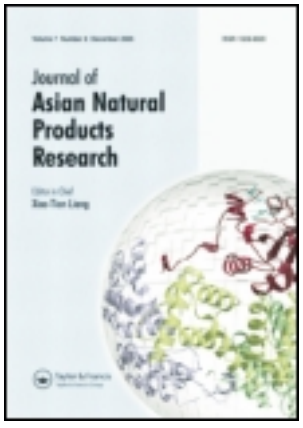

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Triterpenoid saponins from *Gynostemma pentaphyllum*

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Four new dammarane-type triterpene saponins, **1**–**4**, were isolated from the aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino. Their structural elucidations were accomplished mainly on the basis of spectroscopic methods, such as IR, HR-TOF-MS, and NMR. Compounds **1**–**4** showed moderate cytotoxic activities against cancer cell lines HL-60, Colon205, and Du145 *in vitro*.

Keywords: *Gynostemma pentaphyllum* (Thunb.) Makino; dammarane-type; triterpenoid saponin; cytotoxic activities

1. Introduction

Gynostemma pentaphyllum (Thunb.) Makino is a herbal medicine with anticancer activity [1], widely distributed in China, Korea, and Japan. The biologically active constituents are dammarane-type glycosides, called gypenosides, which are structurally correlated to the ginseng saponins [2–5]. In our previous 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, *G. pentaphyllum* was investigated.

From the extract of the aerial parts of this plant, four new dammarane saponins were characterized as (23*S*)-21*R*-*O*-*n*-butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside (**1**), (23*S*)-21*S*-*O*-*n*-butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside (**2**), (23*S*)-21*R*-*O*-*n*-butyl-19-oxo-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside (**3**), and (23*S*)-21*S*-*O*-*n*-butyl-19-oxo-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside (**4**). In this paper, we report the structural elucidation of the new dammarane-type saponins (Figure 1).

2. Results and discussion

Compound **1** was obtained as white amorphous powder with mp 208–210°C and $[\alpha]_D^{20}$ +11.9 ($c = 0.14$, MeOH). Its molecular formula, $C_{51}H_{86}O_{17}$, was deduced from the HR-TOF-MS at *m/z* 993.5742 $[M + Na]^+$. The IR spectrum (KBr) showed absorption at 3418 cm^{-1}

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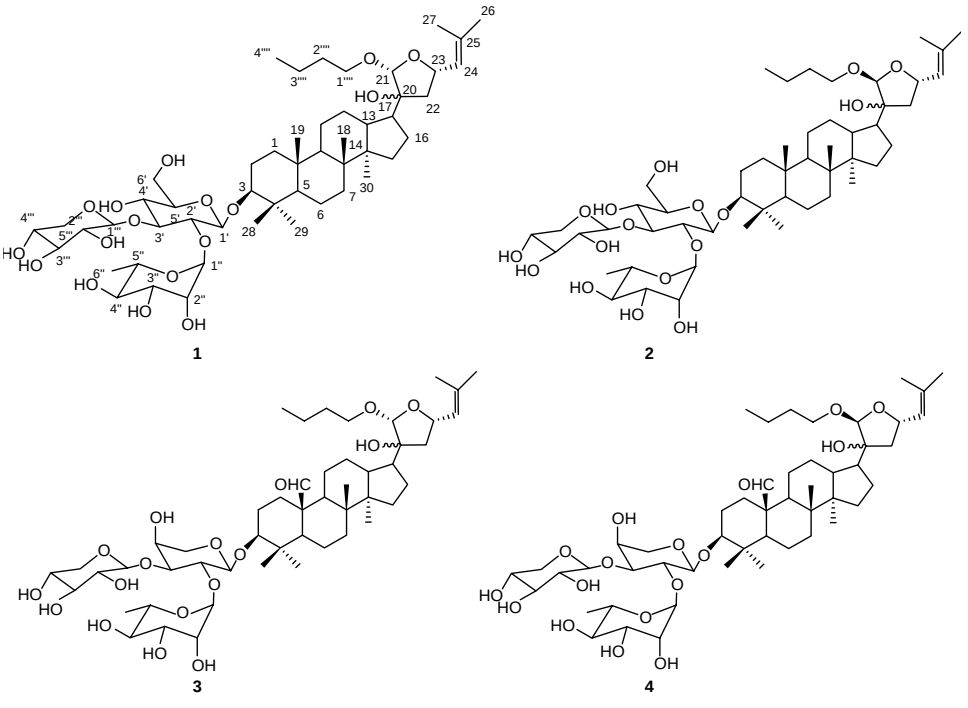

Figure 1. Structures of compounds **1**–**4**.

