Spirostanol Sapogenins from the Underground Parts of *Tupistra chinensis*

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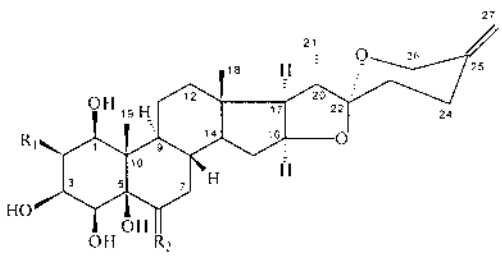
Chemical examination of the underground parts of *Tupistra chinensis* led to the isolation of two new 5β-spirostane type steroidal sapogenins, tupichigenin B (1) and C (2), together with two known steroidal sapogenins, ranmogenin A (3) and $\Delta^{25(27)}$-pentrogenin (4). The structures of 1 and 2 were established as spirost-25(27)-ene-1β,3β,4β,5β,6β-pentaol and 1β,2β,3β,4β,5β-pentahydroxyspirost-25(27)-en-6-one, respectively, on the basis of detailed analysis of their physical and spectral data.

Key words *Tupistra chinensis*; Liliaceae; steroidal sapogenin; spirostanol sapogenin; tupichigenin B, C

Tupistra chinensis BAKER (Liliaceae) is mainly distributed in southwestern China.[1)] As a folkloric Chinese medicine, this species has usually been used to treat rheumatic diseases and snake-bite.[1)] This species is used as a substitute for *Euphorbia helioscopia* L. (Euphorbiaceae) in Taiwan. However, to our knowledge, these two species show different chemical constituents. Therefore, the biological activities of these two species need to be further investigated. In a previous paper, we have reported the isolation and structural elucidation of two steroidal sapogenins, tupichigenin A and 1β,2β,3β,4β,5β,7β-hexahydroxyspirost-25(27)-en-6-one from *T. chinensis*.[2)] In a continuation of our investigation of the constituents of *T. chinensis*, we describe here the isolation and structural elucidation of two new 5β-spirostane type steroidal sapogenins, spirost-25(27)-ene-1β,3β,4β,5β,6β-pentaol (**1**) and 1β,2β,3β,4β,5β-pentahydroxyspirost-25(27)-en-6-one (**2**), as well as two known steroidal sapogenins, spirost-25(27)-ene-1β,3β,4β,5β-tetraol (ranmogenin A) (**3**)[3,4)] and spirost-25(27)-ene-1β,2β,3β,4β,5β-pentaol ($\Delta^{25(27)}$-pentrogenin) (**4**).[3,4)] Steroidal sapogenins are of great commercial utility as starting materials in the synthesis of a variety of steroid hormones.[5)] The 5β-spirostanol sapogenins and saponins (AB *cis* ring junction) which are widely distributed in a large number of higher plants, especially in Liliaceae, have attracted great research interests not only for their chemistry but also for such biological activities as inhibitory effects on human spermatozoa,[6)] inhibition of platelet aggregation,[7)] reduction of blood glucose level,[8)] molluscicidal activities,[9,10)] spasmolytic activity in rat duodenum,[11)] and *in vitro* inhibitory activity on cAMP phosphodiesterase.[12)]

Compound **1**, obtained as white microneedles, $[\alpha]_D^{24}$ −0.1° (c=0.002, pyridine), showed in the HR-FAB-MS (positive mode) a *pseudo*molecular $[M+Na]^+$ peak at m/z 501.2832 (Calcd 501.2829), consistent with the molecular formula $C_{27}H_{42}O_7$. The IR spectrum showed a strong absorption at 3374 cm^{-1} due to hydroxyl groups, but lacked the characteristic bands of the spirostane ring.

