

Flavonoids from the Stem-bark of *Oroxylum indicum*

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Abstract – Two new flavonoid compounds, 8,8"-bisbaicalein **1** and baicalein-7-*O*-caffeate **2** along with six known flavonoids, baicalein, chrysin, scutellarein, 6-hydroxyluteolin, 6-methoxyluteolin and baicalein-7-*O*-glucoside and β -sitosterol have been isolated from the stem-bark of *Oroxylum indicum* (Bignoniaceae) and identified on the basis of spectroscopic and chemical studies. 6-Hydroxyluteolin and 6-methoxyluteolin are reported for the first time from this plant.

Keywords – *Oroxylum indicum* (Bignoniaceae), flavonoids, 8,8"-bisbaicalein, baicalein-7-*O*-caffeate, 6-hydroxyluteolin, 6-methoxyluteolin, baicalein-7-*O*-glucoside.

Introduction

Oroxylum indicum (L.) Vent. (Bignoniaceae), a medium sized tree, is cultivated in India for its timber and use in traditional medicine and tanning industry. The stem-bark is useful for tanning and dyeing as well as in bitter tonics, acute rheumatism and as a remedy for scorpion-sting (Deb, 1983; Chopra *et al.*, 1992). Earlier investigation on the stem-bark reported the presence of chrysin, oroxylin-A, baicalein, scutellarein, baicalein-7-*O*-glucuronide and scutellarein-7-*O*-rutinoside (Subramanian and Nair, 1972). Our reinvestigation on the stem-bark has resulted in the isolation of eight flavonoids among which only three have been reported earlier. Herein we report the isolation and structure elucidation of these compounds.

Experimental

Plant material – The stem-bark of *Oroxylum indicum* in flowering stage was collected from Dhalai Tripura and was identified by Dr. B. K. Datta, taxonomist, Department of Life Sciences, Tripura University. A voucher specimen of the flowering plant (#BD-02/07) has been deposited in the National Herbarium, Govt. of India, Shibpur, Howrah.

Extraction and isolation of phytochemicals – Fresh air-dried stem-bark of *O. indicum* was dried in shaded floor and crushed in to coarse powder. Dried coarse powder (3.0 kg) was extracted with MeOH (3 × 5 L) by percolation

process at room temperature. The combined MeOH extract was concentrated in a rotavapour to a residue (30.5 g), dissolved in a little water (ca. 50 mL) and fractionated into benzene, chloroform and *n*-butanol soluble fractions by partition between water and benzene, water and chloroform, water and *n*-butanol, successively. Each fraction was concentrated and subjected to column chromatography (CC) with silica-gel (60 - 120 mesh, Merck). The eluates of the column were monitored by thin layer chromatography (TLC) on silica gel G (Merck) coated on glass plates in different solvent systems.

Phytochemicals from benzene soluble fraction – The benzene fraction (3.8 g) on CC gave a residue from petroleum ether (PE) bp 60-80°-benzene (PhH) (3 : 7) eluate. This residue on repeated crystallization from PE-EtOAc (6 : 1) mixture afforded colorless needles of β -sitosterol **3** (50 mg). The elution of the column with PE-EtOAc (4 : 1) gave a residue of a single component, which on repeated crystallization from PE-EtOAc mixture afforded brown needles of baicalein **4** (640 mg).

Phytochemical from chloroform soluble fraction – The chloroform fraction (8.2 g) on CC gave a residue from PE-CHCl₃ (1 : 4) eluate. This residue was homogeneous on TLC and on repeated crystallization from PE-CHCl₃ mixture gave yellow needles of chrysin **5** (600 mg).

