Three New Triterpene Saponins from *Bolbostemma paniculatum*

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Two novel noroleanane saponins, tubeimoside A (**1**) and tubeimoside B (**2**), and a new dammarane triterpene saponin, tubeimoside C (**3**), together with two known compounds, **4** and **5**, were isolated from the bulbs of *Bolbostemma paniculatum* (MAXIM.) FRANQUET. Compound **4** was found in this genus for the first time. Based on spectroscopic methods, including IR, NMR (DEPT, COSY, HMQC, HMBC, and TOCSY), and MS experiments, and chemical reactions, the structures of the new compounds were elucidated as 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyloxy]-2β,23-dihydroxy-28-norolean-12-en-16-one (**1**), 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyloxy]-2β,23-dihydroxy-28-norolean-12-en-22-one (**2**), (3β,7β)-7,18,20-trihydroxydammar-24-en-3-yl 2-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (**3**).

Introduction. – The bulb of *Bolbostemma paniculatum* (MAXIM.) FRANQUET, one of the Chinese folk medicinal plants named as '*Tu-Bei-Mu*', is often used for the treatment of tumors and warts as well as for detoxication [1][2]. Previous research indicated that the main constituents of this plant are triterpenoid saponins, which have anticancer and antivirus activities [3–8]. Considering the obvious activities of '*Tu-Bei-Mu*', finding further bioactive compounds, particularly constituents with cytotoxic activities, is desirable. In the current study, a systematic chemical investigation was carried out on the bulbs of *Bolbostemma paniculatum*, and three new triterpenoid saponins and two known ones were isolated and characterized.

From the extract of the bulbs of this plant, three new triterpene saponins, 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyloxy]-2β,23-dihydroxy-28-norolean-12-en-16-one (**1**), 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyloxy]-2β,23-dihydroxy-28-norolean-12-en-22-one (**2**), and (3β,7β)-7,18,20-trihydroxydammar-24-en-3-yl 2-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (**3**), together with two known compounds, lobatoside A (**4**) [9] and tubeimoside IV (**5**) [5] (*Fig. 1*), were isolated. Compound **4** was found in this genus for the first time.

Herein, we report the structural elucidation of the three new triterpene saponins **1**–**3**.

Results and Discussion. – Tubeimoside A (**1**), was obtained as a white amorphous powder. Its HR-ESI-MS showed *quasi*-molecular-ion peak at *m/z* 783.4526 ($[M+H]^+$, calc. for $C_{41}H_{67}O_{14}$ 783.4531) in agreement with the molecular formula $C_{41}H_{66}O_{14}$. The

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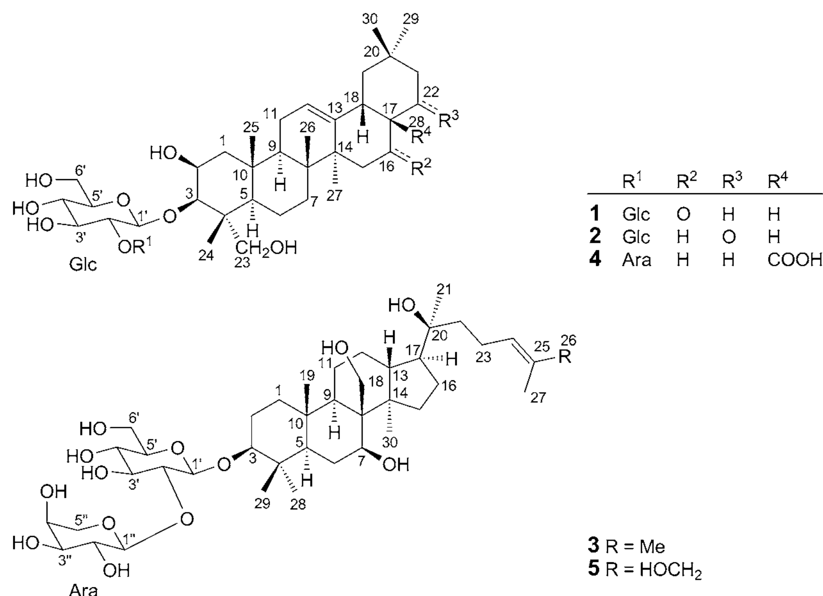

