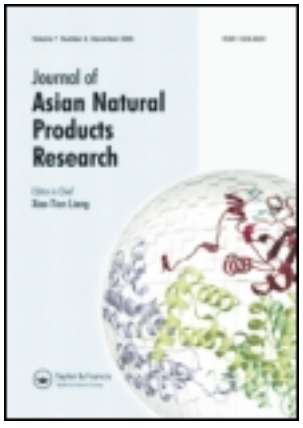

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New oleanane-type triterpenoid saponins isolated from the seeds of *Celosia argentea*

Xu Pang[a1], Hai-Xia Yan[b1], Zhen-Fang Wang[a], Miao-Xuan Fan[b], Yang Zhao[a], Xin-Tong Fu[b], Cheng-Qi Xiong[a], Jie Zhang[a], Bai-Ping Ma[a]* and Hong-Zhu Guo[b]*

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Three new oleanane-type triterpenoid saponins named celosins H (**1**), I (**2**), and J (**3**) were isolated from the seeds of *Celosia argentea* L. Their structures were characterized as 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucuronopyranosyl-polygalagenin 28-*O*-β-D-glucopyranosyl ester, 3-*O*-β-D-glucuronopyranosyl-medicagenic acid 28-*O*-β-D-xylcopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester, and 3-*O*-β-D-glucuronopyranosyl-medicagenic acid 28-*O*-α-L-arabinopyranosyl-(1 → 3)-[β-D-xylcopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester by NMR, MS, and chemical evidences, respectively. In our opinion, celosins H–J could be used as chemical markers for the quality control of *C. argentea* seeds.

Keywords: *Celosia argentea*; triterpenoid saponins; celosins H–J; NMR

1. Introduction

Celosia argentea L. (Amaranthaceae) is an annual herbaceous plant widely distributed in China, especially in southern China. The seeds of *C. argentea* (Semen Celosiae) are used as a traditional Chinese medicine, named 'Qing Xiang Zi' in Chinese, for removing liver-heat, improving eyesight, clearing wind-heat, and lowering the blood pressure [1]. The pharmacological investigation on the extract of *C. argentea* seeds showed that it had hepatoprotective effect and antitumor activity [2,3]. To our knowledge, in many places of China, the seeds of *Amaranthus tricolors*, which also belong to Amaranthaceae family, are always misused as the *C. argentea* seeds or are incorporated unlawfully as adulterants into *C. argentea* seeds due to their almost identical physical features. By RP-HPLC analyses of their constituents, we found the obvious difference between these two kinds of seeds. For the purpose of obtaining chemical markers for the quality control of *C. argentea* seeds, phytochemical investigation was carried out on the extract of the *C. argentea* seeds, and three new compounds named celosins H–J (**1**–**3**) were isolated as the main ingredients in these seeds, and their structures were characterized by NMR, MS, and chemical evidences (Figure 1). This paper mainly presented the isolation and structure elucidation of these new compounds.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula $C_{47}H_{72}O_{20}$ was determined by HR-ESI-MS ion $[M - H]^-$ at *m/z* 955.4583. By combined analyses of the ^1H NMR, ^{13}C NMR, and HSQC spectra, three anomeric proton signals at δ 4.99 (1H, d, $J = 7.8$ Hz, H-1′), 5.31 (1H, d, $J = 7.8$ Hz, H-1″), 6.31 (1H, d, $J = 7.8$ Hz, H-1‴) and

