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Atropurosides A–G, new steroidal saponins from *Smilacina atropurpurea*

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ABSTRACT

Atropurosides A–G (**1**–**7**), seven new steroidal saponins, which possess new polyhydroxylated aglycones, were isolated from the rhizomes of *Smilacina atropurpurea* (Convallariaceae), together with a known saponin, dioscin (**8**). Their structures were elucidated on the basis of detailed spectroscopic analysis, including 1D and 2D NMR techniques and chemical methods. Antifungal testing of the eight compounds indicated that atropurosides B (**2**) and F (**6**) were fungicidal against *Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* with minimum fungicidal concentrations (MFCs) $\leq$20 μg/ml, while dioscin (**8**) was selectively active against *C. albicans* and *C. glabrata* (MFC $\leq$ 5.0 μg/ml). Furthermore, the antifungal saponins **2**, **6**, and **8** were evaluated for their *in vitro* cytotoxicities in a panel of human cancer cell lines (SK-MEL, KB, BT-549, SK-OV-3, and HepG2) and noncancerous Vero cells. All showed moderate cytotoxicities. It appears that the antifungal activity of these steroidal saponins correlates with their cytotoxicity against mammalian cells.

1. Introduction

Smilacina atropurpurea (Franch) Wang et Tang (Convallariaceae) is a perennial plant distributed mainly in the southwest region of the People's Republic of China [1]. The tender aerial part is edible and has been used as a wild vegetable by Lisu, Naxi, and Tibetan people. The rhizome, as a folk traditional medicine, has been used for the treatment of lung ailment, rheumatism, menstrual disturbance, cuts, and bruises [2]. While three nucleosides (thymidine, adenosine, and 2′-deoxyadenosine) have been isolated from the aerial part [3], no chemical study has been reported on its rhizome. Our detailed chemical investigation on the fresh rhizome of *S. atropurpurea* led to the isolation of seven new steroidal saponins with polyhydroxylated aglycones, atropurosides A–G (**1**–**7**), together with a known saponin, dioscin (**8**). In the present paper, we report the isolation and structure elucidation of the seven new compounds by detailed spectroscopic analysis, including 2D NMR techniques, and chemical methods. In addition, the *in vitro* antifungal activity of the eight isolated compounds and the *in vitro* cytotoxicity of the antifungal saponins are also described.

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2. Experimental

2.1. General methods

Optical rotations were measured on a SEPA-3000 automatic digital polarimeter. IR spectra were conducted on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were measured in pyridine-d_5 and recorded on a Bruker AM-400 (for ^{1}H NMR and ^{13}C NMR) and DRX-500 (for 2D NMR) instrument with TMS as internal standard. FABMS (negative ion mode) and HRESIMS (negative ion mode) spectra were recorded on VG AutoSpe 3000 and API Qstar Pulsar LC/TOF spectrometers, respectively. GC analysis was run on Agilent Technologies HP5890 gas chromatograph equipped with an H_2 flame ionization detector. The column was 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180 °C/280 °C; programmed increase, 3 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl, split ratio: 1/50. Silica gel (200–300 mesh and 10–40 μm) and reversed phase silica gel RP-8 (40–63 μm) were used for column chromatography.

2.2. Plant material

The fresh rhizomes of *S. atropurpurea* were collected from Zhongdian, Yunnan, China, and a voucher specimen is deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and Isolation

The fresh rhizomes (20 kg) of *S. atropurpurea* were extracted with MeOH (5 L × 3) under reflux. After removal of the solvent under reduced pressure, the concentrate (400 g) was suspended into H_2O and partitioned with $CHCl_3$ and *n*-BuOH, successively. The *n*-BuOH fraction (100 g) was subjected to column chromatography (CC) using silica gel (3 kg), eluting with $CHCl_3$–MeOH–H_2O (7:3:0.5) to give six fractions (Fr. 1–6). Fr. 3 (8 g) was subjected to silica gel CC, eluting with a gradient solvent system of $CHCl_3$–MeOH (12:2–10:3) to give **1** (105 mg), **3** (500 mg), **4** (23 mg) and **5** (15 mg). Fr. 4 (10 g) was subjected to silica gel CC eluting with $CHCl_3$–MeOH (10:2–9:2) followed with reverse phase RP-8 CC eluting with MeOH–H_2O (4:6–1:0) to afford **1** (20 mg), **2** (40 mg), **7** (1 g) and **8** (300 mg). Fr. 5 (12 g) was applied to a silica gel column and eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2–7:3:0.5) to afford **6** (155 mg).

2.3.1. Atropuroside A (1)

White amorphous powder $[\alpha]_D = -76.5°$ ($c = 0.22$, MeOH). FABMS (negative ion mode): *m/z* 723 $[M-H]^-$, 577 [M-146(Rha)−H]$^-$, 445 [M-146(Rha)-132(Xyl)−H]$^-$. HRESIMS (negative ion mode): *m/z* 723.3930 [M($C_{38}H_{60}O_{13}$)−H]$^-$ (calcd. for 723.3955). ^{1}H NMR (pyridine-d_5): δ 0.67 (d, $J = 5.4$ Hz, CH_3-27), 0.91 (s, CH_3-18), 1.10 (d, $J = 6.9$ Hz, CH_3-21), 1.17 (m, H-14), 1.35 (s, CH_3-19), 1.51 (m, H-15β), 1.61 (br d, $J = 9.6$ Hz, H-11β), 1.79 (d, $J = 6.2$ Hz, Rha CH_3-6), 1.95 (m, H-20), 2.10 (m, H-15α), 2.59 (dd, $J = 5.7$, 11.9 Hz, H-4α), 2.76 (br t, $J = 11.9$ Hz, H-4β), 2.88 (br d, $J = 9.6$ Hz, H-11α), 3.48 (t, $J = 10.5$ Hz, H-26β), 3.56 (2H, m, H-

Table 1 – ^{13}C NMR spectral data for the aglycone moieties of 1–7, 1a, 3a, 3b, and 7b (in pyridine-d_5)

