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NEW FLAVONOID FROM *MENTHA LONGIFOLIA*

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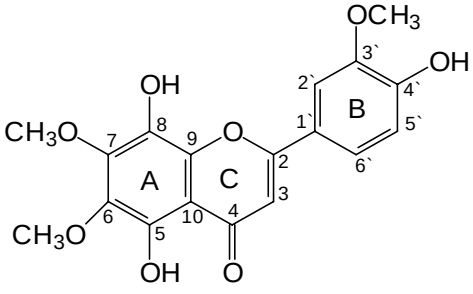
Abstract- 5,8,4`-Trihydroxy-6,7,3`-trimethoxyflavone (**1**) was isolated from *Mentha longifolia*, along with three known compounds(**2-4).** The structure of **1** was established through the extensive spectroscopic studies including 2D-NMR.

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 $C_{18}H_{16}O_8$ was established on the basis of molecular ion peak in HRFAB-MS at m/z 359.0762 [M^+–H] calcd. 359.0767 for $C_{18}H_{15}O_8$. 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 ^{1}H-NMR spectrum, the phenyl ring protons appeared at δ 6.9 1(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 ^{1}H-NMR spectrum and a peak at δ 102.4 in ^{13}C-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 ^{1}H-^{1}H COSY experiments. The substitution pattern of fragment "B" was further confirmed by comparing its ^{13}C-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 (A_1^+) and at *m/z* 148 (B_1^+) as well as peaks at *m/z* 133 [B_1^+-Me] and *m/z* 151 $[B_2]^+$ 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 %), $[M-H_2O-Me]^+$ (26.6 %) and $[M-H_2O]^+$ (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 ^{13}C-NMR spectra (BB and DEPT) reveled 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 $^{3}J$ 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**).



(**1**)

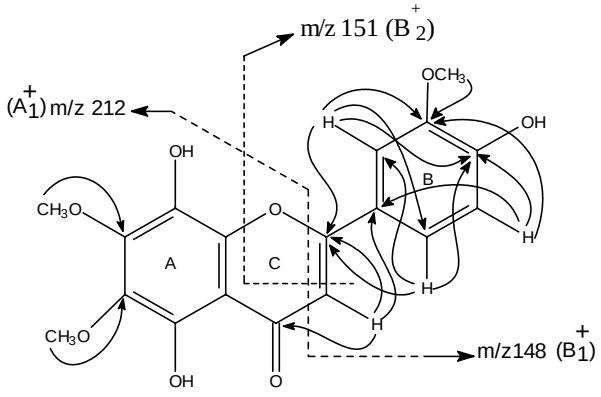
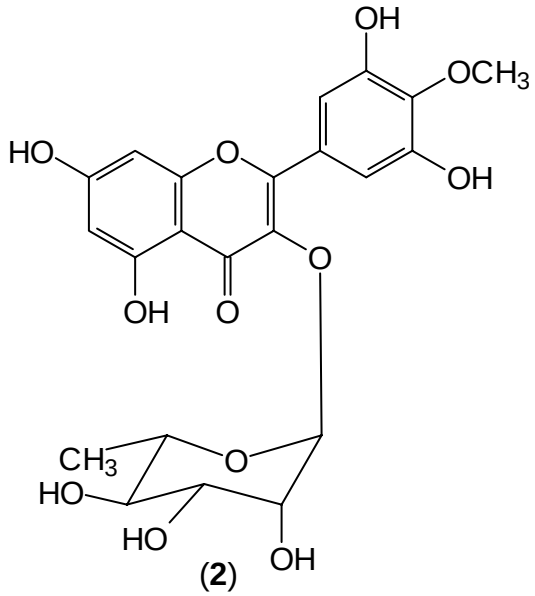
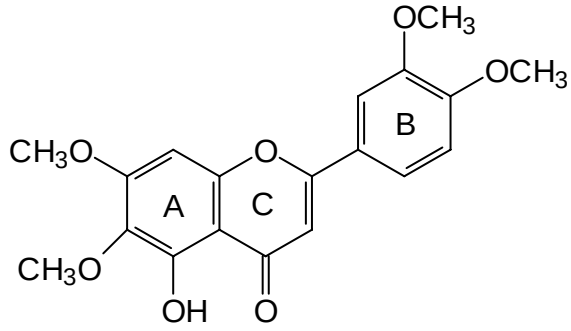


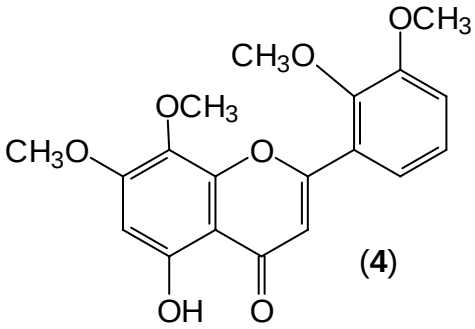
Figure 1. Important HMBC Correlations and Mass fragmentation in1.



(**2**)



(**3**)



(**4**)

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^{o}C and probe temp. 280^{o}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 $CHCl_3$ fraction (30 g) was subjected to medium pressure liquid chromatography with hexane/$CHCl_3$ and $CHCl_3$/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_2CO 3:2 was the binary mixture of two UV active compounds. It was purified by preparative thin layer chromatography using hexane/ $CHCl_3$/Me_2CO (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'-trimethoxyflovone (**1**) (45 mg). The tail fractions provided a mixture, from which **2** (35 mg) could be obtained by PTLC using EtOAc /MeOH/H_2O (90:5:5).

5, 8, 4`-Trihydroxy-6, 5, 3`-trimethoxy flavone (1)

Yellow crystalline powder; mp 228-231^oC, UV λ_{max}^{MeOH} nm: 285 (log ε 3.81), 315 (log ε 3.72), 339 (log ε 3.85); IR $\nu_{max}^{(KBr)}$ 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 $[M-H_2O]^+$ (6.9 %), 326.9 $[M-Me-H_2O]^+$ 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); ^1H and ^{13}C-NMR see Table.

Table ^1H and ^{13}C-NMR assignments of **1** in $CDCl_3$-CD_3OD.

Carbon	**(δ) ^{13}C (m*)**	**δ ^1H (*J* = Hz)**
2	164.5 (C)	
3	102.4 (CH)	6.50
4	182.9 (C)	
5	150.3 (C)	
6	133.4 (C)	
7	147.4 (C)	
8	132.8 (C)	
9	142.4 (C)	
10	106.2 (C)	
1`	122.2 (C)	
2`	108.9 (CH)	7.33 d (2.1)
3`	147.7 (C)	
4`	150.3 (C)	
5`	115.3 (CH)	6.91 d (8.4)
6`	120.3 (CH)	7.42 dd (8.4, 2.1)
3`-OCH_3	55.5(CH_3)	3.97
6-OCH_3	60.8(CH_3)	3.86
7-OCH_3	61.6(CH_3)	3.85

* Multiplicity determined by DETP experiments

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