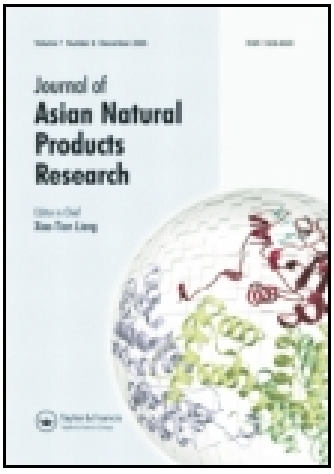

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Jiang-Ming Cui [a b], Li-Ping Kang [b c], He-Shui Yu [b c], Yang Zhao [b], Cheng-Qi Xiong [b], Chao Liu [b] & Bai-Ping Ma [b]

[a] School of Pharmaceutical Sciences, Central South University, Changsha, 410013, China

[b] Department of Biotechnology, Beijing Institute of Radiation Medicine, Beijing, 100850, China

[c] Graduate School of Tianjin University of Traditional Chinese Medicine, Tianjin, 300193, China

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Two new furostanol saponins from *Aspidistra typica*

Jiang-Ming Cui[ab], Li-Ping Kang[bc], He-Shui Yu[bc], Yang Zhao[b], Cheng-Qi Xiong[b], Chao Liu[b]* and Bai-Ping Ma[b]*

[a]*School of Pharmaceutical Sciences, Central South University, Changsha 410013, China;*
[b]*Department of Biotechnology, Beijing Institute of Radiation Medicine, Beijing 100850, China;*
[c]*Graduate School of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China*

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Two new furostanol saponins (**1** and **2**), along with one known saponin (**3**), were obtained from the rhizomes of *Aspidistra typica* Baill. Their structures were elucidated as (25*R*)-26-*O*-β-D-glucopyranosyl-furost-5-ene-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**1**, typaspidoside A), (25*S*)-26-*O*-β-D-glucopyranosyl-furost-5-ene-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**2**, 25*S*-typaspidoside A), and timosaponin H_1 (**3**), based on the integrative spectroscopic analysis of 1D- and 2D-NMR experiments, ESI-MS data and chemical evidence. The investigation on the chemical components of this plant is reported for the first time.

Keywords: *Aspidistra typica*; furostanol saponins; typaspidoside A; 25*S*-typaspidoside A

1. Introduction

The family Convallariaceae, mainly consisting of *Speirantha*, *Convallaria*, *Theropogon*, *Rohdea*, *Reineckia*, *Aspidistra,* and *Tupistra*, is known for its rich source of steroidal saponins. To our best knowledge, many steroidal saponins with the characterized skeleton of polyhydroxylated A/B ring were reported from Convallariaceae [1]. *Aspidistra typica* Baill. is a perennial herbaceous plant which belongs to the genus *Aspidistra* with approximately 50 species [2]. It is being used to treat fractures, congestion, and snakebite as a folk herbal medicine. However, no more than 25 compounds were isolated from the genus *Aspidistra* [3–12]. With a purpose of searching for steroidal saponins with novel structures and bioactivities, studies on the constituents of *A. typica* were carried out. It was described here that two new steroidal saponins, along with one known compound, were isolated from the dried rhizomes of *A. typica*, and their structures were characterized by 1D-NMR (^{1}H and ^{13}C NMR), 2D-NMR (correlated spectrometry (COSY), HSQC, and heteronuclear multiple bond coherence (HMBC)), MS data, and chemical evidence.

2. Results and discussion

The ethanol extracts of *A. typica* were extracted with *n*-BuOH to obtain the total saponins. The *n*-BuOH fraction was chromatographed on silica gel, MCI CHP20/P50, and octadecylsilyl (ODS) silica gel columns, and finally purified by semi-preparative high performance liquid chromatography (HPLC) to give three compounds **1**–**3** (Figure 1). By comparing the NMR and MS data with those in the literature [13], compound **3** was determined as timosaponin H_1.

