Two New Furanoid Norditerpenes from *Dioscorea bulbifera*

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Two new furanoid norditerpenes (1, 2) were isolated from the root tubers of *Dioscorea bulbifera* L. Their structures were established on the basis of extensive spectroscopic analysis.

Key words *Dioscorea bulbifera* L.; furanoid norditerpene; diosbulbin I; diosbulbin J

Plants of the Dioscoreaceae are known as a source of diosgenin and related steroid saponins, which occur mainly in the underground parts.[1,2] *Dioscorea bulbifera* L. is widely distributed in China. It has been used to treat a variety of diseases, mainly for the treatment of thyroid disease and tumors, and so on.[3] Previous phytochemical investigations on the root tubers of the Japanese *Dioscorea bulbifera* L. have revealed no steroid sapogenins but instead eight furanoid norditerpenes, the diosbulbins A—H[4—6] and the enol glucosides of two of these, the diosbulbinosides D and F.[7] From tubers of *D. bulbifera* L. var *sativa* in Bangladesh, 8-epidiosbulbin E acetate was isolated.[8] As part of our effort to discover structurally diverse and biologically active secondary metabolites from local medicinal plants, the re-investigation of the root tubers of *D. bulbifera* led to the isolation of two new furanoid norditerpenes.

Compound **1** was obtained as colorless needles. Its molecular formula was determined to be $C_{29}H_{30}O_8$ on the basis of the quasi-molecular ion peak at *m/z* 529.1836 $[M+Na]^+$ (Calcd for $C_{29}H_{30}O_8Na$, 529.1838) in the HR-ESI-MS, in combination with the ^{13}C-NMR (distortionless enhancement by polarization transfer (DEPT)) spectrum. The IR spectrum was consistent with the presence of a furan ring (3145, 1604, 1512, 875 cm^{-1}), a benzene ring (1634, 1575, 1462, 829, 750 cm^{-1}) and three carbonyl functions, a γ-lactone (1778 cm^{-1}), a δ-lactone (1745 cm^{-1}) and an ester (1708 cm^{-1}). A furan ring was confirmed from the ^{1}H-NMR ($CDCl_3$) spectrum (δ 6.43, 1H, dd, J=0.8, 2.0 Hz, δ 7.43, 1H, dd, J=1.6, 2.0 Hz, δ 7.48, 1H, m). The ^{13}C-NMR spectrum (Table 1) exhibited 29 carbon signals, including three carbonyl resonances at δ 165.4 (s), 173.4 (s), and 175.8 (s), an oxygen-bearing methyl signal at δ 55.3 (q), and an upfield methyl signal at δ 18.4 (q). The presence of a 1′,4′-disubstituted benzene ring was also indicated from the ^{1}H-NMR spectrum δ 6.89 (H-2′, H-6′, 2H, d, J=8.8 Hz) and δ 7.51 (H-3′, H-5′, 2H, d, J=8.8 Hz).

Analysis of the NMR spectrum (Table 1) suggested the norditerpenoid skeleton for compound **1**. This norclerodane structure was previously assigned to diosbulbin D (**3**) isolated from the same plant.[5] The structure of our compound was similar to that of **3** and 8-epidiosbulbin E acetate (**4**).[8] Signals at δ 5.41 (H-12) and δ 4.85 (H-2) in the ^{1}H-NMR spectrum of **1** revealed that each lactone ring was linked through a secondary hydroxyl group as in **3**. Comparison with the spectrum of **3** and **4** revealed that the seven protons attached to C-1, C-2, C-3, C-4 and C-10 had similar chemical shifts and coupling patterns and thus the γ-lactone function was fused to ring A in the same manner in these compounds. The only difference is that an acetoxyl group at C-6 in **4** was replaced by a 3-(4-methoxyphenyl) acryloxyl group in **1**.

