New Flavans, Spirostanol Sapogenins, and a Pregnane Genin from *Tupistra chinensis* and Their Cytotoxicity

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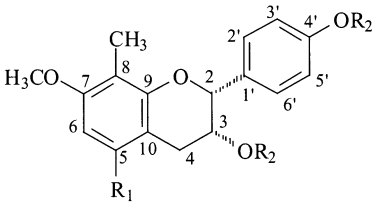
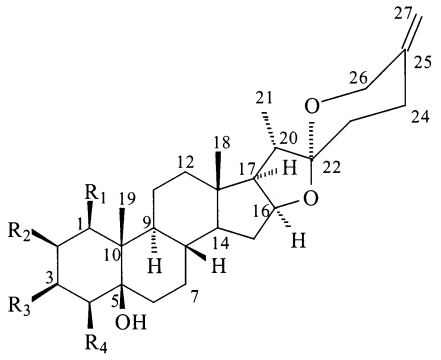
Seven new compounds, including three new flavans [tupichinol A−C (**1**−**3**)], three new spirostanol sapogenins [tupichigenin D−F (**4**−**6**)], and one new pregnane genin [tupipregnenolone (**7**)], together with 18 known compounds, were isolated from the underground parts of *Tupistra chinensis*. The structures of the new compounds were elucidated by spectroscopic analysis and chemical evidence. The structures and relative stereochemistry of **1** and **9** were further confirmed by single-crystal X-ray crystallographic analysis. Compounds $\Delta^{25(27)}$-pentrogenin, **10**, and ranmogenin A showed 100%, 96%, and 80% inhibition, respectively, against human gastric tumor (NUGC) cells at a concentration of 50 μM. $\Delta^{25(27)}$-pentrogenin showed 100% inhibition against human nasopharyngeal carcinoma (HONE-1) cells at a concentration of 50 μM.

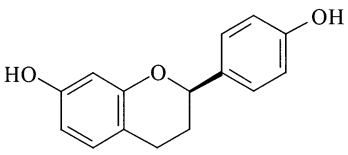
Tupistra chinensis Baker (Liliaceae) is endemic to southwestern regions of the People's Republic of China.[1] In Chinese folk medicine, this species has usually been used for treatment of rheumatic diseases and snake-bite.[1] In previous studies of this species, we described the isolation of several 5β-spirostane type steroidal sapogenins, (20*S*,22*R*)-spirost-25(27)-ene-1β,2β,3β,5β-tetraol (tupichigenin A),[2] (20*S*,22*R*)-spirost-25(27)-ene-1β,2β,3β,4β,5β,7α-hexaol-6-one,[2] spirost-25(27)-ene-1β,3β,4β,5β,6β-pentaol (tupichigenin B),[3] 1β,2β,3β,4β,5β-pentahydroxyspirost-25(27)-en-6-one (tupichigenin C),[3] ranmogenin A,[3] and $\Delta^{25(27)}$-pentrogenin.[3] Further phytochemical investigation of the Chinese folk medicine *T. chinensis* led to the isolation of two new flavan-3-ols, tupichinol A [(2*R*,3*R*)-3,4′-dihy-



1	R_1 = H	R_2= H
1a	R_1 = H	R_2= Ac
2	R_1= OCH_3	R_2= H
2a	R_1= OCH_3	R_2= Ac



4: R_1=β-OH, R_2=H, R_3=α-OH, R_4=β-OH
5: R_1=β-OH, R_2=H, R_3=β-OH, R_4=H
11: R_1=β-OH, R_2=β-OH, R_3=β-OH, R_4=β-OH
12: R_1=β-OH, R_2=H, R_3=β-OH, R_4=β-OH
13: R_1=β-OH, R_2=β-OH, R_3=β-OH, R_4=H
13a: R_1=β-OAc, R_2=β-OAc, R_3=β-OAc, R_4=H
13b: R_1=β-OH, R_2=β-OAc, R_3=β-OAc, R_4=H
13c: R_1=β-OAc, R_2=β-OAc, R_3=β-OH, R_4=H



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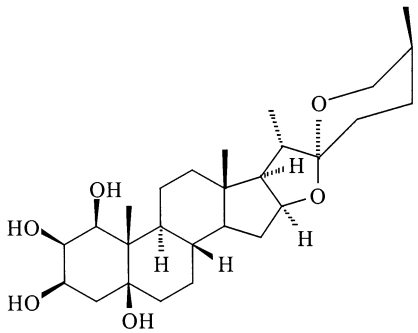

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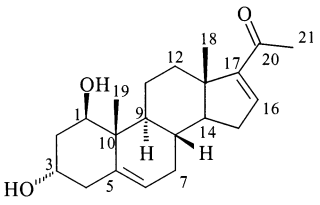
Table 1. ^{1}H NMR Data (δ, ppm) for Compounds **1**, **1a**, **2**, **2a**, and **3**

proton	**1**[a]	**1a**[b]	**2**[a]	**2a**[b]	**3**[a]
H-2	5.03 (s)	5.15 (s)	4.97 (s)	5.05 (s)	4.90 (dd, J = 10.4, 2.4 Hz)
H-3	4.23 (br s)	5.44 (br s)	4.25 (br s)	5.48 (br s)	1.94−2.17 (m)
H-4eq	2.78 (dd, J = 16.4, 3.6 Hz)	2.96 (dd, J = 17.2, 2.4 Hz)	2.78 (ddd, J = 16.8, 3.2, 0.8 Hz)	2.95 (dd, J = 17.6, 2.4 Hz)	2.65 (ddd, J = 12.0, 5.2, 3.2 Hz)
H-4ax	3.16 (dd, J = 16.4, 4.0 Hz)	3.27 (dd, J = 17.2, 4.4 Hz)	2.88 (dd, J = 16.8, 4.4 Hz)	3.01 (dd, J = 17.6, 8.8 Hz)	2.84 (m)
H-5	6.86 (d, J = 8.4 Hz)	6.89 (d, J = 8.8 Hz)			6.83 (d, J = 8.0 Hz)
H-6	6.51 (d, J = 8.4 Hz)	6.53 (d, J = 8.8 Hz)	6.27 (s)	6.16 (s)	6.33 (dd, J = 8.0, 2.4 Hz)
H-8					6.28 (d, J = 2.4 Hz)
H-2′,6′	7.39 (d, J = 8.0 Hz)	7.50 (d, J = 8.4 Hz)	7.38 (d, J = 8.0 Hz)	7.49 (d, J = 8.4 Hz)	7.22 (d, J = 8.8 Hz)
H-3′,5′	6.85 (d, J = 8.0 Hz)	7.12 (d, J = 8.4 Hz)	6.83 (d, J = 8.0 Hz)	7.11 (d, J = 8.4 Hz)	6.81 (d, J = 8.8 Hz)
OH-3	3.72 (br s)		3.65 (br s)		
OMe-5			3.81 (s)	3.82 (s)	
OMe-7	3.78 (s)	3.83 (s)	3.82 (s)	3.85 (s)	
OH-7					8.11 (br s)*
Me-8	2.10 (s)	2.18 (s)	2.02 (s)	2.11 (s)	
OH-4′	8.37 (br s)		8.38 (br s)		8.36 (br s)*
AcO-4′		2.31 (s)		2.31 (s)	
AcO-3		1.87 (s)		1.87 (s)	

[a] Measured in acetone-d_6 at 400 MHz and assignments (*) may be interchangeable. [b] Measured in $CDCl_3$ at 400 MHz.

droxy-7-methoxy-8-methylflavan] (**1**) and tupichinol B [(2*R*,3*R*)-3,4′-dihydroxy-5,7-dimethoxy-8-methylflavan] (**2**), one new 2β-flavan, tupichinol C [(2*R*)-7,4′-dihydroxyflavan] (**3**), three new spirostanol sapogenins, tupichigenins D−F (**4**−**6**), and one new pregnane genin, tupipregnenolone (**7**), together with 18 known compounds, (25*R*)-spirost-5-en-1β,3α-diol (3-epiruscogenin) (**8**),[4] spirost-5,25(27)-dien-1β,3α-diol (3-epi-neoruscogenin) (**9**),[4] 4-allylpyrocatechol



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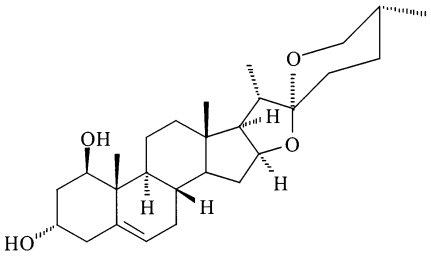

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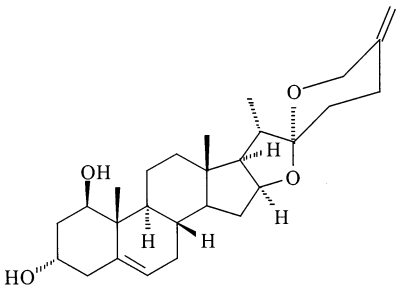

