Chemical Constituents of *Tupistra chinensis* Rhizomes

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A new pregnane glycoside, a dibenzylbutyrolactone lignan, 5-hydroxymatairesinol dimethyl ether, and three new flavonoids, including one 8-methylflavan-3-ol, and two 8-methylflavones, together with five known flavonoids and two known alkaloids were isolated from the rhizomes of *Tupistra chinensis*. The structures of all compounds were elucidated by spectral studies.

Key words *Tupistra chinensis*; Liliaceae; pregnane glycoside; lignan; flavonoid; alkaloid

Tupistra chinensis BAKER (Liliaceae) is endemic to southwestern regions of the People's Republic of China.[1)] As a Chinese folk medicine, this species has usually been used for treatment of rheumatic diseases and snake-bite.[1)] In previous investigations,[2—4)] we have reported the isolation and structural elucidation of several steroidal sapogenins, flavans, and a pregnane genin from this species. Our current phytochemical study for new efficient agents has led to the isolation of a new pregnane glycoside, namely tupichinin A (**1**) and a first naturally-occurring lignan possessing a hydroxyl group at the benzyl position, 5-hydroxymatairesinol dimethyl ether, namely tupichilignan A (**2**), three new flavonoids, tupichinol D (**3**), tupichinol E (**4**), and tupichinol F (**5**), together with five known flavonoids, 3-hydroxy-2-(4-hydroxyphenyl)-7-methoxychromen-4-one (**6**),[5)] rhamnocitrin (**7**),[6)] 3,7-dihydroxy-2-(4-hydroxyphenyl)-chromen-4-one (**8**),[7)] 2-(4-hydroxyphenyl)-4*H*-chromen-7-ol (**9**),[8)] and 3,5,7,8-tetramethoxy-2-(4-methoxyphenyl)-chromen-4-one (**10**),[9,10)] and two known alkaloids, oxoglaucine (**11**)[11)] and oxopurpureine (**12**).[11)] The characterization and structure elucidation of **1**—**5** are reported herein.

Tupichinin A (**1**) was obtained as colorless oil, $[\alpha]_D^{24}$ −12.6° (c=2.30, MeOH), showed in the HR-FAB-MS (positive mode) a pseudomolecular $[M+Na]^+$ peak at m/z 515.2832 (Calcd 515.2829), consistent with the molecular formula $C_{27}H_{40}O_8$, suggesting a pregnane glycoside skeleton with eight degrees of unsaturation.

Unambiguous full assignments for the ^{1}H- and ^{13}C-NMR signals were listed in Table 1 based on an analysis of the combination of distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY), heteronuclear chemical shift correlation (HETCOR), long range-heteronuclear chemical shift correlation (LR-HETCOR), and nuclear Overhauser and exchange spectroscopy (NOESY) spectra data. In the ^{1}H-NMR spectrum in CD_3OD of **1**, signals that are characteristic of the pregnane glycoside skeleton were observed. The ^{1}H-NMR spectrum showed the presence of two methyl groups at δ 0.93 (3H, s, Me-18) and 1.06 (3H, s, Me-19) and an anomeric proton at δ 4.33 (1H, d, J=8.0 Hz). 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 2.26 and two vinylic proton signals at δ 5.49 (1H, d, J=5.6 Hz) and 6.90 (1H, dd, J=3.2, 1.6 Hz), respectively. Two oxymethine proton resonances at δ 3.78 (1H, dd, J=12.0, 4.4 Hz) and 4.05 (1H, br s) were assigned to H-1 and H-3, respectively. The fully decoupled ^{13}C- and DEPT NMR spectra of **1** exhibited 27 carbon signals, which consisted of three methyls, seven methylenes, 12 methines, and five quaternary carbons. One carbonyl carbon at δ 199.5 (C-20); two vinylic carbons at d 140.2 (C-5) and 125.3 (C-6); an anomeric carbon at δ 102.9 (C-1′ of glucose); and two methyl groups at d 16.2 (C-18) and 13.2 (C-19) were also confirmed in ^{13}C-NMR spectra. In the ring D of **1**, two vinylic carbon signals at δ 147.2 (CH) and 156.7 (C) were assigned to the C-16 and C-17, respectively. The two signals at δ 27.1 (Me) and 199.5 (C) arose from the methyl ketone, which was attached to ring D. In the NOESY spectrum (Fig. 1), the three-proton signal at δ 2.26 (Me-21) showed correlations with the proton signals at δ 6.90 (H-16) and 0.93 (Me-18). Moreover, in the LR-HETCOR spectrum (Fig. 2), one carbonyl carbon at δ 199.5 (C-20) exhibited correlation with the proton signal at δ 6.90 (H-16), and the ^{13}C signal at δ 156.7 (C-17) showed correlation with the proton signal at δ 0.93 (Me-18). These findings further support the methyl ketone was attached to the C-17 position. The methylene protons at δ 2.00 (1H, m, H-2α) and δ 2.71 (1H, m, H-2β) were