The following key heteronuclear multiple bonding connectivity (HMBC) correlations (Fig. 2) were observed: from H-1, H-5 to C-9, from H-2, H-5 to C-19, from H-6, H-7′ to C-9′, from H-7 to C-17, from H-12 to C-14, C-16, respectively. The key rotating frame Overhauser enhancement spectroscopy (ROESY) correlations (Fig. 2) between H-7β, H-11β and H-20, between H-8 and H-12 were also observed. The proton at C-10 was coupled to the protons at C-5 and C-

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** in $CDCl_3$ (500 MHz) and **2** in CD_3COCD_3 (400 MHz)

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	28.7 t	2.16 (m)	27.9 t	2.01 (m)
		1.45 (m)		1.57 (m)
2	76.2 d	4.85 (m)	65.6 d	4.18 (m)
3	39.0 t	1.78 (m)	28.5 t	2.04 (m)
		2.50 (m)		2.06 (m)
4	42.1 d	2.69 (m)	45.3 d	2.97 (m)
5	41.7 d	2.22 (ddd, 1.7, 2.7, 12.6)	76.1 s	
6	69.2 d	5.48 (m)	209.0 s	
7	27.1 t	2.25 (m)	29.3 t	2.37 (m)
		1.92 (m)		2.26 (m)
8	41.8 d	3.01 (dd, 3.5, 12.3)	42.9 d	2.01 (dd, 3.5, 12.3)
9	35.7 s		35.7 s	
10	40.9 d	2.37 (ddd, 5.3, 12.2, 12.6)	44.6 d	2.97 (dd, 4.5, 12.1)
11	42.2 t	1.85 (dd, 11.2, 14.2)	43.3 t	2.17 (dd, 11.0, 14.2)
		1.88 (dd, 5.9, 14.2)		1.78 (dd, 6.1, 14.2)
12	70.1 d	5.41 (ddd, 0.6, 5.9, 11.2)	70.6 d	5.62 (ddd, 0.6, 6.1, 11.0)
13	124.0 s		126.1 s	
14	108.4 d	6.43 (dd, 0.8, 2.0)	109.8 d	6.57 (m)
15	143.7 d	7.43 (dd, 1.6, 2.0)	144.6 d	7.58 (m)
16	139.5 d	7.48 (m)	141.0 d	7.67 (m)
17	173.4 s		173.7 s	
19	175.8 s		175.8 s	
20	18.4 q	1.04 (s)	20.4 q	1.08 (s)
1′	161.2 s			
2′,6′	114.1 d	6.89 (d, 8.8)		
3′,5′	129.8 d	7.51 (d, 8.8)		
4′	127.3 s			
7′	144.7 d	7.68 (d, 16.0)		
8′	115.6 d	6.35 (d, 16.0)		
9′	165.4 s			
OCH_3	55.3 q	3.83 (s)		

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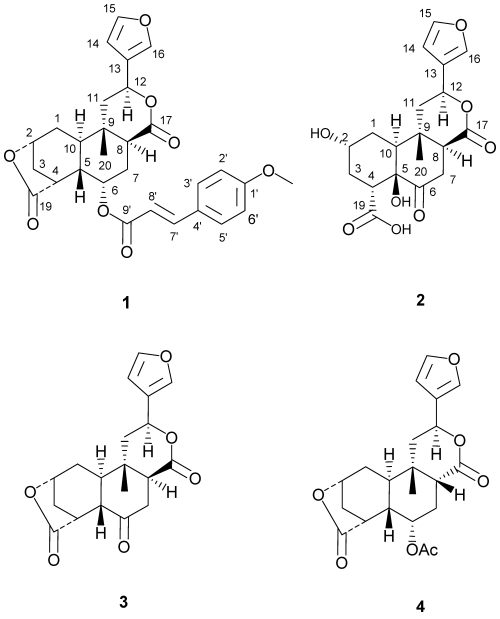

