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Polyhydroxylated steroidal constituents from the fresh rhizomes of *Tupistra yunnanensis*

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Abstract

Six new polyhydroxylated steroidal saponins, tupistrosides A–F (**1**–**6**), together with nine known steroids, were isolated from the fresh rhizomes of *Tupistra yunnanensis*. Their structures were elucidated to be (25*S*)-1β,4β,5β-trihydroxy-spirostane-3β-yl *O*-α-L-arabinopyranoside (**1**), 1β,24β-dihydroxy-spirost-5,25(27)-dien-3α-yl *O*-β-D-glucopyranoside (**2**), (22*S*,25*S*)-1α,2β,3α,5α-tetrahydroxy-furo-spirostane-26-yl *O*-β-D-glucopyranoside (**3**), 1β,3α,22 ξ-trihydroxy-furost-5,25(27)-dien-26-yl *O*-β-D-glucopyranoside (**4**), 26-*O*-β-D-glucopyranosyl-1β,22-dihydroxy-furost-5-en-3α-yl *O*-β-D-glucopyranoside (**5**) and 22-methoxy-1β,2β,3β,4β,5β,7α-hexahydroxy-furost-25(27)-en-6-one-26-yl *O*-β-D-glucopyranoside (**6**), respectively, by means of spectroscopic analysis and the results of acid hydrolysis.

Keywords: *Tupistra yunnanensis*; Liliaceae; Steroidal saponins; Tupistrosides A–F; NMR spectroscopy; Acid hydrolysis

1. Introduction

The genus *Tupistra* (Liliaceae), comprising about 26 species is mainly distributed in Asia. Among which, about 17 species are growing in the People's Republic of China, particularly in the southwestern part [1]. Because of their similar morphologic characteristics, some species were substituted for each other as a traditional Chinese medicine, called "Xin-Bu-Gan" [2]. *Tupistra yunnanensis* Wang et Tang is endemic to Yunnan province of China. Its rhizomes are used as a remedy for stomach upset, injuries from falls and fracture. To the best of our knowledge, no chemical investigation appears to have been performed on this plant, although some species of genus *Tupistra* were studied for their chemical components and bioactivities [3–16]. As a part of a continuing study for the discovery of novel secondary metabolites from liliaceous plants [7–10,17–20], the fresh rhizomes of *Tupistra yunnanensis* were investigated. Here, the isolation and chemical characterization of six new polyhydroxylated steroidal saponins, named tupistrosides A–F (**1**–**6**), together with nine known steroids from this plant are presented.

2. Experimental

2.1. General methods

Optical rotations were measured on a SEPA-3000 automatic digital polarimeter. NMR spectra were measured in pyridine-d_5 unless otherwise noted 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; IR spectra were measured on a Bio-Rad FTS-135 spectrometer in KBr pellets. FABMS (negative ion mode) spectra were recorded on a VG Auto Spec-300 spectrometer.

2.2. Plant material

The rhizomes of *Tupistra yunnanensis* Wang et Tang was collected at Luquan, in the northeast of Yunnan province,

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China, and identified by Prof. Li Heng (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen is deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The fresh rhizomes of *Tupistra yunnanensis* (10.0 kg) were extracted with MeOH under reflux (3 L × 30 L) for three times (4, 3 and 3 h, respectively). After removal of the solvent, the concentrated residue was partitioned between *n*-BuOH (3 mL × 800 mL) and H_2O (3 L). The *n*-BuOH phase (96 g) was fractionated on a silica gel column with $CHCl_3$–MeOH–H_2O (7:3:1, lower layer) and gave four fractions (1–4). Fr. 1 (12.5 g) was chromatographed on silica gel (375 g) with a gradient of $CHCl_3$–MeOH (20:1–10:1) to give $\Delta^{25(27)}$ pentologenin (50 mg), convallagenin B (60 mg), epiruscogenin (50 mg) and tupisgenin (80 mg). Fr. 2 (20.0 g) and Fr. 3 (10.5 g) were supplied separately to silica gel column (400 and 200 g, respectively) chromatography (CC) eluting with $CHCl_3$–MeOH–H_2O (8:2:1, lower layer and 7:2:1) and repeated Rp-8 CC with 70–90% MeOH to yield **1** (200 mg), **2** (50 mg), 1β,2β,3β,4β,5β,7α-hexahydroxy-spirost-25(27)-en-6-one (100 mg) and 25(*R*)-1β-hydroxy-spirost-5-en-3α-yl *O*-β-D-glucopyranoside (1.0 g) from Fr. 2 and 3 (50 mg), 25(*S*)-22-methoxy-1β,3β,4β,5β,26-pentahydroxy-furost-26-yl *O*-β-D-glucopyranoside (150 mg), and a mixture from Fr. 3, respectively. The mixture was refluxed in 30% aqueous acetone to give **4** (40 mg). Fr. 4 (24.0 g) was subjected to silica gel CC (360 g) eluting with $CHCl_3$–MeOH–H_2O (7:2:1) and repeated Rp-8 CC eluting with 50–70% MeOH to furnish tupstroside G (30 mg), wattoside D (50 mg), **6** (60 mg), and a mixture, which yielded **5** (3.0 g) by refluxing in 30% aqueous acetone.

