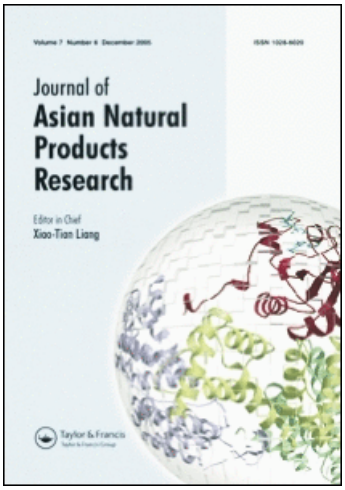

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Six new cycloartane triterpene glycosides from *Actaea asiatica*

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Six new cycloartane triterpene glycosides, (3′,12β)-*O*-diacetyl-cimigenol-3-*O*-β-D-xylopyranoside (**1**), (4′,25)-*O*-diacetyl-cimigenol-3-*O*-β-D-xylopyranoside (**2**), 2′-*O*-acetyl-25-*O*-methyl-cimigenol-3-*O*-β-D-xylopyranoside (**3**), 2′-*O*-acetyl-25-*O*-ethyl-cimigenol-3-*O*-β-D-xylopyranoside (**4**), 3′-*O*-acetyl-cimicifugoside (**5**), and 4′-*O*-acetyl-23-*epi*-26-deoxycimifugoside (**6**), were isolated from the rhizomes of *Actaea asiatica*. Their structures were elucidated on the basis of chemical methods and spectroscopic analysis. Compounds **1**, **2**, **4**–**6** exhibited positive cytotoxic activities.

Keywords: Ranunculaceae; *Actaea asiatica*; cycloartane triterpene glycosides; cytotoxic activities

1. Introduction

Actaea asiatica Hara (Ranunculaceae) is widely distributed in the southwest and northwest of China. As a Chinese folk medicine, the rhizome of *A. asiatica* is used to treat headache, sore throat, measles, pertussis, and prolapse of uterus [1]. Many 9,19-cycloartane triterpene glycosides, as well as their cytotoxic activities, have been reported in previous studies [2–7]. In this paper, six new cycloartane triterpene glycosides (**1**–**6**) were isolated from the rhizomes of *A. asiatica* and their structures were determined by chemical methods and spectroscopic analysis, including 2D NMR spectra. Their cytotoxic activities of the isolated compounds were also researched.

2. Results and discussion

The ethyl acetate extract of the rhizomes of *A. asiatica* was separated by repeated silica gel column chromatography, Toyopearl HW-40C, and preparative HPLC to give compounds **1**–**6**.

Compound **1** was isolated as an amorphous powder, and its high-resolution positive FTMS revealed a quasi-molecular ion at *m/z* 743.3902 $[M+Na]^+$, indicating a molecular formula of $C_{39}H_{60}O_{12}$. Its IR spectrum showed absorption bands at 3442, 1735, and 1734 cm^{-1}, indicating the presence of hydroxyl and carboxyl groups. The ^1H NMR spectrum of **1** revealed the characteristic cyclopropane methylene signals at δ 0.33 and 0.58 (each 1H, d, $J = 3.6$ Hz), two acetyl methyls at δ 2.13, 1.98 (each 3H, s), a secondary methyl at δ 0.95 (3H, d, $J = 6.0$ Hz), six methyl singlets at δ 0.99, 1.22, 1.26, 1.33, 1.50, 1.51 (each 3H, s), and an anomeric proton at δ 4.85 (1H, d, $J = 7.0$ Hz). The ^{13}C NMR spectral data of **1** (Table 2) showed a methylene carbon of cyclopropane ring at

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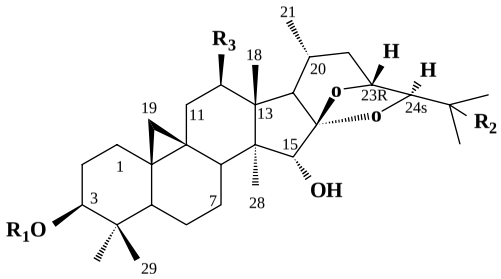
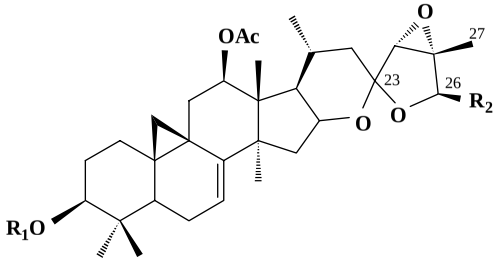


