Three New Triterpene Saponins from *Gynostemma pentaphyllum*

by **Lin Shi**, **Jia-Qing Cao**, **Wei Li**, and **Yu-Qing Zhao***

School of Traditional Chinese Materia Medica 51#, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, P. R. China
(phone: +86-24-23986522; fax: +86-24-23986522; e-mail: zyq4885@126.com)

Three new dammarane-type triterpene saponins, **1**–**3**, together with three known compounds, **4**–**6**, were isolated from the aerial parts of *Gynostemma pentaphyllum* (THUNB.) MAKINO. By means of chemical and spectroscopic methods, their structures were established as (20*S*)-3β,20,21-trihydroxydammara-23,25-diene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-21-*O*-β-D-glucopyranoside (**1**), (20*R*,23*R*)-3β,20-dihydroxy-19-oxodammar-24-en-21-oic acid 21,23-lactone 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside (**2**), and (21*S*,23*S*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside (**3**).

Introduction. – *Gynostemma pentaphyllum* (THUNB.) MAKINO is a herbal medicine with anticancer activity [1], wildly distributed in China, Korea, and Japan. The biologically active constituents are dammarane-type glycosides, called gypenosides, which are structurally related with the ginseng saponins [2–5]. In our series of studies on the anticancer natural medicines, *Panax ginseng* and *Panax notoginseng*, we have found some active compounds [6][7]. As a continuation of our work for discovering more effective components, we have now investigated *G. pentaphyllum* (THUNB.) MAKINO, which is the first example containing ginsenosides (Rb_1, Rb_3, Rd, *etc.*) ever found from a plant not belonging to the Araliaceae.

From the extract of the aerial parts of this plant, three new dammarane saponins, (20*S*)-3β,20,21-trihydroxydammara-23,25-diene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-21-*O*-β-D-glucopyranoside (**1**), (20*R*,23*R*)-3β,20-dihydroxy-19-oxodammar-24-en-21-oic acid 21,23-lactone 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside (**2**), and (21*S*,23*S*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside (**3**) were isolated, together with three known compounds, (20*S*)-3β,20,21-trihydroxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-21-*O*-β-D-glucopyranoside (**4**) [8], (20*R*,23*R*)-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranoside (**5**) [9], and (23*S*)-3β,20ξ,21ξ-trihydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside (**6**) [10] (see *Fig. 1*).

Here, we report the structure elucidation of the three new dammarane-type saponins **1**–**3**.