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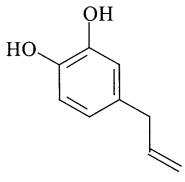

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(**10**),[5] β-sitosterol,[6] β-sitosteryl-3-*O*-β-D-glucoside,[6] stigmasterol,[7] syringic acid,[8] benzoic acid,[9] *p*-hydroxybenzaldehyde,[10] 1-phenylhexan-1-ol,[11] methylparaben,[12] vanillin,[13] vanillic acid,[14] isovanillic acid,[15] *trans*-*p*-hydroxycoumaric acid,[16] *cis*-*p*-hydroxycoumaric acid,[16] *trans*-methyl-*p*-coumarate,[17] and (+)-syringaresinol.[18] Among them, compound **3** possesses an unusual 2β and equatorial aryl skeleton. Pregnanes, which are the biological precursors of cardenolides, remained uninvestigated for a long time. To date, there are over 60 natural plant pregnanes reported, and the C-3 hydroxyl group is always β-oriented like in many other naturally occurring steroidal compounds,[19] while those of compounds **4** and **7**−**9** showed the C-3 hydroxyl group in an α-orientation. Only a few pregnanes have shown specific biological activitiy.[19] In this paper, we report the identification of these isolated compounds and their cytotoxic activities against human gastric tumor (NUGC) cells and nasopharyngeal carcinoma (HONE-1) cells.

Results and Discussion

Compound **1** was obtained as colorless prisms, $[\alpha]^{24}_D$ −40.1° (*c* 0.034, MeOH). The HREIMS showed a $[M]^+$ ion at *m*/*z* 286.1205 (calcd 286.1205), consistent with the molecular formula $C_{17}H_{18}O_4$. The ^{1}H NMR spectra of **1** and its acetate (**1a**) proved the presence of two hydroxyl groups in **1**. In the ^{1}H NMR spectrum (Table 1) of **1**, signals that are characteristic of the 8-methylflavan-3-ol skeleton were observed.[20] Two signals at δ 2.10 (3H, s) and 3.78 (3H, s) were assigned to the methyl group on C-8 and the methoxyl group attached to C-7 in ring A, respectively. The oxymethine protons at δ 5.03 (1H, s) and 4.23 (1H, br s) were assigned to H-2 and H-3, respectively. The signals at δ 8.37 (br s) and 3.72 (br s), which disappeared on addition of D_2O, were assignable to the protons of two hydroxyl groups attached to C-4′ and C-3, respectively.[21] The protons at δ 6.86 (1H, d, J = 8.4 Hz) and 6.51 (1H, d, J = 8.4 Hz) were assigned to H-5 and H-6, respectively.[20] Furthermore, the aromatic protons at δ 7.39 (2H, d, J = 8.0 Hz) and 6.85 (2H, d, J = 8.0 Hz) were assigned to H-2′/6′ and H-3′/5′, respectively.[20] The ^{13}C NMR spectrum (Table 2) showed the characteristic flavan-3-ol signals at δ 80.1, 67.6, and 34.9, corresponding to C-2 (OCH), C-3 (OCH), and C-4 (CH_2), respectively.[21,22] Moreover, this spectrum also indicated the required 12 aromatic carbons (δ 104.7−158.3), one methoxyl carbon at δ 56.6, and one methyl carbon at δ 9.2.

Table 2. ^{13}C NMR Data (δ, ppm) for Compounds **1**, **1a**, **2**, **2a**, and **3**

carbon	**1**[a]	**1a**[b]	**2**[a]	**2a**[b]	**3**[c]
C-2	80.1 d	76.7 d	80.0 d	76.6 d	77.6 d
C-3	67.6 d	68.0 d	67.1 d	67.6 d	24.5 t
C-4	34.9 t	30.4 t	30.0 t	29.6 t	29.9 t
C-5	128.6 d	126.5 d	158.3 s	156.1 s	130.2 d
C-6	104.7 d	103.7 d	89.6 d	99.9 d	107.9 d
C-7	158.3 s	157.0 s	158.4 s	157.0 s	154.8 s
C-8	114.0 s	113.8 s	106.4 s	115.1 s	103.5 d
C-9	154.4 s	152.3 s*	158.2 s	152.8 s*	155.3 s
C-10	113.6 s	110.4 s	102.5 s	106.1 s	114.2 s
C-1′	132.1 s	135.7 s	132.2 s	135.7 s	133.9 s
C-2′,6′	129.5 d	127.3 d	129.5 d	127.3 d	127.6 d
C-3′,5′	116.2 d	121.2 d	116.2 d	121.2 d	115.3 d
C-4′	158.2 s	150.2 s*	154.9 s	150.2 s*	155.9 s
OMe-5			56.4 q	55.4 q	
OMe-7	56.6 q	55.6 q	56.7 q	55.9 q	
Me-8	9.2 q	8.2 q	8.8 q	7.7 q	
CH_3CO		170.3 s		170.3 s	
CH_3CO		21.0 q		21.1 q	
CH_3CO		169.3 s		169.3 s	
CH_3CO		20.8 q		20.9 q	

[a] Measured in acetone-d_6 at 100 MHz. The assignments were made from the DEPT, COSY, NOESY, HETCOR, and long-range HETCOR spectra. [b] Measured in $CDCl_3$ at 100 MHz and assignments (*) may be interchangeable within each column. [c] Measured in $CDCl_3$ at 125 MHz.

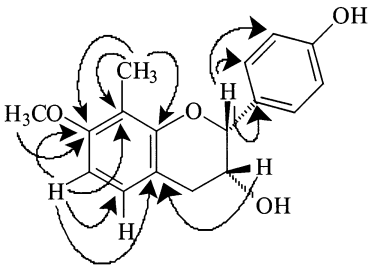


Figure 1. Critical long-range HETCOR correlations for **1**.

Unambiguous assignments for the ^1H and ^{13}C NMR signals in **1** were made by combination of the DEPT, NOESY, $^1H-^1H$ COSY, HETCOR, and long-range HETCOR spectra. The structure of **1** reconciles these data. In the $^1H-^1H$ COSY spectrum, the oxymethine proton at δ 4.23 (H-3) was coupled to the oxymethine proton at δ 5.03 (H-2) and two methylene protons at δ 2.78 (H-$4_{\alpha-eq}$) and 3.16 (H-$4_{\beta-ax}$), establishing the connectivity of H-2, H-3, and H-4. The oxymethine proton at δ 5.03 (H-2) showed correlations with C-1′, C-2′, and C-6′ signals in the long-range HETCOR spectrum (Figure 1) and showed correlations with aromatic proton at δ 7.39 (H-2′, H-6′) in the NOESY spectrum (Figure 2). These findings also supported the B-ring attachment to the C-2 position. In the long-range HETCOR spectrum, the methylene proton at δ 2.78 showed correlations with C-2, C-3, C-5, C-9, and C-10, and the other methylene proton at δ 3.16 showed correlations with C-3, C-9, and C-10. As a result, the methylene protons at δ 2.78 and 3.16 were determined to be at the C-4 position. Furthermore, in the long-range HETCOR spectrum, there were long-range correlations between H-5 and C-6, C-7, and C-9 and between H-6 and C-5, C-7, C-8, and C-10. In the NOESY spectrum, the cross-peaks between H-4/H-5 and H-5/H-6, and H-6/OMe-7, were observed. From the above evidence, the aromatic protons at δ 6.86 and 6.51 were assigned to be at the C-5 and C-6 positions, respectively. Moreover, the methyl protons at δ 2.10 showed correlations with carbon signals C-7, C-8, and C-9 in the long-range HETCOR spectrum, and the cross-peaks between the signals of the methyl protons at δ 2.10 and H-2′/OMe-7 in

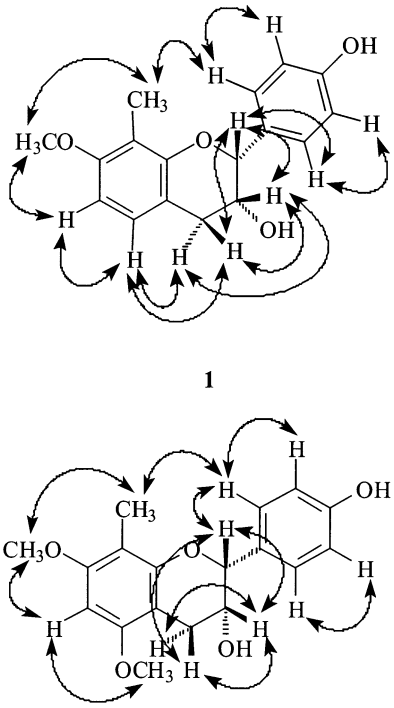


Figure 2. NOESY correlations for **1** and **2**.

