Six New Triterpene Saponins with a 21,23-Lactone Skeleton from *Gynostemma pentaphyllum*

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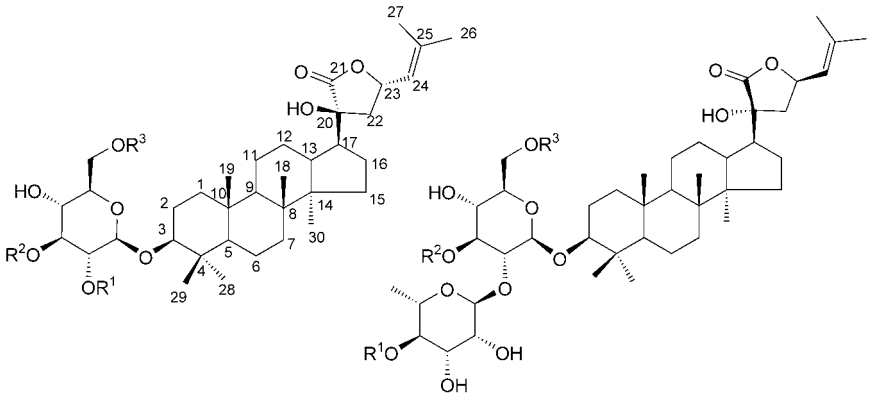
The six new triterpene saponins **1**–**6** with a 21,23-lactone skeleton were isolated from the MeOH extract of the aerial parts of *Gynostemma pentaphyllum*. Their structures were elucidated by 1D- and 2D-NMR-spectra interpretation as well as by chemical degradation.

Introduction. – *Gynostemma pentaphyllum* (THUNB.) MAKINO (Cucurbitaceae) is a well-known ingredient of Chinese traditional medicine used for a variety of reasons that keep people in good health condition especially when getting older [1]. It is a perennial creeping herb distributed in Japan, Korea, the People's Republic of China, and Southeast Asia, was once used as a sweetener in Japan, and has been used as a folk medicine in the People's Republic of China. Its taste is sweet and aromatic, and it can be taken either as tea or in alcohol. Previous investigations of this plant have shown the occurrence of dammarane-type glycosides called the gypenosides that are structurally related to ginseng saponins. Because ginsenosides are well-known biologically active principles in Korean ginseng [1] and have been regarded as the principal bioactive ingredients of *Panax ginseng* C. A. MEYER (Araliaceae) [2], *G. pentaphyllum* has received much attention. 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 [3].

In our study on saponin constituents of the plant *Gynostemma pentaphyllum*, we examined the MeOH extract of the aerial parts of *Gynostemma pentaphyllum* and isolated the six new triterpene saponins **1**–**6** having a 21,23-lactone skeleton. In this paper, we report their isolation and structure characterization. Their structure 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 data.

Results and Discussion. – Compound **1** was obtained as an amorphous powder. The molecular formula was determined as $C_{49}H_{78}O_{18}$ from its quasimolecular-ion peak at *m*/*z* 977.5097 ($[M+Na]^+$) in the HR-ESI-MS. On the basis of its ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*), the aglycon of **1** was identified as (3β)-3,20,23-trihydroxydammar-24-en-24-oic acid 21,23-lactone [4]. Glycosidation of the alcoholic function at C(3) was indicated by the significant downfield shift observed for the C(3) signal of **1**, compared with the corresponding signal of a model compound reported in [5]. Acid hydrolysis of **1** yielded D-glucose, D-xylose, and L-rhamnose (=6-deoxy-L-mannose) in



1 R^1 = Rha, R^2 = Xyl, R^3 = MeCO
2 R^1 = H, R^2 = Xyl, R^3 = MeCO
3 R^1 = Rha, R^2 = Xyl, R^3 = H
4 R^1 = H, R^2 = Xyl, R^3 = H
5 R^1 = Rha, R^2 = Glc, R^3 = H
6 R^1 = MeCO, R^2 = Xyl, R^3 = MeCO

a ratio of 1:1:1, as established by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the standard sugars. The structure of **1** was established as (3*β*,20*S*,23*S*)-3-{{*O*-6-deoxy-*α*-L-manno-pyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-6-*O*-acetyl-*β*-D-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic acid 21,23-lactone.

The ^{13}C-NMR and DEPT spectra of **1** gave 49 signals, of which 19 were assigned to the sugar moiety and 30 to a triterpene moiety. The ^{1}H-NMR spectrum of **1** showed 7 *s* at *δ* 0.80–1.71 assignable to the aglycon Me groups, two of which were diagnostic for Me groups linked to an sp^2 C-atom (*δ* 1.71 and 1.64). The ^{1}H-NMR spectrum of **1** also showed one acetyl methyl *s* at *δ* 2.02, and an olefinic proton at *δ* 5.60 (*d*, *J* = 8.8 Hz). A 3*β*-oxy substitution was evident from the chemical shift and the *J* values of the proton ascribable to H_α–C(3) at *δ* 3.32 (*dd*, *J* = 11.6, 3.9 Hz).

