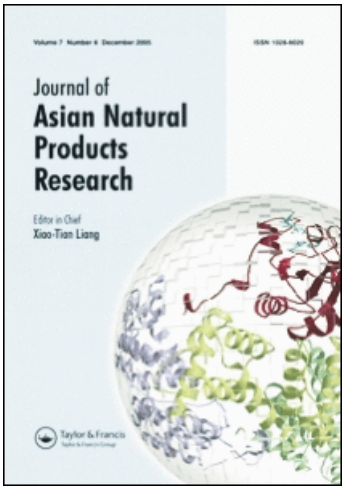

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 12 May 2010

To cite this Article Jin, Jian-Ming , Liu, Xi-Kui and Yang, Chong-Ren(2003) 'Three new hecogenin glycosides from fermented leaves of *Agave americana*', Journal of Asian Natural Products Research, 5: 2, 95 — 103
To link to this Article: DOI: 10.1080/1028602021000054946
URL: http://dx.doi.org/10.1080/1028602021000054946

Journal of Asian Natural Products Research, 2003 VOL. 5 (2), pp. 95–103

THREE NEW HECOGENIN GLYCOSIDES FROM FERMENTED LEAVES OF *AGAVE AMERICANA*

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(Received 24 June 2002; Revised 19 August 2002; In final form 9 September 2002)

Eight steroidal compounds, including three new hecogenin glycosides, agamenosides D–F, were isolated from the fermented leaves of *Agave americana*. The structures of the new steroidal saponins were elucidated by spectroscopic data and chemical methods. The activity of the isolated compounds on deformations of mycelia germinated from conidia of *Pyricularia oaryza*e P-2b was evaluated.

Keywords: *Agave americana*; Fermented leaves; Steroidal saponins; Agamenosides D–F

INTRODUCTION

Agave americana L. is native to Mexico and widely cultivated in the south of China. The leaves of this plant are used as fiber and as a folk medicinal herb; they are also used to produce steroidal sapogenins such as hecogenin [1]. Several steroidal saponins have been isolated from this genus [2–4]. Hecogenin isolated from the plant showed anti-inflammatory activity [5]. Previously our group has reported on several tigogenin glycosides isolated from leaf-juice fermented residues of a cultivated *A. sisalana* [6,7]. We also investigated the steroidal compounds of the fermented residues of leaf-juices of *A. americana*. This paper describes the isolation and structural elucidation of three new hecogenin glycosides together with five known steroidal compounds from fermented leaves of *A. americana*, on the basis of chemical methods and spectral analysis, especially by 2D NMR techniques. Compounds **1**–**5** are known compounds and identified as (25*R*)-5α-spirostan-3β-ol-12-one (hecogenin) (**1**) [8], hecogenin 3-*O*-β-D-galactopyranoside (agaveside A) (**2**) [2,8], hecogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (agaveside B) (**3**) [2,8], hecogenin 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**4**) [8], hecogenin 3-*O*-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**5**) [9] (Fig. 1).

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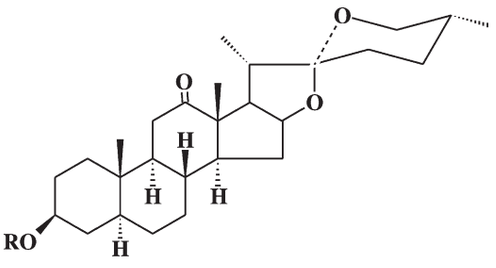
ISSN 1028-6020 print/ISSN 1477-2213 online
DOI: 10.1080/1028602021000054946



1: R=H

2: R=Gal
3: R=Gal(4-1)Glc
4: R=Gal(4-1)Glc(2-1)Glc
5: R=Gal(4-1)Glc[(3-1)Xyl](2-1)Glc
6: R=Gal(4-1)Glc(2-1)Glc(3-1)Rha
7: R=Gal(4-1)[(3-1)Xyl]Glc(2-1)Glc(3-1)Rha
8: R=Gal(4-1)[(3-1)Glc]Glc(2-1)Glc(3-1)Xyl