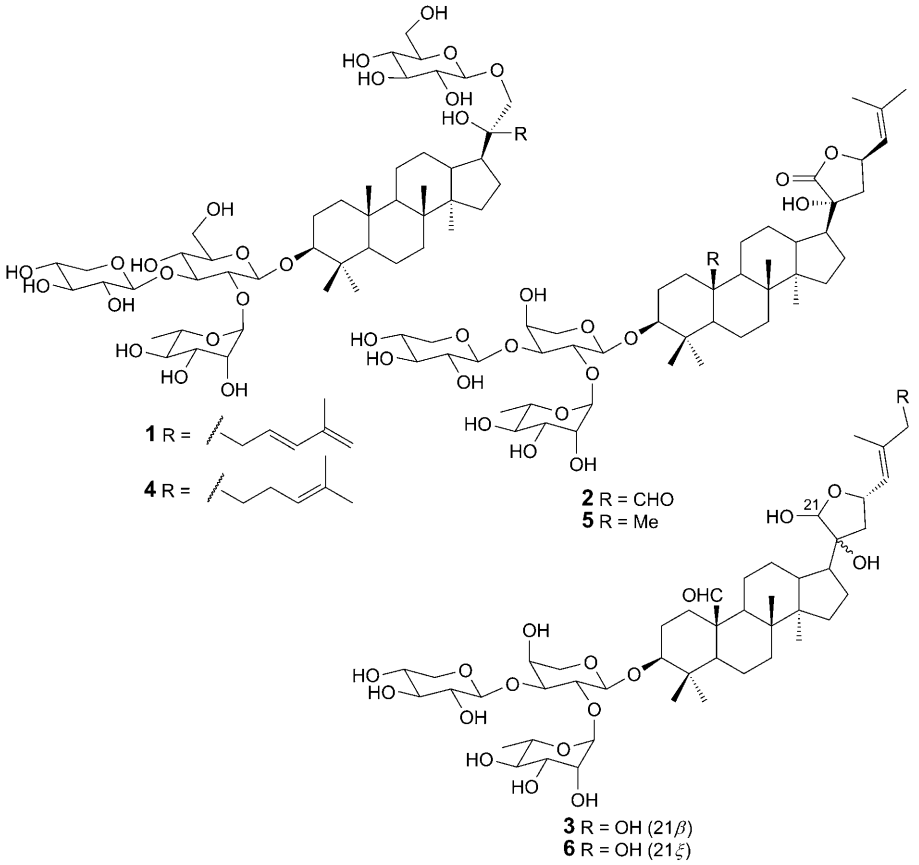

Fig. 1. *Structures of Compounds* **1**–**6**

Results and Discussion. – Compound **1** was obtained as a white amorphous powder, and its molecular formula, $C_{53}H_{88}O_{21}$, was deduced from the HR-TOF-MS (*m*/*z* 1083.5722 ($[M + Na]^+$)). The IR spectrum (KBr) showed absorptions at 3423 cm^{-1} (OH) and 1639 cm^{-1} (C=C). The ^{1}H-NMR spectrum (*Table 1*) showed signals of six Me groups (δ(H) 0.74 (*s*), 0.94 (*s*), 0.96 (*s*), 1.14 (*s*), 1.22 (*s*), 1.84 (*s*)), four olefinic H-atom signals at δ(H) 6.18 (*m*, 1 H), 6.46 (*m*, 1 H), 5.01 (*m*, 1 H), 4.92 (*m*, 1 H), and signals due to two β-D-glucopyranosyl moieties (δ(H) 4.86 (*d*, $J = 7.8$, 1 H), 5.03 (*s*, 1 H)), a β-D-xylopyranosyl moiety (δ(H) 5.01 (*d*, $J = 7.8$, 1 H)), and an α-L-rhamnopyranosyl moiety (δ(H) 6.47 (*s*, 1 H), 1.68 (*d*, $J = 6.0$, 3 H)). The C-atom signals of the aglycon part in the ^{13}C-NMR spectra closely resembled those of **4**, except for a few signals due to the side chain. The structure of the side chain was determined by an HMBC experiment, which showed long-range correlations between CH_2(22), and C(20) and C(17); H–C(23) and C(22); H–C(24), and C(22) and C(26); CH_2(26) and C(27); and Me(27) and C(24) (*Fig. 2*). Up to this point, it could be confirmed that the C=C bonds might be located at C(23)/C(24) and C(25)/C(26). The absolute

Table 1. *[1]H- and [13]C-NMR Data for* **1**. At 600 and 150 MHz, respectively, in C_5D_5N, *J* in Hz.