(OH). On acid hydrolysis, it yielded glucose, xylose, and rhamnose, which were identified by TLC comparison. The ^{1}H NMR spectrum (Table 1) showed three anomeric protons at δ 4.90 (1H, d, $J = 7.8$ Hz, H-1′), 5.00 (1H, m, H-1‴)], and 6.46 (1H, br s, H-1″), a trisubstituted olefin signal at δ 5.63 (1H, d, $J = 8.4$ Hz), and seven methyl proton signals at δ 0.75 (3H, s), 0.92 (3H, s), 0.94 (3H, s), 1.17 (3H, s), 1.23 (3H, s), 1.66 (3H, s), and 1.68 (3H, s). The carbon signals assignable to the sugar moiety and the sapogenin part in the ^{13}C NMR spectrum (Table 1) were almost superimposable on those of (23*S*)-3β,20ξ,21ξ-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside [8], except for the presence of the *n*-butyl group. In HMBC spectrum, long-range correlations between H-1⁗ and C-21, C-2⁗, and C-3⁗ could testify the presentation of *n*-butoxy group located at C-21 (Figure 2). The absolute configuration for C-23 of **1** was deduced to be *S* on the basis of the literature [8]. In the NOESY spectrum, NOESY cross-peaks were observed between the olefenic proton signal at δ 5.08 (H-21) and the signal at δ 5.01 (H-23) (Figure 3). Up to this, the aglycon part of **1** was determined as (23*S*)-21*R*-*O*-*n*-butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene.

By gas chromatography (GC) analysis of the trimethylsilyl ether derivatives of the component monosaccharides (t_R: 8.84, 9.76, and 26.59 min, respectively) and comparing with the standard monosaccharides under the same condition, together with the analysis of the coupling constants in ^{1}H NMR spectrum, it was clear that **1** contained one unit of β-D-glucose, one of β-D-xylose, and one of α-L-rhamnose. The linkage sites and sequences of the three saccharides and of the aglycon were confirmed by the 2D NMR experiments. In the HMBC spectrum (Figure 2), the cross-peaks between H-1′ of the glucose and C-3 of the aglycon, H-1″ of

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **1** in C_5D_5N.

Position	δ_C	δ_H	HMBC correlations
1	39.4	1.41 (m), 0.82 (m)	
2	26.6	2.27 (m), 1.83 (m)	
3	88.6	3.38 (dd, $J = 11.8$, 3.7 Hz)	C-1′, C-4, C-28, C-29
4	39.3		
5	56.3	0.73 (m)	C-3, C-4, C-9, C-10
6	18.1	1.41 (m), 1.39 (m)	
7	35.9	1.49 (m), 1.21 (m)	
8	40.3		
9	50.5	1.26 (m)	C-8, C-10
10	36.5		
11	22.1	1.38 (m), 1.29 (m)	
12	25.3	2.10 (m), 1.97 (m)	
13	41.5	2.26 (m)	
14	50.5		
15	30.9	1.72 (m), 1.12 (m)	
16	27.3	2.05 (m), 1.38 (m)	
17	45.7	2.07 (m)	C-14
18	15.4	0.94 (s)	C-7, C-8, C-9, C-14
19	16.2	0.75 (s)	C-1, C-5, C-9, C-10
20	82.7		
21	104.1	5.08 (br s)	C-20, C-23, *n*-butyl-C-1′′′′
22	43.6	2.43 (dd, $J = 7.2$, 12.6 Hz), 2.13 (dd, $J = 7.4$, 12.6 Hz)	C-20, C-21, C-24
23	73.4	5.01 (m)	
24	129.0	5.63 (d, $J = 8.4$ Hz)	C-26, C-27
25	133.5		
26	25.4	1.66 (s)	C-24, C-25, C-27
27	17.6	1.68 (s)	C-24, C-25, C-26
28	27.5	1.23 (s)	C-3, C-4, C-5, C-29
29	16.5	1.17 (s)	C-3, C-4, C-5, C-28
30	16.5	0.92 (s)	C-8, C-13, C-14, C-15
3-*O*-glc-1′	104.6	4.90 (d, $J = 7.8$ Hz)	C-3
2′	76.6	4.24 (m)	C-1′′
3′	87.9	4.22 (m)	C-1′′′
4′	69.3	4.76 (dd, $J = 7.0$, 3.0 Hz)	
5′	77.8	3.91 (m)	
6′	62.2	4.51 (dd, $J = 11.7$, 1.8 Hz), 4.29 (m)	
rha(1 → 2)-1′′	101.5	6.46 (br s)	C-2′
2′′	72.2	4.60 (dd, $J = 9.6$, 3.6 Hz)	
3′′	72.2	4.81 (dd, $J = 9.6$, 3.6 Hz)	
4′′	73.7	4.30 (m)	
5′′	69.5	4.13 (s)	
6′′	18.4	1.71 (s)	
xyl(1 → 3)-1′′′	104.6	5.00 (m)	C-3′
2′′′	74.5	3.95 (m)	
3′′′	78.0	4.19 (m)	
4′′′	70.3	4.11 (m)	
5′′′	67.0	4.24 (m), 3.69 (t, $J = 10.2$ Hz)	
21-*O*-*n*-butyl-1′′′′	67.3	3.96 (m), 3.57 (m)	C-21, *n*-butyl-C-2′′′′, 3′′′′
2′′′′	32.4	1.61 (m), 1.58 (m)	*n*-butyl-C-1′′′′, 3′′′′, 4′′′′
3′′′′	20.0	1.48 (m), 1.34 (m)	*n*-butyl-C-1′′′′, 2′′′′, 4′′′′
4′′′′	14.1	0.81 (t, $J = 7.5$ Hz)	*n*-butyl-C-2′′′′, 3′′′′