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*-D-configuration for the glucosyl unit (*δ* 4.83 (*d*, *J* = 8.3 Hz, H–C(1) of Glc); *δ* 105.0 (C(1) of Glc)), a *β*-D-configuration for the xylosyl unit (*δ* 4.99 (*d*, *J* = 7.7 Hz, H–C(1) of Xyl); *δ* 105.0 (C(1) of Xyl)), and an *α*-L-configuration for the rhamnosyl unit (*δ* 6.40 (br. *s*, H–C(1) of Rha); *δ* 101.9 (C(1) of Rha)). The ^{13}C-NMR data allowed the assignment of the pyranose forms of D-glucose, D-xylose, and L-rhamnose. All ^{1}H- and ^{13}C-NMR signals of the three sugar units in **1** were assigned by ^{1}H,^{1}H-COSY, HMQC, and HMBC data. The linkage sites and sequences of the three saccharide units, of the Ac group, and of the aglycon were deduced from an HMBC experiment. Correlations were observed between H–C(1) of Glc and C(3) of the aglycon, H–C(1) of Rha and H–C(2) of Glc, H–C(1) of Xyl and C(3) of Glc, and H–C(6) of Glc and the C=O of the Ac group (*Fig. 1*).

The absolute configuration at C(20) of gypenosides has been determined by their ^{13}C-NMR chemical shift value ((*S*) configuration, *δ*(C(20)) *ca.* 79.1; (*R*) configuration, *δ*(C(20)) *ca.* 81.3) [4]. According to this rule, the configuration at C(20) of **1** was determined as (*S*) (*δ*(C(20)) 79.1). Therefore, the *β*-configuration for the OH group was established (*Fig. 1*). The configuration at C(23) of **1** was derived from NOESY interactions (*Fig. 2*) and NMR analysis. The chemical environment of H_β–C(22) and H_α–C(22) is very similar to each other except for the impact of OH–C(20), which shifted the signal of H_β–C(22) to a lower field than that of H_α–C(22). NOESY Correlations H–C(23)/H_β–C(22), H–C(24)/H_α–C(22), Me(26)/H–C(24) and Me(27)/H–C(23) were observed, suggesting that H–C(23) should be located on the *β*-side and the 2-methylpropenyl group on the *α*-side. Thus, the absolute configuration at C(23) was established as (*S*).

Table 1. *^{1}H-NMR* (500 MHz) *Data of* **1**–**3** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm, J in Hz.

