

## 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.**

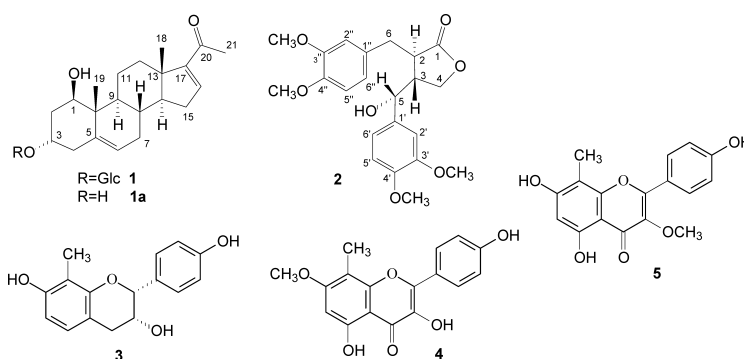
**Key words** *Tupistra chinensis*; Liliaceae; pregnane glycoside; lignan; flavonoid; alkaloid

*Tupistra chinensis* BAKER (Liliaceae) is endemic to southwestern regions of the People's Republic of China.<sup>1)</sup> As a Chinese folk medicine, this species has usually been used for treatment of rheumatic diseases and snake-bite.<sup>1)</sup> In previous investigations,<sup>2–4)</sup> 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**),<sup>5)</sup> rhamnocitrin (**7**),<sup>6)</sup> 3,7-dihydroxy-2-(4-hydroxyphenyl)-chromen-4-one (**8**),<sup>7)</sup> 2-(4-hydroxyphenyl)-4*H*-chromen-7-ol (**9**),<sup>8)</sup> and 3,5,7,8-tetramethoxy-2-(4-methoxyphenyl)-chromen-4-one (**10**),<sup>9,10)</sup> and two known alkaloids, oxoglucaine (**11**)<sup>11)</sup> and oxopurpureine (**12**).<sup>11)</sup> The characterization and structure elucidation of **1**–**5** are reported herein.

Tupichinin A (**1**) was obtained as colorless oil,  $[\alpha]_D^{24} -12.6^\circ$  ( $c=2.30$ , 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  $^1H$ - and  $^{13}C$ -NMR signals were listed in Table 1 based on an analysis of the combination of distortionless enhancement by polarization transfer (DEPT),  $^1H$ - $^1H$  correlated spectroscopy ( $^1H$ - $^1H$  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  $^1H$ -NMR spectrum in  $CD_3OD$  of **1**, signals that are characteristic of the pregnane glycoside skeleton were observed. The  $^1H$ -NMR spectrum showed the presence of two methyl groups at  $\delta$  0.93 (3H, s, Me-18) and 1.06 (3H, s, Me-19) and an anomeric proton at  $\delta$  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  $\delta$  2.26 and two vinylic proton signals at  $\delta$  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  $\delta$  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  $\delta$  199.5 (C-20); two vinylic carbons at  $\delta$  140.2 (C-5) and 125.3 (C-6); an anomeric carbon at  $\delta$  102.9 (C-1' of glucose); and two methyl groups at  $\delta$  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  $\delta$  147.2 (CH) and 156.7 (C) were assigned to the C-16 and C-17, respectively. The two signals at  $\delta$  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  $\delta$  2.26 (Me-21) showed correlations with the proton signals at  $\delta$  6.90 (H-16) and 0.93 (Me-18). Moreover, in the LR-HETCOR spectrum (Fig. 2), one carbonyl carbon at  $\delta$  199.5 (C-20) exhibited correlation with the proton signal at  $\delta$  6.90 (H-16), and the  $^{13}C$  signal at  $\delta$  156.7 (C-17) showed correlation with the proton signal at  $\delta$  0.93 (Me-18). These findings further support the methyl ketone was attached to the C-17 position. The methylene protons at  $\delta$  2.00 (1H, m, H-2 $\alpha$ ) and  $\delta$  2.71 (1H, m, H-2 $\beta$ ) were

