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Novel Dammarane-Type Glycosides from *Gynostemma pentaphyllum*

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Three new dammarane glycosides (1—3), together with five known compounds, gypenoside LXIX (4), gylongiposide I (5), gypenoside XLVIII (6), allantion (7) and vitexin (8) were isolated from the MeOH extract of the aerial parts of *Gynostemma pentaphyllum*. Compounds 5, 7, and 8 were isolated from this plant for the first time. Their structures were elucidated by 1D and 2D NMR spectra interpretation as well as by chemical degradation.

Key words *Gynostemma pentaphyllum*; Cucurbitaceae; dammarane glycoside

Gynostemma pentaphyllum MAKINO (Cucurbitaceae), also called Jiaogulan, praised in China as xiancao, the herb of immortality, a perennial creeping herb distributed in Japan, Korea, China, and Southeast Asia, was once used as a sweetener in Japan, and has been used as a folk medicine in China. Its usages include prevention of growth of cancer and of high blood fat and arteriosclerosis, cure of bronchial asthma and hepatitis, strengthening of the body and prevention of senility. Its taste is sweet and aromatic, and it can be taken either as tea or in alcohol. Previous investigations of this species have shown the occurrence of dammarane-type glycosides structurally related to the ginseng saponins.[1] Recently, certain gypenosides were reported to inhibit the proliferation of Hep-3B and HA22T cells, by affecting calcium and sodium currents in a dose-dependent manner.[2]

In the present study, we have isolated six dammarane-type glycosides, one heterocyclic compound and one flavonoid glycoside from the title plant, comprising three new compounds (**1**—**3**) and five known compounds. Their structural elucidation was accomplished mainly on the basis of the interrelation of 2D NMR spectral data, including ^{1}H–^{1}H and ^{1}H–^{13}C chemical shift correlation spectroscopy.

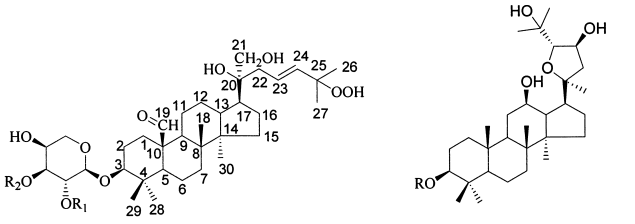
Results and Discussion

The methanol extract of the plant was chromatographied repeatedly on silica gel, RP-18, Sephadex LH-20 and MCI to afford compounds **1**—**8**.

The known compounds gypenoside LXIX (**4**),[3] gylongiposide I (**5**),[4] gypenoside XLVIII (**6**),[5] allantion (**7**)[6] and vitexin (**8**)[7] were identified by comparison of their spectral data with those described in the literature.

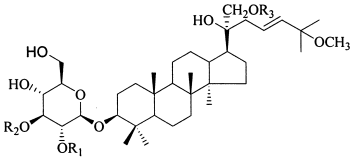
Compound **1** was obtained as an amorphous powder. It gave a positive reaction to KI-starch test paper, indicating the presence of a hydroperoxyl function.[8] Compared to gypenoside LXIX (**4**),[3] the ^{13}C-NMR spectrum of **1** disclosed the opposite shift for the oxygenated carbons C-25 ($\Delta\delta$+10.8) and C-24 ($\Delta\delta$−4.3) and an upfield shift for the C-25 geminal methyls ($\Delta\delta$−5.2 for C-26 and −5.3 for C-27). These observations suggested that **1** had a hydroperoxyl group at C-25 instead of a tertiary hydroxyl at C-25 in gypenoside LXIX (**4**).[9] The molecular weight was determined from the positive HR-ESI-MS at *m*/*z* 939.4918 for the $[M+Na]^+$ ion (Calcd for $C_{46}H_{76}O_{18}Na$, 939.4929). The ^{13}C and DEPT NMR spectra gave 46 signals, of which 16 were assigned to the sugar moiety and 30 to a triterpene moiety. The ^{1}H-NMR spectrum of **1** showed six singlets, assignable to the aglycon methyls at δ 0.89—1.59, one aldehyde proton singlet at δ 10.30, and two olefinic protons at δ 6.24 and δ 6.10. A 3β-hydroxyl substitution was evident from the chemical shift, and the *J* values of the proton ascribable to H-3α at δ 3.31 (1H, br d, *J*=10.0 Hz). On the basis of its ^{1}H- and ^{13}C-NMR data, the aglycon of **1** was identified as 19-oxo-3β,20*S*,21-trihydroxy-25-hydroperoxydammar-23-ene.[8] Glycosidation of the alcoholic function at C-3 was deduced from the significant downfield shift observed for C-3 in **1**, compared with the corresponding signals in model compounds reported in the literature.[10]

