FULL PAPER

Five New Oleanane Triterpenoid Saponins from the Aerial Parts of *Elsholtzia bodinieri*

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Five new oleanane triterpenoid saponins, bodiniosides H – L (**1** – **5**, resp.), were isolated from the aerial parts of *Elsholtzia bodinieri*. Their structures were elucidated on the basis of spectroscopic techniques, including HSQC, HMBC, and HSQC-TOCSY experiments, together with acid hydrolysis and GC analysis.

Keywords: *Elsholtzia bodinieri*, Oleanane triterpenoid saponins, Bodiniosides H – L.

Introduction

The genus *Elsholtzia* (Lamiaceae) consists of at least 33 species, which have been widely distributed and applied in East Asia, Africa, North America, and European countries for centuries [1]. *Elsholtzia bodinieri* Vaniot (Chinese name 'Dongzisu'), commonly known as 'yashuacao', is an annual herbaceous plant distributed in the northwest and southwest districts of China, especially in Yunnan and Guizhou Provinces. It has been used as herbal tea or traditional folk medicine for the prophylaxis and treatment of cough, headache, pharyngitis, fever, and hepatitis [2]. Previous studies on *E. bodinieri* have led to the isolation of triterpenoid saponins [3][4], sesquiterpene glycosides [5], clerodane diterpenoid glycosides [6], and phenolic constituents [7]. As a continuation of our investigation on *E. bodinieri* [8 – 11], five new oleanane triterpenoid saponins, bodiniosides H – L (**1** – **5**) (*Fig. 1*), were isolated from the AcOEt extract of the aerial parts of this plant. In this paper, we report the isolation and structural elucidation of compounds **1** – **5**.

Results and Discussion

Compound **1** was obtained as white amorphous powder, and its negative HR-ESI-MS spectrum indicated the molecular formula to be $C_{60}H_{96}O_{27}$, by the observation of the quasi-molecular ion peak ($[M-H]^-$) at *m/z* 1247.6051 and with the help of NMR spectroscopic data, requiring thirteen degrees of unsaturation. The IR spectrum exhibited the presence of OH (3441 cm^{-1}), C=O (1722 cm^{-1}), and olefinic (1635 cm^{-1}) groups. Acid hydrolysis of **1** with 1M HCl produced L-rhamnose (Rha), D-glucose (Glc), and D-xylose (Xyl) as sugar residues as determined by GC analysis of their corresponding trimethylsilylated L-cysteine derivatives.

The ^{1}H-NMR spectrum (*Table 1*) of **1** showed the existence of six tertiary Me groups at δ(H) 0.81 (*s*, Me(24)), 1.00 (*s*, Me(25)), 0.78 (*s*, Me(26)), 1.14 (*s*, Me(27)), 0.91 (*s*, Me(29)), and 0.94 (*s*, Me(30)); an acetyl methyl at δ(H) 2.10 (*s*); an oxygenated CH_2 at δ(H) 3.98 – 4.00 (*m*, H_a–C(23)), and 4.19 – 4.21 (*m*, H_b–C(23)); an olefinic CH_2 at δ(H) 5.26 (br. *s*, H–C(12)); and an oxygenated CH at δ(H) 3.56 (*dd*, *J* = 8.9, 3.0 Hz, H–C(3)). Additionally, a series of signals at δ(H) 5.48 (br. *s*)/1.35 (*d*, *J* = 6.1 Hz), 5.39 (*d*, *J* = 7.7 Hz), 4.50 (*d*, *J* = 7.7 Hz), 4.22 (*d*, *J* = 7.7 Hz), and 4.28 (*d*, *J* = 7.7 Hz), gave HSQC correlations with the carbons at δ(C) 101.5 (*d*)/18.8 (*q*), 95.3 (*d*), 107.1 (*d*), 107.4 (*d*), and 105.5 (*d*), respectively, which further supported the presence of one rhamnopyranosyl (Rha), two glucopyranosyl (Glc and Glc′), and two xylopyranosyl (Xyl and Xyl′) units. Since NMR signals of five monosaccharides overlapped undesirablely, the HSQC-TOCSY experiment was successfully used to distinguish and assign the ^{1}H- and ^{13}C-NMR signals of each sugar moiety. For example, the correlations from the anomeric H-atom signal at δ(H) 4.22 to five C-atom signals at δ(C) 107.4 (anomeric carbon), 78.4, 75.7, 71.4, and 67.3, as well as from five H-atom signals at δ(H) 4.22, 3.80 – 3.82, 3.47 – 3.49, 3.25 – 3.27, and 3.15 – 3.18 to the anomeric carbon, suggested the presence of β-D-xylopyranose. Except for the signals belonging to five monosaccharides and an acetyl group, the ^{13}C-NMR spectrum (*Table 2*) of **1** exhibited 30 signals for aglycone including six Me groups at δ(C) 13.6 (*q*, C(24)), 16.9 (*q*, C(25)), 18.3 (*q*, C(26)), 26.5 (*q*, C(27)), 34.9 (*q*, C(29)), and 24.7 (*q*, C(30)), 11 CH_2 groups including an oxygenated one (δ(C) 66.7, *t*, C(23)), five CH groups containing one