FIGURE 1

RESULTS AND DISCUSSION

Compound **6** was obtained as an amorphous powder. It gave a positive anisaldehyde–sulfuric acid reaction and showed an accurate negative ion at *m/z* 1061.5205 $[M - H]^-$ in the HRFABMS, corresponding to the empirical molecular formula $C_{51}H_{82}O_{23}$, which was also deduced on the basis of the ^{13}C DEPT NMR spectrum. Negative-ion FABMS showed *m/z* at 1061 $[M - H]^-$, 915 $[M - Rha - H]^-$, 753 $[M - Rha - Glc - H]^-$ and 591 $[M - Rha - Glc - Glc - H]^-$. The IR spectrum gave characteristic absorption bands at 3426 (hydroxyl groups), 982, 920, 897 and 867 cm^{-1} (intensity: 920 $<$ 897 cm^{-1}), which indicated the presence of a (25*R*)-spirostanol steroidal skeleton in the aglycone. The ^1H NMR spectrum of **6** showed signals for two tertiary methyl groups at δ 1.09 (s) and 0.70 (s), three secondary methyl groups at δ 1.63 (d, $J = 6.2$ Hz), 1.33(d, $J = 6.8$ Hz), and 0.71(d, $J = 5.6$ Hz), four anomeric protons at δ 6.01 (br s), 5.04 (d, $J = 7.4$ Hz), 5.02 (d, $J = 7.2$ Hz), 4.84 (d, $J = 7.8$). A broad singlet peak of δ 6.01 indicated the α-orientation at the anomeric center of L-rhamnose. Four anomeric carbon signals of sugar were also observed at δ_C 102.6, 105.2, 106.5 and 102.7 from ^{13}C NMR spectrum. The *J* values (>7 Hz) of the other three anomers of the sugar moieties indicated the β-orientation at the anomeric centers of the D-pyranoses. The signal at δ 1.63 was due to the methyl group of 6-deoxyhexopyranose. Acid hydrolysis of **6** with 0.1 mol L^{-1} HCl in dioxane gave hecogenin as the aglycone and a sugar residue which was identified as D-galactose, D-glucose and L-rhamnose by GC. The structure of the aglycone was confirmed by ^1H and ^{13}C NMR spectra [8]. The above evidence suggested that **6** was a hecogenin tetrasaccharide. The sugar linkages were determined by analysis of 2D NMR experiments. The ^{13}C chemical shifts of the sugar moieties were assigned by HMQC-TOCSY spectrum (Table II below). In the HMBC spectrum, the cross signals were at δ 4.84 (H-1 of inner galactopyranosyl residue) and 77.5 (C-3 of aglycone), 5.02 (H-1 of inner glucopyranosyl residue) and 80.8 (C-4 of inner galactopyranosyl residue), 5.04 (H-1 of outer glucopyranosyl residue) and 85.3 (C-2 of inner glucopyranosyl residue), as well as 6.01 (H-1 of terminal rhamnopyranosyl residue) and 83.0 (C-3 of outer glucopyranosyl residue).

Thus, the structure of **6** was determined to be hecogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (Fig. 1), and it was named agamenoside D.

Compound **7** was obtained as an amorphous powder. Its molecular formula ($C_{56}H_{90}O_{27}$) was determined by ^{13}C DEPT NMR and negative-ion FABMS, which showed a quasi-molecular ion peak at *m/z* 1193 ($[M - H]^-$). Five fragment ion peaks were observed at *m/z* 1061, 1047, 885, 753 and 591 in the negative-ion FABMS spectrum. The IR spectrum also showed that **7** was spirostan glycoside and that the aglycone of **7** has a 25*R* configuration [characteristic absorption bands at 981, 920, 898 and 867 cm^{-1} in the IR spectrum (intensity: 920 < 898 cm^{-1})]. In comparing the whole ^{13}C NMR spectrum of **7** with that of **6**, a set of additional five signals corresponding to a terminal D-xylose moiety appeared at 106.1 (CH), 77.8 (CH), 75.5 (CH), 70.8 (CH), 67.0 (CH_2). The ^1H and ^{13}C NMR spectra confirmed that **7** has the same sapogenol structure as **2**–**6**, and the same oligoside constituent as tigogenin, 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside [10]. In the HMBC spectrum, the anomeric proton signals also exhibited correlations with the carbon signals at δ 4.85 (H-1 of inner galactopyranosyl residue) and δ 77.4 (C-3 of aglycone), δ 5.16 (H-1 of inner glucopyranosyl residue) and δ 79.8 (C-4 of inner galactopyranosyl residue), δ 5.46 (H-1 of outer glucopyranosyl residue) and δ 81.0 (C-2 of inner glucopyranosyl residue), δ 6.07 (H-1 of terminal rhamnopyranosyl residue) and δ 83.8 (C-3 of outer glucopyranosyl residue), δ 5.11 (H-1 of xylopyranosyl residue) and δ 87.4 (C-3 of inner glucopyranosyl residue). Acid hydrolysis of **7** gave hecogenin as the aglycone and the sugar residues which were identified as D-galactose, D-glucose, D-xylose and L-rhamnose by GC. Therefore, the structure of **7** was elucidated as hecogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (Fig. 1), and was named agamenoside E.