*Corresponding authors. Email: liuchao9588@sina.com; mabaiping@sina.com

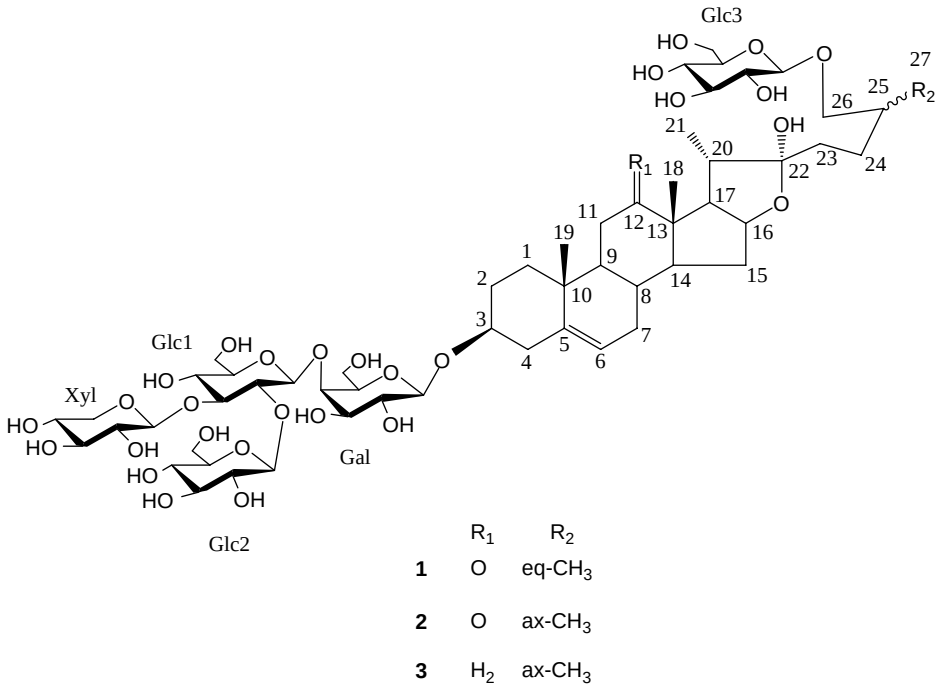

Figure 1. The structures of compounds **1**–**3**.

Compound **1** was isolated as a white amorphous solid, which showed positive color reaction in Liebermann–Burchard and Ehrlich reagent tests, indicating a furostanol saponin. Its molecular formula $C_{56}H_{90}O_{29}$ was deduced from the pseudomolecular ion peak at *m/z* 1225.5457 $[M-H]^-$ in the HR-ESI-MS, which was further supported by the ^1H and ^{13}C NMR data. The existence of four hexose units and one pentose unit was proved by the fragment ion peaks in the negative-ion ESI-MS at *m/z* 1093 $[M-H-132]^-$, 1063 $[M-H-162]^-$, 931 $[M-H-132-162]^-$, 751 $[M-H-132-162-162-18]^-$, 589 $[M-H-132-162-162-162-18]^-$, and 427 $[M-H-132-162-162-162-18-162]^-$. The sugar moieties consisting of one D-galactose, three D-glucoses, and one D-xylose were established by acid hydrolysis experiment. Five anomeric proton signals at δ 4.80 (d, $J = 7.8$ Hz, H-1 of Glc3), 4.86 (d, $J = 7.8$ Hz, H-1 of Gal), 5.18 (d, $J = 8.4$ Hz, H-1 of Glc1), 5.22 (d, $J = 7.8$ Hz, H-1 of Xyl), and 5.57 (d, $J = 7.8$ Hz, H-1 of Glc2) were observed, and the β-configuration of each sugar residue was confirmed by the $J_{1,2}$ value (>7.0 Hz) in the ^1H NMR spectrum [14]. Meanwhile, five anomeric carbon signals at δ 105.0 (C-1 of Glc3), 102.8 (C-1 of Gal), 105.2 (C-1 of Glc1), 105.0 (C-1 of Xyl), and 104.9 (C-1 of Glc2) were assigned in the HSQC spectrum.

