Six New Triterpenoid Glycosides from *Gynostemma pentaphyllum*

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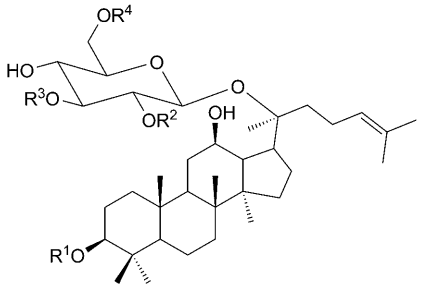
Six new triterpenoid glycosides, gynosaponins I–VI (**1**–**6**, resp.), together with three known compounds, ginseng Rb_1 (**7**), gypenoside XLIX (**8**), and gylongiposide I (**9**), were isolated from the aerial parts of *Gynostemma pentaphyllum*. Based on ESI-MS, IR, 1D- and 2D-NMR data (HMQC, HMBC, COSY, and TOCSY), the structures of the new compounds were determined as (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 20-*O*-[*α*-rhamnopyranosyl-(1→2)]-*β*-glucopyranoside (**1**), (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 20-*O*-[*α*-rhamnopyranosyl-(1→2)][*α*-rhamnopyranosyl-(1→3)]-*β*-glucopyranoside (**2**), (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 3-*O*-*β*-glucopyranosyl-20-*O*-[*α*-rhamnopyranosyl-(1→2)]-*β*-glucopyranoside (**3**), (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 3-*O*-*β*-glucopyranosyl-20-*O*-[*α*-rhamnopyranosyl-(1→2)][*α*-rhamnopyranosyl-(1→3)]-*β*-glucopyranoside (**4**), (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 3-*O*-{[*β*-glucopyranosyl-(1→2)]-*β*-glucopyranosyl}-20-*O*-[*α*-rhamnopyranosyl-(1→2)]-*β*-glucopyranoside (**5**), and (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 3-*O*-{[*β*-glucopyranosyl-(1→2)]-*β*-glucopyranosyl}-20-*O*-[*α*-rhamnopyranosyl-(1→2)][*α*-rhamnopyranosyl-(1→3)]-*β*-glucopyranoside (**6**).

Introduction. – *Gynostemma pentaphyllum* MAKINO (Cucurbitaceae), a Chinese herb called *jiao-gu-lan*, is a perennial creeping herb distributed in Asian countries such as China, Japan, Korea, *etc*. Its usages include preventing of growth of cancer and of high blood fat and arteriosclerosis, curing of bronchial asthma and hepatitis, and strengthening of the body and delay of senility [1]. A H_2O extract of *G. pentaphyllum* has been described as having insignificant toxicity [2]. Previous investigations of this species resulted in the occurrence of dammarane-type glycosides structurally related to the ginseng saponins [3]. According to a review covering the literature until 2004 [4], more than 100 saponins, called gypenosides or gynosaponins, have been isolated from *G. pentaphyllum*. More recently, *Yin et al.* have characterized nine further dammarane saponins from the aerial part of *G. pentaphyllum* [5]. The dammarane-type glycosides are believed to be the active principles responsible for its biological activities [6]. The extract of *G. pentaphyllum* has been reported to possess clinical efficacy for the treatment of cardiovascular diseases and related disorders [7].

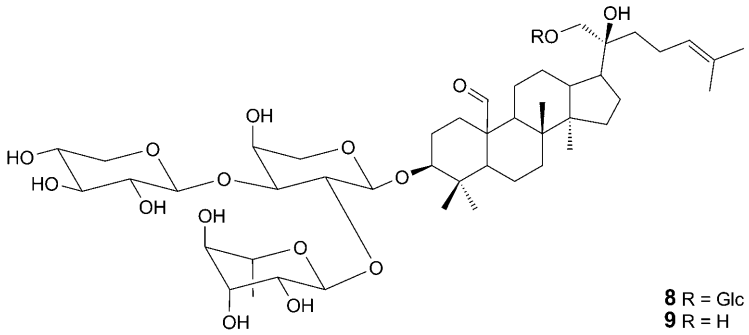
In our research for anti-hyperlipidemic dammarane glycosides, nine analogues were obtained from the aerial parts of *G. pentaphyllum*, including six new ones, **1**–**6**, as well as three known compounds. The three known triterpenoid glycosides, **7**–**9**, were determined as ginseng Rb_1 [8], gypenoside XLIX [9], and gylongiposide I [10], respectively, by comparing their physical properties and the spectral data with those

reported in the literature. The isolation of a large number of new saponins from this well investigated plant species may be due to a different geographical origin of the plant material. This article deals with the isolation and structural elucidation of the new gynosaponins.



