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Steroidal saponins from *Tacca plantaginea*

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Two new steroidal saponins, taccaoside C (**1**) and taccaoside D (**3**), along with one known saponin (**2**) have been isolated from the methanol extracts of *Tacca plantaginea*. Their structures have been elucidated by spectroscopic and chemical methods.

Keywords: *Tacca plantaginea*; Steroidal saponins; Taccaoside C; Taccaoside D

1. Introduction

Tacca plantaginea (Hance) is a folk medicine used as analgesic, anti-pyretic, anti-inflammatory agents and for the treatment of incised wounds [1]. Previously, we have reported two new steroidal saponins from this plant [2]. Further chemical investigation on the methanol extracts of this plant resulted in the isolation of three other saponins, including two new saponins, taccaosides C and D (**1** and **3**). On the basis of spectral and chemical analysis, their structures have been determined as (25*S*)-3β-hydroxy-spirost-5-ene 3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (1), 26-O-β-D-glucopyranosyl-(25*S*)-3β,22ξ,26-triol-furost-5-ene 3-*O*-α-L-rhamnopyranosyl(1 → 2)-[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (2) and 26-*O*-β-D-glucopyranosyl-(25*S*)-3β,22ξ,26-triol-furost-5-ene 3-*O*-α-L-rhamnopyranosyl (1 → 2)-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (**3**) (figure 1).

2. Results and discussion

Taccaoside C (**1**) was obtained as colourless needles. Negative HRESI-MS gave a $[M - 1]^-$ peak at *m/z* 1029.5287, corresponding to a molecular formula of $C_{51}H_{82}O_{21}$. The IR spectrum of 1 gave characteristic absorption bands at 3243 (hydroxyl groups), 1065, 988, 920, 895, 848 and

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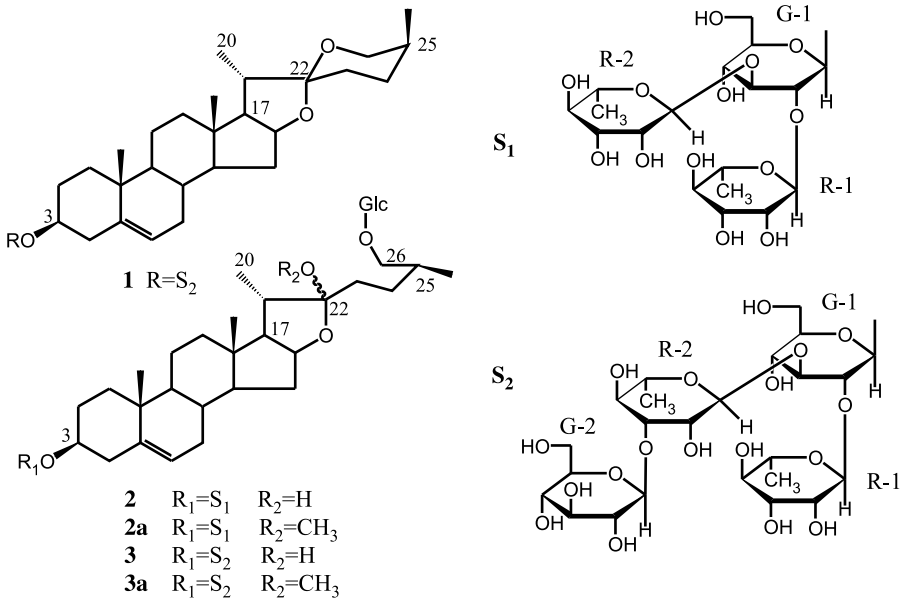

Figure 1. Structures of compounds **1**–**3**.

839 cm^{-1} (intensity: 920 > 895 cm^{-1}), which indicated the presence of a (25*S*)-spirostanol steroidal skeleton in the aglycone [3–5]. Acid hydrolysis of **1** afforded glucose and rhamnose as comparison with authentic samples on TLC and an aglycone. The chemical shifts due to the aglycone were in good agreement with yamogenin [6]. The ^{1}H NMR spectrum of **1** displayed four anomeric proton signals at δ 5.79 (brs), 5.73 (brs), 5.22 (d, $J = 7.75$ Hz) and 4.88 (d, $J = 7.75$ Hz), correlating with the anomeric carbon signals of those sugar moieties at δ 102.6, 103.2, 106.5 and 100.0 in HMQC spectrum, respectively. The linkage sites of each sugar were determined by an HMBC spectrum, which showed long-range correlations between the anomeric proton (δ4.88) of G-1 and C-3 (δ78.4) of the aglycone, the anomeric proton (δ5.79) of R-1 and C-2 (δ 77.9) of G-1, the anomeric proton (δ 5.73) of R-2 and C-3 (δ 86.5) of G-1, the anomeric proton (δ 5.79) of G-2 and C-3 (δ 84.1) of R-2 (figure 2). Each sugar was pyranosyl with β configuration for glucosyl and α configuration for rhamnosyl from the NMR data. Therefore, the structure of **1** was established as (25*S*)-3β-hydroxy-spirost-5-ene 3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside, and was named taccaoside C (**1**).