2.3.1. Tupistroside A (*1*)

White amorphous powder, $[\alpha]_D = -56.4^\circ$ ($c = 0.4$, MeOH). FABMS (negative ion mode): *m/z* 595 $[M-H]^-$, 463 $[M-\text{Ara}-H]^-$. HRFABMS (negative ion mode): *m/z* 595.3430 [*M* ($C_{32}H_{52}O_{10}$)− H]$^-$ (Calc. 595.3482). IR (KBr): ν_{max} 3410 (*br*, OH), 2951 (C–H), 920, 878, 850 (intensity 920 > 878, 25(*S*) spirostanol) cm^{-1} [21]. ^{1}H NMR (pyridine-d_5): δ 0.81 (s, CH_3-18), 1.02 (d, $J = 7.0$ Hz, CH_3-27), 1.14 (d, $J = 6.8$ Hz, CH_3-21), 1.20 (s, CH_3-19), 2.50 (dd, $J = 13.1$, 13.2 Hz, H-2), 3.35 (d, $J = 10.9$ Hz, H-26), 4.06 (d, $J = 10.7$ Hz, H-26), 3.78 (brd, $J = 11.3$ Hz, H-5′), 4.08 (dd, $J = 11.0$, 2.0 Hz, H-26), 4.16 (dd, $J = 8.6$, 7.6 Hz, H-2′), 4.42 (dd, $J = 11.3$, 5.1 Hz, H-5′), 4.54 (*m*, H-16), 4.95 (brs, H-1), 5.05 (d, $J = 7.6$ Hz, H-1′). ^{13}C NMR (pyridine-d_5); see Table 1.

2.3.2. Acid hydrolysis of *1*

A solution of **1** (60 mg) in 2N HCl–MeOH (1:1, 20 mL) was heated at 95 °C for 5 h. The reaction mixture was neutralized with $NaHCO_3$ (1N) and extracted with $CHCl_3$ three times. The $CHCl_3$ phase (40 mg) was chromatographed on silica gel CC (4.8 g) eluting with $CHCl_3$–MeOH (20:1–10:1) to give a pure compound, convallagenin B (**1a**, 10 mg), identified by the comparison with authentic samples through spectral and TLC properties. The aqueous layer was concentrated to dryness and then chromatographed over a silica gel column with $CHCl_3$–MeOH–H_2O (7:3:0.5) to yield L-arabinose [(3.2 mg), $[\alpha]_D = +23.5^\circ$ ($c = 0.17$, H_2O), R_f 0.2 [$CHCl_3$–MeOH–H_2O, 7:3:0.5].

Table 1
^{13}C NMR spectral data of compounds **1**–**6** (pyridine-d_5)