Phytochemicals from *n*-butanol soluble fraction – The *n*-butanol fraction (5.5 g) on CC gave a residue from EtOAc eluate. This residue was a mixture of four compounds as revealed on TLC. This residue on repeated CC afforded scutellarein **6** (100 mg), 6-hydroxyluteolin **7** (15 mg), 6-methoxyluteolin **8** (10 mg) and 8,8"-bisbaicalein

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1 (12 mg). The elution of the column with EtOAc-MeOH (9 : 1) gave a gummy residue of two components. The major part of this residue was acetylated with Ac₂O and pyridine at room temperature for 48 hr and worked up as usual to get a solid residue. This residue on repeated CC gave baicalein-7-*O*-glucoside pentaacetate **9a** (90 mg) and baicalein-7-*O*-caffeate triacetate **2a** (30 mg).

Characterization of phytochemicals

General – All the phytochemicals were characterized after meticulous purification of homogeneity on thin layer chromatography (TLC) in different solvent systems followed by crystallization, study of physical constant and spectral data. In case of known compounds, consultation of literature was carried out for their identification. Melting points were determined by open capillary method and are uncorrected. UV spectrum were recorded on a Perkin UV-Vis spectrophotometer in MeOH and IR spectra on a Shimadzu FT-IR spectrophotometer in KBr pellets. ¹H-, ¹³C- and 2D-NMR spectra were taken on a Varian XL-400 spectrometer with 400 MHz for ¹H- and 100 MHz for ¹³C- spectra using TMS as an internal reference (chemical shifts are expressed in δ , ppm). EI- and FAB-MS were recorded on a Jeol JMS-AX 505 HA mass spectrometer. ¹³C-NMR-DEPT experiments were carried out with flip angle θ of 45°, 90° and 135°.

8,8''-Bisbaicalein (1) – Light brown powder, mp 238 °C; UV (MeOH) λ_{\max} nm (log ϵ): 245sh (5.40), 278 (5.26), 328 (4.72); IR (KBr) ν_{\max} cm⁻¹: 3410, 1658, 1622, 1587, 1501, 1472, 1160, 1085, 830, 681, 640; ¹H-NMR (400 MHz, DMSO-*d*₆) δ : 6.76 (2H, s, H-3''), 12.94 (2H, s, HO-5, 5''), 8.70 (2H, br s, HO-6, 6''), 10.20 (2H, br s, HO-7, 7''), 7.99 (4H, dd, J = 8.0 and 1.5 Hz, H-2', 6', 2''', 6'''), 7.24 (4H, t-like, J = 8.0 Hz, H-3', 5', 3''', 5'''), 7.57 (2H, m, H-4', 4'''); ¹³C-NMR (100 MHz, DMSO-*d*₆) δ : 163.52 (s, C-2, 2''), 102.92 (d, C-3, 3''), 181.83 (s, C-4, 4''), 152.35 (s, C-5, 5''), 128.48 (s, C-6, 6''), 161.42 (s, C-7, 7''), 99.83 (s, C-8', 8''), 152.74 (s, C-9, 9''), 103.85 (s, C-10, 10''), 130.02 (s, C-1', 1'''), 126.34 (d, C-2', 6', 2''', 6'''), 130.95 (d, C-3', 5', 3''', 5'''), 132.36 (d, C-4', 4'''); FAB-MS (+ve) m/z (rel. int.): 539 [M + H]⁺ (42), 271 (100), 270 (61), 168 (50), 105 (12), 77 (85); FAB-MS (-ve) m/z (rel. int.): 537 [M, C₃₀H₁₈O₁₀-H]⁻ (100%). *Anal.* Found : C, 66.83; H, 3.29. Calcd for C₃₀H₁₈O₁₀ : C, 66.92; H, 3.37%.

Baicalein-7-*O*-caffeate triacetate (2a) – Yellow amorphous powder; UV (MeOH) λ_{\max} nm (log ϵ): 240 (4.48), 278 (4.26), 327 (4.21); IR (KBr) ν_{\max} cm⁻¹: 3415, 1725, 1654, 1618, 1587, 1510, 1165, 1080, 825, 635; ¹H-NMR (400 MHz, CDCl₃) δ : 6.78 (1H, s, H-3), 12.77 (1H, s, HO-5), 6.69 (1H,