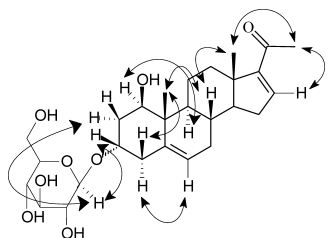
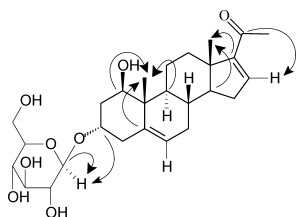


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Table 1.  $^{13}\text{C}$ - and  $^1\text{H}$ -NMR Data for **1**<sup>a)</sup> and **1a**<sup>b)</sup> (100, 400 MHz)

| Position | <b>1</b>            |                                   | <b>1a</b>           |  |
|----------|---------------------|-----------------------------------|---------------------|--|
|          | $\delta_{\text{C}}$ | $\delta_{\text{H}}, J$ (Hz)       | $\delta_{\text{C}}$ | $\delta_{\text{H}}, J$ (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, H $\alpha$               | 40.8, t             | 2.39, dd (14.8, 4.4), H $\alpha$       |
|          |                     | 1.71, m, H $\beta$                |                     | 2.16, dd (4.8, 11.6), H $\beta$        |
| 3        | 75.4, d             | 4.05, br s                        | 66.5, d             | 4.37, br s                             |
| 4        | 39.1, t             | 2.28, m, H $\alpha$               | 41.1, t             | 2.40, br d (14.4), H $\alpha$          |
|          |                     | 2.51, d (14.4), H $\beta$         |                     | 2.73, br d (14.4), H $\beta$           |
| 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, H $\alpha$               | 31.9, t             | 1.70, m, H $\alpha$                    |
|          |                     | 1.95, m, H $\beta$                |                     | 1.96, m, H $\beta$                     |
| 8        | 32.4, d             | 1.65, m, H $\beta$                | 31.5, d             | 1.65, m, H $\beta$                     |
| 9        | 52.1, d             | 1.35, td, (12.0, 8.0), H $\alpha$ | 51.8, d             | 1.62, m, H $\alpha$                    |
| 10       | 46.9, s             |                                   | 46.0, s             |  |
| 11       | 24.8, t             | 2.38, m, H $\alpha$               | 24.3, t             | 2.98, ddd (14.4, 4.8, 4.0), H $\alpha$ |
|          |                     | 1.62, m, H $\beta$                |                     | 1.90, m, H $\beta$                     |
| 12       | 36.5, t             | 1.35, m, H $\alpha$               | 35.8, t             | 1.52, td, (14.4, 4.0), H $\alpha$      |
|          |                     | 2.28, m, H $\beta$                |                     | 2.67, ddd, (14.4, 4.8, 2.8), H $\beta$ |
| 13       | 44.9, s             |                                   | 44.7, s             |  |
| 14       | 58.0, d             | 1.47, m, H $\alpha$               | 56.8, d             | 1.43, m, H $\alpha$                    |
| 15       | 33.5, t             | 2.30, m                           | 32.5, t             | 2.13, m, H $\alpha$                    |
|          |                     |                                   |                     | 1.94, dd, (12.0, 1.6), H $\beta$       |
| 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), H $\alpha$        |                     |  |
| 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  $\text{CD}_3\text{OD}$ . b) Spectra were measured in  $\text{C}_2\text{D}_2\text{N}_4$ .

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  $\delta$  3.78 (H-1) and  $\delta$  4.05 (H-3) in the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum. The methylene protons at  $\delta$  2.28 (H-4 $\alpha$ ) and  $\delta$  2.51 (H-4 $\beta$ ) were in turn coupled with the oxygenated methine proton at  $\delta$  4.05 (H-3). These findings sup-