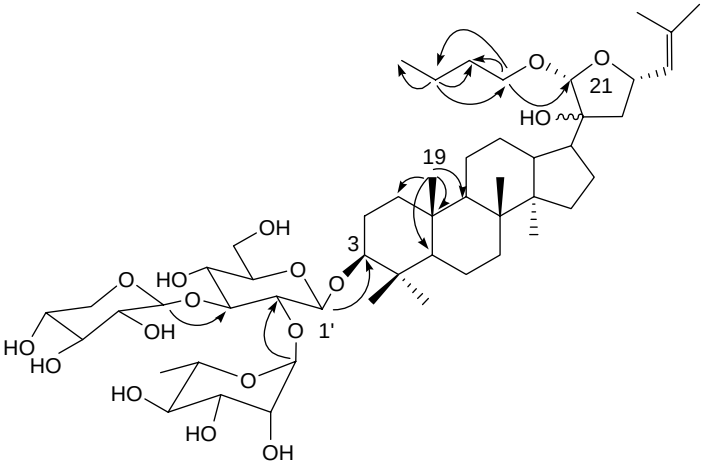

Figure 2. The structure and key HMBC correlations of compound **1**.

the rhamnose and C-2′ of the glucose, H-1‴ of the xylose and C-3′ of the glucose were displayed.

Thus, the structure of **1** was elucidated as (23*S*)-21*R*-*O*-*n*-butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside.

Compound **2**, a white amorphous powder with mp 205–207°C and $[\alpha]_D^{20}$ −14.3 ($c = 0.49$, MeOH), showed an ion peak at *m/z* 993.5748 $[M + Na]^+$ in the HR-TOF-MS, pointing to the molecular formula $C_{51}H_{86}O_{17}$. The IR spectrum (KBr) showed absorption at 3439 cm^{-1} (OH). On acid hydrolysis, it yielded glucose, xylose, and rhamnose, which were identified by TLC comparison. Comparison of the ^1H and ^{13}C NMR spectra (Table 2) of **1** with those of **2** indicated that they had the same aglycon and sugar moieties, and the only difference between them was the absolute configuration of C-21. In the NOESY spectrum, no cross-peak was observed between the olefenic proton signal at δ 5.26 (H-21) and the signal at δ 5.12 (H-23). Moreover, comparing with **1**, the downfield shifts at C-20 ($\Delta\delta + 1.5$), C-21 ($\Delta\delta + 4.1$), and C-22 ($\Delta\delta + 1.8$), and the upfield shift at C-17 ($\Delta\delta - 0.8$) could testify the difference. On the basis of the above description, the absolute configuration at C-21 of **2** was deduced to be *S*. Thus, **2** was elucidated as (23*S*)-21*S*-*O*-*n*-butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside.

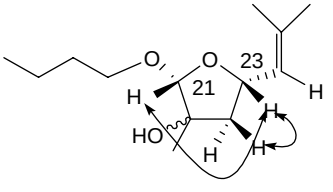

Figure 3. Key NOE correlations in compound **1**.