Fig. 1. *Structures of compounds* **1**–**5**

IR spectrum exhibited strong absorptions at 3423 and 1699 cm^{-1} evidencing the presence of the OH groups and a C=O group, respectively. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed six tertiary Me signals at δ(H) 0.82, 0.87, 0.99, 1.15, 1.46, and 1.58, one olefinic H-atom signal at δ(H) 5.38 (t, J = 3.0), and two anomeric H-atom signals at δ(H) 5.13 (d, J = 7.8) and 5.35 (d, J = 7.8). The ^{13}C-NMR spectrum (*Table 1*), in combination with DEPT spectra, of **1** displayed signals of 41 C-atoms, of which 29 were ascribed to the aglycone moiety, *i.e.*, six Me, nine sp^3-CH_2 groups, six sp^3 CH, five sp^3 quaternary C-atoms, one sp^2-CH group, one sp^2 quaternary C-atom, and one ketone C=O C-atom, among which one sp^3 CH_2 C-atom (δ(C) 65.3) and two sp^3-CH groups (δ(C) 70.1 and 82.3) were oxygenated. This information indicated that the aglycon of **1** possessed a noroleanane triterpene skeleton. The NMR data of the aglycon part of **1** were similar to those of 3β-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyloxy]-2β,17,23-trihydroxy-28-norolean-12-en-16-one reported in [10]. The obvious distinction between the two compounds was the chemical shift value of C(17), which corresponded to a CH group (δ(C) 46.7) for **1** instead of an O-bearing quaternary C-atom (δ(C) 76.5) for the reported compound. Thus, the aglycon of **1** was identified as 2β,3β,23-trihydroxy-28-norolean-12-en-16-one, which could be confirmed through long-range correlations. Namely, the correlation between H–C(17) (δ(H) 2.51) and C(16) (δ(C) 213.3), CH_2(15) (δ(H) 1.94, 2.58), and C(16) (δ(C) 213.3), and between CH_2(15) (δ(H) 1.94, 2.58) and C(17) (δ(C) 46.7) were observed in HMBC (*Fig. 2*). ROESY Correlations between CH_2(23) and H–C(3) (δ(H) 4.21 (m)), and Me(24) and between Me(25) (δ(H) 1.58 (s)) established that H–C(3) has an α-axial orientation. H–C(2) (δ(H) 4.80 (m)) was also determined as α-axial due to a ROESY correlation between H–C(2) and H–C(3) (*Fig. 2*).

Table 1. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp., in C_5D_5N) *Data for* **1**. δ in ppm, J in Hz.