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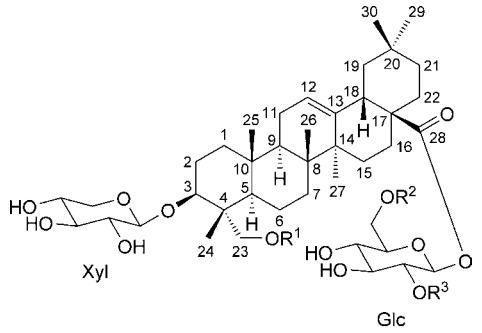

	R^1	R^2	R^3
1	Ac	Xyl'	Glc'-(1→4)-Rha–
2	Ac	Xyl'	Rha
3	Ac	H	Glc'-(1→4)-Rha–
4	H	H	Rha
5	H	Xyl'	H

Fig. 1. Structures of **1** – **5**

oxygenated (δ(C) 83.8, *d*, C(3)), and one unsaturated (δ(C) 124.1, *d*, C(12)), and eight quaternary carbons including one C=O (δ(C) 178.4, *s*, C(28)) and an unsaturated one (δ(C) 145.1, *s*, C(13)). Considering the spectroscopic characteristic mentioned above and the degrees of unsaturation, compound **1** was assumed to be a pentacyclic triterpenoid saponin.

Careful comparison of the 1D- and 2D-NMR data of **1** with those of bodinioside C [10] led to the deductions that they were similar, except for the appearance of an acetyl group (δ(C) 173.5 (*s*) and 21.4 (*q*); δ(H) 2.10 (*s*)) in **1** and the variation of a monosaccharide attached to C(28). The acetyl group was attached to C(23) of the aglycone according to the HMBC correlations (*Fig. 2*) from the H-atom at δ(H) 2.10 (*s*, Ac), 3.98 – 4.00 (*m*, H_a–C(23)), and 4.19 – 4.21 (*m*, H_b–C(23)) to the C=O carbon at δ (C) 173.5. The HMBC correlation between H–C(1) (δ(H) 4.22) of Xyl and C(3) of the aglycone elucidated that the Xyl moiety was located at C(3). While, the HMBC correlation between H–C(1) (δ(H) 4.28) of Xly′ and C(6) (δ(C) 69.5) of Glc, revealed that Glc′ unit attached to C(6) of Glc in bodinioside C had been replaced by Xyl′ unit in **1**. In combination with the following HMBC correlations, from H–C(1) (δ(H) 5.39) of Glc to C(28) of the aglycone, from H–C(1) (δ(H) 5.48) of Rha to C (2) (δ(C) 76.7) of Glc, along with from H–C(1) (δ(H) 4.50) of Glc′ to C(4) (δ(C) 85.3) of Rha, the glycosidic linkages at C(28) was established as depicted in *Fig. 1*. The β-pyranosyl configurations of the glycosidic bonds of glucopyranosyl and xylopyranosyl moieties were deduced from their $^3J_{H1,H2}$ coupling constants (^3J = 7.7 Hz), while the anomeric configuration of rhamnopyranosyl moiety was determined to be α according to the ^{13}C-NMR chemical shifts [10].

The NOE correlations (*Fig. 3*) between H–C(3) and H_b-C(23) with H–C(5) (δ(H) 1.87) in the ROESY spectrum indicated the α-configuration for H–C(3) and H_b–C(23). From the foregoing evidences, the structure of **1** was unequivocally determined to be [(3β)-23-(acetyloxy)-28-oxo-3-(β-D-xylopyranosyloxy)olean-12-en-28-yl] β-D-gluco pyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranose, and named as bodinioside H.

Compound **2** was isolated as white amorphous powder, possessing a molecular formula of $C_{54}H_{86}O_{22}$ as established by its negative HR-ESI-MS (*m/z* 1085.5521, $[M - H]^-$), indicating twelve degrees of unsaturation. Detailed comparison of the ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*) of **2** with those of **1** revealed highly structural similarity, except for the absence of the terminal glucose moiety (Glc′) at C(28). Acid hydrolysis of **2** also yielded L-rhamnose, D-glucose, and D-xylose as sugar residues as determined by GC analysis. In the ^{1}H-NMR spectrum of **2**, four anomeric H-atom at δ(H) 5.36 (br. *s*), 5.43 (*d*, *J* = 7.5 Hz), 4.20 (*d*, *J* = 7.6 Hz), and 4.28 (*d*, *J* = 7.5 Hz) indicated the presence of four sugar residues: a rhamnopyranosyl (Rha), a glucopyranosyl (Glc), and two xylopyranosyl (Xyl and Xyl′) units. The Xyl unit was still linked to C(3) (δ(C) 83.5) of the aglycone based on the HMBC correlation between H–C(1) (δ(H) 4.20) of Xyl and C(3). A series of HMBC correlations from H–C(1) (δ(H) 5.43) of Glc to C(28) (δ(C) 178.0) of the aglycone, from H–C(1) (δ(H) 4.28) of Xyl′ to C(6) (δ(C) 69.1) of Glc, and from H–C(1) (δ(H) 5.36) of Rha to C(2) (δ(C) 77.6) of Glc, enabled the sugar chain at C(28) to be assigned as α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl. Thus, the structure of **2** was adequately illustrated as [(3β)-23-(acetyloxy)-28-oxo-3-(β-D-xylopyranosyloxy)olean-12-en-28-yl] α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranose, and named bodinioside I.

