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DAMMARANE-TYPE GLYCOSIDES FROM *GYNOSTEMMA PENTAPHYLLUM*

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Key Word Index—*Gynostemma pentaphyllum*; Cucurbitaceae; triterpenoid saponins.

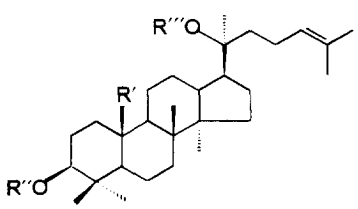
Abstract—Three novel dammarane glycosides together with six known compounds were isolated from a methanol extract of the aerial parts of *Gynostemma pentaphyllum*. Their structures were elucidated by one- and two-dimensional NMR experiments, including ^1H–^1H correlation spectroscopy (DQFCOSY and TOCSY) and ^1H–^{13}C heteronuclear correlation (HMQC and HMBC). Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Gynostemma pentaphyllum is a perennial liana growing wild throughout Japan, China and Korea, once used for its sweet properties [1]. Previous investigations of this species have shown the occurrence of dammarane-type glycosides structurally related to the ginseng saponins [2–4]. Since ginsenosides are the well-known biologically active principles in Korean ginseng, it has received much attention. In a previous paper, we reported the isolation and structural elucidation of several new saponins [5]. As part of our continuing chemical studies of *G. pentaphyllum*, nine compounds have been isolated and identified on the basis of spectral methods. Among them, six were known compounds (**4**–**9**); the other three were new dammarane-type glycosides (**1**–**3**).

RESULTS AND DISCUSSION

Aerial parts of *G. pentaphyllum* were extracted successively with petrol, $CHCl_3$ and MeOH. The MeOH extract was partitioned into a mixture of *n*-butanol and H_2O to yield the *n*-butanol-soluble portion, which was subjected to silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 7:3:0.1). The fractions obtained were further chromatographed on a RP-18 lobar column eluted with methanol–water or acetonitrile–water to yield compounds **1** (50 mg), **2** (34 mg), **3** (20 mg), **4** (550 mg), **5** (30 mg), **6** (25 mg), **7** (30 mg), **8** (27 mg) and **9** (750 mg). The known compounds, Gyp-XXXXIII (**4**), Gyp-XXXXVI (**5**), Gyp-LXIII (**6**), GypIV (**7**), GypV (**8**) and ombuoside (**9**), were identified by comparison of their spectral data with those described in the literature [6–9].



1 R′=CH_2OH, R″=Glc(2→1)Glc, R‴=Glc(6→1)Rha
2 R′=CHO, R″=Glc(2→1)Glc, R‴=Glc(6→1)Rha
3 R′=CHO, R″=Ara(2→1)Glc, R‴=Glc

Compound **1** was obtained as an amorphous powder. Its IR spectrum showed characteristic absorptions for hydroxyl (3400 cm^{-1}) and a glycosidic linkage (1000–1100 cm^{-1}). The FAB-mass spectrum exhibited three pseudomolecular ion peaks at *m/z* 1115 $[M+Na]^+$, 953 $[M+Na-162]^+$, and 791 $[M+Na-162*2]^+$, suggesting the molecular formula $C_{54}H_{92}O_{22}$. On the basis of ^1H and ^{13}C NMR data, the aglycone of compound **1** was identified as 3β,19,20(*S*)-trihydroxydammaran-24-ene, the same aglycone as that of Gyp-LXIII [7]. Comparison of their NMR and FAB-mass spectra revealed that compound **1** contained three hexose and one 6-deoxyhexose moieties, whereas Gyp-XXXVI contained one pentose, two hexose and one 6-deoxyhexose units. Hydrolysis of compound **1** yielded L-rhamnose and D-glucose. Therefore, there were one L-rhamnose and three D-glucose units per molecule of **1**. The glucose units were identified as having a β linkage on the basis of *J* values of their anomeric protons (7.4 Hz); the rhamnose unit had an α-linkage according to ^{13}C NMR data (see

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Table 1. ^{1}H NMR data of compounds **1–3** in C_5D_5N*

