Results and Discussion

Vitex peduncularis Wall. ex Schauer (Verbenaceae), locally known as “Awal”, is a large evergreen tree and is distributed in the region of Eastern Himalaya, Myanmar, Vietnam and Malaysia. The leaves, root bark and young stem bark or whole plant have been used traditionally as folk herbal remedies for malarial and black-water fevers and pains in chest. Earlier studies on different parts of the plant reported the isolation of flavonoids-vitexin, pachypodol, peduncularisin; iridoids-pedunculariside, agnuside; and triterpenes-ursolic acid and 2α-hydroxyursolic acid. In this report, is described the isolation and structure elucidation of a new compound, which on DEPT experiments, represented 3 methine, 4 methyl, 10 quaternary carbons. Possibly two carbon signals represented four methine carbons. The presence of one downfield methoxyl at δ 3.79 (3H, s) and 3.86 (3H, s), one acetoxymethyl at δ 2.38 (3H, s) and a phenolic hydroxyl proton at δ 12.68 (1H, s). The high chemical resonance of phenolic-OH proton suggested its location at C-5 position. The 13C NMR spectral data of compound 1 in DMSO-d6 (Table I) showed 17 carbon signals, which on DEPT experiments, represented 3 methyl, 4 methine and 10 quaternary carbons. Possibly two carbon signals represented four methine carbons. The presence of one downfield methoxyl signal at δ 59.2 (3H, q) indicated its location between two ortho-disubstituted carbons and hence its position was assigned at C-6. The 13C NMR spectral data of compound 1 was very similar to that of salvigenin (= 5-hydroxy-6,7,4′-trimethoxyflavone) except for the methoxyl signal at C-4′ (Ref 8). The NOESY correlation (Figure 2) between H-8 proton (δH 6.74) and methoxyl proton (δH 3.79) indicated the location of another methoxyl group at C-7 position. Therefore, the position of the acetoxyl group was assigned at C-6. The FAB-MS of the compound recorded significant mass ions at m/z 357 [M+H]+, 314 [M]+, 287, 197, 163 and 121 corroborating the 4′-acetoxy-5-hydroxy-.
Deacetylation of compound 1 with 0.5 N methanolic NaOH solution afforded 4',5-dihydroxy-6,7-dimethoxyflavone, cirsimaritin 2, C_{17}H_{14}O_{6}, (M^+ 314). Thus, the structure of compound 1 was established as 4'-acetoxy-5-hydroxy-6,7-dimethoxyflavone.

Compound 2 (Figure 1), C_{17}H_{14}O_{6} (M^+ 314) was isolated as light yellow needles, m.p. 263°C. It was identified as 4',5-dihydroxy-6,7-dimethoxyflavone (cirsimaritin) 2 by comparison of its spectral data with literature.

Compound 3 (Figure 1), C_{17}H_{14}O_{6} (M^+ 314) was isolated as light-brown needles, m.p. 290°C. It was identified as 4',5-dihydroxy-7-methoxyflavone (= genkwanin) 3 by comparison of its spectral data with literature.

Compound 4 (Figure 1), C_{30}H_{52}O (M^+ 428) was isolated as colourless crystals, m.p. 280°C. It was identified as 3α-friedelinol 4 by comparison of its physical constants and spectral data with literature.

Compound 5 (Figure 1), C_{30}H_{52}O (M^+ 428) was isolated as colourless crystals, m.p. 168°C (dec). It was identified as 3β-friedelinol 5 by comparison of its physical constants and spectral data (including 2D-NMR) with literature. The detailed NMR data are provided in the Experimental Section.