Bodinioside J (**3**), was obtained as white amorphous powder, and the molecular formula $C_{55}H_{88}O_{23}$ was deduced from pseudomolecular ions $[M + Na]^+$ and $[M + K]^+$ at *m/z* 1139 and 1155, respectively, in positive ESI-MS, and further confirmed by the negative HR-ESI-MS (*m/z* 1115.5630, $[M - H]^-$), requiring twelve degrees of unsaturation. Detailed analysis of the 1D- and 2D-NMR spectra (*Tables 1* and *2*) of **3** and **1** made it clear that the two compounds were identical, except for the absence of signals for Xyl′ moiety on C(28), which led to 132 mass units less than that of compound **1**. GC analysis after acid hydrolysis of **3** as the same manner with **1** gave D-glucose, D-xylose, and L-rhamnose in a ratio of 2:1:1. In addition, C(6) (δ(C) 62.1) of Glc in **3** evidently moved to upfield when compared with that in **1**, which further demonstrated the above deduce. Accordingly, compound **3** was elucidated as [(3β)-23-(acetyloxy)-28-oxo-3-(β-D-xylopyranosyloxy)olean-12-en-28-yl]-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose.

Compound **4** was obtained as white amorphous powder. It exhibited a quasi-molecular ion peak at *m/z* 911.5001 $[M - H]^-$ in the negative HR-ESI-MS spectrum, suggesting a molecular formula $C_{47}H_{76}O_{17}$. Besides the absence of two terminal glucose moieties (Glc′ and

Table 1. ^{1}H-NMR Data of **1** – **5**. δ in ppm, J in Hz.

