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Chemical constituents from the roots of *Biondia chinensis*

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Two new triterpenoid saponins, biondianosides F and G, together with 13 known compounds, were isolated from the ethanolic extract of the roots of *Biondia chinensis* Schltr. (Asclepiadaceae). Their structures were characterized as 3-*O*-β-D-glucopyranosyl-2α,3β-dihydroxyurs-12-en-28-oic acid-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester (biondianoside F) (**14**) and 3-*O*-β-D-glucopyranosyl-2α,3β,23-trihydroxyolean-12-en-28-oic acid-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl ester (biondianoside G) (**15**) by spectral and chemical evidence.

Keywords: *Biondia chinensis*; Asclepiadaceae; Triterpenoid saponin; Biondianosides F and G

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1. Introduction

Biondia chinensis Schltr. (Asclepiadaceae), an endemic plant mainly distributed in south-western China, is used as a folk medicine for the treatment of traumatic injury [1,2]. Five new pregnane glycosides, biondianosides, A–E, have been isolated from the two closely related species, *B. hemsleyana* [3,4] and *B. chinensis* [5]. In a continuation of our study on chemical constituents of *B. chinensis*, two new triterpenoid saponins were isolated and named biondianosides F and G (**14** and **15**). Together with the new compounds, 13 known ones were isolated and identified as acetyl ursolic acid (**1**) [6], ethyl caffeate (**2**) [7], 12β-hydroxy-androsta-1,4,6-triene-3,17-dione (**3**) [8], 12β-hydroxy-pregna-4,6-diene-3,20-dione (**4**) [9], pregn-5-ene-3β,14β,17β-trihydroxy-21-methoxy-20-one (**5**) [10], periplogenin (**6**) [11], 17β H-periplogenin (**7**) [11,12], methyl β-D-digitalopyranosyl-(1 → 4)-β-D-cymaropyranoside (**8**) [13], periplogenin-3-*O*-β-D-cymaropyranoside (peliprocymarin) (**9**) [14], periplogenin-3-*O*-β-D-glucopyranoside (**10**) [15], pregn-5-ene-3β,20(S)-diol-3-*O*-[2-*O*-acetyl-β-D-digitalopyranosyl-(1 → 4)-β-D-cymaropyranosyl]-20-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-β-D-digitalopyranoside (**11**) [13], pregn-5-ene-3β,16α,20(S)-triol-3-*O*-[2-*O*-acetyl-β-D-digitalopyranosyl-(1 → 4)-β-D-cymaropyranosyl]-20-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-β-D-digitalopyranoside (**12**) [13] and biondianoside D (**13**) [4], respectively. All compounds above were isolated from the title plant for the first time.

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2. Results and discussion

Compound **14** was obtained as an amorphous white powder and its molecular formula $C_{54}H_{88}O_{24}$ was determined from quasi-molecular ion peaks at *m/z* 1121 $[M + H]^+$ and 1143 $[M + Na]^+$ of ESI-MS spectroscopy with ^{1}H NMR and ^{13}C NMR. Other significant ions visible at *m/z* 959 $[M + H - 162]^+$, 797 $[M + H - 2 \times 162]^+$, 635 $[M + H - 3 \times 162]^+$ and 495 $[M + Na - 4 \times 162]^+$ corresponded to the loss of one, two, three and four hexosyl units.

The anomeric proton and carbon signals at δ_H 6.05, 5.58, 4.99, 4.93 (each 1H, d, 8.0 Hz) and δ_C 93.7, 104.8, 105.4, 106.6 (d) showed that compound **14** contained four sugar units. Complete assignments of the ^{1}H NMR for all monosaccharide moieties were achieved by ^{1}H—^{1}H COSY and ROESY spectra, while the ^{13}C NMR data were obtained from HMBC and HSQC analysis (figure 1 and table 1). Only glucose was detected by acid hydrolysis on thin-layer chromatography and the coupling constant of each anomeric proton revealed that all sugar units have β-configuration.

