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# Naturally Occurring Bioactive *o*-Heterocycles: A Quest For New Sources

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## ABSTRACT

*Limnophila heterophylla* and *L. rugosa* (Scrophulariaceae) were established as new sources of natural flavonoids, respectively of nevadensin (5,7-dihydroxy-6,8,4'-trimethoxyflavone) and salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone). Their structures were characterized on the basis of spectral studies. 

## KEYWORDS

*Limnophila heterophylla*;
*L. rugosa*;
Scrophulariaceae;
Flavonoids; Nevadensin;
Salvigenin; Spectral studies.

## INTRODUCTION

Among the naturally occurring heterocyclic compounds, O-heterocycles are mostly abundant and are of importance and interest to a wide variety of scientists from interdisciplinary fields[1,2]. In continuation of our search for natural O-heterocycles[3-7], we have found *limnophila heterophylla*[8] and *limnophila rugosa* (family: Scrophulariaceae)[9,10] as new and rich sources, respectively for nevadensin[11,12] and salvigenin [13]. Both are the well-known natural bioactive flavonoids. As reported so far, nevadensin possesses anti-mycobacterial and anti-inflammatory activities [14], hypotensive property[15], and moderate cytotoxic and antitubercular activities also[16]. Salvigenin is also an important bioactive bioflavonoid[17]. *L.heterophylla* and *L.rugosa,* both locally available herbs, are important Indian medicinal plants and find a lot of applications in the traditional system of medicine against various ailments[4, 9, 18-20]. The petrol extracts of both the plant materials afforded the flavonoids characterized on the basis of detailed spectral studies.

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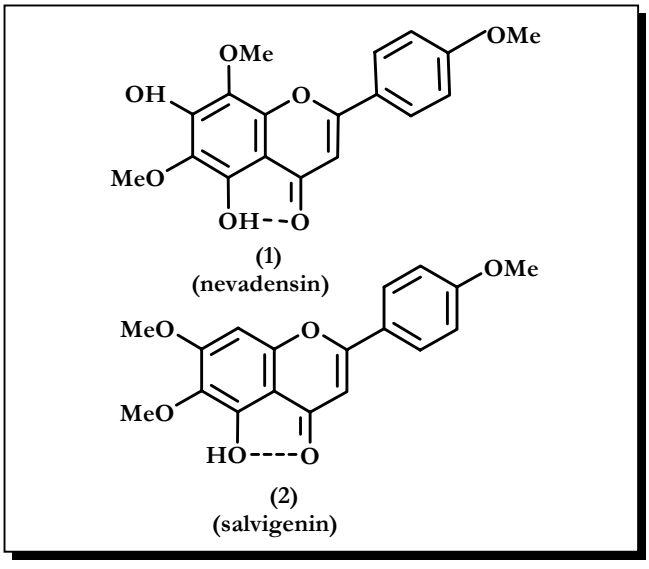


## RESULTS AND DISCUSSION

The dihydroxytrimethoxy flavone **(1)**, $C_{18}H_{16}O_7$ ($[M]^+$ at *m/z* 344), responded positively towards flavonoid colour reactions and ferric chloride solution, and exhibited characteristic UV absorptions. The infrared absorption bands of **(1)** are also of expected outcome. In the $^1$H NMR spectrum of nevadensin, we observed for the first time that the B-ring protons appeared as double doublets (dd) — d 7.89 (2H, dd, *J*= 2.7 Hz, 11.7 Hz, H-2' & H-6') and d 7.045 (2H, dd, *J*= 3 Hz, 11.7 Hz, H-3' & H-5') — thereby supporting the proposed B-ring substitution pattern.