Position	1	1a	2	3	3a	3b	4	5	6	7	7b
1	88.7	82.6	89.0	92.5	82.5	82.5	93.0	93.0	93.2	92.9	93.0
2	78.4	78.2	78.5	78.3	78.1	78.2	78.3	78.3	78.3	78.3	78.3
3	73.2	73.2	73.1	72.8	73.2	73.2	72.8	72.8	72.8	72.8	72.8
4	40.8	41.3	40.8	40.7	41.2	41.2	40.7	40.2	40.8	40.3	40.3
5	138.1	139.2	138.1	138.0	139.4	139.4	138.0	138.0	138.0	138.1	138.0
6	124.9	124.5	125.1	125.2	124.3	124.4	125.1	125.2	125.3	125.2	125.2
7	31.6	31.8	31.8	31.7	31.9	31.9	31.6	31.6	31.6	31.6	30.0
8	33.0	32.4	33.6	33.7	31.7	32.1	33.0	33.1	33.0	33.0	33.0
9	50.1	51.4	49.9	50.2	51.1	51.4	50.5	50.5	50.5	50.5	50.4
10	43.3	43.6	43.4	43.5	43.5	43.6	43.4	43.5	43.5	43.5	43.5
11	24.3	24.0	23.9	23.8	23.6	23.9	24.1	24.1	24.2	24.0	24.1
12	40.4	40.5	32.2	32.5	39.3	41.2	40.2	40.5	38.0	40.8	40.3
13	40.3	40.3	45.2	45.2	41.5	40.4	40.4	40.7	40.8	40.8	40.7
14	57.1	56.8	53.1	53.1	51.3	55.6	57.2	57.3	57.2	56.9	57.0
15	32.4	32.4	31.9	31.9	37.6	34.3	32.3	32.4	32.7	31.7	32.3
16	81.2	81.1	89.9	90.0	213.3	119.3	81.5	81.9	81.4	81.5	81.6
17	63.1	63.1	90.3	90.3	66.6	70.3	63.2	62.9	64.1	64.3	63.1
18	17.0	16.6	17.8	17.9	13.1	15.7	17.1	17.1	17.3	17.0	17.0
19	16.2	15.1	16.3	16.2	15.6	15.1	16.2	16.2	16.3	16.2	15.1
20	43.0	42.0	44.8	44.9	43.8	42.7	41.9	42.1	40.8	40.5	41.9
21	15.1	15.1	10.0	9.9	15.0	15.1	14.9	14.9	16.4	16.2	16.2
22	109.3	109.3	109.9	109.9	217.8	111.1	109.4	111.9	110.5	112.5	109.5
23	31.9	32.2	32.2	32.2	40.4	34.5	33.2	67.1	40.4	32.3	33.2
24	29.3	29.3	28.9	28.9	27.6	29.5	29.0	43.6	28.4	28.1	29.0
25	30.6	30.6	30.5	30.5	36.2	30.5	144.6	149.4	147.3	146.9	144.5
26	66.9	66.9	66.7	66.7	67.4	68.8	65.0	64.7	72.2	72.1	65.0
27	17.3	17.4	17.4	17.3	17.3	17.4	108.6	106.6	110.8	111.1	108.7
OCH_3						50.6				47.4	

Table 2 – ^{13}C NMR spectral data for the sugar moieties of 1–7 and 7b (in pyridine-d_5)

Position	1	2	3	4	5	6	7	7b
Xyl-1	104.2	104.3	107.0				107.2	107.3
2	77.9	77.9	75.9				73.6	73.6
3	79.6	79.7	79.0				75.2	75.1
4	71.7	71.6	71.1				70.0	70.0
5	67.4	67.3	67.7				68.2	68.2
Gal-1				107.0	107.0	107.1		
2				73.7	73.6	73.6		
3				75.6	75.6	75.7		
4				69.7	69.7	69.7		
5				76.7	76.8	76.8		
6				61.9	61.9	61.8		
Glc-1						104.0	103.9	
2						75.2	75.2	
3						78.6	78.6	
4						71.7	71.8	
5						78.6	78.6	
6						62.8	62.9	
Rha-1	102.2	102.3						
2	72.2	72.2						
3	72.6	72.6						
4	74.1	74.1						
5	70.0	70.0						
6	19.0	19.1						

26α, Xyl H-5a), 3.71 (d, J = 9.6 Hz, H-1), 3.85 (m, H-3), 4.13 (2H, m, Xyl H-3,4), 4.27 (t, J = 8.1 Hz, Xyl H-2), 4.34 (t, J = 9.2 Hz, Rha H-4), 4.49 (t, J = 9.6 Hz, H-2), 4.52 (m, H-16), 4.72 (dd, J = 3.3, 9.2 Hz, Rha H-3), 4.79 (dd, J = 1.3, 3.3 Hz, Rha H-2), 4.90 (m, Rha H-5), 5.57 (d, J = 7.9 Hz, Xyl H-1), 5.59 (br d, J = 5.7 Hz, H-6), 6.51 (br s, Rha H-1). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.2. *Acid hydrolysis of* **1**

A solution of **1** (40 mg) in 2 M HCl-dioxane (1:1, 8 ml) was heated at 95 °C for 6 h. The reaction mixture was diluted with 2 ml H_2O and extracted with $CHCl_3$ (6 ml × 3). The $CHCl_3$ phase (20 mg) was chromatographed on silica gel column eluting with $CHCl_3$–MeOH (9:1) to give compound, **1a** (16 mg): white amorphous powder $[\alpha]_D = -47.3°$ (c = 0.27, MeOH). FABMS (negative ion mode): m/z 445 $[M-H]^-$. HRESIMS (negative ion mode): m/z 445.2947 $[M\ (C_{27}H_{42}O_5)-H]^-$ (calcd. for 445.2953). ^{1}H NMR (pyridine-d_5): δ 0.67 (d, J = 4.1 Hz, CH_3-27), 0.89 (s, CH_3-18), 1.09 (d, J = 6.6 Hz, CH_3-21), 1.33 (s, CH_3-19), 1.47 (m, H-15β), 1.62 (m, H-11β), 1.91 (m, H-20), 2.03 (m, H-15α), 2.65 (dd, J = 5.0, 11.9 Hz, H-4α), 2.80 (br t, J = 11.9 Hz, H-4β), 2.88 (br d, J = 9.6 Hz, H-11α), 3.48 (t, J = 10.2 Hz, H-26β), 3.57 (br d, J = 10.2 Hz, H-26α), 3.64 (d, J = 9.1 Hz, H-1), 3.91 (m, H-3), 4.04 (t, J = 9.1 Hz, H-2), 4.52 (m, H-16), 5.59 (br d, J = 5.7 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Table 1. The aqueous layer was passed through an Amberlite IRA-401 (OH^- form) column, and the eluate was concentrated to dryness to give a monosaccharide mixture (15 mg), in which rhamnose and xylose were detected by direct TLC analysis on a HPTLC silica gel 50,000 F_{254} plate using n-BuOH-i-PrOH-H_2O (10:5:4, homogenous) as development and anisaldehyde-H_2SO_4 as detection, comparing with the authentic samples: rhamnose (R_f 0.49) and xylose (R_f 0.42). The absolute configurations of monosaccharides were further determined by GC analysis of its derivatives [4] to be D-xylose and L-rhamnose.

A solution of the sugar residue (7.5 mg) in pyridine (2 ml) was added to L-cysteine methyl ester hydrochloride (10.3 mg) and kept at 60 °C for 1 h. Then trimethylsilylimidazole (1.5 ml) was added to the reaction mixture and kept again at 60 °C for 30 min. The supernatant (4 μl) was analyzed by GC, and the retention times of D- and L-xylose, and L-rhamnose were 13.35, 14.01, and 14.97 min, respectively.