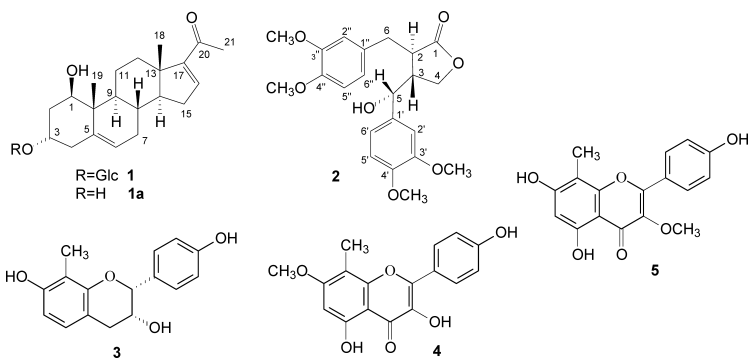

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Table 1. ^{13}C- and ^{1}H-NMR Data for **1**[a] and **1a**[b] (100, 400 MHz)

Position	**1**		**1a**	
	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)
1	76.0, d	3.78, dd (12.0, 4.0)	75.1, d	4.51, dd (11.6, 4.4)
2	36.7, t	2.00, m, Hα	40.8, t	2.39, dd (14.8, 4.4), Hα
		1.71, m, Hβ		2.16, dd (4.8, 11.6), Hβ
3	75.4, d	4.05, br s	66.5, d	4.37, br s
4	39.1, t	2.28, m, Hα	41.1, t	2.40, br d (14.4), Hα
		2.51, d (14.4), Hβ		2.73, br d (14.4), Hβ
5	140.2, s		140.5, s	
6	125.3, d	5.49, d (5.6)	124.3, d	5.69, d (6.0)
7	32.5, t	1.78, m, Hα	31.9, t	1.70, m, Hα
		1.95, m, Hβ		1.96, m, Hβ
8	32.4, d	1.65, m, Hβ	31.5, d	1.65, m, Hβ
9	52.1, d	1.35, td, (12.0, 8.0), Hα	51.8, d	1.62, m, Hα
10	46.9, s		46.0, s	
11	24.8, t	2.38, m, Hα	24.3, t	2.98, ddd (14.4, 4.8, 4.0), Hα
		1.62, m, Hβ		1.90, m, Hβ
12	36.5, t	1.35, m, Hα	35.8, t	1.52, td, (14.4, 4.0), Hα
		2.28, m, Hβ		2.67, ddd, (14.4, 4.8, 2.8), Hβ
13	44.9, s		44.7, s	
14	58.0, d	1.47, m, Hα	56.8, d	1.43, m, Hα
15	33.5, t	2.30, m	32.5, t	2.13, m, Hα
				1.94, dd, (12.0, 1.6), Hβ
16	147.2, d	6.90, dd, (3.2, 1.6)	144.5, d	6.57, dd, (3.2, 1.6)
17	156.7, s		155.6, s	
18	16.2, q	0.93, s	16.2, q	1.03, s
19	13.2, q	1.06, s	13.2, q	1.35, s
20	199.5, s		196.3, s	
21	27.1, q	2.26, s	27.0, q	2.21, s
1′	102.9, d	4.33, d, (8.0), Hα		
2′	74.9, d	3.16, t, (8.0)		
3′	78.2, d	3.35, m		
4′	71.7, d	3.30, m		
5′	77.9, d	3.27, m		
6′	62.8, t	3.66, dd (11.6, 5.2)		
		3.86, dd (11.6, 2.0)		

a) Spectra were measured in CD_3OD. *b*) Spectra were measured in C_5D_5N.[4]