The mass spectral fragmented ion-peaks of compound **(1)** clearly suggest that two methoxyl and two hydroxyl functions are attached to the ring-A, while the remaining methoxyl group is linked with the ring-B, and unambiguously it must be placed at C-4' as evidenced from the $^1$H NMR spectral analysis. The appearance of intense green colour with ferric chloride imparted by the compound locates one of the hydroxyls at C-5 position[21] as also revealed from IR and $^1$H NMR spectra. Again, the bathochromic shift of Band I by 20 nm (335→355) in the UV spectrum of **(1)**, in the presence of aluminium chloride that remained unchanged on addition of hydrochloric acid, confirmed the presence of a hydroxyl function at C-5 and one of the methoxyls at C-6 position[22, 23]. That the C-6 position is blocked by a methoxyl function, is evidenced by its characteristic mass fragmentation pattern[24,25] as well as the negative response of the compound towards the colour reaction with *o*-dinitrobenzene[11]. A negative gossypetone reaction[26] suggested the presence of a methoxyl group at C-8 as well, and hence, the remaining hydroxyl group must be located at C-7 position, although there is no characteristic bathochromic shift of Band II in the UV spectrum upon addition of sodium acetate/ethanol ¾ which is the peculiarity of nevadensin itself[11]. This structural formulation has been confirmed by $^{13}$C NMR and HMQC spectral studies (TABLE 1). The HMQC spectrum exhibited expected heteronuclear cross-peaks, for eight protonated carbons, showing one-bond heteronuclear correlations ($^1$H – $^{13}$C) between $C_{2'}$ and $C_{6'}$ protons at d7.89 (2H, dd, *J*= 2.7 Hz, 11.7 Hz) with 2' and 6' carbons at d127.8; $C_{3'}$ and $C_{5'}$ protons at d7.045 (2H, dd, *J*= 2.7 Hz, 11.7 Hz) with 3' and 5' carbons at d115.0; $C_3$-proton at d 6.585 (1H,s) with $C_3$-carbon at d104.2, and the methoxy protons at d3.90, 4.02 and 4.04 of three methoxyl functions respectively with the methoxyl carbons at d56.0 ($C_{4'}$-O*C*H$_3$), 61.4 ($C_{8'}$-O*C*H$_3$) and 62.3 ($C_{6'}$-O*C*H$_3$).

**TABLE 1: $^{13}$C NMR data and HMQC results for nevadensin**

| C-atom | $\delta_C$- value | HMQC |
|---|---|---|
| 2 | 164.2 | - |
| 3 | 104.2 | $\delta_{H\text{-}3}$ 6.585 |
| 4 | 183.4 | - |
| 5 | 148.8 | - |
| 6 | 131.5 | - |
| 7 | 149.2 | - |
| 8 | 128.5 | - |
| 9 | 146.2 | - |
| 10 | 105.0 | - |
| $1^1$ | 124.0 | - |
| $2^1$,$6^1$ | 127.8 | $\delta_{H\text{-}2^1,6^1}$ 7.89 |
| $3^1$,$5^1$ | 115.0 | $\delta_{H\text{-}3^1,5^1}$ 7.045 |
| $4^1$ | 163.1 | - |
| 8-O*C*H$_3$ | 61.4 | $\delta_H$ 4.02 |
| 6-O*C*H$_3$ | 62.3 | $\delta_H$ 4.04 |
| $4^1$-O*C*H$_3$ | 56.0 | $\delta_H$ 3.90 |

The flavonoid **(2)**, $C_{18}H_{16}O_6$ ($[M]^+$ (EIMS) m/z 328) exhibited UV, IR, NMR and mass spectral properties, which are in excellent conformity with

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the reported values for salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone)[27,28].

## EXPERIMENTAL

Melting points are uncorrected. TMS has been used as int. standard in recording NMR spectra. Whole plants of *Limnophila heterophylla* and *L. rugosa* were collected from Santiniketan and their identities were verified by Dr. H R Chowdhury and Dr. S Mondal (Visva-Bharati University). Voucher specimens have been deposited in the Natural Product Laboratory of this University.