Compound **3**, a white amorphous powder with mp 201–203°C and $[\alpha]_D^{20}$ +21.7 ($c = 0.83$, MeOH), showed an ion peak at *m/z* 977.5448 $[M + Na]^+$ in the HR-TOF-MS, pointing to the molecular formula $C_{50}H_{82}O_{17}$. The IR spectrum (KBr) showed absorption at 3438 (OH) and 1702 (C=O) cm^{-1}. On acid hydrolysis, it yielded arabinose, xylose, and rhamnose, which were identified by TLC comparison. The ^1H NMR spectrum (Table 3) showed three anomeric protons at δ 4.88 (1H, d, $J = 5.4$ Hz, H-1′), 5.00 (1H, m, H-1‴)], and 6.15 (1H, br s, H-1″), a trisubstituted olefin signal at δ 5.61

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **2** in C_5D_5N.

Position	δ_C	δ_H	HMBC correlations
1	39.7	1.44 (m), 0.85 (m)	
2	26.9	2.28 (m), 1.85 (m)	
3	88.9	3.38 (dd, $J = 11.4$, 3.5 Hz)	C-1′, C-4, C-28, C-29
4	39.7		
5	56.7	0.74 (m)	C-4, C-9, C-10
6	18.5	1.49 (m), 1.38 (m)	
7	35.8	1.53 (m), 1.22 (m)	
8	40.7		
9	51.0	1.30 (m)	C-8, C-10
10	37.1		
11	21.8	1.51 (m), 1.43 (m)	
12	24.9	2.02 (m), 1.89 (m)	
13	41.4	2.27 (m)	
14	50.2		
15	31.7	1.74 (m), 1.13 (m)	
16	27.0	1.47–1.50 (m)	
17	44.9	2.46 (m)	
18	15.9	0.92 (s)	C-7, C-8, C-14
19	16.6	0.74 (s)	C-1, C-5, C-9, C-10
20	84.2		
21	108.2	5.26 (br s)	C-22, C-23, *n*-butyl-C-1′′′′
22	45.4	2.51 (m), 1.96 (m)	C-20, C-21, C-24
23	73.6	5.12 (dt, $J = 7.2$, 3.6, 11.8 Hz)	
24	129.5	5.84 (d, $J = 7.2$ Hz)	C-26
25	133.1		
26	25.6	1.58 (s)	C-24, C-25, C-27
27	18.0	1.65 (s)	C-24, C-25, C-26
28	27.9	1.23 (s)	C-3, C-4, C-5, C-29
29	16.9	1.17 (s)	C-3, C-4, C-5, C-28
30	16.7	1.01 (s)	C-8, C-13, C-14, C-15
3-*O*-glc-1′	105.1	4.89 (d, $J = 7.8$ Hz)	C-3, C-5′
2′	77.0	4.25 (m)	C-1′′, C-3′
3′	88.3	4.18 (m)	C-1′′′, C-2′
4′	69.9	4.01 (m)	
5′	78.1	3.91 (m)	
6′	62.6	4.51 (m), 4.30 (m)	
rha(1 → 2)-1′′	101.8	6.48 (br s)	C-2′
2′′	72.6	4.61 (m)	
3′′	72.5	4.82 (m)	
4′′	73.9	4.31 (m)	
5′′	69.8	4.78 (m)	
6′′	18.7	1.68 (d, $J = 6.0$ Hz)	
xyl(1 → 3)-1′′′	104.9	5.01 (d, $J = 7.8$ Hz)	C-3′
2′′′	74.9	3.96 (m)	
3′′′	78.3	4.08 (m)	
4′′′	70.7	4.10 (m)	
5′′′	67.3	4.24 (m), 3.71 (m)	
21-*O*-*n*-butyl-1′′′′	66.4	3.90 (m), 3.45 (m)	C-21, *n*-butyl-C-2′′′′, 3′′′′
2′′′′	32.4	1.63–1.65 (m)	*n*-butyl-C-1′′′′, 3′′′′, 4′′′′
3′′′′	20.0	1.48 (m), 1.45 (m)	*n*-butyl-C-1′′′′, 2′′′′, 4′′′′
4′′′′	14.1	0.90 (t, $J = 7.5$ Hz)	*n*-butyl-C-2′′′′, 3′′′′

Table 3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **3** in C_5D_5N.