Position	δ(H)[a]	δ(C)	HMBCs[b]
1	1.16 (*dd*, J = 13.8, 1.8), 2.26 (*dd*, J = 13.8, 1.8)	43.5	
2	4.80 (*dd*, J = 6.0, 1.8)	70.1	
3	4.21 (*d*, J = 6.0)	82.3	C(1′)
4		42.5	
5	1.67 (*d*, J = 12.0)	47.8	C(4), C(10)
6	1.58–1.60 (*m*), 1.80 (*d*, J = 12.0)	17.7	
7	1.16–1.18 (*m*), 1.48–1.49 (*m*)	32.5	
8		39.6	
9	1.59–1.60 (*m*)	47.3	
10		36.6	
11	2.00–2.01 (*m*), 2.06–2.08 (*m*)	23.5	C(8), C(9)
12	5.38 (*t*, J = 3.0)	122.9	
13		142.4	
14		47.6	
15	1.94 (*d*, J = 14.4), 2.58 (*d*, J = 14.4)	46.7	C(13), C(14), C(16)
16		213.3	
17	2.51 (*t*, J = 4.8)	46.7	C(16), C(18)
18	2.78–2.82 (*m*)	44.6	C(16)
19	1.19 (*d*, J = 13.8), 1.43 (*d*, J = 13.8)	46.5	C(18), C(20)
20		30.8	
21	1.11–1.13 (*m*), 1.59–1.61 (*m*)	34.5	
22	1.32–1.35 (*m*), 2.11–2.13 (*m*)	21.0	
23	3.73 (*d*, J = 10.8), 4.39 (*d*, J = 10.8)	65.3	
24	1.46 (*s*)	14.6	C(3), C(4), C(5), C(23)
25	1.58 (*s*)	16.9	C(1), C(5), C(9), C(10)
26	0.99 (*s*)	17.5	C(7), C(8), C(9), C(14)
27	1.15 (*s*)	26.9	C(8), C(13), C(14), C(15)
29	0.82 (*s*)	33.2	C(19), C(20), C(21), C(30)
30	0.87 (*s*)	23.3	C(19), C(20), C(21), C(29)
Glc–O–C(3)			
1′	5.13 (*d*, J = 7.8)	102.9	C(3)
2′	4.09–4.10 (*m*)	83.4	
3′	4.20–4.23 (*m*)	78.0	
4′	4.26–4.27 (*m*)	71.1	
5′	3.82–3.85 (*m*)	77.8	
6′	4.11–4.12 (*m*), 4.53–4.55 (*m*)	62.3	
Glc–O–C(2′)			
1″	5.35 (*d*, J = 7.8)	105.6	C(2′)
2″	4.11–4.12 (*m*)	76.6	
3″	4.20–4.22 (*m*)	77.8	
4″	4.13–4.15 (*m*)	70.8	
5″	3.93–3.95 (*m*)	78.2	
6″	4.44–4.46 (*m*)	62.2	

[a]) According to the HMQC spectrum (C_5D_5N). [b]) According to the HMBC spectrum (C_5D_5N).

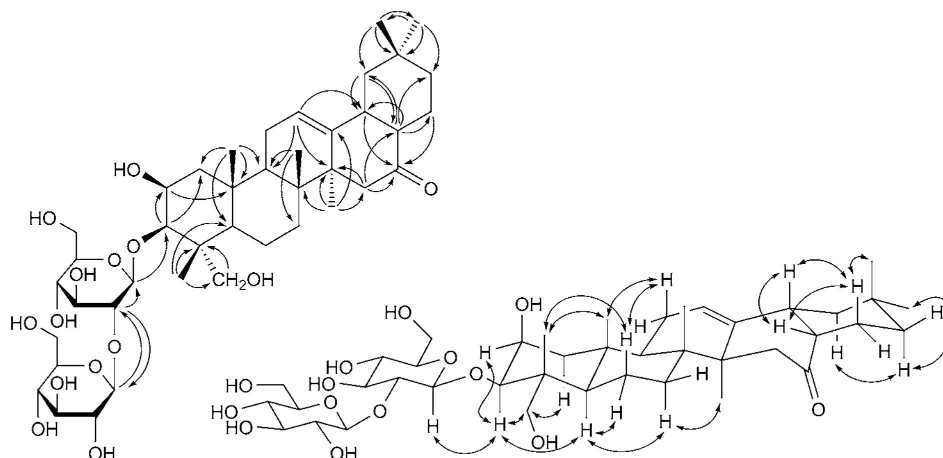

Fig. 2. *Selected HMBCs* (H → C) *and ROESY* (H ↔ H) *correlations of* **1**

The C-atom signals assignable to the sugar moieties were in accordance with the presence of two hexose units, identified as glucoses after analysis of COSY and HSQC spectra (*Table 1*). The 1,2-diaxial coupling of the anomeric H-atoms with signals at δ(H) 5.13 and 5.35, were unambiguously correlated by HSQC experiment to the corresponding C-atom resonances at δ(C) 102.9 and 105.6, respectively. Complete assignments of the resonances of each sugar unit were accomplished by extensive 1D- (^{1}H, ^{13}C) and 2D- (HSQC, HMBC) NMR analyses. These data revealed the presence of two β-glucopyranosyl units (δ(H) 5.13 (*d*, *J* = 7.8) and 5.35 (*d*, *J* = 7.8)). The sugar units were identified as D-glucose after hydrolysis of **1** with 2M HCl, trimethylsilylation, and determination of retention time by GC.

