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A New Cyclic Bisdesmoside from Tubers of *Bolbostemma paniculatum*

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Abstract: The structure of tubeimoside V (**1**), a new cyclic bisdesmoside, isolated from tubers of *Bolbostemma paniculatum* (Tu Bei Mu), was established by means of 2D NMR spectral and chemical methods. Compound **1** has inter-saccharide chain bridging by a dicrotalic acid to form a unique macrocyclic structure.

Keywords: *Bolbostemma paniculatum*, cyclic bisdesmoside, triterpenoid saponin, tubeimoside V.

The tuber of *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae), a Chinese folk medicine named as "Tu Bei Mu", was listed in the Supplement to the Compendium of Materia Medica, compiled in the Qing Dynasty[1]. Tubeimosides I, II and III, isolated from the folk medicine, are the only three examples of saponins having novel cyclic structures with dicrotalic acid bridge and the name "cyclic bisdesmoside" has been proposed for this type of saponin[2-4]. All the three compounds showed significant antitumor, anti-inflammatory and antitumor-promoting effects[5-8]. We report here the isolation and structural elucidation of the fourth cyclic bisdesmoside, a new minor constituent named tubeimoside V (**1**), from the ethanol extracts of tubers of *B. paniculatum*.

The ethanol extracts of the powdered tubers were suspended in water and then partitioned with petroleum ether and *n*-BuOH successively. The *n*-BuOH fraction was subjected to repeated column chromatography on silica gel, Sephadex LH-20 and HPLC to afford compound **1**.

Compound **1**, a white amorphous solid, mp 230°C (dec.), $[\alpha]_D^{20}$ +14 (*c* 0.105, methanol), was positive to Liebermann-Burchard and Molish tests. The positive HRESI-MS showed quasi molecular ion peak at *m/z* 1371.6128 ($[M+Na]^+$, calcd. 1371.6197), which, together with the quasi molecular ion peak at *m/z* 1347$[M-H]^-$ in negative ESI- MS and the NMR data, enabled the molecular formula to be determined as $C_{64}H_{100}O_{30}$. The IR spectrum of **1** revealed the presence of the hydroxyl (3416 cm^{-1}), ester carbonyl (1735 cm^{-1}) and double bond (1639 cm^{-1}) functionalities. It was evident that **1** was a triterpenoid saponin related to oleanolic acid based on the ^{1}H-NMR spectral signals assigned to six tertiary methyl groups at δ 0.70, 0.74, 1.17, 1.19, 1.28 and 1.46,

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together with the ^{13}C-NMR signals for olefinic carbons at δ 123.0 and 144.1, and a carbonyl carbon at δ 176.3. Comparison of the signals from the aglycone of **1** in the ^{13}C-NMR spectrum with the literature[2,3,10,11] showed that the aglycone of **1** was bayogenin. And the glycosidation shifts for the signals due to C-2 (-0.9), C-3 (+9.6), C-23 (-3.5), C-28 (-3.7) indicated that **1** was a bisdesmoside of bayogenin with glycosyl linkage at both 3-OH and 28-COOH.

The ^{1}H and ^{13}C-NMR spectra of **1** indicated the presence of five sugar units attributed to the sugar residues, and suggested they were two β-D-glucopyranosyl groups [anomeric protons at δ 4.89 (d, J=7.5 Hz) and 5.17 (d, J=7.5 Hz); anomeric carbons at δ 102.6 and 105.6], a α-L-arabinopyranosyl group [anomeric proton at δ 5.74 (d, J=7.0 Hz); anomeric carbon at δ 94.5], a α-L-rhamnopyranosyl group [anomeric proton at δ 6.29 (s), secondary methyl group at δ 1.34 (d, 3H, J=6.5 Hz); anomeric carbon at δ 102.1] and a β-D-xylopyranosyl group [anomeric proton at δ 4.98 (d, J=7.5 Hz); anomeric carbon at δ 106.4]. The above suggestion was confirmed by acid hydrolysis followed by GC-MS analysis of the corresponding alditol peracetates. Alkaline hydrolysis of **1** afforded a prosapogenin and trisaccharide, which was originally linked to C-28. The prosapogenin was subjected to acid hydrolysis to give bayogenin and glucose, while the trisaccharide gave arabinose, rhamnose and xylose.

Assignment of the sugar moieties was performed by the ^{1}H-^{1}H COSY, HSQC, TOCSY spectra combined with HMBC spectrum. Starting from the easily distinguished anomeric protons, it led to the total assignment of each proton and carbon signal. Detail inspection of HMBC spectrum led to the determination of conjuction of sugar chain. In the HMBC spectrum (see **Figure 1**), long range correlations from GlcI H-1 to C-3, GlcII H-1 to GlcI C-2, Ara H-1 to C-28, Rha H-1 to Ara C-2 and Xyl H-1 to Rha C-3 unequivocally revealed that a disaccharide β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl moiety and a trisaccharide β-D-arabinopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl moiety were located at C-3 and C-28 of the aglycone, respectively.

