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Two novel furostanol saponins from the rhizomes of *Dioscorea panthaica* Prain et Burkill and their cytotoxic activity

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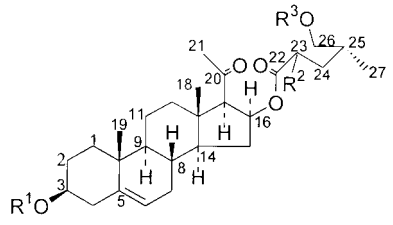
Abstract—Dioscoresides A and B were isolated from the rhizomes of *Dioscorea panthaica* and shown to possess a novel furostanol skeleton. Their structures were fully elucidated as 3-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-26-*O*-β-20, 22-*seco*-D-glucopyranosyl-25 (*R*)-furosta-5-en-20, 22-dione-3β, 26-diol and 3-*O*-[bis-α-L-rhamnopyranosyl-(1→4 and 1→2)-β-D-glucopyranosyl]-26-*O*-β-D-glucopyranosyl-20, 22-*seco*-25 (*R*)-furosta-5-en-20, 22-dione-3β, 23(*S*), 26-triol by spectroscopic techniques and chemical means. These compounds exhibited cytotoxic activity tested in vitro on A375-S2, L929 and Hela cell lines.

1. Introduction

The medicinal plant *Dioscorea panthaica* is widely distributed in Yunnan, Sichuan, Guizhou and Hunan provinces of People's Republic of China. Its rhizomes have long been known in traditional Chinese medicine as a saponin-rich herbal medicine used for the treatment of gastropathy, anthrax, rheumatic heart disease and rheumarthritis.[1] In the course of our screening for biologically active products from *Dioscorea panthaica*, two new furostanol saponins, Dioscoresides A (**1**) and B (**2**), along with five known saponins, progenin II (**3**), progenin III (**4**), dioscin (**5**), gracillin (**6**) and pseudo-protodioscin (**7**)[2–6] were isolated from the rhizomes of *Dioscorea panthaica*. The structures of **1** and **2** were assigned by one- and two-dimensional NMR techniques (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, NOESY) and chemical evidence. In this paper, we describe their isolation, structural elucidation and cytotoxic activity.

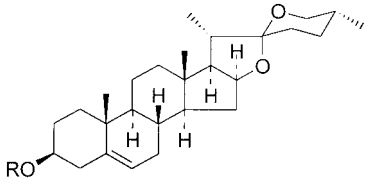
2. Results and discussion

An ethanolic extract of the rhizomes of *Dioscorea panthaica* was processed as described in the experimental section to afford two novel furostanol saponins **1** and **2**.



1 R^1 = β-D-Glc (3) / α-L-Rha (2) → β-D-Glc-, R^2 = H, R^3 = β-D-Glc-

2 R^1 = α-L-Rha (4), α-L-Rha (2) → β-D-Glc-, R^2 = OH, R^3 = β-D-Glc-



3 R = α-L-Rha (4) → β-D-Glc-

4 R = α-L-Rha (2) → β-D-Glc-

5 R = α-L-Rha (4), α-L-Rha (2) → β-D-Glc-

6 R = β-D-Glc (3), α-L-Rha (2) → β-D-Glc-

Keywords: *Dioscorea panthaica*; furostanol saponins; cytotoxic activity.
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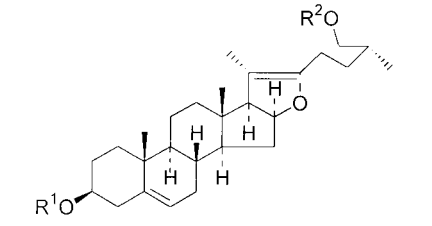
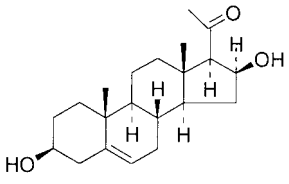
Table 1. ^{13}C NMR spectral data for compounds **1**, **2**, **7** (in C_5D_5N) and **1a** (in $CDCl_3$)