	1	**2**	**3**
CH_2(1)	1.61 (*m*), 0.98 (*m*)	1.62 (*m*), 1.00 (*m*)	1.52 (*m*), 0.90 (*m*)
CH_2(2)	2.31 (*m*), 1.91 (*m*)	2.35 (*m*), 1.93 (*m*)	2.33 (*m*), 1.92 (*m*)
H–C(3)	3.32 (*dd*, *J* = 11.6, 3.9)	3.32 (*dd*, *J* = 11.6, 3.9)	3.49 (*dd*, *J* = 11.7, 4.0)
H–C(5)	0.78 (*d*, *J* = 11.7)	0.80 (*d*, *J* = 11.4)	0.80 (*d*, *J* = 11.3)
CH_2(6)	1.50 (*m*), 1.39 (*m*)	1.50 (*m*), 1.39 (*m*)	1.56 (*m*), 1.47 (*m*)
CH_2(7)	1.50 (*m*), 1.23 (*m*)	1.50 (*m*), 1.25 (*m*)	1.56 (*m*), 1.31 (*m*)
H–C(9)	1.32 (*m*)	1.35 (*m*)	1.32 (*m*)
CH_2(11)	1.58 (*m*), 1.30 (*m*)	1.58 (*m*), 1.30 (*m*)	1.50 (*m*), 1.31 (*m*)
CH_2(12)	2.08 (*m*), 1.73 (*m*)	2.10 (*m*), 1.75 (*m*)	2.10 (*m*), 1.75 (*m*)
H–C(13)	2.08 (*m*)	1.85 (*m*)	2.15 (*m*)
CH_2(15)	1.65 (*m*), 1.12 (*m*)	1.60 (*m*), 1.11 (*m*)	1.72 (*m*), 1.20 (*m*)
CH_2(16)	2.42 (*d*, *J* = 11.1), 1.35 (*m*)	2.51 (*d*, *J* = 11.5), 1.49 (*m*)	2.50 (*d*, *J* = 11.5), 1.42 (*m*)
H–C(17)	2.52 (*td*, *J* = 10.0, 5.0)	2.70 (*m*)	2.61 (*m*)
Me(18)	0.94 (*s*)	1.01 (*s*)	1.01 (*s*)
Me(19)	0.80 (*s*)	0.88 (*s*)	0.85 (*s*)
CH_2(22)	2.70 (*dd*, *J* = 13.3, 7.4), 2.30 (*dd*, *J* = 13.3, 6.0)	2.60 (*dd*, *J* = 13.2, 5.5), 2.12 (*m*)	2.80 (*dd*, *J* = 13.2, 7.3), 2.39 (*dd*, *J* = 13.2, 5.8)
H–C(23)	5.48 (*m*)	5.71 (*m*)	5.54 (*m*)
H–C(24)	5.60 (*d*, *J* = 8.8)	5.41 (*d*, *J* = 8.7)	5.62 (*d*, *J* = 8.9)
Me(26)	1.71 (*s*)	1.70 (*s*)	1.71 (*s*)
Me(27)	1.64 (*s*)	1.65 (*s*)	1.78 (*s*)
Me(28)	1.26 (*s*)	1.25 (*s*)	1.32 (*s*)
Me(29)	1.17 (*s*)	1.18 (*s*)	1.22 (*s*)
Me(30)	0.91 (*s*)	0.91 (*s*)	0.98 (*s*)
Glc–O–C(3)			
H–C(1)	4.83 (*d*, *J* = 8.3)	4.84 (*d*, *J* = 7.4)	5.00 (*d*, *J* = 7.3)
H–C(2)	4.16 (*m*)	4.20 (*t*, *J* = 7.8)	4.30 (*m*)
H–C(3)	4.11 (*m*)	4.18 (*m*)	4.25 (*m*)
H–C(4)	3.82 (*t*, *J* = 9.1)	3.81 (*t*, *J* = 9.0)	4.08 (*m*)
H–C(5)	3.92 (*m*)	3.94 (*m*)	3.98 (*m*)
CH_2(6)	4.80 (*m*), 4.65 (*m*)	4.82 (*m*), 4.69 (*m*)	4.61 (*d*, *J* = 10.6), 4.37 (*m*)
AcO–C(6)	2.02 (*s*)	2.02 (*s*)	
Rha			
H–C(1)	6.40 (br. *s*)	6.41 (br. *s*)	6.50 (br. *s*)
H–C(2)	4.77 (br. *s*)	4.78 (br. *s*)	4.88 (*m*)
H–C(3)	4.58 (*dd*, *J* = 9.2, 2.8)	4.58 (*dd*, *J* = 9.2, 2.8)	4.68 (*dd*, *J* = 9.2, 3.1)
H–C(4)	4.29 (*m*)	4.29 (*m*)	4.36 (*m*)
H–C(5)	4.70 (*m*)	4.71 (*m*)	4.82 (*m*)
Me(6)	1.68 (*d*, *J* = 6.0)	1.68 (*d*, *J* = 6.0)	1.74 (*d*, *J* = 6.0)
Xyl			
H–C(1)	4.99 (*d*, *J* = 7.7)	4.98 (*d*, *J* = 7.5)	5.09 (*d*, *J* = 7.5)
H–C(2)	3.94 (*m*)	3.95 (*m*)	4.02 (*m*)
H–C(3)	4.06 (*m*)	4.08 (*m*)	4.15 (*m*)
H–C(4)	4.10 (*m*)	4.12 (*m*)	4.08 (*m*)
CH_2(5)	4.26 (*m*), 3.70 (*t*, *J* = 10.6)	4.29 (*m*), 3.70 (*t*, *J* = 10.6)	4.35 (*m*), 3.78 (*t*, *J* = 10.6)

Compound **2** was isolated as an amorphous powder. Its molecular formula was established as $C_{49}H_{78}O_{18}$ from the quasimolecular-ion peak at m/z 977.5098 ($[M+Na]^+$) in the HR-ESI-MS. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** (*Tables 1* and *2*) indicated an identical saccharide part and structural similarity in the

Table 2. *^{13}C-NMR* (125 MHz) *Data of* **1**–**6** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm.