Acid hydrolysis of **1** yielded L-arabinose, D-xylose, and L-rhamnose in a ratio of 1 : 1 : 1 by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the standard sugars. The chemical shifts, the signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^{1}H-NMR spectrum, as well as the ^{13}C-NMR data, indicated an

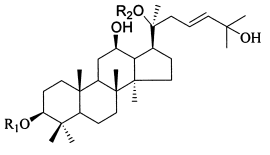


1 R_1 = Rha, R_2 = Xyl

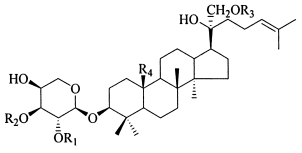
2 R = Glc(2←1)Xyl



3 R_1 = Rha, R_2 = Xyl, R_3 = Xyl'

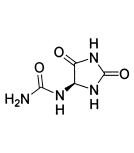


4 R_1 = Glc(2←1)Glc', R_2 = Glc''(6←1)Xyl



5 R_1 = Rha, R_2 = Xyl, R_3 = H, R_4 = CHO

6 R_1 = Rha, R_2 = Glc, R_3 = Glc', R_4 = CHO



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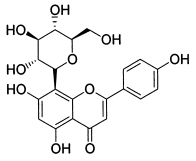

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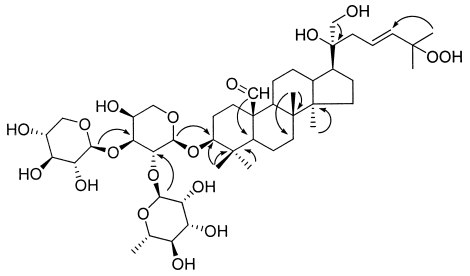

Fig. 1. Key HMBC Correlations of **1**

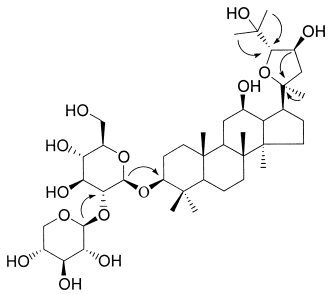

Fig. 2. Key HMBC Correlations of **2**

α-configuration for the arabinosyl units [δ 4.90 (1H, d, J=5.4 Hz, H-1 of ara); δ 105.0 (C-1 of ara)], a β-configuration for the xylosyl unit [δ 5.01 (1H, d, J=7.2 Hz, H-1 of xyl); δ 105.5 (C-1 of xyl)], and an α-configuration for the rhamnosyl uint [δ 6.12 (1H, br s, H-1 of rha); δ 102.3 (C-1 of rha)]. The ^{13}C-NMR data allowed the assignment of the pyranose forms of L-arabinose, D-xylose, and L-rhamnose. All ^{1}H- and ^{13}C-NMR signals of the three sugar units in **1** were assigned using ^{1}H–^{1}H COSY, HMQC, and HMBC spectra. The linkage sites and sequences of the three saccharides, and of the aglycon were deduced from an HMBC experiment. Correlations were observed between H-1 of the arabinose and C-3 of the aglycon, H-1 of the rhamnose and C-2 of the arabinose, H-1 of the xylose and C-3 of the arabinose (Fig. 1). Thus, the structure of **1** was elucidated as 19-oxo-3β,20S,21-trihydroxy-25-hydroperoxydammar-23-ene 3-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside.