Position	δ_C	δ_H	HMBC correlations
1	33.6	2.61 (m), 0.70 (m)	
2	27.4	2.08 (m), 1.68 (m)	
3	87.2	3.31 (dd, $J = 11.8$, 3.9 Hz)	C-1′, C-29
4	40.1		
5	54.9	1.16 (m)	C-4, C-19, C-28, C-29
6	17.7	1.86 (m), 1.55 (m)	
7	34.7	1.62 (m), 1.34 (m)	
8	40.4		
9	52.8	1.67 (m)	C-10, C-19
10	52.8		
11	22.1	1.71 (m), 1.17 (m)	
12	25.6	1.95 (m), 1.97 (m)	
13	41.7	2.16 (m)	
14	50.6		
15	31.7	1.64 (m), 1.17 (m)	
16	27.3	1.98 (m), 1.31 (m)	
17	46.1	2.05 (m)	
18	17.3	0.84 (s)	C-7, C-8, C-9, C-14
19	205.5	10.26 (s)	C-1, C-9, C-10
20	82.9		
21	104.4	5.00 (br s)	C-17, C-20, C-22, C-23, *n*-butyl-C-1′′′′
22	43.6	2.40 (dd, $J = 6.9$, 11.7 Hz), 2.12 (dd, $J = 7.8$, 11.7 Hz)	C-20, C-21, C-24
23	73.7	4.99 (m)	C-17, C-20, C-21, C-22, C-24
24	129.4	5.61 (d, $J = 8.3$ Hz)	C-26, C-27
25	133.9		
26	25.7	1.65 (s)	C-24, C-25, C-27
27	17.9	1.67 (s)	C-24, C-25, C-26
28	26.4	1.28 (s)	C-3, C-4, C-5, C-29
29	16.0	1.07 (s)	C-3, C-4, C-5, C-28
30	16.7	0.96 (s)	C-8, C-13, C-14, C-15
3-*O*-ara-1′	104.8	4.88 (d, $J = 5.4$ Hz)	C-3
2′	74.5	4.64 (dd, $J = 7.2$, 6.0 Hz)	C-1′′
3′	81.4	4.28 (m)	C-1′′′
4′	68.4	4.46 (m)	
5′	65.2	4.27 (m), 3.79 (d, $J = 9.6$ Hz)	
rha(1 → 2)-1′′	102.1	6.15 (br s)	C-2′
2′′	72.6	4.56 (m)	
3′′	72.5	4.74 (m)	
4′′	73.9	4.27 (m)	
5′′	70.1	4.57 (m)	
6′′	18.6	1.58 (d, $J = 6.0$ Hz)	
xyl(1 → 3)-1′′′	105.2	5.00 (m)	C-3′
2′′′	74.7	3.90 (t, $J = 7.8$ Hz)	
3′′′	77.8	4.07 (m)	
4′′′	70.9	4.10 (m)	
5′′′	67.0	4.30 (m), 3.64 (t, $J = 11.1$ Hz)	
21-*O*-*n*-butyl-1′′′′	67.7	3.97 (m), 3.56 (m)	C-21, *n*-butyl-C-2′′′′, 3′′′′
2′′′′	32.2	1.57 (m), 1.34 (m)	*n*-butyl-C-1′′′′, 3′′′′, 4′′′′
3′′′′	19.7	1.33 (m), 1.36 (m)	*n*-butyl-C-1′′′′, 2′′′′, 4′′′′
4′′′′	14.0	0.80 (t, $J = 7.2$ Hz)	*n*-butyl-C-2′′′′, 3′′′′

(1H, d, $J = 8.3$ Hz), and six methyl proton signals at δ 0.84 (3H, s), 0.96 (3H, s), 1.07 (3H, s), 1.28 (3H, s), 1.65 (3H, s), and 1.67 (3H, s). The carbon signals assignable to the sugar moiety and the sapogenin part in the ^{13}C NMR spectrum (Table 3) were superimposable on those of (23*S*)-3β,20*ξ*,21ξ-trihydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-arabinopyranoside [8], except for the presence of the *n*-butyl group. The HMBC correlations between the H-1$''''$ and C-21, C-2$''''$, and C-3$''''$ could testify the presence of *n*-butoxyl group located at C-21 (Figure 4). The absolute configuration at C-23 of **3** was deduced to be *S* on the basis of the literature [8]. In addition, comparable to **1**, the absolute configuration at C-21 of **3** was deduced to be *R*. Up to this, the aglycon part of **3** was determined as (23*S*)-21*R*-*O*-*n*-butyl-19-oxo-3β,20*ξ*,21-trihydroxy-21,23-epoxydammar-24-ene.