	1	**2**	**3**	**4**	**5**	**6**
C(1)	39.7	39.9	39.8	39.8	40.0	40.0
C(2)	26.9	26.9	27.0	27.0	27.2	27.2
C(3)	89.6	89.6	89.0	89.0	89.0	89.8
C(4)	39.8	39.8	39.8	39.8	40.0	40.0
C(5)	56.8	56.9	56.8	56.8	56.9	57.1
C(6)	18.5	18.6	18.5	18.5	18.7	18.8
C(7)	35.7	35.8	35.8	35.8	36.0	35.8
C(8)	40.9	40.9	40.8	40.8	41.0	41.1
C(9)	51.3	51.3	51.2	51.2	51.4	51.5
C(10)	37.1	37.3	37.1	37.2	37.3	37.5
C(11)	21.8	21.9	21.8	21.9	22.0	22.1
C(12)	25.8	26.4	25.9	26.3	26.1	26.6
C(13)	43.4	45.1	43.4	45.1	43.6	45.3
C(14)	50.7	50.3	50.7	50.3	50.9	50.5
C(15)	31.7	31.9	31.8	31.8	32.0	32.0
C(16)	27.4	28.1	27.4	28.1	27.6	28.2
C(17)	46.0	45.5	45.9	45.4	46.1	45.6
C(18)	15.7	15.8	15.7	15.7	15.9	16.0
C(19)	16.6	16.7	16.7	16.7	16.8	17.0
C(20)	79.1	81.2	79.1	81.2	79.3	81.5
C(21)	179.4	178.4	179.5	178.4	179.7	178.6
C(22)	40.7	39.2	40.8	39.2	41.0	39.4
C(23)	74.2	75.3	74.2	75.3	74.2	75.3
C(24)	125.5	124.1	125.5	124.1	125.7	124.2
C(25)	138.5	139.6	138.5	139.5	138.7	139.8
C(26)	25.6	25.7	25.7	25.7	25.9	25.7
C(27)	18.2	18.3	18.2	18.3	18.4	18.3
C(28)	27.8	28.0	28.0	28.0	28.2	28.2
C(29)	16.8	16.9	16.9	17.0	17.1	16.9
C(30)	16.6	16.4	16.6	16.4	16.8	16.6
Glc–O–C(3)						
C(1)	105.0	105.01	105.1	105.1	105.2	105.0
C(2)	76.8	76.8	77.1	77.1	77.1	75.8
C(3)	87.9	87.9	88.3	88.3	89.8	88.3
C(4)	69.8	70.0	69.9	69.9	70.0	70.0
C(5)	74.5	74.5	78.1	78.1	78.1	74.8
C(6)	64.2	64.2	62.7	62.7	62.6	64.3
Me*C*OO	170.7	170.8				171.0
*Me*COO	20.8	20.9				21.2
Rha						
C(1)	101.9	101.9	101.9	101.9	101.8	101.4
C(2)	72.6	72.6	72.7	72.7	72.6	72.2
C(3)	72.4	72.4	72.5	72.5	72.6	70.1
C(4)	74.0	74.0	74.0	74.0	74.1	76.1
C(5)	69.9	70.0	69.9	69.9	70.1	67.1
C(6)	18.7	18.7	18.7	18.7	18.8	18.2
Me*C*OO						171.0
*Me*COO						20.9
Xyl or Glc	Xyl	Xyl	Xyl	Xyl	Glc	Xyl
C(1)	105.0	105.0	105.0	105.0	104.1	105.3
C(2)	74.9	74.9	75.0	75.0	75.4	74.6
C(3)	78.4	78.4	78.4	78.4	78.6	78.8
C(4)	70.7	70.7	70.7	70.7	71.7	70.7
C(5)	67.3	67.4	67.4	67.4	78.8	67.4
C(6)					62.9	

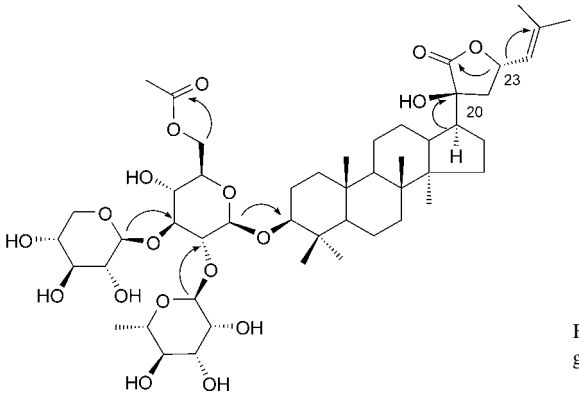


Fig. 1. *Key HMBC correlations in* **1**. Analogously for **2** having inverted configuration at C(20) and C(23).

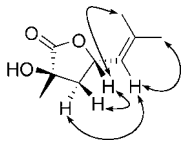


Fig. 2. *Key NOE correlations in* **1**

aglycon moiety. The structure of **2** was established as the diastereoisomeric (3β,20*R*,23*R*)-3-{{*O*-6-deoxy-α-L-mannopyranosyl-(1 $\rightarrow$ 2)-*O*-[β-D-xylopyranosyl-(1 $\rightarrow$ 3)]-6-*O*-acetyl-β-D-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic acid 21,23-lactone.

