Steroidal Sapogenins and Glycosides from the Rhizomes of *Dioscorea bulbifera*

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The MOE Key Laboratory for Standardization of Chinese Medicines, Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201210, People's Republic of China, Shanghai R&D Centre for Standardization of Chinese Medicines, 1200 Cailun Road, Shanghai 201210, People's Republic of China, and Shanghai Mass Spectrometry Center, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, People's Republic of China

Four new steroidal sapogenins (**1**−**4**), named diosbulbisins A−D, two new spirostane glycosides, diosbulbisides A (**5**) and B (**6**), one new cholestane glycoside, diosbulbiside C (**7**), and the known compounds **8**−**10** were isolated from rhizomes of *Dioscorea bulbifera*. Their structures were elucidated by 1D and 2D NMR techniques, HRFTMS, and chemical methods. The unusual furospirostanol sapogenin skeletons, as found in compounds **3** and **4**, are reported in the family Dioscoreaceae for the first time. Cytotoxicity of compounds **1**−**10** was evaluated using two human hepatocellular carcinoma cell lines (Bel-7402 and SMMC7721).

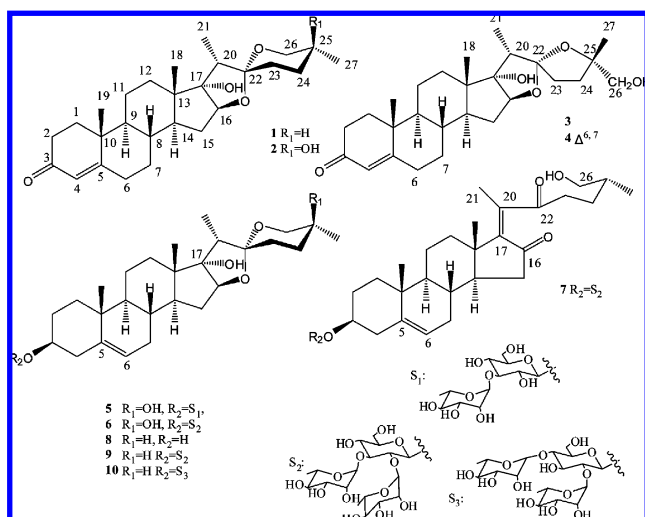Diosgenin and related steroidal saponins commonly found in *Dioscorea* plants have shown antitumor, antifungal, and anti-inflammatory activities.[1−3] The rhizome of *Dioscorea bulbifera* L. (Dioscoreaceae), known as "Huang Yao Zi" in traditional Chinese medicine, has been used to cure thyroid diseases and cancer.[4−6] In the northern districts of Bangladesh, tribal people use rhizomes of this herb for treatment of leprosy and tumors.[7] Earlier chemical investigations of *D. bulbifera* yielded flavonoids,[5,8−10] clerodane diterpenoids,[7,11−14] and a few steroidal saponins.[15,16] Although clerodane diterpenoids were usually described as the primary and characteristic constituents of this herb, they showed no remarkable antitumor activities based on in vitro assays.[13]

In a search for compounds with potential antitumor and antithyroid effects, the present study describes the isolation, structure elucidation, and biological activity screening of compounds from *D. bulbifera*. Four new steroidal sapogenins (**1**−**4**), three new steroidal glycosides (**5**−**7**), and three known compounds (**8**−**10**) were obtained. Cytotoxic activity of these compounds was assayed against Bel-7402 and SMMC7721 cancer cell lines.

5 R₁=OH, R₂=S₁,
6 R₁=OH, R₂=S₂
8 R₁=H, R₂=H
9 R₁=H R₂=S₂
10 R₁=H R₂=S₃

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Results and Discussion

Dried and pulverized rhizomes of *D. bulbifera* were extracted with 95% EtOH. The extract suspended in water was successively extracted with petroleum ether and ethyl acetate. The residual water-soluble portion was subjected to D101 macroporous resin column chromatography (CC) to afford a saponin-rich fraction. This fraction and the EtOAc-soluble portion were further fractionated by silica gel CC and Sephadex LH-20 followed by ODS to give seven new compounds (**1**−**7**) and three known ones (**8**−**10**). Their structures were elucidated by analysis of their molecular mass, sugar components, and 1D and 2D NMR spectroscopy. The sugar residues of compounds **5**−**7** were determined to be D-glucose and L-rhamnose by GC analyses of chiral derivatives. The ¹H NMR coupling constants ($^3J_{1, 2} > 7$ Hz) were consistent with β-D-glucose, and α-L-rhamnose was deduced by comparing the ¹³C NMR spectroscopic data for C-3 and C-5 of rhamnose with those of the literature reports.[17] Compounds **8**−**10** were identified by comparison of their physical and spectroscopic data with published data as pennogenin (**8**),[18,19] pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**9**),[15] and pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**10**).[19,20]

