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Note

Two new clerodane diterpenoid glucosides and other constituents from the roots of *Elsholtzia bodinieri* Van′t

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Two new clerodane diterpenoid glucosides, 6-hydroxy-(-)-hardwickiic acid 2′-β-D-glucopyranosylbenzyl ester **1** and 6,7-dihydroxy-(-)-hardwickiic acid 2′-β-D-glucopyranosylbenzyl ester **2**, together with six known compounds, 5,7- dihydroxyflavone **3**, 5,8,11,13-tetraene-18-oic acid **4**, allonhimachol **5**, oleanolic acid **6**, daucosterol **7** and bodinioside B **8**, have been isolated from the roots of *Elsholtzia bodinieri* Van′t. Their structures have been elucidated by extensive spectroscopic and chemical methods, in particular by using 2D-NMR methods. All compounds except **3, 6, 7** and **8** have been first isolated from *Elsholtzia bodinieri* Van′t.

Keywords: Constituent, Clerodane diterpenoid glucoside, *Elsholtzia bodinieri* Van′t, Labiatae

In recent years, Chinese medicinal herbs and their extracts have received great attention[1-3]. Many investigations have focused on them and explored some pharmacological agents. *Elsholtzia bodinieri* Van′t (Labiatae) belongs to the genus *Elsholtzia* wild, which is widely distributed in the south and southwest regions of China (Chinese name "fengweicha" or "dongzisu")[4]. As a Chinese medicinal plant, it is used in the treatment of diaphoresis, headache, fever, cough, pharyngitis, dyspepsia and hepatitis[5]. Its useful properties have prompted a search for the relative bioactive constituents. However, only a few reports on its chemical constituents are known so far[6-8]. In a previous paper[9], the isolation and characterization of ten compounds from the EtOH extract has been reported. In continuing phytochemical investigations on this plant, eight compounds were again isolated by repeated column chromatography over silica gel and preparative TLC from the root extract. Their structures were identified by spectral and chemical methods as follows: 6-hydroxy-(-)-hardwickiic acid 2′-β-D- glucopyranosylbenzyl ester **1**, 6,7-dihydroxy-(-)-hardwickiic acid 2′-β-D-glucopyranosylbenzyl ester **2**, 5,7-dihydroxyflavone **3** (Ref. 10), 5,8,11,13-tetraene-18-oic acid **4** (Ref. 11), allonhimachol **5** (Ref. 12), oleanolic acid **6**, daucosterol **7**, bodinioside B **8** (Ref. 7). Among them, compounds **1** and **2** were new (**Figure 1**), whereas compounds **4** and **5** were obtained for the first time from this plant. In the present communication, herein is reported the isolation and structural elucidation of two new compounds.

Compound 1 was obtained as a white amorphous powder from MeOH, and gave a positive Molisch reaction. Its molecular formula $C_{33}H_{44}O_{10}$ was deduced from HR-FAB-MS at *m/z* 601.3021 $[M+H]^+$ (calcd. for 601.3013), with twelve degrees of unsaturation. The IR spectrum (KBr) revealed the presence of hydroxyl groups (3340 cm^{-1}, br), aromatic ring (1608, 1582 and 1437 cm^{-1}), α,β-unsatured ester group (1700 and 1635 cm^{-1}) and pyranose function (1088, 1070 and 1036 cm^{-1}) as well as furan moiety (958 and 886 cm^{-1}), respectively. The ^1H NMR spectrum exhibited 44 proton signals and revealed the presence of three angular methyl groups [δ_H 0.83 (3H, d, J = 6.5 Hz), 1.30 (3H, s) and 0.81 (3H, s)], along with four olefinic protons [δ_H 6.61 (1H, t, J = 3.6 Hz), 6.25 (1H, brs), 7.35 (1H, s) and 7.15 (1H, brs)], one 1,2-disubstituted benzyl group [δ_H 7.13 (1H, dd, J = 7.8, 1.6 Hz), 7.25 (1H, m), 7.04 (1H, m), 7.40 (1H, dd, J = 7.5, 1.3 Hz) and 5.40/5.37 (1H×2, d, J = 13.4 Hz)], a pyranose unit [δ_H 4.85 (1H, d, J = 7.5 Hz),