**Table I** — ^1H and ^13C NMR spectral data^a of compound 1 (in DMSO-d_6)

<table>
<thead>
<tr>
<th>No.</th>
<th>H/C</th>
<th>δ_H b</th>
<th>δ_C c</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>165.1 (C)</td>
</tr>
<tr>
<td>3</td>
<td>6.36 s</td>
<td>103.8 (CH)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>181.3 (C)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>153.3 (C)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>131.0 (C)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>161.2 (C)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.74 s</td>
<td>92.1 (CH)</td>
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</tr>
<tr>
<td>9</td>
<td>–</td>
<td>151.2 (C)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>106.2 (C)</td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>–</td>
<td>130.1 (C)</td>
<td></td>
</tr>
<tr>
<td>2', 6'</td>
<td>7.95 d (8.5)</td>
<td>128.0 (CH)</td>
<td></td>
</tr>
<tr>
<td>3', 5'</td>
<td>7.26 d (8.5)</td>
<td>119.3 (CH)</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>–</td>
<td>156.1 (C)</td>
<td></td>
</tr>
<tr>
<td>MeO-6</td>
<td>3.86 s</td>
<td>59.2 (CH_3)</td>
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<tr>
<td>MeO-7</td>
<td>3.79 s</td>
<td>56.1 (CH_3)</td>
<td></td>
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<tr>
<td>AcO-4'</td>
<td>2.38 s</td>
<td>21.1 (CH_3), 170.2 (C=O)</td>
<td></td>
</tr>
<tr>
<td>HO-5</td>
<td>12.68 s</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

^600 MHz for ^1H and 150 MHz for ^13C NMR data
^b J values in Hz in parenthesis
^c Assignment based on HSQC and HMBC data (Figure 2)

6,7-dimethoxyflavone structure for it. Melting points of compound 1 were determined with a Köfler type melting point apparatus and are uncorrected. UV and IR spectra were obtained on a Perkin Elmer Lambda 25 spectrometer and a Perkin Elmer FTIR-100 spectrometer, respectively. ^1H and ^13C NMR spectra were obtained on a Bruker 600 spectrometer using TMS as the internal standard. Mass spectra were obtained on a Jeol JMS-303 spectrometer. Optical rotations were determined on a Jasco P-1020 polarimeter. Column chromatography (CC) was carried out over silica gel (60-120 mesh, Merck, India) and TLC using silica gel G (Merck, India).
Plant Material

Fresh leaves of Vitex peduncularis were collected from Dharmanagar, North Tripura in July 2012 and identified by Prof. B. K. Datta, Plant Taxonomist, Department of Botany, Tripura University. A voucher specimen (TU/H/1510) is deposited in the laboratory of B. K. D.

Extraction and Isolation

Air-dried leaves (2 kg) of Vitex peduncularis were extracted two times consecutively (6 days each time) with MeOH (2×6L). A semi-solid residue (160 g) was obtained after removal of the solvent by evaporation. The residue was suspended in H2O (80 mL) and extracted successively with petroleum ether, CHC13, EtOAc and n-BuOH (each 3×150 mL). The petroleum ether extract (15 g) was subjected to CC over silica gel CC afforded compounds 4 (5 mg) and 5 (42 mg). The CHC13 extract (35 g) was subjected to silica gel CC. Elution of the column with 5% EtOAc in CHC13 gave a colourless solid, which on repeated CC afforded compounds 1 (10 mg) and 2 (65 mg).

4'-Acetoxy-5-hydroxy-6,7-dimethoxyflavone, 1: Light yellow amorphous solid, UV Vis (MeOH): 272 and 310 nm; (+NaOMe): 263, 286 sh and 311 nm; IR (KBr): 3467, 1755, 1643, 1358, 1499, 1254, 1086, 469 cm−1; 1H and 13C NMR (DMSO-d6): Table 1; FAB-MS: m/z (%) 357 [M+H]+ (6), 329 (3), 315 [M-29]+ (100), 197 (6), 163 (12), 121 (8), 42 (30).

Alkaline hydrolysis of compound 1

Compound 1 (3.5 mg) was heated with 0.5 N methanolic NaOH at 35-40°C with stirring for 2 hr. The reaction mixture was neutralized with Amberlite IRA-120 (H+form) and the residue was removed by filtration. The filtrate was concentrated and subjected to silica gel CC to get compound 2 (1 mg), C17H14O6 (M+ 314).