2.3.3. *Atropuroside B* (**2**)

White amorphous powder $[\alpha]_D = -70.3°$ (c = 0.38, MeOH). FABMS (negative ion mode): m/z 739 $[M-H]^-$, 593 $[M-146(Rha)-H]^-$; HRESIMS (negative ion mode): m/z 739.3925 $[M(C_{38}H_{60}O_{14})-H]^-$ (calcd. for 739.3904). ^{1}H NMR (pyridine-d_5): δ 0.66 (d, J = 5.5 Hz, CH_3-27), 1.05 (s, CH_3-18), 1.23 (d, J = 7.1 Hz, CH_3-21), 1.40 (s, CH_3-19), 1.51 (m, H-15β), 1.69 (2H, m, H-9, 11β), 1.80 (d, J = 6.1 Hz, Rha CH_3-6), 2.20 (2H, m, H-14, 15α), 2.29 (q, J = 7.1 Hz, H-20), 2.59 (dd, J = 5.6, 12.3 Hz, H-4α), 2.76 (t, J = 12.3, H-4β), 3.03 (br d, J = 10.0 Hz, H-11α), 3.47–3.51 (3H, m, H-26, Xyl H-5a), 3.71 (d, J = 9.5 Hz, H-1), 3.82 (m, H-3), 4.04 (2H, m, Xyl H-3,4), 4.07 (dd, J = 5.6, 11.0 Hz, Xyl H-5b), 4.28 (t, J = 7.9 Hz, Xyl H-2), 4.35 (t, J = 9.4 Hz, Rha H-4), 4.51 (t, J = 9.5 Hz, H-2), 4.73 (dd, J = 3.3, 9.4 Hz, Rha H-3), 4.79 (dd, J = 1.3, 3.3 Hz, Rha H-2), 4.90 (m, Rha H-5), 5.55 (d, J = 7.9 Hz, Xyl H-1), 5.58 (br d, J = 5.5 Hz, H-6), 6.51 (br s, Rha H-1). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.4. *Atropuroside C* (**3**)

White amorphous powder $[\alpha]_D = -62.1°$ (c = 0.33, MeOH). FABMS (negative ion mode): m/z 593 $[M-H]^-$, 462 $[M-132\ (Xyl)]^-$. HRESIMS (negative ion mode): m/z 593.3333 $[M(C_{32}H_{50}O_{10})-H]^-$ (calcd. for 593.3325). ^{1}H NMR (pyridine-d_5): δ 0.67 (d, J = 5.7 Hz, CH_3-27), 1.02 (s, CH_3-18), 1.20 (d, J = 7.1 Hz, CH_3-21), 1.29 (s, CH_3-19), 1.53 (m, H-15β), 1.63 (2H, m, H-9, 11β), 1.92 (dd, J = 5.8, 12.7 Hz, H-7), 2.21 (2H, m, H-14, 15α), 2.27 (q, J = 7.1 Hz, H-20), 2.38 (m, H-12), 2.61 (dd, J = 5.8, 12.7 Hz, H-4α), 2.79 (t, J = 12.7 Hz, H-4β), 3.12 (br d, J = 9.9 Hz, H-11α),3.51(3H, m, H-26, Xyl H-5a), 3.59 (d, J = 9.2 Hz, H-1), 3.88 (m, H-3), 4.06–4.11 (4H, m, Xyl H-2,3,4,5b), 4.30 (t, J = 9.2 Hz, H-2), 4.48 (t, J = 6.5 Hz, H-16), 5.38 (d, J = 7.5 Hz, Xyl H-1), 5.63 (br d, J = 6.0 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.5. *Acid hydrolysis of* **3**

A solution of **3** (60 mg) was subjected to acid hydrolysis as described for **1**. The $CHCl_3$ residue (40 mg) was chromatographed on silica gel column eluting with $CHCl_3$–MeOH (100:0 → 86:14) to yield two new sapogenins, **3a** (10 mg) and **3b** (16 mg). **3a**: white amorphous powder $[\alpha]_D = -47.9°$ (c = 0.23, $CHCl_3$). IR ν_{max}(KBr) 3440 (OH), 2926 (CH), 1734 (br) (C=O), 1631 (C=C), 1038 (C–O) cm^{-1}. ESIMS (negative ion mode): m/z 462 $[M]^-$. HRESIMS (negative ion mode): m/z 461.2915 $[M(C_{27}H_{41}O_6)-H]^-$ (calcd. for 461.2903). ^{1}H NMR (pyridine-d_5): δ 0.75 (s, CH_3-18), 1.01 (d, J = 6.6 Hz, CH_3-21), 1 × 10 (d, J = 6.7 Hz, CH_3-27), 1.33 (s, CH_3-19), 1.48 (m, H-12a), 1.72 (2H, m, H-11β, 15β), 1.75 (2H, m, H-7, 24a), 1.90 (2H, m, H-12b, 25), 2.06 (dd, J = 7.1, 15.3 Hz, H-15α), 2.16 (m, H-24b), 2.65 (dd, J = 5.3, 13.3 Hz, H-4α), 2.76 (4H, m, H-4β, 17, 20, 23a), 2.90 (br d, J = 11.2 Hz, H-11α), 3.01 (m, H-23b), 3.65 (d, J = 9.2 Hz, H-1), 3.73 (dd, J = 6.2, 10.5 Hz, H-26a), 3.79 (dd, J = 5.7, 10.5 Hz, H-26b), 3.91 (m, H-3), 4.04 (t, J = 9.2 Hz, H-2), 5.58 (d, J = 5.6 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Table 1. **3b**: white amorphous powder $[\alpha]_D = -49.4°$ (c = 0.35, $CHCl_3$). FABMS (negative ion mode):

m/z 475 [M−H]$^-$. HRESIMS (negative ion mode): *m/z* 475.3053 [M($C_{28}H_{43}O_6$)−H]$^-$ (calcd. for 475.3059). ^{1}H NMR (pyridine-d_5): δ 0.72 (d, J = 4.8 Hz, CH_3-27), 0.87 (s, CH_3-18), 1.15 (d, J = 5.9 Hz, CH_3-21), 1.33 (s, CH_3-19), 1.39 (2H, m, H-9, 15β), 1.49 (m, H-14), 1.74 (m, H-11β), 1.90 (m, H-7), 2.07 (2H, m, H-17, 20), 2.20 (dd, J = 5.0, 12.3 Hz, H-15α), 2.64 (dd, J = 5.2, 13.1 Hz, H-4α), 2.79 (br t, J = 13.1 Hz, H-4β), 2.90 (m, H-11α), 3.45 (s, OCH_3-16), 3.63 (d, J = 9.0 Hz, H-1), 3.70 (2H, m, H-26), 3.91 (m, H-3), 4.03 (t, J = 9.0 Hz, H-2), 5.59 (d, J = 4.4 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Table 1.