The ^{13}C NMR spectrum showed 30 carbon signals besides the signals of four sugar units, indicating that it has a triterpene aglycone. The ^{13}C NMR spectrum of compound **14** was very similar to that of methyl 2α-hydroxyl ursolic acid ester [6], except for C-2 (δ_C 67.0, d) and C-3 (δ_C 95.5, d) which were shifted − 1.9 and + 11.7 ppm, respectively, indicating the presence of a glycosidic linkage at C-3 and the other at C-28. The ^{1}H NMR spectrum of **14** also showed signals

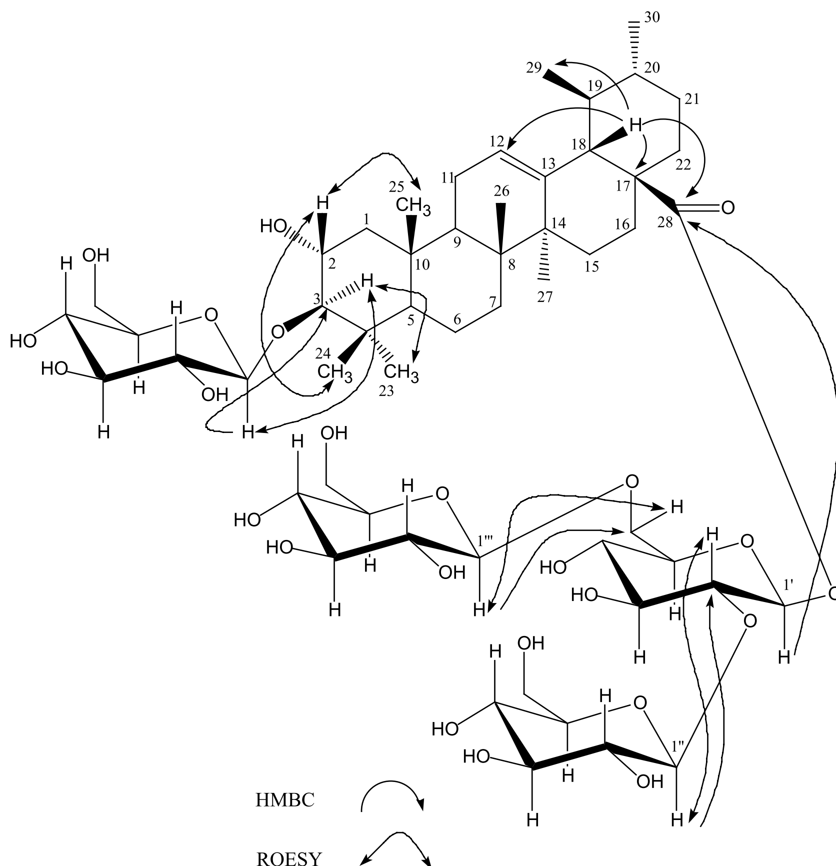

Figure 1. The structure and key correlations of HMBC and ROESY of **14**.

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Table 1. ^{1}H NMR and ^{13}C NMR data for compounds **14** and **15** (in pyridine-d_5).

