

Flavonoid and Steroids from *Polygonum viscosum*

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Abstract

Repeated chromatographic separation and purification of the *n*-hexane-ethyl acetate (3:1) extract of aerial parts of *Polygonum viscosum* provided a flavonoid- ternatin (1) and two steroids- stigmasterol (2) and stigmasta-4,22-dien-3-one (3). The structure of these compounds were determined on the basis of extensive NMR and other spectroscopic techniques and comparison with published data. The identity of steroids 2 and 3 was confirmed by co-TLC with authentic sample.

Key words: *Polygonum viscosum*, Polygonaceae, NMR

Introduction

Polygonum viscosum Buch-Ham ex D. Don. (Polygonaceae), Bengali name- Biskatali, is an annual herb which is indigenous to Nepal and also widely distributed in Bangladesh, northeast India, Japan and China (Datta *et al.*, 2004). Previous phytochemical studies of different species of *Polygonum* revealed a variety of secondary metabolites like hydrocarbons, sterols, acetophenones, coumarins, terpenoids and flavonoids (DNP on CD ROM 9.1, 2000; Ezhilan and Neelamegam, 2012; Das and Ganapaty, 2015).

The ethanol extract of whole plant of *P. viscosum* led to the isolation of several flavonoids and terpenoids (Datta *et al.*, 2000, 2001, 2002), some of which showed significant biological activities (Datta *et al.*, 2004). As part of our continuing studies with *Polygonum* species growing in Bangladesh, we, here in, report the isolation and characterization a flavonoid and two steroid derivatives for the first time.

Materials and Methods

Plant materials: The aerial parts of *Polygonum viscosum* Buch-Ham ex D. Don. were collected from Panchori, Chittagong Hill Tracts, Bangladesh in the month of January, 2014 and authenticated by Professor Md. Abul Hassan, Department of Botany, University of

Dhaka, where a voucher specimen has been maintained under the accession number DUSH-764.

Extraction and fractionation: The air dried and coarse powder (800 gm) was extracted with combination of *n*-hexane and ethyl acetate (3:1, 5.0 L) in a clean flat-bottomed and air tight container for 15 days at room temperature with occasional shaking. The extract was then filtered through a fresh cotton plug followed by Whatman No. 1 filter paper. The filtrate was concentrated with a rotary evaporator at 40-45°C and reduced pressure. The crude extract obtained (30 gm) and stored in refrigerator until used.

General experimental procedures: UV (methanol) and IR (KBr) spectra were obtained on Beckman DU 640 and Perkin Elmer FTIR spectrometer, respectively. ¹H NMR (400 MHz either C₅D₅N or CDCl₃) and ¹³C NMR (100 MHz, C₅D₅N) were acquired on Varian Inova 400 instrument and HR-EIMS have been recorded with a VG Micromass 2AB mass spectrometer. Vacuum Liquid Chromatography (VLC) column was run on Kieselgel 60H (100-200 mesh) and analytical and preparative thin layer chromatography (TLC and PTLC) were performed on glass plates coated with silica gel (Kieselgel 60 PF₂₅₄). Solvent systems ranging from non-polar to polar were used. UV light (254 nm and 365 nm) and vanillin/H₂SO₄ reagents (1:100) (Stahl, 1966) were used to visualize the spots as

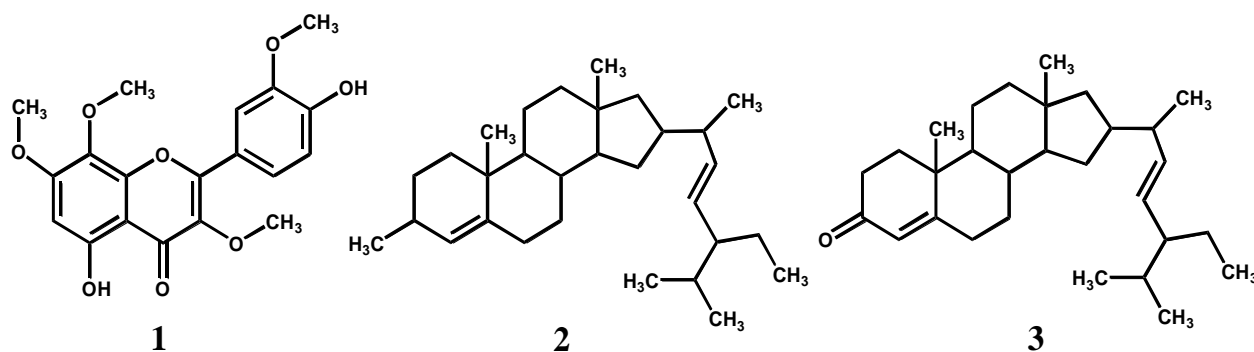
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appropriate. Other reagents and chemicals were of analytical grade.