Position	**1**	**2**[a]	**3**	**4**	**5**[a]	**6**
1	73.5	76.2	77.9	75.5	75.0	76.5
2	32.9	36.7	68.2	38.1	36.7	67.9
3	78.0	75.4	71.9	66.6	74.9	75.5
4	67.5	39.1	39.3	40.8	39.0	71.2
5	78.4	139.9	74.7	140.1	139.8	86.3
6	29.4	125.5	36.0	124.7	125.5	211.1
7	28.5	33.0	28.9	32.5	32.3	75.5
8	34.9	32.9	34.9	32.9	32.9	37.9
9	44.9	51.6	45.5	51.4	51.5	41.1
10	42.5	44.9	45.7	44.7	44.8	50.2
11	22.0	24.9	21.8	24.4	24.8	21.9
12	40.8	39.1	40.1	40.6	41.4	39.3
13	40.1	41.3	40.7	41.1	41.2	40.9
14	56.4	58.0	56.1	57.1	57.9	49.7
15	32.2	32.9	32.2	32.7	32.9	31.6
16	81.3	82.8	81.0	81.2	82.4	81.4
17	62.9	63.8	62.7	64.1	65.2	64.1
18	16.6	16.9	16.5	16.8	16.9	16.3
19	14.9	13.3	13.9	13.3	13.3	11.6
20	42.3	42.9	38.9	40.8	41.2	40.8
21	16.3	14.8	15.2	16.4	16.1	16.1
22	109.8	112.6	120.3	110.8	113.6	112.5
23	26.4	41.3	33.1	30.2	31.4	31.9
24	26.2	67.8	33.9	28.5	28.7	28.2
25	27.6	148.6	83.9	147.3	34.9	146.9
26	65.2	65.2	77.5	72.2	75.9	72.1
27	17.6	106.7	24.4	110.4	17.3	111.3
22-O$\underline{C}$H$_3$						47.4
3-*O*-Glc	(Ara)					
1′	102.6	102.9			103.2	
2′	73.2	74.9			75.0	
3′	75.2	78.2			78.1	
4′	71.2	71.7			71.6	
5′	67.5	77.9			77.8	
6′		62.9			62.8	
26-*O*-Glc-1′			105.4	103.9	104.5	103.9
2′			75.3	75.2	75.4	75.2
3′			78.4	78.6	78.1	78.5
4′			71.6	71.8	71.6	71.8
5′			78.5	78.6	77.8	78.7
6′			62.6	62.8	62.8	62.9

[a] Measured in CD_3OD.

2.3.3. Tupistroside B (*2*)

White amorphous powder, $[\alpha]_D = -60.5^\circ$ ($c = 0.4$, MeOH). FABMS (negative ion mode): *m/z* 605 $[M-H]^-$. HRFABMS (negative ion mode): *m/z* 605.3326 [*M* ($C_{33}H_{50}O_{10}$)− H]$^-$ (Calc. 605.3325). IR (KBr): ν_{max} 3410, 2939, 1650, 1100, 980, 835 cm^{-1}. ^{1}H NMR (CD_3OD): δ 0.84 (s, CH_3-18), 0.98 (d, $J = 6.8$ Hz, CH_3-21), 1.03 (s,

CH_3-19), 3.15 (t, J = 7.8 Hz, H-2′), 3.64 (dd, J = 5.4, 11.7 Hz, H-6′), 3.76 (dd, J = 4.3, 11.7 Hz, H-1), 3.85 (dd, J = 2.4, 11.7 Hz, H-6′), 3.92 (d, J = 12.2 Hz, H-26), 4.04 (dd, J = 3.0, 3.9 Hz, H-3), 4.20 (d, J = 12.2 Hz, H-26), 4.32 (d, J = 7.8 Hz, H-1′), 4.40 (dd, J = 3.0, 7.4 Hz, H-24), 4.87 (brs, H-27), 5.08 (brs, H-27), 5.47 (d, J = 4.7 Hz, H-5). ^{13}C NMR (CD_3OD); see Table 1.

2.3.4. Acid hydrolysis of **2**

A solution of **2** (2 mg) in 2N HCl–MeOH (1:1, 1 mL) was heated at 95 °C for 5 h. The reaction mixture was neutralized with $NaHCO_3$ (1N) and evaporated. TLC analysis on a silica gel HPTLC silica gel 50000 F_{254} plate using *n*-BuOH-*i*-PrOH–H_2O (10:5:4, homogenous) as development and anisaldehyde-H_2SO_4 as detection, compared with the authentic samples, indicated the presence of D-glucose (R_f 0.29).

