Tupichigenin A, a New Steroidal Sapogenin from *Tupistra chinensis*

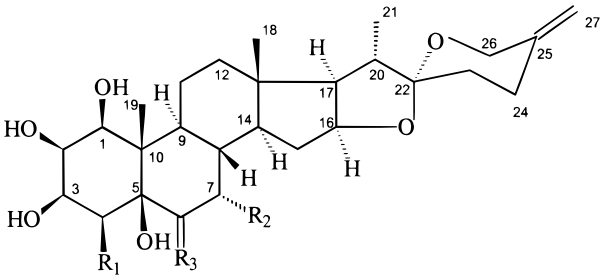
Wen-Bin Pan,[†,‡] Fang-Rong Chang,[‡] and Yang-Chang Wu*[,‡]

Department of Applied Chemistry, Fooyin Institute of Technology, Kaohsiung County 831, Taiwan, and Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan,

Received July 9, 1999

From the underground parts of *Tupistra chinensis*, a novel polyhydroxylated spirostanol sapogenin, tupichigenin A [(20*S*,22*R*)-spirost-25(27)-ene-1*β*,2*β*,3*β*,5*β*-tetraol] (**1**), was isolated and determined structurally on the basis of spectroscopic methods. Also isolated was the known steroidal sapogenin (20*S*,-22*R*)-spirost-25(27)-ene-1*β*,2*β*,3*β*,4*β*,5*β*,7α-hexaol-6-one (**2**).

Tupistra chinensis Baker (Liliaceae) is endemic to southwestern regions of the People's Republic of China.[1] To the best of our knowledge, no detailed chemical investigation appears to have been performed on this plant, which has been used to substitute for *Euphorbia helioscopia* L. (Euphorbiaceae) as a medicinal plant in Taiwan. A survey of the literature shows that the related species, *T. aurantiaca* Wall et Backer, was reported to exhibit cardiotonic action[2] and was found to contain several steroidal sapogenins.[3,4] In the present investigation on the constituents of *T. chinensis*, a novel C-25(27) unsaturated spirostane-type steroidal sapogenin, (20*S*,22*R*)-spirost-25(27)-ene-1*β*,2*β*,3*β*,5*β*-tetraol (**1**), and a known analogue, (20*S*,22*R*)-spirost-25(27)-ene-1*β*,2*β*,3*β*,4*β*,5*β*,7α-hexaol-6-one (**2**), were isolated and characterized from the underground parts of *T. chinensis*, and the structure determination of **1** is described herein. Although **2** has been previously isolated from *T. aurantiaca*, only limited ^{13}C NMR data were reported.[3,4] This is the first report of the complete ^{1}H and ^{13}C NMR spectral assignments for this compound.



1 $R_1 = R_2 = H, R_3 = H_2$

2 $R_1 = R_2 = OH, R_3 = O$

Compound **1** was obtained as white needles, $[\alpha]^{24}_D$ −18° (*c* 0.004, pyridine). The HREIMS showed the $[M]^+$ ion at *m*/*z* 462.2972 (calcd 462.2982), consistent with a molecular formula of $C_{27}H_{42}O_6$. The IR spectrum of **1** lacked the characteristic bands of a spirostane ring, but exhibited a hydroxyl group at 3369 cm^{-1} and an exocyclic methylene group at 1655, 938, and 878 cm^{-1}.[5] The ^{13}C NMR spectrum (Table 1) revealed 27 carbon signals, which were assigned by DEPT as three methyl (*δ* 16.5, 13.8, and 14.9), nine methylene, five methine, four oxygenated methine, four quaternary, and two olefinic [*δ* 108.7 (t) and 144.4 (s)] carbons. The ketal carbon resonance at *δ* 109.4 was assigned to a spiroketal carbon with two oxygens attached. The (20*S*,22*R*)-spirost-25(27)-ene-1*β*,2*β*,3*β*,4*β*,5*β*-pentaol, $\Delta^{25(27)}$-pentrogenin,[3,4] is an example of a C-25(27)-unsaturated 5*β*-spirostane in which C-27 appears as a triplet at *δ* 108.7 while C-25 is a singlet at *δ* 144.4.[4] On comparison of the ^{13}C NMR data of compound **1** and $\Delta^{25(27)}$-pentrogenin, it was found that the structural features of these sub-