Position	1	2	7	1a	Position	1	2	7
1	37.5(t)	37.5(t)	37.6(t)	37.1(t)	Sugar part			
2	30.1(t)	30.2(t)	30.3(t)	27.8(t)	Glc (inner)			
3	77.6(d)	78.0(d)	78.1(d)	73.9(d)	1	99.9(d)	100.3(d)	100.4(d)
4	38.7(t)	38.9(t)	39.1(t)	38.2(t)	2	77.0(d)	78.2(d)	78.6(d)
5	140.9(*S*)	141.0(*S*)	140.9(*S*)	139.8(*S*)	3	89.6(d)	76.9(d)	76.9(d)
6	121.6(d)	121.6(d)	121.9(d)	122.3(d)	4	69.6(d)	78.6(d)	78.7(d)
7	31.9(t)	31.9(t)	32.5(t)	31.9(t)	5	77.9(d)	77.8(d)	77.8(d)
8	30.9(d)	30.8(d)	31.6(d)	31.4(d)	6	61.3(t)	61.3(t)	61.4(t)
9	50.4(d)	50.4(d)	50.4(d)	50.0(d)	Rha (1→2)			
10	37.1(*S*)	37.1(*S*)	37.2(*S*)	36.7(*S*)	1	102.2(d)	102.1(d)	102.0(d)
11	20.6(t)	20.6(t)	21.4(t)	21.1(t)	2	72.5(d)	72.6(d)	72.6(d)
12	38.1(t)	38.6(t)	39.8(t)	38.9(t)	3	72.8(d)	72.8(d)	72.9(d)
13	42.3(*S*)	42.3(*S*)	43.5(*S*)	44.0(*S*)	4	74.1(d)	74.2(d)	74.2(d)
14	54.1(d)	54.1(d)	55.2(d)	56.9(d)	5	69.6(d)	69.6((d)	69.6(d)
15	32.3(t)	32.2(t)	34.6(t)	32.8(t)	6	18.7(q)	18.7(q)	18.7(q)
16	74.1(d)	74.1(d)	84.6(d)	74.3(d)	Rha (1→4)			
17	66.8(d)	66.8(d)	64.6(d)	67.3(d)	1		102.9(d)	102.9(d)
18	13.8(q)	13.8(q)	14.2(q)	13.7(q)	2		72.6(d)	72.6(d)
19	19.4(q)	19.4(q)	19.5(q)	19.3(q)	3		72.8(d)	72.8(d)
20	205.5(*S*)	205.4(*S*)	103.7(*S*)	208.6(*S*)	4		74.0(d)	73.9(d)
21	30.4(q)	30.3(q)	11.9(q)	21.3(q)	5		70.5(d)	70.5(d)
22	173.3(*S*)	175.3(*S*)	152.5(*S*)		6		18.5(q)	18.5(q)
23	35.5(t)	69.4(d)	33.6(t)		Glc (1→3)			
24	29.0(t)	38.1(t)	23.8(t)		1	104.6(d)		
25	33.4(d)	30.9(d)	31.6(d)		2	75.0(d)		
26	74.7(t)	75.6(t)	75.0(t)		3	78.5(d)		
27	16.9(q)	16.6(q)	17.4(q)		4	71.5(d)		
					5	78.0(d)		
					6	62.4(t)		
					26-Glc			
					1	104.9(d)	105.0(d)	104.9(d)
					2	75.2(d)	75.1(d)	75.2(d)
					3	78.7(d)	78.5(d)	78.6(d)
					4	71.7(d)	71.7(d)	71.8(d)
					5	78.0(d)	78.0(d)	78.0(d)
					6	62.8(t)	62.9(t)	62.9(t)