Position	**1**[a][b]	**2**[a][b]	**3**[c][d]	**4**[c][d]	**5**[c][e]
1	1.60 – 1.64 (*m*), 0.93 – 0.95 (*m*)	1.60 – 1.63 (*m*), 0.94 – 0.96 (*m*)	1.51 – 1.56 (*m*), 0.96 – 1.00 (*m*)	1.52 – 1.55 (*m*), 1.03 – 1.07 (*m*)	1.55 – 1.60 (*m*), 1.05 – 1.09 (*m*)
2	1.83 – 1.86 (*m*), 1.70 – 1.73 (*m*)	1.84 – 1.87 (*m*), 1.71 – 1.74 (*m*)	2.20 – 2.26 (*m*), 1.90 – 1.92 (*m*)	2.20 – 2.27 (*m*), 2.00 – 2.03 (*m*)	2.23 – 2.29 (*m*), 2.00 – 2.05 (*m*)
3	3.56 (*dd*, *J* = 8.9, 3.0)	3.51 – 3.58 (*m*)	3.94 – 3.99 (*m*)	4.30 – 4.32 (*m*)	4.29 – 4.33 (*m*)
5	1.86 – 1.88 (*m*)	1.57 – 1.60 (*m*)	1.71 – 1.74 (*m*)	1.67 – 1.69 (*m*)	1.67 – 1.71 (*m*)
6	1.43 – 1.46 (*m*)	1.35 – 1.37 (*m*)	1.64 – 1.69 (*m*), 1.32 – 1.38 (*m*)	1.60 – 1.66 (*m*), 1.30 – 1.33 (*m*)	1.66 – 1.69 (*m*), 1.34 – 1.38 (*m*)
7	1.76 – 1.81 (*m*), 1.50 – 1.55 (*m*)	1.80 – 1.83 (*m*), 1.51 – 1.54 (*m*)	1.86 – 1.89 (*m*)	1.82 – 1.87 (*m*)	1.73 – 1.78 (*m*)
9	1.64 – 1.66 (*m*)	1.54 – 1.57 (*m*)	1.74 – 1.78 (*m*)	1.70 – 1.76 (*m*)	1.78 – 1.82 (*m*)
11	1.87 – 1.91 (*m*)	1.92 – 1.95 (*m*), 1.87 – 1.90 (*m*)	1.94 – 1.96 (*m*)	1.90 – 1.97 (*m*)	1.96 – 2.00 (*m*), 1.91 – 1.94 (*m*)
12	5.26 (br. *s*)	5.27 (br. *s*)	5.45 (br. *s*)	5.44 (br. *s*)	5.41 (br. *s*)
15	1.55 – 1.58 (*m*), 1.24 – 1.26 (*m*)	1.64 – 1.67 (*m*), 1.15 – 1.18 (*m*)	2.07 – 2.11 (*m*), 1.58 – 1.61 (*m*)	2.04 – 2.07 (*m*), 1.57 – 1.60 (*m*)	2.29 – 2.33 (*m*), 1.08 – 1.11 (*m*)
16	2.03 – 2.07 (*m*), 1.58 – 1.61 (*m*)	2.02 – 2.05 (*m*), 1.63 – 1.64 (*m*)	1.93 – 1.99 (*m*)	2.11 – 2.18 (*m*), 2.07 – 2.11 (*m*)	2.05 – 2.08 (*m*), 1.87 – 1.91 (*m*)
18	2.83 (*dd*, *J* = 13.7, 3.9)	2.84 (*dd*, *J* = 13.6, 3.9)	3.12 (*dd*, *J* = 13.8, 5.3)	3.09 (*dd*, *J* = 13.9, 4.0)	3.19 (*dd*, *J* = 13.7, 4.1)
19	1.69 – 1.73 (*m*), 1.10 – 1.13 (*m*)	1.67 – 1.70 (*m*), 1.10 – 1.13 (*m*)	1.69 – 1.73 (*m*), 1.19 – 1.24 (*m*)	1.70 – 1.73 (*m*), 1.18 – 1.22 (*m*)	1.69 – 1.72 (*m*), 1.19 – 1.21 (*m*)
21	1.21 – 1.24 (*m*)	1.21 – 1.24 (*m*)	1.26 – 1.32 (*m*), 1.05 – 1.08 (*m*)	1.30 – 1.35 (*m*), 1.03 – 1.08 (*m*)	1.30 – 1.34(*m*), 1.06 – 1.09 (*m*)
22	1.37 – 1.41 (*m*)	1.36 – 1.40 (*m*)	1.65 – 1.69 (*m*)	1.63 – 1.68 (*m*)	1.60 – 1.64 (*m*)
23	4.19 – 4.21 (*m*), 3.98 – 4.00 (*m*)	4.07 – 4.13 (*m*), 4.00 – 4.05 (*m*)	4.56 – 4.60 (*m*), 4.45 – 4.48 (*m*)	4.35 – 4.39 (*m*), 3.63 – 3.66 (*m*)	4.36 – 4.39 (*m*), 3.71 – 3.75 (*m*)
24	0.81 (*s*)	0.80 (*s*)	0.81 (*s*)	0.91 (*s*)	0.97 (*s*)