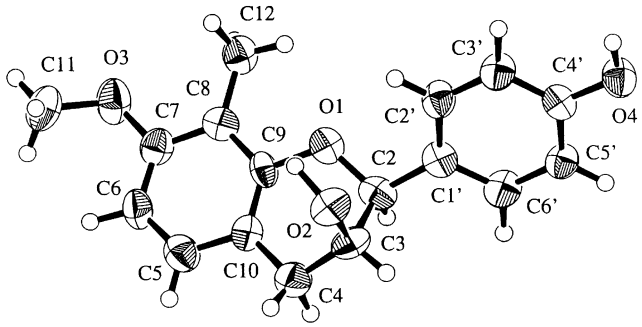


Figure 3. ORTEP representation of **1**.

the NOESY spectrum, indicating that the methyl group must be at the C-8 position and the methoxyl group must be at the C-7 position.

The structure of **1** was further confirmed by the mass spectrum. Prominent fragment ions in the EIMS of **1** were observed at *m*/*z* 151 and 136 arising from rings A and B, respectively, by retro-Diels−Alder (RDA) cleavage, which was consistent with a flavan-3-ol structure with one methoxyl group and one methyl group in the A-ring and one hydroxyl group in the B-ring, respectively.[20,21]

The heterocyclic coupling constant ($J_{2,3}$ < 2 Hz) confirmed the relative 2,3-*cis* configuration, while the optical rotation $[\alpha]^{24}_D$ −40.1° (*c* 0034, MeOH) verified the 2*R*, 3*R* absolute configuration in **1**.[23,24] The relative stereochemistry of compound **1** was also determined by analyzing the NOESY NMR data. The NOESY spectrum showed correlations between H-2 and H-3/H-4_{ax} (δ 3.16), with no correlation observed between H-2 and H-4_{eq} (δ 2.78), indicating that H-2, H-3, and H-4_{ax} were *cis* to each other and the flavan skeleton with a *cis* configuration at the C-2 and C-3 positions. The X-ray crystallographic data (Figure 3) of **1** further confirmed the structure and established its relative stereochemistry. These results indicate unambiguously

that compound **1** is (2*R*,3*R*)-3,4′-dihydroxy-7-methoxy-8-methylflavan, which we have named tupichinol A.

Compound **2** was obtained as colorless prisms, $[\alpha]^{24}_D$ −64.5° (*c* 0.048, MeOH). The HREIMS showed the $[M]^+$ ion at *m*/*z* 316.1313 (calcd 316.1311), consistent with the molecular formula $C_{18}H_{20}O_5$. The IR spectrum and the UV absorption maximum of **2** were very similar to those of **1**, which suggested that both compounds possess the same flavan-3-ol skeleton. On acetylation, **2** gave a diacetate (**2a**). The ^{1}H NMR spectrum (Table 1) of **2** is similar to that of **1**, except for the presence of one additional methoxyl signal at δ 3.81 and the absence of an aromatic signal at δ 6.86 (1H, d, J = 8.4 Hz). Furthermore, a singlet (1H) at δ 6.27 due to an aromatic proton is present in the ^{1}H NMR spectrum. This suggests that three singlets each for three protons at δ 3.82, 3.81, and 2.02 for two methoxyl groups and one methyl group should be located at C-7, C-5, and C-8, respectively, with the oxygenation at C-5 and C-7.[25−27] The singlet at δ 6.27 was assigned to the proton at C-6 and not at C-8 since the signal for the 8-position generally appears slightly downfield.[25,26] This is further supported by the ^{13}C NMR spectrum (Table 2) of **2**, which showed signals similar to those of **1**, except for the presence of one more methoxyl signal at δ 56.4, and the chemical shift of C-5, which showed a downfield shift from δ 128.6 (CH) in **1** to δ 158.3 (C) in **2**. The chemical shift of C-6 showed an upfield shift from δ 104.7 (CH) in **1** to δ 89.6 (CH) in **2**, and the chemical shift of C-10 an upfield shift from δ 113.6 (C) in **1** to δ 102.5 (C) in **2**.

In the NOESY spectrum (Figure 2) of **2**, the methoxyl protons at δ 3.82 showed correlations with the aromatic methyl protons at δ 2.02 (Me-8) and the aromatic proton at δ 6.27 (H-6). This confirmed the position of the methoxyl group (δ 3.82) at C-7. In turn, the aromatic proton at δ 6.27 showed a correlation with the methoxyl protons at δ 3.81. Therefore, the additional methoxyl group (δ 3.81) is located at the C-5 position. The EIMS of **2** exhibited two peaks due to retro-Diels−Alder fragmentations at *m*/*z* 180 and 136 together with a peak at *m*/*z* 150, which was consistent with a flavan-3-ol structure with two methoxyl groups and one methyl group in the A-ring and one hydroxyl group in the B-ring, respectively. On the basis of the above spectroscopic evidence, it is concluded that the structure of **2** is (2*R*,3*R*)-3,4′-dihydroxy-5,7-dimethoxy-8-methylflavan, which we have named tupichinol B.

Compound **3** was obtained as colorless plates, $[\alpha]^{24}_D$ +190.0° (*c* 0.04, $CHCl_3$). The HREIMS showed the $[M]^+$ ion at *m*/*z* 242.0941 (calcd 242.0943), consistent with the molecular formula $C_{15}H_{14}O_3$. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of **3** were similar to those of (2*S*)-7,4′-dihydroxylflavan,[28] except for the presence of the nonequivalent methylene protons at δ 2.65 (H-4_{eq}) and 2.84 (H-4_{ax}) and the two nonequivalent phenolic hydroxyl protons at δ 8.11 and 8.36 in the ^{1}H NMR spectrum. According to the literature,[24,28] a negative $[\alpha]_D$ value indicates that C-2 possesses an *S* configuration. However, **3** had a positive $[\alpha]_D$ value, indicating an unusual *R* configuration at C-2. Consequently, the structure of **3** was probably (2*R*)-7,4′-dihydroxyflavan. The 2-aryl ring in most naturally occurring flavans is α and equatorial.[22] Thus, compound **3** contained the unusual 2β and equatorial aryl skeleton.

In the literature, two reports described the naturally occurring 6,8-dimethylflavan-4-ol glycosides[29] and flavan-3,4-diols,[30] which contained a β and equatorial aryl ring. Compound **3** contained an unusual 2β and equatorial aryl skeleton. The methyl derivatives of flavan-3-ols are rarely found in nature, whereas many flav-4-ones substituted with methoxyl groups have been isolated from a variety of plants, although they are presumed to be biosynthetically related to each other.[22]

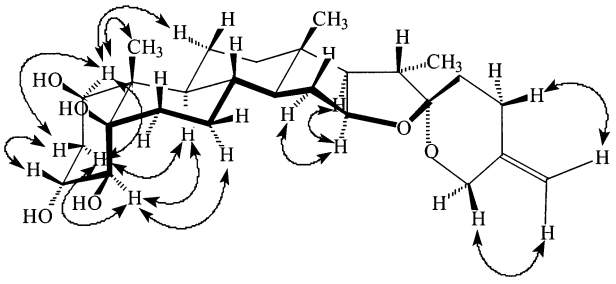
Table 3. ^{13}C NMR Data (δ, ppm) for Compounds **4**, **5**, **6**, and **7**

carbon	**4**[a]	**5**[a]	**6**[b]	**7**[b]
C-1	75.3 d	74.6 d	77.9 d	75.1 d
C-2	36.9 t	40.0 t	68.1 d	40.8 t
C-3	69.4 d	68.4 d	71.8 d	66.5 d
C-4	74.6 d	37.1 t	39.2 t	41.1 t
C-5	77.4 s	75.9 s	74.7 s	140.5 s
C-6	30.6 t	35.6 t	35.9 t	124.3 d
C-7	28.7 t	30.3 t	28.8 t	31.9 t
C-8	35.6 d	34.9 d	34.8 d	31.5 d
C-9	46.0 d	46.4 d	45.6 d	51.8 d
C-10	45.0 s	44.6 s	45.5 s	46.0 s
C-11	21.7 t	22.2 t	21.7 t	24.3 t
C-12	40.4 t	40.7 t	40.0 t	35.8 t
C-13	41.3 s	41.3 s	40.6 s	44.7 s
C-14	56.7 d	56.9 d	56.2 d	56.8 d
C-15	32.7 t	32.8 t	32.2 t	32.5 t
C-16	82.0 d	82.2 d	81.1 d	144.5 d
C-17	63.5 d	63.6 d	62.8 d	155.6 s
C-18	17.2 q	17.2 q	16.5 q	16.2 q
C-19	14.5 q	14.4 q	13.8 q	13.2 q
C-20	42.4 d	42.6 d	42.5 d	196.3 s
C-21	15.6 q	15.7 q	14.8 q	27.0 q
C-22	110.0 s	110.2 s	109.7 s	
C-23	33.8 t	33.8 t	26.3 t	
C-24	29.5 t	29.6 t	26.1 t	
C-25	144.9 s	144.9 s	27.5 d	
C-26	65.6 t	65.7 t	65.1 t	
C-27	109.3 t	109.6 t	16.2 q	

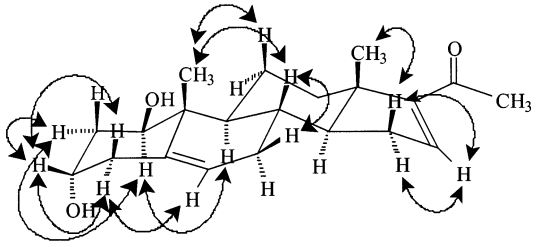
[a] Measured in C_5D_5N at 125 MHz. [b] Measured in C_5D_5N at 100 MHz.