Unambiguous complete assignments for the ^{1}H- and ^{13}C-NMR signals were made by combination of distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY), heteronuclear chemical shift correlation (HETCOR) and nuclear Overhauser and exchange spectroscopy (NOESY) spectra. The ^{1}H-NMR spectrum (Table 1) in pyridine-d_5 of **1** showed signals for two tertiary methyl groups at δ 1.90 (3H, s, Me-19) and 0.89 (3H, s, Me-18), and a secondary methyl group at δ 1.10 (3H, d, J=6.8 Hz, Me-21). The ^{13}C-NMR spectrum (Table 1) showed a total of 27 carbon signals, which were assigned by DEPT as three methyls, nine methylenes, ten methines (including five oxygenated methines), and five quaternary carbons. The carbonyl resonance at δ 109.4 (C) was assigned to C-22 of the spirostanol skeleton. Two signals at δ 144.4 (C) and 108.7 (CH_2) were assigned to the C-25 and C-27 positions,[13)] respectively. Three diagnostic signals at δ 81.4 (CH), 65.0 (CH_2) and 63.1 (CH) were assigned to the C-16, C-26, and C-17 positions, respectively.[14)] These ^{1}H-NMR data and ^{13}C-NMR signals suggested that **1** is a C-25(27) unsaturated spirostane type steroidal sapogenin. The oxygenated methine protons at δ 4.17 (1H, br s), 4.56 (1H, dd, J=3.6, 2.8 Hz), 4.21 (1H, d, J=3.6 Hz) and 4.90 (1H, br s) were assigned to H-1, H-3, H-4, and H-6, respectively. The methylene protons at δ 2.13 (1H, m, H-2$_\alpha$) and δ 2.53 (1H, dt, J=14.8, 2.8 Hz, H-2$_\beta$) were determined, and were shown to be coupled to both of the two oxygenated methine protons at δ 4.17 (H-1) and δ 4.56 (H-3) in the ^{1}H–^{1}H COSY spectrum. The oxygenated methine proton at δ 4.21 (H-4) was in turn coupled with the oxygenated methine proton at δ 4.56 (H-3). The oxygenated methine proton at δ 4.90 was assigned to H-6, which was coupled with two methylene protons at δ 1.56 (1H, td, J=14.0, 3.6 Hz, H-7$_\alpha$) and δ 2.09 (1H, m, H-7$_\beta$). These findings supported the placement of four hydroxyl groups on C-1, C-3, C-4, and C-6 positions. Furthermore, four signals at δ 75.0 (CH), 71.1 (CH), 69.5 (CH), and 69.5 (CH) were assigned to the C-1, C-3, C-4, and C-6 positions, respectively, by HETCOR spectrum. The coupling patterns of H-1 at δ 4.17 (br s), H-3 at δ 4.56 (dd, $J_{3\alpha,4\alpha}$=3.6,



1: R_1=H, R_2=β-OH, H **3**: R_1=H, R_2=H, H
2: R_1=β-OH, R_2=O **4**: R_1=β-OH, R_2=H, H

Chart 1

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Table 1. ^{13}C-NMR and ^{1}H-NMR Data for **1** and **2** (100 and 400 MHz in Pyridine-d_5)