	δ(C)	δ(H)	HMBC (H → C)
CH_2(1)	39.8	1.37–1.41 (*m*), 0.76–0.82 (*m*)	
CH_2(2)	26.4	2.20–2.24 (*m*), 1.79–1.83 (*m*)	
H–C(3)	89.2	3.33 (*dd*, *J* = 11.8, 3.7)	1′
C(4)	40.0		
H–C(5)	56.7	0.68–0.71 (*m*)	4, 10
CH_2(6)	18.7	1.46–1.50 (*m*), 1.34–1.38 (*m*)	
CH_2(7)	35.7	1.46–1.48 (*m*), 1.19–1.21 (*m*)	
C(8)	40.8		
H–C(9)	51.2	1.22–1.25 (*m*)	18
C(10)	37.1		
CH_2(11)	21.8	1.24–1,27 (*m*)	8, 9
CH_2(12)	24.8	1.89–1.93 (*m*)	
H–C(13)	42.0	2.07–2.10 (*m*)	
C(14)	50.5		
CH_2(15)	31.6	1.61–1.64 (*m*), 1.08–1.10 (*m*)	
CH_2(16)	27.9	1.20–1.23 (*m*), 1.15–1.18 (*m*)	
H–C(17)	46.6	2.20–2.23 (*m*)	
Me(18)	15.9	0.94 (*s*)	7, 8, 14
Me(19)	16.7	0.74 (*s*)	1, 5, 10
C(20)	77.0		
CH_2(21)	76.2	4.31–4.35 (*m*), 3.99–4.01 (*m*)	
CH_2(22)	39.9	2.92–2.95 (*m*), 2.71 (*dd*, *J* = 14.0, 8.4)	17, 20, 21, 23, 24
H–C(23)	128.2	6.18 (*dt*, *J* = 15.6, 8.4, 6.0)	20, 22, 25
H–C(24)	135.1	6.46 (*dd*, *J* = 15.6)	22, 25, 26, 27
C(25)	142.8		
CH_2(26)	114.9	5.00–5.03 (*m*), 4.91–4.93 (*m*)	24, 25, 27
Me(27)	19.0	1.84 (*s*)	24, 25, 26
Me(28)	27.9	1.22 (*s*)	3, 4, 5, 29
Me(29)	17.0	1.14 (*s*)	3, 4, 5, 28
Me(30)	16.7	0.96 (*s*)	8, 13, 14, 15
Glc–O–C(3)			
H–C(1′)	105.0	4.86 (*d*, *J* = 7.8)	3
H–C(2′)	77.2	4.20–4.23 (*m*)	1″
H–C(3′)	88.3	4.15–4.17 (*m*)	1‴
H–C(4′)	69.9	3.97–4.00 (*m*)	
H–C(5′)	78.3	3.87–3.90 (*m*)	
CH_2(6′)	62.8	4.50–4.53 (*m*), 4.35–4.37 (*m*)	
Rha–O–C(2′)			
H–C(1″)	101.8	6.40 (br. *s*)	2′
H–C(2″)	72.6	4.56–4.59 (*m*)	
H–C(3″)	72.5	4.79 (br. *s*)	
H–C(4″)	74.0	4.27–4.29 (*m*)	
H–C(5″)	69.9	4.56–4.60 (*m*)	
Me(6″)	18.7	1.68 (*d*, *J* = 6.0)	
Xyl–O–C(3′)			
H–C(1‴)	105.0	4.98 (*d*, *J* = 7.8)	3′
H–C(2‴)	74.9	4.07–4.10 (*m*)	
H–C(3‴)	78.3	4.08–4.11 (*m*)	
H–C(4‴)	70.7	4.10–4.12 (*m*)	
CH_2(5‴)	67.3	4.23–4.26 (*m*), 3.67–3.70 (*m*)	

Table 1 (cont.)

	δ(C)	δ(H)	HMBC (H → C)
Glc–O–C(21)			
H–C(1‴)	106.2	5.03 (*s*)	21
H–C(2‴)	75.5	4.51–4.53 (*m*)	
H–C(3‴)	78.6	4.22–4.25 (*m*)	
H–C(4‴)	71.7	4.20–4.22 (*m*)	
H–C(5‴)	78.6	3.94–3.97 (*m*)	
CH_2(6‴)	62.8	4.35–4.38 (*m*)	

configuration at C(20) of **1** was deduced to be (*S*) on the basis of the chemical shifts of C(20) at δ(C) 77.0, and of C(17) at δ(C) 46.6 [3]. Accordingly, by comparing its NMR spectral data with those in the literature [11], the aglycone was identified as (20*S*)-3β,20,21-trihydroxydammara-23,25-diene. Acid hydrolysis of **1** yielded D-glucose, D-xylose, and L-rhamnose in a ratio of 2:1:1, and GC analysis of the trimethylsilyl ether derivatives of the component monosaccharides provided their configurations. The C-atom signals assignable to the the sugar moieties, and to C(3) and C(21) of **1** were very similar to those of **4**. The linkage sites, and sequences of the three saccharides and of the aglycon were confirmed by the 2D-NMR experiments. The HMBC displayed the cross-peaks between H–C(1′) of the glucose and C(3) of the aglycon, H–C(1″) of the rhamnose and C(2′) of the glucose, H–C(1‴) of the xylose and C(3′) of the glucose. Thus, **1** was elucidated as (20*S*)-3β,20,21-trihydroxydammara-23,25-diene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-21-*O*-β-D-glucopyranoside.