	R^1	R^2	R^3	R^4
1	H	Rha	H	H
2	H	Rha	Rha	H
3	Glc	Rha	H	H
4	Glc	Rha	Rha	H
5	Glc(2→1)Glc	Rha	H	H
6	Glc(2→1)Glc	Rha	Rha	H
7	Glc(2→1)Glc	H	H	Glc

Glc = Glucopyranosyl
Rha = Rhamnopyranosyl



8 R = Glc
9 R = H

Results and Discussion. – Gynosaponin I (**1**) was obtained as amorphous powder, and had a molecular formula $C_{42}H_{72}O_{12}$, deduced from the HR-ESI-MS (*m/z* 803.4704, $[M + Cl]^-$; calc. 803.4718). The ^{13}C-NMR and DEPT NMR spectra gave 42 signals, of which twelve were assigned to the sugar moieties and 30 to a triterpene skeleton. Comparison of the ^{13}C-NMR spectrum (*Table 1*) with those of (20*S*)-protopanaxadiol [11] showed that the two compounds were very similar except for two additional sugar units in **1**. In addition, the C-atom signal at δ(C) 84.2 (C(20)) was shifted downfield compared to the (20*S*)-protopanaxadiol (C(20) δ(C) 72.9), suggesting that the saccharide moiety was linked at C(20) of the aglycone in **1**.

Acid hydrolysis of **1** yielded one glucose and one rhamnose. The ^{13}C-NMR spectrum showed two signals of anomeric C-atoms at δ(C) 96.9 (C(1′)) and 101.4

Table 1. *^{13}C-NMR Data of* **1**–**6**. In C_5D_5N solution at 125 MHz; δ in ppm.

	1	**2**	**3**	**4**	**5**	**6**
C(1)	39.3	39.3	39.1	39.0	39.1	39.1
C(2)	28.2	28.3	26.8	26.7	26.7	26.8
C(3)	78.1	77.0	88.8	88.7	89.0	88.9
C(4)	39.6	39.6	39.7	39.7	39.7	39.7
C(5)	56.4	56.4	56.4	56.3	56.4	56.4
C(6)	18.8	18.8	18.5	18.8	18.5	19.6
C(7)	35.2	35.2	35.1	35.0	35.1	35.1
C(8)	40.1	40.1	40.1	39.9	40.0	40.0
C(9)	50.2	50.1	50.1	50.0	50.0	50.0
C(10)	37.4	37.4	36.9	36.9	36.9	36.9
C(11)	31.0	31.1	31.0	30.9	31.0	31.0
C(12)	70.9	70.9	70.9	70.7	70.9	70.7
C(13)	49.2	49.3	49.3	49.2	49.2	49.3
C(14)	51.7	51.7	51.7	51.7	51.7	51.7
C(15)	30.9	31.0	30.9	30.9	30.9	31.0
C(16)	26.6	26.7	26.6	26.7	26.6	26.7
C(17)	53.3	52.8	53.3	52.7	53.3	52.8
C(18)	16.4	16.4	16.9	16.8	16.6	16.6
C(19)	16.4	16.4	16.4	16.3	16.3	16.3
C(20)	84.2	84.5	84.2	84.4	84.2	84.5
C(21)	25.8	25.8	25.8	25.8	25.8	25.8
C(22)	35.8	35.9	35.8	35.8	35.8	35.8
C(23)	22.9	22.7	22.9	22.7	22.9	22.7
C(24)	125.9	125.8	125.9	125.8	125.9	125.8
C(25)	131.0	131.1	131	131.0	131.0	131.1
C(26)	24.1	24.1	24.1	23.9	24.1	24.0
C(27)	18.0	18.0	18.0	17.9	18.0	17.9
C(28)	15.9	15.9	15.8	15.9	15.8	15.8
C(29)	28.8	28.7	28.2	28.1	28.2	28.2
C(30)	17.1	17.2	17.2	17.1	17.1	17.2
20-Glc						
C(1′)	96.9	96.6	97.0	96.5	97.0	96.6
C(2′)	76.6	87.7	76.4	87.5	76.5	87.7
C(3′)	79.8	76.5	79.8	76.2	79.8	76.3
C(4′)	71.2	70.7	71.3	70.7	71.2	70.7
C(5′)	78.5	78.1	78.4	78.1	78.0	78.0
C(6′)	62.2	62.0	62.3	62.0	62.2	61.8
Rha						
C(1″)	101.4	101.8	101.3	101.7	101.4	101.8
C(2″)	72.6	72.6	72.4	72.5	72.6	72.6
C(3″)	72.4	72.4	72.6	72.2	72.4	72.4
C(4″)	74.1	73.8	74.1	73.6	74.1	73.9
C(5″)	69.4	69.9	69.4	69.9	69.4	69.6
C(6″)	19.0	18.4	18.9	18.4	18.9	18.4
Rha						
C(1‴)		103.6		103.5		103.5
C(2‴)		72.5		72.5		72.5
C(3‴)		72.3		72.3		72.2
C(4‴)		73.6		73.8		73.6

Table 1 (cont.)

