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Cholestane and spirostane glycosides from the rhizomes of *Dioscorea septemloba*

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Abstract

Cholestane glycosides, dioseptemlosides A (**1**) and B (**2**), together with six spirostane glycosides, dioseptemlosides C–H (**3**–**8**), were isolated from the rhizomes of *Dioscorea septemloba*. Their structures were established on the basis of physical data, spectroscopic analysis (HRESIMS, 1D and 2D NMR), and chemical methods. Spirostane aglcones containing hydroxyl group at C-7, as found in compounds **4**–**7**, were reported in the family Dioscoreaceae for the first time. These compounds did not show considerable inhibitory anti-tumor activities at a concentration of 10 μM.

Keywords: *Dioscorea septemloba*; Dioscoreaceae; Cholestane glycoside; Spirostane glycoside; Steroid

1. Introduction

Dioscorea septemloba Thunb., belonging to the family Dioscoreaceae is distributed mainly in the east and south of China, and was used for treatments of urethral and renal infections, as well as rheumatism in traditional Chinese medicine. Many *Dioscorea* species have been studied and found to contain steroids as their characteristic secondary metabolites, with the latter showing broad biological activities, e.g. as anti-tumor, anti-fungal and anti-inflammatory agents (He et al., 2006; Hu et al., 1996; Hu and Yao, 2002; Kawasaki et al., 1962; Yin et al., 2003). As part of our search for anti-tumor substances from *Dioscorea* species, the isolation and biological activity of *D. septemloba* was studied.

In this study, eight new steroidal glycosides (**1**–**8**) (Fig. 1) were obtained from a rhizome extract of *D. septemloba*. Their structures were determined by analysis of physical data, spectroscopic analysis (HRESIMS, 1D and 2D NMR), and chemical methods. All of the compounds were tested against growth of three tumor cell lines, but none showed inhibitory activities at a concentration of 10 μM.

2. Results and discussion

An aqueous ethanol extract of the rhizomes was subjected to D101 macroporous resin column chromatography to afford a saponin-rich fraction, with this fraction further separated using a series of chromatographic separations on silica gel and Sephadex LH-20 followed by MPLC (RP-18 silica gel) to afford eight new steroidal saponins named dioseptemlosides A–H (**1**–**8**). Their structures were determined from analysis of their molecular mass, sugar components, as well as by 1D NMR, and 2D NMR spectroscopy. The absolute configurations of the sugar residues were determined to be D for glucose and L for rhamnose by means of GC analysis of chiral derivatives of the sugars

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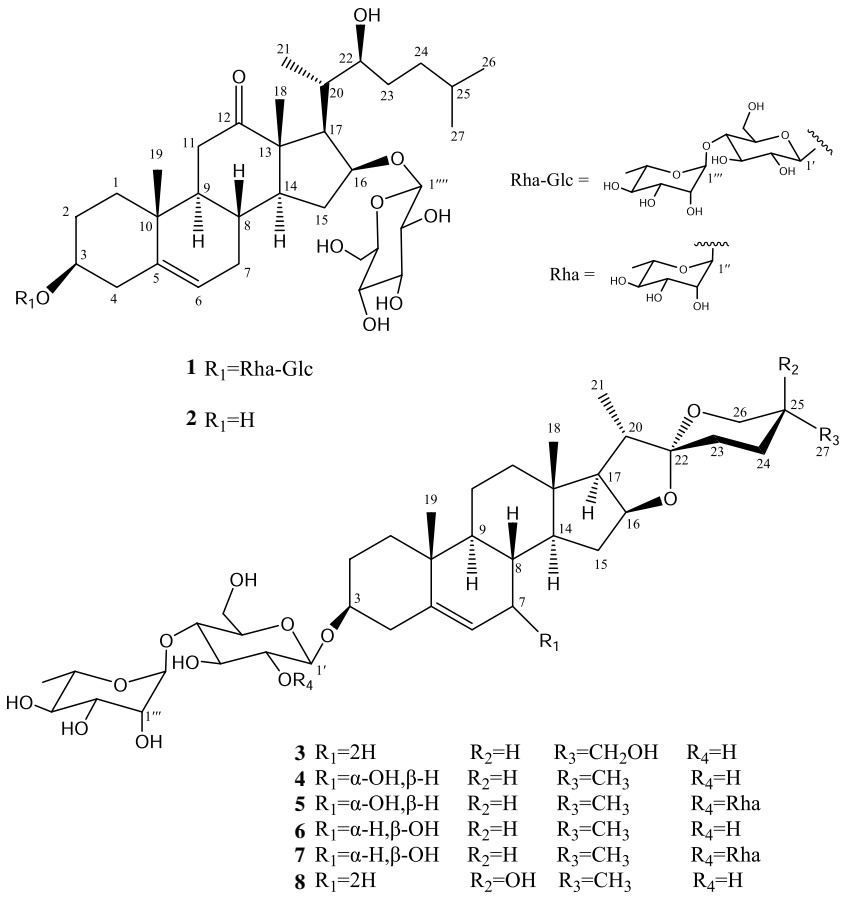

Fig. 1. Structures of compounds **1**–**8**.

in an acidic hydrolysate, as previously reported (Liu et al., 2006, 2007). The configurations of the anomeric carbons of D-glucopyranosyl and L-rhamnopyranosyl units were determined as β and α, respectively, according to the corresponding NMR coupling constants of anomeric protons as well as from the chemical shifts of the anomeric carbons. All of the ^{1}H and ^{13}C NMR signals of compounds **1**–**8** were assigned unambiguously (Tables 1 and 2).