Compound **2**, a white amorphous powder, showed a peak at *m*/*z* 919.4702 ([*M* + Na]$^+$) in the HR-TOF-MS, indicating the molecular formula $C_{46}H_{72}O_{17}$. The IR spectrum (KBr) showed absorptions at 3429 cm^{-1} (OH), 1670 cm^{-1} (C=O), and 1644 cm^{-1} (C=C). The ^{1}H-NMR spectrum (*Table 2*) showed six Me signals (δ(H) 0.81 (*s*), 0.90 (*s*), 1.07 (*s*), 1.23 (*s*), 1.66 (*s*), 1.68 (*s*)), an olefinic H-atom signal at δ(H) 5.38 (*d*, *J* = 9.0, 1 H), and a CHO H-atom signal at δ(H) 10.34 (*s*), and signals due to an α-L-arabinopyranoside moiety (δ(H) 4.87 (*d*, *J* = 5.4, 1 H)), a β-D-xylopyranosyl moiety (δ(H) 5.01 (*d*, *J* = 7.2, 1 H)), and an α-L-rhamnopyranosyl moiety (δ(H) 6.16 (*d*, *J* = 3.6, 1 H), 1.58 (*s*, 3 H)). Analysis of the ^{1}H- and ^{13}C-NMR spectra established that **2** was a triterpene saponin with a 21,23-lactone skeleton. It showed a close similarity to **5**, except that the C(19) signal (δ(C) 16.7) due to the Me group of **5** was replaced by a signal (δ(C) 205.7) of an CHO function, which could be identified by an HMBC experiment. Namely, long-range correlations were observed between the H–C(19) and C(10) (*Fig. 2*). In addition, compared to **5**, the upfield shifts at C(1) (Δδ −6.3), C(5) (Δδ −1.8), and the downfield shift at C(10) (Δδ +15.8), C(9) (Δδ +1.8) evidenced the presence of a C(19)HO function. The absolute configuration at C(20) of **2** was determined as (*R*), based on the signals of C(20) at δ(C) 81.3, and C(17) at δ(C) 45.3 given in the literature [5]. Therefore, the aglycon part of **2** was determined as (20*R*,23*R*)-19-oxo-3β,20-dihydroxydammar-24-en-21-oic acid 21,23-lactone. Acid hydrolysis of **2** yielded L-arabinose, D-xylose, and L-rhamnose in a ratio of 1:1:1, and GC

Table 2. *[1]H- and [13]C-NMR Data for* **2**. At 600 and 150 MHz, respectively, in C_5D_5N, *J* in Hz.

	δ(C)	δ(H)	HMBC (H → C)
CH_2(1)	33.7	2.62 – 2.65 (*m*), 0.70 – 0.74 (*m*)	
CH_2(2)	27.6	2.46 – 2.48 (*m*), 2.07 – 2.10 (*m*)	
H–C(3)	87.2	3.32 (br. *dt*, *J* = 12.0, 4.2)	1′
C(4)	40.1		
H–C(5)	54.9	1.14 – 1.17 (*m*)	4, 10
CH_2(6)	17.7	1.88 – 1.91 (*m*), 1.66 – 1.69 (*m*)	
CH_2(7)	34.7	1.61 – 1.63 (*m*), 1.31 – 1.34 (*m*)	
C(8)	40.5		
H–C(9)	52.9	1.67 – 1.70 (*m*)	
C(10)	52.9		
CH_2(11)	22.3	1.75 – 1.77 (*m*)	
CH_2(12)	25.7	2.25 – 2.28 (*m*), 1.33 – 1.36 (*m*)	
H–C(13)	44.8	2.66 – 2.69 (*m*)	
C(14)	50.0		
CH_2(15)	32.1	1.55 – 1.58 (*m*), 1.12 – 1.15 (*m*)	
CH_2(16)	27.8	1.42 – 1.44 (*m*)	
H–C(17)	45.3	2.50 – 2.53 (*m*)	20, 21
Me(18)	16.7	0.90 (*s*)	7, 8, 9, 13, 14
H–C(19)	205.7	10.34 (*s*)	10
C(20)	81.1		
C(21)	178.3		
CH_2(22)	39.1	2.50 – 2.53 (*m*), 2.07 – 2.09 (*m*)	20
H–C(23)	75.3	5.67 – 5.70 (*m*)	
H–C(24)	124.0	5.38 (*d*, *J* = 9.0)	25
C(25)	139.4		
Me(26)	25.7	1.66 (*s*)	27
Me(27)	18.2	1.68 (*s*)	26
Me(28)	26.4	1.23 (*s*)	3, 4, 5, 29
Me(29)	16.1	1.07 (*s*)	3, 4, 5, 28
Me(30)	17.1	0.81 (*s*)	7, 8, 9, 14
Ara–O–C(3)			
H–C(1′)	104.8	4.87 (*d*, *J* = 5.4)	3
H–C(2′)	74.5	4.64 – 4.67 (*m*)	1″
H–C(3′)	81.7	4.25 – 4.27 (*m*)	1‴
H–C(4′)	68.5	4.44 – 4.47 (*m*)	
CH_2(5′)	65.1	4.21 – 4.24 (*m*), 3.65 (*t*, *J* = 9.1)	
Rha–O–C(2′)			
H–C(1″)	102.1	6.16 (*d*, *J* = 3.6)	2′
H–C(2″)	72.6	4.56 – 4.59 (*m*)	
H–C(3″)	72.5	4.75 (br. *s*)	
H–C(4″)	74.0	4.27 – 4.30 (*m*)	
H–C(5″)	70.1	4.55 – 4.58 (*m*)	
Me(6″)	18.6	1.58 (*s*)	
Xyl–O–C(3′)			
H–C(1‴)	105.3	5.01 (*d*, *J* = 7.2)	3′
H–C(2‴)	74.7	3.91 – 3.93 (*m*)	
H–C(3‴)	77.8	4.06 – 4.09 (*m*)	
H–C(4‴)	71.0	4.09 – 4.12 (*m*)	
CH_2(5‴)	67.0	4.24 – 4.27 (*m*), 3.67 (*t*, *J* = 10.2)	