2.3.5. Tupistroside C (**3**)

White amorphous powder, $[\alpha]_D = -67.2°$ (c = 0.4, MeOH). FABMS (positive ion mode): m/z 643 $[M+H]^+$, 625 $[M–H_2O+H]^+$, 607 $[M–2H_2O+H]^+$, 481 $[M–Glc+H]^+$, 463 $[M–Glc–H_2O+H]^+$. HRFABMS (negative ion mode): m/z 641.3537 [M ($C_{33}H_{54}O_{12}$)− H]$^-$ (Calc. 641.3537). IR (KBr): ν_{max} 3449, 3263, 2936, 1665, 1407, 1043 cm^{-1}. ^{1}H NMR (pyridine-d_5): δ 0.80 (s, CH_3-18), 1.06 (d, J = 6.5 Hz, CH_3-21), 1.39 (s, CH_3-27), 1.55 (s, CH_3-19), 2.20 (dd, J = 2.9, 14.9 Hz, Ha-4), 2.50 (dd, J = 3.8, 14.9 Hz, Hb-4), 4.11 (dd, J = 2.9, 3.8 Hz, H-2), 4.31 (dd, J = 1.8, 3.8 Hz, H-1), 4.38 (dd, J = 5.1, 11.9 Hz, H-6′), 4.52 (dd, J = 2.0, 11.9 Hz, H-6′), 4.67 (td, J = 2.6, 3.8 Hz, H-3), 4.75 (m, H-16), 4.94 (d, J = 7.48 Hz, H-1′). ^{13}C NMR (pyridine-d_5); see Table 1.

2.3.6. Acid hydrolysis of **3**

Compound **3** (2 mg) was subjected to acid hydrolysis as described for **2**. TLC analysis indicated the presence of D-glucose.

2.3.7. Tupistroside D (**4**)

White amorphous powder, $[\alpha]_D = -50.6°$ (c = 0.3, pyridine). FABMS (negative ion mode): m/z 607 $[M-H]^-$, 445 $[M–Glc-H]^-$. HRFABMS (negative ion mode): m/z 607.3436 [M ($C_{33}H_{52}O_{10}$)− H]$^-$ (Calc. 607.3482). IR (KBr): ν_{max} 3430, 2942, 1651, 1450, 1080 cm^{-1}. ^{1}H NMR (pyridine-d_5): 0.96 (s, CH_3-18), 1.26 (d, J = 6.9 Hz, CH_3-21), 1.34 (s, CH_3-19), 4.04 (dd, J = 7.4, 7.8 Hz, H-2′), 4.20 (overlapped, H-26), 4.59 (d, J = 13.0 Hz, H-26), 4.87 (d, J = 7.4 Hz, H-1′), 5.03 (brs, H-27), 5.32 (brs, H-27), 5.63 (d, J = 5.3 Hz, H-6). ^{13}C NMR (pyridine-d_5); see Table 1.

2.3.8. Acid hydrolysis of **4**

Compound **4** (2 mg) was subjected to acid hydrolysis as described for **2**. TLC analysis indicated the presence of D-glucose.

2.3.9. Tupistroside E (**5**)

White amorphous powder, $[\alpha]_D = -48.5°$ (c = 0.3, pyridine). FABMS (negative ion mode): m/z 772 $[M]^-$, 609 $[M–Glc-H]^-$. HRFABMS (negative ion mode): m/z 771.4150 [M ($C_{39}H_{64}O_{15}$)− H]$^-$ (Calc. 771.4163). IR (KBr): ν_{max} 3418, 2902, 1650, 1455, 1078, 1024 cm^{-1}. ^{1}H NMR (CD_3OD): δ 0.83 (d, J = 5.5 Hz, CH_3-27), 0.86 (s, CH_3-18), 1.00 (d, J = 6.7 Hz, CH_3-21), 1.10 (s, CH_3-19), 4.20 (d, J = 7.8 Hz, 3-*O*-Glc H-1′), 4.24 (d, J = 7.5 Hz, 26-*O*-Glc H-1′), 5.47 (d, J = 5.1 Hz, H-6). ^{13}C NMR (CD_3OD); see Table 1.

2.3.10. Acid hydrolysis of **5**

Compound **5** (80 mg) was subjected to acid hydrolysis as described for **1** to give epiruscogenin (**5a**, 14 mg), by comparison with an authentic samples through spectral and TLC properties and D-glucose [(4.4 mg), $[\alpha]_D$ = +26.1° (c = 0.2, H_2O), R_f 0.29 (*n*-BuOH-*i*-PrOH–H_2O (10:5:4, homogenous))].