By GC analysis of the trimethylsilyl ether derivatives of the component monosaccharides (t_R: 6.20, 8.84, and 9.76 min, respectively) and the standard monosaccharides under the same condition, together with the coupling constants in ^{1}H NMR spectrum, it was clear that **3** contained one unit of α-L-arabinose, one of β-D-xylose, and one of α-L-rhamnose. The linkage sites and the sequence of three saccharides and of the aglycon were confirmed by the 2D NMR experiments. In the HMBC spectrum (Figure 4), the cross-peaks between H-1′ of the arabinose and C-3 of the aglycon, H-1″ of the rhamnose and C-2′ of the arabincose, H-1‴ of the xylose and C-3′ of the arabinose were displayed. Thus, the structure of **3** was elucidated as (23*S*)-21*R*-*O*-*n*-butyl-19-oxo-3β,20*ξ*,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside.

Compound **4**, a white amorphous powder with mp 203–205°C and $[\alpha]_D^{20}$ −11.0 ($c = 0.46$, MeOH), showed a peak at *m/z* 977.5443 $[M + Na]^+$ in the HR-TOF-MS, pointing to the molecular formula $C_{50}H_{82}O_{17}$. The IR spectrum (KBr) showed absorption at 3430 (OH), and 1707 (C═O) cm^{-1}. On acid hydrolysis, it yielded arabinose, xylose, and rhamnose, which were identified by TLC comparison. Comparison of the ^{1}H and ^{13}C NMR spectra (Table 4) of **3** with those of **4** indicated that they had the same aglycon and sugar moieties, and the only difference between them was the absolute configuration at C-21. Namely, comparable to **3**, the downfield shifts at C-20 ($\Delta\delta + 1.2$), C-21 ($\Delta\delta + 3.7$), and C-22 ($\Delta\delta + 1.7$), and the upfield shift at C-17 ($\Delta\delta - 1.3$)

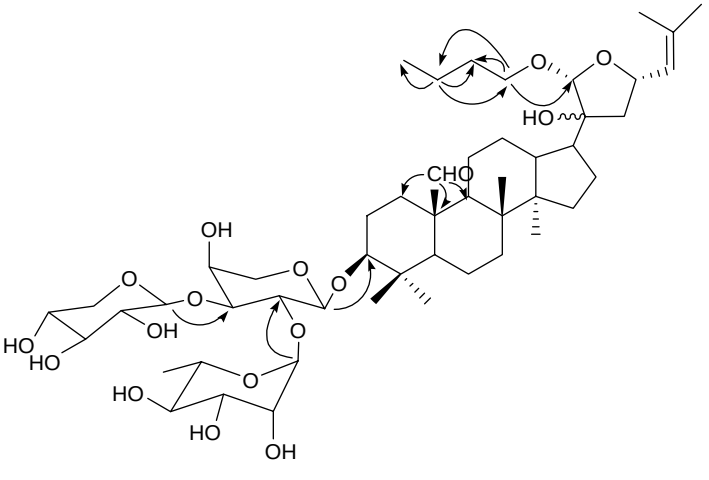

Figure 4. The structure and key HMBC correlations of compound **3**.

Table 4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **4** in C_5D_5N.