Position	**1**		**2**	
	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)
1	75.0, d	4.17, br s	75.8, d	4.28, br s
2	33.1, t	2.53, dt (14.8, 2.8), H_α 2.13, m, H_β	67.3, d	4.33, br s
3	71.1, d	4.56, dd (3.6, 2.8)	74.9, d	4.79, br s
4	69.5, d	4.21, d (3.6)	70.8, d	4.83, d (3.6)
5	79.2, s		85.5, s	
6	69.5, d	4.90, br s, H_α	210.7, s	
7	35.4, t	1.56, td (14.0, 3.6), H_α 2.09, m, H_β	42.1, t	2.50, dd (13.6, 4.0)
8	30.3, d	2.40, qd (11.2, 3.6)	37.3, d	2.01, m
9	45.7, d	1.30, td (11.2, 4.0)	44.4, d	1.89, m
10	45.5, s		49.6, s	
11	21.3, t	1.40, d (14.0), H_α 1.50, m, H_β	21.6, t	1.58, m, H_α 1.50, m, H_β
12	40.0, t	1.14, m, H_α 1.71, m, H_β	39.0, t	1.12, m, H_α 1.67, m, H_β
13	40.7, s		40.6, s	
14	56.1, d	1.18, m	55.6, d	1.46, m
15	32.2, t	2.07—2.10, m, H_α 1.45, m, H_β	31.5, t	1.97, m, H_α 1.39, m, H_β
16	81.4, d	4.61, m	80.8, d	4.52, m
17	63.1, d	1.88, m	62.2, d	1.81, t (8.0)
18	16.5, q	0.89, s	16.0, q	0.72, s
19	16.3, q	1.90, s	12.8, q	1.31, s
20	42.0, d	1.98, quin. (6.8)	41.5, d	1.91, m
21	15.0, q	1.10, d (6.8)	14.5, q	1.02, d (6.8)
22	109.4, s		109.4, s	
23	33.2, t	1.74, m	32.7, t	1.74, m
24	28.9, t	2.70, td (13.2, 5.6), H_{ax} 2.23, td (13.2, 2.4), H_{eq}	28.5, t	2.61, d (12.8), H_{ax} 2.22, d (12.8), H_{eq}
25	144.4, s		143.7, s	
26	65.0, t	4.47, d (12.0), H_{ax} 4.04, d (12.0), H_{eq}	64.8, t	4.38, d (12.0), H_{ax} 3.99, d (12.0), H_{eq}
27	108.7, t	4.78, s, H_A 4.82, s, H_B	108.8, t	4.75, s, H_A 4.79, s, H_B

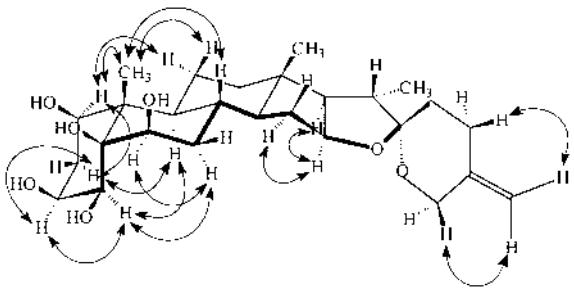

Fig. 1. NOESY Correlations of **1**

$J_{3\alpha,2\alpha}$=2.8 Hz) and H-6 at δ 4.90 (br s) indicated that H-1, H-3 and H-6 are α-equatorial.

The relative stereochemistry of **1** was also established by NOESY correlation, as shown in Fig. 1. NOESY correlations between H-4$_\alpha$ and H-7$_\alpha$/H-9$_\alpha$, between H-1$_\alpha$ and Me-19, and between H-2$_\alpha$ and H-9$_\alpha$ supported the A/B *cis* ring junction pattern and also indicated α-axial configurations of H-2, H-4, H-7, and H-9. Thus, the hydroxyl group at C-5 has a β-orientation and the signal at δ 79.2 (C) was assignable to the C-5 position. The proton at δ 4.61 (1H, m) was assigned to the H-16 position.[15] NOESY correlations between H-16 and H-15$_\alpha$/H-17 indicated that H-16, H-15$_\alpha$ and H-17 were *cis* to each other and oriented α. This fact also supported the D/E *cis* ring junction pattern. The protons at δ 4.04 (1H, d, J=12 Hz) and δ 4.47 (1H, d, J=12 Hz) were assigned to H-26$_{eq}$ and H-26$_{ax}$,[15] respectively. The geminal protons at C-27 were observed at δ 4.78 and δ 4.82 as two singlets, and coupling constants of approximately 0 Hz were characteristic of an exocyclic methylene.[15] The methylene group at C-26 appeared as two doublets. In the NOESY spectrum, cross peaks were observed between δ 4.78 (H-27$_A$) and δ 2.23 (H-24$_{eq}$), and between δ 4.82 (H-27$_B$) and δ 4.04 (H-26$_{eq}$). These properties further confirmed the presence of an exocyclic methylene group at C-25. On the basis of the above spectroscopic evidence, the structure of compound **1** was deduced to be spirost-25(27)-ene-1β,3β,4β,5β,6β-pentaol, which we have named Tupichigenin B.