In the HMBC spectrum of **1** (*Fig. 2*), the long-range correlations between H–C(1)$_{glcI}$ (δ(H) 5.13) and C(3) (δ(C) 82.3) confirmed the mono-desmosidic nature of **1**. The sequence of the disaccharidic chain of **1** was determined by the HMBC spectrum, which showed key cross-peaks between H–C(1)$_{glcII}$ (δ(H) 5.35) and C(2)$_{glcI}$ (δ(C) 83.4), evidencing a β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyloxy chain attached to C(3).

Therefore, the new compound **1** was identified as 3β-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-2β,23-dihydroxy-28-norolean-12-en-16-one, named tubeimoside A.

Tubeimoside B (**2**) was shown to have the molecular formula $C_{41}H_{66}O_{14}$ on the basis of its HR-ESI-MS data, confirmed by ^{13}C-NMR and DEPT analysis, as in the case of **1**. The IR spectrum exhibited strong absorptions at 3415 and 1708 cm^{-1}. The spectroscopic properties of **2** were similar to those of **1**. The ^{1}H-NMR spectrum of **2** (*Table 2*) exhibited six three-H-atom *singlet*s at δ(H) 0.86, 0.88, 0.95, 1.02, 1.48, and 1.58, an olefinic H-atom signal at δ(H) 5.45 (br. *s*), and two anomeric H-atom signals at δ(H) 5.18 (*d*, *J* = 7.8) and 5.39 (*d*, *J* = 7.8). The ^{13}C-NMR spectrum of **2** (*Table 2*) exhibited signals for 41 C-atoms, of which 29 accounted for the aglycone moiety. The twelve remaining signals were in accordance with the presence of two hexose units, identified

Table 2. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp., in C_5D_5N) *Data for* **2**. δ in ppm, *J* in Hz.

Position	δ(H)[a])	δ(C)	HMBCs[b])
1	1.17–1.19 (*m*), 2.27–2.28 (*m*)	43.6	
2	4.83 (*dd*, *J* = 6.0, 1.8)	70.4	
3	4.26 (*d*, *J* = 6.0)	82.7	C(1′)
4		42.8	
5	1.72–1.74 (*m*)	48.3	C(4), C(10)
6	1.42–1.44 (*m*), 1.82–1.84 (*m*)	18.1	
7	1.22–1.24 (*m*), 1.43–1.44 (*m*)	33.0	
8		38.9	
9	1.58–1.60 (*m*)	48.0	
10		37.2	
11	2.02–2.04 (*m*), 2.11–2.13 (*m*)	23.7	C(8), C(9)
12	5.45 (br. *s*)	118.1	
13		142.7	
14		43.0	
15	0.95–0.97 (*m*), 1.89–1.91 (*m*)	42.9	
16	1.40–1.42 (*m*), 2.33–2.35 (*m*)	23.9	C(15), C(17)
17	1.92–1.94 (*m*)	49.7	C(18), C(22)
18	2.38–2.39 (*m*)	37.1	C(13), C(17), C(19)
19	1.12–1.14 (*m*), 1.40–1.42 (*m*)	38.7	
20		30.7	
21	2.16 (*d*, *J* = 15.6), 2.67 (*d*, *J* = 15.6)	44.2	C(20), C(22)
22		213.9	
23	3.74–3.76 (*m*), 4.41–4.43 (*m*)	65.6	
24	1.48 (*s*)	14.7	C(3), C(4), C(5), C(23)
25	1.58 (*s*)	17.1	C(1), C(5), C(9), C(10)
26	0.86 (*s*)	16.9	C(7), C(8), C(9), C(14)
27	1.02 (*s*)	25.7	C(8), C(13), C(14), C(15)
29	0.95 (*s*)	33.4	C(19), C(20), C(21), C(30)
30	0.88 (*s*)	24.8	C(19), C(20), C(21), C(29)
Glc–O–C(3)			
1′	5.18 (*d*, *J* = 7.8)	103.2	C(3)
2′	4.13–4.15 (*m*)	83.7	
3′	4.25–4.26 (*m*)	78.3	
4′	4.29–4.31 (*m*)	71.4	
5′	3.88–3.89 (*m*)	78.1	
6′	4.30–4.32 (*m*), 4.55–4.57 (*m*)	62.6	
Glc–O–C(2′)			
1″	5.39 (*d*, *J* = 7.8)	105.9	C(2′)
2″	4.12–4.14 (*m*)	76.9	
3″	4.16–4.18 (*m*)	78.1	
4″	4.17–4.19 (*m*)	71.1	
5″	3.95–3.97 (*m*)	78.5	
6″	4.46–4.48 (*m*)	62.5	