1 R= -H **2** R= -OH

Figure 1 — Chemical structures of compounds **1** and **2**

Table I — ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (δ, J_{Hz}, CD_3OD, TMS)[a]

No.	1				2			
	δ_H	δ_C	DEPT	HMBC	δ_H	δ_C	DEPT	HMBC
1	1.58/1.29(1H×2,m)	17.2	CH_2	3,10	1.57/1.28(1H×2,m)	17.1	CH_2	3,10
2	2.28/2.15(1H×2,m)	27.2	CH_2	3,10	2.29/2.17(1H×2,m)	27.4	CH_2	3.10
3	6.61(1H,t,3.6)	137.0	CH		6.64(1H,t,3.6)	136.9	CH	
4		142.6	C	19		142.8	C	19
5		38.3	C	1,3,6,19		38.2	C	1,3,6,19
6	3.61(1H,dd,10.0,3.5)	75.5	CH	19	3.46(1H,d,3.5)	76.5	CH	7-OH,19
7	1.67/1.56(1H×2,m)	37.1	CH_2	6-OH,17	3.96(1H,t,3.4)	77.4	CH	6-OH,17
8	1.62(1H,m)	35.4	CH	20	1.59(1H,m)	38.5	CH	7-OH,20
9		38.6	C	5,7,11,12,20		38.9	C	5,7,11,12,20
10	1.32(1H,dd,10.0,3.5)	45.7	CH	2,19,20	1.35(1H,dd,10.0,3.5)	46.1	CH	2,19,20
11	1.70/1.58(1H×2,m)	35.2	CH_2	20	1.68/1.52(1H×2,m)	35.6	CH_2	20
12	2.42/2.11(1H×2,m)	17.9	CH_2	16	2.40/2.12(1H×2,m)	18.0	CH_2	16
13		125.8	C	11,15		126.4	C	11,15
14	6.25(1H,brs)	110.2	CH	16	6.24(1H,brs)	110.8	CH	16
15	7.35(1H,s)	143.2	CH		7.34(1H,s)	143.3	CH	
16	7.15(1H,brs)	138.7	CH	14,15	7.16(1H,brs)	139.1	CH	14,15
17	0.83(3H,d,6.5)	16.4	CH_3		0.85(3H,d,6.5)	15.6	CH_3	
18		169.5	C	3,7′		170.4	C	3,7′
19	1.30(3H,s)	19.5	CH_3	6,10	1.29(3H,s)	19.8	CH_3	6
20	0.81(3H,s)	18.4	CH_3	10	0.80(3H,s)	18.7	CH_3	10
6-OH	5.04(1H,brs)				6.08(1H,brs)			
7-OH					3.01(1H,brs)			
1′		125.3	C	7′,3′,5′		125.4	C	7′,3′,5′
2′		155.0	C	7′,6′,4′,3′,1″		154.9	C	7′,6′,4′,3′,1″
3′	7.13(1H,dd,7.8,1.6)	117.2	CH	5′	7.14(1H,dd,7.8,1.6)	117.3	CH	5′
4′	7.25(1H,m)	130.7	CH	6′	7.26(1H,m)	130.7	CH	6′
5′	7.04(1H,m)	123.5	CH	3′	7.04(1H,m)	125.0	CH	3′
6′	7.40(1H,dd,7.5,1.3)	129.1	CH	4′,7′	7.41(1H,dd,7.5,1.4)	128.9	CH	4′,7′
7′	5.40/5.37(1H×2,d,13.4)	62.9	CH_2	6′	5.41/5.38(1H×2,d,13.4)	63.1	CH_2	6′
Glu-1″	4.85(1H,d,7.5)	101.6	CH	2″, 3″	4.84(1H,d,7.5)	101.8	CH	2″, 3″
2″	3.36(1H,dd,9.0,7.5)	73.9	CH		3.37(1H,dd,9.0,7.5)	73.9	CH	
3″	3.42(1H,t,9.0)	77.1	CH		3.42(1H,t,9.0)	77.2	CH	
4″	3.22(1H,t,9.0)	70.6	CH	2″, 3″	3.23(1H,t,9.0)	70.5	CH	2″, 3″
5″	3.41(1H,m)	77.8	CH	1″, 6″	3.40(1H,m)	77.8	CH	1″, 6″
6″	3.78(1H,dd,12.1,5.2) 3.51(1H,dd,11.8,2.3)	61.6	CH_2		3.76(1H,dd,12.1,5.2) 3.50(1H,dd,11.8,2.3)	61.5	CH_2	

[a] Assigned by the ^{1}H-^{1}H COSY, HMBC and HMQC spectra.