	14		***15***	
No.	δ_C	δ_H *(Hz)*	δ_C	δ_H *(Hz)*
1	47.6 (t)	2.21 (1H, m, 8.1), 1.11 (1H, m, 7.1)	47.3 (t)	
2	67.0 (d)	4.01 (1H, m)	67.0 (d)	
3	95.5 (d)	3.26 (1H, d, 9.4)	88.3 (d)	4.23 (1H, m)
4	40.9 (s)		44.6 (s)	
5	55.9 (d)	0.84 (1H, m)	47.3 (d)	
6	18.7 (t)	1.48 (1H, m), 1.22 (1H, m)	18.2 (t)	
7	33.4 (t)	1.57 (1H, s), 0.87 (1H, s)	32.8 (t)	
8	40.4 (s)		40.0 (s)	
9	48.2 (d)	1.62 (1H, m)	48.1 (d)	
10	38.0 (s)		37.8 (s)	
11	24.0 (t)	1.97 (1H, m), 0.82 (1H, m)	23.9 (t)	
12	125.9 (d)	5.40 (1H, br.s)	122.5 (d)	5.38 (1H, br.s)
13	138.9 (s)		144.4 (s)	
14	42.7 (s)		42.1 (s)	
15	29.6 (t)	2.36 (1H, m), 1.37 (1H, m)	29.0 (t)	
16	24.5 (t)	2.29 (1H, m, 11.0), 2.10 (1H, m, 13.1)	23.0 (t)	
17	48.6 (s)		46.9 (s)	
18	53.4 (d)	2.46 (1H, d, 11.1)	41.7 (d)	3.10 (1H, d, 13.0)
19	39.5 (d)	1.37 (1H, m)	46.1 (t)	
20	39.3 (d)	0.89 (1H, m)	30.7 (s)	
21	31.0 (t)	1.29 (2H, m)	34.0 (t)	
22	36.6 (t)	1.86 (1H, m, 13.3), 1.73 (1H, m, 12.3)	32.2 (t)	
23	28.8 (q)	1.32 (3H, s)	63.8 (t)	4.46 (1H, m, 11.0), 3.57 (1H, m, 11.0)
24	18.4 (q)	0.97 (3H, s)	14.7 (q)	0.92 (3H, s)
25	17.1 (q)	0.87 (3H, s)	17.4 (q)	0.94 (3H, s)
26	17.7 (q)	1.02 (3H, s)	17.5 (q)	1.02 (3H, s)
27	24.1 (q)	1.16 (3H, s)	26.1 (q)	1.12 (3H, s)
28	176.7 (s)		176.4 (s)	
29	17.6 (q)	0.92 (3H, d, 6.3)	33.1 (q)	0.85 (3H, s)
30	21.5 (q)	0.85 (3H, d, 6.4)	23.7 (q)	0.85 (3H, s)
3-*O*-glc				
1	106.6 (d)	4.93 (1H, d, 7.7)	105.6 (d)	5.18 (1H, d, 7.8)
2	75.3 (d)	4.09 (1H, m)	75.4 (d)	4.00 (1H, m)
3	78.5 (d)	4.23 (1H, m)	78.5 (d)	4.16 (1H, m)
4	71.7 (d)	4.20 (1H, m)	71.3 (d)	4.20 (1H, m)
5	78.6 (d)	4.05 (1H, m)	78.3 (d)	3.91 (1H, m)
6	62.8 (t)	4.47, 4.34 (each 1H, m)	62.3 (t)	4.45, 4.35 (each 1H, m)
28-*O*-glc				
1′	93.7 (d)	6.05 (1H, d, 8.2)	93.6 (d)	6.16 (1H, d, 8.2)
2′	78.8 (d)	4.37 (1H, m)	78.1 (d)	4.50 (1H, m)
3′	78.5 (d)	4.10 (1H, m)	78.7 (d)	4.29 (1H, m)
4′	70.5 (d)	4.19 (1H, m)	70.6 (d)	4.25 (1H, m)
5′	77.9 (d)	4.00 (1H, m)	79.7 (d)	3.92 (1H, m)
6′	69.4 (t)	4.62, 4.30 (each 1H, m)	61.9 (t)	4.64, 4.36 (each 1H, m)
1″	104.5 (d)	5.59 (1H, d, 7.7)	104.6 (d)	5.70 (1H, d, 7.2)
2″	75.7 (d)	4.04 (1H, m)	75.9 (d)	4.08 (1H, m)
3″	78.8 (d)	4.08 (1H, m)	78.2 (d)	4.27 (1H, m)
4″	71.7 (d)	4.19 (1H, m)	72.8 (d)	4.05 (1H, m)
5″	78.8 (d)	3.88 (1H, m)	78.1 (d)	4.00 (1H, m)
6″	62.7 (t)	4.61, 4.32 (each 1H, m)	63.8 (t)	4.63, 4.39 (each 1H, m)
1‴	105.4 (d)	4.99 (1H, d, 7.8)		
2‴	76.0 (d)	4.01 (1H, m)		
3‴	78.6 (d)	4.24 (1H, m)		
4‴	72.9 (d)	4.07 (1H, m)		
5‴	78.8 (d)	3.87 (1H, m)		
6‴	63.9 (t)	4.63, 4.39 (each 1H, m)		

