures of compounds **1**–**10**.

spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AM-400 NMR, with TMS as an internal standard. MS were recorded on a VG Auto Spec-3000 spectrometer. Column chromatography (CC) was performed using silica gel (SiO_2; 200–300 mesh; Qingdao Meigao Chemical Company, Qingdao, China), Sephadex LH-20 (20–150 μm; Pharmacia Fine Chemical Co. Ltd, Sweden), MCI gel CHP-20P (70–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Lichroprep Rp-18 gel (40–63 μm, Merck, Germany). The authentic sugars including D-glucose, D-xylose and D-apiose were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

1.2. Plant material

The rhizomes of *Ficus tikoua* were collected in Chuxiong, Yunnan province, PR China, in September 2010, and identified by Professor Deding Tao from the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (2010-09-06) was deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities.

1.3. Extraction and isolation

The dried and powdered rhizomes of *Ficus tikoua* (11 kg) were extracted with 90% EtOH (80 L) under reflux three times. The extract was concentrated in vacuo and partitioned between water, petroleum ether, EtOAc and *n*-BuOH to provide petroleum ether (320 g), EtOAc (280 g) and *n*-BuOH (368 g) fractions. The *n*-BuOH fraction was subjected to silica gel column chromatography (CC) with a gradient elution of $CHCl_3$/MeOH/H_2O (90:10:0, 80:20:2, 70:30:3, 65:35:5 (v/v/v)) to yield seven fractions (Frs. 1–7). Fr. 2 (15 g) was subjected to silica gel CC (200 g) and eluted with $CHCl_3$/MeOH/H_2O (90:10:1) to afford five fractions (Frs. 2a–2e). Fr. 2c (3.2 g) was subjected to MCI gel CC (80 g, MeOH/H_2O 75:25) to give three fractions. The third fraction was further purified by Rp-18 CC (MeOH/H_2O 75:25) to yield compounds **3** (130 mg) and **9** (270 mg). Fr. 2d (2.1 g) was also subjected to MCI gel CC (80 g,

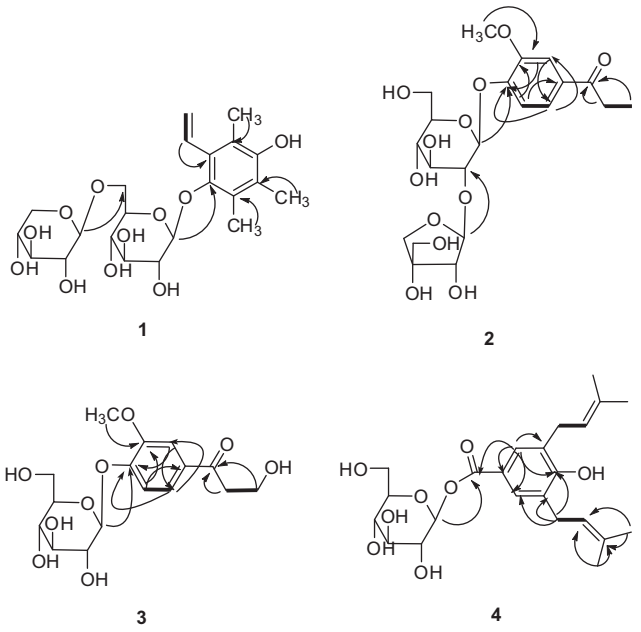

Figure 2. Key HMBC (→) and ^{1}H–^{1}H COSY (▬) correlations of compounds **1**–**4**.

MeOH/H_2O 70:30) and subsequently purified on Rp-18 (MeOH/H_2O 70:30) and Sephadex LH-20 (eluted with MeOH) to afford compounds **1** (65 mg), **6** (201 mg) and **10** (70 mg). Fr. 3 (20.6 g) was purified by silica gel CC eluting with $CHCl_3$/MeOH/H_2O (90:10:1→80:20:2) to provide five fractions (Frs. 3a–3e). Fr. 3b (3.7 g) was decoloured by MCI gel CC and further purified by successive Rp-18 CC to furnish compounds **4** (87 mg), **2** (118 mg) and **8** (360 mg). Fr. 3c (2.5 g) was separated by MCI gel CC, followed by Rp-18 and Sephadex LH-20 CC (eluted with MeOH) to yield compounds **7** (245 mg) and **5** (160 mg).