Compound **8** was also isolated as an amorphous solid. Its molecular formula was determined as $C_{56}H_{90}O_{28}$ by ^{13}C DEPT NMR and negative-ion FABMS, which showed a quasi-molecular ion peak at *m/z* 1209 ($[M - H]^-$). There are six additional fragment ion peaks at *m/z* 1077, 1047, 915, 885, 753 and 591, besides the molecular ion peak in the negative-ion FABMS spectrum. The ^1H NMR spectrum showed the following representative signals: four steroidal methyl protons at δ 0.63 (s), 0.69 (d, $J = 5.7$ Hz), 0.82 (s) and 1.13 (d, $J = 7.3$ Hz); five anomeric protons at δ 4.86, 5.07, 5.09, 5.18 and 5.50. The IR spectrum also showed characteristic absorption bands at 981, 920, 898 and 867 cm^{-1} (intensity: 898 > 920) suggesting that the aglycone of **8** has a 25*R* configuration. Acid hydrolysis of **8** gave hecogenin as the aglycone as well as D-glucose, D-galactose, and D-xylose as the sugar groups, which were confirmed by NMR spectra and GC, respectively. The ^1H and ^{13}C NMR assignments of the sugar groups of **8** were carried out by analysis of the HMQC-TOCSY spectrum followed by HMQC data. Three-bond ^1H–^{13}C long-range correlations were observed in the HMBC spectrum. The HMBC correlations were observed between δ 4.82 (H-1 of galactopyranosyl residue) and 77.3 (C-3 of aglycone), δ 5.10 (H-1 of inner glucopyranosyl residue) and δ 80.0 (C-4 of galactopyranosyl residue), δ 5.54 (H-1 of middle glucopyranosyl residue) and δ 80.9 (C-2 of inner glucopyranosyl residue), δ 5.05 (H-1 of xylcopyranosyl residue) and δ 86.9 (C-3 of middle glucopyranosyl residue), δ 5.18 (H-1 of outer glucopyranosyl residue) and δ 88.5 (C-3 of inner glucopyranosyl residue). Thus, the structure of **8** was showed to be hecogenin 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosy-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (Fig. 1), and it was named agamenoside F.

The activity of the isolated compounds on deformations of mycelia germinated from conidia of *Pyricularia oryzae* P-2b was evaluated. The mycelia morphological deformation concentrations of compounds **5**–**8** on mycelia of *Pyricularia oryzae* P-2b were 4.69, 4.69, 2.34 and 2.93 $\mu g\,mg^{-1}$, respectively. The other compounds did not induce the mycelia morphological deformation of *oryzae* P-2b.We suggest that the sugar linkage of the steroidal saponin is important for biological activity.

EXPERIMENTAL

General Procedures

Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra (KBr) were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were recorded in pyridine-d_5, on a Bruker DRX-500 or AM-400 instrument at 25°C, using TMS as an internal standard. The negative ion and high-resolution FAB mass spectra were recorded on a VG AutoSpec-3000 mass spectrometer using glycerol as matrix. GC was run on Fisons MD800 GC/MS. Silica gel (Qingdao Haiyang Chemical Co.) and RP-8 (Merck) were used for column chromatography; D-glucose (Merck), D-galactose (Merck), D-xylose (Merck) and L-rhamnose (Merck) were used as standard reagents for GC. Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was carried out by spraying the plates with 10% H_2SO_4, followed by heating.

Plant Material

The dried residues of fermented leaves of *A. americana* L. were collected from a factory in Ruili County of Yunnan Province in January 2000. They were industrially produced in Ruili County.

