

## Note

### Three new apianen lactones from *Dioscorea bulbifera* L.

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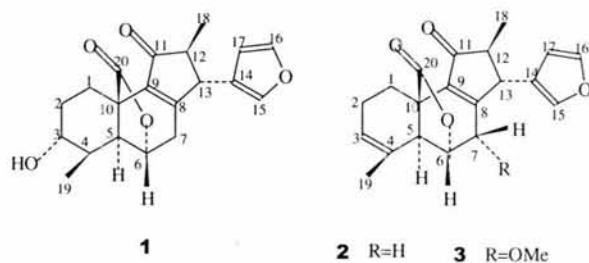
From the root of *Dioscorea bulbifera* L. three new compounds have been isolated along with four known compounds and their structures elucidated as 3 $\alpha$ -hydroxy-13 $\beta$ -furan-11-keto-apian-8-en-(20, 6)-olide **1**, 13 $\beta$ -furan-11-keto-apian-3(4), 8-dien-(20, 6)-olide **2** and 7 $\alpha$ -methoxy-13 $\beta$ -furan-11-keto-apian-3(4), 8-dien-(20, 6)-olide **3**, respectively, on the basis of spectral data analyses and chemical means.

*Dioscorea bulbifera* L. which is a species of the Dioscorea, grows abundantly in southern and eastern China. Its roots are used in Chinese medicine as a remedy for various diseases, especially for tumours and cancers<sup>1</sup>. This prompted us to search for its chemical constituents. Continuing our previous work<sup>2-5</sup>, further studies on the constituents of the drug yielded three new compounds along with three known compounds in the EtOAc fraction of EtOH extraction.

The known compounds were identified by comparing their spectral data with those of authentic samples or with data reported in the literature as 3, 7-dimethoxy-5, 3', 4'-trihydroxy-flavone **4**<sup>6</sup>, morin-7-*O*- $\beta$ -D-glucoside **5**<sup>7</sup> and 5-hydroxy-7-methoxy-6-*O*-[ $\alpha$ -L-rhamnopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-fucopyranosyl]flavone **6**<sup>8</sup>. The paper reports the three new compounds

### Results and Discussion

Compound **1**, was obtained as colourless amorphous powder, analysed for the molecular formula C<sub>20</sub>H<sub>22</sub>O<sub>5</sub> by the HREIMS, indicating ten degree of unsaturation. <sup>1</sup>H and <sup>13</sup>C NMR (Tables I and II) of **1** revealed signals due to two methyls, three methylenes, nine methines and six quaternary carbons, which suggest the presence of twenty carbons. Its IR spectrum showed bands assigned to a carbonyl



group (1720 cm<sup>-1</sup>) and a  $\beta$ -substituted furan ring (1520, 833cm<sup>-1</sup>). The <sup>13</sup>C resonance at  $\delta$ 198.25 and IR band at 1750 cm<sup>-1</sup> indicated the presence of one five-membered ring ketones. The signals at  $\delta$ c 142.25 and 161.42 were attributed to a tetrasubstituted double bond. In the <sup>13</sup>C NMR, signals typical of a lactone group were observed at  $\delta$ 175.80, which were supported by IR spectrum absorption (1760 cm<sup>-1</sup>). An IR absorption (3480 cm<sup>-1</sup>) indicated the presence of a hydroxy group. This was further confirmed by the disappearance of the resonance peak at  $\delta$ H3.62 on addition of D<sub>2</sub>O in the <sup>1</sup>H NMR spectrum. It was also supported by the chemical shift of C<sub>3</sub> ( $\delta$  72.7). The lack of a C-10 methyl signal suggested that C-10 is a part of the lactone group. The downfield appearance of C-6 and H-6 ( $\delta$ c:72.72d;  $\delta$ H:4.60d;  $J=2.2$ Hz) fixed the position of the lactonic oxygen to C-6.

The structure of **1** was deduced on the basis of <sup>1</sup>H-<sup>1</sup>H COSY and <sup>1</sup>H-<sup>13</sup>C long range couplings. The relative stereochemistry of **1** was established from extensive examination of the NOESY spectrum. The orientations of H-3 $\beta$ , H-4 $\alpha$ , H-5 $\alpha$ , H-6 $\alpha$ , H-12 $\alpha$ , H-13 $\beta$  were determined to be the same as those in typical limonoids<sup>9</sup>. The most important correlations in the NOESY spectrum are summarized in Table III. It showed cross-peaks of H-6/H-4, H-5/H-4 and H-12 $\alpha$ /H-5 which established the axial orientation( $\alpha$ -H) of H-5 and a trans-junction between A and B ring. NOESY correlations for H-3/Me-19 and H-3/H-2 implied the  $\alpha$ -orientation of C-3-OH. Thus, we conclude that compound **1** is 3 $\alpha$ -hydroxy-13 $\beta$ -furan-11-keto-apian-8-en-(20, 6)-olide.