	1	**2**	**3**	**4**	**5**	**6**
C(5‴)		69.6		69.5		69.9
C(6‴)		19.2		19.1		19.1
3-Glc						
C(1⁗)			107.0	107.0	105.1	105.1
C(2⁗)			75.8	75.8	83.5	83.5
C(3⁗)			78.8	78.7	78.5	78.4
C(4⁗)			71.9	71.8	71.7	71.7
C(5⁗)			78.5	78.4	78.3	78.2
C(6⁗)			63.1	63.0	62.8	62.8
Glc						
C(1⁗′)					105.9	106.1
C(2⁗′)					77.1	77.2
C(3⁗′)					78.2	78.1
C(4⁗′)					71.6	71.7
C(5⁗′)					78.4	78.3
C(6⁗′)					62.8	62.9

(C(1″)). In the ^{1}H-NMR spectrum of **1** (*Table 2*), a Me signal (δ(H) 1.60 (*d*, $J = 5.5$)) was assignable to rhamnose. The two anomeric H-atom signals of H−C(1′) at δ(H) 5.17 (*d*, $J = 9.2$) and of H−C(1″) at δ(H) 6.52 (br. *s*), respectively, suggested the presence of a β-glucopyranosyl and a α-rhamnopyranosyl moiety, respectively. In the HMBC spectrum, the long-range correlations from H−C(1′) to C(20) (δ(C) 84.2) and from H−C(1″) to C(2′) (δ(C) 76.6) were observed, which revealed that the glucose unit was linked at C(20), and the rhamnose unit was positioned at C(2′) of the glucose. Moreover, 1D-NMR signals of the two sugar units were assigned by COSY, HMQC, and TOCSY spectra (*Tables 1* and *2*). So the structure of **1** was deduced as (3β,12β,20*S*)-trihydroxydammar-24-ene 20-*O*-[α-rhamnopyranosyl-(1 → 2)]-β-glucopyranoside.

Gynosaponin II (**2**) was purified as an amorphous powder. The HR-ESI-MS exhibited a *pseudo*-molecular ion peak at *m*/*z* 949.5304 ([*M* + Cl]$^-$), in accordance with an empirical molecular formula $C_{48}H_{82}O_{16}$. The ^{13}C-NMR spectral features of **1** and **2** were nearly identical to each other, except for one additional sugar unit in **2**.

Hydrolysis of **2** produced glucose and rhamnose in a ratio of 1:2. The three anomeric-H-atom signals of H−C(1′) at δ(H) 5.07 (*d*, $J = 9.0$), H−C(1″) at δ(H) 5.91 (br. *s*), and H−C(1‴) at δ(H) 5.64 (br. *s*) were assignable to a β-glucopyranosyl and two α-rhamnopyranosyl moieties. The positions of the three saccharide units were deduced from HMBC correlations of H−C(1′) of the glucosyl unit with C(20) (δ(C) 84.5), of H−C(1″) of the rhamnose with C(2′) (δ(C) 87.7) of the glucose, and of H−C(1‴) of the rhamnose with C(3′) (δ(C) 76.5) of the glucose. Based on the above results, the structure of **2** was established as (3β,12β,20*S*)-trihydroxydammar-24-ene 20-*O*-[α-rhamnopyranosyl-(1 → 2)][α-rhamnopyranosyl-(1 → 3)]-β-glucopyranoside.

Gynosaponin III (**3**), isolated as an amorphous powder, possessed a molecular formula $C_{48}H_{82}O_{17}$, as deduced from the HR-ESI-MS (negative-ion mode; *m*/*z* 965.5265 ([*M* + Cl]$^-$)). The NMR data (*Table 1* and *2*) of **3** were closely related to those of **1**

Table 2. *^{1}H-NMR Data of the Sugar Moieties of Compounds* **1**–**6**. At 500 MHz in C_5D_5N; δ in ppm, J in Hz. Assignments based on HMQC, HMBC, COSY, and TOCSY correlations.

