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A New Steroidal Saponin from *Dioscorea deltoidea* Wall var. orbiculata

Ping SHEN[1], San Long WANG[3], Xi Kui LIU[2], Chong Ren YANG[2], Bing CAI[3], Xin Sheng YAO[1]*

[1]Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang 110016
[2]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204
[3]Beijing Institute of Biomedicine, Beijing 100091

Abstract: Bioactivity-guided fractionation led to the isolation of a new steroidal saponin, orbiculatoside B, together with a pair of furostanol saponins, protobioside and methyl protobioside, from the fresh rhizomes of *Dioscorea deltoidea* Wall var. *orbiculata.* The new compound was identified to be isonarthogenin 3 – O - α - L - rhamnopyranosyl (1→2) - [α - L - rhamnopyranosyl - (1→ 4)] - β - D - glucopyranoside by various NMR techniques in combination with chemical methods. The three saponins showed strong activity against *Pyricularia oryzae*, and were cytotoxic to cancer cell line K562, HCT-15, A549, and A2780a *in vitro*.

Keywords: *Dioscorea deltoidea* Wall var. *Orbiculata*, orbiculatoside B, *Pyricularia oryzae*, cytotoxic.

During the course of screening bioactive natural products from traditional Chinese medicines (TCMs) under the guidance of *Pyricularia oryzae* bioassay[1], Dioscorea family were found to show significantly strong activities against *Pyricularia oryzae* mycelia among more than 300 species of TCMs. In this report, we described the structure determination of a new spirostanol saponin, orbiculatoside B (**1**) (**Figure 1**), and a pair of known furostanol saponins, protobioside (**2**) [2], and its artifact methyl protobioside (**3**), isolated from the ethanol extract of the fresh rhizomes of *Dioscorea deltoidea* Wall var. *orbiculata,* which showed strong activity against *Pyricularia oryzae*, with a MMDC (minimum morphological deformation concentration) value of 28.04 μmol/L.

The ethanol extract of the fresh rhizome of *Dioscorea deltoidea* Wall var. *orbiculata* was chromatographed on Diaion HP-20, silica gel ($CHCl_3$: MeOH: H_2O 7:3:0.5) and RP-8 silica gel (65%, 70% MeOH in H_2O) to afford orbiculatoside B (9.5mg), and protobioside (16.4mg), which could be transformed to methyl protobioside in the existence of MeOH.

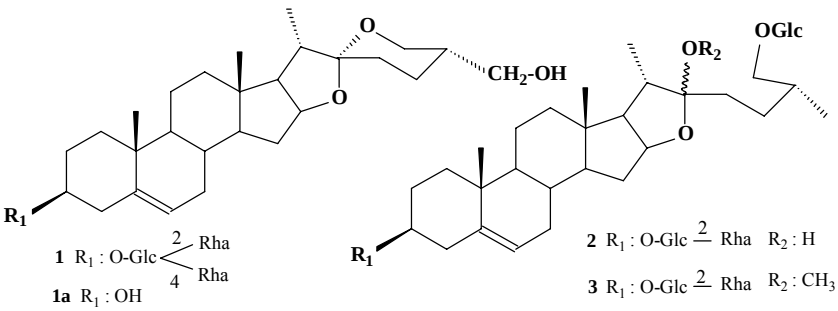
Orbiculatoside B (**1**) , a white powder, [mp 268-270°C (dec.); $[\alpha]_D^{20}$-86.2 (*c* 0.006, C_5H_5N)], was positive to Liebermann-Burchard and Molish tests. The molecular formula of $C_{45}H_{72}O_{17}$ was deduced from its negative FABMS (*m/z* 883[M-H]$^-$), and the

*E-mail: yaoxinsheng@hotmail.com

^{13}C NMR (DEPT) spectrum.

The ^{1}H NMR spectrum of **1** exhibited signals attributed to anomeric protons [δ 6.39 (*br s*), 5.90 (*br s*), and 4.95 (*d*, *J*=7.8Hz)], one olefinic proton (δ 5.32 *br d*, *J*=4.6Hz), two angular methyl protons (δ 1.03 and 0.79) and three secondary methyl protons [δ 1.14 (*d*, *J*=6.9Hz) , 1.61 (*d*, *J*=6.0Hz) , 1.72 (*d*, *J*=6.2Hz)]. The ^{13}C NMR spectrum showed 27 carbons due to the aglycone moiety, with one quaternary carbon signal at δ 109.8 with oxygen atoms and two olefinic carbon signals at δ 140.9 and 121.9, indicating **1** possessed a Δ^5-spirostanol skeleton. By analyzing the HMQC, HMBC, and H-H COSY spectra of **1**, the five carbon signals at δ 15.0, 16.4, 18.6, 18.6, and 19.5 were assigned to be C-21, C-18, rhamnose-6″, rhamnose-6‴, and C-19, respectively. But the lack of C-27 methyl and the presence of an oxymethylene signal (δ 64.2) showed that there was a hydroxyl group linked to the C-27 position. On acid hydrolysis with 1mol/L HCl, **1** gave an aglycone which was identified to be isonarthogenin (**1a**) [3] by comparison with the authentic sample, and the hydrolysate was trimethylsilated and the GC retention time of the derived sugars were compared with those of the standard sugars prepared by the same manner, indicating the sugars were D-glucose and L-rhanmnose, with the ratio of 1:2. Comparing the NMR data of **1** with those of dioscin[4], a known steroid saponin, showed **1** was 27-hydroxy-dioscin, since they had the identical NMR data due to ring A, B, C, D and E. In the HMBC spectrum, the correlation between the anomeric proton (δ 4.95, Glucose-1') and the C-3 signal (δ 78.3) also approved the above conclusion. On the basis of the above analysis, compound **1** was consequently identified to be isonarthogenin 3 – O - α - L – rhamnopyranosyl (1→2) - [α - L – rhamnopyranosyl - (1→ 4)]- β - D – glucopyranoside, and named as orbiculatoside B. The detailed NMR data are listed in **Table 1**.

