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A New Aromatic Glycoside from *Morina longifolia* Wall

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A new aromatic glycoside characterized as 2,6-dihydroxy-5-methoxy-(3-C-glucopyranosyl) benzoic acid (**1**), was isolated along with four known compounds from aerial parts of *Morina longifolia* Wall. Its structure was determined on the basis of chemical and spectroscopic methods.

Key Words: *Morina longifolia* Wall, *Dipsacaceae*, Aromatic glycoside.

INTRODUCTION

Morina longifolia Wall (*Dipsacaceae*) is a tall spinous herb, 60-120 cm high found in temperate and alpine regions of Himalayas from Kashmir to Bhutan at an altitude of 2400-4200 m is commonly known as "Whorl flower". Its stem, leaves and flowers are used in Tibetan medicine. They are said to have a sweet and astringent taste with a heating potency. They are digestive, emetic and stomachic and are used in the treatment of stomach disorders[1]. The plant possesses strong aromatic properties, used as incense and in the preparation of dhup, agarbatties, *etc*[2]. Phytochemical studies have demonstrated that *M. longifolia* Wall contain morinoursolic acids A and B, *n*-triacont-3-one, 8-methylditriacont-7-ol and β-sitosterol[3]. We report herein the isolation and characterization of a new aromatic C-glycoside along with known compounds β-sitosterol, *p*-hydroxybenzoic acid, E-caffeic acid and oleonolic acid from aerial parts of *M. longifolia* Wall.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500 MHz and 125 MHz in C_5D_5N at 30 ºC with TMS as standard. Two-dimensional spectra were measured using standard methods of Bruker. The accuracy of the ^{1}H and ^{13}C chemical shifts were 0.01 ppm; ^{1}H/^{1}H spin-spin coupling constants 0.2 Hz. IR spectra were recorded on a Shimadzu FTIR 8400S in KBr pellets. Column chromatography (CC) was carried out on silica gel (Kieselgel 60-120 and 70-230 mesh, Merck). Sugars were chromatographed on plates impregnated with 0.3 M solution of NaH_2PO_4. The phenolic hydroxy groups and glycosides were detected by alcoholic ferric chloride and Molisch test, respectively.

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The aerial parts of *M. longifolia* Wall were collected from Tungnath (at an altitude of 3500-3600 m) District Chamoli, Uttarakhand, India, in August 2006 and was identified by Sumer Chand, Deptartment of Systematic Botany, Forest Research Institute, Dehradun, India. A voucher specimen (SLT-Med. Plant.-521) was deposited in the SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur, India.

Extraction and isolation: The air-dried and powdered aerial parts of *M. longifolia* Wall (3.0 kg) were washed with light petroleum ether (60-80 ºC). The petroleum free mass was extracted with 70 % ethanol. The crude ethanolic extract (21 g) was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O (6:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give chloroform fraction (5.50 g). The $CHCl_3$ fraction (4.5 g) was subjected to column chromatography (CC) over Si-gel-G using gradient elution with C_6H_6-EtOAc (10:0→9:1) afforded compound **2** (105 mg), compound 3 (250 mg) and various fractions having mixture of compounds. The C_6H_6-EtOAc (9:1) fraction (3.0 g) was subjected to column chromatography over Si-gel using gradient elution with C_6H_6-EtOAc (98:2→9:1) afforded compound **4** (167 mg). The aqueous layer was extracted with *n*-butanol (saturated with water). The butanol layer was evaporated and concentrated under reduced pressure to give butanol fraction (8.4 g). The butanol fraction was further digested with methanol: water (8:2) and filtered. The filtrate was evaporated to dryness under reduced pressure to give methanol extract (5.20 g). The methanol extract (6.0 g) was column chromatographed over Si-gel successively eluted with $CHCl_3$ and $CHCl_3$-MeOH (2:1) afforded compound **5** (105 mg) and fraction **A**. Fraction **A** on column chromatography over Si-gel eluted with $CHCl_3$-MeOH (4:1) afforded compound **1** (80 mg).