Compound **4** was obtained as a white amorphous powder, $[\alpha]^{24}_D$ −83.6° (*c* 0.160, $CHCl_3$). The HREIMS showed the $[M + H]^+$ ion at *m*/*z* 463.3051 (calcd 463.3060), consistent with the molecular formula $C_{27}H_{42}O_6$. Unambiguous complete assignments for the ^{1}H and ^{13}C NMR signals were made by combination of DEPT, ^{1}H−^{1}H COSY, HMQC, and NOESY spectra. The ^{1}H NMR spectrum showed three methyls at δ 0.90 (3H, s, Me-18), 1.55 (3H, s, Me-19), and 1.14 (3H, d, J = 6.5 Hz, Me-21). The carbinol methine protons at δ 4.24 (1H, br s), 4.79 (1H, m), and 4.40 (1H, d, J = 8.8 Hz) were assigned to H-1, H-3, and H-4, respectively. The proton at δ 4.62 (1H, q, J = 7.5 Hz) was assigned to H-16 at the remaining monooxygenated methine carbon. The protons at δ 4.07 (1H, d, J = 12.5 Hz) and 4.51 (1H, d, J = 12.5 Hz) were assigned to H-26_{eq} and H-26_{ax}, respectively. The geminal protons at C-27 were observed at δ 4.79 and 4.82 as two singlets, which are characteristic of an exocyclic methylene. These observations confirmed the presence of an exocyclic methylene group at C-25. The ^{13}C NMR spectrum (Table 3) showed a total of 27 carbon signals, which were assigned by DEPT as three methyls, 10 methylenes, nine methines (including four oxygenated methines), and five quaternary carbons. The ketal resonance at δ 110.0 (C) was assigned to C-22 of the spirostanol skeleton. Two signals at δ 144.9 (C) and 109.3 (CH_2) were assigned to the C-25 and C-27 positions, respectively. These ^{1}H and ^{13}C NMR signals suggested that **4** is a C-25(27) unsaturated spirostane steroidal sapogenin.

The ^{1}H−^{1}H COSY spectrum showed that the two methylene protons at δ 2.20 (H-2_{ax}) and 2.63 (H-2_{eq}) were coupled to two oxymethine protons at δ 4.24 (H-1) and 4.79 (H-3), and the oxymethine proton at δ 4.40 (H-4) was coupled to the oxymethine proton at δ 4.79 (H-3). These findings supported the placement of three hydroxyl groups at C-1, C-3, and C-4, respectively. Furthermore, three



4



7

Figure 4. NOESY correlations for **4** and **7**.

signals at δ 75.3 (CH), 69.4 (CH), and 74.6 (CH) were assigned to C-1, C-3, and C-4 positions from the HMQC spectrum. The coupling patterns of H-1 at δ 4.24 (br s), H-2$_{\alpha-ax}$ at δ 2.20 (dd, $J_{2\alpha,2\beta} = 14.0$, $J_{2\alpha,3\beta} = 14.0$ Hz), H-2$_{\beta-eq}$ at δ 2.63 (dd, $J_{2\alpha,2\beta} = 14.0$, $J_{2\beta,1\alpha} = 4.8$ Hz), H-3$_\beta$ at 4.79 (m), and H-4$_\alpha$ at δ 4.40 (d, $J_{3\beta,4\alpha} = 8.8$ Hz) indicated that H-1, H-3, and H-4 are α-equatorial, β-axial, and α-axial, respectively.

The relative stereochemistry of **4** was further determined by analyzing the NOESY NMR data (Figure 4). In the NOESY spectrum, the crucial correlations between H-4$_\alpha$ and H-2$_\alpha$/H-7$_\alpha$/H-9$_\alpha$, 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-4 and H-9. Therefore, the hydroxyl group at C-5 has a β-orientation, and the signal at δ 77.4 (C) was assignable to the C-5 position. Furthermore, the NOESY spectrum indicated correlation between H-3$_\beta$ and H-2$_\beta$, with no evidence of any correlation between H-3$_\beta$ and H-2$_\alpha$/H-4$_\alpha$, suggesting that the hydroxyl group at C-3 has an α-orientation. On the basis of the above spectroscopic evidence, the structure of **4** was deduced to be spirost-25-(27)-en-1β,3α,4β,5β-tetraol, which we have named tupichigenin D.

Compound **5** was obtained as a white amorphous powder, $[\alpha]^{24}_D$ −24.2° (*c* 0.180, $CHCl_3$). The HRFABMS showed the $[M + H]^+$ ion at *m/z* 447.3109 (calcd 447.3110), consistent with the molecular formula $C_{27}H_{42}O_5$. Unambiguous complete assignments for the ^{1}H and ^{13}C NMR signals were made by a combination of DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra. The ^{1}H NMR spectrum of **5** is similar to that of **4**, except for the absence of one oxymethine proton. This suggests that both compounds possess the same C-25(27) unsaturated spirostane type skeleton. The ^{1}H NMR spectrum showed three methyls at δ 0.89 (3H, s, Me-18), 1.57 (3H, s, Me-19), and 1.14 (3H, d, $J = 7.0$ Hz, Me-21). The carbinol methine protons at δ 4.27 (1H, br s) and 4.62 (1H, br s) were assigned to H-1 and H-3, respectively. The proton at δ 4.66 (1H, q, $J = 7.0$ Hz) was assigned to H-16. The protons at δ 4.10 (1H, d, $J =$ 12.0 Hz) and 4.52 (1H, d, $J = 12.0$ Hz) were assigned to H-26$_{eq}$ and H-26$_{ax}$, respectively. The geminal protons at C-27 were observed at δ 4.84 and 4.87 as two singlets. The proton signal at δ 4.27 (1H, br s) showed correlation to δ 14.1 (C-19) in the HMBC spectrum, supporting the assignment of the signal at δ 4.27 to H-1. The ^{13}C NMR spectrum (Table 3) showed a total of 27 carbon signals, which were assigned by DEPT as three methyls, 11 methylenes, eight methines (including three oxygenated methines), and five quaternary carbons. The ketal resonance at δ 110.2 (C) was assigned to C-22 of the spirostanol skeleton. Two signals at δ 144.9 (C) and 109.6 (CH_2) were assigned to the C-25 and C-27 positions, respectively. Three signals at δ 74.6 (CH), 68.4 (CH), and 75.9 (C) were assigned to the C-1, C-3, and C-5 positions, respectively. The coupling patterns of H-1 at δ 4.27 (1H, br s) and H-3 at 4.62 (1H, br s) indicated α-equatorial configurations of H-1 and H-3. On the basis of the above spectroscopic evidence, the structure of **5** is spirost-25(27)-en-1β,3β,5β-triol, which we have named tupichigenin E.

Compound **6** was obtained as a white amorphous powder, $[\alpha]^{24}_D$ −61.8° (*c* 0.170, $CHCl_3$). The HREIMS showed the $[M]^+$ ion at *m/z* 464.3145 (calcd 464.3137), consistent with the molecular formula $C_{27}H_{44}O_6$. The ^{13}C NMR spectrum (Table 3), which showed 27 carbon signals, was very similar to that of tupichigenin A with the exception of the signals due to the F-ring part (C-22−C-27). The ^{1}H NMR data are reported in the Experimental Section. On comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of tupichigenin A,[2] the signals due to the C-25(27) exomethylene group in tupichigenin A were replaced by the signals assigned to the Me-CH group at δ_H 1.14 (3H, d, $J = 6.4$ Hz) and 1.60 (1H, m) and δ_C 16.2 (Me) and 27.5 (CH) in **6**. Furthermore, the molecular formula of **6** was greater by 2 amu than that of tupichigenin A. These data strongly indicated that **6** was 25(27)-dihydrotupichigenin A. The coupling pattern of H-26$_{ax}$ (1H, dd, $J = 10.8$, 3.2 Hz), the ^{13}C NMR chemical shifts of the F-ring, and the NOESY correlation between Me-27 and H-26$_{eq}$ (1H, d, $J = 10.8$ Hz) gave evidence for the 25*S* configuration. In view of the spectroscopic evidence, the structure of **6** was deduced to be (25*S*)-spirost-1β,2β,3β,5β-tetraol, which we have named tupichigenin F.