ported the placement of two hydroxyl groups on C-1 and C-3 positions. Furthermore, two signals at  $\delta$  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  $\delta$  3.78 (1H, dd,  $J_{1\alpha,2\beta}=12.0$ ,  $J_{1\alpha,2\alpha}=4.4$  Hz) and H-3 at  $\delta$  4.05 (1H, br s) indicated that H-1 and H-3 are  $\alpha$ -axial and  $\beta$ -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  $^{13}\text{C}$  signals of **1** with those of pregnane **1a**,<sup>4)</sup> a set of additional six signals, corresponding to a  $\beta$ -D-glucopyranosyl unit appeared. The signal due to the C-3 carbon, which was observed at  $\delta$  66.5 in **1a**, downfield shift to  $\delta$  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  $^1\text{H}$  and  $^{13}\text{C}$  signals due to the saccharide moiety were as shown in Table 1. In the LR-HETCOR spectrum, the anomeric proton signal at  $\delta$  4.33 exhibited correlations with the  $^{13}\text{C}$  signals at  $\delta$  75.4 (C-3 of aglycone) and 102.9 (C-1' of glucose). In the NOESY spectrum, the anomeric proton signal at  $\delta$  4.33 exhibited correlations with the proton signals at  $\delta$  4.05 (H-3 of aglycone) and 2.00 (H-2 $\alpha$  of aglycone). The  $\alpha$ -configuration of the anomeric carbon of glucopyranosyl unit was determined by  $J_{\text{H1-H2}}$  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-1 $\alpha$  and H-9 $\alpha$ , and between H-6 and H-4 $\alpha$  indicated  $\alpha$ -axial and  $\beta$ -equatorial configurations of H-1 and H-3, respectively. Based on the above spectroscopic evidence, the structure of **1** was established as 1 $\beta$ -hydroxypregna-5,16-dien-3- $\beta$ -ol-20-one 3-O- $\beta$ -D-glucopyranoside, namely tupichinin A.

Tupichilignan A (**2**) was isolated as colorless oil,  $[\alpha]_D^{24} -2.8^\circ$  ( $c=1.45$ , acetone). Its molecular formula C<sub>22</sub>H<sub>26</sub>O<sub>7</sub> was established by EI-MS ( $[M]^+$ ,  $m/z$  402) and HR-EI-MS ( $m/z$  402.1683). The <sup>1</sup>H-NMR spectrum of **2** displayed signals for two methine protons at  $\delta$  2.97 (1H, m, H-2) and  $\delta$  2.62 (1H, quint.,  $J=7.2$  Hz, H-3), an oxygenated methylene at  $\delta$  3.92 (1H, m, H-4 $\beta$ ) and 3.83 (1H, m, H-4 $\alpha$ ), two set of ABX systems of the phenyl protons at  $\delta$  6.63–6.81 (6H), an oxymethine proton at  $\delta$  4.64 (1H, d,  $J=7.2$  Hz, H-5), and two benzylic protons at  $\delta$  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 <sup>1</sup>H-NMR spectrum showed strong singlets at  $\delta$  3.82, 3.85, 3.87, and 3.88 associated with aromatic methoxy groups. Lopes *et al.*<sup>12</sup> reported that *trans*-dibenzylbutyrolactone tented to show the distinct nonequivalence of the protons of the C-4 methylene group ( $\delta$  3.9, 4.2) in the <sup>1</sup>H-NMR spectrum. In contrast, the *cis*-derivative, the hydrogens of the C-methylene group were almost equivalent in the  $\delta$  4.0–4.1 range. The <sup>1</sup>H-NMR spectrum of **2** showed the characteristic signals (H-4;  $\delta$  3.83, 3.92) of a *trans*-2,3-dibenzylbutyrolactone lignan. The presence of a  $\gamma$ -lactone was suggested by a <sup>13</sup>C-NMR shift at  $\delta$  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.*<sup>13</sup> The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of **2** were quite similar to those of 5-hydroxymatairesinol dimethyl