In the ^1H NMR spectrum, two singlet signals at δ 1.14 (3H, s, CH_3-18) and 0.89 (3H, s, CH_3-19) and two doublet signals at δ 1.53 (3H, d, $J = 6.6$ Hz, CH_3-21) and 0.97 (3H, d, $J = 7.2$ Hz, CH_3-27) were suggested to be characteristic steroidal methyls. A series of typical carbon signals at δ 140.8, 121.4, 110.8, and 212.8 revealed the presence of $\Delta^{5(6)}$, α-orientation of 22-OH [15], and one ketonic carbonyl group in the furostanol skeleton. The ketonic carbonyl group at C-12 was confirmed by the HMBC correlations between C-12 at δ 212.8 and H_a-11 at δ

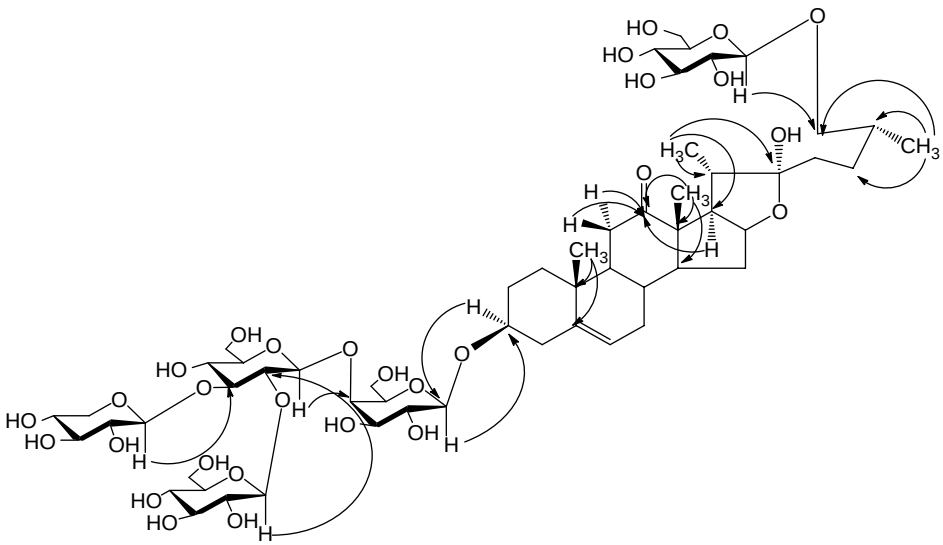

Figure 2. Key HMBC correlations for compound **1**.

2.27, H_b-11 at δ 2.51, H-18 at δ 1.14, and H-17 at δ 2.93 (Figure 2). According to the chemical shift difference between two proton signals of C-26 ($\Delta_{ab} = 0.34$), the C-25 configuration was recognized as *R* [16]. By comparing the NMR data with those in the literature, aglycone of **1** was elucidated as (25*R*)-furost-5-ene-3β,22α,26-triol-12-one [17,18].

Connections among sugars and the sugar linkage sites to the aglycone were validated by the HMBC correlations of H-1 of Glc3 at δ 4.80 with C-26 at δ 75.3, H-1 of Xyl at δ 5.22 with C-3 of Glc1 at δ 86.8, H-1 of Glc2 at δ 5.57 with C-2 of Glc1 at δ 81.4, H-1 of Glc1 at δ 5.18 with C-4 of Gal at δ 79.9, and H-1 of Gal at δ 4.86 with C-3 at δ 77.9 (C-3) (Figure 2). Finally, all analyses above led us to determine the structure of **1** as (25*R*)-26-*O*-β-D-glucopyranosyl-furost-5-en-12-one-3β,22α,26-triol-3-O-*β*-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, named typaspidoside A.