Dioseptemloside A (**1**) was isolated as an amorphous solid with the molecular formula $C_{45}H_{74}O_{18}$, as determined by HRESIMS data exhibiting an $[M+H]^+$ peak at *m/z* 903.4841. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **1** showed signals for five typical steroidal methyl groups at δ_H 1.39 (*s*), 1.33 (*d*, $J = 7.1$ Hz), 1.03 (*s*), 1.01 (*d*, $J = 6.2$ Hz), and 1.01 (*d*, $J = 6.2$ Hz), an olefinic group (δ_H 5.29/δ_C 140.7, 121.9), a ketone carbon (δ_C 214.0), three oxygenated methane carbons (δ_C 78.0, 81.9, and 73.4), and three anomeric protons [δ_H 5.01 (*d*, $J = 7.8$ Hz), 5.96 (*br s*), and 4.84 (*d*, $J = 7.7$ Hz). The combined analysis of the 1D NMR spectra suggested that **1** was a cholestane glycoside with three oxygen substitutions at C-3 (δ_C 78.0), C-16 (δ_C 81.9), and C-22 (δ_C 73.4), one ketone group, and three sugar residues (two β-glucopyranosyl and one α-rhamnopyranosyl units) (Kuroda et al., 2004; Mimaki et al., 2001). The carbonyl carbon (δ_C 214.0) was determined to be C-12 by the appearance of the HMBC correlations of H-9/C-12, H-11a/C-12, and H-8/C-12. In the HMBC spectrum of **1**, the anomeric proton signals of the two glucosyl groups at δ 5.01 and δ 4.84 showed long-range correlations with the C-3 and C-16 carbons of the aglycone, respectively. The location of the rhamnosyl group was suggested to be at C-4′ of the glucose unit attached at C-3 of the aglycone by the HMBC correlation of the anomeric proton H-1‴ (δ 5.96) with C-4′ of the inner glucose. The stereochemistry of **1** could be established by the NOESY experiments. The NOESY correlations of H-17/H-14 could be observed, which indicated that the α-orientation of H-16. The *S*-configuration of C-22 was deduced from an agreement of its ^{1}H and ^{13}C NMR spectroscopic data with those of (22*S*)-16β-[(6-*O*-acetyl-β-D-glucopyranosyl) oxy]-22-hydroxy-3β-[(*O*-α-L-rhanmopyranosyl-(1 → 2)-*O*-[α-L-rhanmopyranosyl-(1 → 4)]-β-D-glucopyranosyl)oxy]-cholest-5-en-12-one (Yin et al., 2003). Therefore, the structure of dioseptemloside A (**1**) was determined as (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-22-hydroxy-3β-[(*O*-α-L-rhanmopyranosyl-(1 → 4)-β-D-glucopyranosyl)oxy]-cholest-5-en-12-one.

Dioseptemlosede B (**2**) analyzed for $C_{33}H_{54}O_9$ by HRESIMS (*m/z* 595.3745 $[M+H]^+$). Its spectroscopic data showed that it was a cholestane glycoside and had the same aglycone as **1**. The ^{1}H and ^{13}C NMR spectra, measured in MeOH-d_4 (Tables 1 and 2), of **2** exhibited signals of one β-glucopyranosyl unit [δ_H 4.20 (*d*, $J = 7.7$ Hz), δ_C 106.9],

Table 1
^{1}H NMR (400 MHz) spectroscopic data [δ (J Hz)] of dioseptemlosides A–H (**1**–**8**)

Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]
1a	1.77 (*m*)	1.76 (*m*)	1.79 (*m*)	1.78 (*m*)	1.82 (*m*)	1.79 (*m*)	1.81 (*m*)	1.79 (*m*)
1b	1.04 (*m*)	1.08 (*m*)	1.05 (*m*)	1.03 (*m*)	1.03 (*m*)	1.01 (*m*)	1.06 (*d*, 3.1)	1.10 (*m*)
2a	2.08 (*m*)	2.10 (*m*)	2.16 (*m*)	2.08 (*m*)	2.06 (*d*, 7.7)	2.19 (*m*)	2.18 (*m*)	2.15 (*m*)
2b	1.70 (*m*)	1.53 (*m*)	1.79 (*m*)	1.78 (*m*)	1.90 (*m*)	1.79 (*m*)	1.90 (*m*)	1.78 (*m*)
3	3.91 (*m*)	3.40 (*m*)	3.96 (*m*)	3.86 (*m*)	3.83 (*m*)	3.99 (*m*)	3.98 (*m*)	3.97 (*m*)
4a	2.78 (*m*)	2.30 (*m*)	2.78 (*dd*, 2.8, 13.3)	2.82 (*dd*, 2.1,13.1)	2.88 (*m*)	2.83 (*dd*, 2.4, 13.6)	2.93 (*dd*, 5.6, 12.8)	2.79 (*dd*, 2.4, 13.0)
4b	2.48 (*m*)	2.20 (*m*)	2.52 (*t*, 11.6)	2.58 (*m*)	2.56 (*m*)	2.54 (*m*)	2.81 (*t*, 12.2)	2.52 (*m*)
5								
6	5.29 (*br s*)	5.38 (*br s*)	5.40 (*br s*)	5.89 (*br s*)	5.86 (*br s*)	5.12 (*br s*)	5.76 (*br s*)	5.39 (*br s*)
7a	1.78 (*m*)	1.78 (*m*)	1.94 (*m*)	4.09 (*m*)	4.09 (*m*)	4.11 (*m*)	4.09 (*m*)	1.93 (*m*)
7b	1.40 (*m*)	1.47 (*m*)	1.60 (*m*)					1.57 (*m*)
8	1.70 (*m*)	1.95 (*m*)	1.84 (*m*)	1.77 (*m*)	1.75 (*m*)	1.90 (*m*)	1.87 (*m*)	1.68 (*m*)
9	1.40 (*m*)	1.42 (*m*)	0.99 (*m*)	1.68 (*m*)	1.70 (*m*)	1.28 (*m*)	1.18 (*dd*, 4.6,11.3)	0.97 (*m*)
10								
11a	2.77 (*m*)	2.82 t (12.5)	1.50 (2H, *m*)	1.59 (*m*)	1.58 (2H, *m*)	1.55 (2H, *m*)	1.54 (2H, *m*)	1.51 (2H, *m*)
11b	2.29 (*m*)	2.07 (*m*)		1.58 (*m*)				
12a			1.78 (*m*)	1.81 (*m*)	1.82 (*m*)	1.81 (*m*)	1.80 (*m*)	1.76 (*m*)
12b			1.18 (*m*)	1.30 (*m*)	1.30 (*m*)	1.23 (*t*, 7.1)	1.25 (*m*)	1.18 (*m*)
13								
14	1.24 (*m*)	1.19 (*m*)	1.15 (*m*)	2.14 (*m*)	2.15 (*m*)	1.45 (*m*)	1.43 (*m*)	1.16 (*m*)
15a	2.48 (*m*)	2.40 (*dd*, 6.2, 13.3)	2.09 (*m*)	2.61 (*m*)	2.64 (*m*)	2.93 (*m*)	2.90 (*m*)	2.14 (*m*)
15b	1.60 (*m*)	1.82 (*dd*, 4.1, 10.1)	1.55 (*m*)	1.65 (*m*)	1.66 (*m*)	2.10 (*m*)	2.09 (*m*)	1.57 (*m*)
16	4.60 (*m*)	4.18 (*m*)	4.65 (*m*)	4.72 (*t*, 7.1)	4.72 (*m*)	4.73 (*m*)	4.72 (*d*, 7.5)	4.66 (*m*)
17	3.09 (*dd*, 7.7, 10.8)	2.44 (*dd*, 7.8, 11.1)	1.09 (*m*)	1.96 (t, 8.0)	1.98 (*dd*, 6.6, 8.3)	1.91 (*dd*, 6.4, 8.0)	1.94 (*d*, 7.9)	1.91 (*m*)
18	1.39 (*s*)	1.31 (*s*)	0.92 (*s*)	1.00 (*s*)	0.99 (*s*)	0.98 (*s*)	0.96 (*s*)	0.93 (*s*)
19	1.03 (*s*)	1.15 (*s*)	1.00 (*s*)	1.00 (*s*)	1.13 (*s*)	0.97 (*s*)	1.09 (*s*)	0.98 (*s*)
20	2.62 (*m*)	2.06 (*m*)	2.08 (*m*)	2.07 (*m*)	2.09 (*d*, 6.7)	2.07 (*dd*, 6.7,13.6)	2.07 (*dd*, 7.1,13.5)	2.10 (*m*)
21	1.33 (*d*, 7.1)	0.80 (*d*, 7.2)	1.24 (*d*, 6.9)	1.23 (*d*, 6.6)	1.22 (*d*, 6.9)	1.24 (*d*, 7.1)	1.24 (*d*, 6.9)	1.26 (*d*, 7.0)
22	4.39 (*m*)	3.71 (*m*)						
23a	1.95 (2H, *m*)	1.43 (2H, *m*)	1.84 (2H, *m*)	1.77 (2H, *m*)	1.75 (2H, *m*)	1.76 (*m*, *m*)	1.75 (2H, *m*)	1.70 (*m*)
23b								2.52 (*m*)
24a	2.03 (*m*)	1.44 (*m*)	1.88 (2H, *m*)	1.65 (*m*)	1.75 (*m*)	1.65 (2H, *m*)	1.64 (2H, *m*)	2.16 (*m*)
24b	1.73 (*m*)	1.24 (*dd*, 6.0, 11.2)		1.64 (*m*)	1.66 (*d*, 5.7)			1.92 (*m*)
25	1.72 (*m*)	0.89 (*m*)	2.14 (*m*)	1.66 (*m*)	1.67 (*m*)	1.68 (*m*)	1.65 (*m*)	
26a	1.01 (*d*, 6.2)	0.90 (*d*, 6.4)	4.20 (*m*)	3.63 (*dd*, 2.5, 9.9)	3.63 (*d*, 9.4)	3.64 (*m*)	3.65 (*d*, 11.8)	4.01 (*d*, 11.4)
26b			3.97 (*m*)	3.55 (*t*, 10.0)	3.55 (*t*, 10.4)	3.60 (*m*)	3.58 (*t*, 10.5)	3.78 (*d*, 13.5)
27a	1.01 (*d*, 6.2)	0.90 (*d*, 6.4)	3.80 (*m*)	0.76 (*d*, 4.9)	0.76 (*d*, 5.3)	0.76 (*d*, 5.3)	0.77 (*d*, 4.9)	1.30 (*s*)
27b			3.73 (*m*)					
3-Glc								
1′	5.01 (*d*, 7.8)		5.33 (*d*, 6.8)	5.05 (*d*, 6.4)	4.94 (*d*, 8.0)	5.04 (*d*, 7.7)	4.99 (*d*, 7.2)	5.05 (*d*, 7.6)
2′	4.05 (*m*)		4.06 (*t*, 8.4)	4.05 (*t*, 8.4)	4.26 (*m*)	4.08 (*m*)	4.25 (*m*)	4.09 (*t*, 8.4)
3′	4.33 (*m*)		4.30 (*d*, 9.0)	4.30 (*t*, 9.2)	4.25 (*d*, 2.4)	4.30 (*m*)	4.25 (*m*)	4.33 (*m*)
4′	4.51 (*d*, 9.2)		4.54 (*t*, 9.3)	4.52 (*t*, 9.2)	4.42 (*m*)	4.43 (*t*, 9.4)	4.41 (*d*, 6.9)	4.58 (*t*, 9.3)
5′	3.80 (*d*, 8.4)		3.80 (*m*)	3.80 (*m*)	3.70 (*d*, 9.0)	3.81 (*d*, 9.2)	3.69 (*d*, 11.2)	3.81 (*d*, 9.4)
6′a	4.33 (*m*)		4.33 (*m*)	4.32 (*d*, 12.1)	4.29 (*m*)	4.35 (*d*, 12.1)	4.26 (*m*)	4.36 (*m*)
6′b	4.24 (*m*)		4.23 (*m*)	4.22 (*dd*, 3.6, 12.1)	4.16 (*dd*, 3.3, 11.9)	4.23 (*dd*, 3.6, 12.1)	4.14 (*dd*, 3.1, 12.4)	4.23 (*dd*, 3.0, 12.1)