Taccaoside D (**3**), which was obtained as homogenous states as described in section 3, gave a red colour with Ehrlich's reagent, which suggested this compound was furostanol [7]. Its molecular formula was $C_{57}H_{94}O_{27}$ from their negative HRESI-MS spectrum. In the ^{13}C NMR spectrum of **3** (table 1), the signals due to its aglycone moiety were indicative of a (25*S*)-3β,22ξ,26-triol-furost-5-ene [5], while the signals due to its sugar moiety were identical to those of **1**, except for a set of additional signals corresponding to a β-glucopyranosyl unit. When allowed standing in methanol, **3** gave **3a**, which showed a typical methoxyl signal at δ 3.25 in the ^{1}H NMR spectrum and characteristic carbon signals of a (25*S*)-22-methoxy-3β,26-diol-furost-5-ene aglycone moiety in the ^{13}C NMR spectrum. Thus, the structure of **3** was proved to be 26-*O*-β-D-glucopyranosyl-(25*S*)-3β,22ξ,26-triol-furost-5-ene 3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside, and was named taccaoside D (**3**).

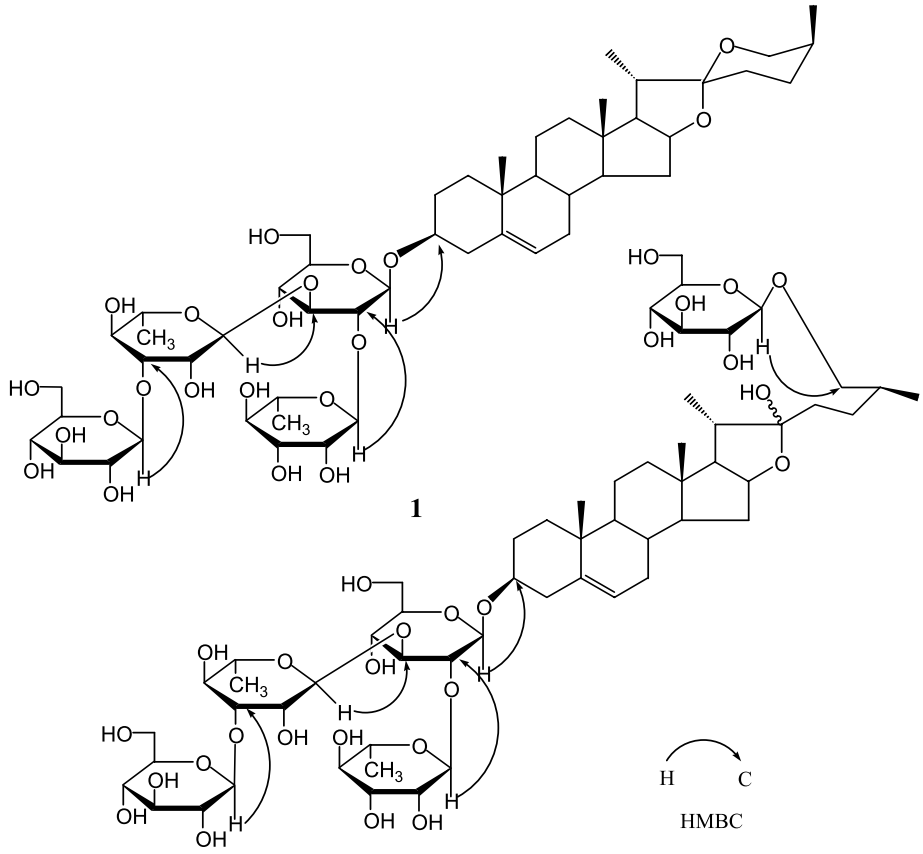

Figure 2. Key HMBC correlations of **1** and **3**.

Saponin **2** was identified as 26-O-β-D-glucopyranosyl-(25*S*)-3β, 22ξ,26-triol-furost-5-ene 3-*O*-α-L-rhamnopyranosyl(1 → 2)-[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside by comparison of its physical and spectral properties with those reported in the literature [8].