Compound **2** was obtained as light yellow needle crystals. The HREIMS gave a molecular formula of

**Table I**— $^1\text{H}$  NMR spectral data of **1**, **2** and **3** in  $\text{CDCl}_3$ 

Proton	1		2		3	
	$\delta_{\text{H}}$ (ppm)	coupling(Hz)	$\delta_{\text{H}}$ (ppm)	coupling(Hz)	$\delta_{\text{H}}$ (ppm)	coupling(Hz)
1 $\alpha$	1.93		1.91		1.90	
$\beta$	2.82		2.82		2.80	
2 $\alpha$	1.94		1.92		1.94	
$\beta$	2.81		2.80		2.82	
3 $\beta$	4.10	3.2	5.24	3.4	5.27	3.6
4 $\alpha$	1.96		—			
5	2.24	10.5	2.46	10.5	2.38	11.2
6	4.60	2.2	4.62	2.3	4.67	2.4
7 $\alpha$	1.98		1.97		—	
$\beta$	2.88		2.87		3.82	
12	1.82		1.84		1.83	
13	3.22		3.25		3.28	
15	7.36	1.5	7.30	1.5	7.31	1.6
16	6.24	0.5, 1.5	6.44	0.5, 1.5	6.45	0.5, 1.5
17	7.22		7.23		7.22	
Me-18	2.09		2.10		2.12	
Me-19	1.22		1.88		1.90	

**Table II**— $^{13}\text{C}$  NMR spectral data of **1**, **2** and **3** in  $\text{CDCl}_3$ 

C	<b>1</b>	DEPT	<b>2</b>	DEPT	<b>3</b>	DEPT
1	22.9	$\text{CH}_2$	21.8	$\text{CH}_2$	21.9	$\text{CH}_2$
2	17.7	$\text{CH}_2$	16.7	$\text{CH}_2$	16.7	$\text{CH}_2$
3	72.7	CH	121.3	CH	122.0	CH
4	30.0	CH	139.9	C	140.4	C
5	51.2	CH	50.2	CH	51.2	CH
6	72.7	CH	74.7	CH	74.7	CH
7	18.2	$\text{CH}_2$	19.2	$\text{CH}_2$	75.8	CH
8	161.4	C	160.4	C	160.4	C
9	142.2	C	145.1	C	145.1	C
10	44.8	C	42.8	C	42.8	C
11	198.2	C	197.2	C	198.1	C
12	31.1	CH	32.4	CH	32.4	CH
13	58.8	CH	57.7	CH	57.8	CH
14	121.2	C	124.2	C	124.2	C
15	141.0	CH	140.4	CH	140.4	CH
16	111.2	CH	113.2	CH	113.2	CH
17	138.5	CH	137.5	CH	136.5	CH
18	30.2	$\text{CH}_3$	30.2	$\text{CH}_3$	30.2	$\text{CH}_3$
19	28.3	$\text{CH}_3$	29.5	$\text{CH}_3$	29.5	$\text{CH}_3$
20	175.8	C	174.6	C	174.5	C
21	—		—		59.5	$\text{OCH}_3$

**Table III**—NOESY correlations of **1**, **2** and **3**

H#	Correlations to H#	H#	Correlations to H#
1	H-2 $\beta$	7	H-6, H-13
2	H-2 $\beta$ , H-19	9	H-5
3	H-7, H-19, H-13	8	H-12, H-13

C<sub>20</sub>H<sub>20</sub>O<sub>4</sub>. Its spectral data were very similar to those of **1**. This suggested that **2** and **1** have a similar skeleton, but **2** featured an additional trisubstituted double bond (C<sub>3</sub>/C<sub>4</sub>,  $\delta_c=121.29, 139.88$ ). Moreover, in compound **2** the typical hydroxy group absorption in IR could not be observed. Consequently, we conclude that compound **2** is 13 $\beta$ -furan-11-keto-apian-3(4), 8-dien-(20, 6)-olide.