Extraction and Isolation

The dried residues of fermented leaves of *A. americana* L.(6.5 kg) were extracted with hot methanol (10 L) three times for 4 h; the combined methanol extract was concentrated under reduced pressure. The concentrated extract was then partitioned between *n*-butanol and water. The *n*-butanol layer was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (7:2.5:0.4), and gave five fractions (I–V). Fraction II was repeatedly chromatographed on Si gel with $CHCl_3$–MeOH–H_2O and RP-8 with MeOH–H_2O to give **1** (393 mg), **2** (393 mg) and **3** (78 mg). Fraction III was subjected to repeated column chromatography of normal and reverse silica gel to afford **4** (470 mg), **5** (200 mg) and **6** (1.22 g). Fraction IV was repeatedly chromatographed on silica gel and RP-8 to produce **7** (190 mg) and **8** (126 mg).

Acid Hydrolysis of 6–8

Each of compounds **6**–**8** (72, 46, 31 mg, respectively) was boiled under reflux with 1 mol L^{-1} HCl–dioxane (1:1, v/v, 4 mL) on a water bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was extracted with $CHCl_3$ four times. The $CHCl_3$ extract was concentrated and chromatographed on Si gel to give **6a** (21 mg), **7a** (11 mg) and **8a** (7 mg), respectively. **6a**, **7a** and **8a** were identified as hecogenin by ^1H and ^{13}C NMR. The sugar residues were trimethylsilylated for GC analysis.

Gas Chromatography

The dry sugar residue was diluted in 5 mL pyridine without water and treated with 0.5 mL trimethylchlorosilane (TMCS, Fluka) at room temperature for 30 min. The reaction mixture was evaporated to dryness under reduced pressure. The mixture of trimethylsilylated derivatives of the monosaccharides was diluted in 0.5 mL MeOMe without water and then analyzed by GC. GC: AC-5 capillary column (30 m × ∅0.25 mm); detector: MS; column temperature: 180–260°C; column head pressure: 12 Pa; carrier gas: He. R_t (s): 692 (D-glucose), 653 (D-galactose), 510 (D-xylose) and 430 (L-rhamnose).

Bioassay

A 90-well flat-bottomed assay plate was used for the bioassay. The first, middle and last columns were preserved for negative and positive controls. Rhizoxin was used for positive control with final concentrations of 1 μmol L^{-1}, 0.5 μmol L^{-1}, 0.25 μmol L^{-1}, 0.125 μmol L^{-1}, 60 nmol L^{-1}, 30 nmol L^{-1}, 16 nmol L^{-1}, and 8 nmol L^{-1}. One column (eight wells) was usually used for one test material with eight different concentrations. Each 50 μL of conidia suspension of *Pyricularia oryza*e P-2b (4×10^4 conidia mL^{-1}) was first poured into each well, and 50 μL of each test solution was then added to the first well. The suspension was mixed and 50 μL taken to the second well. The procedure was repeated to the last well of the column. For negative control, 50 μL of water was added to the first well followed by the procedure described above. The assay plates were incubated at 27°C for 16 h, and the shape of mycelia germinated from conidia was observed and compared with controls under an inverted microscope [11].

Hecogenin (1)

A white amorphous powder; $[\alpha]_D^{28.5}$ −10.7 (*c* 0.36, $CHCl_3$). IR (KBr) ν_{max} 3500, 2930, 2865, 1705, 1455, 1376, 1242, 1157, 1075, 1056, 980. 918, 897 and 862 cm^{-1} (intensity: 897 > 918). EI-MS *m/z* 430, 358, 316, 273, 139 (base peak), 126, 93 and 69. ^{1}H NMR (400 MHz, δ in $CDCl_3$): δ 3.44 (1H, m, H-3); 4.37 (1H, m, H-16); 1.04 (3H, s, H-18); 0.89 (3H, s, H-19); 1.06 (3H, d, $J = 7.0$ Hz, H-21); 3.55 (dd, $J = 3.7$ Hz, 11.4 Hz), 3.46 (t, 11.4 Hz), (1H each, H-26); 0.75 (3H, d, $J = 6.3$ Hz, H-27). For ^{13}C NMR data see Table I.

Agavoside A (2)

A white amorphous powder; $[\alpha]_D^{19.2}$ −24.2 (*c* 0.43, pyridine). IR (KBr) ν_{max} 3421, 2927, 2864, 1706, 1455, 1375, 1157, 1074, 1058, 981, 919, 899, 866 cm^{-1} (intensity: 899 > 919). Negative ion FAB-MS *m/z* 591 $[M - H]^-$, 429 $[M - H - 162]$. ^{1}H NMR (500 MHz, δ in pyridine-d_5): δ 3.93 (1H, m, H-3); 4.44 (1H, m, H-16); 1.08 (3H, s, H-18); 0.68 (3H, s, H-19); 1.35 (3H, d, $J = 7.0$ Hz, H-21); 3.58 (dd, 3.5 Hz, 11.0 Hz), 3.48 (t, 11.0 Hz), (1H each, H-26); 0.69 (3H, d, $J = 5.8$ Hz, H-27); 4.92 (1H, d, $J = 7.6$ Hz, H-Gal-1). For ^{13}C NMR data see Table I.