s, H-8), 7.88 (2H, dd, J = 8.0 and 2.0 Hz, H-2', 6'), 7.54 (2H, t-like, J = 8.0 Hz, H-3', 5'), 7.60 (1H, m, H-4'), 7.01 (1H, d, J = 2.0 Hz, H-2''), 7.20 (1H, d, J = 8.0 Hz, H-5''), 7.36 (1H, dd, J = 8.0 and 2.0 Hz, H-6''), 7.58 (1H, d, J = 16.0 Hz, H-7''), 6.42 (1H, d, J = 16.0 Hz, H-8''), 2.11, 2.34, 2.36 (each 3H, s, 3 × Ac); ¹³C-NMR (100 MHz, CDCl₃) δ : 162.74 (s, C-2), 106.0 (d, C-3), 182.91 (s, C-4), 153.25 (s, C-5), 132.01 (s, C-6), 154.37 (s, C-7), 95.10 (d, C-8), 153.72 (s, C-9), 107.40 (s, C-10), 131.22 (s, C-1'), 126.08 (d, C-2', 6'), 129.10 (d, C-3', 5'), 132.08 (d, C-4'), 127.21 (s, C-1''), 115.80 (d, C-2''), 148.10 (s, C-3''), 149.80 (s, C-4''), 115.93 (d, C-5''), 121.98 (d, C-6''), 146.20 (d, C-7''), 114.10 (d, C-8''), 167.46 (s, C-9''), 20.66, 20.74, 20.79 (each, q), 170.23, 170.55, 170.62 (each s) (3 × Ac); FAB-MS m/z (rel. int.): 581 [M + Na]⁺ (17), 559 [M + H]⁺ (18), 313 [M-diacetylcaffeoyl + 2H]⁺ (46), 271 [aglycone + H]⁺ (63), 270 (47), 247 [diacetylcaffeoyl]⁺ (10), 168 (75), 105 (23), 77 (47), 43 (100). *Anal.* Found: C, 64.41; H, 3.90. Calcd for C₃₀H₁₂O₁₁ : C, 64.52; H, 3.97%.

Deacetylation of 2a – A solution of **2a** (8 mg) in 0.1 *N* aqueous methanolic NaOH (5 mL) was stirred at 40 °C for 2 hr. The reaction mixture was evaporated to a residue in a rotavapour. The residue was dissolved in a little H₂O and neutralized with dil H₂SO₄ at 0 °C. The neutralized solution was concentrated and column purified to get a semi-solid residue of **2** (3.5 mg), which was identical on TLC with one of the spots of the gummy residue used for acetylation.

Alkaline hydrolysis of 2a – A solution of **2a** (8 mg) in 2 *N* aqueous methanolic NaOH (5 ml) was refluxed for 2 hr. The reaction mixture was evaporated to a residue and dissolved the residue in a little H₂O. The aqueous solution was neutralized with dil H₂SO₄ and extracted with CHCl₃. The CHCl₃ extract was concentrated and column purified to get baicalein **3** (2 mg) and *trans*-caffeic acid (1.5 mg). Both the hydrolysis products were identified by direct comparison (mixed-mp and *co*-TLC) with authentic samples.