7 R^1 = α-L-Rha (4→2) α-L-Rha, R^2 = β-D-Glc-



1a

Compound **1** was isolated as a white amorphous solid, mp 178–180°C (dec.), $[\alpha]_D^{25}=-50.2$ (pyridine, *c* 0.003). High resolution FAB-MS indicated the molecular formula $C_{51}H_{82}O_{24}$ (found *m/z* 1078.5184; calcd *m/z* 1078.5196), and this was supported by the ^1H NMR, ^{13}C NMR and DEPT spectra (Table 1). The ESI-MS spectrum showed *m/z* 1101 $(M+Na)^+$, 1079 $(M+H)^+$, 1077 $(M-H)^-$, 931 $(M-H-Rha)^-$, 769 $(M-H-Rha-Glc)^-$. The IR spectrum showed characteristic absorptions for hydroxyl (3417 cm^{-1}), carbonyl ester (1735 cm^{-1}), carbonyl group (1715 cm^{-1}), and a glycosidic linkage (1000–1100 cm^{-1}). Its spectral features and physicochemical properties suggested **1** to be a furostanol saponin. Among the 51 carbon signals in the ^{13}C NMR spectrum, 27 signals were assigned to the aglycone (see Table 1); the remaining 24 signals were indicative of the presence of four hexoses, in good agreement with the four anomeric signals appearing at δ 6.38 (br s), 5.11 (d, J=7.0 Hz), 4.94 (d, J=7.8 Hz), 4.79 (d, J=7.2 Hz) in the ^1H NMR spectrum and the four anomeric carbons observed at δ 104.9, 104.6, 102.2 and 99.9 in the ^{13}C NMR spectrum. The methyl carbon signal at δ 18.7 and proton signal at δ 1.73 (d, J=6.0 Hz) indicated that compound **1** bears one deoxy sugar. When **1** was submitted to acid hydrolysis with 1 M hydrochloric acid in dioxane–H_2O (1:1), it was hydrolysed to yield **1a** identified as 16 β- hydroxypregnenolone based on the NMR and MS data.[7] The monosaccharides of the acidic hydrolysate of **1** were identified as glucose and rhamnose based on the GLC analysis.

The ^1H NMR spectrum revealed the presence of four methyl groups on the aglycone [δ 0.88 (3H, d, J=6.3 Hz, CH_3-27), 1.04 (3H, s, CH_3-19), 1.20 (3H, s, CH_3-18), 2.11 (3H, s, CH_3-21)], and one olefinic methine group [δ 5.30 (1H, br s, H-6)]. The resonances of the protons and carbons (C-24, C-25, C-26, and C-27) around the C-25 centre and the $^3J_{HH}$

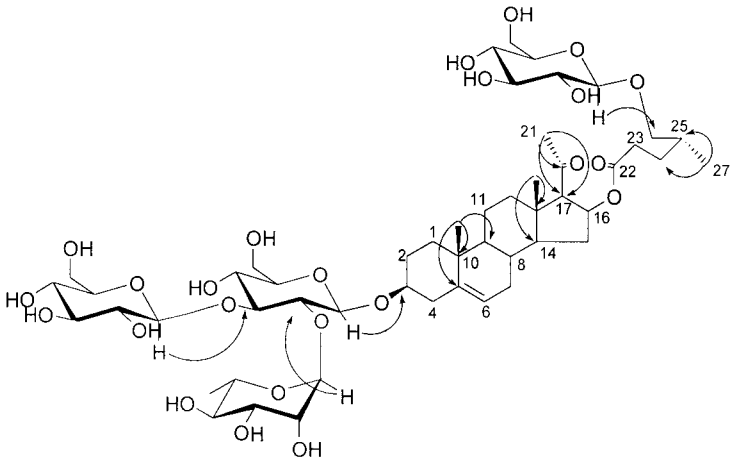
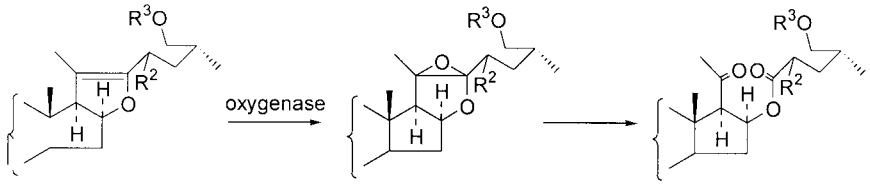


Figure 1.