Agavoside B (3)

A white amorphous powder; $[\alpha]_D^{19.2}$ −37.4 (*c* 0.32, pyridine). IR (KBr) ν_{max} 3392, 2934, 2867, 1704, 1453, 1374, 1158, 1071, 982, 922, 900, 866 cm^{-1} (intensity: 900 > 922). Negative ion FAB-MS *m/z* 753 $[M - H]^-$, 591 $[M - H - 162]^-$. ^{1}H NMR (500 MHz, δ in pyridine-d_5): δ 3.87 (1H, m, H-3); 4.48 (1H, m, H-16); 1.08 (3H, s, H-18); 0.67 (3H, s, H-19);

TABLE I ^{13}C NMR data for compounds **1**–**5**

Position	**1***	**2**	**3**	**4**	**5**	*Position*	**2**	**3**	**4**	**5**
1	36.5 (t)	36.7(t)	36.7 (t)	36.8 (t)	36.8 (t)	Gal-1	102.8 (d)	102.6(d)	102.5 (d)	102.5 (d)
2	31.1 (t)	30.0 (t)	29.8 (t)	29.9 (t)	29.8 (t)	2	72.7 (d)	73.5 (d)	73.4 (d)	73.2 (d)
3	70.7 (d)	77.0 (d)	77.1 (d)	77.4 (d)	77.4 (d)	3	75.5 (d)	75.5 (d)	75.2 (d)	75.4 (d)
4	37.8 (t)	34.7 (t)	34.7 (t)	34.8 (t)	34.8 (t)	4	70.4 (d)	80.0 (d)	81.1 (d)	80.0 (d)
5	44.6 (d)	44.5 (d)	44.6 (d)	44.6 (d)	44.6 (d)	5	76.9 (d)	75.9 (d)	75.7 (d)	75.6 (d)
6	28.3 (t)	28.6 (t)	28.7 (t)	28.7 (t)	28.7 (t)	6	62.7 (t)	61.1 (t)	60.7 (t)	60.8 (t)
7	31.4 (t)	31.5 (t)	31.5 (t)	31.6 (t)	32.0 (t)	Glc-1		107.1 (d)	105.2 (d)	105.1 (d)
8	34.3 (d)	34.4 (d)	34.5 (d)	34.5 (d)	34.5 (d)	2		75.1 (d)	86.0 (d)	81.4 (d)
9	55.5 (d)	55.6 (d)	55.7 (d)	55.5 (d)	55.7 (d)	3		78.5 (d)	78.5 (d)	87.0 (d)
10	36.0 (s)	36.3 (s)	36.4 (s)	36.4 (s)	36.4 (s)	4		72.4 (d)	71.9 (d)	72.5 (d)
11	37.7 (t)	38.0 (t)	38.0 (t)	38.2 (t)	38.2 (t)	5		78.7 (d)	78.2 (d)	77.6 (d)
12	213.4 (s)	212.7 (s)	212.7 (s)	213.2 (s)	213.2 (s)	6		63.2 (t)	61.8 (t)	63.1 (t)
13	55.0 (s)	55.4 (s)	55.4 (s)	55.6 (s)	55.5 (s)	Glc-1			107.0 (d)	104.9 (d)
14	55.8 (d)	56.0 (d)	56.0 (d)	56.1 (d)	56.1 (d)	2			76.8 (d)	76.2 (d)
15	31.5 (t)	31.8 (t)	31.9 (t)	31.6 (t)	31.6 (t)	3			79.0 (d)	77.8 (d)
16	79.1 (d)	79.7 (d)	79.7 (d)	79.8 (d)	79.9 (d)	4			70.5 (d)	71.1 (d)
17	53.5 (d)	54.4 (d)	54.4 (d)	54.4 (d)	54.4 (d)	5			77.7 (d)	78.7 (d)
18	15.9 (q)	16.1 (q)	16.1 (q)	16.3 (q)	16.3 (q)	6			61.8 (t)	62.5 (t)
19	11.9 (q)	11.8 (q)	11.8 (q)	11.9 (q)	11.9 (q)	Xyl-1				105.0 (d)
20	42.2 (d)	42.7 (d)	42.7 (d)	42.8 (d)	42.8 (d)	2				75.1 (d)
21	13.1 (q)	13.9 (q)	13.9 (q)	14.1 (q)	14.1 (q)	3				78.7 (d)
22	109.1(s)	109.3(s)	109.4(s)	109.5 (s)	109.7 (s)	4				70.8 (d)
23	31.1(t)	31.7(t)	31.8 (t)	31.9 (t)	32.0 (t)	5				67.4 (t)
24	28.7 (t)	29.2 (t)	29.3 (t)	29.3 (t)	29.4 (t)					
25	30.1 (d)	30.6 (d)	30.6 (d)	30.7 (d)	30.7 (d)					
26	66.8 (t)	67.0 (t)	67.0 (t)	67.1 (t)	67.1 (t)					
27	17.0 (q)	17.3 (q)	17.3 (q)	17.5 (q)	17.5 (q)					