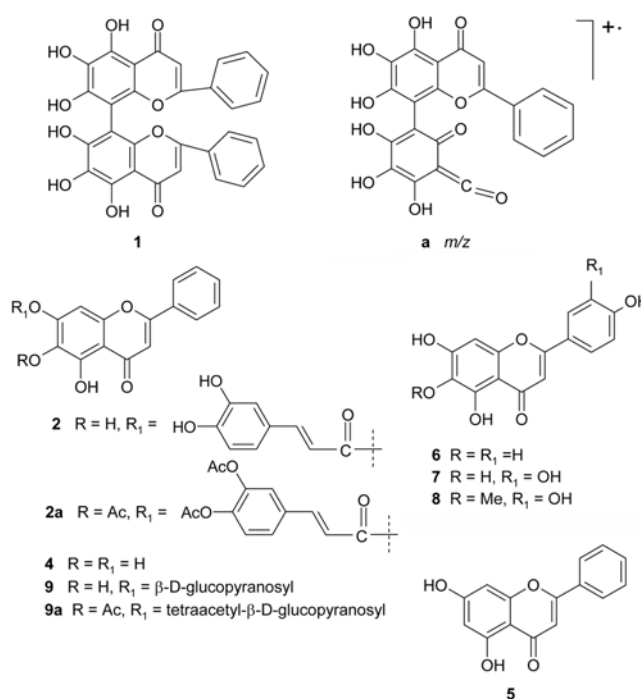
β -Sitosterol (3) – Colorless needles, mp 142 °C; C₂₉H₅₀O (M⁺ 414). It was identified by direct comparison mixed-mp (undepressed), *co*-TLC and GC analysis; RRt (relative retention time) of β -sitosterol is 1.23 i.e. the ratio of retention time of β -sitosterol to the retention time of cholesterol sample is 1.23 under the same column parameters of GC analysis.

Baicalein (4) – Brown needles, mp 260 °C [lit. (Merck Index, 1989) mp 264-265 °C (dec)]; UV (MeOH) λ_{\max} nm (log ϵ): 247 sh (4.83), 273 (5.04), 323 (4.73); IR (KBr) ν_{\max} cm⁻¹: 3412, 1655, 1618, 1585, 1506, 1472, 1163, 1086, 899, 852, 827, 683, 640; ¹H-NMR (400 MHz,

DMSO- d_6) δ : 6.91 (1H, s, H-3), 12.65 (1H, s, HO-5), 8.81 (1H, s, HO-6), 10.57 (1H, s, HO-7), 6.61 (1H, s, H-8), 8.04 (2H, dd, J = 8.0 and 1.5 Hz, H-2', 6'), 7.55 (2H, t-like, J = 8.0 Hz, H-3', 5'), 7.58 (1H, m, H-4'); ^{13}C -NMR (100 MHz, DMSO- d_6) δ : 162.88 (s, C-2), 104.46 (d, C-3), 180.10 (s, C-4), 153.62 (s, C-5), 129.31 (s, C-6), 146.96 (s, C-7), 94.0 (d, C-8), 149.82 (s, C-9), 104.27 (s, C-10), 130.94 (s, C-1'), 126.28 (d, C-2', 6'), 129.08 (d, C-3', 5'), 131.79 (d, C-4'); EI-MS m/z (rel. int.): 270 (M^+ , 100), 269 (4), 242 (M-CO, 4), 168 (40), 140 (9), 105 (7), 102 (4), 77 (8); HR-EI-MS m/z (rel. int.): 270.0540 (100%), calcd for $\text{C}_{15}\text{H}_{10}\text{O}_5$: 270.0528. It was identified by comparison of physical constant and spectral data with literature (Mabry *et al.*, 1970; Chen *et al.*, 2003).

Chrysin (5) – Yellow needles, mp 270 °C [lit. (Merck Index, 1989) 285 °C]; UV (MeOH) λ_{max} nm (log ϵ): 247 sh (4.58), 268 (4.90), 314 (4.48); IR (KBr) ν_{max} cm^{-1} : 3427, 1653, 1611, 1578, 1555, 1499, 1448, 1356, 1313, 1169, 806, 781, 732, 675; ^1H -NMR (400 MHz, DMSO- d_6) δ : 6.93 (1H, s, H-3), 12.80 (1H, s, HO-5), 6.19 (1H, d, J = 2.0 Hz, H-6), 10.89 (1H, s, HO-7), 6.50 (1H, d, J = 2.0 Hz, H-8), 8.04 (2H, dd, J = 8.0 and 2.0 Hz, H-2', 6'), 7.54 (2H, t-like, J = 8.0 Hz, H-3', 5'), 7.59 (1H, m, H-4'); ^{13}C -NMR (100 MHz, DMSO- d_6) δ : 163.12 (s, C-2), 105.14 (d, C-3), 181.83 (s, C-4), 161.43 (s, C-5), 98.98 (d, C-6), 164.40 (s, C-7), 94.04 (d, C-8), 157.42 (s, C-9), 103.93 (s, C-10), 130.68 (s, C-1'), 126.37 (d, C-2', 6'), 129.09 (d, C-3', 5'), 131.96 (d, C-4'); EI-MS m/z (rel. int.): 254 (M^+ , 100), 253 (21), 226 (M-CO, 48), 152 (40), 124 (26), 113 (21), 105 (6), 102 (8), 77 (11); HR-EI-MS m/z (rel. int.): 254.0579 (100%), calcd. for $\text{C}_{15}\text{H}_{10}\text{O}_4$: 254.0579. It was identified by comparison of its physical constant and spectral data with literature (Mabry *et al.*, 1970; Markham and Chari, 1982).