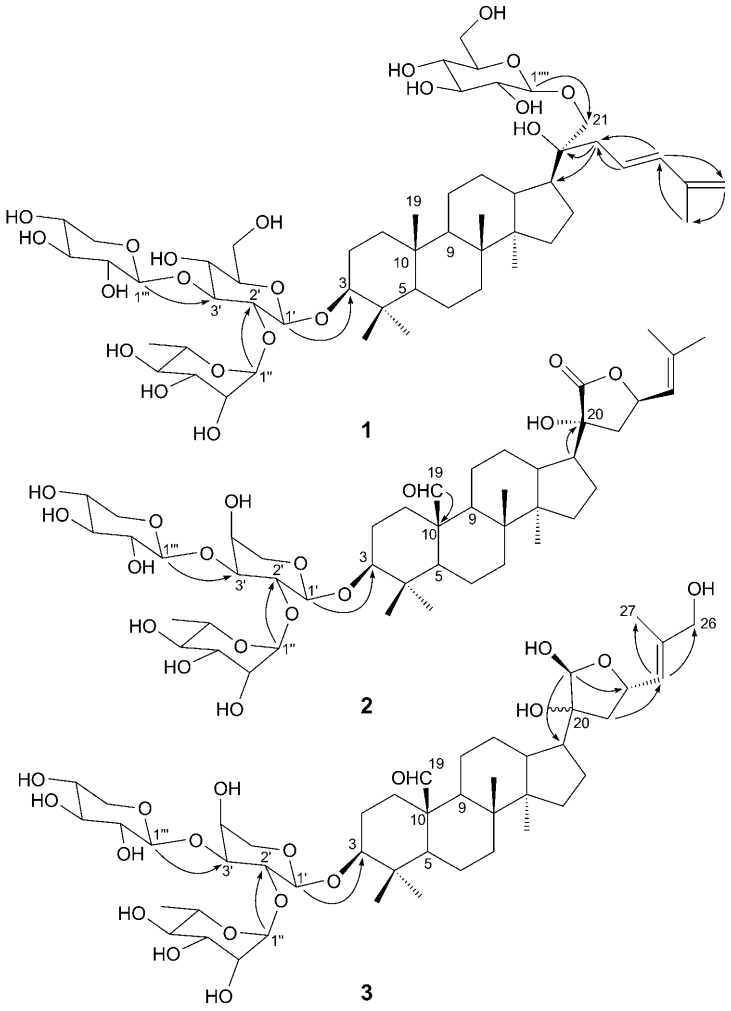

Fig. 2. *Key HMBCs of Compound* **1**–**3**

analysis of the trimethylsilyl ether derivatives of the component monosaccharides led to their configurations. The C-atom signals assignable to the sugar moieties and to C(3) in the ^{13}C-NMR spectrum were superimposable with those of **6**. The linkage sites, and sequences of the three saccharides and of the aglycon were confirmed by the 2D-NMR experiments. The HMBC exhibited the cross-peaks between H−C(1′) of the arabinose and C(3) of the aglycon, H−C(1″) of the rhamnose and C(2′) of the glucose, and H−C(1‴) of the xylose and C(3′) of the glucose. Consequently, **2** was deduced as (20*R*,23*R*)-3*β*,20-dihydroxy-19-oxodammar-24-en-21-oic acid 21,23-lactone 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)][*β*-D-xylopyranosyl-(1 → 3)]-*α*-L-arabinopyranoside.