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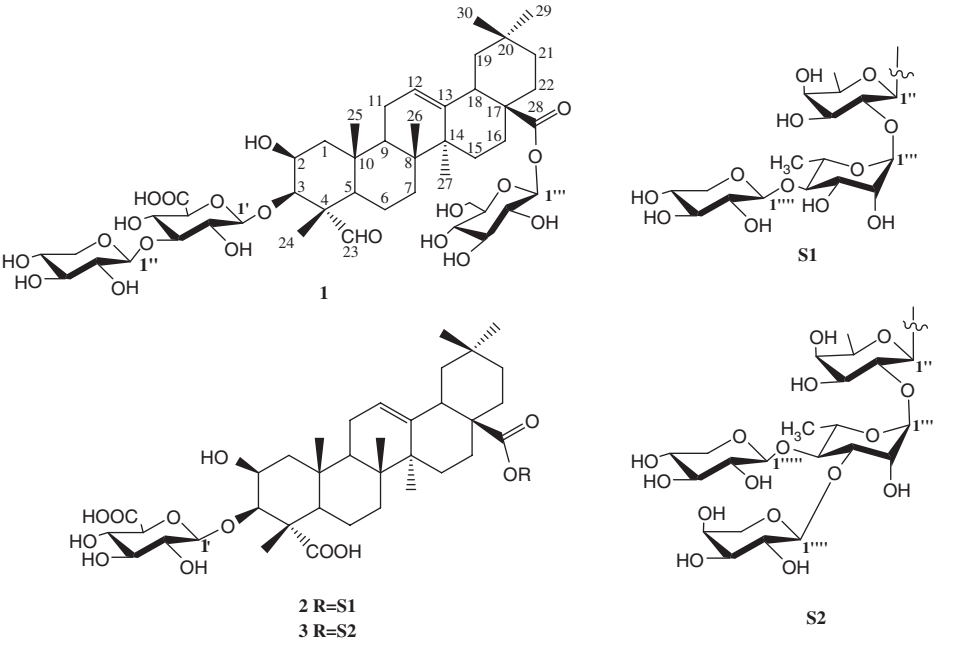

Figure 1. The structures of compounds **1**–**3**.

three corresponding anomeric carbon signals at δ 105.1 (C-1′), 106.2 (C-1″), 95.8 (C-1‴) were observed, which revealed **1** contained three sugar units. Acid hydrolysis of **1** followed by GC analysis of its derivative enabled further identification of the existence of D-glucuronic acid, D-xylose, and D-glucose. The β-configurations of D-glucuronic acid, D-xylose, and D-glucose were determined by the $J_{1,2}$ values of 7.8 Hz, 7.8 Hz, and 7.8 Hz, respectively [4,5]. Comparison of aglycone ^{13}C NMR data of **1** with those of 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl gypsogenin 28-*O*-α-L-arabinopyranosyl-(1 → 4)-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester, isolated from *Gypsophila pacifica* Kom. [5], showed that the aglycone structure of **1** was almost identical to gypsogenin, the aglycone of the reference compound, except the distinguished difference of C-1 to C-3 in A ring portion. The carbon signal at δ 44.0 (C-1, +6.1 ppm) and 69.9 (C-2, +44.9 ppm) deduced the aglycone of **1** and had a hydroxyl group at C-2. In ROESY spectrum, the correlation between H-24 at δ 1.73 and H-25 at δ 1.48 (H-25) revealed the β-configuration of C_{24}-Me. H-3 at δ 4.15 had correlation with H-2 at δ 4.74 and did not show correlation with H-24 at δ 1.73, suggesting the β-configurations of C_2-OH and C_3-OH. By combined analyses of the ^{1}H NMR, ^{13}C NMR, HSQC, ^{1}H–^{1}H COSY, HMBC, and ROESY spectra, the aglycone structure of **1** was identified as polygalagenin [6], and all their proton and carbon signals were assigned (Table 1). The ^{1}H and ^{13}C NMR data of each sugar unit were assigned starting from the identifiable anomeric protons by means of the ^{1}H–^{1}H COSY, HSQC–TOCSY, HSQC, and HMBC spectra (Table 2). The glycosylation positions and sugar sequence of **1** were confirmed due to the following long-range HMBC correlations of H-1′ of GlcA at δ 4.99 with C-3 at δ 83.3, H-1″ of Xyl at δ 5.31 with C-3′ of GlcA at δ 85.9, and H-1‴ of Glc at δ 6.31

Table 1. ^{1}H and ^{13}C NMR spectral data for aglycone moieties of **1**–**3**.