	1	**2**	**3**	**4**	**5**	**6**
20-Glc						
H–C(1′)	5.17 (*d*, *J* = 9.2)	5.07 (*d*, *J* = 9.0)	5.19 (*d*, *J* = 8.9)	5.11 (*d*, *J* = 9.0)	5.18 (*d*, *J* = 8.9)	5.07 (*d*, *J* = 8.8)
H–C(2′)	4.23–4.25 (*m*)	4.07–4.09 (*m*)	4.25–4.27 (*m*)	4.10–4.13 (*m*)	4.23–4.25 (*m*)	4.08–4.10 (*m*)
H–C(3′)	4.28–4.30 (*m*)	4.00–4.02 (*m*)	4.26–4.28 (*m*)	4.04–4.06 (*m*)	4.27–4.29 (*m*)	3.98–4.00 (*m*)
H–C(4′)	4.21–4.23 (*m*)	3.97–3.99 (*m*)	4.12–4.14 (*m*)	3.96–3.98 (*m*)	4.17–4.19 (*m*)	4.00–4.02 (*m*)
H–C(5′)	3.79–3.80 (*m*)	3.69–3.71 (*m*)	3.79–3.81 (*m*)	3.73–3.75 (*m*)	3.80–3.82 (*m*)	3.70–3.72 (*m*)
CH_2(6′)	4.37–4.39 (*m*), 4.28–4.30 (*m*)	4.34–4.36 (*m*), 4.23–4.25 (*m*)	4.35–4.37 (*m*), 4.25–4.27 (*m*)	4.30–4.32 (*m*), 4.22–4.24 (*m*)	4.29–4.31 (*m*), 4.35–4.38 (*m*)	3.82–3.84 (*m*), 4.28–4.30 (*m*)
Rha						
H–C(1″)	6.52 (br. *s*)	5.91 (br. *s*)	6.55 (br. *s*)	5.94 (br. *s*)	6.54 (br. *s*)	5.91 (br. *s*)
H–C(2″)	4.77–4.79 (*m*)	4.81–4.83 (*m*)	4.76–4.78 (*m*)	4.66–4.68 (*m*)	4.77–4.79 (*m*)	4.67–4.68 (*m*)
H–C(3″)	4.61–4.62 (*m*)	4.47–4.49 (*m*)	4.60–4.62 (*m*)	4.48–4.50 (*m*)	4.62–4.64 (*m*)	4.49–4.51 (*m*)
H–C(4″)	4.37–4.39 (*m*)	4.29–4.31 (*m*)	4.36–4.38 (*m*)	4.32–4.34 (*m*)	4.38–4.40 (*m*)	4.32–4.34 (*m*)
H–C(5″)	4.84–4.86 (*m*)	4.73–4.75 (*m*)	4.86–4.88 (*m*)	4.72–4.74 (*m*)	4.85–4.87 (*m*)	4.74–4.76 (*m*)
Me(6″)	1.60 (*d*, *J* = 5.5)	1.70 (*d*, *J* = 5.3)	1.61 (*d*, *J* = 5.4)	1.69 (*d*, *J* = 5.3)	1.61 (*d*, *J* = 5.4)	1.69 (*d*, *J* = 5.3)
Rha						
H–C(1‴)		5.64 (br. *s*)		5.67 (br. *s*)		5.64 (br. *s*)
H–C(2‴)		4.66–4.68 (*m*)		4.84–4.86 (*m*)		4.82–4.84 (*m*)
H–C(3‴)		4.48–4.50 (*m*)		4.50–4.52 (*m*)		4.47–4.49 (*m*)
H–C(4‴)		4.31–4.33 (*m*)		4.32–4.34 (*m*)		4.30–4.32 (*m*)
H–C(5‴)		4.67–4.69 (*m*)		3.95–3.97 (*m*)		4.65–4.67 (*m*)
Me(6‴)		1.76 (*d*, *J* = 5.6)		1.73 (*d*, *J* = 5.5)		1.74 (*d*, *J* = 5.6)
3-Glc						
H–C(1⁗)			4.94 (*d*, *J* = 9.1)	4.96 (*d*, *J* = 9.2)	4.91 (*d*, *J* = 9.0)	4.92 (*d*, *J* = 9.0)
H–C(2⁗)			4.03–4.05 (*m*)	4.05–4.07 (*m*)	4.27–4.29 (*m*)	4.26–4.28 (*m*)
H–C(3⁗)			4.19–4.21 (*m*)	4.26–4.28 (*m*)	4.13–4.15 (*m*)	4.11–4.13 (*m*)
H–C(4⁗)			4.58–4.60 (*m*)	4.60–4.62 (*m*)	4.11–4.13 (*m*)	4.10–4.12 (*m*)
H–C(5⁗)			4.00–4.02 (*m*)	4.02–4.04 (*m*)	4.22–4.24 (*m*)	4.23–4.25 (*m*)
CH_2(6⁗)			4.59–4.61 (*m*), 4.40–4.42 (*m*)	4.60–4.62 (*m*), 4.41–4.43 (*m*)	4.43–4.45 (*m*), 4.35–4.37 (*m*)	4.43–4.45 (*m*), 4.34–4.36 (*m*)
Glc						
H–C(1⁗′)					5.39 (*d*, *J* = 9.2)	5.39 (*d*, *J* = 8.9)
H–C(2⁗′)					4.13–4.15 (*m*)	4.13–4.15 (*m*)
H–C(3⁗′)					4.05–4.07 (*m*)	4.06–4.08 (*m*)
H–C(4⁗′)					4.12–4.14 (*m*)	4.13–4.15 (*m*)
H–C(5⁗′)					4.23–4.25 (*m*)	4.30–4.32 (*m*)
CH_2(6⁗′)					4.54–4.56 (*m*), 4.50–4.52 (*m*)	4.57–4.59 (*m*), 4.51–4.53 (*m*)