values (10.5, 3.5 Hz) between H-25 and H-26 provided the evidence for the C-25 *R* configuration of **1** as described in the previous report.[8,9] Comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with that of pseudo-protodioscin (**7**) indicated the same furostanol skeleton with rings A, B, C, D, and the difference between them was in ring E. A feature differing from that of **7** was the substitution of the double bond between C-20 and C-22 in **7** by the carbonyl groups [δ 205.5, C-20; 173.3, C-22] in **1**. The HMBC spectrum (Fig. 1) indicated long-range correlations between the methyl protons (δ 2.11, CH_3-21) and the carbon signals (δ 205.5, C-20; 66.8, C-17), and between the methine group (δ 2.47, H-17) and the carbon signals (δ 13.8, C-18; 205.5, C-20). These data showed that the aglycone was a derivative of **7** with the double bond between C-20 and C-22 changed into two carbonyl groups. The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined by the analysis of a combination of DEPT, COSY, HMQC, HMBC and NOESY NMR spectra. The ^{13}C NMR spectroscopic data for the sugar moieties indicated that all the monosaccharides were in pyranose forms. The α-anomeric configuration for the rhamnose was determined by its C_5 data (δ 69.6).[10] The β- anomeric configurations for the three glucoses were determined from their large $^{3}J_{1,2}$ coupling constants (J=7.2, 7.8 Hz). In the HMBC spectrum, the anomeric proton signals at δ 4.94 (H-1 of the inner glucose attached to C-3 of the aglycone), 5.11 (H-1 of the terminal glucose attached to C-3 of the inner glucose), 6.38 (H-1 of the terminal rhamnose attached to C-2 of the inner glucose), and 4.79 (H-1 of the terminal glucose attached to C-26 of the aglycone) showed cross-peaks with the carbon signals at δ 77.6 (C-3 of the aglycone), 89.6 (C-3 of the inner glucose attached to C-3 of the aglycone), 77.0 (C-2 of the inner glucose attached to C-3 of the aglycone), and 74.7 (C-26 of the aglycone) respectively (Fig. 1, Table 1). These signals provide ample evidence to determine the linkages by which the sugars were connected. In the NOESY spectra, many similar correlations indicated the same relative stereochemistry of **1** and **7**. The NOESY spectrum of **1** showed NOE correlations between H-14 (δ 0.81), H-16 (δ 5.67, m) and H-17 (δ 2.47, d, J=7.5 Hz), which indicated the α-configurations of H-16 and H-17. Based on the coupling constants and NOESY spectrum, the protons of 16 and 17 were assigned to be α-oriented, having the same configurations as that in **7**.

By analogy with **7**, the absolute stereochemistry of the aglycone of **1** is therefore assumed to be the same as in the aglycone of **7** with *R* configuration of C-17 and *S* configuration of C-16. On the basis of the foregoing evidence, the structure of **1** was determined as 3-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-26-*O*-β-D-glucopyranosyl-20, 22-*seco*-25 (*R*)-furosta-5-en-20, 22-dione-3β, 26-diol, named dioscoreside A.

Compound **2**, a white amorphous solid, mp 186-188°C(dec.), $[\alpha]_D^{25}=-69.1$ (pyridine; *c* 0.005), had a molecular formula of $C_{51}H_{82}O_{24}$ established by HRFAB-MS as well as from analysis of its ^{13}C NMR and DEPT data. The ESI-MS spectrum showed the pseudomolecular ion peaks and the fragment ion peaks at *m/z* 1101 $(M+Na)^+$, 955$(M+Na-Rha)^+$, 809$(M+Na-Rha\times2)^+$, 791$(M+Na-Rha\times2\text{-}H_2O)^+$, 1079$(M+H)^+$, 1077 $(M-H)^-$, 931$(M-H-Rha)^-$ and 785$(M-H-Rha\times2)^-$. Its IR spectrum showed characteristic absorptions for hydroxyl (3420 cm^{-1}), carbonyl ester (1740 cm^{-1}), carbonyl group (1715 cm^{-1}) and a glycosidic linkage (1000–1100 cm^{-1}). Of the 51 carbons observed, 27 were assigned to the aglycone, 24 to the oligosaccharide moieties (see Table 1). Acid hydrolysis of **2** afforded **1a** identified as 16 β-hydroxypregnenolone and the sugar components were identified as glucose and rhamnose by GLC analysis. The ^{1}H NMR spectrum of **2** contained four steroid methyl groups [δ 1.03 (3H, s, CH_3-19), 1.04 (3H, d, J=5.0 Hz, CH_3-27). 1.20 (3H, s, CH_3-18), 2.10 (3H, s, CH_3-21)] and one olefinic methine group [5.28 (1H, br s, H-6)]. The anomeric proton signals appeared at δ 6.41 (br s), 5.87 (br s), 4.95 (d, J=6.9 Hz) and 4.80 (d, J=7.2 Hz) in the ^{1}H NMR spectrum and the anomeric carbons observed at δ 105.0, 102.9, 102.1, and 100.3 in the ^{13}C NMR spectrum showed that compound **2** contained four sugar moieties. The coupling constants of the anomeric signals revealed the configurations for the corresponding sugars. The (25*R*) configuration of **2** was deduced from the resonances of protons and carbons (C-25, C-26, and C-27) and the $^{3}J_{HH}$ values (10.0, 3.5 Hz) between H-25