Compound **2** was isolated as an amorphous powder. Its molecular formula was established as $C_{41}H_{70}O_{14}$ from the positive HR-ESI-MS quasi-molecular ion at m/z 809.4672 (Calcd for $C_{41}H_{70}O_{14}Na$, 809.4663 $[M+Na]^+$). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** and model compounds[11] indicated that the aglycon of **2** was 3β,12β,23S,25-tetrahydroxy-20S,24S-epoxydammarane. Hydrolysis of compound **2** yielded D-glucose and D-xylose. By GC analysis of the leucine derivatives of the component monosaccharides, it was clear that **2** contained one unit of D-glucose and one of D-xylose. The linkage sites and sequences of the two saccharides and of the aglycon were also determined by an HMBC experiment (Fig. 2). Based on the above results, the structure of **2** was elucidated as 3β,12β,23S,25-tetrahydroxy-20S,24S-epoxydammarane 3-*O*-[β-D-xylopyranosyl(1→2)]-β-D-glucopyranoside.

The positive HR-ESI-MS quasi-molecular ion of **3** at m/z 1085.5864 established its formula as $C_{53}H_{90}O_{21}$ (Calcd for $C_{53}H_{90}O_{21}Na$, 1085.5872 $[M+Na]^+$). The ^{13}C and DEPT

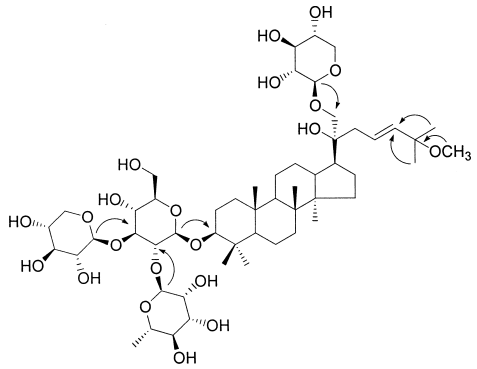

Fig. 3. Key HMBC Correlations of **3**

NMR spectra gave 53 signals, of which 22 were assigned to the sugar moiety and 31 to the aglycon moiety. The ^{1}H-NMR spectrum of **3** showed seven singlets assignable to the aglycon methyls at δ 0.78—1.32. The ^{1}H-NMR spectrum of **3** also showed one methoxy function singlet at δ 3.21, and two olefinic proton at δ 6.10 (1H, m) and δ 5.77 (1H, d, J=15.7 Hz). A 3β-hydroxyl substitution was evident from the chemical shift, and the J values of the proton ascribable to H-3α at δ 3.38 (1H, br d, J=11.3 Hz). On the HMBC spectrum. The correlation was observed between the methoxyl protons at 3.21 ppm and the quaternary carbon at 75.3 ppm (C-25). Thus, it was deduced that the methoxyl group was linked to C-25 of the triterpene moiety. On the basis of its ^{1}H- and ^{13}C-NMR data, the aglycon of **3** was identified as 3β,20S,21-trihydroxy-25-methoxydammar-23-ene.[3]