Scutellarein (6) – Orange needles, mp > 300 °C [lit. (Merck Index, 1989) > 300 °C]; UV (MeOH) λ_{max} nm (log ϵ): 286 (4.94), 336 (5.00); IR (KBr) ν_{max} cm^{-1} : 3443, 1662, 1618, 1587, 1495, 1364, 1273, 1246, 1070, 831, 792, 721; ^1H -NMR (400 MHz, DMSO- d_6) δ : 6.72 (1H, s, H-3), 12.78 (1H, s, HO-5), 8.73 (1H, br s, HO-6), 10.30 (2H, br s, HO-7, 4'), 6.56 (1H, s, H-8), 7.88 (2H, dd, J = 8.0 and 1.5 Hz, H-2', 6'), 6.90 (2H, dd, J = 8.0 and 1.5 Hz, H-3, 5'); ^{13}C -NMR (100 MHz, DMSO- d_6) δ : 163.48 (s, C-2), 102.24 (d, C-3), 182.0 (s, C-4), 147.02 (s, C-5), 129.12 (s, C-6), 149.64 (s, C-7), 93.83 (d, C-8), 153.29 (s, C-9), 103.95 (s, C-10), 121.44 (s, C-1'), 128.33 (d, C-2', 6'), 115.92 (d, C-3', 5'), 160.98 (s, C-4'); EI-MS m/z (rel. int.): 286 [M^+ , $\text{C}_{15}\text{H}_{10}\text{O}_6$] (100), 285 (5), 258 (M-CO, 4), 168 (35), 140 (7), 121 (11), 93 (4). It was identified by comparison of its physical constant and spectral data with literature (Markham and Chari, 1982; Peng *et al.*, 2003).



6-Hydroxyluteolin (7) – Yellow needles, mp 282 °C [lit. (Buckingham, 1994a) 284 °C]; UV (MeOH) λ_{max} nm (log ϵ): 268 (4.64), 346.5 (5.10); IR (KBr) ν_{max} cm^{-1} : 3420, 1662, 1618, 1587, 1558, 1472, 1273, 1178, 831, 792, 569; ^1H -NMR (400 MHz, DMSO- d_6) δ : 6.65 (1H, s, H-3), 12.53 (1H, s, HO-5), 6.61 (1H, s, H-8), 7.31 (1H, d, J = 2.0 Hz, H-2'), 6.91 (1H, d, J = 8.5 Hz, H-5'), 7.39 (1H, dd, J = 8.5 and 2.0 Hz, H-6'); EI-MS m/z (rel. int.): 302 [M^+ , $\text{C}_{15}\text{H}_{10}\text{O}_7$] (100), 168 (40), 140 (10), 137 (8). It was identified by comparison of its physical constant and spectral data with those of literature (Peng *et al.*, 2003).