[a]) According to the HMQC spectrum (C_5D_5N). [b]) According to the HMBC spectrum (C_5D_5N).

as two glucoses, which was further confirmed by acid hydrolysis and GC analysis. The NMR spectra indicated that aglycone moiety of **2** was also a noroleanane triterpene that was the regioisomer of **1** with respect to the position of the C=O group. When the ^{1}H- and ^{13}C-NMR spectra of **2** were compared with those of **1**, the signals attributable to H–C(21) and C(21) were moved downfield at δ(H) 2.16 (*d*, $J = 15.6$; +1.04 ppm), and 2.67 (*d*, $J = 15.6$; +1.07 ppm), and δ(C) 44.2 (+9.7 ppm), respectively. Therefore, it was confirmed that the C=O group was C(22)=O, which was confirmed by the 2D-NMR. The correlations between the signals corresponding to the following atoms were observed in the HMBC spectrum (*Fig. 3*): H–C(21) (δ(H) 2.16, 2.67) and C(22) (δ(C) 213.9); H–C(29) (δ(H) 0.95) and C(19) (δ(C) 38.7); H–C(29) and C(21) (δ(C) 44.2); and H–C(17) (δ(H) 1.92–1.94) and C(22).

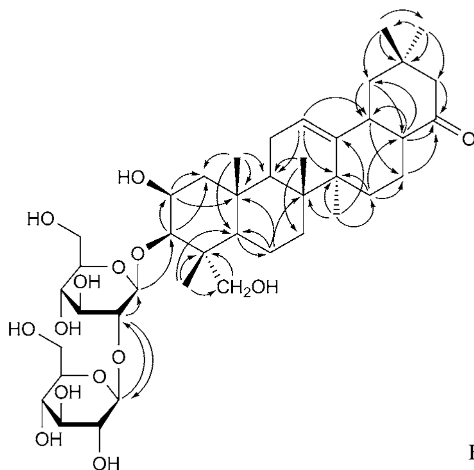

Fig. 3. *Selected HMBCs* (H → C) *of* **2**

Accordingly, the structure of **2** was determined to be 3β-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-2β,23-dihydroxy-28-norolean-12-en-22-one, named tubeimoside B.

Tubeimoside C (**3**) was obtained as colorless needles. HR-ESI Mass spectrum displayed a *quasi*-molecular-ion peak at *m/z* 793.4720 ($[M + Na]^+$) in agreement with the molecular formula $C_{41}H_{70}O_{13}$, which was also confirmed by ^{13}C-NMR and DEPT analysis. The ^{13}C-NMR spectrum of **3** exhibited signals for 41 C-atoms, of which 30 were assigned to the aglycone moiety, and the eleven remaining signals were in accordance with the presence of one hexose and one pentose unit, identified as glucose and arabinose, respectively. Furthermore, the ^{1}H- and ^{13}C-NMR spectrum (*Table 3*) of the aglycone moiety of **3** displayed seven dammarane-type Me *singlet*s (δ(H) 1.01, 1.14, 1.28, 1.34, 1.48, 1.66, and 1.70; δ(C) 16.7, 16.9, 16.9, 17.7, 25.9, 26.5 and 27.9) and the characteristic resonances of the C(24)=C(25) bond (δ(H) 5.32 (*t*, $J = 7.2$); δ(C) 126.2 and 130.8). The NMR spectroscopic data were similar to those of tubeimoside IV reported in [5] except that the CH_2(26)OH group of the reported compound was replaced by a Me group, which was confirmed by HMBC and NOESY experiments, as shown in *Fig. 4*. H–C(3) (δ(H) 3.37 (*dd*, $J = 4.2$, 12.0 Hz)) was determined to be in α-

Table 3. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp., in C_5D_5N) *Data for* **3**. δ in ppm, J in Hz.