	1		**2**		**3**	
No	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	44.0	2.18 m, 1.16 m	44.5	2.27 d (13.2), 1.16 m	44.5	2.28 d (12.6), 1.28 m
2	69.9	4.74 m	70.6	4.82 m	70.6	4.85 br s
3	83.3	4.15 m	86.3	4.75 d (3.6)	86.2	4.74 d (3.0)
4	54.8	–	53.0	–	53.0	–
5	48.4	1.47 m	52.6	3.00 m, 2.07 m	52.7	3.00 m
6	20.4	1.46 m	21.3	1.78 m	21.3	1.86 m, 1.82 m
7	32.5	1.76 m, 1.24 m	33.1	1.69 m, 0.90 m	33.3	1.66 m, 0.91 m
8	40.3	–	40.5	–	40.5	–
9	48.4	1.67 m	48.7	1.75 m	48.7	1.77 m
10	36.3	–	36.9	–	40.0	–
11	23.9	2.04 m	24.0	1.98 m, 0.98 m	24.0	2.13 m, 2.00 m
12	122.8	5.39 t (3.0)	122.8	5.38 br s	122.8	5.38 br s
13	144.2	–	144.0	–	144.0	–
14	42.4	–	42.3	–	42.3	–
15	28.2	2.30 dt (3.0, 13.2),1.10 m	28.3	2.08 m, 1.39 m	28.1	2.32 m, 1.39 m
16	23.4	1.94 m, 1.00 m	23.4	2.09 m, 1.89 m	23.5	1.92 m, 0.97 m
17	47.0	–	47.0	–	47.0	–
18	42.4	3.16 dd (9.6, 4.2)	42.1	3.08 dd (10.8, 3.0)	42.2	3.11 dd (13.3, 3.0)
19	46.2	1.74 m, 1.22 m	46.2	1.72 m, 1.18 m	46.3	1.73 m, 1.18 m
20	30.8	–	30.8	–	30.8	–
21	34.0	1.08 m	34.0	1.26 m, 1.16 m	33.9	1.28 m
22	32.5	1.82 m, 1.47 m	32.4	2.05 m, 1.68 m	32.5	2.10 m, 1.76 m
23	207.0	9.73	180.7	–	180.7	–
24	11.8	1.73 s	14.2	1.99 s	14.3	2.02 s
25	16.8	1.48 s	17.0	1.55 s	17.0	1.61 s
26	17.6	1.09 s	17.4	1.12 s	17.5	1.18 s
27	26.2	1.25 s	26.1	1.23 s	26.2	1.25 s
28	176.4	–	176.6	–	176.7	–
29	33.1	0.89 s	33.1	0.82 s	33.2	0.82 s
30	23.7	0.86 s	23.8	0.87 s	23.9	0.90 s

with C-28 at δ 176.4. Consequently, the structure of **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucuronopyranosyl-polygalagenin 28-*O*-β-D-glucopyranosyl ester, named celosin H.

Compound **2** was obtained as a white amorphous powder with a molecular formula $C_{53}H_{82}O_{24}$ determined by HR-ESI-MS ion $[M - H]^-$ at *m/z* 1101.5189. The ^{1}H NMR spectrum showed four anomeric proton signals at δ 5.24 (1H, d, $J = 7.8$ Hz, H-1′), 6.00 (1H, d, $J = 8.4$ Hz, H-1″), 6.40 (1H, br s, H-1‴), and 5.02 (1H, d, $J = 7.2$ Hz, H-1⁗), and four corresponding anomeric carbon signals at δ 105.9 (C-1′), 94.8 (C-1″), 101.4 (C-1‴), and 107.7 (C-1⁗) were observed by the use of HSQC spectrum, which revealed that **2** contained four sugar units. Acid hydrolysis of **2** followed by GC analysis of its derivative identified the sugar units as D-glucuronic acid, D-fucose, L-rhamnose, and D-xylose. The β-configurations of D-glucuronic acid, D-fucose, and D-xylose were determined by the $J_{1,2}$ values of 7.8 Hz, 8.4 Hz, and 7.2 Hz, respectively, and the α-configuration of L-rhamnose was determined by the carbon signals at δ 68.3 (C-5‴) [4,5]. Compound **2** had the almost same aglycone structure as 3-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-β-D-galactopyranosyl-(1 → 6)-β-D-gluco-

Table 2. ^{1}H and ^{13}C NMR spectral data for sugar moieties of **1**–**3**.