The presence of a dicrotalic acid moiety[12] was suggested from the NMR data [^{1}H-NMR: δ 3.64 and 2.72 (each 1H, AB system, J=15.5 Hz, H_2-2'), 3.03 and 2.58 (each 1H, AB system, J=16.5 Hz, H_2-4'), 1.68 (3H, s, H_3-6'); ^{13}C-NMR: δ 171.8 (s, C-1'), 171.3 (s, C-5'), 70.0 (s, C-3'), 46.5 (t, C-2'), 46.3 (t, C-4'), 26.5 (q, C-6')], combined with spectroscopic evidences obtained by 2D NMR experiments. The allocation of the acyl moiety was determined on the basis of HMBC spectrum. A cross peak of long range coupling was observed between a proton signal at δ 5.96 (Rha H-4) and a carbonyl signal at δ 171.8 (C-1'), whereas the proton signals at δ 4.78 and 4.47 (GlcII H_2-6) were correlated with carbon signal at δ 171.3 (C-5'), indicating that one end of the dicrotalic acid bridge was bound to the *O*-4 of the rhamnose unit, and the other end, to the *O*-6 of the terminal glucose unit.

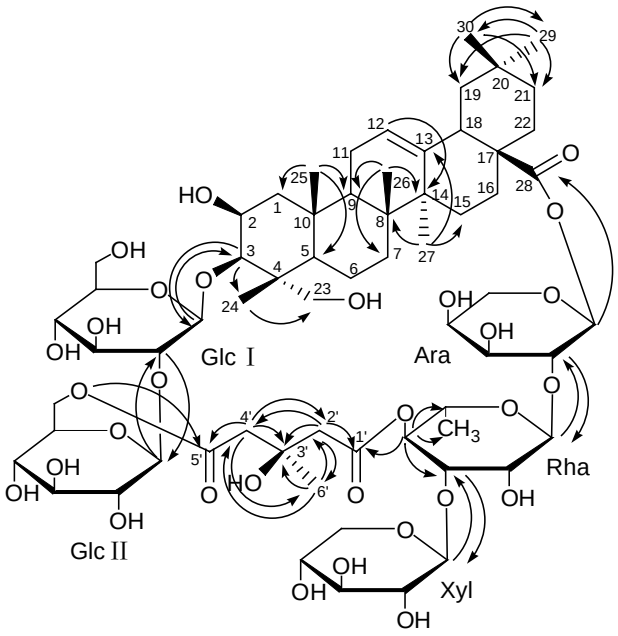
Accordingly, the structure of **1** was established as shown. In fact, tubeimoside V (**1**) is the 16-dehydroxylated derivative of tubeimoside III.

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data[a] of compound **1** (in C_5D_5N, δ ppm, J_{Hz})

Position	δ_H mult[b]	δ_C mult[c]	Position	δ_H mult[b]	δ_C
1	2.14 m, 1.06 m	44.1 t	GlcI		
2	4.57 m	69.5 d	1	4.89 d (7.5)	102.6
3	4.06 d (3.0)	83.0 d	2	3.91 t (8.0)	84.4
4		42.3 s	3	4.06 m	78.3
5	1.69 m	47.8 d	4	3.95 m	69.7
6	2.13 m, 1.72 m	18.4 t	5	3.67 m	78.1
7	1.80 m, 1.76 m	33.8 t	6	4.25 d (11.0), 4.07 d (11.0)	62.5
8		40.1 s	GlcII		
9	1.70 m	48.7 d	1	5.17 d (7.5)	105.6
10		36.9 s	2	3.93 m	76.9
11	1.98 m, 1.78 m	22.7 t	3	3.97 m	77.4
12	5.30 m	123.0 d	4	4.01 m	70.7
13		144.1 s	5	3.83 dd (9.5, 2.5)	75.6
14		42.3 s	6	4.78 d (11.4), 4.47 dd (12.0, 4.5)	64.3
15	1.90 m, 1.23 m	29.3 t	Ara		
16	2.00 m,1.88 m	24.0 t	1	5.74 d (7.0)	94.5
17		47.3 s	2	4.54 t (7.5)	75.8
18	3.00 dd (13.5, 3.5)	41.4 d	3	3.94 m	75.0
19	1.58 m, 1.10 m	46.2 t	4	3.92 m	70.6
20		30.7 s	5	3.99 m, 3.54 d (12.5)	67.4
21	1.22 m, 0.98 m	34.1 t	Rha		
22	1.65 m, 1.59 m	32.5 t	1	6.29 s	102.1
23	4.16 m, 3.57 d (11.5)	64.1 t	2	4.92 brs	72.5
24	1.28 s	15.1 q	3	4.52 dd (10.5, 3.0)	78.3
25	1.46 s	17.4 q	4	5.96 t (10.0)	73.5
26	1.17 s	17.7 q	5	4.32 dq (10.0, 6.5)	67.8
27	1.19 s	26.1 q	6	1.34 d (6.5)	18.1
28		176.3 s	Xyl		
29	0.74 s	33.0 q	1	4.98 d (7.5)	106.4
30	0.70 s	23.5 q	2	3.66 m	74.8
Acyl moiety			3	3.94 m	77.9
1'		171.8 s	4	3.95 m	70.9
2'	3.64 d (15.5), 2.72 d (15.5)	46.5 t	5	3.99 m, 3.49 d (10.5)	67.0
3'		70.0 s			
4'	3.03 d (16.5), 2.58 d (16.5)	46.3 t			
5'		171.3 s			
6'	1.68 s	26.5 q			
3'-OH	5.79 s				

[a]Assignments aided by ^{1}H-^{1}H COSY, TOCSY, HSQC and HMBC experiments.
[b]coupling constants (in Hz) are given in parentheses.
[c]multiplicity by DEPT.

Figure 1 The structure and key HMBC correlatios of **1** (H→C)



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