Scheme 1.

and H-26 which corresponded to those present in **1**.[8,9] Comparison of its ^{1}H and ^{13}C NMR spectra with that of compound **1** indicated the presence of a hydroxyl group; the signals due to C-22, C-23 and C-24 were shifted to lower fields by 2.0 ppm (β effect), 33.9 ppm (α effect) and 9.1 ppm (β effect), whereas the signal due to C-25 was moved to a higher field by 2.5 ppm (γ effect). This led to the assignment of the location of a hydroxyl group at C-23. In addition, the H-23 proton (δ 4.68) showed correlations with the βH-24 proton (δ 2.39) in the COSY spectrum. HMBC correlations between the methyl proton signals (δ 2.10, CH_3-21) and the carbon signals (δ 205.4, C-20; 66.8, C-17) and between the methine group signals (δ 2.48, H-17) and the carbon signals (δ 13.8, C-18; 205.4, C-20) were observed. The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined from the DEPT, COSY, HMQC, HMBC and NOESY spectra as described for compound **1** above. The α-anomeric configurations for the rhamnoses were determined by their C-5 data (δ 69.6, 70.5), and the β-anomeric configurations for the glucoses were determined from their large $^{3}J_{H1,\ H2}$ coupling constants (6.9, 7.2 Hz). From the HMBC spectrum, it was observed that $H_{R(1-2)}$-1 (δ 6.41)with C_G-2 (δ 78.2), $H_{R(1-4)}$-1 (δ 5.87) with C_G-4 (δ 78.6), H_G-1 (δ 4.95) with C-3 (δ 78.0), $H_{26\text{-}G}$-1 (δ 4.80) with C-26 (δ 75.6) had cross peaks. The stereochemistry of **2** was defined by a NOESY experiment. Significant NOEs were observed between the following signals: (i) H-14 (δ 0.91), H-16 (δ 5.75, m) and H-17 (δ 2.49, d, J=8.4 Hz), and (ii) H-23 (δ 4.68), H-25 (δ 1.86) and βH-26 (δ 4.09). Thus, protons 16 and 17 were proved to have α configurations, as in **1**, and the hydroxyl group of C-23 also had the α configuration. So the absolute configuration of **2** was established to be the same as in **1,** being 17*R*, 16*S*, and 23*S*. Therefore, the structure of **2** was established as 3-*O*-[bis-α-L-rhamnopyranosyl-(1→4 and 1→2)-β-D-glucopyranosyl]-26-*O*-β-D-glucopyranosyl-20, 22-*seco*-25 (*R*)-furosta-5-en-20, 22-dione-3β, 23(*S*), 26-triol, called dioscoreside B. Dioscoresides A and B are the first example of E-*seco* furostanol saponins from natural sources.