The main difference between **1** and **2** were the ^{1}H-NMR chemical shifts of CH_2(22) (δ 2.12 and 2.60 in **2** *vs.* δ 2.30 and 2.70 in **1**), H–C(23) (δ 5.71 in **2** *vs.* δ 5.48 in **1**), and H–C(24) (δ 5.41 in **2** *vs.* δ 5.60 in **1**), and the ^{13}C-NMR signals of C(20), C(21), and C(22). These NMR difference established the configuration at C(20) of **2** as (*R*) [4]. The configuration at C(23) of **2** was also derived from NOESY interactions (*Fig. 3*) and NMR analysis. The α-positioned OH–C(20) shifted the signal of H_α–C(22) to a lower field than that of H_β–C(22). NOESY Correlations H–C(23)/H_α–C(22), H–C(24)/H_β–C(22), Me(26)/H–C(24), and Me(27)/H–C(23) were observed, suggesting that H–C(23) should be located on the α-side and the 2-methylpropenyl group on the β-side. Thus, the absolute configuration at C(23) was established as (*R*).

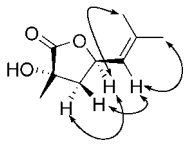


Fig. 3. *Key NOE correlations in* **2**

The quasimolecular-ion peak of compound **3** at *m*/*z* 935.4992 ([M + Na]$^+$) in the HR-ESI-MS established its formula as $C_{47}H_{76}O_{17}$. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** and **1** (*Tables 1* and *2*) indicated that they had the same aglycon moiety. Hydrolysis of compound **3** yielded D-glucose, D-xylose, and L-rhamnose in a ratio of 1:1:1. The only difference between **3** and **1** was that the 6-*O*-acetyl group of Glc in **1** was replaced by a free OH–C(6) of Glc in **3**. The linkage sites and sequences of the saccharide units and of the aglycon were also determined by an HMBC experiment

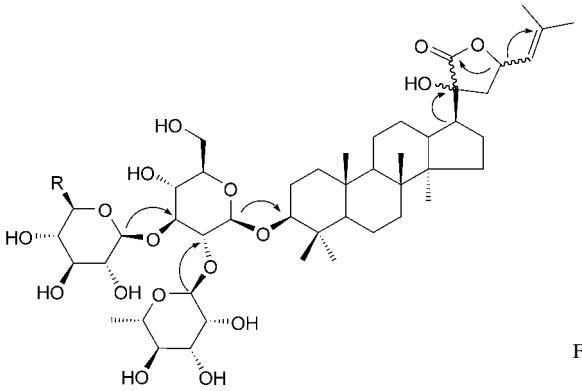


Fig. 4. *Key HMBC correlations in* **3** (R = H), **4** (R = H), *and* **5** (R = CH_2OH)

(*Fig. 4*). Therefore, **3** was identified as (3*β*,20*S*,23*S*)-3-{{*O*-6-deoxy-*α*-L-mannopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic acid 21,23-lactone.

The molecular formula of **4** was established as $C_{47}H_{76}O_{17}$ from the quasimolecular-ion peak at *m*/*z* 935.4994 ($[M + Na]^+$) in the HR-ESI-MS. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** and **2** (*Tables 1–3*) indicated that they had an identical aglycon moiety. The major difference between **4** and **2** was that the 6-*O*-acetyl group of Gly in **2** was replaced by a free OH−C(6) of Glc in **4**. Hydrolysis of compound **4** yielded D-glucose, D-xylose, and L-rhamnose in a ratio of 1:1:1. The linkage sites and sequences of the three saccharide units and of the aglycon were also determined by an HMBC experiment (*Fig. 4*). Thus, the structure of **4** was determined as (3*β*,20*R*,23*R*)-3-{{*O*-6-deoxy-*α*-L-mannopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic acid 21,23-lactone.

The quasimolecular-ion peak of **5** at *m*/*z* 965.5098 ($[M + Na]^+$) in the HR-ESI-MS established its formula as $C_{48}H_{78}O_{18}$. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **5** and **3** (*Tables 1–3*) indicated that they had the same aglycon moiety. Hydrosis of compound **5** yielded D-glucose and L-rhamnose in a ratio of 2:1. The differences between **5** and **3** were that the xylosyl group in **3** was replaced by a glucosyl group in **5**. The *β*-D-glucosyl linkage was defined on the basis of the *J* value of its anomeric proton (*J* = 7.5 Hz) [7]. The linkage sites and sequences of the three saccharide units and of the aglycon were also determined by an HMBC experiment (*Fig. 4*). Thus, the structure of **5** was determined as (3*β*,20*S*,23*S*)-3-{{*O*-6-deoxy-*α*-L-mannopyranosyl-(1 → 2)-*O*-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic acid 21,23-lactone.