2.3.6. *Atropuroside D (4)*

White amorphous powder $[\alpha]_D = -55.3^\circ$ (c = 0.18, MeOH). FABMS (negative ion mode): *m/z* 605 [M−H]$^-$. HRESIMS (negative ion mode): *m/z* 605.3314 [M($C_{33}H_{50}O_{10}$)−H]$^-$ (calcd. for 605.3325). ^{1}H NMR (pyridine-d_5): δ 0.87 (s, CH_3-18), 0.96 (d, J = 6.8 Hz, CH_3-21), 1.25 (s, CH_3-19), 1.48 (m, H-11β), 1.53 (m, H-15β), 1.61 (m, H-9), 1.86 (m, H-17), 1.90 (m, H-20), 2.03 (m, H-15α), 2.23 (br d, J = 10.9 Hz, H-24β), 2.63 (dd, J = 5.7, 13.2 Hz, H-4α), 2.71 (br t, J = 10.9 Hz, H-24α), 2.79 (br t, J = 13.2 Hz, H-4β), 3.10 (br d, J = 12.4 Hz, H-11α), 3.56 (d, J = 9.2 Hz, H-1), 3.92 (m, H-3), 4.02 (2H, br d, J = 12.4 Hz, H-26a, Gal H-5), 4.14 (br d, J = 8.6 Hz, Gal H-3), 4.29 (t, J = 9.2 Hz, H-2), 4.40 (m, Gal H-6a), 4.44 (d, J = 12.4 Hz, H-26b), 4.48 (m, H-16), 4.55 (2H, m, Gal H-2,6b), 4.61 (br s, Gal H-4), 4.77 (br s, H-27a), 4.81 (br s, H-27b), 5.35 (d, J = 7.9 Hz, Gal H-1), 5.64 (br d, J = 5.5 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.7. *Acid hydrolysis of 4*

Compound **4** (20 mg) was subjected to acid hydrolysis as described for **1** to give spirost-5,25(27)-diene-1β,2α,3β-triol (**4a**, 8 mg) [5]. The aqueous layer was treated and analyzed as described for **1**, in which D-galactose was detected by HPTLC (R_f 0.28), and GC analysis (retention times of D- and L-galactose were 19.07 and 19.66 min, respectively).

2.3.8. *Atropuroside E (5)*

White amorphous powder $[\alpha]_D = -42.3^\circ$ (c = 0.06, MeOH). FABMS (negative ion mode): *m/z* 621 [M−H]$^-$. HRESIMS (negative ion mode): *m/z* 621.3274 [M($C_{33}H_{50}O_{11}$)−H]$^-$ (calcd. for 621.3274). ^{1}H NMR (pyridine-d_5): δ 0.86 (s, CH_3-18), 0.99 (d, J = 10.7 Hz, CH_3-21), 1.23 (s, CH_3-19), 1.51 (m, H-15β), 1.58 (2H, m, H-9, 11β), 2.01 (2H, m, H-20, 15α), 2.02 (m, H-8), 2.05 (t, J = 11.8 Hz, H-24α), 2.42 (dd, J = 5.8, 11.8 Hz, H-24β), 2.63 (dd, J = 5.6, 12.9 Hz, H-4α), 2.78 (br t, J = 12.9 Hz, H-4β), 3.07 (br d, J = 10.8 Hz, H-11α), 3.54 (d, J = 10.0 Hz, H-1), 3.91 (m, H-3), 4.03 (m, Gal H-5), 4.13 (br d, J = 9.5 Hz, Gal H-3), 4.22 (br d, J = 12.1 Hz, H-26a), 4.28 (br t, J = 10.0 Hz, H-2), 4.37 (br d, J = 12.1 Hz, Gal H-6a), 4.48–4.55 (4H, m, H-16,26b, Gal H-2,6b), 4.61 (br s, Gal H-4), 5.03 (dd, J = 5.8, 11.8 Hz, H-23), 5.04 (br s, H-27a), 5.33 (d, J = 7.9 Hz, Gal H-1), 5.63 (br d, J = 5.5 Hz, H-6), 5.66 (br s, H-27b). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.9. *Atropuroside F (6)*

White amorphous powder $[\alpha]_D = -27.4^\circ$ (c = 0.57, MeOH). FABMS (negative ion mode): *m/z* 785 [M−H]$^-$, 623 [M-162(Glc or Gal)−H]$^-$. HRESIMS (negative ion mode): *m/z* 785.3982 [M($C_{39}H_{62}O_{16}$)−H]$^-$ (calcd. for 785.3959). ^{1}H NMR (pyridine-d_5): δ 0.93 (s, CH_3-18), 1.19 (d, J = 6.8 Hz, CH_3-21), 1.24 (s, CH_3-19), 1.50 (m, H-15β), 1.58 (2H, m, H-9, 11β),1.85 (m, H-7), 2.00 (2H, m, H-17, 15α), 2.19 (2H, m, H-20,23), 2.19 (m, H-23), 2.66 (dd, J = 6.3, 12.6 Hz, H-4α), 2.67 (m, H-24), 2.78 (br d, J = 12.6 Hz, H-4β), 3.10 (br d, J = 9.8 Hz, H-11α), 3.56 (d, J = 9.2 Hz, H-1), 3.92 (2H, m, H-3, Glc H-5), 4.01 (m, Glc H-2), 4.04 (m, Gal H-5), 4.28 (t, J = 9.2 Hz, H-2), 4.33 (t. J = 9.7 Hz, H-26a), 4.56 (br d, J = 9.7 Hz, H-26b), 4.61 (br s, Gal H-4), 4.87 (d, J = 7.8 Hz, Glc H-1), 4.91 (m, H-16), 5.02 (br s, H-27a), 5.32 (br s, H-27b), 5.34 (d, J = 7.9 Hz, Gal H-1), 5.62 (br d, J = 5.1 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.10. *Enzymatic hydrolysis of 6*

A solution of **6** (29 mg) in H_2O (2 ml) was hydrolyzed with β-glucosidase at 37 °C for 48 h. The reaction mixture was partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract (18 mg) was chromatographed on a silica gel column to afford **4** (12 mg), which was identified by comparison of its NMR spectra with those of the authentic sample isolated from the title plant. The H_2O layer was treated and analyzed as described for **1**, in which D-glucose was detected from TLC (R_f 0.26) and GC analysis (retention times of D- and L-glucose were 18.29 and 18.87 min, respectively).