ether.<sup>13</sup> The signals assigned to C-2 and C-3 were proposed at  $\delta$  45.2 and 43.9 by Nishibe *et al.*<sup>13</sup> However, careful examination of the spectroscopic data revealed the significant reassignment of <sup>13</sup>C-NMR signals at C-2 and C-3 as  $\delta$  43.8 and 45.1, respectively. In the COSY spectrum, the proton signal at  $\delta$  2.97 (H-2) exhibited correlations with the proton signals at  $\delta$  2.62 (H-3), 2.92 (H-6), and 3.07 (H-6). The proton signal at  $\delta$  2.62 (H-3) exhibited correlations with the proton signals at  $\delta$  3.83 (H-4 $\alpha$ ), 3.92 (H-4 $\beta$ ), and 4.64 (H-5). In the NOESY spectrum, the proton signal at  $\delta$  2.97 (H-2) exhibited correlations with the proton signals at  $\delta$  4.64 (H-5), 2.92 (H-6), and 3.07 (H-6). The proton signal at  $\delta$  2.62 (H-3) exhibited correlations with the proton signals at  $\delta$  3.92 (H-4 $\beta$ ), and 4.64 (H-5). These observations support the above assignment. Thus the previously reported assignment is unconvincing. Unambiguous assignments for the <sup>1</sup>H- and <sup>13</sup>C-NMR signals in **2** were made by combination of the DEPT, NOESY, <sup>1</sup>H–<sup>1</sup>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,  $[\alpha]_D^{24} -8.3^\circ$  ( $c=1.20$ , MeOH). The HR-EI-MS showed a  $[M]^+$  ion at  $m/z$  272.1053 (Calcd 272.1048), consistent with the molecular formula, C<sub>16</sub>H<sub>16</sub>O<sub>4</sub>. The <sup>1</sup>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),<sup>4</sup> except for the presence of one additional hydroxyl group signal at  $\delta$  8.00 (br s) and the absence of a methoxyl signal at  $\delta$  3.78 (1H, s). This suggests that a singlet at  $\delta$  8.00 for one hydroxyl group should be located at C-7, with the oxygenation at C-7 only in ring A. In the <sup>1</sup>H-NMR spectrum (Table 2) of **3**, signals that are characteristic of the 8-methylflavan-3-ol skeleton were observed.<sup>14</sup> A signal at  $\delta$  2.01 (3H, s) was assigned to the methyl group on C-8 in ring A. The oxymethine protons at  $\delta$  5.02 (1H, s) and 4.22 (1H, brs) were assigned to H-2, and H-3, respectively. The methylene protons at  $\delta$  2.73 (1H, dd,  $J=16.2, 4.4$  Hz) and  $\delta$  3.14 (1H, dd,  $J=16.2, 4.4$  Hz) were assignable

Table 2. <sup>1</sup>H- and <sup>13</sup>C-NMR Data for Compounds **3**, **4**, and **5** in Acetone-*d*<sub>6</sub>