2,6-Dihydroxy-5-methoxy-(3-C-glucopyranosyl)benzoic acid (1): Crystalline solid m.p., 140 ºC (MeOH); IR (KBr, ν_{max}, cm^{-1}): 3390, 2815, 2723, 1700, 1612, 1530, 1462 1345, 1230, 1130, 990, 960, 760, 715; FAB MS m/z: 347 $[M]^+$, 219, 184, 149, 121, 109, 93; (elemental anal. C = 48.55 %, H = 5.20 %; calcd for $C_{14}H_{18}O_{10}$); ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectra are given in Table-1.

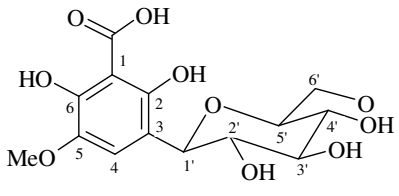
RESULTS AND DISCUSSION

The ethanol extract of aerial parts of *M. longifolia* Wall on repeated column chromatography over Si-gel successively eluted with $CHCl_3$ and $CHCl_3$-MeOH (4:1) afforded compound **1**. It was obtained from methanol as crystalline solid. Its elemental analysis corresponded to molecular formula $C_{14}H_{18}O_{10}$, which was substantiated by presence of molecular ion peak $[M]^+$ at m/z 346 in EI-mass spectrum. The IR spectrum displayed a broad absorption band at 3390 cm^{-1} for OH group and at 1700 cm^{-1} for -C=O group, indicating the presence of carboxylic group in the molecule. IR spectrum also displayed characteristic absorption bands for aromatic ring at 1612 and 1530 cm^{-1} and for alkyl group at 2815 and 2723 cm^{-1}.

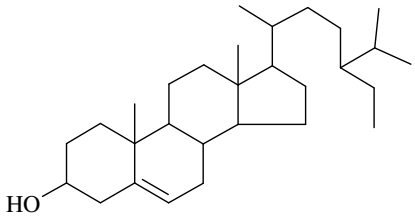
TABLE-1
NMR SPECTRAL DATA OF COMPOUND **1**

C/H	$^{\delta}$C*	$^{\delta}$H	HMBC correlation
1	119.55^{s}	–	
2	149.40^{s}	–	–
3	116.68^{s}	–	–
4	111.49^{d}	7.75, 1H, s	3, 5, 2, 6, 1’
5	141.88^{s}	–	–
6	152.77^{s}		
1’	73.92^{d}	5.24, 1H, d (*J* = 8.0 Hz)	2’, 3’, 5’, 1, 3
2’	81.34^{d}	4.62, 1H, dd (*J* = 8.0, 10.2 Hz)	1’, 3’, 3
3’	75.57^{d}	4.43, 1H, t (*J* = 10.2 Hz)	2’, 4’
4’	72.15^{d}	4.15, 1H, dd (*J* = 10.2, 8.4 Hz)	3’, 5’, 6’
5’	83.53^{d}	4.24, 1H, m	4’, 6’
6’a	62.63^{t}	4.67, 1H, dd (*J* = 10.2, 2.3 Hz)	4’
6’b		4.20, 1H, dd (*J* = 10.2, 7.3 Hz)	5’
-C=O	164.41^{s}	–	–
-OMe	60.28^{q}	3.93	5
-OH		9.82	–
-OH		12.03	–

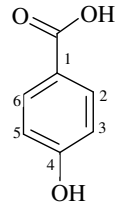
*Multiplicity of carbon signals were determined by DEPT spectrum.