1.4. Identification

1.4.1. Compound 1

White amorphous powder; $[\alpha]_D^{25.8}$ −58.8 (*c* 0.171, MeOH); UV: λ_{max}^{MeOH} (log ε) 247 (3.03), 292 (2.91); IR (KBr) cm^{-1}: 3447, 2976, 1646, 1601, 1505, 1457, 1263, 1127, 1060, 993, 864; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (neg.): *m*/*z* 471 $[M-H]^-$. ESIMS (pos.): *m*/*z* 495 $[M+Na]^+$; HRESIMS (pos.): *m*/*z* 495.1851 $[M+Na]^+$ (calcd for $C_{22}H_{32}O_{11}Na^+$, 495.1842).

1.4.2. Compound 2

White amorphous powder; $[\alpha]_D^{26.4}$ −80.5 (*c* 0.225, MeOH); UV: $[\lambda_{max}^{MeOH}$ (log ε) 275 (2.87); IR (KBr) cm^{-1}: 3444, 2978, 1679, 1595, 1511, 1461, 1267, 1130, 1069; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (pos.): *m*/*z* 497 $[M+Na]^+$; HRESIMS: *m*/*z* 497.1643 $[M+Na]^+$ (calcd for $C_{21}H_{30}O_{12}Na^+$, 497.1634).

1.4.3. Compound 3

White amorphous powder; $[\alpha]_D^{25.0}$ −62.5 (*c* 0.110, MeOH); UV: $[\lambda_{max}^{MeOH}$ (log ε) 278 (2.90); IR (KBr) cm^{-1}: 3445, 2975, 1684, 1592, 1514, 1463, 1274, 1108, 1067; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (pos.): *m*/*z* 381 $[M+Na]^+$; HRESIMS: *m*/*z* 381.1169 $[M+Na]^+$ (calcd for $C_{16}H_{22}O_9Na^+$, 381.1162).

1.4.4. Compound 4

White amorphous powder; $[\alpha]_D^{24.8}$ −28.9 (*c* 0.160, MeOH); UV: $[\lambda_{max}^{MeOH}$ (log ε) 266 (3.05); IR (KBr) cm^{-1}: 3446, 2978, 1650, 1598, 1450, 1267, 1122, 1070, 995, 863; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (pos.): *m*/*z* 459 $[M+Na]^+$; HRESIMS: *m*/*z* 459.1986 $[M+Na]^+$ (calcd $C_{23}H_{32}O_8Na^+$, 459.1994).

1.5. Sugar identification

1.5.1. Acidic hydrolysis of compounds 1–3

Each solution of compounds **1**–**3** (each 5 mg) in a mixture of MeOH (1.0 mL) and 10% HCl (1.0 mL) was stirred at reflux for 4 h. The hydrolysate was allowed to cool, diluted twofold with H_2O, and extracted with EtOAc (3 × 2 mL). The aqueous layer was neutralised with 2 M ammonium hydroxide and concentrated in vacuo to give a residue in which D-glucose and D-xylose (from **1**), D-glucose and D-apiose (from **2**), and D-glucose (from **3**) were identified by comparison with authentic sugar samples (*n*-BuOH/AcOH/H_2O 4:1:5, upper layer; PhOH/H_2O, 4:1) on TLC (sprayed with aniline phthalate reagent, followed by heating).

1.5.2. Alkaline methanolysis of compound 4

Compound **4** (5 mg) was treated with methanolic 3% NaOMe at room temperature and stirred for 4 h. After being extracted with EtOAc (3 × 2 mL), the aqueous layer was neutralised and concentrated in vacuo to afford a residue, where D-glucose was identified

Table 1
The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data for the aglycone moieties of compounds **1**–**4** in CD_3OD