| Position | <b>3</b>  |            | <b>4</b>           |            | <b>5</b>           |            |
|----------|---|------------|--------------------|------------|--------------------|------------|
|          | $\delta_H, J$ (Hz)  | $\delta_C$ | $\delta_H, J$ (Hz) | $\delta_C$ | $\delta_H, J$ (Hz) | $\delta_C$ |
| 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), H $\alpha$<br>3.14, dd (16.2, 4.4), H $\beta$ | 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-4 $\alpha$  and H-4 $\beta$ , respectively.<sup>4)</sup> The signals at  $\delta$  3.65 (br s), 8.00 (br s), and 8.35 (br s) which disappeared on addition of D<sub>2</sub>O, was assignable to three protons of the hydroxyl groups attached to C-3, C-7, and C-4', respectively.<sup>4,15)</sup> The protons at  $\delta$  6.71 (1H, d,  $J=8.2$  Hz) and  $\delta$  6.32 (1H, d,  $J=8.2$  Hz) were assigned to H-5 and H-6, respectively.<sup>4,15)</sup> Furthermore, the aromatic protons at  $\delta$  7.38 (2H, d,  $J=8.8$  Hz) and  $\delta$  6.83 (2H, d,  $J=8.8$  Hz) were assigned to H-2'/6' and H-3'/5', respectively.<sup>14)</sup> The heterocyclic coupling constant ( $J_{2,3} < 2$  Hz) confirmed the relative 2,3-*cis* configuration, while the optical rotation  $[\alpha]_D^{24} -8.3^\circ$  ( $c=1.20$ , MeOH) verified the 2*R*, 3*R* absolute configuration in **3**.<sup>4)</sup> 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<sub>17</sub>H<sub>14</sub>O<sub>6</sub>. In the <sup>1</sup>H-NMR spectrum (Table 2) of **4**, signals that are characteristic of the 8-methylflavone skeleton were observed.<sup>14,15)</sup> Two signals at  $\delta$  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  $\delta$  8.11 (br s), 9.24 (br s), and 12.19 (br s) which disappeared on addition of D<sub>2</sub>O, were assignable to the protons of three hydroxyl groups attached to C-4', C-3, and C-5, respectively.<sup>15)</sup> The proton at  $\delta$  6.49 (1H, s) was assigned to H-6.<sup>14)</sup> Furthermore, the aromatic protons at  $\delta$  8.21 (2H, d,  $J=9.2$  Hz) and  $\delta$  7.04 (2H, d,  $J=9.2$  Hz) were assigned to H-2'/6' and H-3'/5', respectively.<sup>14)</sup> The <sup>13</sup>C-NMR spectrum (Table 2) showed the characteristic 8-methylflavone signals at  $\delta$  177.8, 160.9, and 104.2, corresponding to C-4 (CO), C-5, and C-8, respectively.<sup>15,16)</sup> Moreover, this spectrum also indicated one methoxyl carbon at  $\delta$  57.4, and one methyl carbon at  $\delta$  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<sub>17</sub>H<sub>14</sub>O<sub>6</sub>. In the <sup>1</sup>H-NMR spectrum (Table 2) of **5**, signals that are characteristic of the 8-methylflavone skeleton were observed.<sup>14)</sup> Two signals at  $\delta$  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  $\delta$  8.01 (br s), 12.69 (s), and 9.21 (br s), which disappeared on addition of D<sub>2</sub>O, were assignable to the protons of two hydroxyl groups attached to C-4', C-5, and C-7, respectively.<sup>14)</sup> The proton at  $\delta$  6.34 (1H, s) was assigned to H-6. Moreover, the aromatic protons at  $\delta$  8.09 (2H, d,  $J=8.8$  Hz) and  $\delta$  7.05 (2H, d,  $J=8.8$  Hz) were assigned to H-2'/6' and H-3'/5', respectively.<sup>14)</sup> The <sup>1</sup>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 <sup>1</sup>H data for some protons. The methoxy signal upfield shift from  $\delta$  3.97 (s) to  $\delta$  3.88 (s), the

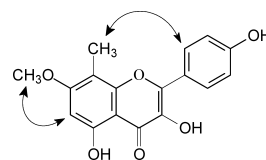


Fig. 3. NOESY Correlations of **4**

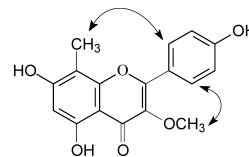


Fig. 4. NOESY Correlations of **5**

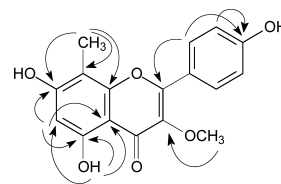


Fig. 5. HMBC Correlations of **5**

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

Unambiguous assignments for the <sup>1</sup>H- and <sup>13</sup>C-NMR signals in **5** were made by combination of the DEPT, NOESY, <sup>1</sup>H-<sup>1</sup>H COSY, HMQC, and HMBC spectra. The structure of **5** reconciles these data. In the <sup>1</sup>H-<sup>1</sup>H COSY spectrum, the aromatic protons at  $\delta$  8.09 (H-2', H-6') were coupled to the protons at  $\delta$  7.05 (H-3', H-5') observed only. The methoxy protons at  $\delta$  3.88 (OMe-3) showed correlations with C-3 signal in the HMBC spectrum (Fig. 5) and showed correlations with aromatic protons at  $\delta$  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  $\delta$  6.34 was assigned to be at the C-6 position. Moreover, the methyl protons at  $\delta$  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  $\delta$  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**)<sup>5)</sup> and 3,5-dihydroxy-2-(4-hydroxy-

phenyl)-7-methoxy-chromen-4-one (7),<sup>6</sup> 3,7-dihydroxy-2-(4-hydroxyphenyl)-chromen-4-one (8),<sup>7</sup> 2-(4-hydroxyphenyl)-4*H*-chromen-7-ol (9),<sup>8</sup> 3,5,7,8-tetramethoxy-2-(4-methoxyphenyl)-chromen-4-one (10),<sup>9,10</sup> oxoglucine (11),<sup>11</sup> and oxopurpureine (12).<sup>11</sup>