	R_1	R_2	R_3
1	3′-acetyl-β/D-xylosyl	OH	OAc
2	4′-acetyl-β/D-xylosyl	OAc	H
3	2′-acetyl-β/D-xylosyl	OCH_3	H
4	2′-acetyl-β/D-xylosyl	OCH_2CH_3	H



	R_1	R_2	C-23
5	3′-acetyl-β/D-xylosyl	OH	R
6	4′-acetyl-β/D-xylosyl	H	S

Figure 1. The structures of compounds **1**–**6**.

Table 1. ^{1}H NMR spectral data of compounds **1**–**6**.

Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
1	1.10, 1.52	1.08, 2.27	Overlapped	Overlapped	1.18, 1.62 m	1.15, 1.52
2	1.90, 2.28	1.98, 2.35	1.70, 1.90	1.70, 1.92	1.90, 2.23 m	1.85, 2.15
3	3.48 dd (4.0, 11.0)	3.49 dd (4.0, 11.0)	3.18 dd (4.5, 11.0)	3.19 dd (4.2, 11.0)	3.42 dd (3.8, 11.0)	3.40 dd (4.0, 11.0)
5	1.28	1.32	1.31	1.30	1.18	1.15
6	0.74 q (11.0), 1.50	0.80 q (11.0), 1.60	0.80, 1.59	0.77, 1.57	1.58, 1.82	1.48, 1.75
7	1.06, 2.07	1.13, 2.16	Overlapped	Overlapped	5.07 br d (6.1)	5.11 d (6.1)
8	1.65	1.70	1.60	1.62	–	–
11	1.15, 2.93	1.03, 2.04	Overlapped	Overlapped	1.29, 2.94 dd (15.0, 9.0)	1.23, 2.92 dd (15.0, 8.5)
12	5.26 br d (8.6)	1.53, 1.62	1.64	1.60	5.22 d (8.2)	5.24 d (8.3)
15	4.40 s	4.27 s	3.88 s	3.88 br d (7.0)	2.17, 1.96	2.12, 1.40
16	–	–	–	–	4.72 q (7.2)	4.32
17	1.62 d (12.5)	1.47 d (11.0)	1.40 d (11.1)	1.38 d (11.0)	1.82	1.81
18	1.26 s	1.16 s	1.07	1.07	1.42 s	1.50 s
19	0.33 d (3.6), 0.58 d (3.6)	0.27 d (4.2), 0.53 d (4.2)	0.36 d (4.0), 0.62 d (4.0)	0.36 d (4.2), 0.63 d (4.2)	0.62 d (4.0), 1.13 d (4.0)	0.52 d (3.6), 1.02 d (3.6)
20	1.65	1.65	1.65	1.67	1.83	2.25
21	0.95 d (6.0)	0.87 d (6.0)	0.88 d (6.4)	0.88 d (6.4)	0.98 d (6.4)	1.03 d (6.3)
22	1.05, 2.15	1.02, 2.34	0.98, 2.32	0.98, 2.31	1.72, 2.25	1.48, 1.60
23	4.77 d (9.0)	4.61 d (8.8)	4.40 d (9.0)	4.44 d (9.0)	–	–
24	3.77 br s	4.13 br s	3.45 br s	3.45 br s	3.94 s	3.68 s
26	1.51 s	1.70 s	1.07	1.07	5.76 s	4.07 d (10.1), 3.63 d (10.1)
27	1.50 s	1.73 s	1.16	1.16	1.81 s	1.48 s
28	1.22 s	1.21 s	0.94	0.94	1.02 s	1.06 s
29	1.33 s	1.33 s	0.94	0.94	1.31 s	1.31 s
30	0.99 s	1.06 s	0.80	0.80	0.98 s	0.98 s
1′	4.85 d (7.0)	4.88 d (7.0)	4.57 d (5.7)	4.59 d (5.7)	4.84 d (7.5)	4.84 d (7.5)
2′	4.07 t (7.5)	4.08 t (7.7)	4.79 t (7.3)	4.80 t (7.3)	4.06 t (7.5)	4.06 t (8.1)
3′	5.75 t (9.1)	4.28	3.61	3.60	5.74 t (9.1)	4.27 t (8.3)
4′	4.20	5.41	3.69	3.68	4.22	5.40
5′	3.72 t (12.0), 4.30	3.62 dd (7.6, 12.0), 4.32 m	3.45, dd (8.5, 12.5), 4.08 dd (4.0, 11.8)	3.45, 4.09 dd (3.8, 11.8)	3.73 t (12.0), 4.33 dd (4.2, 8.5)	3.60 t (10.3), 4.34

[a] C_5D_5N.
[b] $CDCl_3$.