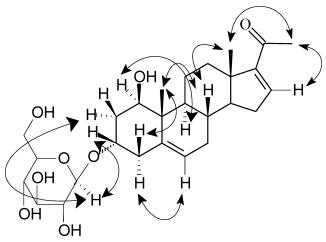

Fig. 1. NOESY Correlations of **1**

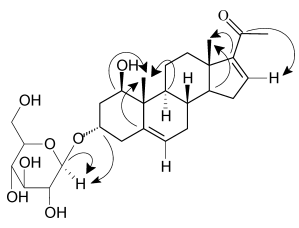

Fig. 2. LR-HETCOR Correlations (C to H) of **1**

determined, and were coupled to both of the two oxygenated methine protons at δ 3.78 (H-1) and δ 4.05 (H-3) in the ^{1}H–^{1}H COSY spectrum. The methylene protons at δ 2.28 (H-4α) and δ 2.51 (H-4β) were in turn coupled with the oxygenated methine proton at δ 4.05 (H-3). These findings supported the placement of two hydroxyl groups on C-1 and C-3 positions. Furthermore, two signals at δ 76.0 (CH), and 75.4 (CH) were assigned to the C-1 and C-3 positions, respectively, from the HETCOR spectrum. The coupling patterns of H-1 at δ 3.78 (1H, dd, $J_{1\alpha,2\beta}$=12.0, $J_{1\alpha,2\alpha}$=4.4 Hz) and H-3 at δ 4.05 (1H, br s) indicated that H-1 and H-3 are α-axial and β-equatorial, respectively. The structure of the saccharide moiety of **1** and its linkage position to the aglycone moiety were determined by the following data. On comparison of the ^{13}C signals of **1** with those of pregnane **1a**,[4] a set of additional six signals, corresponding to a β-D-glucopyranosyl unit appeared. The signal due to the C-3 carbon, which was observed at δ 66.5 in **1a**, downfield shift to δ 75.4, accompanied by upfield shifts of the signal due to C-2 and C-4 by 4.1 and 2.0 ppm, respectively, indicating the sugar moiety was linked at C-3 position. The assignments of the ^{1}H and ^{13}C signals due to the saccharide moiety were as shown in Table 1. In the LR-HETCOR spectrum, the anomeric proton signal at δ 4.33 exhibited correlations with the ^{13}C signals at δ 75.4 (C-3 of aglycone) and 102.9 (C-1′ of glucose). In the NOESY spectrum, the anomeric proton signal at δ 4.33 exhibited correlations with the proton signals at δ 4.05 (H-3 of aglycone) and 2.00 (H-2α of aglycone). The α-configuration of the anomeric carbon of glucopyranosyl unit was determined by $J_{H1–H2}$ value (>7.0 Hz).

To confirm the nature of the sugar unit and to determine its absolute configuration, compound **1** was subjected to acid hydrolysis (4 N HCl), followed by HPLC analysis on a chiral column in comparison with D-(+)-glucose. By this procedure the sugar was identified to belong to the common D-series.

The relative stereochemistry of **1** was also established from the NOESY spectrum. NOESY correlations between H-1α and H-9α, and between H-6 and H-4α indicated α-axial and β-equatorial configurations of H-1 and H-3, respectively. Based on the above spectroscopic evidence, the structure of **1** was established as 1β-hydroxypregna-5,16-dien-3-β-ol-20-one 3-*O*-β-D-glucopyranoside, namely tupichinin A.