Compound **3**, a white amorphous powder, showed a peak at *m*/*z* 937.4787 ([*M* + Na]$^+$) in the HR-TOF-MS, suggesting the molecular formula $C_{46}H_{74}O_{18}$. The IR

spectrum (KBr) showed absorptions at 3439 cm^{-1} (OH), 1703 cm^{-1} (C=O), and 1643 cm^{-1} (C=C). Analysis of the ^{1}H- and ^{13}C-NMR spectra established that **3** was a dammarane-type triterpene saponin, too. The ^{1}H-NMR spectrum (*Table 3*) showed signals of five Me groups (δ(H) 0.87 (*s*), 1.07 (*s*), 1.16 (*s*), 1.24 (*s*), 1.81 (*s*)), an olefinic H-atom signal at δ(H) 6.44 (*d*, $J = 8.4$, 1 H), an CHO H-atom signal at δ(H) 10.26 (*s*), and signals due to an α-L-arabinopyranoside moiety (δ(H) 4.87 (*s*, 1 H)), a β-D-xylopyranosyl moiety (δ(H) 4.99 (*d*, $J = 4.8$, 1 H)), an α-L-rhamnopyranosyl moiety (δ(H) 6.14 (*s*, 1 H), 1.56 (*s*, 3 H)). Compound **3** showed a close similarity to **6** in the ^{13}C-NMR spectra. The difference between them was observed in the side chain. The spectrum of **3** exhibited opposite shifts for the O-bearing C-atoms C(26) ($\Delta\delta$ +41.9) and the geminal Me(27) ($\Delta\delta$ −3.8), and a downfield shift for the C(25) ($\Delta\delta$ +4.8), an upfield shift for the C(24) ($\Delta\delta$ −0.9). The differences between a OH function at C(27) or at C(26) are that the signals of C(26) (δ(C) 21.8) and C(27) (δ(C) 60.9) [12] were not observed, and instead, the signals of a Me group (δ(C) 14.2) and a HO−CH_2 group (δ(C) 67.6) were detected in the spectrum of **3**. Moreover, the structure of the side chain also could be confirmed by the HMBC spectrum; the olefinic H-atom signal at δ(H) 6.44 (*d*, $J = 8.4$, H−C(24)) correlated not only with C(26) (δ(C) 67.6), but also with C(27) (δ(C) 14.2) (*Fig. 2*). The NMR data were very similar to those of **6**, and the configuration at C(23) of **3** was established to be (*S*). Derived from ROESY interactions (*Fig. 3*), the configuration at C(21) was (*S*). In the ROESY spectrum, cross-peaks were observed between the olefinic H-atom signal at δ(H) 6.44 (H−C(24)) and the signal at δ(H) 4.22–4.25 (Me(26)), between the signals at δ(H) 5.46–5.49 (H−C(23)) and δ(H) 1.81 (Me(27)). Accordingly, it could be concluded that the aglycon part was (21*S*,23*S*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene. The chemical shifts of the C-atom signals assignable to the sugar moieties closely corresponded to those of **2**. Thus, the structure of **3** was determined as (21*S*,23*S*)-3β,20ξ,21,26-tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside.

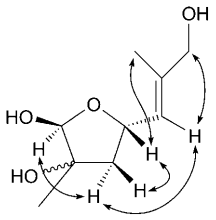

Fig. 3. *Key ROESY correlations in* **3**

The known compounds, **4**–**6**, were determined by physical and spectroscopic evidences, and confirmed by comparing the data with those in the literature.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Group, Co.*), macroporous resin *D101* (*Hebei, Co.*), or *Sephadex LH-20* (*Pharmacia, Co.*). GC: *Agilent Technologies 6890N* apparatus, *OV-17* (30 m × 0.32 mm) column. Prep. HPLC (*Beijing CXTH3000 system*): *P3000* pump, *UV3000* spectrophotometric detector at 203 nm, *Daisogel* C_{18} reversed-phase (RP)

Table 3. *^{1}H- and ^{13}C-NMR Data for* **3**. At 600 and 150 MHz, respectively, in C_5D_5N, *J* in Hz.