(*continued on next page*)

Table 1 (*continued*)

Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]
2′-Rha								
1″					6.46 (*br s*)		6.43 (*br s*)	
2″					4.95 (*m*)		4.94 (*m*)	
3″					4.69 (*dd*, 3.0,5.9)		4.68 (*dd*, 3.3,9.2)	
4″					4.49 (*t*, 9.3)		4.49 (*t*, 9.4)	
5″					5.00 (dd, 3.3,9.5)		5.02 (*m*)	
6″					1.82 (*d*, 6.1)		1.81 (*d*, 6.4)	
4′-Rha								
1‴	5.96 (*br s*)		5.98 (*br s*)	5.97 (*br s*)	5.91 (*br s*)	5.97 (*br s*)	5.90 (*br s*)	5.96 (*br s*)
2‴	4.77 (br *s*)		4.79 (*br s*)	4.79 (*br s*)	4.79 (*br s*)	4.78 (*br s*)	4.77 (*br s*)	4.81 (*br s*)
3‴	4.65 (*dd*, 2.8, 9.2)		4.67 (*m*)	4.66 (*dd*, 3.4, 9.2)	4.62 (*dd*, 3.3, 10.2)	4.65 (*dd*, 3.5, 9.3)	4.61 (*dd*, 3.5, 9.2)	4.69 (*dd*, 3.1,9.2)
4‴	4.41 (*m*)		4.43 (*t*, 9.3)	4.43 (*t*, 9.4)	4.44 (*t*, 9.4)	4.43 (*t*, 9.4)	4.43 (*t*, 7.1)	4.45 (*t*, 9.5)
5‴	5.07 (*dd*, 2.2, 9.4)		5.09 (*dd*, 6.3, 9.5)	5.08 (*dd*, 6.4, 9.5)	4.96 (*m*)	5.08 (*dd*, 6.1, 9.5)	4.96 (*dd*, 6.1, 9.5)	5.15 (*dd*, 6.2, 9.2)
6‴	1.80 (*d*, 6.1)		1.80 (*d*, 6.0)	1.80 (*d*, 6.2)	1.82 (*d*, 6.1)	1.80 (*d*, 6.1)	1.81 (*d*, 6.2)	1.82 (*d*, 6.1)
16-Glc								
1⁗	4.84 (*d*, 7.7)	4.20 (*d*, 7.7)						
2⁗	4.10 (*dd*, 7.7, 8.9)	3.16 (*dd*, 8.0, 16.5)						
3⁗	4.24 (*m*)	3.30 (*m*)						
4⁗	4.36 (*dd*, 8.9, 9.0)	3.32 (*m*)						
5⁗	3.92 (*m*)	3.24 (*m*)						
6⁗a	4.57 (*dd*, 2.5, 11.2)	3.84 (*dd*, 2.1, 11.7)						
6⁗b	4.05 (*dd*, 4.5,11.2)	3.67 (*dd*, 5.0,11.7)						

[a] Measured in pyridine-d_5.

[b] Measured in MeOH-d_4.

Table 2
^{13}C NMR (100 MHz) spectroscopic data (δ) of dioseptemlosides A–H (**1**–**8**)