Compound **2** was obtained as a white amorphous powder. It was also suggested to be a furostanol saponin based on its positive Liebermann–Burchard and Ehrlich reactions. The HR-ESI-MS showed a quasi-molecular ion peak at *m/z* 1225.5453 $[M-H]^-$, corresponding to the molecular formula $C_{56}H_{90}O_{29}$. All the MS and NMR data of **1** and **2** were closely similar, except that the chemical shifts of two protons of C-26 were slightly but distinctly different between **1** and **2** in the ^{1}H NMR spectrum. The signals at δ 4.07 (1H, 26-H_a) and 3.49 (1H, 26-H_b) of **2** were instead of the signals at δ 3.94 (1H, 26-H_a) and 3.60 (1H, 26-H_b) of **1**. So the C-25 configuration of **2** should be *S* [16]. Accordingly, the structure of **2** was determined as (25*S*)-26-*O*-β-D-glucopyranosyl-furost-5-en-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, an epimer of **1**, named 25*S*-typaspidoside A.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 343 polarimeter (Perkin-Elmer, Waltham, MA, USA). IR spectra were recorded on a Vertex 70 spectrometer (Bruker Optics, Billerica, Germany). The

NMR spectra were recorded with a Varian ^{UNITY}*INOVA* 600 (599.8 MH_Z for ^1H NMR and 150.8 MH_Z for ^{13}C NMR; Palo Alto, CA, USA), with tetramethylsilane as internal standard and pyridine-d_5 as solvent. The HR-ESI-MS was recorded on Synapt MS system (Waters Corporation, Milford, MA, USA). Gas chromatographic analyses were carried out by using an Agilent 6890 system equipped with a 5973 mass spectrograph detector and an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, Milford, MA, USA). MCI CHP20/P50 (Mitsubishi Chemical, Tokyo, Japan), ODS silica gel (120 Å, 50 μm, YMC, Kyoto, Japan), and silica gel H (Qingdao marine Chemical, Qingdao, China) were used for chromatography. Thin layer chromatography was carried out on silica gel 60 plates (Merck, Darmstadt, Germany). HPLC (Agilent Technologies, Santa Clara, CA, USA) was carried out using Agilent 1100 system: an analytical column, ODS (5 μm, 4.6 × 250 mm, Agela, Tianjin, China); preparative column, an Agela Venusil XBP C18 (5 μm, 10.0 × 250 mm, Agela); Shodex RID 102 detector (Showa Denko Group, Tokyo, Japan); and Alltech evaporative light scattering detector (ELSD) (evaporative light-scattering detector) 2100.

3.2 Plant material

The rhizomes of *A. typica* were harvested from Xishuangbanna, Yunnan Province, China in August 2011, and identified by Prof. Li-xia Zhang (Yunnan Branch of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences). A voucher specimen (No. 110820) has been deposited in the Herbarium of Beijing Institute of Radiation Medicine, Beijing.

3.3 Extraction and isolation

The air-dried rhizome slices of *A. typica* (2.0 kg) were extracted with 60% EtOH–H_2O for three times (2 h each time) by reflux. The combined extracts were evaporated to about 2 liters under reduced pressure and the condensate was extracted with isovolumetric *n*-BuOH for three times. After removing the solvent from the *n*-BuOH layer by concentrating and freeze drying, the powder of total saponin (92.0 g) was obtained. It was initially separated by a silica gel and eluted with a gradient mixture of $CHCl_3$–MeOH (3:1, 2:1, 1:1) to give five fractions (A–E).

A part of fraction D (7.9 g) was subjected to MCI CHP20/P50 column chromatography and eluted with 30% EtOH–H_2O to produce 14 subfractions (D_1–D_{14}). Fraction D_1 (1.12 g) was chromatographed on an ODS silica gel column with a gradient mixture of MeOH–H_2O (3:7, 7:13, 2:3, 9:11, and 8:2; 50 ml each tube) as eluent. A total of 150 tubes were collected. Then, tubes 74–86 (94 mg) were combined and further isolated by semi-preparative HPLC (column: 10 × 250 mm, RP-18, 5 μm) with MeOH–H_2O (41:59, 4.0 ml/min) to give compound **1** (34.9 mg, t_R = 42.9 min) and compound **2** (15.3 mg, t_R = 39.4 min). A part of fraction D_6 (128.6 mg) was purified by semi-preparative HPLC with ACN–H_2O (27:73, 4.0 ml/min) to afford compound **3** (80.9 mg, t_R = 21.6 min).