25	1.00 (*s*)	1.00 (*s*)	0.86 (*s*)	0.94 (*s*)	0.99 (*s*)
26	0.78 (*s*)	0.80 (*s*)	1.08 (*s*)	1.10 (*s*)	1.14 (*s*)
27	1.14 (*s*)	1.15 (*s*)	1.27 (*s*)	1.18 (*s*)	1.18 (*s*)
29	0.91 (*s*)	0.90 (*s*)	0.85 (*s*)	0.83 (*s*)	0.86 (*s*)
30	0.94 (*s*)	0.94 (*s*)	0.82 (*s*)	0.76 (*s*)	0.87 (*s*)
23-AcO	2.10 (*s*)	2.06 (*s*)	2.14 (*s*)		
3-O-*Xyl*					
1	4.22 (*d*, *J* = 7.7)	4.20 (*d*, *J* = 7.6)	4.87 (*d*, *J* = 7.5)	5.08 (*d*, *J* = 8.5)	5.08 (*d*, *J* = 7.4)
2	3.15 – 3.18 (*m*)	3.12 – 3.15 (*m*)	4.03 – 4.04 (*m*)	4.03 – 4.07 (*m*)	4.00 – 4.05 (*m*)
3	3.25 – 3.27 (*m*)	3.44 – 3.46 (*m*)	3.77 – 3.79 (*m*)	4.09 – 4.13 (*m*)	4.10 – 4.12 (*m*)
4	3.47 – 3.49 (*m*)	3.61 – 3.66 (*m*)	4.35 – 4.37 (*m*)	4.20 – 4.26 (*m*)	4.19 – 4.23 (*m*)
5	3.80 – 3.82 (*m*), 3.15 – 3.18 (*m*)	3.80 – 3.83 (*m*), 3.14 – 3.18 (*m*)	4.40 – 4.42 (*m*), 3.79 – 3.80 (*m*)	4.32 – 4.35 (*m*), 3.65 – 3.68 (*m*)	4.30 – 4.33 (*m*), 3.63 – 3.68 (*m*)
28-O-*Sugar*					
Glc					
1	5.39 (*d*, *J* = 7.7)	5.43 (*d*, *J* = 7.5)	6.22 (*d*, *J* = 8.0)	6.20 (*d*, *J* = 8.1)	6.31 (*d*, *J* = 8.2)
2	3.61 – 3.63 (*m*)	3.56 – 3.60 (*m*)	4.10 – 4.11 (*m*)	4.03 – 4.06 (*m*)	4.12 – 4.16 (*m*)
3	3.25 – 3.27 (*m*)	3.53 – 3.56 (*m*)	4.32 – 4.34 (*m*)	4.35 – 4.38 (*m*)	4.23 – 4.27 (*m*)
4	3.45 – 3.47 (*m*)	3.43 – 3.48 (*m*)	4.22 – 4.26 (*m*)	4.17 – 4.23 (*m*)	4.38 – 4.41 (*m*)
5	3.47 – 3.48 (*m*)	3.25 – 3.27 (*m*)	4.16 – 4.18 (*m*)	4.10 – 4.13 (*m*)	4.14 – 4.16 (*m*)
6	3.99 – 4.01 (*m*), 3.69 – 3.71 (*m*)	4.99 – 4.00 (*m*), 3.70 – 3.73 (*m*)	4.41 – 4.43 (*m*), 4.35 – 4.37 (*m*)	4.43 – 4.46 (*m*), 4.37 – 4.41 (*m*)	4.73 – 4.77 (*m*), 4.37 – 4.39 (*m*)
Rha					
1	5.48 (br. *s*)	5.36 (br. *s*)	6.54 (br. *s*)	6.63 (br. *s*)	
2	4.48 – 4.50 (*m*)	3.92 – 3.94 (*m*)	4.24 – 4.26 (*m*)	4.38 – 4.41 (*m*)	
3	3.83 – 3.85 (*m*)	3.62 – 3.64 (*m*)	4.73 – 4.76 (*m*)	4.55 – 4.59 (*m*)	
4	3.53 – 3.55 (*m*)	3.36 – 3.38 (*m*)	3.97 – 4.00 (*m*)	4.41 – 4.43 (*m*)	
5	3.79 – 3.81 (*m*)	3.70 – 3.72 (*m*)	4.23 – 4.24 (*m*)	4.54 – 4.60 (*m*)	
6	1.35 (*d*, *J* = 6.1)	1.23 (*d*, *J* = 6.1)	1.82 (*d*, *J* = 6.1)	1.75 (*d*, *J* = 6.0)	
Xyl′					
1	4.28 (*d*, *J* = 7.7)	4.28 (*d*, *J* = 7.5)			4.96 (*d*, *J* = 7.5)
2	3.18 – 3.21 (*m*)	3.18 – 3.20 (*m*)			4.00 – 4.04 (*m*)
3	3.45 – 3.48 (*m*)	3.58 – 3.59 (*m*)			4.12 – 4.14 (*m*)
4	3.92 – 3.97 (*m*)	3.91 – 3.92 (*m*)			4.23 – 4.26 (*m*)
5	3.81 – 3.83 (*m*)	3.78 – 3.85 (*m*)			4.33 – 4.36 (*m*)