Compound **7** was obtained as a white amorphous solid, $[\alpha]^{24}_D$ −19.3° (*c* 0.880, $CHCl_3$). The HREIMS showed the $[M]^+$ ion at *m/z* 330.2202 (calcd 330.2195), consistent with the molecular formula $C_{21}H_{30}O_3$, suggesting a pregnane skeleton with seven degrees of unsaturation. In the ^{1}H NMR spectrum of **7**, signals that are characteristic of the pregnane skeleton were observed. Evidence for the presence of a methyl ketone and two double bonds at C-5 and C-16 came from a three-proton singlet at δ 2.21 and two vinylic proton signals at δ 5.69 (1H, d, $J = 6.0$ Hz) and 6.57 (1H, dd, $J = 3.2$, 1.6 Hz), respectively. Two oxymethine proton resonances at δ 4.51 (1H, dd, $J = 11.6$, 4.4 Hz) and 4.37 (1H, br s) were assigned to H-1 and H-3, respectively. The ^{13}C NMR spectrum (Table 3) showed 21 carbon signals: three methyls, six methylenes, seven methines, and five quaternary carbons. The chemical shift values of the carbon atoms of rings A, B, and C of **7** were found to be very similar to those of **8** and **9**, whereas those of ring D showed some differences. Two vinylic carbon signals at δ 144.5 (CH) and 155.6 (C) were assigned to C-16 and C-17, respectively. The two signals at δ 27.0 (Me) and 196.3 (C) arose from the methyl ketone, which was attached to ring D. The methylene protons at δ 2.39 (1H, dd, $J = 14.8$, 4.4 Hz, H-2$_\alpha$) and 2.16 (1H, dd, $J = 14.8$, 11.6 Hz, H-2$_\beta$) were determined and were coupled to both of the two oxygenated methine protons at δ 4.51 (H-1) and 4.37 (H-3) in the ^{1}H−^{1}H COSY spectrum. The methylene protons at δ 2.40 (H-4$_\alpha$) and 2.73 (H-4$_\beta$) were in turn coupled with the oxygenated methine proton at δ 4.37 (H-3). These findings

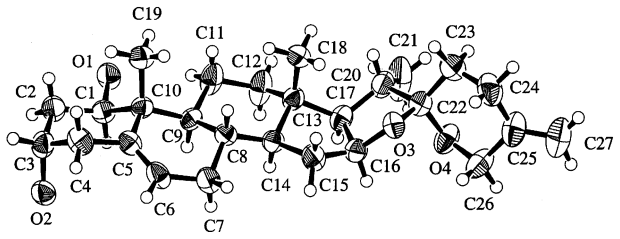


Figure 5. ORTEP representation of **9**.

supported the placement of two hydroxyl groups on the C-1 and C-3 positions. Furthermore, two signals at δ 75.1 (CH), and 66.5 (CH) were assigned to the C-1 and C-3 positions, respectively, from the HETCOR spectrum. The coupling patterns of H-1 at δ 4.51 (1H, dd, $J_{1\alpha,2\beta} = 11.6$, $J_{1\alpha,2\alpha} = 4.4$ Hz) and H-3 at δ 4.37 (1H, br s) indicated that H-1 and H-3 are α-axial and β-equatorial, respectively.

The relative stereochemistry of **7** was also established from the NOESY spectrum, as shown in Figure 4. NOESY correlations between H-1_α and H-9_α/H−2_α, between H-3_β and H-2_α/H-4_α/H-4_β, and between H-6 and H-4_α indicated α-axial and β-equatorial configurations of H-1 and H-3, respectively. On the basis of the spectroscopic evidence, the structure of **7** was shown to be 1β,3α-dihydroxypregna-5,-16-dien-20-one, which we have named tupipregnenolone.

Compounds **8** and **9** are known spirostanol sapogenins and were identified by FAB-MS, ^{1}H and ^{13}C NMR spectra, and two-dimensional NMR spectral data as (25*R*)-spirost-5-en-1β,3α-diol (3-epiruscogenin) and spirost-5,25(27)-dien-1β,3α-diol (3-epi-neoruscogenin), respectively.[4] In the literature, only the ^{13}C NMR data of compounds **8** and **9** were reported, and there were several mistakes in assignment of C-2 (CH_2), C-12 (CH_2), C-13 (C), C-23 (CH_2), and C-24 (CH_2) signals of **9**.[4] We established unambiguous assignments of the ^{1}H and ^{13}C NMR signals, which are reported in the Experimental Section. A comparison of the ^{1}H NMR chemical shifts of ring F resonances for **8** with 25*R* configuration and **6** with 25*S* configuration reveals that H-26_{eq} resonates 0.21 ppm to lower field in **8** than in **6**, and H-26_{ax} resonates 0.59 ppm to higher field. An axial orientation of the Me-27 group is reflected not only in its appearance at 0.45 ppm to lower field but also in the spread of the ^{1}H NMR chemical shifts of the methylene protons occupying β- and γ-positions. In addition, the difference between the chemical shifts for geminal protons at position C-26 was eight times higher in **6** than in **8**. A similar comparison of the ^{13}C NMR chemical shifts of ring F resonances for **8** and **6** showed that all the carbon resonances except C-22 occur at lower field in **8** than in **6**. It is worth noting that substitutions in rings A−D does not usually affect the chemical shifts of ring F and the ^{1}H NMR shielding behavior described will be of general applicability for ring-F-unsubstituted 22α-spirostanoids.[31]

The structure and relative stereochemistry of **9** was also confirmed by single-crystal X-ray analysis. Crystals were obtained by very slow evaporation of a $CHCl_3$−MeOH (5:1) solution of **9**. A computer-generated drawing of the final X-ray model of **9** is given in Figure 5. No absolute configuration is implied.

The known compounds 4-allyl-pyrocatechol (**10**),[5] β-sitosterol,[6] β-sitosteryl-3-*O*-β-D-glucoside,[6] stigmasterol,[7] syringic acid,[8] benzoic acid,[9] *p*-hydroxybenzaldehyde,[10] 1-phenylhexan-1-ol,[11] methylparaben,[12] vanillin,[13] vanillic acid,[14] isovanillic acid,[15] *trans*-*p*-hydroxycoumaric acid,[16] *cis*-*p*-hydroxycoumaric acid,[16] *trans*-methyl-*p*-coumarate,[17] and (+)-syringaresinol[18] were also isolated and characterized from the underground parts of *T. chinensis*. The structures of these known compounds were identified by comparison of their spectroscopic data (UV, IR, NMR, EIMS) with literature values or with authentic samples. All the isolated compounds and previously reported compounds[2,3] were evaluated for their cytotoxicity against human gastric tumor (NUGC) cells and nasopharyngeal carcinoma (HONE-1) cells. Compounds **1**, **7**, **10**, $\Delta^{25(27)}$-pentrogenin (**11**),[3] ranmogenin A (**12**),[3] tupichigenin A (**13**),[2] tupichigenin Aa (**13a**), and tupichigenin Ac (**13c**) exhibited cytotoxicity against the NUGC cell line with inhibition of 30%, 25%, 96%, 100%, 80%, 42%, 50%, and 40%, respectively, at a concentration of 50 μM of the test compound. Compounds **3**, **9**, and **11** exhibited cytotoxicity against the HONE-1 cell line with inhibition of 50%, 45%, and 100%, respectively, at a concentration of 50 μM of the test compound.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The UV spectra were obtained on a JASCO V-530 spectrophotometer; IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 MHz, acetone-d_6), ^{13}C NMR (100, 125 MHz), DEPT, HETCOR, COSY, NOESY, and long-range HETCOR 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 with 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 followed by heating on a hot plate. HPLC was performed using an ODS column (HYPERSIL, 250 mm × 21.2 mm, 5 μm, 2 mL/min).