Compound **1** had the molecular formula $C_{27}H_{40}O_4$ as determined by positive-ion HRFTMS (*m/z* 429.3014 [M + H]⁺, calcd 429.3004) and the NMR (Tables 1 and 2) data. Two methyl singlet signals at δ_H 1.07 and 1.12 and two methyl doublet signals at δ_H 0.78 (*J* = 5.3 Hz) and 1.31 (*J* = 7.2 Hz) in the ¹H NMR spectrum and a quaternary carbon at δ_C 109.9 in ¹³C NMR spectrum indicated a characteristic spirostanol skeleton. Comparison of the ¹H and ¹³C NMR spectra of **1** with those of pennogenin (**9**) revealed that the C−F rings of **1** were identical to that of pennogenin,[19] but that there was a difference in the A and B rings. Compound **1** had an α,β-unsaturated keto group [δ_H 5.91 (s)/δ_C 198.4, 170.6, 124.1], which was assigned at C-3, -4, and -5 of ring A, as confirmed by HMBC correlations of H-2 signals at δ_H 2.49 and 2.37 with the carbonyl carbon signal at δ_C 198.4, the H-19 signal at δ_H 1.12 with the olefinic quaternary carbon at δ_C 170.6, and the olefinic proton signal at δ_H 5.91 with the carbon signals at δ_C 34.4 (C-2), 32.9 (C-6), and 38.8 (C-10). Further analysis of the HMBC spectrum of **1** assigned the oxy-quaternary carbon at C-17 due to the long-range correlations of its signal (δ_C 89.9) with the proton signals at δ_H 4.51 (H-16), 1.07 (H-18), and 1.31 (H-21). The key HMBC correlations of **1** are shown in Figure 1. The methyl group at C-25 was assigned as α-oriented using the chemical shifts of C-23, -24, -25, and -27 by referring to reported data for pennogenin[19] and by

Table 1. ^{1}H NMR (500 MHz) Data of Compounds **1−7** in Pyridine-d_5 (δ values; *J* values in parentheses)