	δ(C)	δ(H)	HMBC (H → C)
CH_2(1)	33.7	2.55–2.62 (*m*), 0.68–0.72 (*m*)	
CH_2(2)	27.7	2.03–2.07 (*m*), 1.65–1.68 (*m*)	
H–C(3)	87.2	3.29–3.32 (*m*)	1′
C(4)	40.1		
H–C(5)	54.9	1.13–1.16 (*m*)	3, 4, 6, 10
CH_2(6)	17.8	1.84–1.87 (*m*), 1.62–1.65 (*m*)	
CH_2(7)	34.9	1.66–1.69 (*m*), 1.33–1.36 (*m*)	
C(8)	40.5		
H–C(9)	53.0	1.68–1.71 (*m*)	
C(10)	52.9		
CH_2(11)	22.3	1.69–1.72 (*m*), 1.08–1.12 (*m*)	
CH_2(12)	25.1	2.07–2.10 (*m*), 1.93–1.96 (*m*)	
H–C(13)	41.4	2.23–2.26 (*m*)	
C(14)	50.2		
CH_2(15)	32.2	1.66–1.74 (*m*), 1.17–1.23 (*m*)	
CH_2(16)	27.1	2.16–2.19 (*m*), 1.57–1.60 (*m*)	
H–C(17)	45.1	2.06–2.07 (*m*)	20
Me(18)	16.7	1.16 (*s*)	8, 14, 15, 30
H–C(19)	205.7	10.26 (*s*)	
C(20)	84.7		
H–C(21)	103.0	5.88 (*s*)	17, 23
CH_2(22)	45.1	2.64–2.67 (*m*), 2.67–2.70 (*m*)	24
H–C(23)	73.3	5.46–5.49 (*m*)	
H–C(24)	129.1	6.44 (*d*, *J* = 8.4)	26, 27
C(25)	137.1		
CH_2(26)	67.6	4.22–4.25 (*m*)	24, 25, 27
Me(27)	14.2	1.81 (*s*)	24, 25
Me(28)	26.5	1.24 (*s*)	3, 4, 5, 29
Me(29)	16.1	1.07 (*s*)	3, 4, 5, 28
Me(30)	17.5	0.87 (*s*)	7, 8, 9, 14
Ara–O–C(3)			
H–C(1′)	104.8	4.87 (*s*)	3
H–C(2′)	74.5	4.61–4.63 (*m*)	1″
H–C(3′)	81.8	4.22–4.25 (*m*)	1‴
H–C(4′)	68.5	4.43–4.46 (*m*)	
CH_2(5′)	65.2	4.24–4.27 (*m*), 3.80 (*d*, *J* = 10.2)	
Rha–O–C(2′)			
H–C(1″)	102.1	6.14 (*s*)	2′
H–C(2″)	72.6	4.55–4.58 (*m*)	
H–C(3″)	72.5	4.74 (br. *s*)	
H–C(4″)	74.0	4.25–4.28 (*m*)	
H–C(5″)	70.1	4.54–4.57 (*m*)	
Me(6″)	18.6	1.56 (*s*)	
Xyl–O–C(3′)			
H–C(1‴)	105.3	4.99 (*d*, *J* = 4.8)	3′
H–C(2‴)	74.7	4.90–4.93 (*m*)	
H–C(3‴)	77.8	4.07–4.10 (*m*)	
H–C(4‴)	71.0	4.09–4.12 (*m*)	
CH_2(5‴)	67.1	4.27–4.30 (*m*), 3.63–3.66 (*m*)	

column (10 μm, 30 × 250 nm; flow rate 14.0 ml/min). Optical rotations: *Perkin-Elmer* polarimeter. UV Spectra: *Shimadzu UV-2201* spectrophotometer; MeOH soln.; in λ_{max} (log ε). IR Spectra: *Bruker IFS-55* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AV-600 and ARX-300* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-TOF-MS: *BIC micro TOF-Q* mass spectrometer; in *m/z* (rel.%).

Plant Material. The aerial parts of *Gynostemma pentaphyllum* (THUNB.) MAKINO were collected from Shaanxi Province in P. R. China by *Xi'an Tianyi Co., Ltd*. A voucher specimen of the plant (No. 2007016) at our laboratory was identified by Prof. *Qishi Sun* of Shenyang Pharmaceutical University.