δ 31.3 (C-19), four oxygenated methine carbons at δ 88.9, 79.6, 71.7, 90.3, and two quaternary carbons at δ 112.4 and 71.6. All the above observations suggested that **1** was a high-oxygenated 9,19-cyclolanostane triterpene glycoside with two acetyl groups, and its ^{13}C NMR spectral data were similar to those of 12β-*O*-acetyl-cimigenol-3-*O*-β-D-xylopyranoside [8], except for an additional acetyl group. In the ^{1}H–^{1}H COSY spectrum, a spin–spin coupling system of H-2′ (δ 4.07)/H-3′ (δ 5.75)/H-4′ (δ 4.20) was observed. Furthermore, the proton at δ 5.75 (H-3′) showed the HMBC correlation with the carbonyl carbon of the acetyl group at δ 171.2 and the proton at δ 4.84 (H-1′) correlated with the carbon at δ 89.1 (C-3). Thus, the acetyl group was located at C-3′ and the xylose moiety was connected with the aglycon at position C-3. In addition, the HMBC correlation between H-12 (δ 5.26) and the carbonyl carbon (δ 170.9, Ac) indicated that another acetyl group was attached to C-12.

In NOESY spectrum, the proton signal at δ 3.48 (H-3) correlated with H-5, H-12 with H_3-28, and H-15 with H-18. Thus, relative configurations of 3-sugar, acetoxy, and hydroxyl groups were elucidated as 3β, 12β, and 15α, respectively. The absolute configurations of C-23 and C-24 were assigned as *R* and *S* by comparing the coupling constants of the H-23 ($J = 9.0$) and H-24 of **1** with those of known 9,19-cyclolanostane triterpene glycosides [2,9]. The sugar was identified as xylose by acid hydrolysis and TLC analysis with an authentic sample, and the coupling constants of H-1′ at δ 4.85 (1H, d, $J = 7.0$ Hz) indicated that it has β configuration. Thus, the structure of **1** was elucidated as (3′, 12β)-*O*-diacetyl-cimigenol-3-*O*-β-D-xylopyranoside (Figure 1).

Compound **2** has a molecular formula $C_{39}H_{60}O_{11}$ from HR-FTMS at *m/z* 727.4022 $[M + Na]^+$. Its IR spectrum showed absorptions of hydroxyl (3431 cm^{-1}) and carbonyl (1738 cm^{-1}) groups. Compound **2** was also a 9,19-cyclolanostane triterpene glycoside, its ^{1}H and ^{13}C NMR spectral data (Table 2) were similar to those of reported compound (25-*O*-acetyl-cimigenol-3-*O*-β-D-xylopyranoside) [9], except for the presence of an additional acetyl group at δ_H 1.98 (3H, s), and δ_C 170.7 (s) and 21.4 (q). The additional acetyl group could be located at C-4′ which was confirmed by the proton signal of H-4′ downfield shifted from δ 4.20 to 5.41, C-3 shifted upfield from δ 79.0 to 75.4, and C-5 shifted upfield from δ 67.6 to 63.6. Its sugar was identified as xylose by acid hydrolysis and TLC analysis with an authentic sample. In the HMBC spectrum, the proton signal at δ 5.41 (H-4′) correlated with the carbon signals at δ 75.4 (C-3′), 63.6 (C-5′), and 170.7 (acetyl group), the signal at δ 4.88 (H-1′) correlated with the signal at δ 89.0 (C-3). Thus, the acetyl group was located at C-4′, and the xylose moiety was connected with the aglycon at position C-3. Therefore, the structure of **2** was elucidated as (4′,25)-*O*-diacetyl-cimigenol-3-*O*-β-D-xylopyranoside (Figure 1).