Position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]
1	37.2	38.5	37.5	37.2	37.2	37.3	37.4	37.5
2	30.1	32.7	30.3	30.1	30.1	30.3	30.3	30.3
3	78.0	72.4	78.3	78.0	78.0	78.2	78.0	78.3
4	39.1	43.0	39.4	39.3	39.0	39.0	38.6	39.4
5	140.7	142.4	141.0	144.0	143.9	141.5	141.6	140.9
6	121.9	122.6	121.8	126.0	126.0	128.7	128.7	121.8
7	31.7	32.3	32.4	64.6	64.6	72.6	72.7	32.3
8	32.0	33.5	31.7	38.0	38.0	40.8	40.9	31.7
9	54.6	56.7	50.4	42.7	42.6	48.7	48.7	50.3
10	38.0	39.0	37.1	37.9	38.0	37.1	37.2	37.1
11	38.2	39.2	21.2	21.0	21.0	21.3	21.3	21.2
12	214.0	218.0	40.0	39.8	39.8	40.0	40.0	39.9
13	57.4	58.6	40.5	40.3	40.4	41.1	41.1	40.5
14	57.3	59.1	56.7	49.9	49.9	56.5	56.5	56.7
15	37.2	38.0	32.3	32.4	32.4	35.3	35.3	32.3
16	81.9	82.3	81.2	81.4	81.5	81.7	81.7	81.4
17	49.4	50.2	63.0	63.1	63.2	62.6	62.9	63.0
18	13.5	13.7	16.4	16.4	16.4	16.5	16.5	16.4
19	19.0	19.7	19.5	18.3	18.3	19.0	19.0	19.5
20	35.5	35.9	42.9	42.1	42.2	42.2	42.2	42.1
21	13.3	12.8	15.1	15.1	15.1	15.2	15.2	15.2
22	73.4	74.5	109.8	109.3	109.3	109.3	109.3	109.7
23	33.8	34.4	31.6	31.9	32.0	31.9	32.0	27.9
24	36.9	37.4	24.1	29.4	29.4	29.3	29.4	33.9
25	29.0	29.9	39.3	30.7	30.7	30.7	30.7	66.0
26	23.1	23.4	64.2	66.9	66.9	66.9	66.9	69.9
27	23.1	23.4	64.5	17.4	17.4	17.4	17.4	27.0
3-Glc								
1′	102.5		102.5	102.6	100.4	102.6	100.4	102.5
2′	75.6		75.6	75.6	77.6	75.6	77.9	75.6
3′	76.8		76.8	76.8	77.9	76.8	77.9	76.8
4′	78.5		78.4	78.5	78.8	78.4	78.7	78.3
5′	77.2		77.2	77.2	77.0	77.2	77.0	77.2
6′	61.6		61.6	61.7	61.4	61.6	61.4	61.6
2′-Rha								
1″					101.8		101.9	
2″					72.6		72.6	
3″					72.9		73.0	
4″					74.1		74.1	
5″					69.5		69.5	
6″					18.5		18.5	
4′-Rha								
1‴	102.9		102.7	102.8	102.93	102.8	102.8	102.8
2‴	72.7		72.7	72.7	72.65	72.7	72.6	72.7
3‴	72.9		72.9	72.9	72.87	72.9	72.8	72.9
4‴	74.1		74.1	74.1	73.96	74.1	73.9	74.1
5‴	70.5		70.4	70.4	70.52	70.4	70.4	70.4
6‴	18.6		18.6	18.6	18.69	18.6	18.7	18.6
16-Glc								
1⁗	107.1	106.9						
2⁗	75.7	75.7						
3⁗	78.9	78.7						
4⁗	71.7	71.9						
5⁗	78.3	78.0						
6⁗	62.9	63.1						

[a] Measured in pyridine-d_5.
[b] Measured in MeOH-d_4.

one ketone carbon (δ_C 218.0), along with resonances for five steroidal methyl groups at δ_H 1.31 (*s*), 1.15 (*s*), 0.90 (*d*, $J = 6.4$ Hz), 0.90 (*d*, $J = 6.4$ Hz), and 0.80 (*d*, $J = 7.2$ Hz). In the HMBC spectrum of **2**, the anomeric proton signal at δ_H 4.20 (H-1⁗) showed a long-range correlation with C-16 (δ 82.3) of the aglycone. The stereochemistry of **2** was determined by a NOESY experiment. Thus, the structure of **2** was (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxy-cholest-5-en-12-one.

Dioseptemloside C (**3**) was obtained as an amorphous solid. Its molecular formula was deduced to be $C_{39}H_{62}O_{13}$ from the HRESIMS data (*m/z* 739.4165 $[M+H]^+$). The ^{1}H NMR spectroscopic data (Tables 1 and 2) of **3** indicated the presence of two anomeric proton signals at δ_H 5.98 (*br s*) and 5.33 (*d*, $J = 6.8$ Hz), two angular methyl groups [δ_H 1.00 (*s*) and 0.92 (*s*)], a secondary methyl group [δ_H 1.24 (*d*, $J = 6.9$ Hz)], a hydroxymethyl group (δ_H 3.80 and 3.73), and an olefinic group [δ_H 5.40 (*br s*)]. The ^{13}C NMR spectrum showed a total of 27 carbons arising from the aglycone moiety. Furthermore, a quaternary carbon signal at δ 109.8 with oxygen atoms and olefinic carbon resonances at δ 141.0 and 121.8 suggested that **3** possessed a $\Delta^{5,6}$-spirotanol skeleton. Comparison of the ^{1}H and ^{13}C NMR spectra of the aglycone moiety of **3** with those of diosgenin revealed that the signals were similar except for the presence of a hydroxymethyl carbon (δ 64.5) and the lack of the C-27 methyl group on **3** (Agrawal et al., 1985; Mimaki and Sashida, 1990; Yang et al., 2005). In the HMBC spectrum of **3**, the long-range correlations of H-25/C-27, H-26b/C-27, and H-27a/C-24 also suggested that the oxygenated carbon was at the C-27 of the aglycone of **3**. The HMBC spectrum also showed correlations of H-1‴ (δ 5.98) of rhamnose with C-4′ (δ 78.4) of glucose as well as the anomeric carbon at δ 102.5 (C-1′) with H-3 (δ 3.96) of the aglycone. The *R* configuration of C-25 could be deduced from the NOESY correlation between H-25 (δ 2.14) with H-23b (δ 1.84). Thus, the structure of **3** could be determined as (25*R*)-27-hydroxy-spirost-5-en-3β-yl-*O*-α-L-rhanmopyranosyl-(1 → 4)-β-D-glucopyranoside.