### Extraction and isolation

**Nevadensin (1)**: Air-dried defatted powered whole plants (1.5 kg) of *L. heterophylla* were extracted with petrol (60-80$^0$) in a Soxhlet apparatus for 56 hr. The extract was concentrated under reduced pressure and then subjected to column chromatography on silica gel (60-120 mesh, 200 g); the petrol (60-80$^0$C): benzene (1:2) eluent afforded nevadensin (5,7-dihydroxy-6,8,4'-trimethoxyflavone) as golden yellow needles (yield 0.8 g), m.p. 184-186$^0$C (from ethanol); UV (ethanol): $\lambda_{max}$ 280, 335 nm; (+$AlCl_3$): 280, 310 (sh), 355 nm; IR (KBr) n cm$^{-1}$: 3407, 3100, 2936, 2840, 1663, 1591, 1508, 1060, 1025; $^1$H NMR ($CDCl_3$, 300MHz; TMS): d 12.78 (1H, s, $C_5$-OH), 7.89 (2H, dd, *J*= 2.7 Hz, 11.7 Hz, H-2' and H-6'), 7.045 (2H, dd, *J*= 3 Hz, 11.7 Hz, H-3' and H-5'), 6.585 (1H, s, $C_3$-H), 4.04 (3H, s, $C_6$-OC$H_3$), 4.02 (3H, s, $C_8$-OC$H_3$) and 3.90 (3H, s, $C_{4'}$-OC$H_3$); EIMS (70 ev): *m/z* 344 ([M]$^+$), 329 (base peak), 316[M-CO]$^+$, 315 [M-CO-H]$^+$, 314 [M-2Me]$^+$, 312[M-2Me -2H]$^+$, 301[M- CO -Me]$^+$, 212 and 132 (retro-Diels-Alder fragmented ion peaks of 1),197 and 132 (retro-Diels-Alder cleavage of mass fragment 329), 169[197-CO]$^+$, 168 [169-H]$^+$, 153[169-Me]$^+$, 141[169-CO]$^+$, 135(fragmented ion peak), 126[ 141-Me]$^+$; $^{13}$C NMR ($CDCl_3$, 75 MHz)and HMQC in TABLE 1.

**Salvigenin (2)**: Air-dried defatted powered whole plants (1.5 kg) of *L. rugosa* were extracted with petrol (60-80$^0$) in a Soxhlet apparatus for 56 hr. The extract was concentrated under reduced pressure and then subjected to column chromatography on silica gel (60-120 mesh, 200 g); the petrol (60-80$^0$C): benzene (1:3) eluent afforded salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone) as yellow cubes (yield 0.65 g), m.p. 184-88$^0$C (from benzene). UV (ethanol): $\lambda_{max}$ 277, 330 nm; IR (KBr) n cm$^{-1}$: 3420, 3080, 3015,2920, 2845, 1650, 1600, 1590, 1570, 1360, 1265, 1120; $^1$H NMR ($CDCl_3$, 300MHz; TMS): d 7.84 (2H, d, *J*= 8.9 Hz, H-2' and H-6'), 7.01 (2H, d, *J*= 8.9 Hz, H-3' and H-5'), 6.58 (1H, s, $C_3$-H), 6.54 (1H, s, $C_8$-H), 4.005 (3H, s, $C_6$-OC$H_3$), 3.976 (3H, s, $C_7$-OC$H_3$) and 3.925 (3H,s,$C_{4'}$-OC$H_3$); EIMS (70 ev): m/ζ 328 ([M]$^+$, base peak), 327 [M-H]$^+$, 313[M-Me]$^+$, 312 [M-Me-H]$^+$, 300[M-CO]$^+$, 299[M-CO-H]$^+$, 196 and 132 (retro-Diels-Alder fragmented ion peaks of 2), 168 [196-CO]$^+$, 167[168-H]$^+$, 153[168-Me]$^+$, 135 (fragmented ion peak), 120[135-Me].

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