Compound **2** was obtained as white microneedles, $[\alpha]_D^{24}$ −10.3° (c=0.02 , $CHCl_3$). The HR-FAB-MS (positive mode) gave a *pseudo*molecular $[M+H]^+$ ion at m/z 493.2810 (Calcd 493.2801), consistent with the molecular formula $C_{27}H_{40}O_8$. The ^{1}H- and ^{13}C-NMR spectral data of **2** are shown in Table 1. All signals were assigned unequivocally according to DEPT, ^{1}H–^{1}H COSY, ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond connectivity (HMBC) and NOESY analysis. The ^{1}H-NMR spectrum in pyridine-d_5 of **2** was similar to that of $\Delta^{25(27)}$-pentrogenin (**4**). Two methylene proton signals (δ 1.47 and

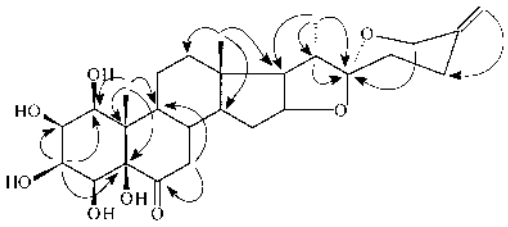

Fig. 2. HMBC Correlations (H to C) of **2**

1.11), assigned to the methylene at C-6 in **4**, disappeared in **2**. The ^{13}C-NMR spectrum of **2** revealed 27 carbon signals, which were assigned by DEPT as three methyls, eight methylenes, ten methines (including five oxygenated methines), and six quaternary carbons. The ^{13}C-NMR spectrum of **2** showed good similarity with that of **4** except the signals of C-5 to C-10. The presence of a carbonyl group in **2** was recognized by the IR (1711 cm^{-1}) and ^{13}C-NMR spectra (δ 210.7). The ketone functionality at C-6 was confirmed by its HMBC correlation to resonance at δ 2.50 (H_2-7). In turn, H_2-7 showed an additional correlation to the methine carbon at δ 44.4 (C-9), as shown in Fig. 2. The downfield shift of the quaternary carbon at C-5 from **4** (δ 77.7) to **2** (δ 85.5) and the downfield shift of the methylene carbon at C-7 from **4** (δ 30.1) to **2** (δ 42.1) also confirmed the ketone functionality at C-6. On the basis of the above spectroscopic evidence, the structure of compound **2** was confirmed to be 1β,2β,3β,4β,5β-pentahydroxyspirost-25(27)-en-6-one, which we have named Tupichigenin C.

Compound **3** and $\Delta^{25(27)}$-pentrogenin (**4**) were known steroidal sapogenins and identified by FAB-MS, IR, ^{1}H- and ^{13}C-NMR spectra, and by two-dimensional NMR spectral data as spirost-25(27)-ene-1β,3β,4β,5β-tetraol and spirost-25(27)-ene-1β,2β,3β,4β,5β-pentaol, respectively.[3,4] The ^{1}H-NMR data of the compounds **3** and **4** were not revealed in the previous report,[3,4] and their chemical shift assignments of C-23 and C-24 in the ^{13}C-NMR data needed to be revised. We reported herein the complete spectral data of **3** and **4** in the experimental section.

Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeOH using a JASCO V-530 spectrophotometer. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. The IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra (all in pyridine-d_5) were recorded with Varian NMR spectrometers, using TMS as an internal standard. LR-FAB-MS and LR-EI-MS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC/MS spectrometer with a direct inlet system. HR-FAB-MS spectra were measured on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Macherey-Nagel, 230—400 mesh) was used for column chromatography, precoated silica gel plates (Macherey-Nagel, SIL G-25 UV_{254}, 0.25 mm) were used for analytical TLC, and precoated silica gel plates (Macherey-Nagel, SIL G/UV_{254}, 0.25 mm) were used for preparative TLC. The spots were detected by spraying with 50% H_2SO_4 followed by heating on a hot plate.