position	1	2	3	4	5	6	7
1a	1.90 m	1.89 m	1.88 m	1.83 m	1.78 m	1.81 m	1.79 m
1b	1.74 m	1.74 m	1.68 m	1.56 m	1.01 m	1.04 m	1.02 m
2a	2.49 m	2.45 m	2.46 m	2.62 m	2.12 m	2.17 m	2.18 m
2b	2.37 m	2.45 m	2.46 m	2.48 m	1.75 m	1.95 m	1.96 m
3					3.93 m	3.97 m	3.98 m
4	5.91 s	5.92 s	5.90 s	5.93 s			
4a					2.70 m	2.79 m	2.82 m
4b					2.70 m	2.79 m	2.82 m
5							
6a	2.29 m	2.30 m	2.28 m				
6b	2.13 m	2.14 m	2.10 m				
6				6.15 d (9.7)	5.37 d (5.1)	5.39 d (4.9)	5.38 d (4.2)
7a	1.73 m	1.77 m	1.69 m		2.02 m	2.04 m	1.88 m
7b	0.96 m	0.96 m	0.90 m		1.66 m	1.74 m	1.58 m
7				6.09 d (9.7)			
8	1.58 m	1.54 m	1.47 m	2.39 m	1.63 m	1.65 m	1.58 m
9	0.93 m	0.90 m	0.86 m	1.18 m	1.03 m	1.05 m	1.06 m
10							
11a	1.63 m	1.58 m	1.59 m	1.55 m	1.64 m	1.66 m	1.59 m
11b	1.48 m	1.46 m	1.48 m	1.46 m	1.61 m	1.61 m	1.59 m
12a	2.29 m	2.20 m	2.20 m	2.21 m	2.33 m	2.35 m	2.23 m
12b	1.67 m	1.60 m	1.59 m	1.61 m	1.62 m	1.64 m	1.58 m
13							
14	2.11 m	2.18 m	2.05 m	2.21 m	2.17 m	2.18 m	1.46 m
15a	2.21 m	2.22 m	2.13 m	2.31 m	2.24 m	2.28 m	2.26 m
15b	1.59 m	1.60 m	1.50 m	1.59 m	1.65 m	1.66 m	2.08 m
16	4.51 t (6.9)	4.58 t (6.9)	4.60 dd (7.5, 6.1)	4.62 m	4.59 m	4.61 m	
17							
18	1.07 s	1.10 s	1.06 s	1.08 s	1.06 s	1.06 s	1.03 s
19	1.12 s	1.13 s	1.10 s	1.09 s	0.99 s	1.16 s	1.13 s
20	2.34 m	2.45 m	2.61 dd (14.3, 7.1)	2.63 m	2.43 m	2.43 m	
21	1.31 d (7.2)	1.37 d (7.3)	1.28 d (7.2)	1.29 d (7.1)	1.36 d (7.1)	1.36 d (7.2)	2.06 s
22							
23a	1.81 m	2.60 m	2.26 m	2.23 m	2.58 m	2.58 m	2.94 m
23b	1.71 m	1.75 m	2.26 m	2.23 m	1.74 m	1.73 m	2.94 m
24a	1.68 m	2.22 m	2.45 m	2.43 m	2.19 m	2.21 m	2.37 m
24b	1.68 m	1.98 m	2.25 m	2.22 m	1.96 m	1.97 m	2.01 m
25	1.67 m						2.04 m
26a	3.60 m	4.03 d (11.3)	3.89 m	3.90 m	4.02 m	4.03 d (11.3)	3.84 m
26b	3.60 m	3.73 dd (11.3, 2.4)	3.89 m	3.90 m	3.70 m	3.70 m	3.80 m
27	0.78 d (5.3)	1.32 s	1.41 s	1.42 s	1.30 s	1.31 s	1.18 d (6.4)
3-Glc							
1′					5.01 m	4.97 d (7.7)	4.99 d (7.6)
2′					4.10 m	4.15 m	4.15 m
3′					4.50 m	4.25 m	4.25 m
4′					4.32 m	4.14 m	4.14 m
5′					3.96 m	3.87 m	3.98 m
6′a					4.54 m	4.52 m	4.54 m
6′b					4.49 m	4.39 m	4.43 m
2′-Rha							
1″						5.94 s	5.95 s
2″						4.84 m	4.83 m
3″						4.58 m	4.61 m
4′						4.38 m	4.41 m
5″						4.94 m	4.93 m
6″						1.83 d (6.2)	1.84 d (6.1)
3′-Rha							
1‴					6.40 s	5.83 s	5.83 s
2‴					4.88 m	4.56 m	4.54 m
3‴					4.67 m	4.95 m	4.91 m
4‴					4.43 m	4.37 m	4.41 m
5‴					5.10 m	4.86 m	4.86 m
6‴					1.80 d (6.2)	1.73 d (6.3)	1.73 d (6.2)

the coupling constants of H-26 signals [δ_H 3.60 (2H, m)]. Thus, **1** was elucidated to be (25*R*)-17α-hydroxyspirost-4-en-3-one, and it was named diosbulbisin A.

Compound **2** was a white powder with a molecular formula of $C_{27}H_{40}O_5$ on the basis of positive-ion HRFTMS (*m/z* 445.2944 [M + H]$^+$, calcd 445.2954) data. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** were very similar to those of **1**, with the exception of an OH group substituted on ring F. The OH group was placed at C-25 from evidence that the H-27 methyl signal became a singlet
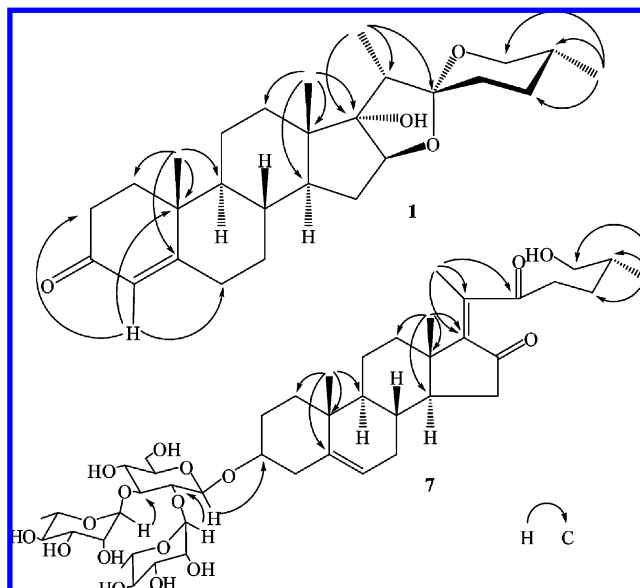
and shifted downfield from δ_H 0.78 (d, *J* = 5.3 Hz) in **1** to 1.32 (s) in **2**. The C-25 signal shifted downfield from δ_C 30.5 (t) in **1** to δ_C 66.0 (q) in **2**, and further confirmation was evident by the HMBC correlations of the proton signals at δ 1.32 (H-27), 1.98 (H-24b), 3.73 (dd, *J* = 11.3, 2.4 Hz) (H-26b), and 4.03 (d, *J* = 11.3 Hz) (H-26a) with the C-25 signal at δ_C 66.0. The configuration of the OH group at C-25 was deduced from the ^{13}C NMR spectrum. The large (−6.7 ppm) γ_g-effect displayed at C-23 was more consistent with an axial OH group at C-25 than with an axial methyl group.