Acid hydrolysis of **3** yielded D-glucose, D-xylose, and L-rhamnose in a ratio of 1 : 2 : 1 by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the standard sugars. The chemical shifts, the signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^{1}H-NMR spectrum, as well as the ^{13}C-NMR data, indicated a β-configuration for the glucosyl units [δ 4.90 (1H, d, J=6.9 Hz, H-1 of glc); δ 105.2 (C-1 of glc)], a β-configuration for the xylosyl unit [δ 5.00 (1H, d, J=7.7 Hz, H-1 of xyl); δ 105.1 (C-1 of xyl)], an α-configuration for the rhamnosyl uint [δ 6.41 (1H, br s, H-1 of rha); δ 102.3 (C-1 of rha)] and a β-configuration for the other xylosyl unit [δ 5.05 (1H, d, J=7.4 Hz, H-1 of xyl′); δ 106.4 (C-1 of xyl′)]. The linkage sites and sequences of the four saccharides, and of the aglycon were deduced from an HMBC experiment. Correlations were observed between H-1 of the glucose and C-3 of the aglycon, H-1 of the rhamnose and C-2 of the glucose, H-1 of the xylose (xyl) and C-3 of the glucose, H-1 of the other xylose (xyl′) and C-21 of the aglycon (Fig. 3). Thus, the structure of **3** was determined as 3β,20S,21-trihydroxy-25-methoxydammar-23-ene 3-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-glucopyranosyl-21-*O*-β-D-xylopyranoside.

Experimental

General Experimental Procedures Optical rotations were measured in MeOH with a Perkin-Elmer model 341 polarimeter. NMR spectra were obtained on a Bruker AMX-500 spectrometer in C_5D_5N solution. Chemical shifts are reported in ppm. ^{1}H-NMR chemical shifts were referenced to the center peak of the residual solvent signal (δ 7.58). ^{13}C-NMR spectra were referenced to the center peak of the solvent at δ 135.9. ESI-MS were run on a Bruker Esquire 3000 plus spectrometer in MeOH and HR–ESI–MS were

Table 1. ^{1}H-NMR Data of Compounds **1**—**3** in C_5D_5N[a)]

Position	**1**	**2**	**3**
1	2.62 m, 0.72 m	1.62 m, 0.88 m	1.47 m, 0.83 m
2	2.10 m	2.27 m, 1.91 m	2.27 m
3	3.31 br d (10.0)	3.39 dd (11.6, 4.2)	3.38 br d (11.3)
5	1.19 m	0.80 d (9.5)	0.74 d (11.5)
6	1.60 m	1.59 m	1.52 m
7	1.66 m, 1.36 m	1.49 m, 1.27m	1.53 m, 1.27 m
9	1.70 m	1.54 m	1.30 m
11	1.61 m	2.12 m	1.49 m
12	1.91 m	3.89 m	1.95 m
13	2.06 m	2.09 m	2.10 m
15	1.62 m, 1.15 m	1.62 m, 1.11 m	1.65 m, 1.12 m
16	2.08 m	1.96 m	1.88 m
17	2.28 m	2.40 m	2.22 m
18	0.97 s	0.96 s	0.97 s
19	10.30 s	0.90 s	0.78 s
21	4.28 m, 3.99 m	1.51 s	4.38 m, 4.03 m
22	2.86 m, 2.61 m	2.51 m, 2.48 m	2.90 m, 2.60 m
23	6.24 m	5.12 m	6.10 m
24	6.10 m	4.30 d (7.9)	5.77 d (15.7)
C-25-OCH_3			3.21 s
26	1.59 s	1.69 s	1.32 s
27	1.58 s	1.71 s	1.32 s
28	1.27 s	1.39 s	1.27 s
29	0.95 s	1.20 s	1.18 s
30	0.89 s	1.02 s	0.93 s
	C-3-Ara	C-3-Glc	C-3-Glc
1	4.90 d (5.4)	5.00 d (7.6)	4.90 d (6.9)
2	3.91 m	4.20 m	4.22 m
3	4.28 m	4.37 m	4.18 m
4	4.47 br s	4.24 m	4.00 m
5	4.25 m, 3.80 d (10.2)	4.22 m	3.96 m
6		4.64 d (10.2), 4.42 m	4.52 m, 4.37 m
Rha			
1	6.12 br s		6.41 br s
2	4.56 m		4.58 br s
3	4.72 m		4.79 m
4	4.25 m		4.28 m
5	4.54 m		4.75 m
6	1.61 d (5.8)		1.69 d (6.2)
Xyl			
1	5.01 d (7.2)	5.32 d (6.8)	5.00 d (7.7)
2	3.91 m	4.20 m	3.96 m
3	4.09 m	4.01 m	4.08 m
4	4.11 m	4.30 m	4.11 m
5	4.30 m, 3.68 t (9.9)	4.46 m, 3.78 t (10.5)	4.26 m, 3.70 m
C-21-Xyl′			
1			5.05 d (7.4)
2			4.10 m
3			4.22 m
4			4.22 m
5			4.32 m, 3.70 m