2.3.11. *Atropuroside G (7)*

White amorphous powder $[\alpha]_D = -22.0^\circ$ (c = 0.28, MeOH). FABMS (negative ion mode): *m/z* 769 [M−H]$^-$, 607 [M-162(Glc)−H]$^-$. HRESIMS (negative ion mode): *m/z* 769.4003 [M($C_{39}H_{62}O_{15}$)−H]$^-$ (calcd. for 769.4010). ^{1}H NMR (pyridine-d_5): δ 0.85 (s, CH_3-18), 1.10 (d, J = 6.7 Hz, CH_3-21), 1.23 (s, CH_3-19), 1.49 (m, H-15β), 1.54 (2H, m, H-9, 11β), 1.77 (dd, J = 6.2, 8.2 Hz, H-17), 1.98 (m, H-15α), 2.20 (m, H-20), 2.38 (m, H-24), 2.63 (dd, J = 5.7, 12.5 Hz, H-4α), 2.78 (t, J = 12.5 Hz, H-4β), 3.07 (br d, J = 10.4 Hz, H-11α), 3.57 (d, J = 9.3 Hz, H-1), 3.74 (br d, J = 12.0 Hz, Xyl H-5a), 3.92 (m, H-3), 4.28 (t, J = 9.3 Hz, H-2), 4.31 (br d, J = 12.0 Hz, Xyl H-5b), 4.35 (br d, J = 12.9 Hz, H-26a), 4.45 (m, H-16), 4.50 (t, J = 7.9 Hz, Xyl H-2), 4.61 (br d, J = 12.9 Hz, H-26b), 4.91 (d, J = 7.7 Hz, Glc H-1), 5.04 (br s, H-27a), 5.26 (d, J = 7.9 Hz, Xyl H-1), 5.34 (br s, H-27b), 5.65 (d, J = 5.8 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.12. *Acid hydrolysis of 7*

Compound **7** (10 mg) was subjected to acid hydrolysis as described for **1** to give spirost-5,25(27)-diene-1β,2α,3β-triol (**4a**, 4 mg) [5], which was identified by direct TLC and NMR comparison with an authentic sample.

2.3.13. *Enzymatic hydrolysis of 7*

Compound **7** (30 mg) was enzymatic hydrolyzed with β-glucosidase as described for **6**. The reaction mixture was dried and applied to silica gel CC eluting with $CHCl_3$–MeOH (8:2) to give **7b** (11 mg): white amorphous powder $[\alpha]_D = -17.0^\circ$ (c = 0.14, MeOH). FABMS (negative ion mode): *m/z* 575 [M−H]$^-$. HRESIMS (negative ion mode): *m/z* 575.3226 [M($C_{32}H_{48}O_9$)−H]$^-$ (calcd. for 575.3220). ^{1}H NMR (pyridine-d_5): δ 0.85 (s, CH_3-18), 1.02 (d, J = 6.9 Hz, CH_3-21), 1.24 (s, CH_3-19), 1.52 (br d, J = 14.1 Hz, H-11β), 1.90 (m, H-20), 2.02 (m, H-15), 2.23 (br d, J = 13.5 Hz, H-24a), 2.64 (dd, J = 5.6, 12.2 Hz, H-4α), 2.70 (m, H-24b), 2.80 (t, J = 12.2 Hz, H-4β), 3.08 (br d, J = 14.1 Hz, H-11α), 3.58 (d, J = 9.3 Hz, H-1), 3.74 (br d, J = 12.1 Hz, Xyl H-5a), 3.93 (m, H-3), 4.02 (br d, J = 12.2 Hz, H-26a), 4.08 (dd, J = 5.4, 12.1 Hz, Xyl H-5b), 4.30 (t, J = 9.3 Hz, H-2), 4.45 (br d, J = 12.2 Hz, H-26b), 4.54 (m, H-16), 5.27 (d, J = 7.9 Hz, Xyl H-1) 5.64 (br d, J = 5.8 Hz, H-6). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

2.3.14. *Antifungal bioassay*

Susceptibility testing was performed using a modified version of the NCCLS methods [6,7] using organisms obtained from the American Type Culture Collection (Manassas, VA) including *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906. Briefly, samples (dissolved in DMSO) are serially diluted using 20%/0.9% DMSO/saline and transferred in duplicate to 96 well flat bottom microplates. Fungal inocula are prepared by diluting saline suspensions of colonies/spores in broth [RPMI 1640/2% dextrose/MOPS at pH 4.5 (Cellgro) for *Candida* spp., Sabouraud Dextrose for *C. neoformans*, RPMI 1640/2% dextrose/MOPS at pH 7.3 + 5% Alamar Blue (Biosource) for *A. fumigatus*] based on OD_{630} readings using the EL-340 Biokinetics Reader (Bio-Tek Instruments, VT) to afford recommended target inocula using the 0.5 McFarland standard as a reference. The inocula are added to the samples to achieve a final volume of 200 μl and final sample concentration starting with 20 μg/ml. Amphotericin B (ICN Biomedicals, OH) is used as a positive control. All organisms are read at either 630 nm or 544ex/590em (*A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG Lab Technologies, Germany) prior to and after incubation. The minimum inhibitory concentration (MIC) is defined as the lowest test concentration that allows no detectable growth. The minimum fungicidal concentration (MFC) is determined by removing 5 μl from each clear well, transferring to agar and incubating. The MFC is defined as the lowest test concentration that kills 100% of the organism (allows no growth on agar).

2.3.15. *In vitro cytotoxicity bioassay [8,9]*

Cytotoxicity of saponins was tested against a panel of mammalian cells that included five human cell lines [SK-MEL (melanoma), KB (epidermal carcinoma, oral), BT-549 (ductal carcinoma, breast), SK-OV-3 (ovary carcinoma), and Hep G2 (hepatic carcinoma)], and one non-cancerous Vero cells (African green monkey kidney fibroblast). All cells were obtained from ATCC (Manassas, VA). The cells were cultured in 75 cm^2 culture flasks in RPMI-1640 medium (Gibco™, Invitrogen Corporation) supplemented with bovine calf serum (10%) and amikacin (60 mg/L), at 37 °C in an atomosphere of 95% humidity and 5% CO_2. The assay was performed in 96-well microplates. Cells were seeded to the wells of the plate at a density of 25,000 cells/well and incubated for 24 h at 37 °C. Samples, diluted appropriately in RPMI-1640 medium, were added to the cells and again incubated for 48 h. The number of viable cells was determined using Neutral Red assay procedure. Briefly, after incubating with the samples, the cells were washed with saline and incubated for 90 min with a solution of Neutral Red (166 μg/ml). Cells were again washed to remove extracellular dye. A solution of acidified isopropanol (0.33% HCl) was then added to lyse the cells. As a result the incorporated dye is liberated from the viable cells, the absorbance of which is read at 540 nm. IC_{50} (the concentration of the test compound that caused a growth inhibition of 50% after 48 h exposure of the cells) was calculated from the dose curves generated by plotting percent viable cells versus the test concentration on a logarithmic scale using Microsoft Excel. Doxorubicin (Sigma) was used as a positive control.