3.3.1 Compound *1*

White amorphous solid; $[\alpha]_D^{20}$ −62.5 (*c* 0.080, pyridine). IR (KBr) ν_{max}: 3413, 2931, 1706, 1647, 1377, and 1073 cm^{-1}. ^1H NMR (pyridine-d_5, 599.8 MHz) and ^{13}C NMR (pyridine-d_5, 150.8 MHz) spectral data, see Table 1. ESI-MS (neg.): *m/z* 1093 $[M-H-132]^-$, 1063 $[M-H-162]^-$, 931 $[M-H-132-162]^-$, 751 $[M-H-132-162-180]^-$, 589 $[M-H-132-162-162-180]^-$, and 427 $[M-H-132-162-162-180-162]^-$. HR-ESI-MS (neg.): *m/z* 1225.5457 $[M-H]^-$ (calcd for $C_{56}H_{89}O_{29}$, 1225.5490).

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (δ in pyridine-d_5).

	1		2	
Position	δ_C	δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)
1	37.0	0.85 m, 1.45 o[a]	37.0	0.85 m, 1.45 o
2	29.9	1.62 m, 2.03 o	29.9	1,63 m, 2.03 o
3	77.9	3.82 m	77.9	3.82 m
4	39.1	2.37 m, 2.65 m	39.1	2.37 m, 2.65 m
5	140.8	–	140.8	–
6	121.4	5.25 br s	121.3	5.25 br s
7	31.8	1.44 o, 1.85 m	31.8	1.45 o, 1.84 m
8	30.9	1.83 m	30.9	1.83 m
9	52.3	1.28 m	52.3	1.28 m
10	37.6	–	37.6	–
11	37.6	2.27 dd (5.7, 14.5), 2.51 dd (13.3, 14.5)	37.6	2.27 dd (5.6, 14.7), 2.51 m
12	212.8	–	212.8	–
13	55.3	–	55.3	–
14	55.9	1.40 o	55.9	1.40 o
15	31.8	1.60 m, 2.06 m	32.7	1.59 m, 2.06 m
16	79.7	4.86 o	79.7	4.85 o
17	54.8	2.93 dd (6.8, 8.4)	54.8	2.92 dd (6.8, 8.6)
18	16.1	1.14 s	16.1	1.13 s
19	18.8	0.89 s	18.8	0.89 s
20	41.3	2.19 m	41.3	2.19 m
21	15.2	1.53 d (6.6)	15.2	1.52 d (6.6)
22	110.8	–	110.8	–
23	37.1	2.03 o	37.1	1.95 m, 2.05 o
24	28.4	1.66 m, 2.03 o	28.4	1.67 m, 2.04 o
25	34.3	1.91 m	34.4	1.92 m
26	75.3	3.60 dd (6.0, 9.6), 3.94 o	75.4	3.49 dd (6.9, 9.3), 4.07 o
27	17.4	0.97 d (7.2)	17.5	1.02 d (7.2)
Sugar part				
Glc3			Glc3	
1	105.0	4.80 d (7.8)	105.2	4.80 d (7.8)
2	75.2	4.01 t (7.8)	75.2	4.01 t (7.8)
3	78.6	4.22 o	78.7	4.23 o
4	71.8	4.22 o	71.7	4.22 o
5	78.5	3.93 o	78.5	3.93 o
6	62.9	4.37 o, 4.54 o	62.9	4.37 o, 4.53 o
Gal			Gal	
1	102.8	4.86 d (7.8)	102.8	4.86 d (7.8)
2	73.2	4.41 o	72.5	4.40 o
3	75.6	4.09 o	75.6	4.08 o
4	79.9	4.58 o	79.9	4.58 o
5	75.4	3.95 o	75.3	3.95 o
6	60.6	4.16 o, 4.65 m	60.6	4.16 o, 4.65 m
Xyl			Xyl	
1	105.0	5.22 d (7.8)	105.0	5.23 d (7.8)
2	75.1	3.95 o	75.1	3.95 o
3	78.7	4.06 o	78.7	4.07 o
4	70.8	4.09 o	70.8	4.10 o
5	67.4	3.66 t (10.5), 4.21 o	67.4	3.66 t (10.6), 4.21 o
Glc1			Glc1	
1	105.2	5.18 d (8.4)	105.2	5.18 d (8.4)
2	81.4	4.42 t (8.4)	81.4	4.42 t (8.4)
3	86.8	4.16 o	86.8	4.16 o
4	70.5	3.81 m	70.5	3.80 m