3.36 (1H, dd, J = 9.0, 7.5 Hz), 3.42 (1H, t, J = 9.0 Hz), 3.22 (1H, t, J = 9.0 Hz), 3.41 (1H, m) and 3.78 (1H, dd, J = 12.1, 5.2 Hz)/3.51 (1H, dd, J = 11.8, 2.3 Hz)]. The ^{13}C NMR and various DEPT spectra of 1 (**Table I**) displayed 33 carbon signals, which were ascribed to three methyls (δ_C 16.4, 19.5 and 18.4),

seven methylenes (δ_C 17.2, 17.9, 27.2, 35.2, 37.1, 61.6 and 62.9), sixteen methines (δ_C 35.4, 45.7, 70.6, 73.9, 75.5, 77.1, 77.8, 101.6, 110.2, 117.2, 123.5, 129.1, 130.7, 137.0, 138.7 and 143.2) and seven quaternary carbons (δ_C 38.3, 38.6, 125.3, 125.8, 142.6, 169.5 and 155.0). On complete acid hydrolysis, compound 1 gave β-D-glucose and saligenol (in the ratio of 1:1), respectively, which were determined by comparison of co-TLC with authentic samples. These were further confirmed from the NMR signals and FAB-MS at *m/z* 439 $[M+H-162]^+$, which showed loss of glucose unit. The glucose was present in pyranose form which was established by comparing ^{13}C NMR data of the glucose moiety with literature values[13], and β-configuration according to a characteristic doublet signal at δ_H 4.85 with coupling constant (J = 7.5 Hz)[14].

Besides signals of saligenol and D-glucose, additional 27 proton and 20 carbon signals, including three methyls [two tertiary methyl groups (δ_H 1.30, 0.81 and δ_C 19.5, 18.4) and one secondary methyl group (δ_H 0.83 and δ_C 16.4)], a hydroxyl group [δ_H 5.04 (1H, brs)], a β-monosubstituted furan ring [δ_H 6.25 (1H, brs), 7.35 (1H, s), 7.15 (1H, brs) and δ_C 125.8, 110.2, 143.2, 138.7] and an α,β-unsatured acyloxy group [δ_H 6.61 (1H, t, J = 3.6 Hz) and δ_C 137.0, 142.6, 169.5] were recognized in ^1H, ^{13}C NMR and DEPT spectra. Analysis of spectral data confirmed that five of the twelve degrees of unsaturation were due to one benzene ring and pyranose ring, and two due to one unsatured ester group, three were satisfied by one furan ring, and the remaining two must be attributed to two rings since no signal arising from multiple bonds was present in any of the spectra. These spectroscopic features were very similar to those of hardwickiic acid and its derivative[15,16]. The relative stereochemistry of the chiral centres at C-5, C-6, C-8, C-9 and C-10 was determined by analysis of the HMBC and NOE spectra (**Figure 2**) and comparison of the 2D-NMR spectral data with those of the reported compounds[17]. The clear correlations of H-6 with C-19 and H-10 with C-19/C-20 in the HMBC spectrum, H-10 with H-6 but Me-19 was not related to H-6/H-10 in the NOE spectrum, together with the ^1H NMR chemical shifts of H-17, H-19 and H-20, indicated an A/B ring *trans* fused clerodane[17]. This conclusion was further supported by the ^{13}C NMR chemical shifts of C-19, which was in the 15-20 ppm range[18-21]. In addition, the Me-19 did not show any correlations with H-6/H-10 but with Me-20/HO-6. Me-20 was not related to H-10 but to Me-17, H-8 correlated with H-6/H-7/H-10 in the NOE spectrum. These correlations suggested that the hydroxyl group at C-6, Me-17, Me-19 and Me-20 are all α-configuration and the proton at C-10 is β-configuration. Moreover, the key correlations of H-3 with C-1/C-2/C-5/C- 18 and the protons of HO-6/Me-17 with C-7 were also clearly observed in the HMBC and NOESY experiment. Therefore, these spectral features and physicochemical properties suggested that compound 1 was an analogue of 6-hydroxy-(-)-hardwickiic acid, a clerodane-type diterpenoid.