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at δ_H 4.01 (1H, m) and 3.26 (1H, d, $J = 9.4$ Hz) ascribable respectively to the 2β- and 3α-protons on the carbons bearing hydroxyl functions [16]. In the ROESY experiment the correlations between signals δ_H 4.01 (1H, m, H-2), 0.97 (3H, s, 24-H) and 0.87 (3H, s, 25-H); δ_H 3.26 (1H, d, $J = 9.4$ Hz, H-3) and 1.32 (3H, s, 23-H), showed H-2 and H-3 should be β- and α-configuration, respectively. After extensive NMR analysis, the aglycone was established as 2α-hydroxyl ursolic acid. Alkaline hydrolysis of **14** performed with 5% KOH yielded a prosapogenin **14a**. In the ESI-MS spectroscopy of **14a** the quasi-molecular ion peak at *m/z* 657 $[M + Na]^+$ and one significant ion peak visible at *m/z* 495 $[M + Na - 162]^+$ corresponded to the loss of one hexosyl unit, so **14** should be a triterpene-bidesmosidic saponin in which one glucose was bound to the aglycone by a glycosidic linkage at C-3, while the remaining three glucose were bound to the genin by a glycosidic ester linkage at C-28.

The sugar chain at C-28 was established from the HMBC correlations between δ_H 4.99 (1H, d, $J = 7.8$ Hz, 28-Glc-H-1‴) and δ_C 69.4 (t, 28-Glc-C-6′); δ_H 5.59 (1H, d, $J = 7.7$ Hz, 28-Glc-H-1″) and δ_C 78.8 (d, 28-Glc-C-2′); δ_H 6.05 (1H, d, $J = 8.2$ Hz, 28-Glc-H-1′) and δ_C 176.7 (d, 28-C). These linkages were confirmed by the ROESY correlations between the signals at δ_H 4.99 (1H, d, $J = 7.8$ Hz, 28-Glc-H-1‴) and 4.30 (1H, m, 28-Glc-H-6″); δ_H 5.59 (1H, d, $J = 7.7$ Hz, 28-Glc-H-1″) and 4.37 (1H, m, 28-Glc-H-2′).

On the basis of the above spectroscopic and chemical evidence, compound **14** was determined as 3-*O*-β-D-glucopyranosyl-2α,3β-dihydroxyurs-12-en-28-oic acid-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester named biondianoside F (**14**).

Compound **15** was obtained as an amorphous white powder and its molecular formula $C_{48}H_{78}O_{20}$ was determined from a quasi-molecular ion peak at *m/z* 997 $[M + Na]^+$ of ESI-MS spectroscopy with ^{1}H- and ^{13}C-NMR data. Other significant ion peaks visible at *m/z* 835 $[M + Na - 162]^+$, 673 $[M + Na - 2 \times 162]^+$, 511 $[M + Na - 3 \times 162]^+$ corresponded to the loss of one, two and three hexosyl units.

From ^{1}H and ^{13}C NMR spectra of anomeric protons and carbons δ_H 6.16, 5.70, 5.18 (each 1H, d, $J = 8.2$, 7.2, 7.8 Hz); δ_C 93.6, 104.6, 105.6 (d), **15** was shown to contain three sugar residues. Only glucose was detected by acid hydrolysis on thin-layer chromatography and the coupling constant of each anomeric proton revealed that all sugar units have β-configuration.

The ^{13}C NMR spectrum showed 30 carbon signals besides the signals of three sugar units, indicating that it has a triterpene aglycone. Comparison of the ^{13}C NMR spectrum of compound **15** with that of 3-*O*-β-D-glucopyranosyl arjunolic acid [17] revealed that the aglycone of these two compounds are very similar, except for δ_C 176.4 (s, C-28) of **15** which was shifted −3.6 ppm, indicating the presence of a glycosidic ester linkage at C-28. Furthermore, anomeric carbon of inner glucose attached to C-28 appear at higher field δ_C 93.6 (d, 28-Glc-C-1′) while the other carbon appear at lower field δ_C 78.1 (d, 28-Glc-C-2′) in comparison with that of oleanolic acid 28-*O*-β-D-glucopyranoside [18], indicating that the terminal glucose was linked to the C-2′ of inner glucose. So **15** should be a triterpene-bidesmosidic saponin in which one glucose was bound to the aglycone by a glycosidic linkage at C-3, while the remaining two glucose were bound to the genin by a glycosidic ester linkage at C-28.