Fig. 1. Structures of Compounds **1**—**4**

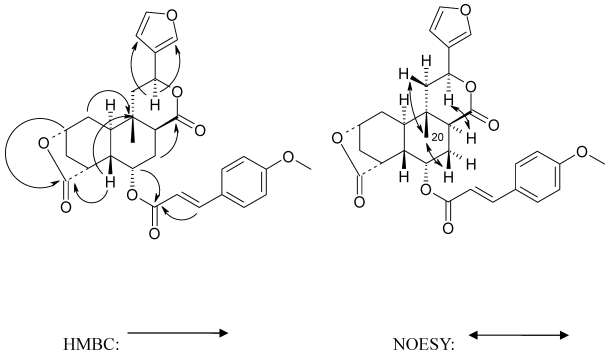

Fig. 2. Key HMBC and ROESY Correlations for Compound **1**

1, respectively. It was axial from its coupling constants of 12.6 Hz (ax–ax) to the axial C-5 proton, 12.2 Hz (ax–ax) to the H-1β, and 5.3 Hz (eq–ax) to the H-1α. The axial C-5 proton showed one coupling (2.7 Hz) with the methine proton at δ 5.48, which was equatorial. The proton at C-8 was coupled only to the protons at C-7 and was axial from its coupling constants of 12.3 Hz (ax–ax) to H-7β and 3.5 (eq–ax) to H-7α. This can help to distinguish the configurations of each geminal proton at C-7. The configuration at C-8 of **1** was thus opposite to that of the **4**, and same to that of **3**. These spectroscopic findings and the ^{13}C-NMR spectrum (Table 1) taken with the co-occurrence of diosbulbin D of known absolute configuration[6] were consistent with structure **1**. Consequently, the structure of **1** was elucidated as shown in Fig. 1 and named diosbulbin I.

Compound **2** was obtained as colorless needles. It had the molecular formula $C_{19}H_{22}O_8$ and was deduced to be a norclerodane diterpenoid. The IR and ^{1}H-NMR spectra confirmed the presence of a β-substituted furan ring (1625, 1506, 875 cm^{-1}) and carbonyl groups (1731 cm^{-1}), included a carboxylic acid, a δ-lactone and a cylcohexanone, as well as one tertiary methyl group.

Signal at δ 5.62 (H-12) in the ^{1}H-NMR spectrum of **2** revealed that the δ-lactone ring was linked through a secondaryl hydroxyl group as in **1**. The δ-lactone in **2** was similarly proximate to the furan ring from the allylic coupling (0.6 Hz) of H-16 with H-12. This, together with a similar isolated ABX system for the C-12 methine and adjacent C-11 methylene, revealed that the six-membered ring lactone was again attached to ring B. But signal at δ 4.18 (H-2) in the ^{1}H-NMR spectrum of **2** appeared in up-field comparison with **1**, **3**, and **4**. Considering nine degrees of unsaturation, including the contribution of rings A and B, a furan ring, a δ-lactone and three carbonyl groups, it suggested that the γ-lactone ring was open, and the secondary hydroxyl group at C-2 and the carboxyl group at C-4 became free, and kept to be α orientation. The proton at C-10 was coupled to the protons at C-1 and was axial from its coupling constants of 12.1 Hz (ax–ax) to the H-1β, and 4.5 Hz (eq–ax) to the H-1α.

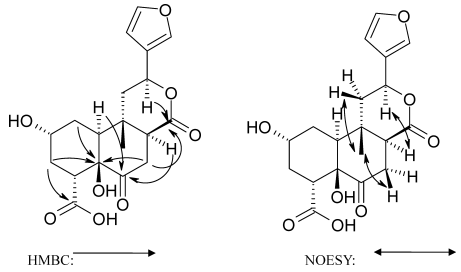

Fig. 3. Key HMBC and ROESY Correlations for Compound **2**

Further analysis of the NMR spectrum (Table 1), signal at δ 76.1 (C-5) in the ^{13}C-NMR (CD_3COCD_3) spectrum of **2** revealed that this carbon must be connected with a hydroxyl group. The linked position of this hydroxyl group was determined at C-5 based on the following important HMBC correlations (Fig. 3): from δ_H 1.57, 2.01 (each m, H-1), δ_H 2.04, 2.06 (each m, H-3), δ_H 2.26, 2.37 (each m, H-7) to δ_C 76.1 (s, C-5). Taken the consideration with the co-occurrence of **1** in the same plant, **2** is probably an oxidation product of **1**. It suggested that the hydroxyl group at C-5 is β orientation, same as the proton at C-5 in **1**. Moreover, other key HMBC correlations (Fig. 3) were also observed: from H-1, H-8 to C-6, from H-3, H-4 to C-19, from H-7, H-12 to C-17, respectively. The significant ROESY correlations (Fig. 3) between H-7β, H-11β and H-20, between H-8 and H-12 were also observed. The proton at C-8 was coupled only to the protons at C-7 and was axial from its coupling constants of 12.3 Hz (ax–ax) to H-7β and 3.5 (eq–ax) to H-7α. This can help to distinguish the configurations of each geminal proton at C-7. The configuration at C-8 of **2** was thus opposite to that of the **4**, and same to that of **3**. Thus, the structure of **2** was established as shown in Fig. 1 and named diosbulbin J.