Plant Material *Tupistra chinensis* was purchased in Kaohsiung, Taiwan, in August 1997, and identified by Professor Yueh-Cherng Li, Sichuan Provincial Laboratory of Drugs, People's Republic of China. A voucher specimen (No. 970808) is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Separation The air-dried underground parts of *T. chinensis* (17 kg) were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and partitioned to yield *n*-hexane (140 g), $CHCl_3$ (60 g), EtOAc (100 g), *n*-BuOH (130 g), and aqueous (280 g) extracts. A portion of the $CHCl_3$ extract was concentrated and chromatographed over silica gel and eluted with *n*-hexane–EtOAc mixtures of increasing polarity to yield eleven fractions. Fraction 6, eluted from *n*-hexane–EtOAc (1 : 7), was further chromatographed on silica gel elution with $CHCl_3$–MeOH (15 : 1) and recrystallized with $CHCl_3$–MeOH (15 : 1) to afford compound **3** (30 mg, 0.05% dry weight), then eluted with $CHCl_3$–MeOH (10 : 1) and recrystallized with $CHCl_3$–MeOH (15 : 1) to afford compound **1** (150 mg, 0.25% dry weight). Fraction 6 was further chromatographed on silica gel elution with $CHCl_3$–MeOH (8 : 1) and recrystallized with $CHCl_3$–MeOH (15 : 1) to afford compound **2** (290 mg, 0.48% dry weight). Fraction 7, eluted from *n*-hexane–EtOAc (1 : 10), was further chromatographed on silica gel elution with $CHCl_3$–MeOH (16 : 1) and recrystallized with $CHCl_3$–MeOH (20 : 1) to afford compound **4** (75 mg, 0.13% dry weight).

Tupichigenin B (**1**): White microneedles, mp 247—248 °C, $[\alpha]_D^{24}$ −0.1° (c=0.002, pyridine). Positive FAB-MS (positive mode) *m/z*: 501 $[M+Na]^+$. HR-FAB-MS *m/z*: Found 501.2832 $[M+Na]^+$ (Calcd for $C_{27}H_{42}O_7Na$ 501.2829). IR ($CHCl_3$) ν_{max} cm^{-1}: 3374 (OH), 3020, 2936, 2400, 1522, 1422, 1216, 1047, 928. ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5) data see Table 1.

Tupichigenin C (**2**): White microneedles, mp 252—253 °C, $[\alpha]_D^{24}$ −10.3° (c=0.02, $CHCl_3$). FAB-MS (positive mode) *m/z*: 515 $[M+Na]^+$. HR-FAB-MS *m/z*: Found 493.28109 $[M+H]^+$ (Calcd 493.28014). IR ($CHCl_3$) ν_{max} cm^{-1}: 3414 (OH), 2945, 2832, 2586, 2517, 2149, 2048, 1711, 1422, 1365, 1224, 1031, 928. ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5) data see Table 1.