In the HMBC spectrum, the correlations of H-7′ with C-1′/C-2′/C-6′ signified the aromatic ring has an *ortho*-oxy substitution, and the long-range correlation of the H-7′ with C-18 established the linkage between salicyl group and acyloxy moiety, the H-1″ of glucose with the C-2′ of aglycone determined the point of attachment of the glucose unit. Based on the above spectral characteristics, the structure of compound **1** was established to be 6-hydroxyl-(-)-hardwickiic acid 2′-β-D-glucopyranosylbenzyl ester, Compound **2** was isolated as a white amorphous powder from MeOH, and gave a positive Molisch reaction. Its molecular formula was decided to be $C_{33}H_{44}O_{11}$ from HR-FAB-MS at *m/z* 617.2971 $[M+ H]^+$ (calcd. for 617.2962), 16 mass units greater than that of **1**, corresponding to twelve degrees of unsaturation. The IR spectrum (KBr) also revealed the presence of hydroxyl groups, aromatic ring, unsatured ester group, pyranose function and furan moiety. The ^1H NMR spectrum exhibited 44 proton signals, of which the chemical shifts and coupling constants were quite similar to those of **1**, differing only at δ_H 3.46 (1H, d, J = 3.5 Hz) and 3.96 (1H, t, J = 3.4 Hz) [two oxygenated

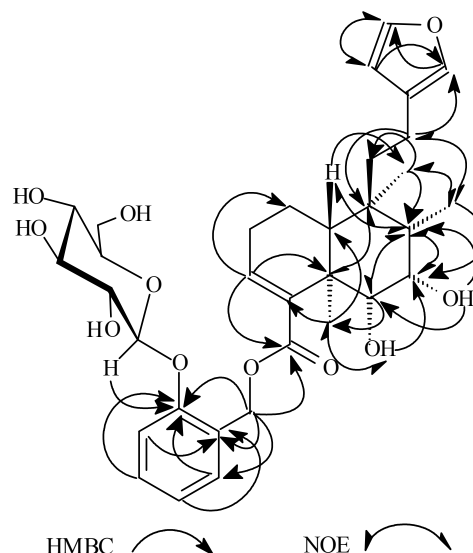

Figure 2 — Selected HMBC and NOE correlations of compound **2**

methine protons], and δ_H 6.08 (1H, brs) and 3.01(1H, brs) [two hydroxyl group]. In addition, the ^{13}C NMR and DEPT spectra of **1** and **2** (**Table I**) were also similar except that the latter possesses an additional teritiary carbon signal (δ_C 77.4) instead of a secondary carbon signal (δ_C 37.1) in **1**. These spectral features suggested that the structure of **2** is closely related to that of **1** except for an additional -OH group at C-7 of **2**. The HMBC experiment decided the positions of the two hydroxyl groups at C-6 and C-7 as H-6 showed correlation with C-19 while H-7 with C-17. H-10 was related to C-20 and H-3 to C-1/C-2/C-18. In the NOESY experiment, H-10 was not correlated to Me-19 but it was related to H-8 which was again related to H-6 and H-7. Therefore, the two hydroxyl groups at C-6 and C-7 possess the α-configuration. Me-20 was also related to Me-19 and Me-17, all having similar α-configuration. The structure of compound **2** was assigned as 6,7-dihydroxy-(-)-hardwickiic acid 2′-β-D-glucopyranosylbenzyl ester based on these spectral data (**Figure 1**).

To the best of the knowledge, **1** and **2** have not been reported previously from any plant source.

The structures of known compounds **3**, **4**, **5** and **8** were identified by detailed spectroscopic analysis and comparison of their spectral data with reported values in the literature mentioned above. However, compounds **6** and **7** were identified by direct comparison of their melting points and R_f values with authentic samples.

Experimental Section

Melting points were measured on an X-4 melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer model 241 automatic polarimeter. IR spectra (KBr disc) were obtained on Alpha-Centauri FT-IR spectrometer. The 1D and 2D-NMR experiments were performed on a Bruker AM-400 MHz or DRX-500 MHz spectrometers using TMS as an internal standard. VG Autospec-300 spectrometer was used to record the FAB-MS spectrum. Column chromatography (CC) was performed over silica gel (200-300 mesh) and TLC were run on silica gel GF_{254} (Qingdao Marine Chemical Inc., China), TLC (0.2 mm thick plates) spots were visualized by spraying with 5% H_2SO_4 in EtOH followed by heating.