Position	δ(H)[a])	δ(C)	HMBCs[b])
1	0.76–0.78 (*m*), 1.49–1.51 (*m*)	39.6	
2	1.89–1.91 (*m*), 2.26–2.28 (*m*)	27.1	
3	3.37 (*dd*, *J* = 4.2, 12.0)	88.8	C(1′)
4		39.7	
5	0.93 (*d*, *J* = 11.4)	54.8	C(4), C(10)
6	2.12–2.14 (*m*), 2.19–2.21 (*m*)	30.1	
7	3.95–3.97 (*m*)	78.2	
8		49.3	
9	1.23–1.25 (*m*)	52.1	C(18)
10		37.5	
11	1.15–1.17 (*m*), 1.46–1.48 (*m*)	22.9	C(8), C(9)
12	1.40–1.42 (*m*), 2.23–2.25 (*m*)	28.3	
13	2.30–2.32 (*m*)	44.6	
14		50.3	
15	2.22–2.24 (*m*), 2.60–2.61 (*m*)	36.6	
16	2.03–2.05 (*m*)	25.9	
17	2.10–2.12 (*m*)	49.5	
18	4.50 (*d*, *J* = 12.0), 4.67 (*d*, *J* = 12.0)	61.7	C(7), C(8), C(9)
19	1.01 (*s*)	16.9	C(1), C(5), C(9), C(10)
20		74.3	
21	1.48 (*s*)	26.5	C(17), C(20), C(22)
22	1.83–1.85 (*m*), 1.88–1.90 (*m*)	41.6	
23	2.40–2.41 (*m*), 2.48–2.50 (*m*)	23.4	
24	5.32 (*t*, *J* = 7.2)	126.2	C(23), C(25)
25		130.8	
26	1.66 (*s*)	17.7	C(24), C(25), C(27)
27	1.70 (*s*)	25.9	C(24), C(25), C(26)
28	1.34 (*s*)	27.9	C(3), C(4), C(5), C(29)
28	1.14 (*s*)	16.7	C(3), C(4), C(5), C(28)
30	1.28 (*s*)	16.9	C(8), C(13), C(14), C(15)
Glc–O–C(3)			
1′	4.97 (*d*, *J* = 7.2)	105.2	C(3)
2′	4.11–4.13 (*m*)	83.9	
3′	4.30–4.32 (*m*)	78.3	
4′	4.18–4.19 (*m*)	71.7	
5′	3.96–4.37 (*m*)	78.2	
6′	4.38–4.39 (*m*), 4.60–4.62 (*m*)	62.9	
Ara–O–C(2′)			
1″	5.19 (*d*, *J* = 7.2)	106.7	C(2′)
2″	4.59–4.61 (*m*)	73.8	
3″	4.22–4.24 (*m*)	74.4	
4″	4.32–4.33 (*m*)	69.2	
5″	3.78 (*dd*, *J* = 12.0, 1.8), 4.39 (*dd*, *J* = 12.0, 3.0)	67.1	

[a]) According to the HMQC spectrum (C_5D_5N). [b]) According to the HMBC spectrum (C_5D_5N).