Plant Material. *T. chinensis* Baker 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 into H_2O-, $CHCl_3$-, and *n*-hexane-soluble parts. The $CHCl_3$ extract was subjected to chromatography over a silica gel column by eluting with gradients of *n*-hexane−EtOAc mixtures of increasing polarity to afford 11 fractions. Fraction 1 was column chromatographed over silica gel using $CHCl_3$−MeOH (100:1) as eluent and was further purified by silica gel column chromatography, eluting with *n*-hexane−EtOAc (6:1) to afford **3** (4 mg, 0.007% dry weight), benzoic acid (4 mg), vanillin (10 mg), methylparaben (5 mg), *trans*-methyl-*p*-coumarate (12 mg), and 1-phenylhexan-1-ol (4 mg). Fraction 2 was rechromatographed on silica gel using $CHCl_3$−MeOH (80:1) and further purified by silica gel column chromatography eluting with *n*-hexane−EtOAc (3:1), then recrystallized with EtOAc to afford compounds **1** (50 mg, 0.083% dry weight) and **2** (40 mg, 0.066% dry weight). Fraction 3 was column chromatographed over silica gel using $CHCl_3$−MeOH (10:1) as eluent to give β-sitosterol (18 mg) and stigmasterol (4 mg) followed by HPLC (RP_{18}, MeOH−H_2O, 97:3) to give **8** (8 mg) and **9** (16 mg). Fraction 4 was column chromatographed over silica gel using $CHCl_3$−MeOH (40:1) as eluent to give **4** (8 mg), β-sitosteryl-3-*O*-β-D-glucoside (10 mg), and (+)-syringaresinol (8 mg). Fraction 6 was column chromatographed over silica gel using $CHCl_3$−MeOH (50:2) as eluent to give **5** (5 mg). Fractions 10 and 11 were column

chromatographed over silica gel using $CHCl_3$−MeOH (8:1) as eluent to give **6** (20 mg), syringic acid (6 mg), and isovanillic acid (12 mg). The EtOAc extract was chromatographed on a silica gel column eluted with a stepwise gradient from $CHCl_3$−MeOH (100:1) to $CHCl_3$−MeOH (100:20) afforded eight fractions. Fractions 1−3 were purified by chromatography over a silica gel column and eluted with $CHCl_3$−MeOH (100:1) to afford *p*-hydroxybenzaldehyde (5 mg) and vanillic acid (8 mg). Fractions 4−6 were purified by chromatography over a silica gel column using $CHCl_3$−MeOH (100:3) as eluent to obtain *trans*-*p*-hydroxycoumaric acid (10 mg), *cis*-*p*-hydroxycoumaric acid (8 mg), and **10** (12 mg). Fractions 7−8 were purified by chromatography over a silica gel column and eluted with $CHCl_3$−MeOH (10:1) to afford **7** (14 mg).

(2*R*,3*R*)-3,4′-Dihydroxy-7-methoxy-8-methylflavan (tupichinol A) (1): colorless prisms (EtOAc); mp 141−142 °C; $[\alpha]^{24}_D$ −40.1° (*c* 0.034, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (3.65), 226 sh (4.31), 206 (4.67) nm; IR ($CHCl_3$) ν_{max} 3360 (OH), 1614, 1517, 1493 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) data, see Table 2; EIMS *m*/*z* 286 $[M]^+$ (32), 151 (100), 136 (42), 107 (59); HREIMS *m*/*z* $[M]^+$ 286.1205 (calcd for $C_{17}H_{18}O_4$ 286.1205).

Crystal data for 1: $C_{17}H_{18}O_4$, MW = 286.33; primitive orthorhombic; $P2_12_12_1$ (No. 19); a = 7.499(4) Å, b = 8.488(3) Å, c = 23.898(3) Å, V = 1521.1(7) Å^3, Z = 4, D_{calc} = 1.250 g/cm^3, Mo Kα (λ = 0.71069 Å), F(000) = 608.00. The R (R_w) value of **1** was 0.064 (0.056). A total of 1594 reflections were collected. The data were collected at a temperature of 25 ±1 °C using a crystal of dimensions of 0.60 × 0.80 × 0.80 mm to a maximum 2θ value of 50.0°, collected on a Rigaku AFC7S diffractometer with graphite-monochromated Mo Kα radiation. The structure was solved by direct methods, expanded using Fourier techniques, and refined by full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. A final electron difference (ΔF) map showed minimal electron density. An ORTEP drawing may be found in Figure 3.

Acetylation of 1. Compound **1** (20 mg) was dissolved in a mixture of pyridine (2 mL) and Ac_2O (2 mL). The reaction mixture was maintained at room temperature for 24 h and then poured into ice water. The product was extracted with $CHCl_3$ and purified by silica gel column chromatography with a gradient of EtOAc in *n*-hexane to give a diacetate (**1a**): ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; EIMS *m*/*z* 370 $[M]^+$ (1), 310 $[M - AcOH]^+$ (84), 268 $[M - AcOH - COCH_2]^+$ (100), 163 (43), 151 (59), 136 (42), 107 (41).

(2*R*,3*R*)-3,4′-Dihydroxy-5,7-dimethoxy-8-methylflavan (tupichinol B) (2): colorless prisms ($CHCl_3$−MeOH); mp 158−159 °C; $[\alpha]^{24}_D$ −64.5° (*c* 0.048, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.61), 226 sh (4.26), 216 (4.63) nm; IR ($CHCl_3$) ν_{max} 3341 (OH), 1614, 1517 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) data, see Table 2; EIMS *m*/*z* 316 $[M]^+$ (42), 180 (100), 150 (81), 136 (58), 107 (74); HREIMS *m*/*z* $[M]^+$ 316.1313 (calcd for $C_{18}H_{20}O_5$ 316.1311).

Acetylation of 2. Compound **2** (10 mg) was acetylated in the same manner as **1** to give a diacetate (**2a**): ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; EIMS *m*/*z* 400 $[M]^+$ (3), 340 $[M - AcOH]^+$ (100), 298 $[M - AcOH - COCH_2]^+$ (86), 193 (43), 181 (50), 107 (42).

(2*R*)-7,4′-Dihydroxylflavan (tupichinol C) (3): colorless plates; $[\alpha]^{24}_D$ +190.0° (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 282 (2.86), 224 sh (3.39), 204 (3.73) nm; IR ($CHCl_3$) ν_{max} 3451 (OH), 1617, 1509 cm^{-1}; ^1H NMR (acetone-d_6 and $CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 2; EIMS *m*/*z* 242 $[M]^+$ (46), 136 (24), 123 (61), 120 (100), 107 (40); HREIMS *m*/*z* $[M]^+$ 242.0940 (calcd for $C_{15}H_{14}O_3$ 242.0943).

Spirost-25(27)-en-1β,3α,4β,5β-tetraol (tupichigenin D) (4): white amorphous powder; $[\alpha]^{24}_D$ −83.6° (*c* 0.160, $CHCl_3$); ^1H NMR (C_5D_5N, 500 MHz) δ 4.24 (1H, br s, H-1), 2.20 (1H, dd, J = 14.0, 14.0 Hz, H-2$_\alpha$), 2.63 (1H, dt, J = 14.0, 4.8 Hz, H-2$_\beta$), 4.79 (1H, m, H-3), 4.40 (1H, d, J = 8.8 Hz, H-4), 1.71 (1H, m, H-6$_\alpha$), 2.43 (1H, dt, J = 12.8, 3.5 Hz, H-6$_\beta$), 1.21 (1H, dd, J = 12.5, 3.5 Hz, H-7$_\alpha$), 1.56 (1H, m, H-7$_\beta$), 1.70 (1H, m, H-8$_\beta$), 1.43 (1H, m, H-9$_\alpha$), 1.42 (2H, m, H-11), 1.01 (1H, m, H-12), 1.65 (1H, m, H-12), 0.95 (1H, m, H-14$_\alpha$), 2.06 (1H, m, H-15$_\alpha$), 1.47 (1H, m, H-15$_\beta$), 4.62 (1H, q, J = 7.5 Hz, H-16$_\alpha$), 1.83 (1H, m, H-17$_\alpha$), 0.90 (3H, s, H-18), 1.55 (3H, s, H-19), 2.01 (1H, p, J = 7.0 Hz, H-20$_\beta$), 1.14 (3H, d, J = 6.5 Hz, H-21), 1.80 (1H, m, H-23), 1.86 (1H, m, H-23), 2.75 (1H, td, J = 11.0, 5.0 Hz, H-24$_\alpha$), 2.30 (1H, m, H-24$_\beta$), 4.51 (1H, d, J = 12.5 Hz, H-26$_\alpha$), 4.07 (1H, d, J = 12.5 Hz, H-26$_\beta$), 4.79 (1H, br s, H-27$_A$), 4.82 (1H, br s, H-27$_B$); ^{13}C NMR (C_5D_5N, 125 MHz) data, see Table 3; FABMS *m*/*z* 463 $[M + H]^+$ (14), 445 (11), 307 (20), 289 (13), 154 (100), 136 (70); HRFABMS *m*/*z* $[M + H]^+$ 463.3051 (calcd for $C_{27}H_{43}O_6$ 463.3060).