Dioscoresides A and B are obviously furostanol saponins which have undergone the opening of ring E. In the course of the former precursor transforming into the final product, two processes could occur: (a) the oxidation of the double bond and (b) the opening of ring E. It is assumed that an enzyme such as oxygenase initiated the biosynthetic step of this pathway (Scheme 1). The chemical correlations confirmed the possible biosynthetic pathway for the formation of **1** and **2** from **7** in the plant organ.[11–13]

Table 2. Cytotoxic activity (IC_{50}, μg/ml)

	1	2	3	4	5	6	7
A375-S2	8.4	7.1	3.6	2.7	2.2	3.4	5.7
L929	8.6	6.7	4.5	3.1	1.8	3.3	5.5
Hela	7.9	6.9	4.2	2.7	2.1	2.8	6.2

2.1. Cytotoxic activity

We evaluated the cytotoxic activity of dioscoreside A and B, and five known saponins (**3**, **4**, **5**, **6**, **7**) against human melanoma A375-S2, murine pheumoepithelial carcinoma L929 and human cervicoma Hela cell lines. The cells were continuously treated with the samples for 72 h, and the cell growth was measured with an MTT assay procedure.[14–15] The compounds showed considerable activities as shown in Table 2.

3. Experimental

3.1. General

Melting points were determined on a Yanaco MP-S3 micro-melting point apparatus. The IR spectra were measured on a Bruker IFS 55 spectrometer. HRFAB-MS in a glycerol matrix were recorded on a VG-Autospec-3000 instrument. All ESI-MS spectra were acquired using a Finnigan MAT LCQ™ ion trap mass spectrometer (San Jose, CA, USA). Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. ^{1}H, ^{13}C NMR, DEPT and 2D-NMR (HMQC, HMBC, ^{1}H–^{1}H COSY, NOESY) spectra were taken on a Bruker ARX-300 MHz spectrometer in C_5D_5N solution, internal reference was tetramethylsilane (TMS). Column chromatography and TLC were carried out on silica gel H (10–40 μm, Qingdao Haiyang Chemical Factory). Compounds were detected by spraying with 10% H_2SO_4/EtOH solution or vanillin/H_2SO_4/EtOH, followed by heating. HPLC: Shimadzu LC-8A (SPD-6AV UV-VIS detector) equipped with an ODS column (Phenomenex, 10.0 mm i.d.×250 mm). GLC: Shimadzu GC-7A, Column: Silicone OV-17 on Uniport HP (80–100 mesh), 3 mm i.d.×2.1 m; column temperature, 160°C; carrier gas, N_2, flow rate 30 ml/min. Cells: obtained from ATCC, USA. RPMI 1640 medium: Gibco, USA. CO_2 incubator: Revco, USA. Microplate reader: Bio-rad Model 450, Richmond, USA. All other chemicals used were of biochemical reagent grade.

Plant material. The rhizomes of *Dioscorea panthaica* were purchased from Sichuan province of China and were identified by Professor Qishi Sun (Division of Pharmacognosy, Shenyang Pharmaceutical University). A voucher specimen is deposited at the herbarium of Shenyang Pharmaceutical University (No. 1068).

Table 3. ^{1}H NMR spectral data for compounds **1**, **2**, **7** (in C_5D_5N) and **1a** (in $CDCl_3$) (all the signals were assigned by 1D and 2D NMR spectra)