Table 2. ^{13}C NMR (125 MHz) Data (δ) of Compounds **1**−**7** in Pyridine-d_5

position	1	2	3	4	5	6	7
1	36.0	36.0	35.9	34.4	37.6	37.6	37.2
2	34.4	34.5	34.4	34.2	30.1	30.2	30.1
3	198.4	198.5	198.3	198.4	78.3	77.9	77.8
4	124.1	123.8	123.8	124.0	39.3	38.8	38.7
5	170.6	170.8	170.6	163.4	140.9	141.0	141.0
6	32.9	32.9	32.8	127.9	121.9	122.0	121.5
7	32.5	32.5	32.5	141.1	32.5	32.6	31.8
8	35.9	36.0	35.9	38.0	32.4	32.5	30.9
9	53.8	53.8	53.8	50.8	50.3	50.3	50.0
10	38.8	38.9	38.8	36.3	37.1	37.2	37.2
11	21.0	21.0	20.9	20.5	21.1	21.0	21.0
12	32.1	32.1	32.1	32.2	31.9	31.9	36.2
13	45.3	45.4	45.4	46.4	45.3	45.3	43.5
14	52.3	52.3	52.1	49.7	53.2	53.2	50.6
15	31.6	31.8	31.5	31.0	32.2	32.2	38.1
16	89.9	90.2	89.9	89.7	90.3	90.4	205.7
17	89.9	90.0	89.5	89.4	90.2	90.3	142.7
18	17.3	17.3	17.2	16.3	17.3	17.2	16.8
19	17.4	17.4	17.3	16.9	19.5	19.5	19.4
20	44.9	45.0	41.6	41.6	44.9	44.9	145.7
21	9.8	9.9	10.1	10.1	9.9	9.9	15.9
22	109.9	110.3	120.6	120.6	110.3	110.3	210.7
23	32.1	28.2	34.4	34.4	28.2	28.2	39.2
24	28.9	33.5	32.4	32.6	33.5	33.5	28.0
25	30.5	66.0	86.2	86.3	65.9	65.9	36.2
26	66.8	69.8	70.1	70.1	69.8	69.8	67.6
27	17.3	27.1	24.1	24.1	27.1	27.0	17.3
3-Glc							
1′					102.4	100.0	100.1
2′					75.8	78.4	78.4
3′					83.7	87.6	87.5
4′					69.8	70.1	70.1
5′					78.4	78.1	78.2
6′					61.6	62.4	62.4
2′-Rha							
1″						102.7	102.7
2″						72.5	72.6
3″						72.9	72.9
4′						73.9	73.9
5″						69.9	70.0
6″						18.7	18.8
3′-Rha							
1‴					103.0	104.0	104.0
2‴					72.7	72.6	72.6
3‴					72.8	72.7	72.7
4‴					74.3	73.7	73.7
5‴					70.3	70.7	70.7
6‴					18.8	18.5	18.5

Figure 1. Key HMBC correlations (arrows) of compounds **1** and **7**.

Compound **4** had the molecular formula $C_{27}H_{38}O_5$ as deduced from the HRFTMS data (m/z 465.2623 [M + Na]$^+$, calcd 465.2616). The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **3**, except that **4** had an additional olefinic group [δ_H 6.09 (d, J = 9.7), 6.15 (d, J = 9.7)/δ_C 141.1, 127.9], which was assigned at C-6 and C-7 on the basis of the upfield shift of C-5 (−7.2 ppm) due to the formation of the conjugated double bond and the HMBC correlations of H-4 [δ_H 5.93 (s)] with the olefinic carbon [δ_C 127.9 (C-6)], the olefinic proton signal (H-6) at δ_H 6.15 with the carbon signals at δ_C 38.0 (C-8) and 36.3 (C-10), and the olefinic proton signal (H-7) at δ_H 6.09 with the carbon signals at δ_C 163.4 (C-5), 50.8 (C-9), and 49.7 (C-14) respectively. Thus, **4** was shown to be (22S,25S)-17α,26-dihydroxy-22,25-epoxyfurost-4,6-dien-3-one, and it was given the trivial name diosbulbisin D.