The molecular formula $C_{38}H_{60}O_{10}$ for **3** was established by the high-resolution positive TOF-ESI-MS. The ^{1}H NMR spectral data of **3** (Table 1) showed the existence of cyclopropane methylene, seven methyls, one acetyl, one methoxy, and an anomeric proton. The ^{13}C NMR spectral data of **3** (Table 2) were similar to those of **2**, except for the presence of methoxyl and the difference from the location of the acetyl group. From the HMBC spectrum, the proton signal at δ 4.79 (H-2′) correlated with the carbon signals at δ 102.2 (C-1′), 73.4 (C-3′), 170.5 (acetyl group), and the methoxyl proton at δ 3.22 correlated with the carbon at δ 75.8 (C-25). Thus, the structure of **3** was elucidated as 2′-*O*-acetyl-25-methoxyl-cimigenol-3-*O*-β-D-xylopyranoside.

The molecular formula $C_{39}H_{62}O_{10}$ for **4** was determined from the HR-FTMS, showing one more methylene than **3**. The ^{1}H and ^{13}C NMR spectral data of **4** (Tables 1 and 2) were similar to those of **3**,

Table 2. ^{13}C NMR spectral data of compounds **1**–**6**.

Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
1	32.8	32.8	31.9	31.9	30.6	30.6
2	30.4	30.5	29.0	29.0	29.9	29.8
3	88.9	89.0	89.6	89.7	88.5	88.3
4	41.6	41.8	40.7	40.7	40.8	40.8
5	47.5	48.0	47.3	47.2	42.8	42.8
6	21.1	21.5	20.7	20.8	22.1	22.2
7	26.4	26.9	26.0	26.1	114.5	114.5
8	47.5	49.1	48.0	48.1	148.1	148.1
9	20.5	20.5	19.8	19.9	21.3	21.6
10	27.2	27.1	26.2	26.2	28.7	28.6
11	37.9	26.8	26.1	26.0	37.1	37.0
12	77.7	34.5	33.6	33.6	77.2	77.2
13	48.8	42.3	41.8	41.8	48.5	48.5
14	46.6	47.6	46.9	46.9	51.0	50.9
15	79.6	80.6	79.6	79.6	42.9	43.4
16	112.4	112.9	111.4	111.4	73.5	74.9
17	59.6	59.8	58.9	58.9	57.2	57.0
18	13.1	20.0	19.4	19.1	15.3	15.2
19	31.3	31.4	30.8	30.8	29.2	29.2
20	24.5	24.4	23.6	23.6	27.1	23.5
21	22.1	20.0	19.0	19.4	22.0	22.0
22	38.9	38.4	37.8	37.8	37.8	37.9
23	71.7	72.1	71.6	71.7	106.3	106.4
24	90.3	87.2	87.8	87.9	63.8	62.7
25	71.6	83.6	75.8	75.5	66.1	62.9
26	25.9	22.8	20.7	20.1	98.8	68.6
27	27.6	23.8	21.7	22.5	13.6	14.6
28	12.3	12.3	11.0	11.0	27.1	27.3
29	26.0	26.1	25.3	25.3	26.0	26.1
30	15.7	15.8	14.9	14.9	14.6	14.7
1′	107.6	107.8	102.2	102.1	107.6	107.6
2′	73.5	76.1	72.9	72.8	73.5	76.1
3′	79.7	75.4	73.4	73.3	79.7	75.3
4′	69.6	73.6	70.0	70.0	69.6	73.5
5′	67.2	63.6	63.6	63.5	67.2	63.6

[a] C_5D_5N.
[b] $CDCl_3$.

except for the C-25 substituted group. Namely, the methoxyl in **3** was substituted by the ethoxyl at δ_H 1.13 (3H, t, $J = 6.9$ Hz), 3.38 (2H, q, $J = 6.9$ Hz), and at δ_C 16.2 (t) and 56.7 (q) in **4**. Moreover, the HMBC correlation from the ethoxy proton at δ 3.38 to C-25 (δ 75.5) indicated that the ethoxy group was located at C-25. Therefore, the structure of **4** was elucidated as 2′-*O*-acetyl-25-*O*-ethyl-cimigenol-3-*O*-β-D-xylopyranoside (Figure 1).