Acetylation of Tupichigenin A. Tupichigenin A (**13**) (120 mg) was acetylated in the same manner as **1** to give a triacetate (tupichigenin Aa) (**13a**): ^1H NMR (C_5D_5N, 400 MHz) δ 5.92 (1H, d, J = 3.6 Hz, H-1), 5.50 (1H, t, J = 3.6 Hz, H-2), 5.84 (1H, ddd, J = 4.0, 3.6, 1.6 Hz, H-3), 2.05 (1H, dd, J = 15.6, 1.6 Hz, H-4$_\alpha$), 2.68 (1H, dd, J = 15.6, 4.0 Hz, H-4$_\beta$), 4.57 (1H, q-like, J = 6.8 Hz, H-16), 0.81 (3H, br s, H-18), 1.24 (3H, br s, H-19), 1.08 (3H, d, J = 7.2 Hz, H-21), 4.04 (1H, d, J = 12.0 Hz, H-26$_{eq}$), 4.47 (1H, d, J = 12.0 Hz, H-26$_{ax}$), 4.79 (1H, br s, H-27$_A$), 4.82 (1H, br s, H-27$_B$), 2.22 (3H, s, AcO), 2.14 (3H, s, AcO), 2.01 (3H, s, AcO); ^{13}C NMR (C_5D_5N, 100 MHz) δ 73.3 (C-1), 67.6 (C-2), 70.2 (C-3), 39.8 (C-4), 75.0 (C-5), 36.5 (C-6), 30.0 (C-7), 34.7 (C-8), 45.9 (C-9), 45.6 (C-10), 21.8 (C-11), 40.4 (C-12), 40.5 (C-13), 55.8 (C-14), 32.1 (C-15), 81.3 (C-16), 63.0 (C-17), 16.4 (C-18), 12.7 (C-19), 41.9 (C-20), 15.0 (C-21), 109.5 (C-22), 34.7 (C-23), 30.0 (C-24), 144.4 (C-25), 65.0 (C-26), 108.8 (C-27), 170.4, 170.2, 169.8 (CH_3CO ×3), 21.1, 20.8, 20.7 (CH_3CO ×3); FABMS *m*/*z* 589 $[M + H]^+$ (6), 511 (1), 447 (1), 391(2), 239 (4), 173 (14), 149 (39), 137 (28), 43 (100). A diacetate (tupichigenin Ab) (**13b**): ^1H NMR (C_5D_5N, 400 MHz) δ 4.37 (1H, d, J = 2.8 Hz, H-1), 5.46 (1H, t, J = 2.8 Hz, H-2), 5.91 (1H, dd, J = 4.4, 2.8 Hz, H-3), 2.17 (1H, br d, J = 16.0 Hz, H-4$_\alpha$), 2.69 (1H, dd, J = 16.0, 4.4 Hz, H-4$_\beta$), 4.61 (1H, q-like, J = 7.2 Hz, H-16), 0.84 (3H, br s, H-18), 1.59 (3H, br s, H-19), 1.11 (3H, d, J = 6.8 Hz, H-21), 4.05 (1H, d, J = 12.0 Hz, H-26$_{eq}$), 4.49 (1H, d, J = 12.0 Hz, H-26$_{ax}$), 4.79 (1H, br s, H-27$_A$), 4.82 (1H, br s, H-27$_B$), 2.05 (3H, s, AcO), 2.03 (3H, s, AcO); ^{13}C NMR (C_5D_5N, 100 MHz) δ 73.8 (C-1), 69.4 (C-2), 69.9 (C-3), 39.2 (C-4), 74.1 (C-5), 36.1 (C-6), 28.1 (C-7), 34.6 (C-8), 44.8 (C-9), 45.0 (C-10), 20.9 (C-11), 40.0 (C-12), 40.0 (C-13), 55.3 (C-14), 32.6 (C-15), 81.0 (C-16), 62.2 (C-17), 15.8 (C-18), 13.0 (C-19), 41.3 (C-20), 14.3 (C-21), 109.3 (C-22), 34.2 (C-23), 31.5 (C-24), 143.9 (C-25), 64.6 (C-26), 108.7 (C-27), 171.2, 171.0 (CH_3CO ×2), 20.8, 20.5 (CH_3CO ×2); FABMS *m*/*z* 547 $[M + H]^+$ (17), 529 (5), 469 (4), 409 (5), 307 (4), 289 (5), 267 (5), 154 (70), 137 (75), 43 (100). A diacetate (tupichigenin Ac) (**13c**): ^1H NMR (C_5D_5N, 400 MHz) δ 5.96 (1H, d, J = 3.2 Hz, H-1), 5.29 (1H, t, J = 3.2 Hz, H-2), 4.60 (1H, ddd, J = 3.6, 3.2, 2.4 Hz, H-3), 2.13 (1H, dd, J = 14.8, 3.6 Hz, H-4$_\alpha$), 2.52 (1H, dd, J = 14.8, 2.4 Hz, H-4$_\beta$), 4.55 (1H, q-like, J = 7.2 Hz, H-16), 0.76 (3H, br s, H-18), 1.21 (3H, br s, H-19), 1.04 (3H, d, J = 7.2 Hz, H-21), 4.02 (1H, d, J = 11.6 Hz, H-26$_{eq}$), 4.43 (1H, d, J = 11.6 Hz, H-26$_{ax}$), 4.76 (1H, br s, H-27$_A$), 4.79 (1H, br s, H-27$_B$), 2.13 (3H, s, AcO), 2.05 (3H, s, AcO); ^{13}C NMR (C_5D_5N, 100 MHz) δ 74.1 (C-1), 69.5 (C-2), 70.6 (C-3), 38.4 (C-4), 74.7 (C-5), 35.6 (C-6), 28.6 (C-7), 34.9 (C-8), 46.5 (C-9), 46.0 (C-10), 22.1 (C-11), 40.5 (C-12), 40.6 (C-13), 56.0 (C-14), 32.2 (C-15), 81.4 (C-16), 63.0 (C-17), 16.4 (C-18), 12.8 (C-19), 41.9 (C-20), 15.0 (C-21), 109.5 (C-22), 33.2 (C-23), 29.0 (C-24), 144.4 (C-25), 65.0 (C-26), 108.7 (C-27), 171.2, 170.5 (CH_3CO ×2), 21.0, 20.9 (CH_3CO ×2); FABMS *m*/*z* 547 $[M + H]^+$ (11), 391 (4), 338 (5), 307 (11), 289 (8), 154 (100), 136 (87).

Spirost-25(27)-en-1β,3β,5β-triol (tupichigenin E) (5): white amorphous powder; $[\alpha]^{24}_D$ −24.4° (*c* 0.180, $CHCl_3$); ^1H NMR (C_5D_5N, 500 MHz) δ 4.27 (1H, br s, H-1), 4.62 (1H, br s, H-3), 4.66 (1H, q, J = 7.0 Hz, H-16), 0.89 (3H, s, H-18), 1.57 (3H, s, H-19), 1.14 (3H, d, J = 7.0 Hz, H-21), 4.10 (1H, d, J = 12.0 Hz, H-26$_{eq}$), 4.52 (1H, d, J = 12.0 Hz, H-26$_{ax}$), 4.84 (1H, s, H-27$_A$), 4.87 (1H, s, H-27$_B$); ^{13}C NMR (C_5D_5N, 125 MHz) data, see Table 3; FABMS *m*/*z* 447 $[M + H]^+$ (1), 307 (23), 154 (100),

137 (64); HRFABMS *m/z* [M + H]$^+$ 447.3109 (calcd for $C_{27}H_{43}O_5$ 447.3110).

(25*S*)-Spirost-1*β*,2*β*,3*β*,5*β*-tetraol (tupichigenin F) (6): white amorphous powder; $[\alpha]^{24}_D$ −61.8° (*c* 0.170, $CHCl_3$); ^{1}H NMR (C_5D_5N, 400 MHz) *δ* 4.35 (1H, br s, H-1), 4.10 (1H, br s, H-2), 4.67 (1H, br s, H-3), 2.53 (1H, dd, *J* = 14.8, 3.2 Hz, H-4$_\alpha$), 2.20 (1H, d, *J* = 14.8 Hz, H-4$_\beta$), 4.58 (1H, q, *J* = 7.2 Hz, H-16), 0.85 (3H, s, H-18), 1.59 (3H, s, H-19), 1.07 (3H, d, *J* = 7.2 Hz, H-21), 1.60 (1H, m, H-25), 3.37 (1H, d, *J* = 10.8 Hz, H-26$_{eq}$), 4.08 (1H, dd, *J* = 10.8, 3.2 Hz, H-26$_{ax}$), 1.14 (3H, d, *J* = 6.4 Hz, H-27); ^{13}C NMR (C_5D_5N, 100 MHz) data, see Table 3; EIMS *m/z* 464 [M]$^+$ (2), 392 (4), 317 (7), 303 (7); HREIMS *m/z* [M]$^+$ 464.3145 (calcd for $C_{27}H_{44}O_6$ 464.3137).