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Koffler melting point apparatus by Sichuan University (China) and are uncorrected. Optional rotations were measured on a Japanese Fasco DIP-370 digital polarimeter. NMR spectra were recorded in C_5H_5N on a Bruker DRX-500 spectrometer at room temperature. MS spectra were run on a VG Auto Spec-3000 spectrometer. IR spectra were carried out on a BIO-RADFTS-135 spectrometer with KBr pellets. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China) and silica gel H (60 μm, Qingdao Haiyang Chemical Co. Ltd., China), RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), respectively. TLC spots were detected by spraying with 10% H_2SO_4 followed by heating.

Table 1. ^{13}C NMR spectral data in pyridine-d_5 at 100 MHz for compounds **1**, **3** and **3a**.

No.	***1***	***3***	***3a***		*No.*	***1***	***3***	***3a***
1	37.6	37.7	37.6	Glc-1	1′	100.0	100.1	100.0
2	30.1	30.2	30.2		2′	77.9	78.1	78.6
3	78.4	78.6	78.0		3′	86.5	87.5	86.8
4	38.7	38.8t	39.8		4′	69.9	70.0	69.9
5	140.9	141.0	140.9		5′	78.4	78.4	78.5
6	121.9	121.9	121.9		6′	62.3	62.4	62.4
7	32.3	32.5	32.3	Rha-1	1″	102.6	102.6	102.6
8	31.8	31.9	31.8		2″	71.5	71.7	72.8
9	50.4	50.5	50.4		3″	72.1	72.2	72.1
10	37.2	37.3	37.2		4″	72.5	72.4	72.3
11	21.2	21.3	21.1		5″	69.8	70.0	69.9
12	39.9	40.1	39.9		6″	18.7	18.7	18.7
13	40.5	40.9	40.9	Rha-2	1‴	103.2	103.3	103.3
14	56.7	56.8	56.7		2‴	72.8	72.9	72.8
15	32.2	32.5	32.4		3‴	84.1	84.3	84.4
16	81.2	81.3	81.4		4‴	72.4	72.4	72.4
17	62.8	63.9	64.3		5‴	68.8	68.9	69.9
18	16.4	16.6	16.3		6‴	18.3	18.4	18.3
19	19.4	19.5	19.5	Glc-2	1⁗	106.5	106.5	106.5
20	42.5	40.8	40.9		2⁗	73.9	73.9	73.9
21	14.9	17.6	17.6		3⁗	78.4	78.4	78.1
22	109.8	110.9	112.8		4⁗	71.5	71.7	71.7
23	26.5	37.3	31.0		5⁗	77.9	78.1	78.5
24	26.3	28.4	28.3		6⁗	62.7	62.8	62.8
25	27.6	34.5	34.5	26-O-Glc	1⁗′		105.1	105.1
26	65.2	75.5	75.1		2⁗′		75.3	75.4
27	16.4	16.6	16.3		3⁗′		78.6	78.5
OCH_3			47.4		4⁗′		71.7	71.7
					5⁗′		78.4	78.0
					6⁗′		63.0	63.0

3.2 Plant material

Whole plants of *Tacca plantaginea* (Hance) were collected from Guilin, Guangxi Zhuang Autonomous Region, China in August 1999 and identified by professor Tao De-Ding at Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

Dried, powdered plants of *T. plantaginea* were extracted with hot EtOH and the extract was concentrated under reduced pressure. The concentrated extract was suspended in water and extracted with petroleum, EtOAc and n-BuOH successively. The n-BuOH extract was repeatedly subjected to silica-gel column chromatography with $CHCl_3$/MeOH/H_2O (from 8:2:0.1 to 7:3:0.5) to give fractions I–VI. Fraction III was purified by Rp-18 column chromatography with MeOH/H_2O gradiently (6:4 or 8:2) to yield compound **1** (326 mg); Fraction IV was purified by Rp-18 column chromatography with MeOH/H_2O (6:4) to afford a mixture showing two spots on TLC. This mixture in 70% Me_2CO (15 ml) was heated at 85°C for 24 h and then concentrated to dryness to give **2** (215 mg); Fraction VI was purified by Rp-18 column chromatography with MeOH/H_2O (5.5:4.5) to afford a mixture showing two spots on TLC. This mixture in 70% Me_2CO (10 ml) was heated at 85°C for 24 h and then concentrated to dryness to give **3** (150 mg).