3. Results and discussion

The *n*-butanol fraction of the methanolic extract of *S. atropurpurea* rhizomes was repeatedly chromatographed on silica gel and reversed phase silica gel (RP-8) columns to afford seven new steroidal saponins (**1**–**7**) and a known compound, dioscin (**8**), which was identified by comparison of its spectroscopic data with reported literature values [10] (Chart 1).

Compound **1** was obtained as a white amorphous powder. The molecular formula was assigned as $C_{38}H_{60}O_{13}$ on the basis of the ^{13}C NMR data (Tables 1 and 2) and negative ion HRESIMS

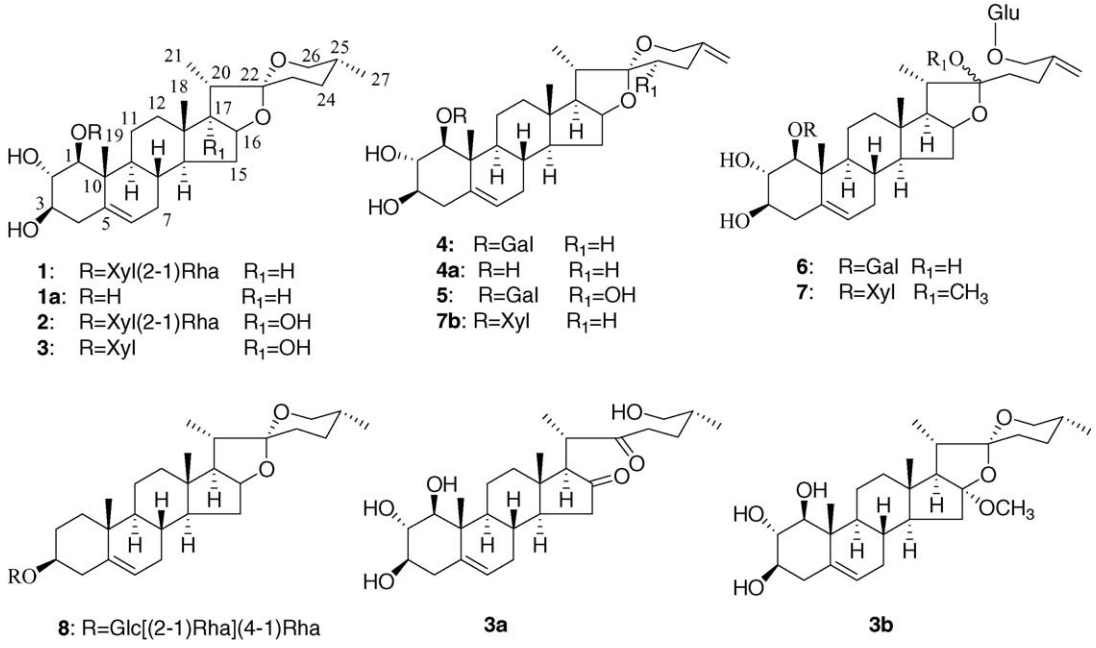

Chart 1 – Steroidal saponins (1–8) isolated from *Smilacina atropurpurea*.

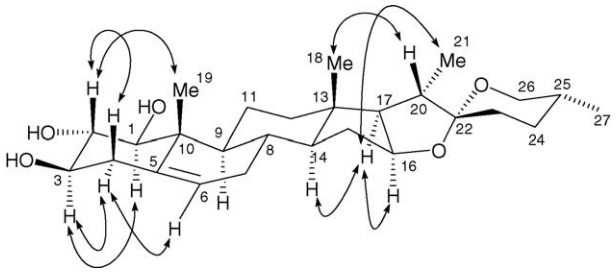

Fig. 1 – Selected ROESY correlations of 1a.

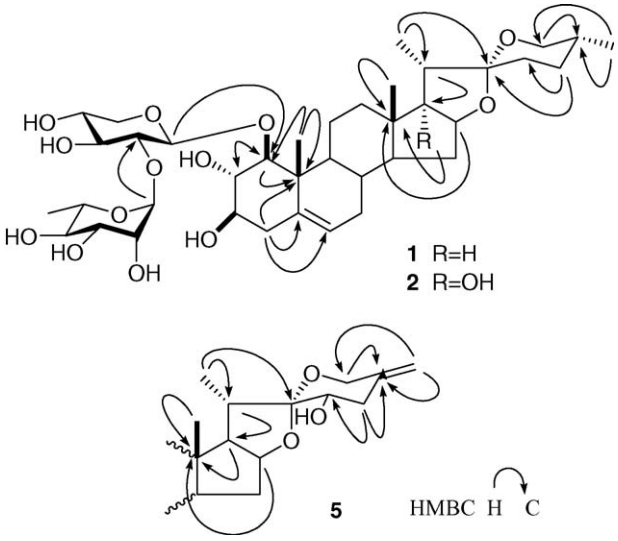

Fig. 2 – Important HMBC correlations of 1, 2 and 5.

([M−H]$^-$, *m*/*z* 723.3930). The negative ion FABMS also showed characteristic fragment ion peaks at *m*/*z* 577 [M-146-H]$^-$, 445 [M-146-132-H]$^-$, suggesting the existence of a terminal deoxyhexosyl unit and a pentosyl unit in the molecule. The ^{1}H NMR spectrum of **1** displayed four methyl proton signals of a typical steroidal skeleton at δ 0.91 (s), 1.35 (s), 0.67 (d, J = 5.4 Hz), and 1.10 (d, J = 6.9 Hz), as well as two anomeric proton signals at δ 5.57 (d, J = 7.9 Hz), and 6.51 (br s). The ^{13}C NMR and DEPT spectrum showed a quaternary carbon signal at δ 109.3, which is the characteristic C-22 of a spirostanol skeleton [11,12]. The ^{1}H NMR and DEPT spectra also exhibited an olefinic proton at δ 5.59 (br d) and two olefinic carbon signals at δ 138.1 (C) and 124.9 (CH), respectively, indicating the presence of a double bond between C-5 and C-6 [13]. These observations suggested that **1** was a spirostanol diglycoside. Acid hydrolysis of **1** produced a new steroidal sapogenin **1a**, and D-xylose and L-rhamnose as sugar residues which were determined by GC analysis of their corresponding trimethylsilylated L-cysteine adducts [4]. The *J* values of the anomeric proton signals indicated the β- and α-configuration at the anomeric centers of D-xylopyranose and L-rhamnopyranose, respectively.