1*β*,3α-Dihydroxypregna-5,16-dien-20-one (tupipregnenolone) (7): white amorphous solid; $[\alpha]^{24}_D$ −19.3° (*c* 0.880, $CHCl_3$); ^{1}H NMR (C_5D_5N, 400 MHz) *δ* 4.51 (1H, dd, *J* = 11.6, 4.4 Hz, H-1), 2.16 (1H, dd, *J* = 14.8, 11.6 Hz, H-2$_\beta$), 2.39 (1H, dd, *J* = 14.8, 4.4 Hz, H-2$_\alpha$), 4.37 (1H, br s, H-3), 2.40 (1H, br d, *J* = 14.4 Hz, H-4$_\alpha$), 2.73 (1H, br d, *J* = 14.4 Hz, H-4$_\beta$), 5.69 (1H, d, *J* = 6.0 Hz, H-6), 1.70 (1H, m, H-7$_\alpha$), 1.96 (1H, m, H-7$_\beta$), 1.65 (1H, m, H-8$_\beta$), 1.62 (1H, m, H-9$_\alpha$), 1.90 (1H, m, H-11$_\beta$), 2.98 (1H, ddd, *J* = 14.4, 4.8, 4.0 Hz, H-11$_\alpha$), 1.52 (1H, td, *J* = 14.4, 4.0 Hz, H-12$_\alpha$), 2.67 (1H, ddd, *J* = 14.4, 4.8, 2.8 Hz, H-12$_\beta$), 1.43 (1H, m, H-14$_\alpha$), 1.94 (1H, dd, *J* = 12.0, 1.6 Hz, H-15$_\beta$), 2.13 (1H, m, H-15$_\alpha$), 6.57 (1H, dd, *J* = 3.2, 1.6 Hz, H-16), 1.03 (3H, s, H-18), 1.35 (3H, s, H-19), 2.21 (3H, s, H-21); ^{13}C NMR (C_5D_5N, 100 MHz) data, see Table 3; EIMS *m/z* 330 [M]$^+$ (4), 312 (8), 105 (34), 91 (52), 55 (100); HREIMS *m/z* [M]$^+$ 330.2202 (calcd for $C_{21}H_{30}O_3$ 330.2195).

(25*R*)-Spirost-5-en-1*β*,3α-diol (3-epiruscogenin) (8): white amorphous powder; $[\alpha]^{24}_D$ −63.8° (*c* 1.280, $CHCl_3$); ^{1}H NMR (C_5D_5N, 400 MHz) *δ* 4.53 (1H, dd, *J* = 12.4, 4.4 Hz, H-1), 2.39 (1H, dd, *J* = 12.4, 4.4 Hz, H-2$_{eq}$), 2.16 (1H, t, *J* = 12.4 Hz, H-2$_{ax}$), 4.37 (1H, br s, H-3), 2.37 (1H, d, *J* = 14.4 Hz, H-4$_{eq}$), 2.73 (1H, d, *J* = 14.4 Hz, H-4$_{ax}$), 5.65 (1H, d, *J* = 5.6 Hz, H-6), 1.66, (1H, m, H-7), 1.95, (1H, m, H-7), 1.66 (1H, m, H-8$_\beta$), 1.57, (1H, m, H-9$_\alpha$), 2.94 (1H, dd, *J* = 10.4, 3.6 Hz, H-11$_\alpha$), 1.71 (1H, m, H-11$_\beta$), 1.25 (1H, m, H-12), 1.68 (1H, m, H-12), 1.16 (1H, m, H-14$_\alpha$), 2.05 (1H, m, H-15$_\alpha$), 1.47 (1H, m, H-15$_\beta$), 4.49 (1H, m, H-16$_\alpha$), 1.80 (1H, m, H-17$_\alpha$), 0.93 (3H, s, H-18), 1.35 (3H, s, H-19), 1.95 (1H, m, H-20$_\beta$), 1.09 (3H, d, *J* = 6.8 Hz, H-21$_\alpha$), 1.68 (2H, m, H-23), 1.55 (2H, m, H-24), 1.57 (1H, m, H-25$_{ax}$), 3.49 (1H, t, *J* = 10.4 Hz, H-26$_{ax}$), 3.58 (1H, dd, *J* = 10.4, 3.2 Hz, H-26$_{eq}$), 0.69 (3H, d, *J* = 5.6 Hz, H-27); ^{13}C NMR (C_5D_5N, 100 MHz) *δ* 75.4 (C-1), 40.8 (C-2), 66.5 (C-3), 41.1 (C-4), 140.1 (C-5), 124.6 (C-6), 32.4 (C-7), 31.8 (C-8), 51.2 (C-9), 44.6 (C-10), 24.4 (C-11), 40.6 (C-12), 40.2 (C-13), 57.0 (C-14), 32.4 (C-15), 81.1 (C-16), 63.1 (C-17), 16.6 (C-18), 13.2 (C-19), 42.0 (C-20), 15.0 (C-21), 109.2 (C-22), 32.9 (C-23), 29.2 (C-24), 30.6 (C-25), 66.8 (C-26), 17.3 (C-27); FABMS (positive mode) *m/z* 431 [M + H]$^+$ (6), 304 (21), 282 (81), 256 (10), 176 (9), 154 (44), 137(44).

Spirost-5,25(27)-dien-1*β*,3α-diol (3-epineoruscogenin) (9): colorless prisms; $[\alpha]^{24}_D$ −61.7° (*c* 1.670, $CHCl_3$); ^{1}H NMR (C_5D_5N, 400 MHz) *δ* 4.53 (1H, dd, *J* = 11.6, 2.0 Hz, H-1), 2.42 (1H, d, *J* = 14.0 Hz, H-2$_\alpha$), 2.17 (1H, ddd, *J* = 14.0, 11.6, 2.0.0 Hz, H-2$_\beta$), 4.37 (1H, br s, H-3), 2.38 (1H, dt, *J* = 14.4, 2.4 Hz, H-4$_\alpha$), 2.73 (1H, d, *J* = 14.4 Hz, H-4$_\beta$), 5.66 (1H, d, *J* = 5.6 Hz, H-6), 2.94 (1H, d, *J* = 11.2 Hz, H-11$_\alpha$), 4.49 (1H, m, H-16), 0.92 (3H, s, H-18), 1.34 (3H, s, H-19), 1.04 (3H, d, *J* = 6.8 Hz, H-21), 4.45 (1H, d, *J* = 12.0 Hz, H-26$_\alpha$), 4.03 (1H, d, *J* = 12.0 Hz, H-26$_\beta$), 4.78 (1H, br s, H-27$_A$), 4.81 (1H, br s, H-27$_B$); ^{13}C NMR (C_5D_5N, 100 MHz) *δ* 75.4 (C-1), 40.8 (C-2), 66.5 (C-3), 41.0 (C-4), 140.0 (C-5), 124.5 (C-6), 32.9 (C-7), 32.3 (C-8), 51.2 (C-9), 44.6 (C-10), 24.3 (C-11), 40.5 (C-12), 40.2 (C-13), 57.0 (C-14), 32.3 (C-15), 81.4 (C-16), 63.1 (C-17), 16.6 (C-18), 13.2 (C-19), 41.8 (C-20), 14.9 (C-21), 109.4 (C-22), 33.2 (C-23), 28.9 (C-24), 144.5 (C-25), 64.9 (C-26), 108.6 (C-27); FABMS (positive mode) *m/z* 429 [M + H]$^+$ (17), 304 (16), 282 (33), 176 (17), 154 (39), 137 (51).

Crystal data for 9: $C_{28}H_{44}O_5$, MW = 460.65; C-centered monoclinic; *C*2 (No. 5); *a* = 20.172(3) Å, *b* = 7.740(5) Å, *c* = 18.403(4) Å, *V* = 2601(2) Å^3, *Z* = 4, D_{calc} = 1.176 g/cm^3, Mo Kα (*λ* = 0.71069 Å), *F*(000) = 1008.00. The *R* (*R*$_w$) value of **9** was 0.045 (0.039). A total of 2556 reflections were collected. The data were collected at a temperature of 25.0 °C using a crystal of dimensions of 0.22 × 0.40 × 0.64 mm to maximum 2*θ* value of 50.0°, collected using the *ω*−2*θ* scan technique on a Rigaku AFC7S diffractometer with graphite-monochromated Mo Kα radiation. A total of 2478 unique reflections were collected, of which 1704 (69%) were judged observed and used in subsequent calculations. The structure was solved by direct methods and expanded using Fourier techniques and refined by full-matrix least-squares calculations. The final cycle of full-matrix least-squares refinement was based on 1704 observed reflections ($I > 3.00\sigma(I)$) and 298 variable parameters. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final (Δ*F*) map showed minimal electron density (+0.15 and −0.13 e/Å^3).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP: The Supporting Information paragraph was not included in the version posted on Dec 20, 2002.

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