6-Methoxyluteolin [Nepetin or eupafolin] (8) – Pale yellow needles, mp 280 °C [lit. (Buckingham, 1994b) 280 °C]; UV (MeOH) λ_{max} nm (log ϵ): 268 (4.62), 348 (5.06); IR (KBr) λ_{max} cm^{-1} : 3440, 1660, 1618, 1585, 1500, 1248, 832, 791; ^1H -NMR (400 MHz, DMSO- d_6) δ : 6.74 (1H, s, H-3), 12.37 (1H, s, HO-5), 3.78 (3H, s, MeO-6), 6.24 (1H, s, H-8), 7.41 (1H, d, J = 2.0 Hz, H-2'), 6.80 (1H, d, J = 8.0 Hz, H-5'), 7.37 (1H, dd, J = 8.0 and 2.0 Hz, H-6'); EI-MS m/z (rel. int.): 316 [M^+ , $\text{C}_{16}\text{H}_{12}\text{O}_7$] (100), 183 (48), 137 (12), 109 (7). Physical and spectral data were in good agreement with those reported in the literature (Ferraro and Coussio, 1973).

Baicalein-7-O-glucoside pentaacetate (9a) – Pale brown needles, mp 275 °C; UV (MeOH) λ_{max} nm (log ϵ): 240 (4.56), 295 (4.46), 325.7 (4.43); IR (KBr) ν_{max} cm^{-1} : 3410, 1654, 1620, 1578, 1339, 1298, 1163, 1086, 900, 852, 640; ^1H -NMR (400 MHz, CDCl_3) δ : 6.71 (1H, s, H-3),

12.82 (1H, s, HO-5), 6.67 (1H, s, H-8), 7.88 (2H, dd, $J=8.5$ and 1.5 Hz, H-2, 6), 7.53 (2H, t, $J=8.5$ Hz, H-3', 5'), 7.57 (1H, m, H-4'), 5.18 (1H, d, $J=7.5$ Hz, H-1''), 5.33 (1H, dd, $J=7.5$ and 9.0 Hz, H-2''), 5.37 (1H, dd, $J=9.0$ and 9.5 Hz, H-3''), 5.16 (1H, dd, $J=9.0$ and 9.0 Hz, H-4''), 4.0 (1H, m, H-5''), 4.26 (1H, dd, $J=12.0$ and 2.5 Hz, H-6''), 4.29 (1H, dd, $J=12.0$ and 4.0 Hz, H-6''), 2.05, 2.07, 2.09 \times 2, 2.11 (each s, $5 \times$ Ac); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 164.70 (s, C-2), 106.0 (d, C-3), 182.60 (s, C-4), 153.22 (s, C-5), 132.08 (s, C-6), 154.41 (s, C-7), 93.34 (d, C-8), 154.45 (s, C-9), 107.45 (s, C-10), 131.03 (s, C-1'), 126.36 (d, C-2', 6'), 129.17 (d, C-3', 5'), 132.21 (d, C-4'), 98.62 (d, C-1''), 70.32 (d, C-2''), 72.27 (d, C-3''), 68.12 (d, C-4''), 72.40 (d, C-5''), 61.89 (t, C-6''), 20.10, 20.58 \times 2, 20.66, 20.79 (each q), 168.54, 169.37, 169.44, 170.09, 170.46 (each s) (5XAc); FAB-MS m/z (rel. int.): 643 [M, $\text{C}_{31}\text{H}_{30}\text{O}_{15}+\text{H}$] $^+$ (33), 615 (M-28, 3), 601 (M-42, 5), 331 (oxonium ion of tetraacetyl glucose, 34), 271 (aglucone + H, 64), 270 (47), 168 (75), 105 (23), 77 (47), 43 (100). It was identified by comparison of its spectral data with those reported for baicalein-7-*O*-glucoside in the literature (Chen *et al.*, 2003). On hydrolysis with 2 *N* aqueous methanolic HCl it gave baicalein and D-glucose.