1.34 (3H, d, $J = 7.2$ Hz, H-21); 3.58 (dd, 3.6 Hz, 11.4 Hz); 3.48 (t, 11.4 Hz), (1H each, H-26); 0.69 (3H, d, $J = 5.9$ Hz, H-27); 4.85 (1H, d, $J = 7.5$ Hz, H-Gal-1); 5.26 (1H, d, $J = 7.8$ Hz, H-Glc-1). For ^{13}C NMR data see Table I.

Compound 4

A white amorphous powder; $[\alpha]_D^{20.4}$ −45.0 (*c* 0.15, pyridine). IR (KBr) ν_{max} 3426, 2932, 2868, 1704, 1456, 1373, 1160, 1072, 982, 921, 899, 867 cm^{-1} (intensity: 899 > 921). Negative ion FAB-MS *m/z* 915 $[M - H]^-$, 753 $[M - H - 162]^-$, 591$[M - H - 162 - 162]^-$, 429 $[M - H - 162 - 162 - 162]^-$. ^{1}H NMR (500 MHz, δ in pyridine-d_5): δ 3.88 (1H, m, H-3); 4.48 (1H, m, H-16); 1.08 (3H, s, H-18); 0.67 (3H, s, H-19); 1.34 (3H, d, $J = 6.9$ Hz, H-21); 3.58 (dd, 3.6 Hz, 10.8 Hz); 3.48 (t, 10.8 Hz), (1H each, H-26); 0.69 (3H, d, $J = 5.8$ Hz, H-27); 4.86 (1H, d, $J = 7.8$ Hz, H-Gal-1); 5.11 (1H, d, $J = 7.8$ Hz, H-Glc-1); 5.19 (1H, d, $J = 7.8$ Hz, H-Glc′-1). For ^{13}C NMR data see Table I.

Compound 5

A white amorphous powder; $[\alpha]_D^{19.7}$ −57.6 (*c* 0.42, pyridine). IR (KBr) ν_{max} 3407, 2931, 2868, 1705, 1455, 1373, 1159, 1071, 1040, 981, 920, 899, 866 cm^{-1} (intensity: 899 > 920). Negative ion FAB-MS *m/z* 1047 $[M - H]^-$, 915 $[M - H - 132]^-$, 885 $[M - H - 162]^-$, 753 $[M - H - 132 - 162]^-$, 591 $[M - H - 132 - 162 - 162]^-$. ^{1}H NMR (500 MHz, δ in pyridine-d_5): δ 3.84 (1H, m, H-3); 4.46 (1H, m, H-16); 1.08 (3H, s, H-18); 0.65 (3H, s, H-19); 1.32 (3H, d, $J = 6.5$ Hz, H-21); 3.57 (dd, $J = 3.6$ Hz, 11.0 Hz); 3.46 (t, 11.0 Hz), (1H each, H-26); 0.69 (3H, d, $J = 5.8$ Hz, H-27); 4.82 (1H, d, $J = 7.8$ Hz, H-Gal-1); 5.12 (1H, d, $J = 7.4$ Hz, H-Glc-1); 5.16 (1H, d, $J = 7.5$ Hz, H-Xyl-1); 5.23 (1H, d, $J = 7.8$ Hz, H-Glc′-1). For ^{13}C NMR data see Table I.