2.3.11. Tupistroside F (**6**)

White amorphous powder, $[\alpha]_D = -51.7°$ (c = 0.2, pyridine). FABMS (negative ion mode): m/z 703 $[M-H]^-$, 541 $[M–Glc-H]^-$. HR FABMS (negative ion mode): m/z 701.3386 [M ($C_{34}H_{54}O_{15}$)− H]$^-$ (Calc. 701.3384). IR (KBr): ν_{max} 3399, 2945, 2913, 1712 (C=O), 1647 (C=C) cm^{-1}. ^{1}H NMR (pyridine-d_5): δ 0.81 (s, CH_3-18), 1.15 (d, J = 6.8 Hz, CH_3-21), 1.43 (s, CH_3-19), 3.22 (s, 22-OCH_3), 4.92 (d, J = 8.0 Hz, H-1′), 5.06 (brs, H-27), 5.33 (brs, H-27). ^{13}C NMR (pyridine-d_5); see Table 1.

2.3.12. Acid hydrolysis of **6**

Compound **6** (80 mg) was subjected to acid hydrolysis as described for **1** to give 1β,2β,3β,4β,5β,7α-hexahydroxy-spirost-25(27)-en-6-one (**6a**, 12 mg), by comparison with an authentic samples through spectral and TLC properties and D-glucose [(2.4 mg), $[\alpha]_D$ = +22.1°(c = 0.1, H_2O), R_f 0.29 (*n*-BuOH-*i*-PrOH–H_2O (10:5:4, homogenous))].

3. Results and discussion

The MeOH extract of the fresh rhizomes of *Tupistra yunnanensis* was suspended in H_2O and extracted with *n*-BuOH. The *n*-BuOH fraction was chromatographed on silica gel and Rp-8 to afford compounds **1**–**6** (Fig. 1) and nine known compounds. On the basis of the spectroscopic evidence and by comparison with the literature values, the known compounds were identified as $\Delta^{25(27)}$ pentologenin [22], epiruscogenin [22], 1β,2β,3β,4β, 5β,7α-hexahydroxy-spirost-25(27)-en-6-one [22], tupisgenin [22], 25(*S*)-22-methoxy-1β, 3β,4β,5β,26-pentahydroxy-furost-26-yl *O*-β-D-glucopyranoside [23], convallagenin B (**1a**) [24], 25(*R*)-1β-hydroxy-spirost-5-en-3α-yl *O*-β-D-glucopyranoside [25], wattoside D [8], tupstroside G [19], respectively.