	1		**2**		**3**	
Position	^{1}H	*J*(Hz)	^{1}H	*J*(Hz)	^{1}H	*J*(Hz)
1	2.49*m*		2.45*m*		2.74d	12.8
	0.79*t*	12.6	0.57*t*	12.6	0.82*t*	12.8
2	2.37*m*		2.18*m*		2.09*m*	
	2.18*m*		1.63*m*		1.66*m*	
3	3.49*dd*	11.3, 3.9	3.32*dd*	11.1, 4.1	3.37*dd*	11.5, 3.8
5	0.88*m*		1.03*m*		1.10*m*	
6	1.52*m*		1.89*m*		1.88*m*	
7	1.47*m*		1.49*m*		1.56*m*	
	1.20*m*		1.25*m*		1.32*m*	
9	1.47*m*		1.60*m*		1.65*m*	
11	2.13*m*		1.65*m*		1.66*m*	
	1.89*m*		1.47*m*		1.47*m*	
12	2.09*m*		2.03*m*		2.01*m*	
	1.87*m*		1.87*m*		1.83*m*	
13	1.84*m*		1.76*m*		1.80*m*	
15	1.58*m*		1.48*m*		1.50*m*	
	1.10*m*		1.10*m*		1.08*m*	
16	1.99*m*		1.87*m*		1.90*m*	
	1.67*m*		1.63*m*			
17	2.23*m*		2.14*m*		2.11*m*	
18	1.42*s*		0.87*s*		0.99*s*	
19	4.41*m*		10.29*s*		10.38*s*	
	4.22*m*					
21	1.51*s*		1.41*s*		1.56*s*	
22	1.84*m*		1.95*m*		1.97*m*	
	1.56*m*		1.82*m*		1.84*m*	
23	2.43*m*		2.43*m*		2.46*m*	
	2.10*m*					
24	5.48*m*		5.39*t*		5.38*t*	
26	1.81*s*		1.73*s*		1.76*s*	
27	1.80*s*		1.70*s*		1.76*s*	
28	1.46*s*		1.32*s*		1.37*s*	
29	1.32*s*		1.07*s*		1.12*s*	
30	1.14*s*		0.99*s*		1.05*s*	
3-*O*-Glc or Ara (inner)						
G_1 or A_1	5.08*d*	7.4	4.93*d*	7.5	5.02*d*	5.9
G_2 or A_2	4.30*m*		4.28*m*		4.67*t*	6.5
G_3 or A_3	4.13*m*		4.00*m*		4.41*m*	
G_4 or A_4	4.22*m*		4.19*m*		4.41*m*	
G_5 or A_5	4.41*m*		4.38*m*		4.29*m*	
					3.85*m*	
G_6	4.62*m*		4.61*m*			
			4.29*m*			
3-*O*-Glc (terminal)						
G_1	5.47*d*	7.3	5.34*d*	7.6	5.24*d*	7.6
G_2	4.21*m*		4.19*m*		4.18*t*	7.6
G_3	4.32*m*		4.31*m*		4.28*m*	
G_4	4.41*m*		4.39*m*		4.39*m*	
G_5	3.98*m*		3.98*m*		3.89*m*	
					4.49*d*	3.6
G_6	4.543*m*		4.52*m*			
20-*O*-Glc (inner)						
G_1	5.13*d*	7.6	5.07*d*	7.6	5.11*d*	7.6
G_2	4.06*m*		4.04*m*		4.15*m*	
G_3	4.28*m*		4.28*m*		4.30*m*	
G_4	4.05*m*		4.03*m*		4.28*m*	
G_5	4.06*m*		4.03*m*		3.99*m*	
G_6	4.70*d*	10.0	4.59*d*	18.9	4.53*dd*	11.2, 5.9
	4.03*m*		4.41*m*			

Table 1. (*Continued*)

Position	1		2		3	
	^{1}H	*J*(Hz)	^{1}H	*J*(Hz)	^{1}H	*J*(Hz)
20-*O*-Rham (terminal)						
R_1	5.56*s*		5.47*s*			
R_2	4.70*m*		4.54*m*			
R_3	4.62*m*		4.50*m*			
R_4	4.36*m*		4.36*m*			
R_5	4.38*m*		4.38*m*			
R_6	1.73*d*	6.0	1.64*d*	5.7		

* 400 MHz, δ referenced to 7.56 (C_5D_5N).

Table 2). All proton and carbon signals of the aglycone and each sugar moiety in compound **1** were assigned using ^{1}H–^{1}H DQFCOSY, TOCSY, HMQC, and HMBC NMR experiments (Tables 1 and 2). The linkage sites and sequences of the two saccharides and the aglycone were established by a HMBC experiment (Table 3). Therefore compound **1** was identified as 3β,19,20(*S*)-dihydroxydammar-24-ene-3-*O*-[β-D-glucopyranosyl(2 → 1)-β-D-glucopyranosyl]-20-*O*-[α-L-rhamnopyranosyl(6 → 1)-β-D-glucopyranoside].