4',5-Dihydroxy-6,7-dimethoxyflavone, 2: Light yellow needles, m.p. 257°C (lit.293-80°C); [α]D25 +18.2° (c = 0.1, CHCl3); 1H and 13C NMR data were similar to reported data10.

Acknowledgement

The authors are thankful to Prof. B. K. Datta, Tripura University for identification of the plant material and Prof. S. Roy, Director, IICB, Kolkata for spectral facility of some samples. PR is thankful to CSIR, New Delhi for the award of JRF. The work was supported by a grant (DST-SR/S1/OC-75/2009) from DST, New Delhi.

References


FAB-MS: m/z (%) 515 [M+H]+ (100), 287 [MH-CO]+ (9), 197 (6), 167 (8), 121 (15).

4',5-Dihydroxy-7-methoxyflavone (genkwanin), 3: Light yellow needles, m.p. 290°C (lit.25 293–94°C); 1H and 13C NMR data were similar to reported data10.

FAB-MS: m/z (%) 285 [M+H]+ (100), 257 [MH-CO]+ (4), 167 (10), 121 (14).

3α-Friedelinol, 4: Colourless crystals, m.p. 280°C (lit.30 278-80°C); [α]D25 +18.2° (c = 0.1, CHCl3); 1H and 13C NMR data were similar to reported data12.

3β-Friedelinol, 5: Colourless crystals, m.p. 168°C (dec); 1H NMR (600 MHz, CDCl3); δH 1.88 (1H, dq, J=13.0, 3.0 Hz, H-2), 1.54 (1H, H-2), 3.73 (1H, q-like, J=2.0 Hz, H-3), 1.72 (1H, dt, J=12.0, 3.0 Hz, H-6), 0.98 (1H, H-6), 0.89 (3H, d, J=7.0 Hz, H-23), 0.91 (3H, s, H2-24), 0.84 (3H, s, H-25), 0.96 (3H, s, H2-26), 0.99 (3H, s, H-27), 1.18 (3H, s, H-28), 0.93 (3H, s, H-29), 0.98 (3H, s, H-30); 13C NMR (150 MHz, CDCl3); δC 15.8 (CH2-3), 36.1 (CH2, C-2), 72.8 (CH, C-3), 49.2 (CH, C-4), 37.9 (C, C-5), 41.7 (CH2, C-6), 17.6 (CH2, C-7), 35.2 (CH, C-8), 37.1 (C, C-9), 61.4 (CH, C-10), 35.6 (CH2, C-11), 30.0 (CH2, C-12), 38.4 (C, C-13), 39.7 (C, C-14), 32.4 (CH2, C-15), 36.1 (CH2, C-16), 30.7 (C, C-17), 42.8 (CH, C-18), 35.4 (CH2, C-19), 28.2 (C, C-20), 32.8 (CH2, C-21), 39.3 (CH2, C-22), 11.6 (CH3, C-23), 16.4 (CH3, C-24), 18.3 (CH3, C-25), 20.3 (CH3, C-26), 18.7 (CH3, C-27), 32.1 (CH3, C-28), 35.1 (CH3, C-29), 31.8 (CH3, C-30); FAB-MS: m/z (%) 429 [M+H]+ (100).

Table 1: FAB-MS: m/z (%) 357 [M+H]+ (6), 329 (3), 315 [M-29]+ (100), 197 (6), 163 (12), 121 (8), 42 (30).

13C NMR (150 MHz, CDCl3): δC 164.1 (C, C-2), 102.7 (CH, C-3), 182.3 (C, C-4), 152.7 (C, C-5), 131.9 (C, C-6), 158.6 (C, C-7), 91.6 (CH, C-8), 152.1 (C, C-9), 105.1 (C, C-10), 121.1 (C, C-1'), 128.6 (CH, C-2' 6'), 116.0 (CH, C-3', 5'), 161.3 (C, C-4'), 60.1 (CH3, MeO-6), 56.5 (CH3, MeO-7); FAB-MS: m/z (%) 315 [M+H]+ (100), 287 [MH-CO]+ (9), 197 (6), 167 (8), 121 (15).

Carbon Numbers were identified by DEPT (Table 1).