Dioseptemloside D (**4**) was obtained as an amorphous solid. Its molecular formula was deduced to be $C_{39}H_{62}O_{13}$ from the HRESIMS data (*m/z* 739.4155 $[M+H]^+$). The ^{1}H NMR spectrum of **4** exhibited signals attributed to two anomeric protons [δ 5.97 (*br s*) and 5.50 (*d*, $J = 6.4$ Hz)], an olefinic proton [δ 5.89 (*br s*)], two angular methyl protons (both δ 1.00), and two secondary methyl protons [δ 1.23 (*d*, $J = 6.6$ Hz) and 0.76 (*d*, $J = 4.9$ Hz)]. The ^{13}C NMR spectrum showed a quaternary carbon at δ 109.3 and olefinic carbon signals at δ 144.0 and 126.0. The above data assumed that **4** consisted of a $\Delta^{5,6}$-spirotanol skeleton and the same disaccharide unit as **3**. The substituted position of the hydroxyl group was different between **3** and **4**. The hydroxyl group was suggested to be at C-7 deducing from the ^{1}H–^{1}H COSY correlations of the olefinic proton signal at δ 5.89 (H-6) with the proton linked to an oxygenated carbon at δ 4.09. The location of 7-OH also could be confirmed by the HMBC correlations of H-6/C-7, H-7/C-5, and H-7/C-9. The α-orientation of 7-hydroxyl group could be determined by the resonance of C-7 (δ 64.6) (Pettit et al., 2005), while the signal for

C-7 would be at δ 72.6 of the 7β-isomer (Blunden et al., 1990). Furthermore, in the ^{13}C NMR spectrum of **4**, the signals of C-9 (δ 42.7) and C-14 (δ 49.9), were up-field of corresponding signals in the spectrum of diosgenin (δ 50.1 and δ 56.5, respectively) (Agrawal et al., 1985; Chen et al., 1995) due to the γ-gauche interactions. This provides additional confirmation of the α-orientation of C_7-OH. The HMBC correlations of C-4′ (δ 78.5) of glucose with the anomeric proton H-1‴ (δ 5.97) of rhamnose, and C-1′ (δ 102.6) of glucose with H-3 (δ 3.86) of the aglycone were also observed. Thus, the structure of dioseptemloside D (**4**) was proposed to be (25*R*)-7α-hydroxy-spirost-5-en-3β-yl-*O*-α-L-rhanmopyranosyl-(1 → 4)-β-D-glucopyranoside.

Dioseptemloside E (**5**) was obtained as an amorphous solid. Its molecular formula was deduced to be $C_{45}H_{72}O_{17}$ by the HRESIMS (*m/z* 907.4785 $[M+Na]^+$). Comparison of the ^{1}H and ^{13}C NMR spectra of **5** with those of **4** showed their considerable structural similarity. The differences consisted only in the signals of one more rhamnopyranosyl unit appearance in **5**. The HMBC spectrum showed the long-range correlations of C-2′ (δ 77.6) of glucose with H-1″ (δ 6.46) of one rhamnose, C-4′ (δ 78.8) of the glucose with H-1‴ (δ 5.91) of the other rhamnose, and C-3 (δ 78.0) of the aglycone with H-1′ (δ 4.94, *d*, $J = 8.0$) of the glucose. The above data allowed the structural assignment of **5** as (25*R*)-7α-hydroxy-spirost-5-en-3β-yl-*O*-α-L-rhanmopyranosyl-(1 → 2)-*O*-[α-L-rhanmopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Dioseptemloside F (**6**) (an amorphous solid) was a spirostane glycoside with the molecular formula $C_{39}H_{62}O_{13}$ deduced from the HRESIMS (*m/z* 761.4169 $[M+Na]^+$). Analysis of the spectroscopic data of **6** implied that it was closely related to that of **4**, except for the ^{13}C NMR signal at δ 72.6 which could be assigned to C-7 by the COSY and HMBC spectra. The β-orientation of 7-hydroxyl group of **6** could be determined by the resonance of C-7 (δ 72.6) (Blunden et al., 1990), which could be further confirmed by the NOESY correlations of H-7/H-9 and H-7/H-14. Therefore, the structure of **6** was shown to be (25*R*)-7β-hydroxy-spirost-5-en-3β-yl-*O*-α-L-rhanmopyranosyl-(1 → 4)-β-D-glucopyranoside.

Dioseptemloside G (**7**) was obtained as an amorphous solid. Its molecular formula was deduced to be $C_{45}H_{72}O_{17}$ from the HRESIMS (*m/z* 885.4738 $[M+H]^+$). Analysis of the ^{1}H and ^{13}C NMR spectra of **7** and comparison with those of **6** indicated that **7** differed from **6** in the presence of one more rhamnopyranosyl unit (the anomeric proton signal at δ 6.43). Thus, **7** contained two rhamnose, one glucose, and the aglycone assigned as 3β,7β-dihydroxyl-(25*R*)-spirost-5-ene (Blunden et al., 1990). The HMBC correlations of C-2′ (δ 77.9)/H-1″ (δ 6.43), C-4′ (δ 78.7)/H-1‴ (δ 5.90) and C-3 (δ 78.0)/H-1′ (δ 4.99) also could be observed. The above data led to the full structure of **7** as (25*R*)-7β-hydroxy-spirost-5-en-3β-yl-*O*-α-L-rhanmopyranosyl-(1 → 2)-*O*-[α-L-rhanmopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Dioseptemloside H (**8**) was obtained as an amorphous solid. Its molecular formula was deduced to be $C_{39}H_{62}O_{13}$ from the HRESIMS (*m/z* 739.4161 $[M+H]^+$). The ^{1}H and ^{13}C NMR spectra of **8** exhibited signals of a glucose and a rhamnose, whose anomeric protons were observed at δ_H 5.05 (*d*, $J = 7.6$ Hz) and 5.96 (*br s*), respectively, together with an aglycone moiety which was closely related to diosgenin (Agrawal et al., 1985; Chen et al., 1995). The ^{13}C NMR spectra of **8** showed the downfield shifts at Me-27 (δ 27.0), C-25 (δ 66.0) and C-24 (δ 33.9) related to diosgenin and indicated that there was a hydroxyl group substituted on the C-25 of **8**. This could be further confirmed by the long-range correlations of H-26a/C-25, H-27/C-25, and H-24b/C-25 in the HMBC spectrum of **8**. The HMBC spectrum also showed the correlations of C-4′ (78.3) of glucose and H-1‴ (δ 5.96) of rhamnose, and C-3 (δ 78.3) of the aglycone with H-1′ (5.05, *d*, $J = 7.6$ Hz) of glucose. The configuration of the hydroxyl group at C-25 could be deduced from the ^{13}C NMR spectrum. The large (−4.1 ppm) γ_g-effect displayed by C-23 was more consistent with an axial hydroxyl group at C-25 instead of an axial methyl group. The latter would show a smaller γ_g-effect, e.g. −1.5 ppm, as is the case in (25*R*)-isonuatigenin. Comparison the chemical shift at C-25 (δ 66.0) of **8** with the corresponding values reported for (25*R*)-isonuatigenin and (25*S*)-isonuatigenin (δ 81.6 and 65.1, respectively) also revealed the axial hydroxyl group, i.e. *S*-configuration at C-25 (Agrawal et al., 1985; Faini et al., 1984). From the above evidence, the C-25 of **8** could be clearly indicated to be *S*-configuration. Thus, the structure of **8** was defined as (25*S*)-25-hydroxy-spirost-5-en-3β-yl-*O*-α-L-rhanmopyranosyl-(1 → 4)-β-D-glucopyranoside.