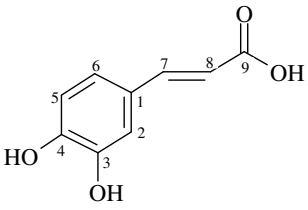
Structure of compound **1**



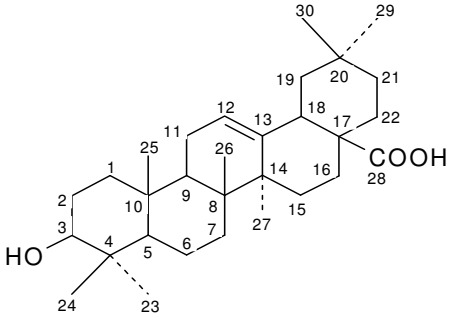
Structure of compound **2**



Structure of compound **3**



Structure of compound **4**



Structure of compound **5**

The ^{1}H NMR spectrum displayed a sharp singlet for one proton in the aromatic region at δ 7.75, two broad singlet at δ 9.82 and 12.03 and a singlet integrating for three protons in the aliphatic region at δ 3.93. In addititon to these the ^{1}H NMR spectrum also displayed a doublet integrated for one proton at δ 5.24 assigned for anomeric proton of hexose sugar alongwith other signals assignable to sugar protons. The ^{13}C NMR spectrum showed presence of 14 carbon atoms while the DEPT spectrum displayed presence of 6 methine, one methylene, 1 methyl and 6 quaternary carbon atoms. The assignment of protons and carbon atoms were made by ^{1}H-^{1}H COSY, HMQC and HMBC spectral data (Table-1).

The integrated singlet for one proton at δ 7.75 assigned for H-4, indicated the presence of penta substituted aromatic ring in the molecule. The presence of two phenolic groups in the molecule was attributed by the presence of two broad singlets at δ 9.82 and 12.03 ppm. The ^{13}C-chemical shifts of three carbon atoms [δ 149.40 (C-2), 141.88 (C-5) and 152.77 (C-6)] which appeared between δ 140-150 ppm indicated that the molecule contains an aromatic oxygen function at 2, 5 and 6-positions[4]. If the hydroxy groups are present at 2,4,6-position then the chemical shift of the oxygen carrying carbon should appear downfield than 150 ppm[4,5]. The presence of carboxylic group was confirmed by ^{13}C-chemical shift of carbonyl carbon at δ 164.41. The presence of a methoxyl group displayed by ^{1}H NMR spectrum at δ 3.93 was confirmed by its ^{13}C-chemical shift at δ 60.28. The downfield chemical shifts of C-2 and C-6 carbons at δ 149.40 and 152.77, as compared with C-5 at 141.88 suggested that the OH groups are attached to these carbon atoms. Thus the position of methoxy group was determined by the upfield ^{13}C-chemical shift of C-5 carbon at δ 141.88. The C-5 position of methoxy group was confirmed by the HMBC correlation of methoxyl protons (δ 3.93) with C-5 carbon.

The ^{1}H NMR spectrum displayed a doublet (J = 8.0 Hz) at δ 5.24 was assigned for anomeric protons of a sugar. The compound was found to be resistant to acid hydrolysis, which is the characteristic of the C-glycoside[6]. The C-glycosidic linkage of the sugar was further confirmed by ^{13}C-chemical shifts of anomeric carbon, which appeared upfield at δ 73.92 ppm[6]. The sugar was identified as β-D-glucose by comparison of its ^{1}H and ^{13}C-data.

The linkages between the structural units identified by above discussed spectral data were determined by the HMBC experiment (Table-1). The methoxyl protons resonating at δ 3.93 showed strong correlation with the C-5 carbon resonating at δ 141.88 in HMBC spectrum confirmed its location at C-5. The aromatic singlet at δ 7.75 showed $^{2}J_{CH}$ correlation with C-5 and C-3, $^{3}J_{CH}$ correlation with the C-2 and C-6 carbon at δ 149.40 and 152.77, respectively, confirmed its location at C-4 position. The position of glucose was determined at C-3 by HMBC spectrum. The anomeric protons resonating at δ 5.24 showed correlation with C-2, C-3 and C-5 carbons of aromatic ring. Furthermore, the aromatic singlet also displayed HMBC correlation with the anomeric carbon at 73.92, which confirmed the C-3 position as well the C-glycosidic nature of the sugar moiety. The other long-range correlations between the protons and carbons of sugar moiety are given in Table-1.

The above discussed spectral evidences led to the identification of a new compound characterized as 2,6-dihydroxy-5-methoxy-(3-C-glucopyranosyl) benzoic acid (**1**). Compound **2** was identified as β-sitosterol by mixed melting point and Co-IR with authentic sample. Compounds **3-5** were identified as *p*-hydroxybenzoic acid[7], E-caffeic acid[8] and oleonolic acid[9], respectively by comparison of their physical spectral data with the literature values.

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