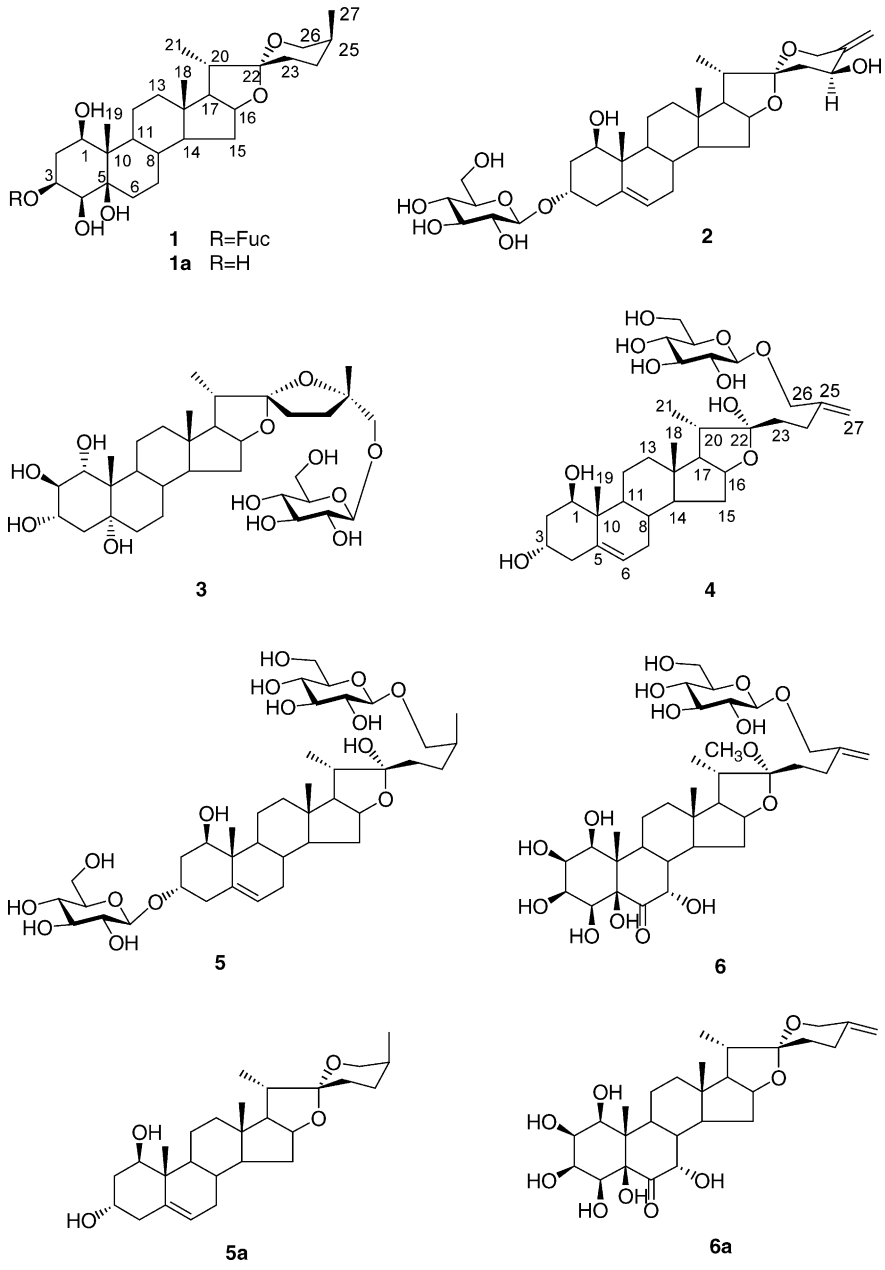

Fig. 1. New polyhydroxylated steroidal saponins isolated from *Tupistra yunnanensis*.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{32}H_{52}O_{10}$ on the basis of the ^{13}C NMR data (Table 1) and negative ion HRFABMS ($[M-H]^-$, *m/z* 595.3430). The ^1H NMR spectrum of **1** showed four steroidal methyl groups at δ 1.14 ($J = 6.8$ Hz), 1.20 (s), 1.02 ($J = 7.0$ Hz) and 0.81 (s), and one doublet anomeric proton signal at δ 5.05 ($J = 7.6$ Hz) [26]. Acid hydrolysis of **1** with 2N HCl afforded convallagenin B (**1a**), which was confirmed by direct comparison of the ^{13}C chemical shifts with literature data [24] and L-arabinose ($[\alpha]_D = +23.5^\circ$). In the ^{13}C NMR spectrum of **1**, the downfield shift of C-3 (δ 78.0) and the upfield shift of C-2 and C-4 (δ 32.9, 67.5) [convallagenin B: δ 71.4, 33.6 and 68.3, respectively] indicated that the arabinopyranosyl unit was linked at C-3 of the aglycone. This was confirmed by the long-range correlation between δ 5.05 (H-1′ of arabinose) and δ 78.0 (C-3 of aglycone) in the HMBC spectrum. Therefore, the structure of **1** was determined to be (25*S*)-1β,4β, 5β-trihydroxy-spirostane-3β-yl *O*-α-L-arabinopyranoside (**1**), named as tupistroside A.

Compound **2** was shown to have the molecular formula $C_{33}H_{50}O_{10}$ on the basis of HRFABMS ($[M-H]^-$ *m/z* 605.3326) and ^{13}C DEPT spectrum. Acid hydrolysis of **2** afforded D-glucose. The typical methyl signals at δ 0.84 (s), 1.03 (s) and 0.95 (d, $J = 6.8$ Hz) in the ^1H NMR spectrum and olefinic carbon signals at δ 139.9 and 125.5 and a quaternary

carbon signal at δ 112.6 (C-22) in the ^{13}C NMR spectrum indicated that **2** was a Δ^5-spirostanol. Moreover, instead of a doublet methyl proton signal, the presence of the olefinic carbon signals at δ 148.6 (q) and 106.7 (d) suggested that an additional olefinic bond was present between C-25 and C-27 [27]. In addition, an anomeric proton signal was also noted at δ 4.32 (d, J = 7.80 Hz) in the ^{1}H NMR spectrum.