	1			**2**			**3**	
No	δ_C	δ_H (J in Hz)	No	δ_C	δ_H (J in Hz)	No	δ_C	δ_H (J in Hz)
GlcA-1′	105.1	4.99 d (7.8)	GlcA-1′	105.9	5.24 d (7.8)	GlcA-1′	105.9	5.26 d (7.8)
2′	73.9	3.98 m	2′	75.0	4.00 t (8.4)	2′	75.0	4.00 overlap
3′	85.9	4.30 m	3′	77.8	4.21 m	3′	77.8	4.22 m
4′	71.1	4.46 m	4′	73.3	4.52 m	4′	73.3	4.51 m
5′	77.3	4.64 d (9.6)	5′	77.5	4.61 m	5′	77.5	4.63 m
6′	172.2	–	6′	172.5	–	6′	172.8	–
Xyl-1″	106.2	5.31 d (7.8)	Fuc-1″	94.8	6.00 d (8.4)	Fuc-1″	94.8	6.03 d (8.4)
2″	75.4	4.00 m	2″	74.1	4.64 m	2″	74.3	4.60 m
3″	78.1	4.14 m	3″	76.3	4.15 dd (6.0, 3.0)	3″	76.2	4.12 m
4″	71.0	4.32 m	4″	73.2	3.93 br d (3.0)	4″	73.2	3.92 br d (3.0)
5″	67.4	4.34 m, 3.68 m	5″	72.4	3.87 q (6.0)	5″	72.4	3.84 q (6.6)
			6″	16.9	1.45 d (6.0)	6″	16.9	1.46 d (6.6)
Glc-1‴	95.8	6.31 d (7.8)	Rha-1‴	101.4	6.40 s	Rha-1‴	101.4	6.32 brs
2‴	74.2	4.20 m	2‴	71.8	4.82 brs	2‴	71.6	5.05 brs
3‴	79.0	4.25 m	3‴	72.5	4.69 dd (9.0, 3.0)	3‴	82.4	4.74 dd (9.0, 3.0)
4‴	71.1	4.35 m	4‴	85.5	4.31 t (9.6)	4‴	78.9	4.51 m
5‴	79.4	4.03 m	5‴	68.3	4.46 m	5‴	68.5	4.45 m
6‴	62.2	4.44 m, 4.38 m	6‴	18.6	1.68 d (6.0)	6‴	18.9	1.67 d (5.4)
			Xyl-1⁗	107.7	5.02 d (7.2)	Ara-1⁗	106.3	5.20 d (7.8)
			2⁗	76.6	4.05 m	2⁗	72.9	4.45 m
			3⁗	78.9	4.04 m	3⁗	74.6	4.08 dd (9.0, 3.0)
			4⁗	71.0	4.19 m	4⁗	69.9	4.24 m
			5⁗	67.6	4.24 m, 3.50 t (10.4)	5⁗	67.6	4.20 m, 3.72 d (12.0)
						Xyl-1⁗′	105.1	5.35 d (7.8)
						2⁗′	75.9	3.88 t (8.4)
						3⁗′	79.7	3.98 m
						4⁗′	71.3	4.13 m
						5⁗′	67.1	4.12 m, 3.22 t (10.2)

pyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl ester [5] except the difference at C-1 and C-3 in A ring by comparing their ^{13}C NMR data. Likewise, the higher carbon signal at δ 44.5 (C-1, + 6.0 ppm) and 70.6 (C-2, + 46.0 ppm) deduced the aglycone of **2** which also had a hydroxyl group at C-2. The correlation between H-24 at δ 1.99 and H-25 at δ 1.55 in ROESY spectrum revealed the β-configuration of C_{24}-Me. H-3 at δ 4.75 had correlation with H-2 at δ 4.82 and did not show a correlation with H-24 at δ 1.99, suggesting the β-configurations of C_2-OH and C_3-OH. By combined use of the ^1H NMR, ^{13}C NMR, HSQC, $^1H-^1H$ COSY, HMBC, and ROESY spectra, the aglycone structure of **2** was elucidated as medicagenic acid [7], and all the proton and carbon signals were assigned (Table 1). Subsequently, the ^1H and ^{13}C NMR data of the sugar moieties were also assigned by means of the $^1H-^1H$ COSY, TOCSY, HSQC, and HMBC spectra (Table 2). The HMBC correlations of H-1′ of GlcA at δ 5.24 with C-3 at δ 86.3, H-1′ of Fuc at δ 6.00 with C-28 at δ 176.6, H-1‴ of Rha at δ 6.40 with C-2″ of Fuc at 74.1, and H-1⁗ of Xyl at δ 5.02 with C-4‴ of Rha at δ 85.5 revealed the glycosylation positions and sugar sequence of **2**. According to the above mentioned information, the structure of **2** was determined as 3-*O*-β-D-glucuronopyranosyl-medicagenic acid 28-*O*-β-D-xylcopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester, named celosin I.

Compound **3** was obtained as a white amorphous powder with a molecular formula $C_{58}H_{90}O_{28}$ determined by HR-ESI-MS ion $[M - H]^-$ at *m/z* 1233.5614. The ^1H NMR spectrum showed five anomeric proton signals at δ 5.26 (1H, d, $J = 7.8$ Hz, H-1′), 6.03 (1H, d, $J = 8.4$ Hz, H-1″), 6.32 (1H, brs, H-1‴), 5.20 (1H, d, $J = 7.8$ Hz, H-1⁗), and 5.35 (1H, d, $J = 7.8$ Hz, H-1⁗′), and five corresponding anomeric carbon signals at δ 105.9 (C-1′), 94.8 (C-1″), 101.4 (C-1‴) 106.3 (C-1‴), and 105.1 (C-1⁗′) were observed by means of HSQC spectrum, which revealed that **3** contained five sugar units. Acid hydrolysis of **3** followed by GC analysis of its derivative identified the sugar units as D-glucuronic acid, D-fucose, L-rhamnose, D-xylose, and L-arabinose. The β-configurations of D-glucuronic acid, D-fucose, D-xylose and the α-configuration of L-arabinose were determined by the $J_{1,2}$ values of 7.8 Hz, 8.4 Hz, 7.8 Hz, and 7.8 Hz, respectively. And the α-configuration of L-rhamnose was identified by the carbon signal at δ 68.5 (C-5‴) [4,5]. The molecular weight of **3** was 132 Da less than that of **2**. Comparison of ^{13}C NMR spectral data of **3** with those of **2** revealed that they had the same aglycone, medicagenic acid, and their structural difference could be deduced to be only in one more terminal arabinose. By combined use of the ^1H NMR, ^{13}C NMR, HSQC, $^1H-^1H$ COSY, TOSCY, HMBC, and ROESY spectra, the structures of aglycone and sugar units were identified, and the proton and carbon signals were fully assigned (Tables 1 and 2). The HMBC spectrum showed the cross peaks between H-1′ of GlcA at δ 5.26 with C-3 at δ 86.2, and H-1″ of Fuc at δ 6.03 with C-28 at δ 176.7, H-1‴ of Rha at δ 6.32 with C-2″ of Fuc at δ 74.3, H-1⁗ of Ara at δ 5.20 with C-3‴ of Rha of 82.4, and H-1⁗′ of Xyl at δ 5.35 with C-4‴ of Rha at δ 78.9, which revealed the glycosylation positions and sugar sequence of **3**. Finally, the structure of **3** was elucidated as 3-*O*-β-D-glucuronopyranosyl-medicagenic acid 28-*O*-α-L-arabinopyranosyl-(1 → 3)-[β-D-xylcopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester, named celosin J.