Tupichilignan A (**2**) was isolated as colorless oil, $[\alpha]_D^{24}$ $-2.8°$ ($c=1.45$, acetone). Its molecular formula $C_{22}H_{26}O_7$ was established by EI-MS ($[M]^+$, *m/z* 402) and HR-EI-MS (*m/z* 402.1683). The ^{1}H-NMR spectrum of **2** displayed signals for two methine protons at δ 2.97 (1H, m, H-2) anδ 2.62 (1H, quint., $J=7.2$ Hz, H-3), an oxygenated methylene at δ 3.92 (1H, m, H-4β) and 3.83 (1H, m, H-4α), two set of ABX systems of the phenyl protons at δ 6.63—6.81 (6H), an oxymethine proton at δ 4.64 (1H, d, $J=7.2$ Hz, H-5), and two benzylic protons at δ 2.92 (1H, dd, $J=14.2$, 5.2 Hz, H-6) and 3.07 (1H, dd, $J=14.2$, 5.2 Hz, H-6). Furthermore, the ^{1}H-NMR spectrum showed strong singlets at δ 3.82, 3.85, 3.87, and 3.88 associated with aromatic methoxy groups. Lopes *et al.*[12] reported that *trans*-dibenzylbutyrolactone tented to show the distinct nonequivalence of the protons of the C-4 methylene group (δ 3.9, 4.2) in the ^{1}H-NMR spectrum. In contrast, the *cis*-derivative, the hydrogens of the C-methylene group were almost equivalent in the δ 4.0—4.1 range. The ^{1}H-NMR spectrum of **2** showed the characteristic signals (H-4; δ 3.83, 3.92) of a *trans*-2,3-dibenzylbutyrolactone lignan. The presence of a γ-lactone was suggested by a ^{13}C-NMR shift at δ 179.1. The chemical shift of the signal due to the H-5 indicates that the configuration at C-5 is *R*, according to Nishibe *et al.*[13] The ^{1}H- and ^{13}C-NMR spectra of **2** were quite similar to those of 5-hydroxymatairesinol dimethyl ether.[13] The signals assigned to C-2 and C-3 were proposed at δ 45.2 and 43.9 by Nishibe *et al.*[13] However, careful examination of the spectroscopic data revealed the significant reassignment of ^{13}C-NMR signals at C-2 and C-3 as δ 43.8 and 45.1, respectively. In the COSY spectrum, the proton signal at δ 2.97 (H-2) exhibited correlations with the proton signals at δ 2.62 (H-3), 2.92 (H-6), and 3.07 (H-6). The proton signal at δ 2.62 (H-3) exhibited correlations with the proton signals at δ 3.83 (H-4α), 3.92 (H-4β), and 4.64 (H-5). In the NOESY spectrum, the proton signal at δ 2.97 (H-2) exhibited correlations with the proton signals at δ 4.64 (H-5), 2.92 (H-6), and 3.07 (H-6). The proton signal at δ 2.62 (H-3) exhibited correlations with the proton signals at δ 3.92 (H-4β), and 4.64 (H-5). These observations support the above assignment. Thus the previously reported assignment is unconvincing. Unambiguous assignments for the ^{1}H- and ^{13}C-NMR signals in **2** were made by combination of the DEPT, NOESY, ^{1}H–^{1}H COSY, and HETCOR spectra. Thus the structure of **2** was determined as 5-hydroxymatairesinol dimethyl ether, which we named tupichilignan A.

Compound **3** was obtained as colorless prisms, $[\alpha]_D^{24}$ $-8.3°$ ($c=1.20$, MeOH). The HR-EI-MS showed a $[M]^+$ ion at *m/z* 272.1053 (Calcd 272.1048), consistent with the molecular formula, $C_{16}H_{16}O_4$. The ^{1}H-NMR spectrum (Table 2) of **3** is similar to that of (2*R*,3*R*)-3,4′-dihydroxy-7-methoxy-8-methylflavan (tupichinol A),[4] except for the presence of one additional hydroxyl group signal at δ 8.00 (br s) and the absence of a methoxyl signal at δ 3.78 (1H, s). This suggests that a singlet at δ 8.00 for one hydroxyl group should be located at C-7, with the oxygenation at C-7 only in ring A. In the ^{1}H-NMR spectrum (Table 2) of **3**, signals that are characteristic of the 8-methylflavan-3-ol skeleton were observed.[14] A signal at δ 2.01 (3H, s) was assigned to the methyl group on C-8 in ring A. The oxymethine protons at δ 5.02 (1H, s) and 4.22 (1H, br s) were assigned to H-2, and H-3, respectively. The methylene protons at δ 2.73 (1H, dd, $J=16.2$, 4.4 Hz) and δ 3.14 (1H, dd, $J=16.2$, 4.4 Hz) were assignable

Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **3**, **4**, and **5** in Acetone-d_6

Position	**3**		**4**		**5**	
	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C
2	5.02, s	80.2		144.0		155.4
3	4.22, br s	67.7		132.7		139.5
4	2.73, dd (16.2, 4.4), Hα 3.14, dd (16.2, 4.4), Hβ	34.9		177.8		179.9
5	6.71, d (8.2)	128.5		160.9		160.7
6	6.32, d (8.2)	109.1	6.49, s	95.7	6.34, s	99.0
7		133.5		164.7		161.1
8		112.0		104.2		102.0
9		131.2		147.9		156.7
10		116.7		105.1		105.0
1′		130.8		124.3		123.1
2′,6′	7.38, d (8.8)	129.5	8.21, d (9.2)	131.2	8.09, d (8.8)	131.3
3′,5′	6.83, d (8.8)	116.2	7.04, d (9.2)	117.1	7.05, d (8.8)	116.6
4′		132.3		148.2		151.8
OH-3	3.65, br s		9.24, br s			
OMe-3					3.88, s	60.3
OH-5			12.19, s		12.69, s	
OH-7	8.00, br s				9.21, br s	
OMe-7			3.97, s	57.4		
Me-8	2.01, s	9.3	2.28, s	8.4	2.27, s	7.9
OH-4′	8.35, br s		8.11, br s		8.01, br s	

to H-4α and H-4β, respectively.[4] The signals at δ 3.65 (br s), 8.00 (br s), and 8.35 (br s) which disappeared on addition of D_2O, was assignable to three protons of the hydroxyl groups attached to C-3, C-7, and C-4′, respectively.[4,15] The protons at δ 6.71 (1H, d, J=8.2 Hz) and δ 6.32 (1H, d, J=8.2 Hz) were assigned to H-5 and H-6, respectively.[4,15] Furthermore, the aromatic protons at δ 7.38 (2H, d, J=8.8 Hz) and δ 6.83 (2H, d, J=8.8 Hz) were assigned to H-2′/6′ and H-3′/5′, respectively.[14] The heterocyclic coupling constant ($J_{2,3}$<2 Hz) confirmed the relative 2,3-*cis* configuration, while the optical rotation $[\alpha]_D^{24}$ −8.3° (c=1.20, MeOH) verified the 2*R*, 3*R* absolute configuration in **3**.[4] Thus the structure of **3** was determined as (2*R*,3*R*)-3,7,4′-trihydroxy-8-methylflavan, which we have named tupichinol D.

Compound **4** was obtained as yellow oil. The HR-EI-MS showed the $[M]^+$ ion at *m/z* 314.0795 (Calcd 314.0790), consistent with the molecular formula, $C_{17}H_{14}O_6$. In the ^{1}H-NMR spectrum (Table 2) of **4**, signals that are characteristic of the 8-methylflavone skeleton were observed.[14,15] Two signals at δ 2.28 (3H, s) and 3.97 (3H, s) were assigned to the methyl group on C-8 and the methoxyl group attached to C-7 in ring A, respectively. The signals at δ 8.11 (br s), 9.24 (br s), and 12.19 (br s) which disappeared on addition of D_2O, were assignable to the protons of three hydroxyl groups attached to C-4′, C-3, and C-5, respectively.[15] The proton at δ 6.49 (1H, s) was assigned to H-6.[14] Furthermore, the aromatic protons at δ 8.21 (2H, d, J=9.2 Hz) and δ 7.04 (2H, d, J=9.2 Hz) were assigned to H-2′/6′ and H-3′/5′, respectively.[14] The ^{13}C-NMR spectrum (Table 2) showed the characteristic 8-methylflavone signals at δ 177.8, 160.9, and 104.2, corresponding to C-4 (CO), C-5, and C-8, respectively.[15,16] Moreover, this spectrum also indicated one methoxyl carbon at δ 57.4, and one methyl carbon at δ 8.4. In the NOESY spectrum (Fig. 3), the cross-peaks between H-6/OMe-7 and Me-8/H-2′/6′ were observed, indicating that the methyl group must be at the C-8 position and the methoxyl group must be at the C-7 position. These results indicate unambiguously that compound **4** is 3,5,4′-trihydroxy-7-methoxy-8-methylflavone, which we have named tupichinol E.