Compound **5** was isolated as an amorphous powder. Its HR-FTMS showed a quasi-molecular ion peak at *m*/*z* 739.3635 $[M+Na]^+$ indicating the molecular formula $C_{39}H_{56}O_{12}$. The ^{1}H NMR spectrum of **5** revealed the cyclopropane methylene signals at δ 0.62, 1.13 (each 1H, d, $J = 4.0$ Hz), five tertiary methyls at δ 0.98, 1.02, 1.31, 1.42, 1.81 (each 3H, s), a secondary methyl at δ 0.98 (d, $J = 5.7$ Hz), two acetyl methyls at δ 2.00, 2.18, one anomeric proton at δ 4.84 (d, $J = 7.5$ Hz), and a vinyl proton at δ 5.07 (br d, $J = 6.1$ Hz). The ^{13}C NMR spectral data of **5** (Table 2) showed five oxygenated

carbons assignable to the xylose moiety at δ 107.6 (C-1′), 73.5 (C-2′), 79.7 (C-3′), 69.6 (C-4′), 67.2 (C-5′), a double bond (δ 114.5, 148.1), five oxygenated methine carbons at δ 88.5 (C-3), 77.2 (C-12), 73.5 (C-16), 63.8 (C-24) and 98.8 (C-26), and two oxygenated quaternary carbons at δ 106.3 (C-23), 66.1 (C-25). The above spectral data showed a very close similarity to those of cimicifugoside [10], suggesting that compound **5** was also a 7-ene-9,19-cyclolanostane triterpene glycoside, and the side chain has 16, 23:23, 26:24, 25-triepoxy structure.

In the HMBC spectrum, the proton at δ 0.98 (H_3-21) correlated with carbons at δ 57.2 (C-17), 27.1 (C-20), and 37.8 (C-22), the proton at δ 2.25 (H-22a) with the carbons at δ 57.2 (C-17) and 106.3 (C-23), the proton at δ 5.76 (H_3-26) with the carbons at δ 63.8 (C-24), 66.1 (C-25), and 13.6 (C-27), and the proton at δ 1.81 (H-27) with the carbons at δ 106.3 (C-23) and 63.8 (C-24). Furthermore, the HR-FTMS showed that there are 11 unsaturated degrees in **5**. To accommodate the HMBC correlations and unsaturated degrees, 16, 23:23, 26:24, 25-triepoxy rings of the side chain must exist. The sugar and two acetyl groups were elucidated by the same method as described above. Therefore, compound **5** was established as 3′-*O*-acetylcimicifugoside.

Compound **6** has the molecular formula $C_{39}H_{56}O_{11}$ assigned by positive HR-FAB-MS. The ^{1}H and ^{13}C NMR spectral data of **6** (Tables 1 and 2) were similar to those of **5**, except for a significant difference in the resonance of C-24, C-26, C-27, and the sugar moiety. According to the literature [11], when the configuration of C-23/C-26 epoxide is α-oriented, H-20 and C-20 were observed at δ 1.80 and 26.0; on the contrary, H-20 and C-20 were observed at δ 2.25 and 23.5, respectively. Because the radius and negative substitute effects of oxygen are larger than those of carbon, changes of these chemical shifts can be explained as being due to the γ-effect of axial oxygen between C-23 and C-26 in **6** instead of an equatorial direction in **5**. Thus, the configuration of C-23/C-26 epoxide is β-oriented, different from that of **5**. The sugar moiety of **6** was identified as 4′-*O*-acetyl-β-D-xylose, as described for **2**. Therefore, compound **6** was established as 4′-*O*-acetyl-23-*epi*-26-deoxycimicifugoside.

Compounds **1**–**6** were assayed for cytotoxicity using reported procedure [12]. The inhibition effects of isolated compounds were shown in Table 3. Compounds **1**, **2**, **4**–**6** exhibited positive cytotoxic activities against these tumor cells *in vitro*.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured with a MC 241 digital polarimeter (Perkin-Elmer). The IR spectra were recorded on a NICOLET 380 FT-IR spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA). NMR spectra were run on a Bruker AVANCE 300 instrument (^{1}H NMR 300 MHz and ^{13}C NMR 75 MHz), both with tetramethylsilane as the internal standard. MS data were obtained on an IonSpec 4.7 Tesla FTMS instrument. HPLC was performed on JASCO Gulliver Series with PU-2089 (pump), RI-2031, and UV-2075 (detector). Preparative HPLC column was used as below: ODS (YMC-Pack ODS-A and SH-343-5). Column chromatography was performed on silica gel (Qingdao Haiyang Chemical Co., Ltd, Qingdao, China) and Toyopearl HW-40 (TOSOH, Tokyo, Japan). Flash chromatography was carried on a column (C18 HS 40M 1621-1, Biotage, Inc., Charlottesville, VA, USA).