Compound **2** was isolated as an amorphous powder, whose IR spectrum showed characteristic absorptions for hydroxyl (3400 cm^{-1}), carbonyl (1700 cm^{-1}) and a glycosidic linkage (1000–1100 cm^{-1}). The FAB-mass spectrum showed a quasi-$[M]^+$ peak at *m/z* 1113, corresponding to $[M(C_{54}H_{90}O_{22})+Na]^+$, indicating the presence of two hydrogen atoms less than in compound **1**. The ^{13}C NMR data were very similar to those of compound **1** except for an aldehyde function (δ 206.2) instead of an oxygen-bearing methyl group (δ 61.6). This diagnostic signal exhibited a connectivity with the one proton singlet signal at δ 10.29 in the HMQC spectrum, which in turn showed cross-peaks with C-1 (33.5), C-5 (54.6), C-9 (52.8) and C-10 (52.8) in a HMBC experiment. These observations clearly suggested an aldehyde function at C-19. Furthermore, on the basis of ^{13}C NMR, ^{1}H–^{1}H DQFCOSY, TOCSY, HMQC and HMBC spectra, all proton and carbon signals in its aglycone were assignable (Tables 1 and 2).

Compound **2** contained four monosaccharide units. Hydrolysis yielded D-glucose and L-rhamnose. As in compound **1**, there were one L-rhamnose and three D-glucose units per molecule of compound **2**. Assignment of all proton and carbon NMR signals of four sugar units were made from ^{1}H–^{1}H DQFCOSY, TOCSY, HMQC and HMBC spectra (Tables 1 and 2). The linkage sites and sequences of the saccharides and the aglycone were also determined using a HMBC experiment (Table 3). Thus, the structure of compound **2** was shown to be 19-oxo-3β,20(*S*)-dihydroxydammar-24-ene-3-*O*-[β-D-glucopyranosyl[2 → 1)-β-D-glucopyranosyl]-20-*O*-[α-L-rhamnopyranosyl [6 → 1)-β-D-glucopyranoside].

Compound **3**, an amorphous powder, exhibited a quasi-molecular ion peak at *m/z* 937, corresponding to $[M(C_{47}H_{78}O_{17})+Na]^+$ in the FAB-mass spectrum. By comparison of its ^{1}H and ^{13}C NMR spectra with those of compound **2**, it was apparent that compound **3** had the same aglycone. Its NMR and FAB-mass spectra revealed that compound **3** contained one pentose and two hexose units. Hydrolysis of compound **3** yielded L-arabinose and D-glucose. Thus, there were one L-arabinose and two D-glucose units per molecule of compound **3**. Chemical shifts, the multiplicity of the signals, the absolute values of the coupling constants and their magnitude in the ^{1}H NMR, and also the ^{13}C NMR data (Tables 1 and 2), indicated a β-configuration at the anomeric positions for the glucopyranosyl units and an α-configuration for the arabinosyl unit. ^{13}C NMR data allowed assignment of the pyranose form to D-glucose and L-arabinose. Assignment of all proton and carbon NMR signals were also made using the NMR experiments described above (Tables 1 and 2). The linkage sites and sequences of the saccharides and the aglycone were also determined using a HMBC experiment (Table 3). Based on the above results, the structure of compound **3** was elucidated to be 19-oxo-3β,20(*s*)-dihydroxydammar-24-ene-3-*O*-[α-L-arabinopyranosyl(2 → 1)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranoside.

EXPERIMENTAL

General. FAB-MS: Finnigan-MAT 8430. ^{1}H and ^{13}C NMR spectra: Bruker AM-400 and Varian Gemini-300. Chemical shifts are reported in ppm.

Plant material. Gynostamma pentaphyllum Makino was collected at Hangzhou, Zhejiang Province, People's Republic of China, in June 1994. A voucher sample is deposited at the Herbarium of the Department of Phytochemistry, Shanghai Institute of Materia Medica, Academia Sinica, Shanghai, People's Republic of China.