All of these compounds were tested against growth of three tumor cell lines, i.e., A549 (human lung carcinoma cell), BGC-823 (human gastric cancer cell), and HGC-27 (human gastric carcinoma cell), following a standard MTT assay with dioscin as a positive control (Mosmann, 1983). However, none displayed considerable inhibitory activities at a concentration of 10 μM.

3. Concluding remarks

In conclusion, as a result of this investigation, the structures of eight new compounds (**1**–**8**) from *D. septemloba* were identified, and among these, spirostane aglycones containing hydroxyl group at C-7 were reported in the family Dioscoreaceae for the first time. This type of compound was reported previously only from the genus *Sansevieria* (Agavaceae). The MTT assay also indicated that all of the compounds were inactive against growth of the tumor cell lines. Since the title plant has been used for the treatments of urethral infection, renal infection, and rheumatism in traditional Chinese Medicines for hundreds of years, further and systematic pharmacological studies on the chemical substances from *D. septemloba* should be carried out in the future.

4. Experimental

4.1. General

Optical rotations were taken in MeOH on a Perkin–Elmer PE 241 polarimeter. The 1D and 2D NMR spectra were acquired on a Bruker AV400 spectrometer. The HRESIMS were obtained on an FTMS-7 spectrometer (Bruker Daltonic). Open column chromatography (CC) was carried out using D101 macroporous resin (Tianjin Pesticide Co., China), silica gel (200–300 mesh, Qingdao Marine Chemical Co., China), ODS-A (50 μm, YMC, Kyoto, Japan), and Sephadex LH-20 (GE Healthcare, Sweden) as stationary phases. TLC was performed on $HSGF_{254}$ (0.2 mm, Qingdao Marine Chemical Co., China) or RP-18 F_{254} (0.25 mm, Merck) plates.

4.2. Plant material

The dried rhizomes of *D. septemloba* Thunb. (Dioscoreaceae) were collected from Lianyungang, Jiangsu Province, PR China, in August 2006, and was identified by Prof. Xue-Hua Song (The Herbarium of China Pharmaceutical University, Nanjing, PR China). A voucher specimen (No. 20061010) was deposited in the State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, PR China.

4.3. Extraction and isolation

Dried rhizomes of *D. septemloba* (10 kg) were extracted with 80% EtOH for three times. The combined extracts were filtered, concentrated, and applied to a D101 macroporous resin column (40–60 mesh, 60 kg) eluted with 95% EtOH/H_2O mixtures to yield a crude saponin mixture (240 g). The latter was subjected to silica gel CC with elution using with gradient of $CHCl_3$–MeOH (99:1, 9:1, 8:2, 7:3, 6:4, 1:1, 1:2, 1:0); TLC analysis was carried out with sulfuric vanillin and heating at 150 °C, which indicates the presence of 25 distinct fractions. Fraction 6 (3.0 g) was fractionated by MPLC (YMC, ODS-A, RP-18, 400 mm × 30 mm, flow rate 10 ml min^{-1}) using MeOH–H_2O (60:40, 65:35 and 70:30, v/v) as eluants, to give sub-fractions 1–12. Sub-fraction 6 (92 mg) was further subjected to silica gel CC with $CHCl_3$–MeOH (100:6) as eluent, followed by purification on Sephadex LH-20 (MeOH) to afford **2** (51 mg). Fractions 11 and 12 (5.0 g) were subjected to silica gel CC using gradients of CH_2Cl_2–MeOH (100:5, 100:7, 100:9, 100:12 and 100:15) to give 12 fractions. Sub-fraction 8 (280 mg) was fractionated by MPLC (YMC, ODS-A, RP-18, 400 mm × 30 mm, flow rate 10 ml min^{-1}) using MeOH–H_2O (80:20, v/v) as eluants further was purified on Sephadex LH-20 (MeOH) to give **8** (5 mg). Sub-fraction 10 (310 mg) were purified by using MPLC (YMC, ODS-A, RP-18, 400 mm × 30 mm, flow rate 10 ml min^{-1}) with gradient elutions of MeOH–H_2O (80:20, 90:10 and 100:0, v/v) to afford **3** (9 mg), **4** (20 mg), and **6** (11 mg). Fraction 16 (5.0 g) was subjected to silica gel CC eluting with gradients of CH_2Cl_2–MeOH–H_2O (85:15:1, 80:20:1, 77:23:1, 74:26:1.5, v/v) to give sub-fractions 1–7. Sub-fraction 5 (1.2 g) was subjected to MPLC (YMC, ODS-A, RP-18, 400 mm × 30 mm, flow rate 10 ml min^{-1}) eluting with gradients of MeOH–H_2O (50:50, 60:40, 70:30, 80:20 and 90:10, v/v), with fractions of interest further purified using Sephadex LH-20 (MeOH) to afford **5** (20 mg) and **7** (30 mg). Sub-fraction 6 (500 mg) was fractionated by MPLC (YMC, ODS-A, RP-18, 400 mm × 30 mm, flow rate 10 ml min^{-1}) eluting with gradients of MeOH–H_2O (60:40, 70:30 and 80:20, v/v), followed by the purification on silica gel CC (CH_2Cl_2–MeOH–H_2O, 80:20:1) and Sephadex LH-20 (MeOH) to give **1** (35 mg).