The sugar chain at C-28 was established from the following HMBC correlations (figure 2): between δ_H 5.70 (1H, d, $J = 7.2$ Hz, 28-Glc-H-1″) and δ_C 78.1 (d, 28-Glc-C-2′); δ_H 6.16 (1H, d, $J = 8.2$ Hz, 28-Glc-H-1′) and δ_C 176.4 (d, 28-C). Complete assignments of all proton and carbon signals of sugar chain at C-28 were achieved by comparison of the ^{1}H and ^{13}C NMR with that of 2α,3β,23-trihydroxyolean-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside [19].

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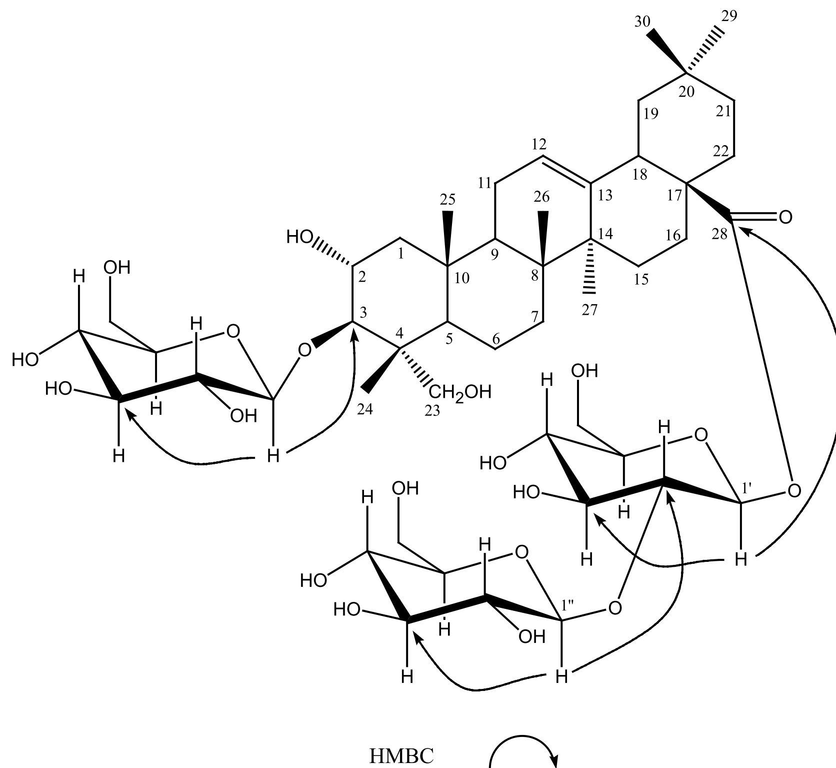

Figure 2. The structure and key correlations of HMBC of **15**.

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On the basis of the above spectroscopic evidence, compound **15** was determined as 3-*O*-β-D-glucopyranosyl-2α,3β,23-trihydroxyolean-12-en-28-oic acid-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl ester named biondianoside G.

3. Experimental

3.1 General experimental procedures

NMR spectra were recorded on Bruker DRX-500 spectrometer using TMS as an internal standard. ESI-MS spectra were obtained on a Finnigan LCQ^{DECA} spectrometer. Optical rotations were taken on a PE-341 polarimeter. Column chromatography was performed on silica gel and TLC was conducted on silica gel GF254 plates (Marine Chemical Factory, Qingdao, China) and reversed phase TLC on Rp-18 F254 plates (Merck). ODS (Cosmosil 75 C_{18}-OPN) (Nacalai Tesque), Lobar LiChroprep Rp-18 and Rp-4 (Merck), Macroporous Resin D101 (Tianjin Pesticide Factory, China). Spot of TLC was detected by spraying with 5% H_2SO_4-EtOH followed by heating. Sugars were detected by spraying with aniline-phthalate reagent.

3.2 Plant material

The roots of *Biondia chinensis* were collected in Summer 1999 from the western part of Sichuan Province, China, and identified by Professor Zuo-Cheng Zhao of Chengdu Institute

of Biology, Chinese Academy of Sciences. The voucher specimen (1999085) is deposited in the Herbarium of the institute.

