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Isolation and structure elucidation of viscoazucine, a novel sesquiterpene from *Polygonum viscosum*

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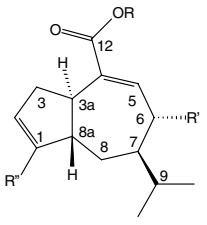
A new sesquiterpene, 1,4-dimethoxycarbonyl-7-(1-methylethyl)-3,3a,6,7,8,8a-hexahydroazulene (viscoazucine) (**1**), and a known flavone, 3′,5,7-trihydoxy-3,4′,5′-trimethoxyflavone (**4**), have been isolated from *Polygonum viscosum.* The structures of these compounds were elucidated by spectroscopic analyses, notably UV, MS and NMR.

1. Introduction

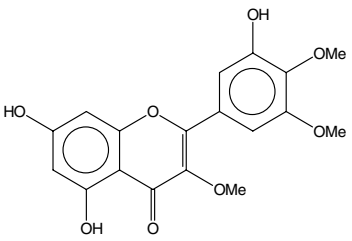
Polygonum viscosum Buch-Ham. Ex D. Don (Polygonaceae), commonly known as "Bishkatali", is a Nepalese annual herb, and widely distributed in Bangladesh, northeast India, China and Japan. Many species from *Polygonum* are pharmacologically active, and used in oriental traditional medicine [1]. The ethanolic extract of shoots of this species was found to have antibacterial activity [2]. Previously, we have reported several flavonoids and sesquiterpenes from *P. viscosum* [3–5]. This paper now reports the isolation and structure elucidation of a further new sesquiterpene, named viscoazucine (**1**) from this species. The complete ^{1}H and ^{13}C NMR spectral assignments for 3′,5,7-trihydroxy-3,4′,5′-trimethoxyflavone (**4**), a known flavone, are also reported here for the first time.

2. Investigations, results and discussion

A combination of vacuum liquid chromatography (VLC), column chromatography (CC) and preparative thin layer chromatography (PTLC) of the *n*-hexane extract of *P. viscosum* resulted in the isolation of the new sesquiterpene, viscoazucine (**1**). Similar treatment of the EtOAc extract produced the known flavone, 3′,5,7-trihydroxy-3,4′,5′-trimethoxyflavone (**4**). HRFABMS of **1** showed a protonated molecular ion peak at m/z 293.1747, which established its molecular formula as $C_{17}H_{24}O_4$. The UV and IR spectra suggested the presence of α, β-unsaturated carbonyl functionalities. The ^{1}H NMR spectrum of **1** showed signals for an isopropyl moiety, two carboxymethyl groups, five methines, including two olefinics (δ 6.98 and 7.04), and three methylenes groups. The deshielded nature of the olefinic methine signals indicated their β positions to the carbonyl groups. The ^{13}C NMR spectrum displayed 17 carbon resonances, while data from the HMQC experiment confirmed that 13 out of the 17 carbons had attached protons. The DEPT experiments revealed the presence of four methyls, three methylenes, six methines, and four quaternary carbons. The ^{1}H and ^{13}C NMR spectral data of **1** revealed close similarities with the spectra recorded for viscozulenic acid (**2**), previously isolated from this plant [5]. However, resonances appropriate for the oxymethine group in **2** were absent from the spectra of **1**, and were replaced by the resonances for a methylene group. The olefinic proton (H-5, δ 6.98) showed coupling with these methylene protons (δ 2.00 and 2.13), both of which had HSQC correlation with a methylene carbon at δ 25.3 (C-6). In addition, there were signals attributable to two carboxymethyl groups in **1**, as compared to one in **2**, which suggested that compound **1** is actually a methyl ester of 6-deoxyviscozulenic acid (**2**). It was possible to trace all of the proton-proton spin systems in **1** with data from a COSY-45 experiment. Heteronuclear HSQC and HMBC experiments allowed unambiguous assignment of all ^{1}H and ^{13}C NMR resonances in **1**. HMBC correlations (^{2}J) from H-5 and H-7 to δ 25.3 (C-6) confirmed the presence of a methylene group in-between C-5 and C-7. Similar HMBC correlations from H-2 to δ_C 167.8, and from the carboxymethyl signal at δ_H 3.71 to δ_C 167.3 (C-12) and 167.8 (C-14) confirmed the esterification sites in compound **1**. The relative stereochemistry of the chiral centres in **1** was determined by selective NOESY experiments (Fig.). The new sesquiterpene was thus identified as 1.4-dimethoxycarbonyl-7-(1-methylethyl)-3,3a,6,7,8,8a-hexahydroazulene and was given a trivial name, viscoazucine (**1**). While sesquiterpene acids containing a substituted azulene skeleton are not very common, a related compound sclerosprin (**3**) having sprogenic activity has been isolated from fungi, in particular *Sclerofinia fructicola* [6].