a) 500 MHz; referenced to δ 7.58 (C_5D_5N); *J* values (Hz) in parentheses.

run on a Bruker Atex III spectrometer in MeOH, respectively. GC: Shimadzu GC-MS-QP5050A; db-1 column, 0.25 mm i.d.×30 m; column temperature, 200 °C; injection temperature, 250 °C; carrier gas N_2 at flow rate of 32.2 ml/min; detector, EI-MS.

Plant Material *Gynostemma pentaphyllum* was collected in Hunan Province, People's Republic of China in May, 2002. A voucher specimen of the plant (No. 2002003) was identified by Mr. Jin-Gui Shen and deposited at the herbarium of Chinese National Center for Drug Screening, Shanghai, People's Republic of China.

Extraction and Isolation The dried and powdered aerial parts of *G. pentaphyllum* (2.0 kg) were extracted successively with petroleum ether (5 l) and MeOH (3×5 l) at room temperature. Removal of MeOH under reduced pressure left a dark residue (70 g). The residue was subjected to silica gel column chromatography, eluted with chloroform–methanol (100 : 10, 100 : 20, 100 : 30, 100 : 50), to yield four fractions (A—D). Fraction B (15 g) was passed through a Sephadex LH-20 (25—100 μm, Pharmacia, Uppsala, Sweden) column, eluted with methanol to remove flavonoids. Then, the fraction was subjected to MCI gel CHP 20P (75—150 μm, Mitsubishi Kasei Industry Co., Ltd., Tokyo, Japan) column chromatography, eluted with water–acetone (9 : 1, 8 : 1, 7 : 1, 6 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1) to yield eight subfractions (B-1-8). Subfraction B-1 (190 mg) was subjected to RP-18 (20—45 μm, Fuji Silysia Chemical Ltd., Fuji, Japan) flash column chromatography, eluted with methanol–water (60 : 40) to give **2** (20 mg). Fraction C (10 g) was passed over a Sephadex LH-20 column, eluted with methanol to remove flavonoids, and then was subjected to MCI gel CHP 20P column chromatography, eluted with water–acetone (9 : 1, 8 : 1, 7 : 1, 6 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1) to yield eight subfractions (C-1-8). Subfraction C-2 (1.3 g) was further purified by silica gel H flash column chromatography, eluted with chloroform–methanol–water (8 : 1 : 0.1), to give **4** (25 mg) and a mixture. This mixture was purified on a RP-18 flash column, eluted with MeOH–H_2O

