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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. © 2006 Trade Science Inc. - INDIA

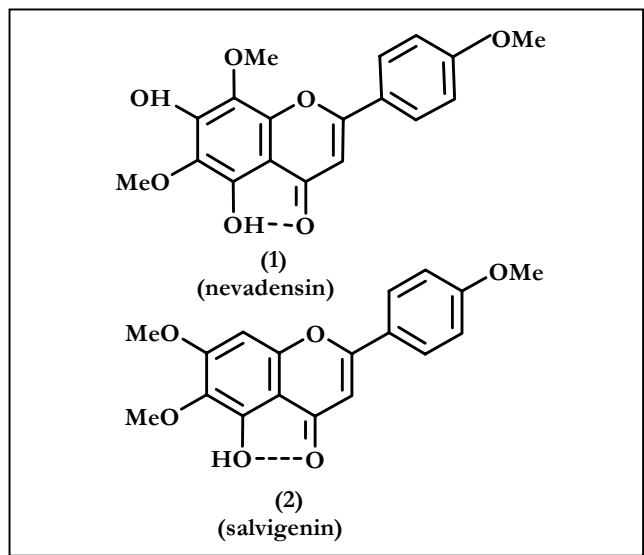
KEYWORDS

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 fla-

vonoids. 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.



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 1H 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 1H 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 1H 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 fragmenta-

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

C-atom	δ_C -value	HMQC
2	164.2	-
3	104.2	δ_{H-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'	124.0	-
2 ^{1,6} 1	127.8	$\delta_{H-2^{1,6}}$ 7.89
3 ^{1,5} 1	115.0	$\delta_{H-3^{1,5}}$ 7.045
4 ¹	163.1	-
8-OCH ₃	61.4	δ_H 4.02
6-OCH ₃	62.3	δ_H 4.04
4 ¹ -OCH ₃	56.0	δ_H 3.90

tion 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 $3/4$ 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 ($^1H - ^{13}C$) between C₂ and C₆' protons at d7.89 (2H, dd, $J= 2.7$ Hz, 11.7 Hz) with 2' and 6' carbons at d127.8; C₃ and C₅' protons at d7.045 (2H, dd, $J= 2.7$ Hz, 11.7 Hz) with 3' and 5' carbons at d115.0; C₃-proton at d 6.585 (1H,s) with C₃-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 (C₄'-OCH₃), 61.4 (C₈'-OCH₃) and 62.3 (C₆'-OCH₃).

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 *Limmophila 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 powdered whole plants (1.5 kg) of *L. heterophylla* were extracted with petrol (60-80^o) 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^oC): 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^oC (from ethanol); UV (ethanol): λ_{\max} 280, 335 nm; (+AlCl₃): 280, 310 (sh), 355 nm; IR (KBr) ν cm⁻¹: 3407, 3100, 2936, 2840, 1663, 1591, 1508, 1060, 1025; ¹H NMR (CDCl₃, 300MHz; TMS): δ 12.78 (1H, s, C₅-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, C₃-H), 4.04 (3H, s, C₆-OCH₃), 4.02 (3H, s, C₈-OCH₃) and 3.90 (3H, s, C₄-OCH₃); 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]⁺; ¹³C NMR (CDCl₃, 75 MHz) and HMQC in TABLE 1.

Salvigenin (2): Air-dried defatted powdered whole plants (1.5 kg) of *L. rugosa* were extracted with petrol (60-80^o) 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^oC): benzene (1:3) eluent afforded salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone) as yellow cubes (yield 0.65 g), m.p. 184-88^oC (from benzene). UV (ethanol): λ_{\max} 277, 330 nm; IR (KBr) ν cm⁻¹: 3420, 3080, 3015, 2920, 2845, 1650, 1600, 1590, 1570, 1360, 1265, 1120; ¹H NMR (CDCl₃, 300MHz; TMS): δ 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, C₃-H), 6.54 (1H, s, C₈-H), 4.005 (3H, s, C₆-OCH₃), 3.976 (3H, s, C₇-OCH₃) and 3.925 (3H, s, C₄-OCH₃); EIMS (70 ev): m/z 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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