Position	δ_C	δ_H	HMBC correlations
1	33.7	2.64 (m), 0.74 (m)	
2	27.6	2.07 (m), 1.68 (m)	
3	87.4	3.32 (dd, $J = 11.7, 3.9$ Hz)	C-1′, C-4, C-28, C-29
4	40.1		
5	54.9	1.19 (m)	C-4, C-6, C-10, C-19
6	17.8	1.87–1.89 (m)	
7	34.8	1.68 (m), 1.37 (m)	
8	40.5		
9	52.9	1.72 (m)	C-8, C-10, C-19
10	52.9		
11	22.3	1.78 (m), 1.13 (m)	
12	24.9	2.02 (m), 1.89 (m)	
13	41.2	2.20 (t, $J = 11.4$ Hz)	
14	50.1		
15	32.1	1.70 (m), 1.21 (m)	
16	27.0	1.80 (m), 1.45 (m)	
17	44.8	2.41 (m)	C-13
18	17.4	0.85 (s)	C-7, C-8, C-9, C-14
19	205.5	10.26 (s)	C-1, C-10
20	84.1		
21	108.1	5.21 (br s)	C-22, C-23, *n*-butyl-C-1′′′′
22	45.3	2.47 (dd, $J = 9.0, 12.6, 3.6$ Hz), 1.94 (dd, $J = 9.0, 12.6, 3.6$ Hz)	C-20, C-21, C-24
23	73.6	5.10 (m)	C-21
24	129.4	5.82 (d, $J = 8.4$ Hz)	C-26, C-27
25	132.9		
26	25.6	1.59 (s)	C-24, C-25, C-27
27	18.0	1.66 (s)	C-24, C-25, C-26
28	26.4	1.21 (s)	C-4, C-5, C-29
29	16.0	1.07 (s)	C-4, C-5, C-28
30	16.5	1.03 (s)	C-8, C-13, C-14, C-15
3-*O*-ara-1′	104.8	4.87 (d, $J = 6.0$ Hz)	C-3
2′	74.5	4.62 (dd, $J = 7.2, 6.0$ Hz)	C-1′′
3′	81.7	4.28 (m)	C-1′′′
4′	68.4	4.30 (m)	
5′	65.1	4.26 (m), 3.79 (d, $J = 9.6$ Hz)	
rha(1 → 2)-1′′	102.1	6.13 (br s)	C-2′
2′′	72.6	4.57 (m)	
3′′	72.5	4.73 (m)	
4′′	73.9	4.25 (m)	
5′′	70.1	4.56 (m)	
6′′	18.6	1.58 (s)	
xyl(1 → 3)-1′′′	105.3	5.00 (d, $J = 7.2$ Hz)	C-3′
2′′′	74.6	3.92 (m)	
3′′′	77.8	4.11 (m)	
4′′′	70.9	4.10 (m)	
5′′′	67.0	4.30 (m), 3.64 (t, $J = 9.0$ Hz)	
21-*O*-*n*-butyl-1′′′′	66.3	3.88 (m), 3.42 (m)	C-21, *n*-butyl-C-2′′′′, 3′′′′
2′′′′	32.3	1.63 (m), 1.43 (m)	*n*-butyl-C-1′′′′, 3′′′′, 4′′′′
3′′′′	19.9	1.41 (m), 1.43 (m)	*n*-butyl-C-1′′′′, 2′′′′, 4′′′′
4′′′′	14.1	0.90 (t, $J = 7.5$ Hz)	*n*-butyl-C-2′′′′, 3′′′′

could also testify the difference. And the absolute configuration at C-21 of **4** was deduced to be *S*. Thus, **4** was elucidated as (23*S*)-21*S*-*O*-*n*-butyl-19-oxo-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-arabinopyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on *Perkin-Elmer* polarimeter. Melting points were determined with the Electrothermal melting point apparatus (Beijing Taike X-4 spectrophotometer) and are uncorrected. UV spectra were measured on Shimadzu UV-2201 spectrophotometer; MeOH soln.; in λ_{max} ($\log \epsilon$). IR spectra were recorded on Bruker IFS-55 spectrophotometer; KBr pellets; ν in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded using Bruker AV-600 and ARX-300 spectrometer; δ in ppm relative to Me_4Si as internal standard, *J* in Hz. HR-TOF-MS were recorded by BIC micro TOF-Q mass spectrometer; in *m/z* (rel. %). Prep. GC were recorded on Agilent technologies 6890N apparatus, OV-17 (30 m × 0.32 mm) column. HPLC (Beijing CXTH3000 System) was performed through P3000 pump, UV3000 spectrophotometric detector at 203 nm, Daisogel C_{18} reversed-phase column (10 μm, 30 × 250 mm; flow rate 14.0 ml/min). Column chromatography was performed on silica gel (SiO_2: 200–300 mesh, Qingdao Marine Chemical Group, Co., Qingdao, China) and macroporous resin D101.

3.2 Plant material

The aerial parts of *G. pentaphyllum* (Thunb.) Makino were collected in July 2006 from Xi'an city, Shanxi province of China by Xi'an Tianyi Co. Ltd. A voucher specimen (No. 2007016) has been deposited at the herbarium of the department.