except that an additional glucopyranosyl functionality was observed, and the ^{13}C-NMR spectral signal at C(3) was shifted downfield from δ(C) 78.1 in **1** to δ(C) 88.8 in **3**. After acid hydrolysis of **3**, two glucose and one rhamnose units were detected by GC, which were determined as β-glucose and α-rhamnose by the coupling constants of the anomeric H-atom signals of H–C(1′) at δ(H) 5.19 (*d*, *J* = 8.9), H–C(1″) at δ(H) 6.55 (br. *s*), and H–C(1⁗) at δ(H) 4.94 (*d*, *J* = 9.1). Through a HMBC experiment, correlations were observed from H–C(1′) of the glucose to C(20) (δ(C) 84.2), from H–C(1″) of the rhamnose to C(2′) (δ(C) 76.4) of the glucose, and from H–C(1⁗) of the other glucose to C(3) (δ(C) 88.8). So the structure of **3** was identified as

(3β,12β,20*S*)-trihydroxydammar-24-ene 3-*O*-β-glucopyranosyl-20-*O*-[α-rhamnopyranosyl-(1 → 2)]-β-glucopyranoside.

Gynosaponin IV (**4**) was obtained as amorphous powder. The HR-ESI-MS *quasi*-molecular ion appeared at *m*/*z* 1111.5811, in accordance with the molecular fomula $C_{54}H_{92}O_{21}$ (calc. for $C_{54}H_{92}ClO_{21}^{-}$, 1111.5819, $[M+Cl]^-$). Comparison of the NMR spectroscopic data of **4** with those of **3** indicated that they had the same aglycone (*Tables 1* and *2*), but that **4** contained an additional rhamnose residue. Acid hydrolysis of **4** yielded two glucose and two rhamnose units. In the ^{1}H-NMR spectrum, the four anomeric H-atom signals of H−C(1′), H−C(1″), H−C(1‴), and H−C(1⁗) at δ(H) 5.11 (*d*, *J* = 9.0), 5.94 (br. *s*), 5.67 (br. *s*), and 4.96 (*d*, *J* = 9.2), respectively, suggested the presence of β-linkages of glucoses and α-linkages of rhamnoses. The linkage sites and sequences of the saccharides and the aglycone were also detemined by HMBC experiments. The long-range correlations between H−C(1′) and C(20) (δ(C) 84.4) were observed, together with the correlations between H−C(1″) and C(2′) (δ(C) 87.5) , between H−C(1‴) and C(3′) (δ(C) 76.2), and between H−C(1⁗) and C(3) (δ(C) 88.7). As a result, compound **4** was established as (3β,12β,20*S*)-trihydroxydammar-24-ene 3-*O*-β-D-glucopyranosyl-20-*O*-[α-rhamnopyranosyl-(1 → 2)][α-rhamnopyranosyl-(1 → 3)]-β-glucopyranoside.

The HR-ESI-MS *quasi*-molecular ion at *m*/*z* 1127.5751 ($C_{54}H_{92}ClO_{22}^{-}$, $[M+Cl]^-$; calc. 1127.5774) of gynosaponin V (**5**) established its molecular formular as $C_{54}H_{92}O_{22}$. Comparison of the NMR data of **5** with those of **3** indicated that they had the same aglycone. Analysis of the NMR spectra and acid hydrolysis of **5** established that compound **5** contained three glucoses and one rhamnose. In the ^{1}H-NMR spectrum, four anomeric H-atom signals of H−C(1′), H−C(1″), H−C(1⁗) and H−C(1⁗′) at δ(H) 5.18 (*d*, *J* = 8.9), 6.54 (br. *s*), 4.91 (*d*, *J* = 9.0), and 5.39 (*d*, *J* = 9.2), respectively, suggested the presence of β-linkages of glucoses and α-linkage of rhamnoses. The following correlations from the HMBC experiment were observed: H−C(1′) of one glucose to C(20) (δ(C) 84.2) of the aglycone, H−C(1″) of one rhamnose to C(2′) (δ(C) 76.5) of the first glucose, H−C(1⁗) of the second glucose to C(3) (δ(C) 89.0) of the aglycone, and H−C(1⁗′) of the third glucose to C(2⁗) (δ(C) 83.5) of the second glucose. Thus, compound **5** was elucidated as (3β,12β,20*S*)-trihydroxydammar-24-ene 3-*O*-{[β-glucopyranosyl-(1 → 2)]-β-glucopyranosyl}-20-*O*-[α-rhamnopyranosyl-(1 → 2)]-β-glucopyranoside.