Table 2. ^{13}C-NMR Data of Compounds **1**—**6** in C_5D_5N (125 MHz)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	33.9	39.4	39.9	39.3	33.9	33.8
2	27.9	27.1	27.1	26.9	27.9	27.8
3	87.5	89.3	89.1	89.2	87.4	87.3
4	40.8	39.9	39.9	39.8	40.7	40.2
5	55.2	56.8	56.8	56.5	55.2	55.1
6	18.2	18.6	18.7	18.6	18.0	17.9
7	35.0	35.3	35.9	35.2	35.0	34.7
8	40.4	40.1	40.9	40.2	40.3	40.6
9	53.3	50.7	51.2	50.3	53.2	52.9
10	53.1	37.1	37.2	37.0	53.1	52.9
11	22.6	32.7	22.0	31.0	22.7	22.5
12	24.8	70.9	24.8	70.7	25.0	24.7
13	41.7	49.8	42.0	49.6	41.9	41.8
14	50.5	52.6	50.7	51.6	50.6	50.4
15	32.3	32.6	31.7	31.0	32.1	32.0
16	28.2	28.9	27.2	26.5	28.2	27.8
17	46.5	50.1	46.4	52.1	46.4	46.3
18	16.3	15.6	16.0	16.1	16.3	16.1
19	205.5	16.7	16.8	16.4	205.9	205.5
20	72.8	85.5	77.0	83.5	76.8	76.3
21	67.2	29.8	76.7	23.3	67.0	76.3
22	40.3	42.2	40.0	39.8	36.9	36.7
23	127.3	70.6	127.3	122.9	23.5	23.4
24	138.1	91.8	138.7	142.4	126.4	126.0
25	81.5	70.3	75.3	70.7	131.1	130.9
C-25-OCH_3			50.7			
26	25.5	27.7	26.5	30.7	26.1	25.9
27	25.4	26.7	26.2	30.7	18.0	17.9
28	26.7	27.9	28.0	28.2	26.7	26.6
29	16.8	16.3	16.9	16.7	16.9	16.6
30	17.6	18.2	16.8	17.3	17.5	17.4
	C-3-Ara	C-3-Glc	C-3-Glc	C-3-Glc	C-3-Ara	C-3-Ara
1	105.0	105.3	105.2	105.2	105.1	104.8
2	74.9	84.3	77.3	83.2	74.7	74.9
3	81.8	78.5	88.5	78.0	82.1	82.2
4	68.7	71.7	70.1	71.7	68.8	68.3
5	65.3	78.3	78.8	78.2	65.5	65.0
6		63.1	63.1	62.9		
	Rha	Xyl	Rha	Glc′	Rha	Rha
1	102.3	107.2	102.1	105.9	102.3	102.0
2	72.8	76.7	72.8	77.1	72.8	72.6
3	72.7	78.2	72.7	78.4	72.7	72.4
4	74.2	71.2	74.2	71.8	74.2	73.9
5	70.3	67.8	70.1	78.4	70.3	70.1
6	18.9		18.9	62.9	18.8	18.6
	Xyl		Xyl		Xyl	Glc
1	105.5		105.1		105.6	104.8
2	74.7		75.1		74.7	74.9
3	78.0		78.5		78.0	78.5
4	71.2		70.9		71.2	71.6
5	67.3		67.5		67.3	78.5
						62.9
C-20-Glc″						
				98.3		
				75.2		
				78.9		
				71.6		
				76.9		
				70.1		
Xyl						
				105.6		
				74.9		
				78.0		
				71.7		
				67.1		
			C-21-Xyl′			C-21-Glc′
1			106.4			106.1
2			75.7			75.5
3			78.9			78.6
4			72.0			72.0
5			67.2			78.6
6						63.1

(63 : 37), to yield compound **1** (16 mg). Subfraction C-3 (260 mg) was subjected to RP-18 flash column chromatography, eluted with methanol–water (50 : 50) to give **5** (30 mg). Subfraction C-4 (200 mg) was further purified by RP-18 flash column chromatography, eluted with methanol–water (63 : 37), to give **7** (22 mg), and eluted with methanol–water (83 : 17) to afford **8** (15 mg). Fraction D (20 g) was passed through a Sephadex LH-20 column, eluted with methanol to remove flavonoids. Then, the fraction rich in saponin was subjected to MCI gel CHP 20P column chromatography, eluted with water–acetone (10 : 1, 9 : 1, 8 : 1, 7 : 1, 6 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1), to yield nine subfractions (D-1-9). Subfraction D-2 (1 g) was further chromatographed on a RP-18 flash column, eluted with MeOH–H_2O (65 : 35) to yield **6** (28 mg), eluted with acetone–water (35 : 65), to yield compounds **3** (27 mg).