Table 1. NMR Spectral Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
1	77.9 d	4.34 (br s)	76.4 d	4.34 (br s)
2	68.1 d	4.10 (br s)	67.8 d	4.41 (br s)
3	71.9 d	4.66 (br s)	75.5 d	4.75 (br s)
4	39.2 t	2.53 (H-4α, d, 15.2) 2.21(H-4*β*, d, 15.2)	71.1 d	5.81 (d, 3.6)
5	74.8 s		86.2 s	
6	36.0 t	1.97 (H-6α, m) 1.61 (H-6*β*, m)	211.0 s	
7	28.9 t	0.97 (H-7α, m) 1.50 (H-7*β*, m)	75.1 d	4.56 (br s)
8	34.9 d	1.68 (d, 11.6)	40.9 d	2.25(td, 10.8, 1.2)
9	45.6 d	1.21 (td, 11.2, 4.0)	37.9 d	2.47(td, 11.2, 4.4)
10	45.5 s		50.1 s	
11	21.7 t	1.43 (m)	21.9 t	1.57 (m)
12	40.0 t	1.04 (H-12α, m) 1.64 (H-12*β*, m)	39.4 t	1.29 (H-12α, td, 12.4, 4.8) 1.68 (H-12*β*, td, 12.4, 4.8)
13	40.6 s		40.7 s	
14	56.2 d	1.96−1.99 m	49.3 d	2.19 (td, 11.2, 5.2)
15	32.2 t	2.05 (H-15α, m) 1.41 (H-15*β*, m)	31.4 t	2.40 (H-15α, m) 1.57 (H-15*β*, m)
16	81.4 d	4.61 (m)	81.3 d	4.59 (m)
17	63.0 d	1.85 (d, 8.0)	62.8 d	1.93 (t, 6.8)
18	16.5 q	0.85 (s)	16.3 q	0.89 (s)
19	13.8 q	1.59 (s)	12.9 q	1.46 (s)
20	41.9 d	1.90−1.93 (m)	42.0 d	1.98 (m)
21	14.9 q	1.09 (d, 6.8)	14.9 q	1.08 (d, 6.8)
22	109.4 s		109.5 s	
23	33.2 t	1.79 (m)	33.2 t[b]	1.78 (m)
24	28.9 t	2.25 (H-24eq, d, 17.2) 2.72 (H-24ax, td, 12.0, 6.0)	28.9 t[b]	2.23 (H-24eq, m) 2.69 (H-24ax, td, 12.8, 5.6)
25	144.4 s		144.4 s	
26	65.0 t	4.05 (H-26eq, d, 12.0) 4.48 (H-26ax, d, 12.0)	65.0 t	4.02 (H-26eq, d, 12.0) 4.43 (H-26ax, d, 12.0)
27	108.7 t	4.80 (H-27 $_A$, s) 4.82 (H-27 $_B$, s)	108.7 t	4.78 (H-27 $_A$, s) 4.81 (H-27 $_B$, s)

[a] Measured in pyridine-d_5, with TMS as internal standard. The assignments were made from the DEPT, COSY, HMQC, and HMBC (J = 8 Hz) spectra. [b] These assignments are different from those in the literature.[3,4]

* Author to whom correspondence should be addressed. Tel.: 886-7-3121101, ext. 2197. Fax: 886−7−3114773. E-mail: yachwu@cc.kmu.edu.tw.
† Fooyin Institute of Technology.
‡ Kaohsiung Medical University.

10.1021/np990337i CCC: $19.00
Published on Web 04/19/2000

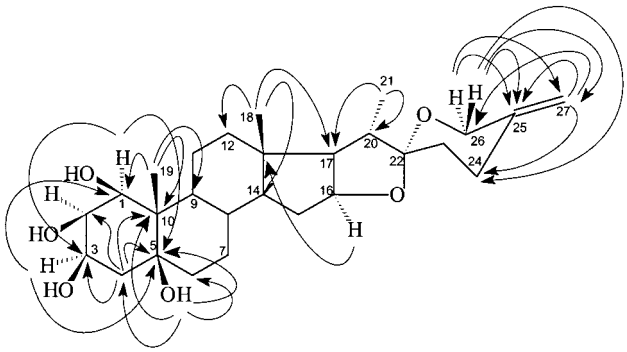


Figure 1. HMBC correlations for **1**.

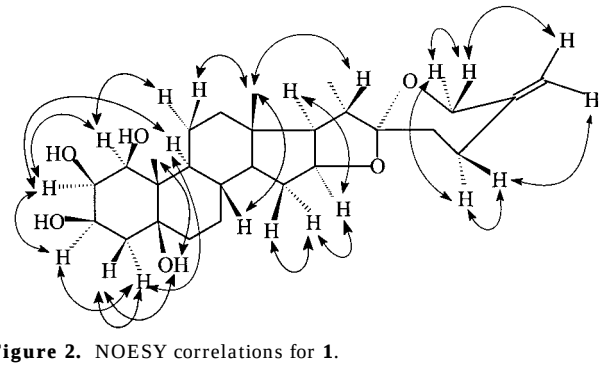


Figure 2. NOESY correlations for **1**.

stances are very close, except for the chemical shifts of C-2 to C-6. Moreover, the chemical shifts of C-2 to C-6 of **1** are similar to those of (25*R*)-spirost-1β,2β,3α,5β-tetraol (kogagenin).[6]