Sapogenin **1a** was a white amorphous powder and had a molecular formula $C_{27}H_{42}O_5$, as deduced by the negative ion HRESIMS ([M−H]$^-$, *m*/*z* 445.2947), which had two more oxygen atoms than that of diosgenin [(25*R*)-3β-hydroxy-spirost-5-ene]. Comparison of the ^{13}C NMR data of **1a** with those of diosgenin [13] indicated that **1a** was 1,2-dihydroxy diosgenin since C-1 and C-2 were shifted to downfield at δ 82.6 (C-1) and 78.2 (C-2) in **1a** from δ 37.2 and 31.6 in diosgenin, respectively. The 1,2,3-trihydroxy substitution on ring-A of this sapogenin was actually confirmed by the COSY experiment of saponin **1**, which established a clear spin network from H-1 → H-2 → H-3 → H-4. The large coupling constants in **1a** between H-1 and H-2 (9.1 Hz), H-2 and H-3 (9.1 Hz), and H-3 and H-4 (11.9 Hz) indicated that three protons at C-1, C-2 and C-3 adopted axial orientations in a chair form conformation of ring-A. This was supported by the ROESY experiment (Fig. 1), in which correlations of H-1 (δ 3.64) with H-3 (δ 3.91), H-3 with H-4α (δ 2.65), H-2 (δ 4.04) with H-4β (δ 2.80) and CH_3-19 (δ 1.33) were observed. Thus, the three hydroxyl groups should be equatorially oriented and possessed the stereochemistry of 1β, 2α, 3β. Consequently, the structure of **1a** was elucidated to be (25*R*)-1β,2α,3β-trihydroxy-spirost-5-ene.

The sequence of the sugars and linkage site to the aglycone of **1** were determined by 2D NMR experiments. In the HMBC spectrum of **1**, correlations of δ 6.51 (Rha H-1) with δ 77.9 (Xyl C-2), and δ 5.57 (Xyl H-1) with δ 88.7 (C-1) were observed. Other key HMBC correlations are shown in Fig. 2. Therefore, **1** was determined to be (25*R*)-2α,3β-dihydroxy- spirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranoside, and named atropuroside A.

The molecular formula of **2** was assigned to be $C_{38}H_{60}O_{14}$ on the basis of its negative ion HRESIMS ([M−H]$^-$, *m*/*z* 739.3925), which has one more oxygen atom than **1**. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were closely related to those of **1**, except for the obvious differences at C-16 (δ 89.9), C-17 (δ 90.3) and C-21 (δ 10.0). In addition, the ^{13}C NMR and DEPT spectra indicated that C-17 in **2** was a quaternary carbon while that in **1** was a tertiary carbon. These observations suggested that **2** had one more hydroxyl group α-oriented at C-17 than **1**, which was supported by comparison of the ^{13}C NMR data of the B–F rings of **2** with those of pennogenin [(25*R*)-3β,17α-dihydroxy-spirost-5-ene] [14]. The HMBC spectrum of **2** (Fig. 2) confirmed the above elucidation. Moreover, the ROESY experiment also proved the 1β,2α,3β- stereochemistry of the hydroxyl groups at C-1, C-2, and C-3. The 17α-OH configuration was further supported by the ROESY correlations between 20-H and 18-CH_3 and between 16-H and 15α-H on the *cis*-fused D/E ring system. Therefore, the structure of atropuroside B (**2**) was assigned to be (25*R*)-2α,3β,17α-trihydroxy-spirost-5-en-1β-y1 *O*-[*O*-α-L-rhamnopyranosyl(1 → 2)]-β-D-xylopyranoside.

Compound **3** had a molecular formula $C_{32}H_{50}O_{10}$, as deduced from the negative ion HRESIMS ([M−H]$^-$, *m*/*z* 593.3333). The ^{1}H and ^{13}C NMR signals of the aglycone moiety of **3** were identical to those of **2**; however, there was only one anomeric proton signal at δ 5.38 (d, J = 7.5 Hz) appearing in the ^{1}H NMR spectrum, and five carbon signals (δ 107.0, 75.9, 79.0, 71.1, 67.7) due to a D-xylose moiety in the ^{13}C NMR spectrum. These observations suggested that **3** lacked L-rhamnose unit in sugar part compared to **2**. Thus, the structure of **3** was deduced to be (25*R*)-2α,3β,17α-trihydroxy-spirost-5-en-1β-y1 *O*-β-D-xylopyranoside, and named atropuroside C.

Saponins **2** and **3** possess the same new aglycone, which has one more hydroxyl group attached at C-17 when compared with atropurogenin A (**1a**), the new aglycone of atropuro-

side A (**1**). When treated with 2 M HCl-dioxane (1:1), saponin **3** gave two new artifact sapogenins **3a** and **3b**. Based on the analysis of IR, HRESIMS, 1D NMR [15] and 2D NMR data, the structures of **3a** and **3b** were readily assigned as 1β,2α-dihydroxy- kryptogenin (**3a**) and (25*R*)-16α-methoxyl-1β,2α,3β-trihydroxy-spirost-5-ene (**3b**), respectively. The formation of similar compounds, kryptogenin and a 16-methoxy analog, has been demonstrated in the case of HCl-methanol hydrolysis of pennogenin bearing a 17α-hydroxy group that is susceptible to acidic treatment [16]. The presence of a methoxy group at C-16 in **3b**, instead of a presumable hydroxy group according to the reaction mechanism as previously described [16] is attributed to the conversion of this C-16 tertiary hydroxy group to the methoxy group in the presence of methanol during the work-up and isolation process, as evidenced by the facile conversion of 22-OH furostanol saponins into corresponding 22-OMe furostanol saponins in the solution of methanol at room temperature [12]. The α-configuration of the 16-OCH_3 in **3b** has been firmly determined by the ROESY spectrum, in which correlations of H-17 (δ 2.07) with CH_3-21 (δ 1.15), H-20 (δ 2.07) with CH_3-18 (δ 0.87), H-15α (δ 2.20) with 16-OCH_3 (δ 3.45) were observed on a *cis*-fused D/E ring system.

Compound **4** was shown to have a molecular formula $C_{33}H_{50}O_{10}$ on the basis of HRESIMS ($[M-H]^-$, *m/z* 605.3314) and ^{13}C NMR spectrum. The typical methyl signals at δ 0.87 (s), 1.25 (s), and 0.96 (d, *J* = 6.8 Hz) in the ^1H NMR spectrum, and olefinic carbon signals at δ 138.0 and 125.1 and a quaternary carbon signal at δ 109.4 (C-22) in the ^{13}C NMR spectrum indicated that **4** was also a Δ^5-spirostanol glycoside. Furthermore, methyl proton doublet of C-27 was replaced by an exocyclic methylene as evidenced by the presence of two olefinic carbons at δ 144.6 (C-25) and 108.6 (CH_2-27) [15]. Only one anomeric proton signal was noted at δ 5.35 (d, *J* = 7.9 Hz) in its ^1H NMR spectrum.