3.2 Plant material

The rhizomes of *A. asiatica* were collected in August 2004 from Hefeng, Hubei Province, and were identified by Prof.

Table 3. Inhibition activities of compounds **1**–**6** on Hela and L929 cell growth.

	Inhibition (%)			
	Hela		L929	
Compound	30 μg/ml	10 μg/ml	30 μg/ml	10 μg/ml
1	7.18	3.59	28.26	16.24
2	13.70	8.35	26.12	14.78
3	2.63	−2.15	24.03	13.54
4	31.54	22.50	13.93	6.96
5	17.99	7.49	27.19	23.34
6	14.42	4.13	46.97	23.05

Ding-Rong Wan (School of Life Sciences, South-Central University for Nationalities). A voucher specimen (D20040901) has been deposited at the School of Pharmacy, Tianjin Medical University, China.

3.3 Extraction and isolation

The rhizomes of *A. asiatica* (2.6 kg) were refluxed three times with 95% EtOH (5000 ml each) for 5 h. The extract was concentrated *in vacuo* to give a residue (600 g), which was suspended in water, and then partitioned with petroleum ether (PE), EtOAc, and *n*-BuOH, successively.

The EtOAc extract (220 g) was chromatographed on a silica gel column, eluted with solvents of increasing polarity (PE–EtOAc (3:1, 1:1, 1:3), EtOAc, EtOAc–MeOH (19:1, 10:1, 5:1)) to give 14 fractions (1–14). Fraction 3 (8.9 g) was chromatographed on MPLC with PE–EtOAc (1:1, 1:2, 1:3), and then EtOAc to give five fractions (3.1–3.5). Fraction 3.3 (2.5 g) was chromatographed on Toyopearl HW-40C ($CHCl_3$–MeOH, 2:1) to give five fractions (3.3.1–3.3.5). Fraction 3.3.2 (702 mg) was separated by HPLC (ODS, MeOH–H_2O, 85:15) to give **1** (10 mg), **3** (12.3 mg), and **4** (5.8 mg). Fraction 3.2 (2.1 g) was separated by Toyopearl HW-40C with $CHCl_3$–MeOH (2:1) to give three fractions (3.2.1–3.2.3). Fraction 3.3.2 (277 mg) was separated by flash chromatography on a column (C18) with MeOH–H_2O (85:15) to give three fractions (3.3.2.1–3.3.2.3). Fraction 3.3.2.1 was separated by HPLC (ODS, MeOH–H_2O, 80:20) to give **5** (24.3 mg) and **6** (8.1 mg). Fraction 3.3.2.3 (15.5 mg) was separated by HPLC (ODS, MeOH–H_2O, 8:2) to give **2** (10.3 mg).

*3.3.1 Compound **1***

White amorphous powder; $[\alpha]_D^{25}$ −5.03 ($c = 5.53$, pyridine); IR (KBr) ν_{max} (cm^{-1}) 3442 (OH), 2937, 1735, 1382, 1242, 1042; ^1H NMR (C_5D_5N) δ: 2.13 (3H, s, 12-$OCOCH_3$), 1.98 (3H, s, 3′-$OCOCH_3$), other spectral data see Table 1; ^{13}C NMR (C_5D_5N) δ: 170.9, 20.3 (12-$OCOCH_3$), 171.2, 21.6 (3′-$OCOCH_3$), other spectral data see Table 2. HR-MOLDI-FTMS *m/z* 743.3902 $[M+Na]^+$ (calcd for $C_{39}H_{60}O_{12}Na$, 743.3982).

*3.3.2 Compound **2***

White amorphous powder; $[\alpha]_D^{25}$ −4.04 ($c = 9.57$, pyridine); IR (KBr) ν_{max} (cm^{-1}) 3431 (OH), 2932, 1738, 1372, 1247, 1042; ^1H NMR (C_5D_5N) δ: 1.98 (3H, s, 25-$OCOCH_3$), 1.98 (3H, s, 4′-$OCOCH_3$), other spectral data see Table 1; ^{13}C NMR (C_5D_5N) δ: 171.1, 22.0 (25-$OCOCH_3$), 170.7, 21.4 (4′-$OCOCH_3$), other spectral data see Table 2. HR-MOLDI-FTMS *m/z* 727.4022 $[M+Na]^+$ (calcd for $C_{39}H_{60}O_{11}Na$, 727.4033).