Gynosaponin VI (**6**), an amorphous powder, displayed a *quasi*-molecular ion peak at *m*/*z* 1273.6318, corresponding to $[M+Cl]^-$ in the HR-ESI-MS spectrum. The ^{13}C-NMR signals of **6** and **4** were almost the same, except for an additional β-glucopyranosyl unit in **6**. Acid hydrolysis of **6** yielded three glucose and two rhamnose units. The ^{13}C-NMR spectrum also showed five signals of anomeric C-atoms at δ(C) 96.6 (C(1′)), 101.8 (C(1″)), 103.5 (C(1‴), 105.1 (C(1⁗)) and 106.1 (C(1⁗′)). In the ^{1}H-NMR spectrum, the five anomeric-H-atom signals of H−C(1′) at δ(H) 5.07 (*d*, *J* = 8.8), H−C(1″) at δ(H) 5.91 (br. *s*), H−C(1‴) at δ(H) 5.64 (br. *s*), H−C(1⁗) at δ(H) 4.92 (*d*, *J* = 9.0), and H−C(1⁗′) at δ(H) 5.39 (*d*, *J* = 8.9) suggested the presence of β-linkages of glucoses and α-linkages of rhamnoses. The linkage sites and sequences of the five saccharide units were determined by HMBC correlations of H−C(1′) with C(20) (δ(C) 84.5), of H−C(1″) with C(2′) (δ(C) 87.7), of H−C(1‴) with C(3′) (δ(C) 76.3), of H−C(1⁗) with C(3) (δ(C) 88.9), and of H−C(1⁗′) with C(2⁗) (δ(C) 83.5),

which revealed that one glucose unit, with two rhamnose units positioned at C(2′) and C(3′), was linked at C(20), and the other glucose unit, with a glucosyl unit linked at C(2‴), was located at C(3), respectively. Based on these facts, compound **6** was identified as (3*β*,12*β*,20*S*)-trihydroxydammar-24-ene 3-*O*-{[*β*-glucopyranosyl-(1 → 2)]-*β*-glucopyranosyl}-20-*O*-[*α*-rhamnopyranosyl-(1 → 2)][*α*-rhamnopyranosyl-(1 → 3)]-*β*-glucopyranoside.

Experimental Part

General. Glucose and rhamnose were purchased from *Sigma* (USA). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Products Industry Factory*, P. R. China); *Sephadex LH-20* (*Pharmacia*) and *RP-18* silica gel (50–80 μm; *Merck*, Germany). TLC: silica gel *G*-precoated plates (*Qingdao Haiyan Chemical Co.*) and *Rp-18-F254S* precoated plates (*Merck*, Germany); spots were visualized by spraying with 10% aq. H_2SO_4 soln., followed by heating. GC: *Shimadzu GC-17A* gas chromatograph equipped with an H_2 flame ionization detector; column: *TC-1* cap. column (30 m × 0.25 mm); detector, *FID*. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Shimadzu IR-450* instrument, with KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AC-400* or *DRX-500* instruments; chemical shifts δ in ppm rel. to Me_4Si, coupling constants *J* in Hz. FAB-MS (neg.-ion mode; glycerol matrix) and HR-ESI-MS: *VG-Auto-Spec-3000* and *Thermo-Finnigan LCQ-Advantage* spectrometer; in *m*/*z* (rel. int. in % of the base peak).

Plant Material. Plants of *Gynostemma pentaphyllum* were bought in An Guo Chinese Medicine Market in Baoding city, Hebei pronvince, P. R. China, in August 2007 (Locality: Pingli county, Ankang city, Shanxi province, P. R. China) and identified by Prof. *Wang Guang Shu*, and a voucher specimen (No. 07081705) was deposited with the Herbarium of the Kunming Institute of Botany.

