NEW FLAVONOID FROM MENTHA LONGIFOLIA

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Abstract- 5,8,4’-Trihydroxy-6,7,3’-trimethoxyflavone (1) was isolated from Mentha longifolia, along with three known compounds (2-4). The structure of 1 was established through the extensive spectroscopic studies including 2D-NMR.

Mentha longifolia belongs to the family Labiatae. It is a perennial herb having white creeping rhizomes with a strong aromatic odor. It is commonly known as horsemint, which is considered carminative, antiseptic and stimulant.¹ Some bioactive aroma containing constituents have previously been reported from M. longifolia.² Herein we report the isolation and structure elucidation of a new flavonoid (1) along with known flavonoids (2-4).³⁻⁵ The structure of 1 is established through NMR.

Compound (1) was obtained as a yellow crystalline powder. It gave positive tests for flavone and showed UV maxima (MeOH) at 339, 315 and 285 nm. The molecular formula C₁₈H₁₆O₈ was established on the basis of molecular ion peak in HRFAB-MS at m/z 359.0762 [M⁺⁺H] calcd. 359.0767 for C₁₈H₁₅O₈. The IR spectrum exhibited absorption bands at 3200-3500, 2845, 1667, 1585, 1375 cm⁻¹ that indicated the presence of chelated hydroxyl, conjugated carbonyl and aromatic functionalities, respectively. In the ¹H-NMR spectrum, the phenyl ring protons appeared at δ 6.91 (d, J = 8.4 Hz), 7.33 (d, J = 2.1 Hz) and 7.42 (dd, J = 8.4, 2.1 Hz) indicating the presence of ortho, meta and ortho-meta coupled protons. The chemical shifts and the coupling constants of protons indicated a 3’, 4’ disubstituted pattern for ring B. A singlet at δ 6.50 in ¹H-NMR spectrum and a peak at δ 102.4 in ¹³C-NMR spectrum were characteristic for H-3 and C-3 of flavones. The singlets at δ 3.97, 3.86 and 3.85 (3H, each) showed the presence of three methoxyl groups. The
same coupling constants for H-5` and H-6` indicated that both were ortho to each other and confirmed by the presence of cross-peak between the signals at δ 6.91 (H-5`) and 7.42 (H-6`) in the 1H-1H COSY experiments. The substitution pattern of fragment “B” was further confirmed by comparing its 13C-NMR data with thymonin.6

The position of the substituents at ring A was determined by MS spectral data. The EIMS of 1 exhibited a molecular ion peak at m/z 360, in accordance with a flavone containing three hydroxyls and three methoxyls. The retro-Diels-Alder (RDA) fragments at m/z 212 (A1+) and at m/z 148 (B1+) as well as peaks at m/z 133 [B1+-Me] and m/z 151 [B2] confirmed the presence of a hydroxyl and a methoxyl group in the ring B and two hydroxyls and two methoxyls at ring A. The most intense peak in the MS spectrum appeared at m/z 345 for [M-Me]+ as a base peak providing strong evidence for location of methoxyl group either at C-6 or C-8 at the ring A.7 The intensity of the [M-H]+ ion was in accordance with the more ready loss of H from the 8-position than from the 6-position of the 5-hydroxyflavones. This is because of the greater stability of p-quinonoid over o-quinonoid forms. The relative abundances were [M]+ (95.97 %), [M-H]+ (37.53 %), [M-Me]+ (100 %), [M-H2O-Me]+ (26.6 %) and [M-H2O]+ (6.9 %), respectively. The last fragment ion is characteristic of flavones with C-8 hydroxyl group.8,9 Thus the substitution pattern of ring A of 1 is same as that of Wessely-Moser flavone derivative obtained on acid treatment of thymonin.8 The 13C-NMR spectra (BB and DEPT) revealed the presence of four methine, three methyl and 11 quaternary carbons in the molecule. The methines at δ 7.42, 7.33 and 6.91 were assigned to the carbons at δ 120.3 (C-6’), 108.9 (C-2’) and 115.3 (C-5’), respectively, with the help of HMQC experiments (Table). The 3J interactions of H-2’, H-6` with C-2 and H-3 with C-1` in the HMBC experiment (Figure 1) revealed that ring “B” was connected to C-2. Therefore, the compound (1) was elucidated the structure 5,8,4’-trihydroxy-6,7,3’-trimethoxyflavone (1).
EXPERIMENTAL