Compound **5** had the molecular formula $C_{39}H_{62}O_{14}$ [HRFTMS (m/z 777.4044 [M + Na]$^+$, calcd 777.4037)]. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **5** showed three methyl singlets (δ_H 1.30, 1.06, and 0.99), one methyl doublet [δ_H 1.36 (d, J = 7.1 Hz)], an olefinic group [δ_H 5.37 (d, J = 5.1)/δ_C 140.9, 121.9], and a quaternary carbon signal at δ_C 110.3, which suggested that **5** was a steroidal glycoside that possessed a Δ5,6-spirotanol skeleton.[19] Three additional oxygen substituents at C-3 (δ_C 78.3), C-17 (δ_C 90.2), and C-25 (δ_C 65.9) and two sugar residues indicated by two aromeric proton signals [δ_H 5.01 (m) and 6.40 (s)] and two aromeric carbon signals (δ_C 103.0 and 102.4) were observed in the NMR spectra of **5**. The ^{1}H and ^{13}C NMR spectra of the E and F ring portions of **5** were similar to those of **2**. In the HMBC spectrum, the anomeric proton signals of rhamnose and of glucose showed long-range correlations with C-3′ (δ_C 83.7) of the glucose and C-3 (δ_C 78.3) of the aglycone, respectively. The configuration of **5** was determined by a NOESY experiment. NOESY correlation of H-3 (δ_H 3.93) with H-9 (δ_H 1.03) suggested that H-3 was α-oriented. Therefore, compound **5** was determined to be (25S)-17α,25-dihydroxyspirost-5-en-3β-yl-O-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, and it was given the trivial name diosbulbiside A.

Compound **6** was obtained as a white, amorphous solid with molecular formula $C_{45}H_{72}O_{18}$ on the basis of the positive-ion HRFTMS (m/z 923.4649 [M + Na]$^+$, calcd 923.4616). Comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of **5** revealed that they were similar except for the presence of an additional rhamnosyl group in **6**. The HMBC spectrum showed correlations of the anomeric proton signal at δ_H 5.94 (H-1″) of rhamnose with C-2′

The latter showed a smaller γ_g-effect, e.g., −1.5 ppm, as the case in (25R)-isonuatigenin.[21,22] Comparison of the chemical shift of C-25 (δ_C 66.0) of **2** with the corresponding values reported for (25R)-isonuatigenin and (25S)-isonuatigenin (δ_C 81.6 and 65.1, respectively) also indicated an axial OH group, i.e., the S-configuration at C-25.[19,21] Thus, **2** was characterized as (25S)-17α,25-dihydroxyspirost-4-en-3-one, and it was named diosbulbisin B.

Compound **3** was obtained as a white powder. Its molecular formula was deduced to be $C_{27}H_{40}O_5$ from the HRFTMS data (m/z 445.2941 [M + H]$^+$, calcd 445.2954). The ^{1}H NMR spectrum of **3** (Table 1) exhibited three methyl singlets (δ_H 1.41, 1.10 and 1.06) and one methyl doublet signal [δ_H 1.28 (J = 7.2 Hz)]. The ^{13}C NMR spectrum of **3** (Table 2) showed a quaternary carbon signal at δ 120.6 typical of 22,25-epoxyfuranols (furospirostanols). The spectroscopic data of **3** were closely related to those of **2**, except for the ring F portion. The ^{1}H and ^{13}C NMR data of the ring F of **3** were similar to those of (22S,25S)-22,25-epoxyfurost-5-ene-3β,26-diol (nuatigenin).[19,23] Accordingly, **3** was elucidated as (22S,25S)-17α,26-dihydroxy-22,25-epoxyfurost-4-en-3-one, and it was given the trivial name diosbulbisin C.

(δ_C 78.4) of glucose and the anomeric proton signal at δ_H 5.83 (H-1‴) of rhamnose with C-3′ (δ_C 87.6) of glucose as well as the anomeric proton at δ_H 4.97 (H-1′) of glucose with C-3 (δ_C 77.9) of the aglycone. Thus, compound **6** was determined to be (25S)-17α,25-dihydroxyspirost-5-en-3β-yl-*O*-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside and was given the trivial name diosbulbiside B.

