Revised structures of the flavonoids from *Linnophila gratissima* (Scrophulariaceae)

Sujatha Krishnan & A G Ramachandran Nair*
Department of Chemistry, Pondicherry University, Pondicherry 605 014, India.

Received 7 September 1998; accepted (revised) 2 June 1999.

7-Desmethyllartemetrin (5,7-dihydroxy-3,6,3',4'-tetramethoxyflavone), a rare flavonoid reported from *Linnophila gratissima*, has been found to be a mixture of two flavones [5,7-dihydroxy-6,8,4'-trimethoxyflavone (nevadensin)] and 5-hydroxy-6,7,4'-trimethoxyflavone (salvigenin)]. Besides the plant contains two phenolic acids; caffieic acid and chlorogentic acid. The present finding is supported by chemotaxonomy of Scrophulariaceae.

A rare methylated flavonol, 7-desmethyllartemetrin (5,7-dihydroxy-3,6,3',4'-tetramethoxyflavone) was reported from *Linnophila gratissima* (Scrophulariaceae). In view of the rare occurrence of flavonols in the family Scrophulariaceae and in continuation of the work on this family in our laboratory, along with the doubt on the reported spectral and chromatographic data on the compound, a re-examination of the flavonoids from this plant was considered desirable and hence taken up. Adopting standard procedures two flavones [5,7-dihydroxy-6,8,4'-trimethoxyflavone (nevadensin)] and 5-hydroxy-6,7,4'-trimethoxyflavone (salvigenin)] and two phenolic acids (caffeic acid and chlorogentic acid) were isolated and characterised. 7-Desmethyllartemetrin was found to be a mixture of these two methylated flavones.

Experimental Section

Melting points were determined in open capillaries and are uncorrected. UV-visible absorption spectra were measured in spectroscopic MeOH and in the presence of diagnostic shift reagents in the range 200-500 nm using Milton-Roy UV-Visible spectrophotometer. The $^1$H NMR spectrum was recorded on Brucker 350 MHz spectrometer using DMSO-$d_6$ as solvent and TMS as internal standard and mass spectra was recorded on Jeol-300 mass spectrometer with a 70 eV ionisation energy.

Plant material

The aerial parts of *L. gratissima* were collected from Mahe region in South India. A voucher specimen is kept in the Dept of Chemistry, Pondicherry University, Pondicherry.

Extraction and isolation

The plant material (2 kg) was refluxed with 90% boiling EtOH and concentrated under reduced pressure. The aqeous crude extract was fractionated into benzene, dichloromethane and ether solubles, of which the latter two tested positive for polyphenolics. The dichloromethane extract was concentrated and subjected to column chromatography (SiO$_2$, 100-200 mesh) with different proportions of hexane, CH$_2$Cl$_2$ and their binary mixtures in increasing order of polarity. 15 fractions of 250 mL each were collected of which fractions 11-13 indicated the presence of two UV active compounds. Further separation of these two compounds (0.25 g) was carried out by preparative polyamide TLC with C$_6$H$_5$-pet-ether:MeOH (5:5:1) as the developing solvent. The eluates from the separated zones, $R_f$: 0.15 (major, designated, A) and 0.37 (minor, designated, B) were concentrated to yield yellow residues. Recrystallisation from MeOH yielded pure compounds A and B.

The residue from the ether extract (100 mg) was subjected to preparative PC (Whatman No.3, 15% AcOH) when two bands separated. The eluates from them, $R_f$: 0.15, designated, C and 0.73, designated, D were concentrated and residue recrystallised from MeOH to yield pure compounds C and D.