Experimental

General The optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired on a Bruker DRX-500 and AV-400 instruments. EI-MS was performed on a Finnigan-MAT 90 instrument. HR-ESI-MS was detected on a API QSTAR Pulsar 1 spectrometer. Silica gel (200—300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by silica gel plates sprayed with vanillin–H_2SO_4 in ethanol, in combination with Agilent 1200 reversed-phase HPLC (Eclipse XDB-C18 column, 5 μm, 4.6×150 mm,

30—100% MeOH in H_2O over 10 min followed by 100% MeOH to 15 min, 1 ml/min, 25 °C).

Plant Material The root tubers of *Dioscorea bulbifera* L. collected in September 2007, from Anhui Province of China, and identified by Prof. Cheng-Wu Fang, Anhui College of Traditional Chinese Medcine. The voucher specimen was deposited in the Herbarium of Anhui College of Traditional Chinese Medcine.

Extraction and Isolation The air-dried, powdered root tubers (2 kg) of *Dioscorea bulbifera* were soaked with 95% ethanol (3×6 l, each soaking for 3 d) at room temperature and filtered. The filtrate was concentrated in vacuum to give a residue (*ca.* 100 g), which was isolated by silica gel column chromatography with a gradient elution system of petroleum ether–acetone (100 : 0→0 : 100) to obtain 20 fractions. Fraction-4 eluted with 85 : 15 and was repeatedly separated by silica gel ($CHCl_3$/MeOH=200 : 1), Sephadex LH-20 ($CHCl_3$/MeOH=1 : 1) and recrystallization techniques to give rise to compound **1** (10 mg). Fraction-6 eluted with 70 : 30 was further separated and purified by silica gel ($CHCl_3$/MeOH=20 : 1), Sephadex LH-20 ($CHCl_3$/MeOH=1 : 1) and recrystallization process to yield **2** (8 mg).

Compound **1**: Colorless needles. mp 190—192 °C ($CHCl_3$/MeOH). $[\alpha]_D^{19.3}$ −21° (*c*=0.33, $CHCl_3$). UV λ_{max} ($CHCl_3$) 312 nm ($\log \varepsilon$ 4.26). IR (KBr) cm^{-1}: ν_{max} 3145, 2957, 1778, 1745, 1708, 1634, 1604, 1575, 1512, 1462, 1389, 1253, 1163, 875, 829, 750. EI-MS *m*/*z* (%): 506 ($[M]^+$, 12), 345 (2), 178 (27), 161 (100). HR-ESI-MS *m*/*z*: 529.1836 ($[M+Na]^+$) (Calcd for $C_{29}H_{30}O_8Na$: 529.1838).

Compound **2**: Colorless needles. mp 197—198 °C ($CHCl_3$/MeOH). $[\alpha]_D^{18.5}$ −5° (*c*=0.20, MeOH). UV λ_{max} (MeOH) 213 nm ($\log \varepsilon$ 3.72). IR (KBr) cm^{-1}: ν_{max} 3435, 2936, 1731, 1625, 1625, 1506, 1389, 875, 740. EI-MS *m*/*z* (%): 378 ($[M]^+$, 38), 360 (13), 342 (8), 237 (51), 219 (95), 142 (48), 94 (100). HR-ESI-MS *m*/*z*: 401.1222 ($[M+Na]^+$) (Calcd for $C_{19}H_{22}O_8Na$: 401.1212).

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