Table 1 – *continued*

	1		2	
Position	δ_C	δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)
5	77.6	3.87 m	77.6	3.87 m
6	63.0	4.04 o, 4.50 m	63.0	4.03 o, 4.51 o
Glc2			Glc2	
1	104.9	5.57 d (7.8)	104.9	5.57 d (7.8)
2	76.2	4.06 o	76.3	4.06 o
3	77.7	4.11 o	77.8	4.11 o
4	71.1	4.19 o	71.1	4.19 o
5	78.8	3.92 o	78.8	3.92 o
6	62.5	4.36o, 4.56 o	62.5	4.36 o, 4.56 o

[a] Overlapped with other signals.

3.3.2 *Compound* **2**

White amorphous solid; $[\alpha]_D^{20}$ −53.3 (*c* 0.060, pyridine). IR (KBr) ν_{max}: 3420, 2931, 1707, 1646, 1377, and 1073 cm^{-1}. ^1H NMR (pyridine-d_5, 599.8 MHz), and ^{13}C NMR (pyridine-d_5, 150.8 MHz) spectral data, see Table 1. ESI-MS (neg.): *m/z* 1093 $[M-H-132]^-$, 1063 $[M-H-162]^-$, 931 $[M-H-132-162]^-$, 751 $[M-H-132-162-180]^-$, 589 $[M-H-132-162-162-180]^-$, and 427 $[M-H-132-162-162-180-162]^-$. HR-ESI-MS (neg.): *m/z* 1225.5453 $[M-H]^-$ (calcd for $C_{56}H_{89}O_{29}$, 1225.5490).

3.4 *Acid hydrolysis of compounds 1 and 2*

Compounds **1** (2.7 mg) and **2** (2.2 mg) were separately treated with 2N aq. CF_3COOH (5 ml) at 95°C for 2.5 h. The cooled hydrolysate was extracted with CH_2Cl_2 (5 ml) for three times. The aqueous phase was concentrated to dryness with EtOH to yield the sugar residue. Then it was dissolved in anhydrous pyridine (2 ml), and L-cysteine methyl ester hydrochloride (12 mg) was added. The mixture was stirred at 60°C for 1 h. Furthermore, (hexamethyldisilazane–trimethylchlorosilane, 2:1; 6 ml) was added and preserved at 60°C for another 0.5 h [19]. In the end, the supernatant was analyzed by GC under the following conditions: Agilent Technologies 6890 gas chromatograph equipment; 5973 mass spectrograph detector; HP-5 capillary column (30 m × 0.25 mm × 0.25 μm); column temperature: 180°C–250°C, programmed increase, 15°C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250°C; injection volume: 1.0 μl; split ratio: 1/50. Ultimately, the D-configurations of glucose, galactose, and xylose were determined by comparing their retention times with those of standard samples, respectively. t_R: D-glucose (17.93, 19.67 min), D-xylose (13.01, 14.77 min), and D-galactose (18.54, 20.24 min).

Acknowledgments

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