3.3 Extraction and isolation

The air-dried and powdered roots (5.5 kg) of *B. chinensis* were extracted three times with 90% EtOH, each for 7 days at room temperature. After filtration and removal of the solvent, the brown residue (587 g) was dissolved in water and successively partitioned with petroleum ether (bp 60–90°C), EtOAc and *n*-BuOH successively. The petroleum ether extract (50 g) was chromatographed on silica gel column eluting with petroleum ether/acetone (50(1 to 5:1) and the fractions was inspected by TLC using petroleum ether/acetone (5:1) as developing solvent. The eluate (Fractions 59–62) was collected, then the solvent was recovered and recrystallized from $CHCl_3$ to provide compound **1** (47 mg). The EtOAc extract (50 g) was fractioned by repeated chromatography on normal silica gel with $CHCl_3$/acetone (50:1 to 3:1) as eluent and the fractions were inspected by TLC using $CHCl_3$/acetone (8:1) and $CHCl_3/CH_3OH$ (5:1) as developing solvent. The eluate ($CHCl_3$/acetone 30:1 to 5:1) was collected and subjected to ODS and Rp-18 silica gel column eluting with CH_3OH/H_2O (4:6 to 6:4) and preparative TLC (petroleum ether/acetone 2:1) yielding several major fractions which were combined respectively and recrystallized from CH_3OH to afford compounds **2** (25 mg), **3** (25 mg), **4** (20 mg), **5** (15 mg), **6** (15 mg), **7** (30 mg), **8** (110 mg) and **9** (10 mg). The *n*-BuOH extract (78 g) was subjected to normal silica gel column with $CHCl_3/CH_3OH$ (30:1 to 2:1) as eluent and the fractions were inspected by TLC using $CHCl_3/CH_3OH$ (5:1) and EtOAc/CH_3OH (3:1) as developing solvent. The eluate ($CHCl_3/CH_3OH$ 20:1 to 3:1) was collected and subjected to ODS, Rp-18 and Rp-4 silica gel column eluted with CH_3OH/H_2O (3:7 to 8:2) to give several major fractions which were combined respectively and recrystallized from CH_3OH to give compounds **10** (15 mg), **11** (160 mg), **12** (250 mg), **13** (15 mg), **14** (53 mg) and **15** (10 mg).

3.3.1 Acid hydrolysis. Compounds **14**, **15** and the sugar authentic samples (glucose and other sugars) were spotted on a silica gel TLC plate and hydrolysed in a water bath by exposure to HCl vapour at 60°C for 30 min. The TLC plate was then developed with $CHCl_3/CH_3OH$ (7:3) and sprayed with aniline-phthalate reagent for detection. Only glucose was detected by direct comparison of R_f values and colours with the sugar authentic samples.

3.3.2 Alkaline hydrolysis. A solution of compound **14** (10 mg) in 5% KOH/CH_3OH sealed in a test tube was heated at 100°C for 4 h. The reaction mixture was cooled to room temperature and neutralized to pH 6 with dilute HCl. After removal of CH_3OH, the remaining mixture was passed through a column of macroporous resin eluted with H_2O and CH_3OH. The CH_3OH eluent was evaporated to dryness. The residue was subjected to silica gel column to afford **14a**.

3.4 Identification

3.4.1 Compound 14. White amorphous powder (53 mg); $[\alpha]_D^{25}$ −19.0 (*c* 0.10, CH_3OH); ^1H NMR and ^{13}C NMR spectra data, see table 1; ESI-MS (positive) *m/z*: 1159 $[M + K]^+$,

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1143 $[M + Na]^+$, 1121 $[M + H]^+$, 959 $[M + H - glc]^+$, 797 $[M + H - 2glc]^+$, 635 $[M + H - 3glc]^+$, 495 $[M + Na - 4glc]^+$.

3.4.2 Compound 15. White amorphous powder (10 mg); $[\alpha]_D^{25} + 0.5$ (c 0.40, CH_3OH); ^{1}H NMR and ^{13}C NMR spectra data, see table 1; ESI-MS (positive) *m/z*: 997 $[M + Na]^+$, 835 $[M + Na - glc]^+$, 673 $[M + Na - 2glc]^+$, 511 $[M + Na - 3glc]^+$.

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