4.4. Dioseptemloside A (*1*)

White amorphous solid; $[\alpha]_D^{25}$ −60.5 (*c* 0.56, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 903.4841 $[M+H]^+$ (calcd. for $C_{45}H_{75}O_{18}$, 903.4875).

4.5. Dioseptemloside B (*2*)

White amorphous solid; $[\alpha]_D^{25}$ −49.2 (*c* 0.25, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 595.3745 $[M+H]^+$ (calcd. for $C_{33}H_{55}O_9$, 595.3767).

4.6. Dioseptemloside C (*3*)

White amorphous solid; $[\alpha]_D^{25}$ −98.0 (*c* 0.51, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 739.4165 $[M+H]^+$ (calcd. for $C_{39}H_{63}O_{13}$, 739.4190).

4.7. Dioseptemloside D (*4*)

White amorphous solid; $[\alpha]_D^{25}$ −103.6 (*c* 0.62, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 739.4155 $[M+H]^+$ (calcd. for $C_{39}H_{63}O_{13}$, 739.4190).

4.8. Dioseptemloside E (*5*)

White amorphous solid; $[\alpha]_D^{25}$ −101.5 (*c* 0.45, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 907.4785 $[M+Na]^+$ (calcd. for $C_{45}H_{72}O_{17}Na$, 907.4769).

4.9. Dioseptemloside F (*6*)

White amorphous solid; $[\alpha]_D^{25}$ −92.7 (*c* 0.52, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 761.4169 $[M+Na]^+$ (calcd. for $C_{39}H_{62}O_{13}Na$, 761.4190).

4.10. Dioseptemloside G (7)

White amorphous solid; $[\alpha]_D^{25}$ −90.9 (*c* 0.72, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 885.4738 $[M+H]^+$ (calcd. for $C_{45}H_{73}O_{17}$, 885.4769).

4.11. Dioseptemloside H (8)

White amorphous solid; $[\alpha]_D^{25}$ −92.5 (*c* 0.68, MeOH); For ^1H and ^{13}C NMR spectroscopic assignments, see Tables 1 and 2; HRESIMS *m/z*: 739.4161 $[M+H]^+$ (calcd. for $C_{39}H_{63}O_{13}$, 739.4190).

4.12. Acid hydrolysis and sugar analysis

Compounds **1**–**8** (each 3 mg) were individually hydrolyzed with 4 M TFA (0.5 ml) at 120 °C for 1 h, whereafter the solvent was evaporated with a stream of N_2. Each of the crude products were dissolved in H_2O (0.5 ml) and the whole neutralized with NH_4OH. To each crude mixtures was added (*S*)-α-methylbenzylamine (5 mg) and $Na[BH_3CN]$ (6 mg) in EtOH (0.5 ml) and the reaction mixtures each heated to 40 °C for 5 h. Excess $Na[BH_3CN]$ was quenched with a few drops of HOAc and the boric acid formed removed by co-distillation with 10% HOAc in MeOH (3 × 0.5 ml) and MeOH (3 × 0.5 ml). Each of the reaction mixtures were acetylated with Ac_2O–pyridine (1:1, 1.0 ml) at 120 °C for 30 min and analysed by GC–MS using an Agilent 6890 Plus GC with 7683 Autoinjector and Agilent 5973 Network MSD detector apparatus, employing an HP-5MS capillary column of 30 m × 0.25 mm, 0.25 μm film thickness, He gas as carrier gas at 1.0 ml min^{-1}, inlet pressure of 12.8 psi, using a temperature program, from 140 °C (1 min) to 230 °C at 3 °C min^{-1}. The diasteromeric alditol derivative of standard sugar of glucose and rhamnose was used as reference (Liu et al., 2006, 2007). The absolute configurations of the sugar residues were determined to be D-glucose (t_R 19.26 min) and L-rhamnose (t_R 15.10 min).

4.13. MTT colorimetric assay

Compounds were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C, protected from light. Human-derived cell lines (A549, BGC-823 and HGC-27) 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. Cells were seeded into 96-well microtiter plates at a density of 5000 per well and allowed 24 h to adhere before drugs were introduced. Serial drug dilutions were prepared in medium immediately prior to each assay. At the time of drug addition (parallel triplicate wells were set) and following 48 h of exposure, MTT was added to each well and reduced by viable cells to an insoluble formazan product. Well contents were aspirated and formazan solubilized by addition of DMSO. Absorbance was read on a systems plate reader at 550 nm as a measure of cell viability. Thus, cell growth or drug toxicity was determined.

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