3.3.1 (25*S*)-3β-hydroxy-spirost-5-ene 3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (1). Colourless needle, mp 236-237°C; $[\alpha]_D^{27}$ −110.0 (*c* 0.10, pyridine). Negative FAB-MS (*m/z*): 1029 $[M]^-$, 867 $[M\text{-Glc-H}]^-$, 721 $[M\text{-Glc-Rha-H}]^-$. HRESI-MS: *m/z* 1029.5287 $[M-1]^-$ (calcd for $C_{51}H_{81}O_{21}$, 1029.5211). IR (KBr) ν_{max} (cm^{-1}): 3423 (OH), 1065, 988, 920, 895, 848, 839 (intensity 920 > 895, (25*S*)-spiroketal). ^{1}H NMR (500 MHz, C_5H_5N) δ: 0.81 (3H, s, Me-18), 1.03 (3H, s, Me-19), 1.07 (3H, d, $J = 7.05$ Hz, Me-27), 1.13 (3H, d, $J = 6.90$ Hz, Me-21), 1.66 (3H, d, $J = 6.05$ Hz, R-2, H-6‴), 1.74 (3H, d, $J = 6.20$ Hz, R-1, H-6″), 4.88 (1H, d, $J = 7.75$ Hz, G-1, H-1′), 5.22 (1H, d, $J = 7.75$ Hz, G-2, H-4⁗), 5.73 (1H, brs, R-2, H-1‴), 5.79 (1H, brs, R-1, H-1″). ^{13}C NMR data: see table 1.

3.3.2 26-*O*-β-D-glucopyranosyl-(25*S*)-3β,22ξ,26-triol-furost-5-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)]-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (3). White powder, mp 236–237°C; $[\alpha]_D^{27}$ −23.5 (*c* 0.17, pyridine). Negative FAB-MS (*m/z*): 1210 $[M]^-$, 1057 $[M\text{-Glc-H}]^-$. HRESI-MS: *m/z* 1209.5932 $[M-1]^-$ (calcd for $C_{57}H_{93}O_{27}$, 1209.5904). IR (KBr) ν_{max} (cm^{-1}): 3422 (OH), 1068, 1046, 914, 894, 838, 812. ^{1}H NMR (500 MHz, C_5H_5N) δ: 0.85 (3H, s, Me-18), 0.94 (3H, s, Me-19), 0.99 (3H, d, $J = 6.92$ Hz, Me-27), 1.29 (3H, d, $J = 6.55$ Hz, Me-21), 1.65 (3H, d, $J = 6.01$ Hz, R-2, H-6‴), 1.71 (3H, d, $J = 6.05$ Hz, R-1, H-6″), 4.77 (1H, d, $J = 7.75$ Hz, 26-G, H-1⁗), 4.81 (1H, d, $J = 7.70$ Hz, G-1, H-1′), 5.20 (1H, d, $J = 7.04$ Hz, G-2, H-1⁗), 5.71 (1H, brs, R-2, H-1‴), 5.75 (1H, brs, R-1,H-1″). ^{13}C NMR data: see table 1.

3.3.3 26-*O*-β-D-glucopyranosyl-22-methoxy-(25*S*)-3β,26-diol-furost-5-ene 3-*O*-[α-L-rhamnopyranosyl(1 → 2)]-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside (3a). White powder, mp 237–238°C; $[\alpha]_D^{27}$ −62.1 (*c* 0.15, pyridine). Negative FAB-MS (*m/z*): 1224 $[M]^-$, 1061 $[M\text{-Glc-H}]^-$, 915 $[M\text{-Glc-Rha-H}]^-$, HRESI-MS: *m/z* 1223.6033 $[M-1]^-$ (calcd for $C_{58}H_{95}O_{27}$, 1223.6060). IR (KBr) ν_{max} (cm^{-1}): 3422 (OH), 1069, 1047, 914, 894, 838, 812). ^{1}H NMR (500 MHz, C_5H_5N) δ: 0.79 (3H, s, Me-18), 1.02 (3H, s, Me-19), 1.04 (3H, d, $J = 7.20$ Hz, Me-27), 1.15 (3H, d, $J = 6.80$ Hz, Me-21), 1.51 (3H, d, $J = 6.00$ Hz, R-2, H-6‴), 1.68 (3H, d, $J = 6.00$ Hz, R-1, H-6″), 3.35 (3H, s, OMe), 4.84 (1H, d, $J = 7.68$ Hz, 26-G, H-1⁗), 4.88 (1H, d, $J = 7.84$ Hz, G-1, H-1′), 5.23 (1H, d, $J = 7.72$ Hz, G-2, H-1⁗), 5.75 (1H, brs, R-2, H-1‴), 5.80 (1H, brs, R-1, H-1″). ^{13}C NMR data: see table 1.

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