Acid Hydrolysis of Compounds 1—3[12] Compounds **1**—**3** (4 mg each) in 10% HCl–dioxane (1 : 1, 1 ml) were heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 ml×3). After concentration, each H_2O layer (monosaccharide portion) was examined by TLC with $CHCl_3$–MeOH–H_2O (55 : 45 : 10) and compared with authentic samples.

Determination of Sugar Components The monosaccharide subunits were obtained by hydrochloric acid hydrolysis as described above. The sugar residue was then dissolved in 1 ml anhydrous pyridine under Ar, 2 mg L-leucine methyl ester hydrocloride was added, and the mixture was warmed at 60 °C for 1 h. Then 2 mg $NaBH_4$ were added, and the mixture was stirred for 1 h at ambient temperature. Then 0.2 ml of trimethylsilylation reagent trimethylchlorosilane (Shengyu Chemical Ltd., Shanghai, China) was added and warming at 60 °C was continued for another 30 min. The leucine derivatives were subjected to GC analysis to identify the sugars. Column temperature 200 °C; injection temperature 250 °C; carrier gas N_2 at flow rate of 32.2 ml/min; derivatives of D-glucose, D-xylose, L-arabinose, and L-rhamnose: 13.95, 8.23, 7.62 and 8.87 min, respectively.

19-Oxo-3β,20*S*,21-trihydroxy-25-hydroperoxydammar-23-ene 3-*O*-α-L-Rhamnopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside (**1**): Amorphous powder; $[\alpha]_D^{20}$ +157.8° (*c*=0.59, MeOH); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-ESI-MS (positive) *m/z* 939.4918 $[M+Na]^+$ (Calcd for $C_{46}H_{76}O_{18}Na$, 939.4929); GC analysis of sugar components, t_R 8.24 (D-xyl), 8.85 (L-rha) and 7.63 (L-ara) min.

3β,12β,23*S*,25-Tetrahydroxy-20*S*,24*S*-epoxydammarane 3-*O*-[β-D-Xylopyranosyl(1→2)]-β-D-glucopyranoside (**2**): Amorphous powder; $[\alpha]_D^{20}$ +70.4° (*c*=0.29, MeOH); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-ESI-MS (positive) *m/z* 809.4672 $[M+Na]^+$ (Calcd for $C_{41}H_{70}O_{14}Na$, 809.4663); GC analysis of sugar components, t_R 8.22 (D-xyl) and 13.98 (D-glc) min.

3β,20*S*,21-Trihydroxy-25-methoxydammar-23-ene 3-*O*-α-L-Rhamnopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-glucopyranosyl-21-*O*-β-D-xylopyranoside (**3**): Amorphous powder; $[\alpha]_D^{20}$ −7.9° (*c*=2.68, MeOH); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-ESI-MS (positive) *m/z* 1085.5864 $[M+Na]^+$ (Calcd for $C_{53}H_{90}O_{21}Na$, 1085.5872); GC analysis of sugar components, t_R 8.87 (L-rha), 8.25 (D-xyl) and 13.97 (D-glc) min.

Gypenoside LXIX (**4**): Amorphous powder; $C_{53}H_{90}O_{23}$; ESI-MS (*m/z*): 1117 $[M+Na]^+$; ^{1}H-NMR (300 MHz, in pyridine-d_5): H-1 of the four sugars: δ 4.91 (1H, d, *J*=7.3 Hz), 4.95 (1H, d, *J*=7.3 Hz), 5.16 (1H, d, *J*=7.7 Hz), 5.37 (1H, d, *J*=7.3 Hz); H-3: 3.23 (1H, dd, *J*=11.7, 4.0 Hz); Methyl signals: 0.83 (1H, s), 0.90 (1H, s), 1.01 (1H, s), 1.10 (1H, s), 1.27 (1H, s), 1.55 (1H, s), 1.56 (1H, s), 1.60 (1H, s); ^{13}C-NMR, see Table 2.