3.3 Extraction and isolation

Dried aerial parts of *G. pentaphyllum* (Thunb.) Makino (8.0 kg) were extracted with 75% EtOH (× 3), and the water soluble extract of the plant was separated by a macroporous resin column to get the 70% EtOH eluates, which upon drying afforded the total saponins (80 g). The total saponins were chromatographed repeatedly over silica gel with $CHCl_3$–MeOH–H_2O (7:2:1–7:4:1) to provide five fractions A–E. Fraction D (1.0 g) was separated into five fractions, D_a–D_e, by HPLC (ODS, 80% MeOH). Fraction D_a (0.3 g) was then subjected to preparative RP-HPLC (75% MeOH) to yield **3** (100 mg, t_R = 18 min) and **1** (25 mg, t_R = 23 min), **2** (30 mg, t_R = 27 min), and **4** (40 mg, t_R = 32 min).

3.3.1 (23S)-21*R*-O-n-Butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-O-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside (**1**)

White amorphous powder, Libermann–Burchard and Molish reactions were positive. MP 208–210°C, $[\alpha]_D^{20}$ +11.9 (c = 0.14, MeOH). IR (KBr): ν_{max} 3418, 2937, 1645, 1452, 1384, 1265, 1097, 1046, 811, 613 cm^{-1}. For ^1H and ^{13}C NMR spectral data see Table 1. HR-TOF-MS: *m/z* 993.5742 $[M + Na]^+$ (calcd for $C_{51}H_{86}O_{17}Na$, 993.5757).

3.3.2 (23S)-21*S*-O-n-Butyl-3β,20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-O-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside (**2**)

White amorphous powder, Libermann–Burchard and Molish reactions were positive. MP 205–207°C, $[\alpha]_D^{20}$ −14.3 (c = 0.49, MeOH). IR (KBr): ν_{max} 3439, 2933, 1645, 1384, 1044, 616 cm^{-1}. For ^1H and ^{13}C NMR spectral data see Table 2.

HR-TOF-MS: *m/z* 993.5748 $[M + Na]^+$ (calcd for $C_{51}H_{86}O_{17}Na$, 993.5757).

*3.3.3 (23S)-21R-*O-n-*Butyl-19-oxo-3β, 20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O-*[α-L-rhamnopyranosyl (1 → 2)][β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside (**3**)*

White amorphous powder, Libermann–Burchard and Molish reactions were positive. MP 201–203°C, $[\alpha]_D^{20}$ +21.7 ($c = 0.83$, MeOH). IR (KBr): ν_{max} 3438, 2942, 1702, 1651, 1540, 1453, 1384, 1259, 1041, 817, 783, 610 cm^{-1}. For ^1H and ^{13}C NMR spectral data see Table 3. HR-TOF-MS: *m/z* 977.5448 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{17}Na$, 977.5444).

*3.3.4 (23S)-21S-*O-n-*Butyl-19-oxo-3β, 20ξ,21-trihydroxy-21,23-epoxydammar-24-ene 3-*O-*[α-L-rhamnopyranosyl (1 → 2)][β-D-xylopyranosyl(1 → 3)]-α-L-arabinopyranoside (**4**)*

White amorphous powder, Libermann–Burchard and Molish reactions were positive. MP 203–205°C, $[\alpha]_D^{20}$ −11.0 ($c = 0.46$, MeOH). IR (KBr): ν_{max} 3430, 2929, 1707, 1637, 1384, 1043, 612 cm^{-1}. For ^1H and ^{13}C NMR spectral data see Table 4. HR-TOF-MS: *m/z* 977.5443 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{17}Na$, 977.5444).

3.4 Acid hydrolysis of 1–4

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

3.5 Determination of sugar components

The monosaccharide subunits were obtained by HCl hydrolysis as described above. The aqueous layer was concentrated to dryness to give a residue which was dissolved in pyridine (1 ml), and then hexamethyldisilazane (0.4 ml) and trimethylchlorosilane (0.2 ml) were added to the solution to obtain the trimethylsilyl (TMS) ethers. The mixture was kept at 20°C for 15 min, and extracted with H_2O (1 ml). Each aqueous layer was examined by GC (H_2 flame ionization detector, column temperature: 100–280°C, programmed increase: 10°C/min, carrier gas: N_2 (1.5 ml/min), injector and detector temperature: 280°C, injection volume: 1 μl, split ratio: 10:1). The derivatives of L-arabinose, D-xylose, L-rhamnose, and D-glucose were detected. t_R: 6.20, 8.84, 9.76, and 26.59 min, respectively. The standard monosaccharides were subjected to the same operation and GC analysis under the same condition.

3.6 Antitumor bioassay

Antitumor activities were evaluated by MTT assay [9] with Rg_3 as the positive control. Compounds **1**–**4** showed moderate antitumor activities against HL-60, Colon205, and Du145.

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