Isolation. Crude saponins (30 g) were subjected to silica gel CC ($CHCl_3$–MeOH–H_2O, 7:3:0.1). The frs obtained were further chromatographed on an RP-18 lobar column eluted with MeOH–H_2O or MeCN–H_2O to yield compounds **1** (50 mg), **2** (34 mg), **3** (20

Table 2. ^{13}C NMR data of compounds **1–3** in C_5D_5N*

Position	1	2	3
1	34.6	33.5	33.6
2	27.5	27.9	27.8
3	89.3	87.7	88.0
4	39.7	40.1	40.1
5	57.1	54.6	54.7
6	18.2	17.8	17.7
7	36.2	34.7	34.8
8	41.0	40.4	40.4
9	53.0	52.8	52.8
10	42.1	52.8	52.8
11	25.1	22.5	22.6
12	25.9	25.9	25.6
13	43.3	42.4	42.2
14	51.1	50.5	50.5
15	32.1	31.9	32.1
16	28.8	28.0	28.0
17	48.6	48.6	48.5
18	16.1	15.9	15.9
19	61.6	206.2	206.1
20	82.5	82.2	82.3
21	20.9	21.2	21.6
22	40.4	40.4	40.4
23	23.1	23.2	23.2
24	126.2	126.1	126.0
25	130.6	130.6	130.6
26	25.9	25.9	25.8
27	18.0	18.6	17.9
28	28.8	26.7	26.7
29	16.9	16.9	16.8
30	17.6	17.2	17.3
3-*O*-Glc or Ara (inner)			
G_1	105.1	105.0	104.9
G_2	83.4	83.6	81.2
G_3	78.1	78.2	73.6
G_4	71.7	71.7	68.5
G_5	78.1	78.2	65.2
G_6	62.8	62.8	
3-*O*-Glc (terminal)			
G_1	106.0	106.2	106.2
G_2	77.1	77.1	76.4
G_3	78.1	78.2	78.0
G_4	71.7	71.6	71.8
G_5	78.1	78.0	78.2
G_6	62.9	62.8	62.6
20-*O*-Glc (inner)			
G_1	98.6	98.6	98.7
G_2	75.8	75.6	75.8
G_3	79.0	79.0	79.2
G_4	71.9	72.0	72.0
G_5	76.5	76.6	78.2
G_6	68.5	68.5	63.1
20-*O*-Rham (terminal)			
R_1	102.4	102.4	
R_2	72.3	72.3	
R_3	72.8	72.8	
R_4	74.1	74.1	
R_5	69.7	69.8	
R_6	18.7	18.7	

* 100 MHz, δ referenced to 135.5 (C_5D_5N).

Table 3. Cross-peaks in HMBC spectra of compounds **1–3** in C_5D_5N

Compounds	C-3	H_{G1} or H_{A1}	C_{G2} or C_{A2}	H_{G1}	C-20	$H_{G''-1}$	$C_{G''-6}$	H_{R-1}
1	89.3	5.08	83.4	5.47	82.5	5.13	68.5	5.56
2	87.7	4.93	83.6	5.34	82.2	5.07	68.5	5.47
3	88.0	5.02	81.2	5.24	82.3	5.11		

mg), **4** (550 mg), **5** (30 mg), **6** (25 mg), **7** (30 mg), **8** (27 mg) and **9** (750 mg).

Compound **1**. Amorphous powder. $[\alpha]_D^{29.5}$ −3.52° (MeOH; *c* 0.0398). IR υ_{max} cm^{-1}: 3420, 1640, 1100–1000. FAB-MS *m/z*: 1115 $[M+Na]^+$, 953 $[M+Na-162]^+$, 791 $[M+Na-162*2]^+$. ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **2**. Amorphous powder. $[\alpha]_D^{29.5}$ +8.08° (MeOH; *c* 0.0379). IR υ_{max} cm^{-1}: 3400, 1700, 1640, 1100–1000. FAB-MS *m/z*: 1113 $[M+Na]^+$, 951 $[M+Na-162]^+$, 789 $[M+Na-162*2]^+$. ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **3**. Amorphous powder. $[\alpha]_D^{29.5}$ +18.08° (MeOH; *c* 0.0262). FAB-MS *m/z*: 937 $[M+Na]^+$. ^1H and ^{13}C NMR: Tables 1 and 2.

Acidic hydrolysis. MeOH solns of **1–3**, together with standard sugar samples, were applied at points *ca* 1 cm from the bottom of a HP-TLC silica gel plates and hydrolysed with HCl vapour for 2 hr at 50°. The plate was then heated at 60° for 2 hr to remove residual HCl and developed using $CHCl_3$–MeOH–H_2O (8:2:0.1). The plate was sprayed with 10% H_2SO_4 in EtOH and then heated to 110°.

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