### 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. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were acquired on a Varian Germini 200 MHz FT-NMR running at 400 Mz (<sup>1</sup>H) or 100 MHz (<sup>13</sup>C), respectively. Chemical shifts (δ) were reported in ppm relative to residual solvent signals. The multiplicities of <sup>1</sup>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. <sup>13</sup>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<sub>254</sub>, 0.25 mm) were used for analytical TLC, and precoated silica gel plates (Macherey-Nagel, SIL G/UV<sub>254</sub>, 0.25 mm) were used for preparative TLC. The spots were detected by spraying with 50% H<sub>2</sub>SO<sub>4</sub> 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<sub>3</sub> (60 g), EtOAc (100 g), *n*-BuOH (130 g), and aqueous (280 g) extracts. The CHCl<sub>3</sub> 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<sub>3</sub>–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<sub>3</sub>–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<sub>3</sub>–MeOH (100:12) to afford compound 2 (15 mg). The EtOAc extract was concentrated and chromatographed over silica gel and eluted with CHCl<sub>3</sub>–MeOH mixtures of increasing polarity to yield 10 fractions. Fraction 2 was rechromatographed on silica gel elution with CHCl<sub>3</sub>–MeOH (100:3) to afford compound 8 (10 mg). Fraction 2 was rechromatographed on silica gel elution with CHCl<sub>3</sub>–MeOH (100:5) to afford compound 5 (10 mg), and 9 (8 mg). Fraction 3 was rechromatographed on silica gel elution with CHCl<sub>3</sub>–MeOH (100:5) to afford compound 3 (13 mg). Fraction 10 was rechromatographed on silica gel elution with CHCl<sub>3</sub>–MeOH (6:1) to afford compound 1 (25 mg).

**Tupichinin A (1):** Colorless oil,  $[\alpha]_D^{24} -12.6^\circ$  (*c*=2.30, MeOH). FAB-MS (positive mode) *m/z*: 515 [M+Na]<sup>+</sup>. HR-FAB-MS *m/z*: Found 515.2832 [M+Na]<sup>+</sup> (Calcd 515.2829). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD) 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<sub>2</sub>CO<sub>3</sub>, 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,  $[\alpha]_D^{24} -2.8^\circ$  (*c*=1.45, acetone). EI-MS *m/z*: 402 [M]<sup>+</sup> (21), 167 (100), 151 (79), 139 (57). HR-EI-MS *m/z*: Found 402.1683 [M]<sup>+</sup> (Calcd for C<sub>22</sub>H<sub>26</sub>O<sub>7</sub> 402.1678). IR (neat)  $\nu_{\max}$  cm<sup>-1</sup>: 3350 (OH), 1750 (CO), 1600, 1580, 1500. UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 233 (4.24), 280 (3.80) nm. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) 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<sub>3</sub>), 3.87 (3H, s, –OCH<sub>3</sub>), 3.85 (3H, s, –OCH<sub>3</sub>), 3.83 (1H, m, H-4α), 3.82 (3H, s, –OCH<sub>3</sub>), 3.07, 2.92 (each 1H, dd, *J*=14.2, 5.2 Hz, H<sub>2</sub>-6), 2.97 (1H, m, H-2), 2.62 (1H, quintet, *J*=7.2 Hz, H-3). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) 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), 149.3 (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,  $[\alpha]_D^{24} -8.3^\circ$  (*c*=1.20, MeOH). EI-MS *m/z*: 257 [M–CH<sub>3</sub>]<sup>+</sup> (100), 222 (65), 207 (92), 179 (34). HR-EI-MS *m/z*: Found 272.1053 [M]<sup>+</sup> (Calcd for C<sub>16</sub>H<sub>16</sub>O<sub>4</sub> 272.1048). <sup>1</sup>H-NMR (400 MHz, acetone-*d*<sub>6</sub>) and <sup>13</sup>C-NMR (100 MHz, acetone-*d*<sub>6</sub>) spectral data see Table 2.