Unambiguous assignments for the ^{1}H and ^{13}C NMR signals in **1** were made by combination of the DEPT, NOESY, ^{1}H−^{1}H COSY, HMBC, and HMQC spectra. The ^{1}H NMR spectrum (Table 1) revealed three methyls at δ 0.85 (3H, s, Me-18), 1.59 (3H, s, Me-19), and 1.09 (3H, d, J = 6.8 Hz, Me-21). The carbinol methine protons at δ 4.34 (1H, br s), 4.10 (1H, br s), and 4.66 (1H, br s) were assigned to H-1, H-2, and H-3, respectively.[7] The sharp singlet at δ 5.87, which was not observed on addition of D_2O, was assigned to the proton of the hydroxyl group attached to C-5 by HMBC correlations. The proton at δ 4.61 (1H, m) was assigned to H-16 at the remaining monooxygenated methine carbon.[8] The protons at δ 4.05 (1H, d, J = 12 Hz) and δ 4.48 (1H, d, J = 12 Hz) were assigned to H-26$_{eq}$ and H-26$_{ax}$,[8] respectively. The geminal protons at C-27 were observed at δ 4.80 and 4.82 as two singlets, and the coupling constants of approximately 0 Hz are characteristic of an exocyclic methylene.[8] The ^{1}H NMR spectrum lacked the doublet normally observed for the Me-27 of a saturated spirostane derivative. Moreover, the methylene group at C-26 appeared as an AB quartet with no other coupling. These observations confirmed the presence of an exocyclic methylene group at C-25.

In the HMBC spectrum of **1**, carbon signals resonating at δ 77.9, 45.6, and 45.5 were correlated with Me-19 (δ 1.59), indicating that these signals are assignable to C-1, C-9, and C-10, respectively. The ^{1}H−^{1}H COSY spectrum revealed that the oxymethine proton at δ 4.10 (H-2) was coupled to two oxymethine protons at δ 4.34 (H-1) and δ 4.66 (H-3), and the oxymethine proton at δ 4.66 (H-3) was coupled to two methylene protons at δ 2.21 (H-4$_{eq}$) and δ 2.53 (H-4$_{ax}$). These findings supported the placement of three hydroxyl groups at C-1, C-2, and C-3, respectively. The HMBC spectrum (Figure 1) supported this conclusion and also suggested sites for the attachment of the two tertiary methyls. Thus, the methyl signal at δ 0.85, which showed correlations with C-12, C-14, and C-17, was placed at C-18, while the other methyl at δ 1.59, which showed correlations with C-1, C-5, C-9, and C-10, was placed at C-19. In the HMBC spectrum, the D_2O-exchangeable hydroxyl proton at δ 5.87 showed correlations with the methylene carbon at δ 39.2 (C-4), the quaternary oxygen-bearing carbon at δ 74.8 (C-5), the methylene carbon at δ 36.0 (C-6), and the quaternary carbon at δ 45.5 (C-10), respectively. These findings indicated that a hydroxyl group (δ 5.87) should be affixed to C-5.

The relative stereochemistry of the molecule of **1** was determined by analyzing the NOESY NMR data (Figure 2). The NOESY spectrum indicated correlations between the proton of the hydroxyl group at C-5 and Me-19/H-4$_{eq}$ (δ 2.21), with no evidence of any correlation between the proton of the hydroxyl group at C-5 and H-9α/H-4$_{ax}$, suggesting that the hydroxyl group at C-5 has a β orientation. In the NOESY spectrum, the correlations between H-4$_{ax}$ and H-9α/H-3 and those between H-2 and H-3/H-1/H-9α indicated that H-1, H-2, H-3, and H-9 were *cis* to each other and oriented in an α fashion. The significant correlations between H-9 and H-2/H-4$_{ax}$ strongly supported a *cis* junction between rings A and B. Moreover, in the NOESY spectrum, the methylene proton H-24$_{ax}$ (δ 2.72) was correlated with the methylene proton H-26$_{ax}$ (δ 4.48), with no correlation observed between H-24$_{eq}$ (δ 2.25) and H-26$_{eq}$ (δ 4.05). The geminal proton H$_A$-27 (δ 4.80) showed a correlation with H-24$_{eq}$ (δ 2.25), while the other geminal proton H$_B$-27 (δ 4.82) showed a correlation with H-26$_{eq}$ (δ 4.05). Therefore, H-24$_{eq}$ (δ 2.25), H-24$_{ax}$ (δ 2.72), H-26$_{eq}$ (δ 4.05), and H-26$_{ax}$ (δ 4.48) were assigned as equatorial, axial, equatorial, and axial, respectively. These results indicate unambiguously that compound **1** is (20*S*,22*R*)-spirost-25(27)-ene-1β,2β,3β,5β-tetraol, which we have named tupichigenin A.