The HR-ESI-MS of **6** showed a quasimolecular-ion peak at *m*/*z* 1019.5199 ($[M + Na]^+$), establishing the molecular formula as $C_{51}H_{80}O_{19}$. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1–3*) established that **6** had the same aglycon and saccharide units as **2**. Comparison of the ^{1}H- and ^{13}C-NMR data of **6** and **2** revealed that the only difference between **6** and **2** was that **6** had one more Ac group. The AcO group was located at C(4) of Rha as deduced from its HMBC cross-peak between the quaternary C=O signal (*δ* 171.0) and H−C(4) of Rha (*δ* 5.78, *t*, *J* = 9.7 Hz) (*Fig. 5*). On the basis of the above results, the structure of **6** was elucidated as (3*β*,20*R*,23*R*)-3-{{*O*-4-*O*-acetyl-6-deoxy-*α*-

Table 3. *^{1}H-NMR* (500 MHz) *Data of* **4**–**6** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm, J in Hz.

	4	**5**	**6**
CH_2(1)	1.46 (*m*), 0.83 (*m*)	1.42 (*m*), 0.81 (*m*)	1.67 (*m*), 1.01 (*m*)
CH_2(2)	2.25 (*m*), 1.82 (*m*)	2.23 (*m*), 1.82 (*m*)	2.23 (*m*), 1.82 (*m*)
H–C(3)	3.40 (br. *d*, J = 11.3)	3.36 (br. *d*, J = 11.3)	3.37 (*dd*, J = 11.7, 4.0)
H–C(5)	0.75 (*d*, J = 11.0)	0.70 (*d*, J = 11.2)	0.82 (*d*, J = 11.3)
CH_2(6)	1.48 (*m*), 1.40 (*m*)	1.47 (*m*), 1.38 (*m*)	1.48 (*m*), 1.38 (*m*)
CH_2(7)	1.48 (*m*), 1.21 (*m*)	1.48 (*m*), 1.21 (*m*)	1.56 (*m*), 1.25 (*m*)
H–C(9)	1.25 (*m*)	1.22 (*m*)	1.37 (*m*)
CH_2(11)	1.44 (*m*), 1.25 (*m*)	1.40 (*m*), 1.23 (*m*)	1.50 (*m*), 1.31 (*m*)
CH_2(12)	2.04 (*m*), 1.65 (*m*)	2.00 (*m*), 1.65 (*m*)	2.08 (*m*), 1.70 (*m*)
H–C(13)	1.81 (*m*)	2.06 (*m*)	1.89 (*m*)
CH_2(15)	1.55 (*m*), 1.10 (*m*)	1.65 (*m*), 1.10 (*m*)	1.61 (*m*), 1.11 (*m*)
CH_2(16)	2.50 (*d*, J = 9.7), 1.42 (*m*)	2.40 (*d*, J = 11.0), 1.35 (*m*)	2.53 (*d*, J = 11.6), 1.47 (*m*)
H–C(17)	2.69 (*m*)	2.52 (*m*)	2.71 (*m*)
Me(18)	0.99 (*s*)	0.92 (*s*)	1.02 (*s*)
Me(19)	0.81 (*s*)	0.77 (*s*)	0.89 (*s*)
CH_2(22)	2.58 (*dd*, J = 13.1, 5.1), 2.11 (*m*)	2.71 (*dd*, J = 12.8, 7.2), 2.30 (*m*)	2.62 (*dd*, J = 13.1, 5.4), 2.15 (*m*)
H–C(23)	5.69 (*m*)	5.46 (*m*)	5.71 (*m*)
H–C(24)	5.44 (*d*, J = 8.6)	5.58 (*d*, J = 8.4)	5.45 (*d*, J = 8.6)
Me(26)	1.69 (*s*)	1.62 (*s*)	1.70 (*s*)
Me(27)	1.63 (*s*)	1.61 (*s*)	1.65 (*s*)
Me(28)	1.20 (*s*)	1.22 (*s*)	1.27 (*s*)
Me(29)	1.18 (*s*)	1.15 (*s*)	1.17 (*s*)
Me(30)	0.91 (*s*)	0.89 (*s*)	0.95 (*s*)
Glc–O–C(3)			
H–C(1)	4.90 (*d*, J = 7.4)	4.82 (*d*, J = 7.3)	4.85 (*d*, J = 8.6)
H–C(2)	4.20 (*m*)	4.21 (*m*)	4.20 (*m*)
H–C(3)	4.12 (*m*)	4.20 (*m*)	4.17 (*m*)
H–C(4)	3.98 (*m*)	4.08 (*m*)	3.82 (*t*, J = 9.8)
H–C(5)	3.88 (*m*)	3.88 (*m*)	3.98 (*m*)
CH_2(6)	4.50 (*m*), 4.28 (*m*)	4.55 (*m*), 4.20 (*m*)	4.82 (*m*), 4.70 (*m*)
Ac–O–C(6)			2.12 (*s*)
Rha			
H–C(1)	6.40 (br. *s*)	6.45 (br. *s*)	6.58 (br. *s*)
H–C(2)	4.78 (*m*)	4.81 (*m*)	4.73 (br. *s*)
H–C(3)	4.56 (*m*)	4.59 (*m*)	4.62 (*m*)
H–C(4)	4.27 (*m*)	4.28 (*m*)	5.78 (*t*, J = 9.7)
H–C(5)	4.71 (*m*)	4.72 (*m*)	4.82 (*m*)
Me(6)	1.66 (*d*, J = 6.0)	1.67 (*d*, J = 6.2)	1.41 (*d*, J = 6.0)
AcO–C(6)			2.01 (*s*)
	Xyl	Glc	Xyl
H–C(1)	5.00 (*d*, J = 7.5)	5.10 (*d*, J = 7.5)	4.96 (*d*, J = 7.5)
H–C(2)	3.92 (*m*)	4.00 (*m*)	3.97 (*m*)
H–C(3)	4.04 (*m*)	4.17 (*m*)	4.07 (*m*)
H–C(4)	4.08 (*m*)	4.07 (*m*)	4.11 (*m*)
H–C(5)	4.21 (*m*), 3.70 (*t*, J = 10.1)	4.00 (*m*)	4.26 (*m*), 3.70 (*t*, J = 10.7)
CH_2(6)		4.48 (*d*, J = 11.1), 4.21 (*m*)	