Gylongiposide I (**5**): Amorphous powder; $C_{46}H_{76}O_{16}$: GESI-MS (*m/z*): F907 $[M+Na]^+$; ^{1}H-NMR (300 MHz, in pyridine-d_5): H-1 of the three sugars: δ 4.88 (1H, d, *J*=5.5 Hz), 5.01 (1H, d, *J*=7.5 Hz), 6.02 (1H, s); H-19: 10.29 (1H, s); H-3: 3.31 (1H, dd, *J*=11.7, 3.9 Hz); Methyl signals: 0.82 (1H, s), 0.92 (1H, s), 1.06 (1H, s), 1.23 (1H, s), 1.62 (1H, s), 1.66 (1H, s), 1.58 (1H, d, *J*=6.1 Hz); ^{13}C-NMR, see Table 2.

Gypenoside XLVIII (**6**): Amorphous powder; $C_{53}H_{88}O_{22}$; ESI-MS (*m/z*): 1099 $[M+Na]^+$; ^{1}H-NMR (300 MHz, in pyridine-d_5): H-1 of the four sugars: δ 6.17 (1H, br s), 5.09 (1H, d, *J*=7.7 Hz), 5.02 (1H, d, *J*=7.7 Hz), 4.83 (1H, d, *J*=5.9 Hz); H-19: 10.27 (1H, s); H-3: 3.31 (1H, dd, *J*=11.3, 3.5 Hz); Methyl signals: 0.86 (1H, s), 0.96 (1H, s), 1.09 (1H, s), 1.26 (1H, s), 1.64 (1H, s), 1.66 (1H, s), 1.59 (1H, d, *J*=5.5 Hz); ^{13}C-NMR, see Table 2.

Allantion (**7**): Khaki powder; $C_4H_6N_4O_3$; HR-EI-MS (positive) (*m/z*): 158.0444 M^+ (Calcd for $C_4H_6N_4O_3$, 158.0440); mp: 226—229 °C (CH_3OH); ^{1}H-NMR (300 MHz, in DMSO-d_6): δ 10.54 (1H, br s, NH-3), 8.05 (1H, br s, NH-1), 6.90 (1H, d, *J*=8.1 Hz, –NH-4), 5.79 (2H, br s, NH_2-6), 5.24 (1H, d, *J*=8.1 Hz, H-4); ^{13}C-NMR (75 MHz, in DMSO-d_6): δ 173.6 (C-5), 157.2 (C-6), 156.8 (C-2), 62.5 (C-4).

Vitexin (**8**): Yellow powder; $C_{21}H_{20}O_{10}$; mp: 185—189 °C (CH_3OH); ^{1}H-NMR (300 MHz, in DMSO-d_6): δ 13.17 (1H, br s, OH-5), 10.85 (1H, br s, OH-7), 10.35 (1H, br s, OH-4′), 8.03 (1H, d, *J*=8.7 Hz, H-2′, 6′), 6.90 (1H, d, *J*=8.7 Hz, H-3′, 5′), 6.78 (1H, s, H-6), 6.28 (1H, s, H-3), 4.69 (1H, d, *J*=9.9 Hz, H-1″), other protons of the sugar: δ 3.26—5.01; ^{13}C-NMR (75 MHz, in DMSO-d_6): δ 164.0 (C-2), 102.5 (C-3), 182.1 (C-4), 156.0 (C-5), 98.1 (C-6), 162.5 (C-7), 104.1 (C-8), 162.5 (C-9), 104.6 (C-10), 121.6 (C-1′), 129.0 (C-2′, 6′), 115.8 (C-3′, 5′), 160.4 (C-4′), 78.6 (C-1″), 73.4 (C-2″), 70.8 (C-3″), 70.5 (C-4″), 81.8 (C-5″), 61.3 (C-6″).

Bioassay. Sensory Testing Sweetness relative to sucrose was evaluated by a human sensory panel.[13] None of the compounds was sweeter than sucrose.

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