3.3.3 Compound **3**

White amorphous powder; $[\alpha]_D^{25} + 15.57$ ($c = 6.93$, pyridine); IR (KBr) ν_{max} (cm^{-1}) 3415 (OH), 2937, 2870, 1740, 1375, 1246, 1073; ^1H NMR ($CDCl_3$) δ: 3.22 (3H, s, 25-OCH_3), 2.13 (3H, s, 2′-$OCOCH_3$), other spectral data see Table 1; ^{13}C NMR ($CDCl_3$) δ: 49.3 (25-OCH_3), 170.5, 21.0 (2′-$OCOCH_3$), other spectral data see Table 2. HR-MOLDI-FTMS *m/z* 699.4073 $[M+Na]^+$ (calcd for $C_{38}H_{60}O_{10}Na$, 699.4084).

3.3.4 Compound **4**

White amorphous powder; $[\alpha]_D^{25} + 0.60$ ($c = 2.65$, pyridine); IR (KBr) ν_{max} (cm^{-1}) 3395 (OH), 2965, 2933, 2869, 1458, 1379, 1254, 1166, 1047; ^1H NMR ($CDCl_3$) δ: 1.13 (3H, t, $J = 6.9$ Hz, 25-OCH_2CH_3), 3.38 (2H, q, $J = 6.9$ Hz, 25-OCH_2CH_3), 2.14 (3H, s, 2′-$OCOCH_3$), other spectral data see Table 1; ^{13}C NMR ($CDCl_3$) δ: 16.2, 56.7 (25-OCH_2CH_3), 170.5, 21.0 (2′-$OCOCH_3$), other spectral data see Table 2. HR-MOLDI-FTMS *m/z* 713.4237 $[M+Na]^+$ (calcd for $C_{39}H_{62}O_{10}Na$, 713.4241).

3.3.5 Compound **5**

White amorphous powder; $[\alpha]_D^{25} - 82.54$ ($c = 10.99$, pyridine); IR (KBr) ν_{max} (cm^{-1}) 3446 (OH), 2964, 1735, 1243, 1042, 988; ^1H NMR (C_5D_5N) δ: 2.18 (3H, s, 12-$OCOCH_3$), 2.00 (3H, s, 3′-$OCOCH_3$), other spectral data see Table 1; ^{13}C NMR (C_5D_5N) δ: 171.2, 21.6 (12-$OCOCH_3$), 171.1, 21.4 (3′-$OCOCH_3$), other spectral data see Table 2. HR-MOLDI-FTMS *m/z* 739.3685 $[M+Na]^+$ (calcd for $C_{39}H_{56}O_{12}Na$, 739.3669).

3.3.6 Compound **6**

White amorphous powder; $[\alpha]_D^{25} - 72.92$ ($c = 7.76$, pyridine); IR (KBr) ν_{max} (cm^{-1}) 3446 (OH), 2932, 1734, 1384, 1245, 1031; ^1H NMR (C_5D_5N) δ: 2.20 (3H, s, 12-$OCOCH_3$), 1.99 (3H, s, 4′-$OCOCH_3$), other spectral data see Table 1; ^{13}C NMR (C_5D_5N) δ: 171.2, 21.8 (12-$OCOCH_3$), 171.1, 21.3 (4′-$OCOCH_3$), other spectral data see Table 2. HR-MOLDI-FTMS *m/z* 723.3671 $[M+Na]^+$ (calcd for $C_{39}H_{56}O_{11}Na$, 723.3720).

3.3.7 Acid hydrolysis of **1–6**

Compounds **1–6** (each 2 mg) were refluxed with 1 M CF_3COOH (1 ml) in EtOH (2 ml) for 6 h. EtOH and CF_3COOH were then removed *in vacuo* from each reaction mixture and a residue was obtained. The sugar was identified from the residue by TLC comparison with authentic samples using $CHCl_3$–MeOH–H_2O–CH_3COOH (6:4:1:0.1) as mobile phases and visualized after spraying with *p*-anisaldehydro-H_2SO_4 reagent followed by heating at 110°C for 5 min. Xylose was observed at R_f values of 0.55.

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