Compound **5** was obtained as yellow oil. The HR-EI-MS showed the $[M]^+$ ion at *m/z* 314.0798 (Calcd 314.0795), consistent with the molecular formula, $C_{17}H_{14}O_6$. In the ^{1}H-NMR spectrum (Table 2) of **5**, signals that are characteristic of the 8-methylflavone skeleton were observed.[14] Two signals at δ 2.27 (3H, s) and 3.88 (3H, s) were assigned to the methyl group on C-8 in ring A and the methoxy group attached to C-3 in ring C, respectively. The signals at δ 8.01 (br s), 12.69 (s), and 9.21 (br s), which disappeared on addition of D_2O, were assignable to the protons of two hydroxyl groups attached to C-4′, C-5, and C-7, respectively.[14] The proton at δ 6.34 (1H, s) was assigned to H-6. Moreover, the aromatic protons at δ 8.09 (2H, d, J=8.8 Hz) and δ 7.05 (2H, d, J=8.8 Hz) were assigned to H-2′/6′ and H-3′/5′, respectively.[14] The ^{1}H-NMR spectrum of **5** is similar to that of **4**, the only difference being due to the existence of a methoxy group on C-3 in **5** instead of a hydroxy group on C-3 in **4**, and the existence of a hydroxy group attached to C-7 in **5** instead of a methoxy group attached to C-7 in **4**, which caused some minor shifts of the ^{1}H data for some protons. The methoxy signal upfield shift from δ 3.97 (s) to δ 3.88 (s), the

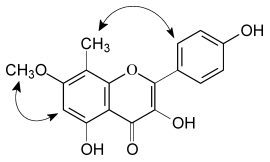

Fig. 3. NOESY Correlations of **4**

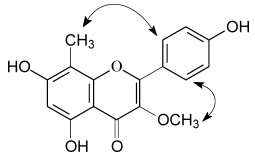

Fig. 4. NOESY Correlations of **5**

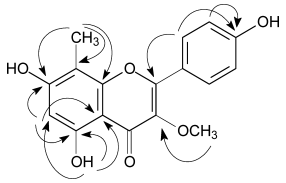

Fig. 5. HMBC Correlations of **5**

hydroxyl proton signal (OH-5) downfield shift from δ 12.19 (s) to δ 12.69 (s), the aromatic proton signal (H-6) upfield shift from δ 6.49 (s) to δ 6.34 (s), and the H-2′ and H-6′ aromatic proton signal upfield shift from δ 8.21 to δ 8.09 were observed. The ^{13}C-NMR spectrum (Table 2) showed the characteristic 8-methylflavone signals at δ 179.9, 102.0, and 7.9, corresponding to C-4 (CO), C-8 (C) and Me-8, respectively.[15,16] Moreover, this spectrum also indicated the required twelve aromatic carbons (δ 99.0—161.1), and one methoxy carbon at δ 60.3.