Compound A (65mg), C$_{16}$H$_{10}$O$_7$, m.p.186-88°C, purple under UV and UV/NH$_3$, red with Mg-HCl and green with Fe$^{3+}$; $\lambda_{max}$ (MeOH): 282, 296sh, 329 (+NaOAc) 282, 304sh, 376 (+NaOAc/H$_2$BO$_3$) 286, 330 (+NaOMe); 284, 304sh, 378 (+AlCl$_3$); 264, 290, 310, 354, 412sh (+AlCl$_3$/HCl); 261, 288sh, 308, 349, 412sh; $^1$H NMR spectrum (350 MHz, $\delta$, ppm) showed the following characteristic signals: $\delta$ 8.02(d, $J$=9.2Hz, 2H, H-2',6'); 7.15(d, $J$=9.2Hz, 2H, H-3'); 6.84(s, 1H, H-3) 3.88, 3.87, 3.78 (s each, 3H each, 3-OMe); MS (EIMS,m/z, intensity as %): 344 (M$^+$, C$_{16}$H$_{10}$O$_7$)2 329 (100) 311 (15) 301 (16) 197 (30) 169 (26) 135 (20) and 132 (17). Compound A on acetylation with acetic anhydride and pyridine yielded the diacetcate, m.p. 172-74°C whose $^1$H NMR showed signals at $\delta$: 8.02 (d, $J$=9Hz, 2H, H-2',6') 7.14 (d, 2H, H-3'), 6.78 (s, 1H, H-3) 2.01, 1.99, 1.98 (t, 3H each, 3-OAc); 1.74 (s, 3H, 3-OAc).
Compound B (35 mg), C₁₈H₁₆O₁₀, m.p. 185-86 °C, purple under UV and UV/NH₃, green with Fe³⁺; λₘₚₙ (MeOH) 276, 329 (+NaOAc) 276, 329 (+NaOAc/H₂BO₃), 276, 330 (+NaOAc), 297, 372s 396sh (+AlCl₃) 262, 290, 299, 354 (+AlCl₃/HCl), 262, 290, 299, 353; ¹H NMR spectrum (350 MHz, δ, ppm) showed signals at δ 8.07 (d, J=9Hz, 2H, H-2',5'), 7.13 (d, J=9Hz, 2H, H-3',5') 6.96 (s, 1H, H-8) 6.91 (s, 1H, H-3) 3.94, 3.88, 3.75 (s each, 3H each, 3-OME); MS (EIMS, m/z, intensity as %): 328 (M⁺, C₁₈H₁₆O₁₀, 100), 314 (14), 313 (85), 299 (25), 181 (23), 153 (31), 135 (15) and 132(10). Compound B on acetylation yielded a monoaacetate, m.p. 166-67 °C. All these data were in good agreement with the earlier report for nevdensin². Thus, compound B was characterised as nevdensin (5,7-dihydrory-6,8,4'-trimethoxyflavone) which was further confirmed by m.p and co-chromatography with an authentic sample from Acanthospermum hispidum.⁶

Compound C (35 mg), C₁₈H₁₆O₉, m.p. 210-12 °C, blue under UV changing to greenish blue under UV/NH₃, produced brisk effervescence with NaHCO₃ solution and green colour with Fe³⁺; λₘₚₙ (MeOH): 245, 325; ¹H NMR spectrum (350 MHz, δ, ppm) 7.53 (d, J=15.9 Hz, 1H, α-H), 7.16 (d, J=2.3Hz, 1H, H-2), 7.03 (dd, J=8.3 & 2.3Hz, 1H, H-6), 6.86 (d, J=8.3Hz, 1H, H-5), 6.26 (d, J=15.9Hz, 1H, β-H); MS (EIMS, m/z, intensity as %): 180 (M⁺, C₁₈H₁₆O₉, 100) 163 (M⁻-17, 30), 136 (M⁻-44, 84), 134 (35), 69 (20), 57 (30), 55(20) and 44 (42). Compound C on acetylation yielded the diacetate, m.p. 201-03 °C. Based on these data the compound was identified as caffeic acid and the identity confirmed by direct comparison (m.p and co-PC) with an authentic sample from Acanthospermum hispidum.⁶

Compound D (10 mg), C₁₆H₁₃O₆, m.p. 199-200 °C, blue under UV changing to yellow green with UV/NH₃, deep blue with Fe³⁺; λₘₚₙ (MeOH): 243, 305sh and 329; ¹H NMR spectrum (350 MHz, δ, ppm): 9.64, 9.22 (each brs, each 1H, 7',8'-OH), 7.41 (d, J=16Hz, 1H, H-3') 7.39 (d, J=2Hz, 1H, H-9') 7.00 (d, J=8 and 2Hz, 1H, H-5') 6.65 (d, J=8Hz, 1H, H-6') 6.15 (d, J=16Hz, 1H, H-2') 5.05 (m, 1H, H-4) 4.95, 4.79, 3.92 (each brs, each 1H, 1, 4, 5-OH), 3.9-3.6 (m, 2H, H-4, -5) 2.05-1.73 (m, 4H, 2-CH₂); MS (EIMS, m/z, intensity as %): 354 (M⁺, C₉H₅O₄, 5) 336 (32), 180 (60); 163 (100). Hydrolysis with 2N NaOH yielded caffeic acid and quinic acid (1:1) identified by co-pc and HPLC (Rt=16.16, phenyl Bondapak column, gradient elution with 5% AcOH-MeOH, 25°C). Thus compound D was characterised as chlorogenic acid⁹ (3-O-cafeoyl quinic acid) which was further confirmed by m.m.p and co-chromatography with an authentic sample from Berberis aristata.⁹

A sample of 7-desmethylartemetin isolated from Limnophila grattissima and kindly supplied by one of the authors¹ was found to be a mixture of the same two flavones nevdensin and slavigenin isolated now. Further, nevdensin has been later isolated from a sister species L. conferta¹⁰ justifying its presence in the genus Limnophila and thus strengthening the present finding.

Acknowledgements
Our thanks are due to Prof. B. Voiron, Laboratoire de Phytochimie, Villeurbanne, France for part of the spectral data and Dr K. K. Srinivasan (Manipal) for the sample of 7-desmethyartemetin. One of the authors (SK) thanks UGC for a research scholarship.

References
7 Herz W & Gibaja S, Phytochemistry, 11, 1972, 928.