3. Experimental

3.1 *General experimental procedures*

Optical rotations were measured with Perkin-Elmer 343 Polarimeter (Perkin-Elmer, Waltham, MA, USA). The

measurements of IR were carried out using Bruker Vertex 70 (Bruker Corporation, Karlsruhe, Germany). Gas chromatographic analyses were carried out using an Agilent 6890N Network GC system equipped with a FID detector and a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Santa Clara, CA, USA). The HR-ESI-MS and ESI-MS were recorded on Synapt Q/TOF MS (Waters Corporation, Milford, MA, USA). The NMR spectra were recorded with Varian SYS-600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR) (Palo Alto, CA, USA). TLC was carried out on silica gel GF254 plates (Qingdao Marine Chemical, Qingdao, China). Macroporous resin SP825 (Mitsubishi Chemical, Tokyo, Japan) and silica-gel H (Qingdao Marine Chemical, Qingdao, China) were used for column chromatography. HPLC analyses were carried out using Agilent 1100 system equipped with an Alltech 2000ES evaporative light scattering detector (temp: 110°C, gas: 2.4 l/min) (Alltech Corporation, Chicago, IL, USA) and Techmate C18 column (4.6 mm × 250 mm, ODS, 5 μm) (Techmate Co. Ltd, Beijing, China). Semi-preparative HPLC separations were carried out using the NP7000 module (Hanbon Co. Ltd, Huaian, China) equipped with a Shodex RID 102 detector (Showa Denko Group, Tokyo, Japan) and a Vensiul C18 column (8.0 mm × 250 mm, ODS, 5 μm) (Bonna-Agela, Tianjin, China).

3.2 Plant material

The seeds of *C. argentea* L. and *A. tricolors* L. were collected from Bozhou, Anhui Province, China in September 2012. The seeds were identified by Prof. Zhi-Guo Tao (Department of Traditional Chinese Medicine, Beijing Institute for Drug Control), and a voucher specimen of *C. argentea* seeds (No. BIDC20120916) was deposited in Herbarium of Department of Traditional Chinese Medicine, Beijing Institute for Drug Control.

3.3 Extraction and isolation

The air-dried *C. argentea* seeds (5 kg) were crushed and extracted with 50% aqueous EtOH at 120°C for three times, which was carried out for 1 h each time. The combined extract was filtered, concentrated under reduced pressure. The extract was then subjected to macroporous resin SP825 column chromatography with a gradient mixture of EtOH–H_2O (0:100 → 15:85 → 60:40 → 95:5) to provide four fractions: A (H_2O elution), B (15% EtOH elution), C (60% EtOH elution), and D (95% EtOH elution). Fr. C (20 g) was separated over silica-gel column (8 cm × 20 cm) with $CHCl_3$–MeOH (5:1 → 3:1) to obtain four important combined fractions: Fr. 60–81, Fr. 82–92, Fr. 116–124, and Fr. 125–144. Among them, Fr. 60–81 was further separated on silica-gel column (8 cm × 20 cm) with $CHCl_3$–MeOH (5:1), and the tubes 28–75 was purified by semi-preparative RP-HPLC with CH_3CN–0.01%TFA (Trifluoroacetic acid) in H_2O (30:70, flow rate 4.5 ml/min) to give **1** (256 mg, t_R 31.7 min). Fr. 82–92 was purified by semi-preparative RP-HPLC with CH_3CN–0.1%TFA in H_2O (33:67, flow rate 4.5 ml/min) to obtain **2** (432 mg, t_R 33.8 min). Fr. 116–124 was separated on ODS silica gel column with MeOH–H_2O, and then was purified by semi-preparative RP-HPLC with CH_3CN–0.1%TFA in H_2O (33:67, flow rate 4.5 ml/min) to give **3** (46 mg, t_R 28.2 min). An additional amount of **3** (47 mg, t_R 29.7 min) was obtained from Fr. 125–144 by semi-preparative RP-HPLC with CH_3CN–0.1%TFA in H_2O (33:67, flow rate 4.5 ml/min).