Extraction and Isolation. The dried and powdered arial parts of *G. pentaphyllum* (5.0 kg) were extracted with 60% EtOH (3 × 10 l) at r.t. for three times in 2 d. After evaporation of the EtOH extract, the residue was suspended in H_2O (1500 ml) and re-extracted successively with petroleum ether (PE; 3 × 2000 ml), AcOEt (3 × 2000 ml), and BuOH (3 × 2000 ml). The BuOH fraction (105 g) was subjected to CC (SiO_2, 200–300 mesh; 1.2 kg; gradient $CHCl_3$/MeOH/H_2O 8:2:0.1 → 6:4:0.5) to yield *Frs. 1–30* (250 ml each). *Frs. 10–15* (with $CHCl_3$/MeOH/H_2O 8:2:0.1 and 7.5:2.5:0.2) gave 20 g of residue, which was repeatedly submitted to CC (SiO_2; 500 g; $CHCl_3$/MeOH/H_2O 8:2:0.1 and 7.5:2.5:0.1) to afford three fractions: *Fr. A* (5 g), *Fr. B* (8 g), and *Fr. C* (5 g). **1** (80 mg) was obtained from *Fr. A* by repeated CC (*RP-18* gel, MeCN/H_2O 48:52) and purified over *Sephadex LH-20* with MeOH as eluent. *Fr. B* was applied to CC (SiO_2; 400 g) eluted with $CHCl_3$/MeOH/H_2O 7:3:1 lower layer and purified by *Sephadex LH-20* with MeOH as eluent to afford **2** (88 mg). *Fr. C* was submitted to CC (SiO_2; 250 g; $CHCl_3$/MeOH/H_2O 7:3:1 lower layer): *Fr. C-1* and **7** (130 mg). *Fr. C-1* was submitted to CC (*RP-18* gel, MeCN/H_2O 44:56): *Fr. C-1-1*. *Fr. C-1-1* was submitted to CC (*Sephadex LH-20*, MeOH): **3** (76 mg). *Frs. 18–30* (with $CHCl_3$/MeOH/H_2O 7.5:2.5:0.2, 7:3:0.3, and 6:4:0.5) gave 42 g of a residue, which was repeatedly submitted to CC (SiO_2; 500 g; $CHCl_3$/MeOH/H_2O 6:3.5:1 lower layer): *Frs. D* (17 g) and *E* (14 g). *Fr. D* was submitted to CC (SiO_2; 850 g; $CHCl_3$/MeOH/H_2O 6.5:3.5:1 lower layer): *Frs. D-1* and *D-2*, **8** (100 mg), and **9** (120 mg). *Fr. D-1* was submitted to CC (SiO_2; 250 g; BuOH/AcOEt/H_2O 4:4:2 upper layer): *D-1-1*. *Fr. D-2* was submitted to CC (SiO_2; 250 g; BuOH/AcOEt/H_2O 4:1:5 upper layer): *Fr. D-2-1*. *Frs. D-1-1* and *D-2-1* were further purified by CC (*Sephadex LH-20*; MeOH): **4** (67 mg) and **5** (73 mg). *Fr. E* was submitted to CC (*RP-18* gel; MeCN/H_2O 38:62): *Fr. E-1*. *Fr. E-1* was submitted to CC (SiO_2; 230 g; BuOH/AcOEt/H_2O 4:1:5 upper layer): **6** (70 mg).

Gynosaponin I (= *(3β,12β,20*S*)-Trihydroxydammar-24-ene 20-*O*-[α-Rhamnopyranosyl-(1 → 2)]-β-glucopyranoside* = *(3β,12β)-3,12-Dihydroxydammar-24-en-20-yl 2-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranoside*; **1**). Amorphous powder. $[\alpha]_D^{25} = -0.7$ ($c = 0.9$, MeOH). IR (KBr): 3400, 2943, 1641, 1452, 1084, 1050. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 767 ($[M-H]^-$). HR-ESI-MS: 803.4704 ($[M+Cl]^-$, $C_{42}H_{72}ClO_{12}^-$; calc. 803.4718). GC Analysis of sugar components: t_R 12.00 and 10.38 min.

Gynosaponin II (= *(3β,12β,20*S*)-Trihydroxydammar-24-ene 20-*O*-[α-Rhamnopyranosyl-(1 → 2)][α-rhamnopyranosyl-(1 → 3)]-β-glucopyranoside* = *(3β,12β)-3,12-Dihydroxydammar-24-en-20-yl 6-Deoxy-*

α-L-mannopyranosyl-(1→2)-[6-deoxy-α-L-mannopyranosyl-(1→3)]-β-D-glucopyranoside; **2**). Amorphous powder. $[\alpha]_D^{25} = -27.1$ ($c = 0.7$, MeOH). IR (KBr): 3400, 2940, 1642, 1452, 1058, 1047. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 913 ($[M-H]^-$). HR-ESI-MS: 949.5304 ($[M+Cl]^-$, $C_{48}H_{82}ClO_{16}^-$; calc. 949.5297). GC Analysis of sugar components: t_R 11.97 and 10.39 min.