Table 1. (cont.)

Position	**1**[a)b)]	**2**[a)b)]	**3**[c)d)]	**4**[c)d)]	**5**[c)e)]
					3.67 – 3.71 (*m*)
Glc′					
1	4.50 (*d*, *J* = 7.7)		5.12 (*d*, *J* = 7.6)		
2	3.61 – 3.64 (*m*)		4.09 – 4.11 (*m*)		
3	3.54 – 3.56 (*m*)		4.16 – 4.18 (*m*)		
4	3.28 – 3.30 (*m*)		4.34 – 4.37 (*m*)		
5	3.36 – 3.39 (*m*)		3.78 – 3.80 (*m*)		
6	3.83 – 3.85 (*m*),		4.45 – 4.49 (*m*),		
	3.68 – 3.69 (*m*)		4.35 – 4.37 (*m*)		

[a]) Recorded in CD_3OD. [b]) Recorded at 400 MHz. [c]) Recorded in (D_5)pyridine. [d]) Recorded at 500 MHz. [e]) Recorded at 600 MHz.

Table 2. ^{13}C-NMR Data of **1** – **5**. δ in ppm, *J* in Hz.

Position	**1**[a)b)]	**2**[a)b)]	**3**[c)d)]	**4**[c)d)]	**5**[c)e)]
1	39.9	39.5	38.6	38.9	39.3
2	26.8	26.4	26.2	26.2	26.7
3	83.8	83.5	82.0	82.1	82.3
4	43.7	43.0	42.2	43.5	42.5
5	48.5	48.3	48.5	47.7	48.0
6	19.6	19.1	18.6	18.3	18.6
7	33.5	33.1	32.3	32.2	33.0
8	41.1	40.8	40.0	40.0	40.4
9	48.8	48.5	48.5	48.2	48.6
10	38.2	37.8	38.6	37.0	37.4
11	25.0	24.6	23.9	23.9	24.3
12	124.1	123.7	122.7	122.7	123.4
13	145.1	144.7	144.1	144.3	144.5
14	43.4	42.9	42.1	42.8	44.0
15	29.7	29.1	28.6	28.7	28.7
16	24.3	24.0	23.3	23.4	23.8
17	48.5	48.1	47.2	47.1	47.5
18	43.2	42.8	42.6	42.1	42.1
19	47.6	47.3	46.3	46.3	46.6
20	32.0	31.6	30.7	30.7	31.2
21	35.3	34.8	34.0	34.0	34.4
22	34.3	33.7	33.2	33.0	33.0
23	66.7	66.3	65.9	64.5	64.8
24	13.6	13.3	13.1	13.6	14.1
25	16.9	16.5	16.0	16.3	16.7
26	18.3	18.3	17.6	17.5	18.0
27	26.5	26.1	25.7	25.9	26.5
28	178.4	178.0	176.5	176.4	177.0
29	34.9	33.5	33.1	33.1	33.6
30	24.7	24.3	23.7	23.7	24.1
23-AcO	173.5	172.6	171.2		
	21.4	20.9	20.9		
3-O-*Xyl*					
1	107.4	107.0	107.3	106.8	107.4
2	75.7	75.3	75.4	75.7	76.2
3	78.4	77.3	78.9	78.6	79.1
4	71.4	71.2	71.8	71.4	71.7
5	67.3	66.8	67.2	67.2	67.7
28-O-*Sugar*					
Glc					
1	95.3	95.0	94.7	94.9	96.1
2	76.7	77.6	76.1	75.6	74.3
3	78.4	79.0	79.6	79.8	79.3
4	71.4	70.9	71.7	71.2	71.3
5	77.9	78.0	78.6	79.0	78.4
6	69.5	69.1	62.1	62.1	69.6

Table 2. (cont.)

Position	**1**[a)b)]	**2**[a)b)]	**3**[c)d)]	**4**[c)d)]	**5**[c)e)]
Rha					
1	101.5	101.6	101.3	101.5	
2	71.5	72.0	71.3	72.3	
3	72.7	72.1	72.5	72.6	
4	85.3	73.7	85.8	73.9	
5	69.2	70.3	71.3	69.8	
6	18.8	17.9	18.7	18.8	
Xyl′					
1	105.5	105.1			106.2
2	75.3	74.9			75.3
3	77.9	77.4			78.7
4	72.2	71.2			71.6
5	67.2	66.9			67.6
Glc′					
1	107.1		107.2		
2	76.7		76.4		
3	79.8		78.7		
4	71.9		71.3		
5	78.9		78.3		
6	63.3		62.9		

[a]) Recorded in CD_3OD. [b]) Recorded at 100 MHz. [c]) Recorded in (D_5)pyridine. [d]) Recorded at 125 MHz. [e]) Recorded at 150 MHz.

Glc″) on C(28), most NMR signals (*Tables 1* and *2*) of **4** were nearly identical to those of bodinioside C [10]. Three anomeric H-atom at δ(H) 5.08 (*d*, *J* = 8.5 Hz), 6.20 (*d*, *J* = 8.1 Hz), and 6.63 (*s*) in the ^{1}H-NMR spectrum were ascribed to D-xylose, D-glucose, and L-rhamnose, respectively, in combination with acid hydrolysis and GC analysis. The Xyl unit was assigned to C(3) (δ(C) 82.1) of the aglycone on the basis of the long range correlation between H–C(1) (δ(H) 5.08) of Xyl and C(3). Meanwhile, the cross-peaks between H–C(1) (δ(H) 6.20) of Glc and C(28) (δ(C) 176.4), as well as between H–C(1) of Rha (δ(H) 6.63) and C(2) (δ(C) 75.6) of Glc in the HMBC spectrum clarified the linkage of Glc and Rha units at C(28) as shown. The structure of compound **4** was thereby concluded to be [(3β)-23-hydroxy-28-oxo-3-(β-D-xylopyranosyloxy)olean-12-en-28-yl] 2-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranose, and named as bodinioside K.