L-mannopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-6-*O*-acetyl-β-D-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic acid 21,23-lactone. The structure was confirmed by HMQC and HMBC experiments.

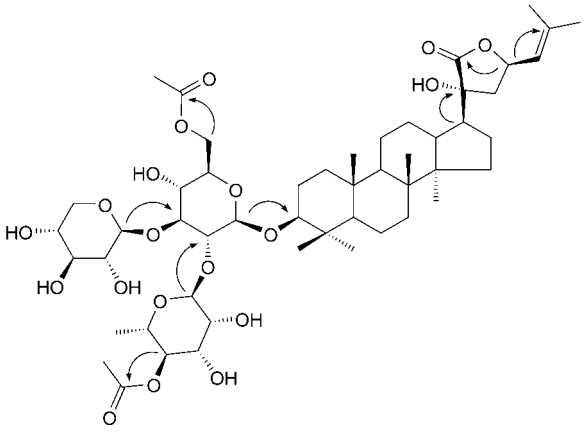


Fig. 5. *Key HMBC correlations in* **6**

Accordingly, as a result of this investigation, the structures of the six new triterpene saponins **1**–**6** with a 21,23-lactone skeleton, obtained from *G. pentaphyllum*, were identified, and their absolute configuration was deduced for the first time.

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Experiment Part

General. All solvents used were of chemical grade (*Shanghai Chemical Plant*). TLC: precoated silica-gel GF_{254} plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): silica gel (200–300 mesh), *MCI Gel CHP20P* (75–150 μm; *Mitsubishi Kasei Chemical Industries*), C_{18} reversed-phase silica gel (20–45 μm; *Fuji Silysia Chemical Ltd.*), or *Sephadex LH-20* (*Pharmacia*); FC = flash chromatography. Optical rotations: *Perkin-Elmer 341* polarimeter. NMR Spectra: *Bruker AMX-500* spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C); conventional pulse sequences for NOESY, HMQC, and HMBC; 200 ms mixing time for NOESY; chemical shifts δ in ppm, J in Hz; (D_5)pyridine solns. (δ(C) 150.3, 155.9, 123.9). HR-ESI-MS (positive mode). *Bruker-Atex-III* spectrometer. GC/EI-MS: *Shimadzu GC-MS-QP5050A*; *db-1* column, 0.25 mm (i.d.) × 30 m; column temp. 200°; injection temp. 250°; carrier gas N_2, flow rate of 32.2 ml/min; EI-MS detector.