Acid hydrolysis of **4** afforded D-galactose as sugar residue and 1β,2α,3β-trihydroxy-spirost-5,25(27)-diene (**4a**) as sapogenin, whose structure was identified by comparison of its spectroscopic data with **1a** and literature values [5]. The location of the galactose unit at C-1 in **4** was readily established by the HMBC spectrum, which exhibited correlations of the anomeric proton (δ 5.35) of galactose unit to C-1 (δ 93.0) of the aglycone. Therefore, the structure of **4** was determined to be 2α,3β-dihydroxy-spirost-5,25(27)-diene-1β-y1 *O*-β-D-galactopyranoside, and named atropuroside D.

Based on the negative ion HRESIMS ($[M-H]^-$, *m/z* 621.3274), the molecular formula of **5** was determined to be $C_{33}H_{50}O_{11}$, which had one more oxygen atom than that of **4**. The NMR data of **5** including the sugar residue were closely related to that of **4**, except for signals arising from F-ring of the aglycone. Instead of a methylene carbon (C-23) at δ 33.2 in **4**, an oxygen-bearing methine signal at δ 67.1 which correlated with a proton signal at δ 5.03 (dd, *J* = 5.8, 11.8 Hz) in the HMQC spectrum. These observations suggested that **5** had one more hydroxyl group substituted in F-ring. The chemical shift values of C/H-23, proton coupling constants of H-23, and detailed 2D NMR analysis of COSY and HMBC correlations (Fig. 2) revealed the hydroxyl group was linked at C-23 position. The big coupling constant (11.8 Hz) assigned the axial orientation of H-23, corresponding to α-configuration of 23-OH, which was consistent with those reported for similar compounds [17], and was confirmed by the ROESY correlations of H-23 (δ 5.03) with H-20 (δ 2.01). Therefore, the structure of atropuroside E (**5**) was assigned as 2α,3β,23α-trihydroxy-spirost-5,25(27)-diene-1β-y1 *O*-β-D-galactopyranoside.

Compound **6** was obtained as a white amorphous powder and showed a positive reaction (red color) to the Ehrlich reagent [11,12]. Its molecular formula was assigned as $C_{39}H_{62}O_{16}$ on the basis of the negative ion HRESIMS ($[M-H]^-$, *m/z* 785.3982). Compound **6** exhibited two anomeric proton signals at δ 4.87 (d, *J* = 7.8 Hz) and 5.34 (d, *J* = 7.9 Hz) in the ^1H NMR spectrum, suggesting that two sugar units existed in the molecule. Enzymatic hydrolysis of **6** yielded D-glucose as the sugar residue and the corresponding spirostanol glycoside which was identified as **4**. These observations suggested that **6** was 26-*O*-D-glucopyranosyl furostanol glycosides [11,12]. Therefore, the structure of atropuroside F (**6**) was determined to be 26-*O*-β-D-glucopyranosyl-2α,3β,22ξ-trihydroxy-furost-5,25(27)-diene-1β-y1 *O*-β-D-galactopyranoside.

Compound **7** was also a furostanol glycoside as indicated by the positive Ehrlich reagent. Its molecular formula was determined as $C_{39}H_{62}O_{15}$ by HRESIMS ($[M-H]^-$, *m/z* 769.4003). Two anomeric proton signals at δ 4.91 (d, *J* = 7.7 Hz) and 5.26 (d, *J* = 7.9 Hz) were observed in the ^1H NMR spectrum. The appearance of a methoxyl signal around δ 47.4 in the ^{13}C NMR spectrum and the downfield shift of C-22 to δ 112.5 suggested that the 22-OH was methylated [11,13]. When treated with 2 M HCl, **7** gave 1β,2α,3β-trihydroxy-spirost-5,25(27)-diene (**4a**) as the aglycone and D-xylose and D-glucose as sugar residues. Enzymatic hydrolysis of **7** gave a new spirostanol glycoside (**7b**), in addition to the D-glucose residue. The location of D-xylose in **7b** was determined to be at C-1 position based on its HMBC spectrum, in which a correlation of the anomeric proton signal at δ 5.27 (d, *J* = 7.9 Hz, Xyl H-1) with C-1 (δ 93.0) was observed. Therefore, atropuroside G (**7**) was determined as 26-*O*-β-D-glucopyranosyl-22ξ-methoxyl-2α,3β-dihydroxy-furost-5,25(27)-diene-1β-y1 *O*-β-D-xylopyranoside.

Saponins **1–8** were examined for their antifungal activity against *C. albicans*, *C. glabrata*, *C. krusei*, *C. neoformans*,

Table 3 – Antifungal activity of saponins 2, 6 and 8 (MIC/MFC, μg/ml)

Compounds	*C. albicans*	*C. glabrata*	*C. krusei*	*C. neoformans*	*A. fumigatus*
2	20/20	NA/NA	NA/NA	10/10	20/20
6	10/10	20/20	NA/NA	5.0/5.0	20/20
8	2.5/2.5	5.0/NA	NA/NA	NA/NA	NA/NA
Amphotericin B	0.16/0.63	0.31/0.63	1.25/1.25	1.25/1.25	0.63/2.50

MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration; NA, not active at the highest test concentration of 20 μg/ml.

Table 4 – Cytotoxicity of saponins 2, 6, and 8 (IC_{50}, μg/ml)

Compounds	SK-MEL	KB	BT-549	SK-OV-3	HepG2	Vero
2	6.4	6.8	5.4	8.5	4.8	5.5
6	5.4	4.0	2.9	6.0	NA	2.8
8	2.8	1.9	3.0	4.8	6.8	3.7
Doxorubicin	0.9	0.16	0.25	1.7	0.6	8.0

IC_{50}: 50% inhibitory concentration; SK-MEL: human malignant, melanoma; KB: human epidermal carcinoma, oral; BT-549: human ductal carcinoma, breast; SK-OV-3: human ovary carcinoma; Hep G2: human hepatic carcinoma; Vero: African monkey kidney fibroblasts; NA: not active.

and *A. fumigatus* using amphotericin B as a positive control. Their minimum inhibitory concentrations (MIC) and minimum fungicidal concentration (MFC) are shown in Table 3. Astropuroside B (**2**) and F (**6**) exhibited moderate inhibitory activity against *C. albicans*, *C. glabrata*, *C. neoformans* and *A. fumigatus*. Dioscin (**8**), one of the major components of *S. atropurpurea*, was more active against *C. albicans* and *C. glabrata* with MICs of 2.5 μg/ml and 5.0 μg/ml, respectively. Dioscin was previously reported to be antifungal against *C. albicans* [18] and *Piricularia pryzae* [19,20]. The remaining compounds (**1**, **3–5**, **7**) were inactive.

The antifungal saponins **2**, **6**, and **8** were further evaluated for their cytotoxicities in human cancer cell lines (SK-MEL, KB, BT-549, SK-OV-3, and HepG2) as well as non-cancerous Vero cells *in vitro*. As shown in Table 4, they exhibited moderate cytotoxicity when compared to the positive control doxorubicin. It appears that the antifungal activity of these steroidal saponins has a correlation with their cytotoxicity against mammalian cells.

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