Figure 1 The structures of compound **1**, **1a**, **2** and **3**.



Compound **3** was refluxed with 40 % aqueous acetone at 90°C for 24 h to give compound **2**, which could be reversed to **3** in MeOH for more than 24 h. The two compounds appeared on SiO_2 TLC as a pair of pink-colored spots after spraying with 10% H_2SO_4 / EtOH followed by heating, with $CHCl_3$: MeOH : H_2O (7 : 3 : 0.5) as the

Table 1 ^{13}C NMR data[a] of compound **1**, **2**, and **3** in Pyridine-d_5 (δ n ppm)

position	**1**	**1a**	**2**	**3**
1	37.6	37.8	37.6	37.8
2	30.3	32.6	30.3	30.5
3	78.3	71.3	78.1	78.4
4	39.1	43.5	39.1	39.3
5	141.0	142.0	141.0	141.2
6	121.9	121.0	121.8	122.2
7	32.4	32.3	32.4	32.5
8	31.7	31.9	31.8	32.0
9	50.3	50.3	50.5	50.6
10	37.2	37.1	37.2	37.5
11	21.1	21.2	21.2	21.4
12	40.5	40.0	40.0	40.1
13	40.8	40.5	40.6	41.2
14	56.7	56.8	56.7	56.9
15	32.4	32.2	32.4	32.7
16	81.4	81.2	81.2	81.7
17	63.0	63.0	63.9	64.5
18	16.4	16.4	16.4	16.7
19	19.5	19.6	19.5	19.8
20	42.0	42.1	40.9	40.8
21	15.0	15.1	16.5	16.7
22	109.8	109.7	110.8	113.2
23	31.7	31.6	37.2	31.1
24	24.1	24.1	28.4	28.5
25	39.2	39.2	34.4	34.7
26	64.1	64.4	75.2	75.4
27	64.2	64.1	17.5	17.5
22-O-CH_3				47.5
3-O-Glc-1'	100.3		100.5	100.6
2'	78.3		79.6	79.7
3'	78.0		78.0	78.4
4'	78.6		71.9	72.0
5'	77.2		78.4	78.7
6'	61.5		62.8	62.9
Rha(1-2)-1″	102.1		102.0	102.4
2″	72.6		72.5	72.7
3″	72.8		72.9	73.1
4″	74.2		74.2	74.3
5″	69.5		69.5	69.9
6″	18.6		18.6	18.9
Rha(1-4)-1‴	102.9			
2‴	72.7			
3‴	72.8			
4‴	74.1			
5‴	70.4			
6‴	18.6			
26-O-Glc-1⁗			105.1	105.1
2⁗			75.2	75.4
3⁗			78.4	78.7
4⁗			71.9	72.1
5⁗			78.6	78.8
6⁗			63.0	63.1

[a] Recorded on a Bruker-500 (^{13}C 125 MHz) spectrometer

developing solvent. They were positive to Ehrlich reagent, indicating they were furostanol saponins. Analysis of the mp, IR, MS, and NMR data of the two compounds indicated **2** and **3** as protobioside and methyl protobioside, respectively. The detailed NMR data are listed in **Table 1**.

The three compounds showed strong activity against P.*oryzae*, and were cytotoxic to cancer cell line K562, HCT-15, A549, and A2780a *in vitro*. The bioactivity results are listed in **Table 2**.

Table 2 Activity data of compound **1**, **2**, and **3**[a]

compound	P. oryzae[b]	K562	A549	HCT-15	A2780a
1	0.04	74.84	51.88	-	165.92
2	15.35	15.10	88.82	37.13	149.17
3	12.12	12.71	7.47	58.25	452.82
cis-DDP[c]	-	69.33	1426.67	69.50	54.81

[a] Use the IC_{50} value (μmol/L) to evaluate the cytotoxicties of the samples.
[b] Use MMDC value (μmol/L) to evaluate the activity against P. *oryzae*.
[c] *cis*-DDP was used as a positive control.

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