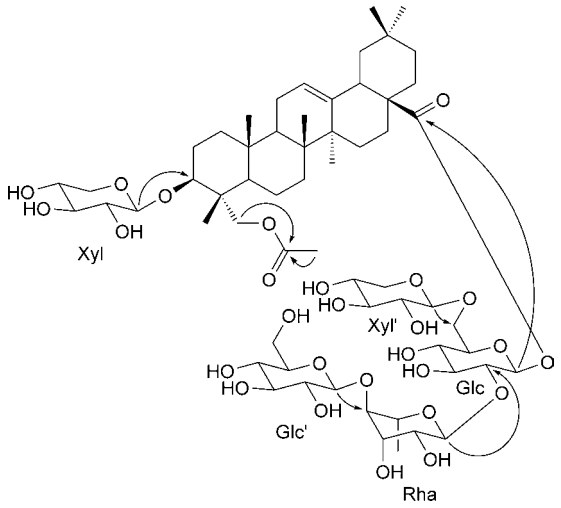

Fig. 2. Key HMB correlations of **1**

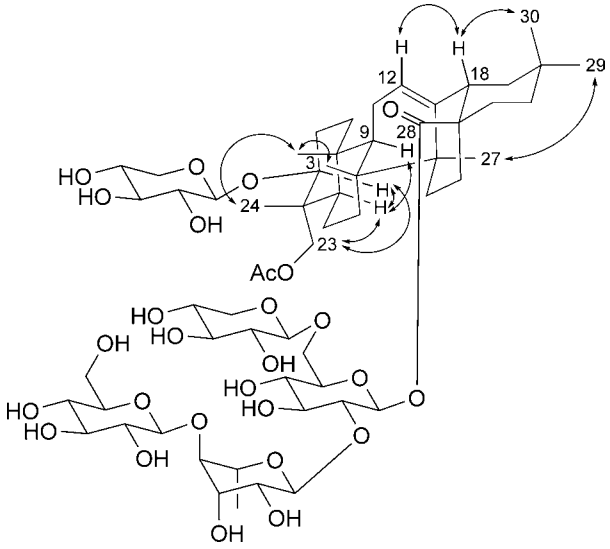

Fig. 3. Key ROESY correlations of **1**

The molecular formula of bodinioside L (**5**) was established as $C_{46}H_{74}O_{17}$ on the basis of the negative HR-ESI-MS from the *quasi*-molecular ion peak at *m/z* 897.4842 ($[M - H]^-$), indicating ten degrees of unsaturation. Interpretation of its NMR data (*Tables 1* and *2*) revealed that the structure of compound **5** was closely related to compound **4**, with the exception of sugar chain at C(28). The absence of the signals belonging to one rhamnose in compound **4**, and the presence of the other xylose (Xyl′) in compound **5** illustrated that the Xyl group attached to Glc in **5** instead of the Rha group in **4**. The HMBC correlation from H–C(1) (δ(H) 4.96, *d*, *J* = 7.5 Hz) of Xyl′ to C(6) (δ(C) 69.6) of Glc, further demonstrated that the Xyl′ moiety was linked to C(6) of Glc moiety, rather than C(2) of Glc moiety. Consequently, the structure of **5** was unambiguously characterized as [(3β)-23-hydroxy-28-oxo-3-(β-D-xylopyranosyloxy)olean-12-en-28-yl] 6-*O*-β-D-xylopyranosyl-β-D-glucopyranose.

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Experimental Part

General

Thin layer chromatography (TLC) and column chromatography (CC): silica gel (SiO_2; 100 – 200 mesh, 200 – 300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China), *Sephadex LH-20* (40 – 70 μm; *Amersham Pharmacia Biotech AB*, Uppsala, Sweden), and *YMC-Gel ODS-A* (50 μm; *YMC*, Milford, MA, USA). HPLC: *Agilent 1200* LC system; *Zorbax SB-C_{18}* (9.4 × 250 mm; 5 μm); flow rate, 3 ml/min. GC: *Agilent Technologies HP5890* gas chromatograph; *30QC2/AC-5* (30 m × 0.32 mm; Massy, France); carrier gas, N_2; flow rate, 1 ml/min. Optical rotations: *Jasco DIP-370* digital polarimeter (MD, USA). UV Spectra: *UV-210A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AM-400* (400 and 100 MHz for ^1H and ^{13}C, resp.), *DRX-500* (500 and 125 MHz for ^1H and ^{13}C, resp.), or *AVANCE III-600* (600 and 150 MHz for ^1H and ^{13}C, resp.) spectrometers (*Bruker BioSpin Group*, Rheinstetten, Germany); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *VG-Autospec-3000* spectrometer; in *m/z*. HR-ESI-MS: *API-QSTAR-Pulsar* instrument; in *m/z*.

Plant Material

The aerial parts of *E. bodinieri* were collected from Honghe, Yunnan Province, P. R. China, in May 2010, and identified by Prof. *Hai-Zhou Li*, Kunming University of Science and Technology. A voucher specimen (KMUST 20100501) was deposited with the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science and Technology.

Extraction and Isolation

The aerial parts of *E. bodinieri* (15 kg) were powdered and extracted with 75% aq. acetone (3 × 35 l, 24 h, each) at room temperature, then concentrated *in vacuo* to yield an extract, which was suspended in H_2O, and successively partitioned with $CHCl_3$, AcOEt, and BuOH. The AcOEt extract (507.0 g) was chromatographed over silica gel CC eluting with $CHCl_3$/acetone (gradient 1:0 – 0:1, each 4 l) to afford six fractions, A – F. *Fr. F* ($CHCl_3$/acetone 0:1, 65.5 g) was separated by *Sephadex LH-20* gel column (eluted with 30%, 60%, and 90% MeOH/H_2O) to obtain subfractions F-1~F-4. *Fr. F-1* (12.5 g) was isolated by *RP-18* CC (eluted with 30%, 60%, and 90% MeOH/H_2O) to get subfractions F-1-1~F-1-6. *Fr. F-1-6* (1.4 g) was sub-