Compound **2** was obtained as white needles. The ^{1}H and ^{13}C NMR data of **2** are presented in Table 1. The ^{13}C NMR chemical shifts of **2** were very close to those of the previously reported values,[3,4] except for the chemical shift of C-2. On the basis of the HRFABMS, DEPT, ^{1}H−^{1}H COSY, HMBC, HMQC, and NOESY spectral data, compound **2** was identified as the known compound (20*S*,22*R*)-spirost-25(27)-ene-1β,2β,3β,4β,5β,7α-hexaol-6-one.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 200−20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 MHz, pyridine-d_5), ^{13}C NMR (100 MHz), DEPT, HETCOR, COSY, NOESY, and HMBC spectra were obtained on a Varian NMR spectrometer (Unity Plus). Low-resolution FABMS and low-resolution EIMS were collected on a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC/MS spectrometer having a direct inlet system. High-resolution EIMS and high-resolution FABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography, precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC, and precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. The spots were detected by spraying with 50% H_2SO_4 and then heating on a hot plate.

Plant Material. *T. chinensis* Bak. 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 Isolation. 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 eluting with *n*-hexanes−EtOAc mixtures of increasing polarity to yield 11 fractions. Fraction 6, eluted with $CHCl_3$−MeOH (10:1), was further purified by silica gel column chromatography using the same solvent system, then recrystallized with $CHCl_3$−MeOH to afford compound **1** (50 mg, 0.007% dry weight). The original residue eluted with $CHCl_3$−MeOH (4:1) was rechromatographed on silica gel using the same solvent system and recrystallized with $CHCl_3$−MeOH to afford compound **2** (60 mg, 0.009% dry weight).

(20*S*,22*R*)-Spirost-25(27)-ene-1β,2β,3β,5β-tetraol (Tupichigenin A) (1): white needles ($CHCl_3$−MeOH); mp 282−283 °C; $[\alpha]^{24}_D$ −18° (*c* 0.004, pyridine); UV (EtOH) λ_{max} (log ϵ) 204 (2.83) nm; IR (neat) ν_{max} 3370, 2950, 1655, 938, 878 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 1; EIMS *m*/*z* 462 $[M]^+$ (11), 444 (10), 426 (8), 405 (6), 395 (33), 392 (27), 332 (62), 314 (50), 139 (85), 137 (100), 124 (10), 113 (16); HREIMS *m*/*z* found 462.2972 $[M]^+$ (calcd for $C_{27}H_{42}O_6$ 462.2982).

(20*S*,22*R*)-Spirost-25(27)-ene-1β,2β,3β,4β,5β,7α-hexaol-6-one (2): white needles ($CHCl_3$−MeOH); mp 222−223 °C; $[\alpha]^{24}_D$ −41.2° (*c* 0.001, pyridine); UV (EtOH) λ_{max} (log ϵ) 208 (3.26), 256 (2.77) nm; IR (neat) ν_{max} 3367, 2949, 1716, 1655, 938, 878 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) data see Table 1; FABMS *m*/*z* 531 $[M + Na]^+$ (8), 307 (8), 289(6), 154 (98), 137 (71), 136 (100), 124 (15), 113 (4); HRFABMS *m*/*z* found 531.2559 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_9Na$ 531.2570).

Acknowledgment. This investigation was supported by a grant from the National Science Council of the Republic of China. We thank Professor Yueh-Cherng Li, Sichuan Provincial Laboratory of Drugs, People's Republic of China, for the identification of plant material.

References and Notes

(1) Jiangsu New Medical College. *A Dictionary of Chinese Medicine*; Shanghai Science Technology Co., Ltd.: Shanghai, 1979; p 907.
(2) Deng, S. H. *Yao Hsueh Hsueh Pao* **1965**, *12*, 788−792.
(3) Renzhou, Y.; Dezu, W.; Jian, F. *Acta Bot. Yunnan.* **1987**, *9*, 374−378.
(4) Agrawal, P. K.; Jain, D. C.; Pathak, A. K. *Magn. Reson. Chem.* **1995**, *33*, 923−953.
(5) Brunengo, M. C.; Tombesi, O. L.; Doller, D.; Gros, E. G. *Phytochemistry* **1988**, *27*, 2943−2945.
(6) Tori, K.; Seo, S.; Terui, Y.; Nishikawa, J.; Yasuda, F. *Tetrahedron Lett.* **1981**, *22*, 2405−2408.
(7) Brunengo, M. C.; Tombesi, O. L.; Doller, D.; Gros, E. G. *Phytochemistry* **1988**, *27*, 2943−2945.
(8) Blunden, G.; Jaffer, J. A.; Jewers, K.; Griffin, W. J. *Tetrahedron* **1981**, *37*, 2911−2915.

NP990337I