	R	R'	R"
1	Me	H	COOMe
2	Me	OH	COOH
3	H	H	Me



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The structure of 3′,5,7-trihydroxy-3,4′,5′-trimethoxyflavone (**4**) was independently determined by extensive NMR analyses. The HSQC and HMBC spectra recorded in acetone-d_6 allowed the first unambiguous assignment of its ^{13}C resonances. Although 3′,5,7-trihydroxy-3,4′,5′-tri-

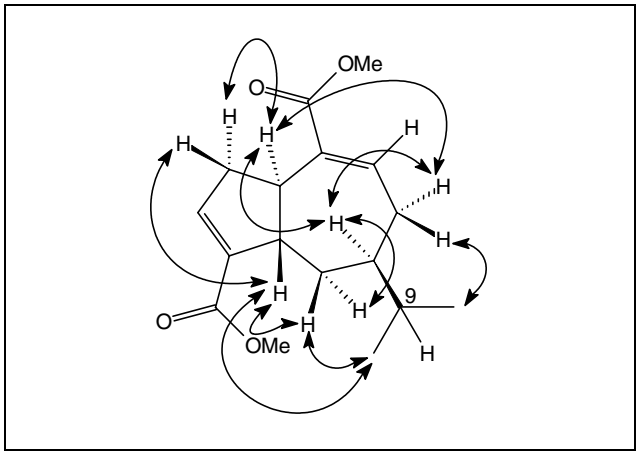

Fig.: Key NOESY interactions observed in **1**

methoxyflavone had previously been isolated from *Cistus monspeliensis* and *Haplopappus integerrimus* [7], this is the first report from a *Polygonum* species.

3. Experimental

3.1. General

Silicagel 60 H, Kieselgel 70–230 mesh and Silicagel 60 PF_{254} were used, respectively, for VLC, CC and PTLC. UV spectrum was taken in MeOH using Beckman DU-640 UV-Vis spectrometer. IR spectrum was obtained in liquid film using Perkin-Elmer 1600 FT-IR spectrometer. Optical rotation was measured in Perkin-Elmer 241 polarimeter. FABMS was taken in JEOL SX102 mass spectrometer. The ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian INOVA 500 spectrometer.

3.2. Plant material

Whole plants of *P. viscosum* were collected from Panchari, Chittagong, Bangladesh and identified by Prof. M. A. Hassan, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh, where a voucher specimen (no 764) has been deposited.

3.3. Extraction and isolation

Ground dried whole plants' parts (2.3 kg) were successively extracted with *n*-hexane and EtOAc. Evaporation of solvents using a rotary evaporator at 45 °C yielded 2.57 g of *n*-hexane and 9.31 g of EtOAc dried extracts. An aliquot (2.06 g) of the n-hexane extract was subjected to VLC eluting with petroleum ether-EtOAc mixtures of increasing polarity. A total of 30 fractions (50 ml each) were collected. Repeated PTLC (toluene : EtOAc : AcOH : 96 : 4 : few drops) of the VLC fraction (10% EtOAc in hexane) yielded **1** (18.5 mg). The EtOAc extract was fractionated by CC using a mixture of solvents, *n*-hexane and EtOAc of increasing polarity, and the fraction eluted with 65% EtOAc in hexane was further purified by PTLC (toluene : EtOAc : AcOH : 70 : 30 : few drops) to yield 3′,5,7-trihydroxy-3,4′,5′-trimethoxyflavone (**4**, 11.5 mg).

3.3.1. Viscoazucine (**1**)

Brownish gum, $[\alpha]_D + 6.73\,^\circ$ (c = 1.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 217 (4.10) nm; IR (film) ν_{max} 2921, 2846, 1717, 1560, 1469, 1432, 1388, 1367, 1255, 1191, 1106, 1082, 1045, 1004, 912, 749 cm^{-1}; HR-FABMS *m/z* $[M + H]^+$ 293.1747 calcd 293.1753 for $C_{17}H_{24}O_4$; ^1H and ^{13}C NMR (Table).

Table: ^1H- and ^{13}C NMR data of 1

	δ_H	δ_C
1	–	130.9
2	7.04 bd (4.0)	141.1
3α	2.26 m	24.8
3β	2.38 bd (18.0)	–
3a (α)	2.61 bd (12.0)	53.3
4	–	132.8
5	6.98 bs	139.7
6α	2.00 m	25.3
6β	2.13 dt (19.5, 5.0)	–
7α	1.57 m	40.3
8α	1.37 ddd (17.0, 12.0, 4.0)	25.0
8β	1.96 m	–
8a (β)	2.22 m	36.5
9	1.99 m	26.7
10	0.89 d (7.0)	21.1
11	0.91 d (7.0)	15.4
12	–	167.3
13-OMe	3.71 s	51.6
14	–	167.8
15-OMe	3.71 s	51.6

(500 and 125 MHz, $CDCl_3$, δ-values in ppm, J in Hz in parenthesis, TMS as internal standard)

3.3.2. 3′,5,7-Trihydroxy-3,4′,5′-trimethoxyflavone (**4**)

Yellow powder, HR-FABMS *m/z* $[M + H]^+$ 361.0913 (calcd 361.0923 for $C_{18}H_{17}O_8$); ^1H NMR (500 MHz, acetone-d_6): δ 12.71 (1 H, bs, 5-OH), 9.72 (1 H, bs, 7-OH), 8.25 (1 H, bs, 3;-OH), 7.34 (1 H, bs, H-6′), 7.31 (1 H, bs, H-2′), 6.54 (1 H, bs, H-8), 6.30 (1 H, bs, H-6), 3.93 (3 H, s, 5-OMe), 3.89 (3 H, s, 3-OMe), 3.87 (3 H, s, 4′-OMe); ^{13}C NMR (125 MHz. acetone-d_6): δ 179.0 (C-4), 165.0 (C-7), 163.2 (C-5), 157.8 (C-9), 156.1 (C-2), 153.9 (C-5), 151.3′ (C-3′), 140.0 (C-3), 139.7 (C-4′), 126.7 (C-1′), 110.1 (C-2′), 105.9 (C-10), 105.2 (C-6′), 99.2 (C-6), 94.5 (C-8), 60.6 (3-OMe), 60.2 (4′-OMe), 56.4 (5′-OMe).

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