Unambiguous assignments for the ^{1}H- and ^{13}C-NMR signals in **5** were made by combination of the DEPT, NOESY, ^{1}H–^{1}H COSY, HMQC, and HMBC spectra. The structure of **5** reconciles these data. In the ^{1}H–^{1}H COSY spectrum, the aromatic protons at δ 8.09 (H-2′, H-6′) were coupled to the protons at δ 7.05 (H-3′, H-5′) observed only. The methoxy protons at δ 3.88 (OMe-3) showed correlations with C-3 signal in the HMBC spectrum (Fig. 5) and showed correlations with aromatic protons at δ 8.09 (H-2′, H-6′) in the NOESY spectrum (Fig. 4). These findings also supported the methoxy group attachment to the C-3 position. Furthermore, in the HMBC spectrum, there were correlations between H-6 and C-5, C-7 and C-10, and between OH-5 and C-5, C-6, and C-10. From the above evidence, the aromatic proton at δ 6.34 was assigned to be at the C-6 position. Moreover, the methyl protons at δ 2.27 showed correlations with carbon signals C-7, C-8, and C-9 in the HMBC spectrum, and the cross-peaks between the signals of the methyl protons at δ 2.27 and H-2′/H-6′ in the NOESY spectrum, indicating that the methyl group must be at the C-8 position. These results indicate unambiguously that compound **5** is 5,7,4′-trihydroxy-3-methoxy-8-methylflavone, which we have named tupichinol F.

Seven of the known compounds were identified by comparison their physical and spectral data with the literature values, namely, 3-hydroxy-2-(4-hydroxyphenyl)-7-methoxychromen-4-one (**6**)[5] and 3,5-dihydroxy-2-(4-hydroxy-

phenyl)-7-methoxy-chromen-4-one (**7**),[6] 3,7-dihydroxy-2-(4-hydroxyphenyl)-chromen-4-one (**8**),[7] 2-(4-hydroxyphenyl)-4*H*-chromen-7-ol (**9**),[8] 3,5,7,8-tetramethoxy-2-(4-methoxyphenyl)-chromen-4-one (**10**),[9,10] oxoglaucine (**11**),[11] and oxopurpureine (**12**).[11]

Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were acquired on a Varian Germini 200 MHz FT-NMR running at 400 Mz (^{1}H) or 100 MHz (^{13}C), respectively. Chemical shifts (δ) were reported in ppm relative to residual solvent signals. The multiplicities of ^{1}H signals are designated by the following abbreviations: s=singlet; d=doublet; t=triplet; q=quartet; br=broad; m=multiplet. All coupling constants, *J*, are reported in Hertz. ^{13}C-NMR spectra were acquired on a broad band decoupled mode and the multiplicities were obtained using DEPT sequences. 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. High-resolution EI-MS was 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. 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 hexane (140 g), $CHCl_3$ (60 g), EtOAc (100 g), *n*-BuOH (130 g), and aqueous (280 g) extracts. The $CHCl_3$ extract was concentrated and chromatographed over silica gel and eluted with hexane–EtOAc mixtures of increasing polarity to yield 11 fractions. Fraction 2, eluted from *n*-hexane–EtOAc (1 : 3), was chromatographed on silica gel elution with $CHCl_3$–MeOH (10 : 1) to afford compound **6** (11 mg), and **4** (8 mg), and **10** (9 mg). Fraction 3, eluted from *n*-hexane–EtOAc (1 : 4), was subjected on silica gel elution with $CHCl_3$–MeOH (100 : 11) to afford compound **11** (15 mg), and **12** (13 mg). Fraction 4, eluted from *n*-hexane–EtOAc (1 : 5), was chromatographed on silica gel elution with $CHCl_3$–MeOH (100 : 12) to afford compound **2** (15 mg). The EtOAc extract was concentrated and chromatographed over silica gel and eluted with $CHCl_3$–MeOH mixtures of increasing polarity to yield 10 fractions. Fraction 2 was rechromatographed on silica gel elution with $CHCl_3$–MeOH (100 : 3) to afford compound **8** (10 mg). Fraction 2 was rechromatographed on silica gel elution with $CHCl_3$–MeOH (100 : 5) to afford compound **5** (10 mg), and **9** (8 mg). Fraction 3 was rechromatographed on silica gel elution with $CHCl_3$–MeOH (100 : 5) to afford compound **3** (13 mg). Fraction 10 was rechromatographed on silica gel elution with $CHCl_3$–MeOH (6 : 1) to afford compound **1** (25 mg).