Ranmogenin A (**3**): White microneedles, $[\alpha]_D^{24}$ −24.4° (c=0.06, pyridine). FAB-MS (positive mode) *m/z*: 485 $[M+Na]^+$, 463 $[M+H]^+$. HR-FAB-MS *m/z*: 463.3052 (Calcd for $C_{27}H_{43}O_6$ 463.3060). IR ($CHCl_3$) ν_{max} cm^{-1}: 3363 (OH), 3019, 2946, 1523, 1424, 1365, 1027, 929. ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 5.50 (1H, br s, OH-5), 4.83, 4.79 (each 1H, s, H_2-27), 4.61 (1H, m, H-16), 4.60 (1H, s, H-3$_\alpha$), 4.49 (1H, d, J=12 Hz, H-26$_{ax}$), 4.28 (1H, d, J=2.8 Hz, H-4), 4.22 (1H, br s, H-1), 4.05 (1H, d, J=12 Hz, H-26$_{eq}$), 2.72 (1H, td, J=12.8, 5.6 Hz, H-24$_{ax}$), 2.54, 2.10 (each 1H, dt, J=15.2, 2.8 Hz, H_2-2), 2.49, 1.70 (each 1H, dt, J=13.2, 3.2 Hz, H_2-6), 2.25 (1H, d, J=12.8 Hz, H-24$_{eq}$), 1.61 (3H, s, H_3-19), 1.09 (3H, d, J=7.2 Hz, H_3-21), 0.89 (3H, s, H_3-18). ^{13}C-NMR (100 MHz, pyridine-d_5) δ: 73.8 (C-1), 33.5 (C-2), 71.2 (C-3), 68.1 (C-4), 78.4 (C-5), 30.4 (C-6), 28.5 (C-7), 35.0 (C-8), 45.7 (C-9), 45.4 (C-10), 21.5 (C-11), 40.1 (C-12), 40.7 (C-13), 56.3 (C-14), 32.2 (C-15), 81.4 (C-16), 63.0 (C-17), 16.6 (C-18), 13.9 (C-19), 41.9 (C-20), 15.0 (C-21), 109.4 (C-22), 33.2 (C-23), 29.0 (C-24), 144.4 (C-25), 65.0 (C-26), 108.7 (C-27).

$\Delta^{25(27)}$-Pentrogenin (**4**): White microneedles, $[\alpha]_D^{24}$ −6.1° (c=0.001, pyridine). FAB-MS (positive mode) *m/z*: 501 $[M+Na]^+$. HR-FAB-MS *m/z*: 501.2839 (Calcd for $C_{27}H_{42}O_7Na$ 501.2828). IR ($CHCl_3$) ν_{max} cm^{-1}: 3419 (OH), 3020, 2949, 2400, 1522, 1434, 1216, 1056, 928. ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 4.82 (1H, dd, J=4.0, 3.2 Hz, H-3), 4.80, 4.77 (each 1H, s, H_2-27), 4.56 (1H, dd, J=14.6, 8.0 Hz, H-16), 4.44 (1H, d, J=12.0 Hz, H-26$_{ax}$), 4.33 (1H, d, J=3.2 Hz, H-1), 4.32 (1H, d, J=4.0 Hz, H-4), 4.18 (1H, t, J=3.2 Hz, H-2), 4.02 (1H, d, J=12.0 Hz, H-26$_{eq}$), 2.69 (1H, td, J=12.4, 6.8 Hz, H-24$_{ax}$), 2.45 (1H, m, H-7$_\alpha$), 2.23 (1H, d, J=13.6 Hz, H-24$_{eq}$), 1.98 (1H, m, H-15$_\alpha$), 1.95 (1H, m, H-20), 1.81 (1H, m, H-17), 1.69 (1H, m, H-8), 1.65 (1H, m, H-7$_\beta$), 1.59 (3H, s, H_3-19), 1.56, 0.99 (each 1H, m, H_2-12), 1.47 (1H, m, H-6$_\alpha$), 1.40 (1H, m, H-15$_\beta$), 1.18 (1H, td, J=11.2, 4.4 Hz, H-9), 1.11 (1H, m, H-6$_\beta$), 1.06 (3H, d, J=7.2 Hz, H_3-21), 1.02 (1H, m, H-14), 0.82 (3H, s, H_3-18). ^{13}C-NMR (100 MHz, pyridine-d_5) δ: 77.9 (C-1), 67.1 (C-2), 75.3 (C-3), 68.0 (C-4), 77.7 (C-5), 28.2 (C-6), 30.1 (C-7), 34.7 (C-8), 45.2 (C-9), 44.9 (C-10), 21.5 (C-11), 39.8 (C-12), 40.5 (C-13), 56.0 (C-14), 31.9 (C-15), 81.3 (C-16), 62.8 (C-17), 16.4 (C-18), 13.6 (C-19), 41.7 (C-20), 14.8 (C-21), 109.4 (C-22), 33.0 (C-23), 28.7 (C-24), 144.1 (C-25), 64.0 (C-26), 108.7 (C-27).

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