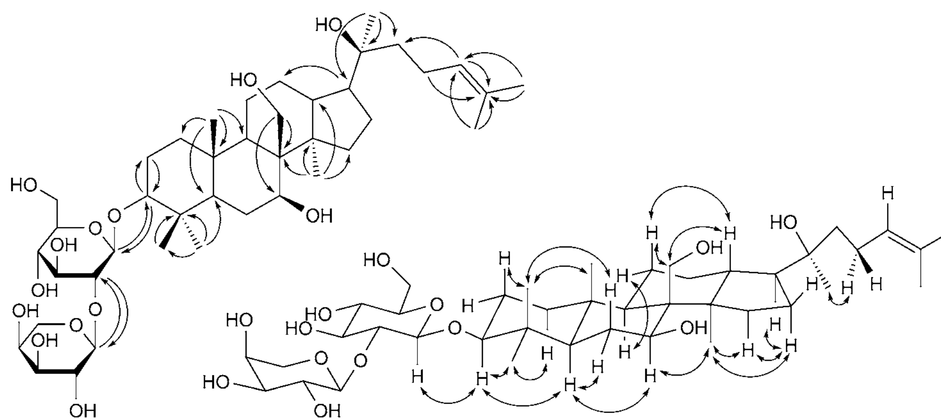

Fig. 4. *Key HMBCs* (H → C) *and NOESY* (H ↔ H) *correlations of* **3**

axial orientation due to its coupling constant, which was confirmed by a ROESY correlation between the signals of Me(28) and H–C(3). In turn, H–C(7) (δ(H) 3.96 (*m*)) of **3** was assigned α-axial position due to its ROESY correlation with Me(30). The chemical shifts of C(13), C(15), C(17), C(21), C(22), and C(23) were quite similar to those of tubeimoside IV [5], of which the configuration of C(20) was established as (*S*). Thus, the configuration at C(20) of **3** was determined to be (*S*).

Therefore, the structure of **3** was identified as (3β,7β)-7,18,20-trihydroxydammar-24-en-3-yl 2-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside, named tubeimoside C.

The known compounds **4** and **5** were identified by physical and spectroscopic evidences, and confirmed by comparing with the literature, data.

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Experimental Part

General. Solvents were distilled prior to use, and spectroscopic grade-solvents were employed. Column chromatography (CC): silica gel (SiO_2; 300–400 mesh, *Qingdao Marine Chemical Co.*). TLC: *GF_{254}* SiO_2 (10–40 µm, *Qingdao Marine Chemical Co.*); detection under UV light and visualization by spraying with 10% H_2SO_4 in EtOH (*v*/*v*), followed by heating. Column chromatography (CC): reversed-phase (RP) *C_{18}* SiO_2 (300–400 mesh; *Agela Technologies Co.*) and *Sephadex TM LH-20* (25–100 µm; *Pharmacia, Co.*). Semi-prep. HPLC: *Beijing CXTH3000* system (*P3000* pump, *UV3000* spectrophotometric detector at 203 nm, *YMC C_{18}* RP column (5 µm, 10 × 250 nm; flow rate, 3.0 ml/min)). GC: *Agilent Technologies 6890 N* apparatus, *OV-17* (30 m × 0.32 mm) column. M.p.: *SGW X-4* electric micro melting point apparatus; uncorrected. Optical rotations: *PerkinElmer 341* digital polarimeter in MeOH. IR Spectra: *Bruker IFS-55* spectrophotometer in KBr; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker micro TOF-Q* mass spectrometer; in *m*/*z*.

Plant Material. The bulbs of *Bolbostemma paniculatum* (MAXIM.) FRANQUET (Cucurbitaceae) were collected in Shaanxi Province of P. R. China. A voucher specimen of this herb (No. 2010090) was identified by Prof. *Jincai Lu* at Shenyang Pharmaceutical University.

Extraction and Isolation. The bulbs of *Bolbostemma paniculatum* (50.0 kg) were extracted with 75% EtOH (80 l × 3) under reflux for 2 h. The alcohol was evaporated off, and the aq. soln. (28 l) was partitioned with petroleum ether, AcOEt, and BuOH, sequentially (3 × 28 l). The BuOH-soluble fraction (500 g) was then subjected to CC (SiO_2 (120 × 1500 mm, 4.0 kg); CH_2Cl_2/MeOH 20 : 1, 10 : 1, 5 : 1, 3 : 1, 1 : 1, and 100% MeOH) to provide 14 fractions, *Frs. A – N*, based on TLC analysis. *Fr. I* was subjected to CC (SiO_2; CH_2Cl_2/MeOH/H_2O (the lower layer) 7 : 2 : 1) to obtain ten subfractions, *Frs. I.1 – I.10*. *Fr. I.8* was submitted to CC (ODS SiO_2; 70% MeOH/H_2O) to afford **3** (25.3 mg). *Fr. J* was purified by CC (SiO_2; CH_2Cl_2/MeOH/H_2O (the lower layer) 7 : 2.5 : 1) to give eleven subfractions, *Frs. J.1 – J.11*. *Fr. J.7* was separated by CC (ODS SiO_2; MeOH/H_2O 3 : 10; 1 : 2, 7 : 10, and 9 : 10) to furnish **4** (20.0 mg) and **2** (28.0 mg) as pure compounds, and **1** with a few impurities. Compound **1** (25.0 mg) was purified by prep. HPLC (75% MeOH/H_2O). *Fr. J.8* was subjected to CC (ODS SiO_2; MeOH/H_2O 3 : 10; 1 : 2, 7 : 10, and 9 : 10) to afford **5** (50.0 mg).