No.	1		2		3		4	
	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)
1	–	147.0 (s)	–	132.4 (s)	–	133.0 (s)	–	121.6 (s)
2	–	130.8 (s)	7.56 (1H, d, 1.6)	112.1 (d)	7.60 (1H, d, 2.0)	112.4 (d)	7.68 (1H, s)	130.5 (d)
3	–	122.4 (s)	–	150.6 (s)	–	150.7 (s)	–	129.5 (s)
4	–	150.4 (s)	–	152.2 (s)	–	152.4 (s)	–	159.0 (s)
5	–	129.6 (s)	7.18 (1H, d, 8.8)	115.6 (d)	7.24 (1H, d, 8.8)	116.2 (d)	–	129.5 (s)
6	–	125.3 (s)	7.63 (1H, dd, 1.6, 8.8)	123.6 (d)	7.66 (1H, dd, 2.0, 8.8)	124.0 (d)	7.68 (1H, s)	130.5 (d)
7	6.94 (1H, dd,11.6,18.4)	134.7 (d)	–	202.2 (s)	–	199.7 (s)	–	167.2 (s)
8	5.48 (1H, dd, 2.4, 11.6) 5.32 (1H, dd, 2.4, 18.4)	120.2 (t)	3.02 (2H, q, 7.2)	32.3 (t)	3.20 (2H, t, 6.0)	41.8 (t)	–	–
9	2.23 (3H, s)	14.3 (q)	1.17 (3H, t, 7.2)	8.9 (q)	3.95 (2H, t, 6.0)	58.7 (t)	–	–
10	2.16 (3H, s)	13.2 (q)	3.89 (3H, s)	56.4 (q)	3.92 (3H, s)	56.7 (q)	–	–
11	2.20 (3H, s)	14.2 (q)	–	–	–	–	–	–
Isopentenyl	–	–	–	–	–	–		
1″a, 1″b	–	–	–	–	–	–	3.32 (4H, d, 4.8)	29.4 (t)
2″a, 2″b	–	–	–	–	–	–	5.30 (2H, m)	123.0 (d)
3″a, 3″b	–	–	–	–	–	–	–	134.1 (s)
4″a, 4″b	–	–	–	–	–	–	1.75 (6H, s)	26.6 (q)
5″a, 5″b	–	–	–	–	–	–	1.72 (6H, s)	17.9 (q)

δ in ppm, *J* in Hz.

Table 2
The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data for the sugar moieties of compounds **1–4** in CD_3OD

No.	1		2		3		4	
	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)
glc-								
1′	4.68 (1H, d, 7.6)	104.8 (d)	5.11 (1H, d, 7.6)	100.2 (d)	5.08 (1H, d, 7.6)	101.9 (d)	5.69 (1H, d, 7.8)	95.9 (d)
2′	3.48 (1H, m)	75.9 (d)	3.76 (1H, overlapped)	77.3 (d)	3.53 (1H, m)	74.7 (d)	3.48 (1H, overlapped)	74.1 (d)
3′	3.39–3.44 (1H, overlapped)	77.8 (d)	3.64 (1H, m)	78.7 (d)	3.48 (1H, m)	77.9 (d)	3.48 (1H, overlapped)	78.2 (d)
4′	3.39–3.44 (1H, overlapped)	71.6 (d)	3.41–3.43 (1H, m)	71.3 (d)	3.41–3.44 (1H, m)	71.2 (d)	3.43 (1H, overlapped)	71.0 (d)
5′	3.23 (1H, m)	77.7 (d)	3.47 (1H, m)	78.2 (d)	3.42 (1H, m)	78.4 (d)	3.43 (1H, overlapped)	78.8 (d)
6′	3.92 (1H,dd, 5.2, 11.6) 3.67 (1H, dd, 1.6, 11.6)	69.5 (t)	3.87–3.91 (1H,dd,2.0,12.0) 3.69 (1H, dd, 5.6, 12.0)	62.4 (t)	3.87–3.90 (1H, dd, 1.6, 12.0) 3.69 (1H, dd, 5.2, 12.0)	62.4 (t)	3.85 (1H, br d, 12.4) 3.63(1H, dd, 2.0, 12.4)	62.3 (t)
xyl- or api-								
1″	4.12 (1H, d, 6.8)	104.6	5.54 (1H, d, 1.2)	110.2 (d)	—	—	—	—
2″	3.06–3.10 (1H, overlapped)	74.7	3.96 (1H, br s)	77.9 (d)	—	—	—	—
3″	3.12–3.16 (1H, overlapped)	77.3	—	80.8 (s)	—	—	—	—
4″	3.43–3.45 (1H, m)	71.1	4.17 (1H, d, 9.6) 3.76 (1H, overlapped)	75.4 (t)	—	—	—	—
5″	3.76 (1H, dd, 5.2, 11.6) 3.03 (1H, m)	66.5	3.53 (2H, br s)	66.1 (t)	—	—	—	—

δ in ppm, J in Hz.