General Experimental Procedure- Column chromatography and medium pressure liquid chromatography were done by using silica gel 70-230 and 230-400 mesh, respectively. The UV spectra were obtained using a Hitachi-UV-3200 spectrophotometer whereas the IR spectra were measured on JASCO-320A spectrophotometer. Electron impact MS spectra were measured on Finnigan MAT-311A spectrometer, with a direct inlet (70 eV, ion source temp. 250°C and probe temp. 280°C). The HR-FABMS was performed on JMS-DA-500 mass spectrometer. 1D and 2D-NMR spectra were recorded on Brucker AMX-400 and 500 MHz spectrometers, respectively.

*Mentha longifolia*, collected from Qamber Swat (Pakistan) in August 1999, was identified by Mr. Habib Ahmed, Plant Taxonomist, Jehanzeb Post Graduate College Swat, where a voucher specimen is deposited in the herbarium.

**Extraction and Isolation**

The air dried ground plant material (20 kg) of *M. longifolia* was extracted with EtOH (80 L) at rt for 10 days. The ethanolic extract (550 g) was suspended in water and extracted with n-hexane and chloroform. The CHCl₃ fraction (30 g) was subjected to medium pressure liquid chromatography with hexane/CHCl₃ and CHCl₃/MeOH gradient systems. The fractions obtained with
chloroform/hexane (95:5) showed three major and two minor spots, and subjected to column chromatography, eluting initially with n-hexane and then with n-hexane/acetone in order of increasing polarity. The fraction eluted with hexane/Me$_2$CO 3:2 was the binary mixture of two UV active compounds. It was purified by preparative thin layer chromatography using hexane/CHCl$_3$/Me$_2$CO (6:3:1) to provide 3 (30 mg) and 4 (25 mg), respectively. The fractions eluted with CHCl$_3$/MeOH (94:6) showed two major spots, and were rechromatographed over silica gel using CHCl$_3$ and increasing the polarity with methanol. The top fraction obtained from CHCl$_3$/MeOH 95:5 provided the new compound 5,8,4'-trihydroxy-6,7,3'-trimethoxyflavone (1) (45 mg). The tail fractions provided a mixture, from which 2 (35 mg) could be obtained by PTLC using EtOAc /MeOH/H$_2$O (90:5:5).

5, 8, 4'-Trihydroxy-6, 5, 3'-trimethoxy flavone (1)
Yellow crystalline powder; mp 228-231°C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 285 (log $\varepsilon$ 3.81), 315 (log $\varepsilon$ 3.72), 339 (log $\varepsilon$ 3.85); IR $\nu_{\text{max}}^{(\text{KBr})}$ cm$^{-1}$: 3500-3200, 2845, 1667, 1585, and 1375. HR-FAB-MS (Neg.) $m/z$ 359.0762 (calcd for C$_{18}$H$_{15}$O$_8$, 359.0767); EIMS $m/z$: (rel. int. %): M$^+$ 360 (95.97), 359 [M-H]$^+$ (37.5), 345 [M-Me]$^+$ (100.0), 342 [M-Me-H$_2$O]$^+$ (6.9 %), 326.9 [M-Me-H$_2$O-Me]$^+$ 317 (2.9), 197 [A$_1$-Me]$^+$ (76.03), 169 [A$_1$-MeCO]$^+$ (27.0), 151 [B$_2$]$^+$ (22.26), 148 [B$_1$]$^+$ (10.86); $^1$H and $^{13}$C-NMR see Table.

<table>
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* Multiplicity determined by DETP experiments
REFERENCES