Compound **7** had the molecular formula $C_{45}H_{70}O_{17}$ (HRFTMS). The ¹H and ¹³C NMR spectra (Tables 1 and 2) suggested a C_{27} steroidal saponin having a cholestane skeleton[24] with two carbonyl groups, an oxymethylene, a methyl group on a double bond, a tetrasubstituted olefinic group, two singlet methyl groups, three doublet methyl groups, a trisubstituted olefinic group, and three anomeric protons. Signals for three sugar groups were also observed. In the HMBC spectrum, correlations from the methylene proton signals [δ_H 2.26 and 2.08 (H-15)] to the carbonyl carbon signal [δ_C 205.7 (C-16)] and from the methyl proton signals [δ_H 2.06 (Me-21)] and two methylenes [δ_H 2.37 and 2.01 (H₂-24) and 2.94 (2H, H₂-23)] to the carbonyl carbon signal at δ_C 210.7 (C-22) gave ample evidence for carbonyl groups at C-16 and C-22, respectively. Further HMBC correlations from Me-18 (δ_H 1.03) to δ_C 142.7 and from Me-21 to δ_C 142.7 and 145.7 were indicative of a double bond between C-17 and C-20. The primary OH was at C-26 as deduced from the HMBC correlations of proton signals at δ_H 3.84 (H-26a) and 3.80 (H-26b) with the ¹³C signals at δ_C 28.0 (C-24), 36.2 (C-25), and 17.3 (C-27). HMBC correlations of C-2′ (δ_C 78.4)/H-1″ (δ_H 5.95), C-3′ (δ_C 87.5)/H-1‴ (δ_H 5.83), and C-3 (δ_C 77.8)/H-1′ (δ_H 4.99) were also observed. The key HMBC correlations of **7** are shown in Figure 1. The 25R-configuration of **7** was deduced from the geminal proton resonances of H-26a and H-26b, which showed a $\Delta_{a\ b}$ ($\delta_{Ha} - \delta_{Hb}$) of 0.04, which was less than 0.48.[25] The above data led to the structure of **7** as (25R)-26-hydroxy-cholesta-5,17-diene-16,22-dione-3β-yl-*O*-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, and it was named diosbulbiside C.

Compounds **1**−**10** were tested against proliferation of Bel-7402 human hepatocellular carcinoma cells at a concentration of 10 μM following a standard MTT assay.[1,26] Only **9** and **10** showed significant cytotoxic activity with 99.1% and 92.6% inhibition, respectively. The compounds were then tested for cell growth inhibition activity toward SMMC7721 human hepatocellular carcinoma cells, and they exhibited IC_{50} values of 4.54 μM (**9**) and 4.85 μM (**10**), respectively.

Experimental Section

General Experimental Procedures. Optical rotations were taken in MeOH on a KRÜSS P8000-T polarimeter (A. KRÜSS Optronic GmbH, Germany). Melting points were determined on a Buchi melting point B-540 (uncorrected). IR spectra were measured as films on KBr pellets using a Thermo Nicolet 380 FT-IR spectrometer (Thermo Electron Corporation, Waltham, MA). The 1D and 2D NMR spectra were acquired on a Bruker AV500 spectrometer. The HRFTMS were obtained on an Ionspec 4.7 T HisRes MALDI-FTMS (Ionspec, Irvine, CA). GC analysis was performed on a Thermo Finnigan Trace DSQ gas chromatograph equipped with a mass spectrometry detector. Open column chromatography (CC) was carried out using D101 macroporous resin (Tianjin Pesticide Co., China), silica gel (200−300 and 300−400 mesh, Qingdao Marine Chemical Co., China), ODS-A (50 μm, YMC, Kyoto, Japan), RP-C₁₈ (4.6 mm × 250 mm, 5 μm, Sepax Technologies Inc., Newark, DE), and Sephadex LH-20 (GE Healthcare, Sweden) as stationary phases. TLC was performed on HSGF₂₅₄ (0.2 mm, Qingdao Marine Chemical Co., China) or RP-18 F₂₅₄ (0.25 mm, Merck) plates.

Plant Material. Dried rhizomes of *D. bulbifera* were collected from Qingyang, Anhui Province, China, in September 2006, and were identified by Professor Shou-Jin Liu (Anhui College of Traditional Chinese Medicine, Anhui Province, China). A voucher specimen (LH0609-1) was deposited in the herbarium of the Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine.