3.3.1 Compound *1*

White amorphous powder, $C_{47}H_{72}O_{20}$, $[\alpha]_D^{20} + 28.8$ ($c = 0.75$, CH_3OH). IR (KBr, cm^{-1}): ν_{max} 3431, 2928, 1726, 1640, 1459, 1367, 1071, 571. ^{1}H NMR

(pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) spectral data, see Tables 1 and 2. HR-ESI-MS (negative): *m/z* 955.4583 $[M - H]^-$ (calcd for $C_{47}H_{71}O_{20}$, 955.4539).

3.3.2 *Compound* **2**

White amorphous powder, $C_{53}H_{82}O_{24}$, $[\alpha]_D^{20} + 8.9$ ($c = 0.80$, CH_3OH). IR (KBr, cm^{-1}): ν_{max} 3426, 2932, 1716, 1455, 1385, 1068, 623. For ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) spectral data, see Tables 1 and 2. HR-ESI-MS (negative): *m/z* 1101.5189 $[M - H]^-$ (calcd for $C_{53}H_{81}O_{24}$, 1101.5118).

3.3.3 *Compound* **3**

White amorphous powder, $C_{58}H_{90}O_{28}$, $[\alpha]_D^{20} + 1.3$ ($c = 0.80$, CH_3OH). IR (KBr, cm^{-1}): ν_{max} 3423, 2934, 1717, 1455, 1386, 1059, 623. For ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) spectral data, see Tables 1 and 2. HR-ESI-MS (negative): *m/z* 1233.5614 $[M - H]^-$ (calcd for $C_{58}H_{89}O_{28}$, 1233.5540).

3.4 *Acid hydrolysis of 1–3 and GC analysis*

Compound **1** (5.0 mg) was treated with 2 N aqueous CF_3COOH (5 ml) at 95°C for 5 h. After extraction with CH_2Cl_2 for three times (5 ml × 3), the aqueous layer was repeatedly evaporated to dryness with EtOH until neutral. After that, the glucose, xylose, and glucuronic acid in monosaccharide mixture were detected by TLC over silica gel ($CHCl_3$–MeOH–H_2O, 8:5:1) by comparison with authentic samples [8]. Likewise, the fucose, rhamnose, xylose, and glucose acid were detected from compound **2**, and the fucose, rhamnose, xylose, arabinose, and glucuronic acid were detected from compound **3**. Furthermore, each residue of sugars was dissolved in anhydrous pyridine (2 ml) and was added L-cysteine methyl ester hydrochloride (5 mg). Then, the mixture was stirred at 60°C for 1 h. After that, hexamethyldisilazane–trimethylchlorosilane (2:1) (3 ml) was added, and kept at 60 °C for 0.5 h. Finally, the supernatant was analyzed by GC: Agilent 6890N Network GC system; FID detector; HP-5 capillary column (30 m × 0.25 mm × 0.25 μm); column temperature: 180°C keep 10 min, 180–250°C, programmed increase, 15°C/min, and 250°C keep 10 min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 270°C; injection volume: 1.0 μl, split ratio: 1/50. Consequently, the D-configurations of glucose, fucose, xylose, and glucuronic acid and the L-configurations of rhamnose and arabinose were confirmed by comparing the retention times with those of standard samples, respectively. The retention times (t_R, min) of monosaccharide derivatives were as follows: D-glucose (24.469, 25.924 min), D-fucose (22.175, 23.494 min), D-xylose (20.621, 22.115 min), D-glucuronic acid (25.222, 26.869 min), L-rhamnose (21.932, 23.325 min), and L-arabinose (20.594, 22.081 min) [9].

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Note

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