Results and Discussion

The positive ion FAB mass spectrum of 8,8''-bisbaicalein **1** showed a quasi-molecular ion peak at m/z 539 [M+H] $^+$ and negative ion FAB mass spectrum showed quasi-molecular ion peak at m/z 537 [M-H] $^-$ suggesting its molecular formula $\text{C}_{30}\text{H}_{18}\text{O}_{10}$. This molecular formula was also supported by its elemental analysis. The UV spectrum in MeOH showed absorption maxima at 245 nm ($\log \epsilon$, 5.04), 278 (5.26) and 328 nm (4.72) characteristic of flavonoids (Mabry *et al.*, 1970). The IR spectrum in KBr exhibited absorption bands for hydroxyl (3410 cm^{-1}), α,β -unsaturated carbonyl (1658 and 1622 cm^{-1}) and aromatic (1587 and 830 cm^{-1}) functions (Markham, 1975). The ^1H - and ^{13}C -NMR data coupled with DEPT experiments (see Experimental) can be explained by considering 8,8''-bisbaicalein structure **1** for it. The attachment of two flavonoid nuclei at C-8,8'' position was considered from the deshielding effect at C-7 and C-9 carbons and shielding of the C-7 hydroxyl group (δ_{H} 10.20). The presence of strong mass ion peak at m/z 271 (baicalein + H) $^+$ and 270 (baicalein) in the positive FAB-MS clearly suggested the dimeric baicalein structure of compound **1**. The appearance of mass ion peak at m/z 436 (**a**) also corroborated the linkage of the monomeric units

in ring A of flavone molecules (Mabry and Markham, 1975). To the best of our knowledge it is a new natural product.

Baicalein-7-*O*-caffeate **2** was isolated as its triacetate **2a** in amorphous powder. The positive ion FAB-MS of the acetate derivative showed quasi-molecular ion at m/z 581 [M + Na] $^+$ and 559 [M + H] $^+$ suggesting its molecular formula $\text{C}_{30}\text{H}_{22}\text{O}_{11}$. The UV spectrum of the compound in MeOH showed absorption maxima at 240 nm ($\log \epsilon$, 4.48), 278 (4.26) and 327 nm (4.21) suggesting its flavonoid nature. The IR spectrum in KBr exhibited the presence of hydroxyl (3415 cm^{-1}), aromatic ester (1725 cm^{-1}), unsaturated carbonyl (1654 and 1618 cm^{-1}) and aromatic (1587 and 825 cm^{-1}) functions. The ^1H - and ^{13}C -NMR spectra in combination with DEPT experiments (see Experimental) supported the presence of acetyl baicalein and diacetylcaffeoyl moieties in the molecule (Kuo *et al.*, 1996). The attachment of diacetyl caffeoyl moiety at C-7 of baicalein unit was assigned on the basis of high chemical shift value of C-7 carbon similar to that in baicalein-7-*O*-glucoside. The FAB-MS recorded mass ions at m/z 313 and 247 supporting the presence of acetyl baicalein and diacetyl-caffeoyl moieties in the molecule. Alkaline hydrolysis of the compound afforded baicalein and *trans*-caffeic acid confirming the presence of these monomeric units. Therefore, the structure of the compound **2a** was established as 6-*O*-acetyl baicalein-7-*O-trans*-diacetylcaffeate. Compound **2a** on deacetylation under mild condition afforded compound **2**, $\text{C}_{24}\text{H}_{16}\text{O}_8$ (M^+ 432) in semi-solid mass, which was identical on TLC with one of the spots of gummy mass used for acetylation. Hence, the structure of **2** was assigned as baicalein-7-*O-trans*-caffeate. It is interesting to note that under ordinary condition of acetylation only C-6 hydroxyl group was acetylated. Possibly C-5-hydroxyl group of baicalein was not acetylated due to its intramolecular hydrogen bonding with carbonyl oxygen. Compound **2** is a new natural product.

Known compounds, baicalein, baicalein-7-*O*-glucoside, chrysin, scutellarein, 6-hydroxyluteolin and 6-methoxy luteolin were identified by comparison of their spectral data with literature (see Experimental) and β -sitosterol by comparison with an authentic sample. The detailed spectral data of baicalein-7-*O*-glucoside pentaacetate was not reported in the literature. The chemical shift value of C-7 carbon in baicalein and its derivatives was found different from that of reported value (Chen *et al.*, 2003).

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