**Tupichinol E (4):** Yellow oil. EI-MS *m/z*: 314 [M]<sup>+</sup> (69), 271 (19), 121 (44), 105 (100). HR-EI-MS *m/z*: Found 314.0795 [M]<sup>+</sup> (Calcd for C<sub>17</sub>H<sub>14</sub>O<sub>6</sub> 314.0790). <sup>1</sup>H-NMR (400 MHz, acetone-*d*<sub>6</sub>) and <sup>13</sup>C-NMR (100 MHz, acetone-*d*<sub>6</sub>) spectral data see Table 2.

**Tupichinol F (5):** Yellow oil. EI-MS *m/z*: 314 [M]<sup>+</sup> (45), 313 (47), 285 (18), 271 (28), 121 (59), 57 (100). HR-EI-MS *m/z*: Found 314.0798 [M]<sup>+</sup> (Calcd for C<sub>17</sub>H<sub>14</sub>O<sub>6</sub> 314.0795). <sup>1</sup>H-NMR (400 MHz, acetone-*d*<sub>6</sub>) and <sup>13</sup>C-NMR (100 MHz, acetone-*d*<sub>6</sub>) spectral data see Table 2.

**Acknowledgments** This work was supported by a grant from the National Science Council of the Republic of China.

### References

- 1) Jiang Su New Medical College (ed.), "Dictionary of Traditional Chinese Crude Drugs," Shanghai Scientific Technologic, Shanghai, 1979, p. 907.
- 2) Pan W. B., Chang F. R., Wu Y. C., *J. Nat. Prod.*, **63**, 861–863 (2000).
- 3) Pan W. B., Chang F. R., Wu Y. C., *Chem. Pharm. Bull.*, **48**, 1350–1353 (2000).
- 4) Pan W. B., Chang F. R., Wei L. M., Wu Y. C., *J. Nat. Prod.*, **66**, 161–168 (2003).
- 5) Camarda L., Merlini L., Nasini G., *Heterocycles*, **20**, 39–43 (1983).
- 6) Gonnet J., *Phytochemistry*, **11**, 2313–2314 (1972).
- 7) Jurd L., *Tetrahedron*, **23**, 1057–1064 (1967).
- 8) Iinuma M., Kakuto Y., Camarda L., Merlini L., Nasini G., *Heterocycles*, **20**, 39–43 (1983).
- 9) Iinuma M., Kakuto Y., Tanida N., Tanaka T., Lang F. A., *Phytochemistry*, **44**, 705–710 (1997).
- 10) Rashid M. A., Armstrong J. A., Gray A. I., Waterman P. G., *Phytochemistry*, **31**, 1265–1270 (1992).
- 11) Chang F. R., Chen C. Y., Wu P. H., Kuo R. Y., Chang Y. C., Wu Y. C., *J. Nat. Prod.*, **63**, 746–748 (2000).
- 12) Lopes L. M. X., Yoshida M., Gottlieb O. R., *Phytochemistry*, **22**, 1516–1518 (1983).
- 13) Nishibe S., Chiba M., Sakushima A., Hisada S., Yamanouchi S., Takido M., Sankawa U., Sakakibara A., *Chem. Pharm. Bull.*, **28**, 850–860 (1980).
- 14) Coxon D. T., O'Neill T. M., Mansfield J. W., Porter A. E. A., *Phytochemistry*, **19**, 889–891 (1980).
- 15) Takasugi M., Niino N., Nagao S., Anetai M., Masamune T., Shirata A., Takahashi K., *Chem. Lett.*, **1984**, 689–692 (1984).
- 16) Morimoto S., Nonaka G. I., Nishioka I., Ezaki N., Takizawa N., *Chem. Pharm. Bull.*, **33**, 2281–2286 (1985).