jected to silica gel CC (eluted with $CHCl_3$/MeOH/H_2O from 8: 2: 0.5 to 6.5: 3.5: 1) to yield F-1-6-1~F-1-6-5. *Fr. F-1-6-4* (185.0 mg) was further purified on semipreparative HPLC (62% MeOH/H_2O) to obtain compounds **4** (t_R = 14.6 min, 11.9 mg) and **5** (t_R = 18.7 min, 3.6 mg). *Fr. F-1-6-5* (350.0 mg) was also purified over semipreparative HPLC (65% MeOH/H_2O) to give compounds **1** (t_R = 15.6 min, 18.9 mg), **2** (t_R = 23.7 min, 25.9 mg), and **3** (t_R = 25.3 min, 4.9 mg).

Bodinioside H (= **[(3*β*)-23-(Acetyloxy)-28-oxo-3-(*β*-D-xylopyranosyloxy)olean-12-en-28-yl] *β*-D-Glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→6)]-*β*-D-glucopyranose**; **1**). White amorphous powder. $[\alpha]_D^{19}$ = −20.1 (*c* = 0.14, MeOH). IR (KBr): 3441, 2942, 1722, 1635, 1384, 1255, 1045. UV (MeOH): 202.8 (3.89). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 1283 ([*M* + Cl]$^-$), 1247 ([*M* − H]$^-$). HR-ESI-MS (neg.): 1247.6051 ([*M* − H]$^-$, $C_{60}H_{95}O_{27}^-$; calc. 1247.6066).

Bodinioside I (= **[(3*β*)-23-(Acetyloxy)-28-oxo-3-(*β*-D-xylopyranosyloxy)olean-12-en-28-yl] *α*-L-Rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→6)]-*β*-D-glucopyranose**; **2**). White amorphous powder. $[\alpha]_D^{23.1}$ = −55.6 (*c* = 0.06, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 1121 ([*M* + Cl]$^-$), 1085 ([*M* − H]$^-$). HR-ESI-MS (neg.): 1085.5521 ([*M* − H]$^-$, $C_{54}H_{85}O_{22}^-$; calc. 1085.5538).

Bodinioside J (= **[(3*β*)-23-(Acetyloxy)-28-oxo-3-(*β*-D-xylopyranosyloxy)olean-12-en-28-yl] *β*-D-Glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranose**; **3**). White amorphous powder. $[\alpha]_D^{22.8}$ = −12.2 (*c* = 0.12, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 1139 ([*M* + Na]$^+$), 1155 ([*M* + K]$^+$). HR-ESI-MS (neg.): 1115.5630 ([*M* − H]$^-$, $C_{55}H_{87}O_{23}^-$; calc. 1115.5644).

Bodinioside K (= **[(3*β*)-23-Hydroxy-28-oxo-3-(*β*-D-xylopyranosyloxy)olean-12-en-28-yl] 2-*O*-(*α*-L-Rhamnopyranosyl)-*β*-D-glucopyranose**; **4**). White amorphous powder. $[\alpha]_D^{23.1}$ = −17.8 (*c* = 0.06, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 935 ([*M* + Na]$^+$). HR-ESI-MS (neg.): 911.5001 ([*M* − H]$^-$, $C_{47}H_{75}O_{17}^-$; calc. 911.5010).

Bodinioside L (= **[(3*β*)-23-Hydroxy-28-oxo-3-(*β*-D-xylopyranosyloxy)olean-12-en-28-yl] 6-*O*-*β*-D-Xylopyranosyl-*β*-D-glucopyranose**; **5**). White amorphous powder. $[\alpha]_D^{18.5}$ = −7.6 (*c* = 0.19, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 921 ([*M* + Na]$^+$). HR-ESI-MS (neg.): 897.4842 ([*M* − H]$^-$, $C_{46}H_{73}O_{17}^-$; calc. 897.4853).

Acid Hydrolysis for Sugar Analysis

Each of **1** – **5** (1.0 mg for each compound) in 1M HCl (0.4 ml) was heated at 90 – 100 °C in a screw-capped vial for 5 h. The mixture was neutralized by addition of *Amberlite IRA400* (OH^- form) and filtered. The filtrate was dried *in vacuo*, dissolved in 0.2 ml of pyridine containing L-cysteine methyl ester (10 mg/ml) and reacted at 60 °C for 1 h. To this mixture, a solution (0.2 ml) of *N*-(trimethylsilyl)imidazole in pyridine (10 mg/ml) was added, and it was heated at 60 °C for 1 h. The final mixture was directly analyzed by GC (*30QC2/AC-5* quartz capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180 °C/280 °C; programmed increase 3 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl; split ratio: 1/50). The standards were prepared following the same procedure. Under these conditions, the retention times of D- and L-glucose, D- and L-xylose, and L-rhamnose were 18.29, 18.87, 13.35, 14.01, and 14.97 min, respectively. During coinjection studies, identical retention times were observed between the different hydrolysates and authentic standards.

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