In the ^{13}C NMR spectrum of **2**, 33 carbon signals were observed, six of which were due to the glucose. This implied a $C_{27}H_{40}O_5$ molecular formula for the aglycone portion, suggesting that the aglycone was a spirostanol with three hydroxyl groups. The locations of the hydroxyl groups, as well as the sugar moiety on the aglycone, were determined by detailed analysis of the HMQC and HMBC spectra. In the HMBC spectrum of **2**, the long-range correlations of δ 1.03 (H-19) with δ 76.2 (C-1), δ 2.23 (H-4) with δ 75.4 (C-3), δ 4.42 (H-24) with δ 148.6 (C-25) and δ 4.21 (H-26) with δ 67.8 (C-24) revealed that the three hydroxyl groups were linked at C-1, C-3 and C-24 of the aglycone. HMBC correlation of the anomeric proton signal at δ 4.32 with δ 75.4 (C-3) proved the location of the glucopyranosyl moiety at C-3 of aglycone. The J coupling patterns (H-1: δ 3.76, dd, J = 4.3, 11.7 Hz; H-3: δ 4.04, dd, J = 3.0, 3.9 Hz; H-24: δ 4.42, dd, J = 3.0, 7.4Hz) measured in the JRES spectrum, determined the configuration of the hydroxyl groups as 1β, 3α and 24β. On the basis of the above evidence, tupistroside B (**2**) was determined to be 1β,24β-dihydroxy-spirost-5,25(27)-dien-3α-yl *O*-β-D-glucopyranoside.

Compound **3** had a molecular formula $C_{33}H_{54}O_{12}$ on the basis of HRFABMS ([M − H]$^-$ m/z 641.3537). The ^{1}H NMR spectrum showed four typical steroidal methyl signals at δ 1.55 (s), 1.39 (s), 1.06 (d, J = 6.5 Hz) and 0.80 (s) [26]. The ^{13}C NMR spectrum exhibited the C-22 carbon of the aglycone at δ 120.3, characteristic of the furospirostanol skeleton [28]. In addition, the observation of an anomeric proton signal at δ 4.94 (d, J = 7.5 Hz), together with acidic hydrolysis of **3** affording D-glucose, suggested that **3** were a furospirostanol glucoside.

In the FAB mass spectrum of **3**, the presence of the fragment ion peaks at m/z 643 [M + H]$^+$, 625 [M–H_2O + H]$^+$, 607 [M–2H_2O + H]$^+$, 481 [M-162 + H]$^+$, 463 [M-162–H_2O + H]$^+$, 445 [M-162–2H_2O + H]$^+$ and 427 [M-3H_2O + H]$^+$ indicated the existence of several hydroxyl groups in the aglycone. The locations of the hydroxyl groups were determined to be at C-1, C-2, C-3, C-5 and C-26, respectively, on the basis of the ^{1}H–^{1}H COSY correlations from δ 4.31 (H-1) to δ 4.11 (H-2), H-2 to δ 4.67 (H-3) and H-3 to δ 2.50 and 2.20 (H-4), and the long-range correlations observed in the HMBC spectrum (Fig. 2). In addition, the HMBC correlation of the anomeric proton at δ 4.94 with δ 77.5 (C-26) revealed that the glucopyranosyl unit was linked to the C-26 OH of the aglycone.

The configurations of C-1, C-2, C-3 and C-5 were identified through the following steps. The observation of the small correlation (J = 1.8 Hz) between H-1 and H-3 suggested that H-1 and H-3 were in a W-coupling system. The

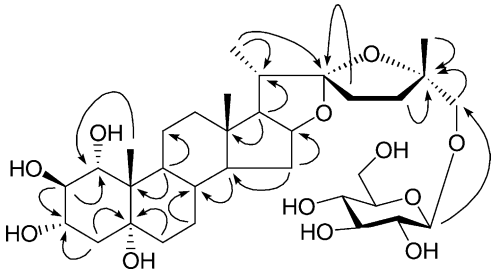

Fig. 2. The important ^{1}H–^{13}C long-range correlations in the HMBC spectrum of **3**.