Collection of plant material. The roots of *E. bodinieri* Van′t were collected in June 2004 from Ziwuling mountainous district of Gansu Province in China, and identified by Prof. YunShan Lian (Department of Biology, Northwest Normal University, China). A voucher specimen (No.304128) of the plant is deposited at the Herbarium of the Botany Department, Northwest Normal University, Lanzhou, 730070, China.

Extraction and isolation. The air-dried and powdered roots of *E. bodinieri* Van′t (7.0 kg) were extracted with 95% EtOH (20 L, 7d × 3) at RT. After filtration and removal of solvent by evaporation *in vacuo*, a residue (280 g) was obtained, which was suspended in warm water (1L). The suspension was defatted with petroleum ether (60-90°C), and concentrated, then extracted successively with EtOAc and *n*-BuOH. The EtOAc extract (95 g) was chromatographed over silica gel column (200-300 mesh) and eluted with petroleum ether-$CHCl_3$, $CHCl_3$-EtOAc and $CHCl_3$-MeOH in order of increasing polarity, and then combined by monitoring with TLC. The fraction of petroleum ether-$CHCl_3$-EtOAc yielded compounds **3** (16 mg) and **5** (27 mg) after purifying twice by silica gel column chromatography. The fraction of $CHCl_3$-EtOAc gave compounds **4** (18 mg) and **7** (12 mg), and a crude fraction after purifying twice by silica gel column chromatography, and the crude fraction gave compound **6** (15 mg) after recrystallization with a mixture of $CHCl_3$ and MeOH. The fraction of $CHCl_3$-MeOH was rechromatographed over a silica gel column to yield **8** (25 mg) and another subfraction. The subfraction was further purified with preparative TLC and developed with Me_2CO-MeOH as eluent to provide compound **1** (15 mg) and **2** (16 mg).

Compound 1. White amorphous powder, m.p. 115-17°C, $[\alpha]_D^{20}$ -35.8° (c = 0.45, MeOH); IR (KBr): 3340, 2960, 1700, 1635, 1608, 1582, 1437, 1088, 1070, 1036, 958, 886 cm^{-1}; HR-FAB-MS (positive-ion mode): *m/z* 601.3021 $[M+H]^+$ (calcd. for $C_{33}H_{44}O_{10}$, 601.3013); FAB-MS: *m/z* 601 $[M+H]^+$, 439 $[M+ H-162]^+$; for ^1H and ^{13}C NMR data see **Table I**.

Compound 2. White amorphous powder, m.p. 121-23°C, $[\alpha]_D^{20}$ -38.2° (c = 0.58, MeOH); IR ν: 3439, 2961, 1700, 1636, 1610, 1582, 1438, 1089, 1070, 1036, 958, 886 cm^{-1}; HR-FAB-MS (positive-ion mode): *m/z* 617.2971 $[M+H]^+$ (calcd. for $C_{33}H_{44}O_{11}$, 617.2962); FAB-MS: *m/z* 617 $[M+H]^+$, 455 $[M+ H-162]^+$; for ^1H and ^{13}C NMR data see **Table I**.

Acid hydrolysis of compound 1. Compound **1** (8 mg) was hydrolyzed by refluxing with 5% H_2SO_4

(5 mL) in MeOH-H_2O (1:1, v/v) for 1.5 hr on a hot water bath. The reaction mixture was cooled and then concentrated under reduced pressure to give a syrup which was partitioned between EtOAc/H_2O. The EtOAc extract was purified by recrystallization from $CHCl_3$-EtOAc to give white crystals, 6-hydroxyl-(-)-hard wicklic acid (2 mg), and the aqueous layer was neutralized with $NaHCO_3$ and concentrated *in vacuo*. Glucose and saligenol were identified by co-TLC with authentic samples using the solvent system [*n*-BuOH : HAc: H_2O (1:1:5)], (R_f = 0.42 for glucose; R_f = 0.35 for saligenol).

Acid hydrolysis of compound 2. Same as for compound **1**.

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