Tupichinin A (**1**): Colorless oil, $[\alpha]_D^{24}$ −12.6° (*c*=2.30, MeOH). FAB-MS (positive mode) *m/z*: 515 $[M+Na]^+$. HR-FAB-MS *m/z*: Found 515.2832 $[M+Na]^+$ (Calcd 515.2829). ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) spectral data see Table 1.

Determination of the Absolute Configuration of Sugar Compound **1** (15 mg) was refluxed for 2 h with 4 N HCl in MeOH (35 ml). The acid hydrolysate was concentrated, extracted with EtOAc. The acidic mother liquor was neutralized with Na_2CO_3, filtered, and evaporated to dryness for examination of the sugar moiety, which proved to D-(+)-glucose by detection on HPLC (HITACHI L7100) eluted with MeOH, refractive index detector (BISCHOFF), using LiChrospher 60 (5 mm) column with a flow rate of 1.0 ml/min. Peak of the hydrolysate of **1** was detected at 2.53 min. Retention time for authentic sample D-(+)-glucose was 2.53 min.

Tupichilignan A (**2**): Colorless oil, $[\alpha]_D^{24}$ −2.8° (*c*=1.45, acetone). EI-MS *m/z*: 402 $[M]^+$ (21), 167 (100), 151 (79), 139 (57). HR-EI-MS *m/z*: Found 402.1683 $[M]^+$ (Calcd for $C_{22}H_{26}O_7$ 402.1678). IR (neat) ν_{max} cm^{-1}: 3350 (OH), 1750 (CO), 1600, 1580, 1500. UV (EtOH) λ_{max} (log ε) 233 (4.24), 280 (3.80) nm. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.81—6.63 (6H, aromatic protons), 4.64 (1H, d, *J*=6.8 Hz, H-5), 3.92 (1H, m, H-4β), 3.88 (3H, s, $-OCH_3$), 3.87 (3H, s, $-OCH_3$), 3.85 (3H, s, $-OCH_3$), 3.83 (1H, m, H-4α), 3.82 (3H, s, $-OCH_3$), 3.07, 2.92 (each 1H, dd, *J*=14.2, 5.2 Hz, H_2-6), 2.97 (1H, m, H-2), 2.62 (1H, quintet, *J*=7.2 Hz, H-3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 179.1 (C-1, s), 43.8 (C-2, d), 45.1 (C-3, d), 68.3 (C-4, t), 75.3 (C-5, d), 34.9 (C-6, t), 149.3 (d), 149.1 (d), 148.9 (d), 147.8 (d), 134.0 (d), 130.1 (d), 121.7 (d), 118.2 (d), 112.8 (d), 111.1 (d), 111.0 (d), 109.0 (d), 55.9 (q), 55.9 (q), 55.9 (q), 55.8 (q).

Tupichinol D (**3**): Colorless needles, $[\alpha]_D^{24}$ −8.3° (*c*=1.20, MeOH). EI-MS *m/z*: 257 $[M-CH_3]^+$ (100), 222 (65), 207 (92), 179 (34). HR-EI-MS *m/z*: Found 272.1053 $[M]^+$ (Calcd for $C_{16}H_{16}O_4$ 272.1048). ^{1}H-NMR (400 MHz, acetone-d_6) and ^{13}C-NMR (100 MHz, acetone-d_6) spectral data see Table 2.

Tupichinol E (**4**): Yellow oil. EI-MS *m/z*: 314 $[M]^+$ (69), 271 (19), 121 (44), 105 (100). HR-EI-MS *m/z*: Found 314.0795 $[M]^+$ (Calcd for $C_{17}H_{14}O_6$ 314.0790). ^{1}H-NMR (400 MHz, acetone-d_6) and ^{13}C-NMR (100 MHz, acetone-d_6) spectral data see Table 2.

Tupichinol F (**5**): Yellow oil. EI-MS *m/z*: 314 $[M]^+$ (45), 313 (47), 285 (18), 271 (28), 121 (59), 57 (100). HR-EI-MS *m/z*: Found 314.0798 $[M]^+$ (Calcd for $C_{17}H_{14}O_6$ 314.0795). ^{1}H-NMR (400 MHz, acetone-d_6) and ^{13}C-NMR (100 MHz, acetone-d_6) spectral data see Table 2.

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