Gynosaponin III (=*(3β,12β,20S)-Trihydroxydammar-24-ene 3-O-β-Glucopyranosyl-20-O-[α-rhamnopyranosyl-(1→2)]-β-glucopyranoside = (3β,12β)-20-{[2-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl β-D-Glucopyranoside*; **3**). Amorphous powder. $[\alpha]_D^{25} = -8.4$ ($c = 0.7$, MeOH). IR (KBr): 3400, 2945, 1657, 1456, 1077, 1047. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 929 ($[M-H]^-$). HR-ESI-MS: 965.5265 ($[M+Cl]^-$, $C_{48}H_{82}ClO_{17}^-$; calc. 965.5246). GC Analysis of sugar components: t_R 12.01 and 10.42 min.

Gynosaponin IV (=*(3β,12β,20S)-Trihydroxydammar-24-ene 3-O-β-D-Glucopyranosyl-20-O-[α-rhamnopyranosyl-(1→2)][α-rhamnopyranosyl-(1→3)]-β-glucopyranoside = (3β,12β)-20-{[6-Deoxy-α-L-mannopyranosyl-(1→2)-[6-deoxy-α-L-mannopyranosyl-(1→3)]-β-D-glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl β-D-Glucopyranoside*; **4**). Amorphous powder. $[\alpha]_D^{25} = -12.2$ ($c = 0.9$, MeOH). IR (KBr): 3400, 2938, 1642, 1452, 1074, 1042. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1075 ($[M-H]^-$). HR-ESI-MS: 1111.5811 ($[M+Cl]^-$, $C_{54}H_{92}ClO_{21}^-$; calc. 1111.5825). GC Analysis of sugar components: t_R 12.00 and 10.42 min.

Gynosaponin V (=*(3β,12β,20S)-Trihydroxydammar-24-ene 3-O-{[β-Glucopyranosyl-(1→2)]-β-glucopyranosyl}-20-O-[α-rhamnopyranosyl-(1→2)]-β-glucopyranoside = (3β,12β)-3-{[2-O-(β-D-Glucopyranosyl)-β-D-glucopyranosyl]oxy}-12-hydroxydammar-24-en-20-yl 2-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside*; **5**). Amorphous powder. $[\alpha]_D^{25} = -24.4$ ($c = 1.0$, MeOH). IR (KBr): 3400, 2944, 1645, 1453, 1080, 1047. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1091 ($[M-H]^-$). HR-ESI-MS: 1127.5751 ($[M+Cl]^-$, $C_{54}H_{92}ClO_{22}^-$; calc. 1127.5774). GC Analysis of sugar components: t_R 11.99 and 10.41 min.

Gynosaponin VI (=*(3β,12β,20S)-Trihydroxydammar-24-ene 3-O-{[β-Glucopyranosyl-(1→2)]-β-glucopyranosyl}-20-O-[α-rhamnopyranosyl-(1→2)][α-rhamnopyranosyl-(1→3)]-β-glucopyranoside = (3β,12β)-20-{[6-Deoxy-α-L-mannopyranosyl-(1→2)-[6-deoxy-α-L-mannopyranosyl-(1→3)]-β-D-glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl 2-O-β-D-Glucopyranosyl-β-D-glucopyranoside*; **6**). Amorphous powder. $[\alpha]_D^{25} = -14.2$ ($c = 0.7$, MeOH). IR (KBr): 3400, 2934, 1641, 1453, 1076, 1043. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1237 ($[M-H]^-$). HR-ESI-MS: 1273.6318 ($[M+Cl]^-$, $C_{60}H_{102}ClO_{26}^-$; calc. 1273.6353). GC Analysis of sugar components: t_R 11.96 and 10.39 min.

Acid Hydrolysis of Compounds **1**–**6**. Compounds **1**–**6** (5 mg each) in 1N HCl/MeOH/BuOH (1:0.5:0.5, 2 ml) were each heated at 90° for 4 h in a water bath. The mixtures were neutralized with $AgCO_3$, filtered, and then extracted with $CHCl_3$ (3 × 2 ml). After concentration, each H_2O layer (monosaccharide portion) was examined by TLC with BuOH/AcOEt/H_2O (4:1:5 upper layer) and compared with authentic samples.

GC Analysis of Sugar Components. Each neutralized hydrolysate of **1**–**6** was dissolved in 0.6 ml of pyridine, then 0.4 ml of hexamethyl disilazane and 0.2 ml trimethyl chlorosilane were added successively. The mixture was kept at 60° for 10 min under water-bath condition. Next, the mixture was centrifuged for 20 min at 1.0×10^4 rpm. The supernatant was subjected to GC analysis under the following conditions: *Shimadzu GC-17A* gas chromatograph equipped with an H_2 flame ionization detector. Column: *TC-1* cap. column (30 m × 0.25 mm). Column temp.: 200°–260°, programmed increase: 3°/min; injection temp. 250°; carrier gas: N_2 (1 ml/min); injection volume: 1 µl; split ratio: 1/50; glucose and rhamnose 11.99 min and 10.42 min, resp.

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