Position	1	2	7	1a	Position	1	2	7
1	0.95, 1.67 o[a]	0.92, 1.67 o	0.95, 1.70 o	0.89, 1.55 o	Sugar part			
2	1.85, 2.06 o	1.83, 2.06 o	1.85, 2.06 o	1.76, 1.94 o	Glc (inner)			
3	3.87 m	3.85 m	3.82 m	3.66 m	1	4.94 d, 7.8	4.95 d, 6.9	4.94 d, 7.2
4	2.70, 2.78 m	2.71, 2.80 m	2.70, 2.78 m	2.67, 2.72 m	2	4.44	4.42	4.43
5					3	4.45	4.22	4.22
6	5.30 br s	5.28 br s	5.32 br s	5.28 br s	4	4.21	4.40	4.41
7	1.85 o	1.87 o	1.85 o	1.82 o	5	3.87	3.85	3.86
8	1.45 o	1.52 o	1.45 o	1.51 o	6	4.10, 4,23	4.12, 4,25	4.11, 4,23
9	0.85 o	0.90 o	0.92 o	0.85 o	Rha (1→2)			
10					1	6.38 br s	6.41 br s	6.39 br s
11	1.44 o	1.48 o	1.45 o	1.40 o	2	4.80	4.81	4.79
12	1.08, 2.12 o	1.10, 2.46 o	1.08, 2.11 o	1.27, 1.65 o	3	4.60	4.60	4.59
13					4	4.35	4.36	4.36
14	0.81 m	0.84 m	0.81 m	0.77 m	5	4.92	4.94	4.93
15	1.31, 2.40 o	1.55, 2.42 o	1.3, 2.40 o	1.28, 2.20 o	6	1.74 d, 6.0	1.75 d, 5.4	1.76 d, 6.3
16	5.67 m	5.75 m	4.76 m	5.36 m	Rha (1→4)			
17	2.47 d, 9.6[b]	2.49 d, 9.0	2.44 d, 10.2	2.42 d, 9.6	1		5.87 br s	5.85 br s
18	1.20 s	1.20 s	0.72 s	1.06 s	2		4.68	4.66
19	1.04 s	1.03 s	1.05 s	0.68 s	3		4.55	4.56
20					4		4.32	4.31
21	2.11 s	2.10 s	1.63 s	2.15 s	5		4.92	4.92
22					6		1.62 d, 5.4	1.62 d, 6.0
23	1.32, 2.43 o	4.68 m	1.37, 2.49 o		Glc (1→3)			
24	1.54, 1.88 o	1.94, 2.39 o	1.45, 1.75 o		1	5.11 d, 7.0		
25	1.80 o	1.86 o	1.76 o		2	4.03		
26	3.46, 3.95 m	4.09, 4.20 m	3.52, 3.98 m		3	4.30		
27	0.88 d, 6.3	1.04 d, 5.7	1.01 d, 6.6		4	4.32		
					5	4.05		
					6	4.28, 4.40		
					26-Glc			
					1	4.79 d, 7.2	4.80 d, 7.2	4.83 d, 7.5
					2	4.01	4.03	4.04
					3	4.27	4.28	4.28
					4	4.32	4.30	4.32
					5	4.00	4.02	4.03
					6	4.34, 4.47	4.36, 4.49	4.37, 4.50

[a] Overlapped signals are indicated by 'o'.
[b] *J* values are reported in Hz.

3.2. Extraction and isolation

The rhizomes (10 kg) of *D. panthaica* were refluxed with 90% EtOH three times and the extract was condensed to residue (440 g) under reduced pressure. The extract (50 g) was subjected to chromatographic separation on a silica gel column (1000 g) (7.5 cm×60 cm). The compounds of the mixture were eluted with $CHCl_3$–MeOH (9:1, 6:1, 4:1, 3:1, 1:1), subsequently 5 fractions (I–V) were obtained. Fr. IV was further separated by preparative HPLC with the mobile phase MeOH–H_2O (85:15, 70:30), and finally compound **1** (48 mg), compound **2** (32.5 mg) were separated.

3.2.1. Compound 1. White amorphous solid; mp 178–180°C (dec.), $[\alpha]_D^{25}=-50.2$ (pyridine, *c* 0.003). HRFAB-MS: *m/z* 1078.5184 (calcd for $C_{51}H_{82}O_{24}$, 1078.5196). Positive-ion ESI-MS: *m/z* 1101 $(M+Na)^+$, 1079 $(M+H)^+$, 955 $(M+Na-Rha)^+$; negative-ion ESI-MS: *m/z* 1077 $(M-H)^-$, 931 $(M-H-Rha)^-$, 769 $(M-H-Rha-Glc)^-$. IR (KBr) υ_{max} 3417, 1735, 1715, 1680, 1100–1000, 840 cm^{-1}. ^{1}H NMR (300 MHz, C_5D_5N) and ^{13}C NMR (75 MHz, C_5D_5N), see Tables 1 and 3.