Extraction and Isolation. Dried aerial parts of *Gynostemma pentaphyllum* (THUNB.) MAKINO (8.0 kg) were extracted with 75% EtOH (3 ×), and the H_2O-soluble extract of the plant was separated by a macroporous resin column to obtain the 70% EtOH eluates which, upon drying, afforded the total saponins (80 g). The total saponins were subjected to CC repeatedly on silica gel to provide five fractions *A* – *E*. *Fr. C* was separated into eight fractions, *Frs.* C_a – C_h, by HPLC (*ODS*, 75% MeOH). From *Fr.* C_f, **1** (30 mg) and **4** (200 mg) were obtained as white amorphous powder. *Fr.* C_d was then subjected to prep. RP-HPLC (70% MeOH) to yield **2** (15 mg; t_R 20 min) and **5** (40 mg; t_R 25 min). *Fr.* C_g was passed through a *Sephadex LH-20* column eluted with MeOH, and finally purified by RP-HPLC (*ODS*, 75% MeOH) **3** (35 mg; t_R 36 min), **6** (40 mg; t_R 43 min).

*(20*S*)-3β,20,21-Trihydroxydammara-23,25-diene 3-*O*-[α-*L*-Rhamnopyranosyl-(1 → 2)][β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranosyl-21-*O*-β-*D*-glucopyranoside* (= *(3β,23*E*)-21-(β-*D*-Glucopyranosyloxy)-20-hydroxydammara-23,25-dien-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranoside*; **1**). White amorphous powder. *Libermann – Burchard* and *Molish* reactions were positive. $[\alpha]_D^{28} = -29.0$ ($c = 0.02$, MeOH). UV: 232 (2.80), 283 (0.37). IR: 3423, 2931, 1639, 1384, 1043, 612. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-TOF-MS: 1083.5722 ($[M + Na]^+$; calc. 1083.5716).

*(20*R*,23*R*)-3β,20-Dihydroxy-19-oxodammar-24-en-21-oic Acid 21,23-Lactone 3-*O*-[α-*L*-Rhamnopyranosyl-(1 → 2)][β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranoside* (= *(3β,20*R*,23*R*)-20-Hydroxy-19,21-dioxo-21,23-epoxydammar-24-en-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-xylopyranoside*; **2**). White amorphous powder. *Libermann – Burchard* and *Molish* reactions were positive. $[\alpha]_D^{28} = +3.5$ ($c = 0.05$, MeOH). UV: 213 (1.07), 275 (0.19). IR: 3429, 2926, 1670, 1644, 1384, 1097, 616. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-TOF-MS: 919.4702 ($[M + Na]^+$; calc. 919.4667).

*(21*S*,23*S*)-3β,20ξ,21,26-Tetrahydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-*L*-Rhamnopyranosyl-(1 → 2)][β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranoside* (= *(3β,20ξ,21*S*,23*S*,24*E*)-20,21,26-Trihydroxy-19-oxo-21,23-epoxydammar-24-en-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranoside*; **3**). White amorphous powder. *Libermann – Burchard* and *Molish* reactions were positive. $[\alpha]_D^{28} = +16.0$ ($c = 0.16$, MeOH). UV: 214 (0.99). IR: 3439, 2944, 1703, 1643, 1384, 1042. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-TOF-MS: 937.4787 ($[M + Na]^+$; calc. 937.4773).

Acid Hydrolysis of **1** – **3**. Each compound (4 mg) was heated in 5 ml of 2M HCl/MeOH 4 : 1 at 90° for 6 h in a H_2O bath. After cooling, the mixture was diluted to 20 ml with H_2O and then extracted with $CHCl_3$ (3 × 20 ml). After concentration, each aq. layer was examined by TLC ($CHCl_3$/MeOH/H_2O 55 : 45 : 10) and compared with authentic samples.

Determination of Sugar Components. The monosaccharide subunits were obtained by HCl hydrolysis as described above. The aq. layer was concentrated to dryness to give a residue and dissolved in pyridine (1 ml), and then hexamethyl disilazane (0.4 ml) and Me_3SiCl (0.2 ml) were added to the soln. to obtain the trimethylsilyl (TMS) ethers. The mixture was stirred at 20° for 15 min, and extracted with H_2O (1 ml). Each aq. layer was examined by GC (H_2 flame ionization detector, column temp.: 100 – 280°, temp. program: 10°/min, carrier gas: N_2 (1.5 ml/min), injector and detector temp.: 280°, injection volume: 1 μl, split ratio: 10 : 1). The derivatives of L-arabinose, D-xylose, L-rhamnose, and D-glucose were detected at t_R [min] 6.20, 8.84, 9.76, and 26.59, resp. The standard monosaccharides were subjected to the same reactions and GC analysis under the same conditions.

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