Compound **3** was obtained as light yellow needle crystals. Its spectra indicated that **3** has an almost similar set of signals as that of **2**, but it showed slight difference in the <sup>1</sup>H NMR spectra owing to the presence of one methoxy group at  $\delta=3.82$ (1H, s). It was further supported by the highfield shifting of C-7 signal by 75.76 in <sup>13</sup>C NMR spectra of **3** (Table II). The NOE interactions for H-7/Me-19 suggested the  $\alpha$ -orientation of C<sub>7</sub>-OMe. Thus, we conclude that **3** is 7 $\alpha$ -methoxy-13 $\beta$ -furan-11-keto-apian-3(4), 8-dien-(20, 6)-olide.

### Experimental Section

Melting points were determined using a Kofler melting point apparatus (uncorrected). IR spectra were recorded on Perkin-Elmer-59913; UV on UV-3400; NMR on 400MHz (<sup>1</sup>H) and 100MHz (<sup>13</sup>C) and MS on MAT-12 (70 ev). Column chromatography was done on silica gel, 160-200, 200-300mesh.

### Collection of plant material

The plant material was collected from Anhui of China in July 1999, and identified in the Botany Department, Northwest Normal University, Lanzhou 730070, People's Republic of China where a voucher specimen is deposited.

### Extraction and isolation

Powdered dried roots (5 kg) were extracted three times with 75% EtOH at room temperature. After removing solvents under reduced pressure, about 500g

residue was obtained. This was divided into four fractions, by pet. ether (30~60°C), CHCl<sub>3</sub>, EtOAc and *n*-C<sub>4</sub>H<sub>9</sub>OH. The EtOAc extract was concentrated to syrup (150g), which was chromatographed on silica gel column (500g, 100-200mesh) using mixture of CHCl<sub>3</sub>-EtOAc and EtOAc-HCOOH of increasing gradient (40:1-0:1, each 500 mL), four major fractions (A 55 g, B 10 g, C 22 g, D 28 g) being obtained. Rechromatography of fraction 1, [CHCl<sub>3</sub>:CH<sub>3</sub>OH (30:1-10:1)] and fraction 4 [CHCl<sub>3</sub>:CH<sub>3</sub>OH (30:1-0:1)] on the same silica gel column (100 mesh) afforded **5** (85 mg) and **6** (0.5g), respectively. Followed by rechromatography from a CHCl<sub>3</sub>-MeOH gradient (10:1-1:10), the fraction 1 gave **3** (16mg, R<sub>f</sub>=4.0). Rechromatography of fraction 3 on silica gel (100 mesh), and CHCl<sub>3</sub>:MeOH (7:2) as eluant, afforded **4** (1.8g). Fraction 2 was purified by preparative TLC (silica GF<sub>254</sub>)[CHCl<sub>3</sub>:EtOAc:CH<sub>3</sub>OH (5:4:1)] to furnish **1** (75mg, R<sub>f</sub>=4.4) and **2** (43mg, R<sub>f</sub>=3.6).

Compound **1**: C<sub>20</sub>H<sub>22</sub>O<sub>5</sub>, colourless amorphous powder, m.p. 234-35°C,  $[\alpha]_D^{20} -30.4^\circ$ ; IR(KBr): 3480, 2960, 2860, 1760, 1720, 1520, 1240, 833 cm<sup>-1</sup>; UV:  $\lambda_{\max}^{\text{CHCl}_3} = 303, 285, 270\text{nm}$ ; HR-MS: Found: 342.6742, required: 342.6738; EI-MS:m/z 324, 298, 286, 105, 95.

Compound **2**: C<sub>20</sub>H<sub>20</sub>O<sub>4</sub>, light yellow needle crystals, m.p. 240-41°C,  $[\alpha]_D^{20} -25.8^\circ$ . IR(KBr): 3010, 2960, 2860, 1760, 1720, 1650, 1580, 833 cm<sup>-1</sup>; UV:  $\lambda_{\max}^{\text{CHCl}_3} = 303, 292, 276\text{nm}$ ; HR-MS: Found:324.0954, required:324.0948; EI-MS:m/z 280, 268, 105, 95.

Compound **3**: C<sub>21</sub>H<sub>22</sub>O<sub>5</sub>, light yellow needle crystals, m.p. 258~259°C,  $[\alpha]_D^{20} -23.5^\circ$ . IR(KBr): 3022, 2930, 2810, 1760, 1700, 1240, 1085 cm<sup>-1</sup>; UV:  $\lambda_{\max}^{\text{CHCl}_3} = 310, 298, 286\text{nm}$ ; HR-MS: Found: 355.1927, required:355.1932; EI-MS:m/z 280, 268, 115, 95, 31.

<sup>1</sup>H and <sup>13</sup>C-NMR data of **1**, **2** and **3** are listed in Tables I and II.

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