Tubeimoside A (= *(2*S*,3*R*,4*R*,4a*R*,6a*R*,6b*S*,8a*S*,12a*R*,14a*R*,14b*R*)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-Icosahydro-2-hydroxy-4-(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-8-oxopicen-3-yl 2-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranoside*; **1**). White amorphous powder. M.p. 228 – 229°. $[\alpha]_D^{28} = +0.41$ ($c = 0.11$, MeOH). IR: 3423, 2926, 1699, 1384, 1079. *Libermann–Burchard* and *Molish* reactions were positive. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 783.4526 ($[M + H]^+$, $C_{41}H_{67}O_{14}^+$; calc. 783.4531).

Tubeimoside B (= *(2*S*,3*R*,4*R*,4a*R*,6a*R*,6b*S*,8a*R*,12a*R*,14a*R*,14b*R*)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-Icosahydro-2-hydroxy-4-(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-9-oxopicen-3-yl 2-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranoside*; **2**). White granulated crystals. M.p. 298 – 299°. $[\alpha]_D^{28} = -0.36$ ($c = 0.11$, MeOH). IR: 3415, 2924, 1708, 1383, 1079. *Libermann–Burchard* and *Molish* reactions were positive. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 805.4358 ($[M + Na]^+$, $C_{41}H_{66}NaO_{14}^+$; calc. 805.4350).

Tubeimoside C (= *(3β,7β)-7,18,20-Trihydroxydammar-24-en-3-yl 2-*O*-α-*L*-Arabinopyranosyl-β-*D*-glucopyranoside*; **3**). Colorless needles. M.p. 196 – 197°. $[\alpha]_D^{28} = +0.35$ ($c = 0.10$, MeOH). IR: 3419, 2926, 1630, 1384, 1084. *Libermann–Burchard* and *Molish* reactions were positive. ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 793.4720 ($[M + Na]^+$, $C_{41}H_{70}NaO_{13}^+$; calc. 793.4714).

Acid Hydrolysis of **1** – **3**. Each compound (2 mg) was hydrolyzed with 2M aq. HCl at 90° for 4 h. After cooling, the mixture was diluted to 10 ml with H_2O and then extracted with CH_2Cl_2 (3 × 10 ml). The H_2O layer was neutralized with 2M aq. KOH. After concentration, each 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 as described above. The aq. layer was concentrated to give a residue. The residue was dissolved in pyridine (0.2 ml), hexamethyl disilazane (0.2 ml), and Me_3SiCl (0.2 ml), and the soln. was stirred at 20° for 15 min. After drying the soln. with a stream of N_2, the residue was partitioned with H_2O and CH_2Cl_2 (1 ml, 1 : 1 (*v*/*v*)). The CH_2Cl_2 layer was analyzed by GC (H_2 flame ionization detector). The column temp. was programmed from 80 to 280° at a rate of 10°/min, and N_2 was used as carrier gas at 1.5 ml/min. The injector and detector temp. was at 280°, and the injection volume was 1 μl with split ratio of 10: 1. The derivatives of L-arabinose and D-glucose were detected: t_R [min]: 6.20 and 26.59 min, resp. The standard monosaccharides were subjected to the same reaction and GC analysis under the same conditions.

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