Compound 6

A white amorphous powder; $[\alpha]_D^{13.2}$ −39.7 (*c* 0.406, MeOH). IR (KBr) ν_{max} 3426, 2932, 2866, 1705, 1456, 1374, 1158, 1072, 982, 920, 899, 867 cm^{-1} (intensity: 899 > 920). HR FAB-MS *m/z* 1061.5205 $[M - H]^-$ (calcd for $C_{51}H_{81}O_{23}$, 1061.5169). Negative ion FAB-MS *m/z* 1061 $[M - H]^-$, 915 $[M - H - 146]^-$, 753 $[M - H - 146 - 162]^-$, 591 $[M - H - 146 - 162 - 162]$. ^{1}H NMR (500 MHz, δ in pyridine-d_5): δ 3.85 (1H, m, H-3); 4.48 (1H, m, H-16); 1.09 (3H, s, H-18); 0.70 (3H, s, H-19); 1.33 (3H, d, $J = 6.8$ Hz, H-21); 3.59 (drd, 10.9 Hz); 3.48 (t, 10.9 Hz), (1H each, H-26); 0.71 (3H, d, $J = 5.6$ Hz, H-27); 1.63 (3H, d, $J = 6.2$ Hz, H-Rha-6); 4.84 (1H, d, $J = 7.8$ Hz, H-Gal-1); 5.02 (1H, d, $J = 7.2$ Hz, H-Glc-1); 5.04 (1H, d, $J = 7.4$ Hz, H-Glc-1); 6.01 (1H, brs, H-Rha-1). For ^{13}C NMR data see Table II.

Compound 7

A white amorphous powder; $[\alpha]_D^{13.4}$ −39.1 (*c* 0.395, MeOH). IR (KBr) ν_{max} 3434, 2932, 2866, 1705, 1454, 1375, 1158, 1039, 981, 920, 898, 866 cm^{-1} (intensity: 898 > 920). HR FAB-MS *m/z* 1193.5527 $[M - H]^-$ (calcd for $C_{56}H_{89}O_{27}$, 1193.5591). Negative ion FAB-MS *m/z* 1193 $[M - H]^-$, 1061 $[M - H - 132]^-$, 1047 $[M - H - 146]^-$, 885 $[M - H - 146 - 162]^-$, 753 $[M - H - 146 - 162 - 132]^-$, 591 $[M - H - 146 - 162 - 132 - 162]^-$. ^{1}H NMR (500 MHz, δ in pyridine-d_5): δ 3.77 (1H, m, H-3); 4.47 (1H, m, H-16); 1.08 (3H, s, H-18); 0.66 (3H, s, H-19); 1.33 (3H, d, $J = 6.8$ Hz, H-21); 0.68 (3H, d, $J = 5.7$ Hz, H-27); 1.61 (3H, d, $J = 6.2$ Hz, H-Rha-6);