NOE between H-1 and H-19 (δ 1.55, s) assigned H-1 as β-orientation. Since only the A/B *trans* fusion and the 3-α OH can satisfy the above properties, H-3 was assigned to have an β-orientation and 5-OH an α-orientation. The α-orientation of H-2 was concluded by the J values observed from the JRES spectrum (H-1: δ 4.31, dd, J = 1.8, 3.8 Hz; H-2: δ 4.11, dd, J = 2.9, 3.8 Hz; H-3: δ 4.67, td, J = 2.9, 3.8 Hz; H-4: δ 2.20, dd, J = 2.9, 14.9 Hz and δ 2.50, dd, J = 3.8, 14.9 Hz), which also confirmed the above conclusion. Moreover, the chemical shift of the C-27 (δ 24.4) and the other carbons in the F-ring (Table 1) suggested that **3** belonged to the 25*S* series [22]. Therefore, **3** was deduced to be (22*S*,25*S*)-1α,2β,3α,5α-tetrahydroxy-furospirostane-26-yl *O*-β-D-glucopyranoside, and named tupistroside C (**3**).

Compounds **4**–**6** were obtained as white amorphous powders and showed a positive reaction to the Ehrlich reagent. The DEPT spectrum showed a characteristic quaternary carbon signal around δ 111. Acid hydrolysis of **4**–**6** yielded D-glucose as the sugar residue. These observations suggested that **4**–**6** were 26-*O*-D-glucopyranosyl furostanols. Their molecular formulae were assigned to be $C_{33}H_{52}O_{10}$, $C_{39}H_{64}O_{15}$ and $C_{34}H_{54}O_{15}$, respectively, on the basis of the negative-ion HRFABMS.

The ^{1}H NMR spectrum of **4** showed one anomeric proton signal at δ 4.87 (d, J = 7.4 Hz), indicating the presence of one glucopyranosyl unit in the molecule. Comparing the ^{13}C NMR spectral data of **4** with those of epiruscogenin [25(*R*)-5-en-spirost-1β, 3α-diol] suggested that the chemical shifts due to the A–D rings were in good agreement. Significant differences were observed for their F-ring. The olefinic carbons signals at δ 147.3 (q) and 110.4 (d) in the ^{13}C NMR spectrum were assigned to C-25 and C-27, which was confirmed by the long range correlations of δ 4.20 (H-26) with δ 147.3 (C-25), δ 5.03 and 5.32 (H-27) with δ 72.2 (C-26), 28.5 (C-24) and C-25 in the HMBC spectrum. Moreover, the HMBC correlations of the anomeric proton signal at δ 4.87 with δ 72.2 (C-26) indicated that the glucopyranosyl unit was attached at C-26 of the aglycone. Thus, tupistroside D (**4**) was deduced to be 1β,3α,22 ξ-trihydroxy- furost-5,25(27)-dien-26-yl *O*-β-D-glucopyranoside.

Compound **5** exhibited two anomeric proton signals at δ 4.20 (d, J = 7.8Hz) and 4.24 (d, J = 7.5Hz) in the ^{1}H NMR spectrum, suggesting that two sugar units existed in the

molecule. Acid hydrolysis of **5** afforded epiruscogenin (**5a**) and D-glucose. The sugar linkage position of **5** was determined by the HMBC spectrum, in which correlations of one anomeric proton signal at δ 4.20 with δ 74.9 (C-3), and another anomeric proton at δ 4.24 with δ 76.0 (C-26) were observed. Thus, tupistroside E (**5**) was determined to be 26-*O*-β-D-glucopyranosyl-1β,22-dihydroxy-furost-5-en-3α-yl *O*-β-D-glucopyranoside.

Compound **6** showed only one anomeric proton signal at δ 4.92 (d, $J = 8.0$ Hz) in the ^{1}H 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 suggested that the 22-OH was methylated. When treated with 2N HCl, **6** gave 1β,2β,3β,4β,5β,7α-hexahydroxy-spirost-25(27)-en-6-one (**6a**) [22] as the aglycone, which were identified on the basis of their spectroscopic evidence and by comparison with literature values. Therefore, tupistroside F (**6**) were deduced to be 22-methoxy-1β,2β,3β,4β,5β,7α-hexahydroxy-furost-25(27)-en-6-one-26-yl *O*-β-D-glucopyranoside.

In this work, 15 polyhydroxylated steroidal saponins and sapogenins were obtained from the fresh rhizomes of *Tupistra yunnanensis* among which **1**–**6** were newly isolated molecules. It was known that genus *Tupistra* was close to genera *Convallaria*, *Aspidistra*, *Rohdea* and *Reineckea*, and all of them are in rich of steroidal saponins with the polyhydroxynated skeletons [24,29–31]. The results discussed in this paper will give some chemical evidences for the chemotaxonomy of Liliaceae.

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