Plant Material. The aerial parts of *Gynostemma pentaphyllum* (THUNB.) MAKINO was collected in Hunan Province, P. R. 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 the Chinese National Center for Drug Screening, Shanghai, P. R. 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 r.t. Evaporation of MeOH left a dark residue (70 g). The residue was subjected to CC (silica gel, $CHCl_3$/MeOH 100:10, 100:20, 100:30, 100:50): *Fractions A–D. Fr. B* (15 g) was passed through a CC (*Sephadex LH-20*, MeOH) to remove flavonoids. Then, the fraction was subjected to CC (*MCI* gel, H_2O/acetone 9:1 → 2:1): *Fr. B.1* to *B.8*). *Fr. B.1* (1.0 g) was subjected to FC (C_{18} SiO_2, MeOH/H_2O 60:40): **1** (320 mg). *Fr. B.2* (1.2 g) was subjected to FC (C_{18} silica gel, MeOH/H_2O 55:45): **2** (260 mg). *Fr. B.3* (900 mg) was subjected to FC (C_{18} silica gel, MeOH/H_2O 60:40): **3** (130 mg). *Fr. B.4* (1.6 g) was subjected to FC (C_{18} silica gel, MeOH/H_2O 70:30): **4** (158 mg) and **6** (10 mg). *Fr. C* (10 g) was passed over CC (*Sephadex LH-20*, MeOH) to remove flavonoids, and then was subjected to CC (*MCI* gel, H_2O/acetone 9:1 → 2:1): *Fr. C.1* to *C.8*. *Fr. C.2* (1.3 g) was further purified by CC (silica gel, $CHCl_3$/MeOH/H_2O 6:1:0.1): **5** (23 mg).

*(3β,20S,23S)-3-{[*O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O*-[β-*D*-xylopyranosyl-(1 → 3)]-6-*O*-acetyl-β-*D*-glucopyranosyl}oxy]-20,23-dihydroxydammar-24-en-21-oic Acid 21,23-Lactone* (**1**): White amorphous powder.

$[\alpha]_D^{20} = -13.5$ ($c = 1.52$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 977.5097 ($[M+Na]^+$, $C_{49}H_{78}NaO_{18}^+$; calc. 977.5085).

*(3β,20*R*,23*R*)-3-{{*O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*[β-*D*-xylopyranosyl-(1 → 3)]-6-*O*-acetyl-β-*D*-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic Acid 21,23-Lactone* (**2**): White amorphous powder. $[\alpha]_D^{20} = +9.9$ ($c = 1.34$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 977.5098 ($[M+Na]^+$, $C_{49}H_{78}NaO_{18}^+$; calc. 977.5085).

*(3β,20*S*,23*S*)-3-{{*O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic Acid 21,23-Lactone* (**3**): White amorphous powder. $[\alpha]_D^{20} = -15.3$ ($c = 1.04$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 935.4992 ($[M+Na]^+$, $C_{47}H_{76}NaO_{17}^+$; calc. 935.4980).

*(3β,20*R*,23*R*)-3-{{*O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic Acid 21,23-Lactone* (**4**): White amorphous powder. $[\alpha]_D^{20} = +136.8$ ($c = 0.54$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. HR-ESI-MS (pos.): 935.4994 ($[M+Na]^+$, $C_{47}H_{76}NaO_{17}^+$; calc. 935.4980).

*(3β,20*S*,23*S*)-3-{{*O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*[β-*D*-glucopyranosyl-(1 → 3)]-β-*D*-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic Acid 21,23-Lactone* (**5**): White amorphous powder. $[\alpha]_D^{20} = -9.3$ ($c = 0.90$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. HR-ESI-MS (pos.): 965.5098 ($[M+Na]^+$, $C_{48}H_{78}NaO_{18}^+$; calc. 965.5086).

*(3β,20*R*,23*R*)-3-{{*O*-4-O-Acetyl-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*[β-*D*-xylopyranosyl-(1 → 3)]-6-*O*-acetyl-β-*D*-glucopyranosyl}oxy}-20,23-dihydroxydammar-24-en-21-oic Acid 21,23-Lactone* (**6**): White amorphous powder. $[\alpha]_D^{20} = 0$ ($c = 0.25$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. HR-ESI-MS (pos.): 1019.5199 ($[M+Na]^+$, $C_{51}H_{80}NaO_{19}^+$; calc. 1019.5191).

Acid Hydrolysis [8]. Compounds **1**–**6** (4 mg each) in 10% HCl soln./dioxane 1:1 (1 ml) were heated at 80° for 4 h in a water bath. The mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (3 × 1 ml). After concentration, each aq. layer (monosaccharide portion) 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 sugar residue was then dissolved in anh. pyridine (1 ml) under Ar, L-leucine methyl ester hydrochloride (2 mg), was added, and the mixture was warmed at 60° for 1 h. Then $NaBH_4$ (2 mg) was added and the mixture stirred for 1 h at r.t. Then chlorotrimethylsilane (0.2 ml; *Shengyu Chemical Ltd.*, Shanghai, China) was added, and warming at 60° was continued for another 30 min. The silylated leucine derivatives were analyzed by GC (column temp. 200°, injection temp. 250°, carrier gas N_2, flow rate of 32.2 ml/min); D-glucose, D-xylose, and L-rhamnose at t_R 13.95, 8.23, and 8.87 min, resp.

Bioassay. Antitumor activities were evaluated by the SRB (sulforhodamine B) assay [9] with 5-FU as the positive control. None of the compounds was active against stomach cancer cells SGC-7901 and liver cancer cells BEL-7402.

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