Extraction and Isolation. The dried rhizomes of *D. bulbifera* (30 kg) were pulverized and extracted three times (each time for 4 h) with 80% ethanol. The filtrate obtained was concentrated under reduced pressure to yield a dark residue, which was suspended in water and extracted with petroleum ether and ethyl acetate successively. After evaporation of solvents, petroleum ether- (160 g) and ethyl acetate (600 g)-soluble portions were obtained. The residual water-soluble portion was applied to a D101 macroporous resin column (40−60 mesh, 50 kg) and eluted with 95% EtOH−H₂O mixtures to afford a saponin-rich fraction (400 g). The EtOAc-soluble portion was subjected to silica gel CC eluting with a gradient of CH₂Cl₂−MeOH (99:1, 20:1, 9:1, 6:1, 8:2, 6:4, 1:1, 0:1); TLC analysis was carried out (visualization with 10% sulfuric acid−ethanol solution and heating at 150 °C), which indicated 28 distinct fractions. Fraction 4 (1.3 g) was separated by silica gel CC with petroleum ether−ethyl acetate (8:2) and further fractionated on Sephadex LH-20 [CH₂Cl₂−MeOH (1:1)] to give **1** (29 mg) and **8** (36 mg). Fraction 7 (0.9 g) was purified using Sephadex LH-20 and RP-HPLC (C₁₈ column, 4.6 mm × 250 mm, flow rate 1.0 mL min⁻¹) using ACN−H₂O (55:45, v/v) as eluants, to afford **2** (23 mg), **3** (19 mg), and **4** (3 mg). The saponin-rich fraction was subjected to silica gel CC eluting with a gradient of CH₂Cl₂−MeOH (99:1, 9:1, 8:2, 7:3, 6:4, 1:1, 0:1) to afford fractions 1−26. Fractions 20 and 21 (3.2 g) were separated by silica gel CC with CH₂Cl₂−MeOH (8:1), followed by chromatography on Sephadex LH-20 (MeOH) and ODS (YMC, ODS-A, RP-18, 400 mm × 30 mm) and elution with MeOH−H₂O (60:40, v/v) to afford **5** (6 mg), **6** (5 mg), **9** (35 mg), and **10** (156 mg). Fraction 24 (3.2 g) was subjected to Sephadex LH-20 (MeOH) and further purified on ODS using MeOH−H₂O (55:45, v/v) as eluants, followed by silica gel CC with CH₂Cl₂−MeOH (7:3) to afford **7** (32 mg).

Diosbulbisin A (1): white powder; mp 185−186 °C; [α]²⁵_D −11.9 (c 0.012, MeOH); IR (KBr) ν_max 3526.9, 2951.0, 2930.9, 2860.0, 1677.6, 1615.9, 1455.6, 1378.2, 1340.2, 1270.4, 1176.3, 1159.0, 1126.8, 1051.0, 979.6, 897.8, 864.7 cm⁻¹; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 429.3014 [M + H]⁺ (calcd. for $C_{27}H_{41}O_4$, 429.3004).

Diosbulbisin B (2): white powder; mp 232−233 °C; [α]²⁵_D −12.1 (c 0.016, MeOH); IR (KBr) ν_max 3530.5, 2962.3, 2950.3, 2931.7, 2875.9, 2862.2, 1674.0, 1610.2, 1456.5, 1375.6, 1242.5, 1176.1, 1128.6, 1064.1, 1036.3, 984.1, 934.6, 889.5, 847.2 cm⁻¹; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 445.2944 [M + H]⁺ (calcd for $C_{27}H_{41}O_5$, 445.2954).

Diosbulbisin C (3): white powder; mp 244−245 °C; [α]²⁵_D −39.7 (c 0.015, MeOH); IR (KBr) ν_max 3414.3 (br), 2949.4, 2908.2, 2852.1, 1675.5, 1610.8, 1458.0, 1383.5, 1326.0, 1269.9, 1228.9, 1184.2, 1124.9, 1060.5, 994.6, 871.6 cm⁻¹; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 445.2941 [M + H]⁺ (calcd. for $C_{27}H_{41}O_5$, 445.2954).