Table 3
The cytotoxic activities of compounds **1–10**

Samples	IC_{50} (μM)			
	Hela	K562	HL60	HepG2
90% Ethanol extract (μg/mL)	17.5 ± 3.1	28.1 ± 5.8	29.0 ± 6.1	18.7 ± 4.1
1	22.3 ± 5.2	76.9 ± 10.1	45.5 ± 8.8	15.1 ± 6.3
2	21.5 ± 6.7	102.5 ± 11.3	72.8 ± 9.3	118.3 ± 11.2
3	73.5 ± 8.9	16.3 ± 3.8	28.1 ± 7.4	16.9 ± 4.9
4	16.0 ± 3.2	89.9 ± 9.7	23.2 ± 4.7	132.4 ± 10.8
5	79.2 ± 9.7	18.5 ± 7.3	20.3 ± 6.2	121.2 ± 9.2
6	68.0 ± 6.6	20.6 ± 4.4	15.1 ± 5.4	105.1 ± 10.6
7	19.6 ± 5.5	76.3 ± 8.1	23.6 ± 4.8	71.1 ± 9.4
8	23.7 ± 6.2	24.8 ± 6.4	41.4 ± 7.3	50.1 ± 8.0
9	49.9 ± 10.5	20.6 ± 5.2	89.8 ± 9.1	44.3 ± 5.9
10	22.4 ± 5.8	75.4± 6.6	47.9 ± 6.7	77.0 ± 8.1
Cisplatin[a]	6.9	12.0	10.1	8.5

[a] Positive control.

by TLC comparison with the authentic sugar sample (*n*-BuOH/AcOH/H_2O 4:1:5, upper layer; PhOH/H_2O, 4:1).

1.5.3. Determination of absolute configuration

After being dried over P_2O_5 for 48 h, the above-mentioned aqueous residue was dissolved in anhydrous pyridine (1.0 mL), and 5 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h. The reaction mixture was then concentrated in vacuo to furnish a dry residue. 0.5 mL of *N*-trimethylsilylimidazole was added, and the reaction mixture was heated at 60 °C for 1 h. Subsequently, the mixture was partitioned between *n*-hexane and H_2O (1:1; v/v). The *n*-hexane extract was directly subjected to GC analysis 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, raised to 280 °C at 5 °C min^{-1} with the final temperature being maintained for 10 min; N_2 gas as carrier. By comparing the retention times (R_t) of the derivatives with those of authentic sugars prepared in a similar way, D-glucose (R_t 20.5 min) and D-xylose (R_t 13.1 min) from **1**, D-glucose and D-apiose (R_t 13.6 min) from **2**, and D-glucose from **3** and **4** were detected.

1.6. Cytotoxicity assay

Cytotoxic activities were evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method[21] using HeLa, K562, HL60 and HepG2 cell lines. Briefly, the cell suspensions (200 mL) at a density of 5 × 10^4 cells mL^{-1} were plated in 96 well microtitre plates and incubated for 24 h at 37 °C in a humidified incubator containing 5% CO_2. The test compound solution (2 mL in DMSO) at different concentrations was added to each well and further incubated for 72 h under the same conditions. The MTT solution (20 mL) was then added to each well and incubated for 4 h. The old medium (150 mL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. The absorbance was then determined with a Spectra Max Plus plate reader at 540 nm. Dose–response curves were generated and the IC_{50} values were defined as the concentration of compound required to inhibit cell proliferation by 50%. Cisplatin,

an approved agent for the treatment of many tumours, was used as the positive control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.09.008.

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