TABLE II ^{13}C NMR data of compounds **6–8**

Position	**6**	**7**	**8**	*Position*	**6**	**7**	**8**
1	36.9 (t)	36.8 (t)	36.7 (t)	Gal-1	102.6 (d)	102.6 (d)	102.5 (d)
2	30.0 (t)	29.7 (t)	29.7 (t)	2	73.4 (d)	73.1 (d)	73.2 (d)
3	77.5 (d)	77.4 (d)	77.3 (d)	3	75.3 (d)	75.3 (d)	75.4 (d)
4	34.8 (t)	34.8 (t)	34.7 (t)	4	80.8 (d)	79.8 (d)	80.0 (d)
5	44.7 (d)	44.6 (d)	44.5 (d)	5	75.8 (d)	75.4 (d)	75.5 (d)
6	28.8 (t)	28.7 (t)	28.7 (t)	6	60.8 (t)	60.8 (t)	60.9 (t)
7	31.9 (t)	31.9 (t)	31.9 (t)	G-1	105.2 (d)	104.7 (d)	104.8 (d)
8	34.6 (d)	34.5 (d)	34.4 (d)	2	85.3 (d)	81.0 (d)	80.9 (d)
9	55.8 (d)	55.7 (d)	55.6 (d)	3	78.8 (d)	87.4 (d)	88.5 (d)
10	36.5 (s)	36.4 (s)	36.3 (s)	4	72.0 (d)	70.4 (d)	70.8 (d)
11	38.2 (t)	38.1 (t)	38.1 (t)	5	78.3 (d)	77.6 (d)	77.5 (d)
12	213.3 (s)	212.8 (s)	212.9 (s)	6	63.3 (t)	63.0 (t)	63.1 (t)
13	55.8 (s)	55.5 (s)	55.4 (s)	G′-1	106.5 (d)	104.3 (d)	104.1 (d)
14	56.2 (d)	56.0 (d)	56.0 (d)	2	76.8 (d)	76.3 (d)	75.1 (d)
15	31.6 (t)	31.5 (t)	31.5 (t)	3	83.0 (d)	83.8 (d)	86.9 (d)
16	79.9 (d)	79.8 (d)	79.8 (d)	4	68.7 (d)	69.8 (d)	69.1 (d)
17	54.5 (d)	54.4 (d)	54.3 (d)	5	78.1 (d)	78.5 (d)	78.2 (d)
18	16.3 (q)	16.1 (q)	16.2 (q)	6	61.7 (t)	62.4 (t)	62.1 (t)
19	12.0 (q)	11.8 (q)	11.8 (q)	R or G″-1	102.7 (d)	102.8 (d)	104.5 (d)
20	42.8 (d)	42.7 (d)	42.7 (d)	2	72.5 (d)	72.3 (d)	75.4 (d)
21	14.1 (q)	13.9 (q)	14.0 (q)	3	72.7 (d)	72.6 (d)	78.6 (d)
22	109.6 (s)	109.4 (s)	109.4 (s)	4	74.1 (d)	74.2 (d)	71.6 (d)
23	31.6 (t)	31.8 (t)	31.8 (t)	5	70.0 (d)	69.8 (d)	78.4 (d)
24	29.4 (t)	29.3 (t)	29.3 (t)	6	18.8 (q)	18.6 (q)	62.4 (t)
25	30.7 (d)	30.6 (d)	30.6 (d)	Xyl 1		104.9 (d)	106.1 (d)
26	67.2 (t)	67.0 (t)	67.1 (t)	2		75.6 (d)	75.4 (d)
27	17.6 (q)	17.4 (q)	17.4 (q)	3		78.4 (d)	77.8 (d)
				4		70.7 (d)	70.8 (d)
				5		67.2 (t)	67.0 (t)

4.85 (1H, d, $J = 7.4$ Hz, H-Gal-1); 5.16 (1H, d, $J = 7.7$ Hz, H-Glc-1); 5.46 (1H, d, $J = 7.9$ Hz, H-Glc′-1); 6.08 (1H, brs, H-Rha-1); 5.11 (1H, d, $J = 7.5$ Hz, H-Xyl-1). For ^{13}C NMR data see Table II.

Compound 8

A white amorphous powder; $[\alpha]_D^{14.1} -31.6$ (*c* 0.31, MeOH). IR (KBr) ν_{max} 3423, 2929, 2870, 1704, 1456, 1374, 1158, 1073, 981, 920, 898, 867 cm^{-1} (intensity: 898 > 920). HR FAB-MS *m/z* 1209.5539 $[M - H]^-$ (calcd for $C_{56}H_{89}O_{28}$, 1209.5540). Negative ion FAB-MS *m/z* 1209 $[M - H]^-$, 1077 $[M - H - 132]^-$, 1047 $[M - H - 162]^-$, 915 $[M - H - 162 - 132]^-$, 885 $[M - H - 162 - 162]^-$, 753 $[M - H - 162 - 132 - 162]^-$, 591 $[M - H - 162 - 132 - 162 - 162]$. ^1H NMR (500 MHz, δ in pyridine-d_5): δ 3.82 (1H, m, H-3); 4.47 (1H, m, H-16); 0.82 (3H, s, H-18); 0.63 (3H, s, H-19); 1.13 (3H, d, $J = 7.3$ Hz, H-21); 0.69 (3H, d, $J = 5.7$ Hz, H-27); 4.82 (1H, d, $J = 7.1$ Hz, H-Gal-1); 5.10 (1H, d, $J = 7.01$ Hz, H-Glc-1); 5.54 (1H, d, $J = 7.1$ Hz, H-Glc′-1); 5.18 (1H, d, $J = 7.5$ Hz, H-Glc″-1); 5.05 (1H, d, $J = 7.04$ Hz, H-Xy-1). ^{13}C NMR data see Table II.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (NSFC) (39969005). The authors are grateful to the analytical group of State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany for

measurements of all spectra. We are also grateful to Professor Xingcong Li (University of Mississippi) for his help.

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