3.2.2. Compound 2. White amorphous solid; mp 186–188°C (dec.). $[\alpha]_D^{25}=-69.1°$ (pyridine, *c* 0.005). HRFAB-MS: *m/z* 1078.5178 (calcd for $C_{51}H_{82}O_{24}$, 1078.5196). Positive-ion ESI-MS: *m/z* 1101 $(M+Na)^+$, 1079 $(M+H)^+$, 955 $(M+Na-Rha)^+$, 809 $(M+Na-Rha\times2)^+$, 791 $(M+Na-Rha\times2\text{-}H_2O)^+$; negative-ion ESI-MS: *m/z* 1077 $(M-H)^-$, 931 $(M-H-Rha)^-$, 785 $(M-H-Rha\times2)^-$. IR (KBr) υ_{max} 3420, 1740, 1715, 1680, 1100–1000, 840 cm^{-1}. ^{1}H NMR (300 MHz, C_5D_5N) and ^{13}C NMR (75 MHz, C_5D_5N), see Tables 1 and 3.

3.2.3. Compound 7. Colorless needles; mp 174–176°C (dec.). Positive-ion ESI-MS: *m/z* 1053 $(M+Na)^+$, 1031 $(M+H)^+$, 907 $(M+Na-Rha)^+$, 761 $(M+Na-Rha\times2)^+$; negative-ion ESI-MS: *m/z* 1029 $(M-H)^-$, 883 $(M-H-Rha)^-$, 737 $(M-H-Rha\times2)^-$. ^{1}H NMR (300 MHz, C_5D_5N) and ^{13}C NMR (75 MHz, C_5D_5N), see Tables 1 and 3.

3.2.4. Acid hydrolysis of 1 and 2. A solution of **1** (20 mg) in 1 M HCl (dioxane–H_2O, 1:1, 10 ml) was heated at 100°C for 2.5 h in a sealed tube. After dioxane was removed, the solution was extracted with EtOAc (3×5 ml). The extraction was washed with H_2O and evaporated to dryness in a vacuum. The residue was chromatographed on silica gel eluting with $CHCl_3$–MeOH (20:1, 1:1) to furnish **1a** (4.3 mg). The monosaccharide portion was neutralised by passing through an exchange resin (Amberlite MB-3)

column, concentrated (dried overnight) and then treated with 1-(trimethylsilyl) imidazole at room temperature for 2 h. After the excess reagent was decomposed with water, the reaction product was extracted with hexane (2×5 ml). The TMS derivatives of the monosaccharides were identified as D-glucose and L-rhamnose in a ratio of (3:1) by GLC analysis with authentic samples.

Compound **2** (20 mg) was subjected to acid hydrolysis as described for **1** to give the same compound **1a** (4.6 mg), and the monosaccharides were identified as D-glucose and L-rhamnose in a ratio of 1:1 by GLC analysis.

3.2.5. Compound 1a. Colorless needles; mp 223–225°C (dec.). ESI-MS: 355 $(M+Na)^+$, 333 $(M+H)^+$. IR (KBr)υ_{max} 3417, 1735, 1715, 1680, 1100–1000, 840 cm^{-1}. ^1H NMR (300 MHz, C_5D_5N) and ^{13}C NMR (75 MHz, C_5D_5N), see Tables 1 and 3.

3.3. Cell culture and assay for cytotoxic activity

All the cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum and 1% L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. After they were digested with 0.25% trypsin solution, the cells were washed, resuspended in the above medium to 5×10^4 cells/ml, then 100 μl of this cell suspension were placed in each well of a 96-well flat-bottom plate. The cells were incubated for 24 h at 37°C in 4% CO_2. After incubation, 4 μl of EtOH–H_2O (1:1) solution containing the sample was added to give final concentrations of 0.01–20 μg/ml; 4 μl EtOH–H_2O (1:1) was added into control wells. The cells were incubated for a further 72 h in the presence of each agent, and then cell growth was evaluated with an MTT assay procedure. After termination of cell culture, 20 μl of 5 mg/ml MTT in phosphate buffered saline was added to every well and the plate was reincubated at 37°C in 4% CO_2 for a further 4 h, then the supernatant was removed from every well, and 150 μl DMSO was added to dissolve the formazan crystals. The plate was shaken on a microshaker for 10 min, and then read on a microplate reader at 490 nm. A dose response curve was plotted for these samples that showed more than 90% of cell growth inhibition at the sample concentration of 20 μg/ml, and a concentration giving 50% inhibition (IC_{50}) was calculated.

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