Diosbulbisin D (4): white powder; mp 211−212 °C; [α]²⁵_D −20.8 (c 0.014, MeOH); IR (KBr) ν_max 3442.1 (br), 2947.8, 2918.8, 2850.4, 1665.7, 1618.2, 1458.6, 1384.1, 1323.6, 1264.7, 1225.0, 1123.5, 1051.6, 1000.6, 876.9 cm⁻¹; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 465.2623 [M + Na]⁺ (calcd for $C_{27}H_{38}O_5Na$, 465.2616).

Diosbulbiside A (5): white, amorphous solid; mp 237−238 °C; [α]²⁵_D −20.2 (c 0.021, MeOH); IR (KBr) ν_max 3423.4 (br), 2961.4, 2929.4, 1637.5, 1457.9, 1379.2, 1261.3, 1060.9, 1038.4, 934.8, 887.6, 803.4 cm⁻¹; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 777.4044 [M + Na]⁺ (calcd for $C_{39}H_{62}O_{14}Na$, 777.4037).

Diosbulbiside B (6): white, amorphous solid; mp 278−279 °C; [α]²⁵_D −33.2 (c 0.028, MeOH); IR (KBr) ν_max 3423.3 (br), 2960.9, 2930.0, 1637.6, 1457.7, 1382.1, 1261.1, 1229.3, 1042.3, 986.8, 934.7, 887.4, 808.4 cm⁻¹; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 923.4649 [M + Na]⁺ (calcd for $C_{45}H_{72}O_{18}Na$, 923.4616).

Diosbulbiside C (7): white, amorphous solid; mp 230−231 °C; [α]²⁵_D −48.0 (c 0.016, MeOH); IR (KBr) ν_max 3423.6 (br), 2931.7, 1716.8, 1701.2, 1630.1, 1383.1, 1064.1, 1042.6; ¹H and ¹³C NMR assignments, see Tables 1 and 2; HRFTMS m/z 905.4516 [M + Na]⁺ (calcd for $C_{45}H_{70}O_{17}Na$, 905.4510).

Acid Hydrolysis and Sugar Analysis. Compounds **5**−**7** (each 1.5 mg) were hydrolyzed with 2 M aqueous CF₃CO₂H (1 mL) at 120 °C for 2.5 h, after which the solvent was evaporated with a stream of N₂. The following solutions were added: (a) 1:8 (S)-1-amino-2-propanol−MeOH (20 μL); (b) 1:4 glacial acetic acid−MeOH (17 μL); and (c) 3% NaBH₃CN in MeOH (13 μL). The vial was capped, and

the mixture was allowed to react for 2 h at 65 °C. After cooling, 2 M aqueous CF_3CO_2H was added dropwise to pH 1−2. The mixture was evaporated and co-evaporated with MeOH (5 × 0.5 mL). The residue was dried overnight in a desiccator and treated with 1:1 pyridine−acetic anhydride for 1 h at 100 °C. After cooling the derivatives were extracted with CH_2Cl_2 and washed with water (3 × 1 mL) and saturated $NaHCO_3$ (3 × 1 mL). The organic phase was dried with anhydrous Na_2SO_4 and subjected to GC-MS using a Thermo TR-5MS column (60 m × 0.25 mm × 2.5 μm) with helium as carrier gas at a flow rate of 1 mL/min to identify the monosaccharides. The oven temperature started at 180 °C and was increased to 220 °C at a rate of 4 °C/min, keeping it at 220 °C for 2 min, and then increased to 270 °C at a rate of 1 °C/min, keeping it at 270 °C for 1 min. Derivatives of D-glucose and L-glucose eluted at 49.50 and 49.61 min,[27] and the derivative of L-rhamnose eluted at 35.94 min.

MTT Colorimetric Assay. Inhibition of cell-growth activity was determined by a MTT assay as previously described.[1,26] Human hepatocellular carcinoma cells (Bel-7402 and SMMC7721) were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in DMEM medium supplemented with 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Tumor cell cultures were diluted with fresh medium to 7500 cells per well and plated into 96-well microtiter plates at 100 μL per well. After 24 h incubation, the test compounds at various concentrations were added to the microtiter plates. Experiments were conducted in triplicate, and following 48 h of exposure, MTT was added to each well and was reduced by viable cells to an insoluble formazan product. Well contents were aspirated and formazan was solubilized by addition of DMSO. Absorbance was read on a Bio-Rad model 550 systems plate reader at 570 nm as a measure of cell viability. 10-Hydroxycamptothecin (HCP) was used as a positive control and showed an IC_{50} value of 0.14 μM (SMMC7721).

Supporting Information Available: ^{1}H and ^{13}C NMR, 2D NMR, and HRFTMS spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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