Properties of isolated compounds

Properties of ternatin (1): Yellow crystals, m.p. 217 °C; Found M^+ 374.1001, $C_{19}H_{18}O_8$, requires 374.1002; UV λ_{max} (MeOH) nm: 254, 272, 336, 368; IR ν_{max} (KBr) cm^{-1} : 3350, 2940, 1650, 1595, 1460, 1360, 1265, 1160, 1045, 920, 820; 1H NMR (400MHz, C_5D_5N): 13.01 (1H, s, OH-5), 8.07 (1H, dd, $J = 8.1, 1.7$ Hz, H-6'), 8.01 (1H, d, $J = 1.7$ Hz, H-2'), δ 7.37 (1H, d

$J = 8.1$ Hz, H-5), 6.71 (1H, s, H-6), 4.04 (3H, s, OMe-3), 4.03 (3H, s, OMe-8), 3.91 (3H, s, OMe-3'), 3.85 (3H, s, OMe-7); ^{13}C NMR (100 MHz, C_5D_5N): 156.9 (C-2), 139.2 (C-3), 179.8 (C-4), 158.3 (C-5), 96.6 (C-6), 159.4 (C-7), 129.8 (C-8), 149.3 (C-9), 106.2 (C-10), 122.4 (C-1'), 112.9 (C-2'), 149.1 (C-3'), 152.2 (C-4'), 117.4 (C-5'), 123.9 (C-6'), 60.4 (OMe-3), 56.9 (OMe-7), 61.9 (OMe-8), 56.3 (OMe-3'); EIMS, m/z (rel. int.): 374 [M] $^+$ (64), 373 (5), 361 (2), 360 (20), 359 (100), 331 (3), 153 (6), 95 (6).



Properties of stigmasterol (2): White needles, m.p. 126-128 °C; Found M^+ 412.7051, $C_{29}H_{48}O$, requires 412.7053; IR ν_{max} (KBr) cm^{-1} : 3450, 2940, 2820, 2450, 1640, 1450, 1360, 1040, 850; 1H NMR (400 MHz, $CDCl_3$): δ 5.37 (1H, m, H-6), 5.16 (1H, dd, $J = 15.2, 8.0$ Hz, H-22), 5.03 (1H, dd, $J = 15.2, 7.2$ Hz, H-23), 3.55 (1H, m, H-3), 1.03 (3H, s, Me-13), 0.90 (3H, d, $J = 6.8$ Hz, Me-20), 0.83 (3H, d, $J = 6$ Hz, Me-25), 0.81 (3H, t, $J = 6.5$ Hz, Me-28), 0.69 (3H, s, Me-10); EIMS, m/z (rel. int.): 412 (19), 271 (14), 213 (29), 173 (14), 133 (30), 91 (46), 55 (73), 43 (100).

Properties of stigmasta-4,22-dien-3-one (3): White gum, FABMS m/z : Found 411.6971 [$M+H$] $^+$, $C_{29}H_{46}O$, requires 411.6974 ($C_{29}H_{46}O + H$); IR ν_{max} (Film) cm^{-1} : 2928, 2852, 1680, 1672, 1649, 1450; 1H NMR (400 MHz, $CDCl_3$): δ 5.73 (1H, s, H-4), 5.16 (1H, dd, $J = 15.2, 8.0$ Hz, H-22), 5.05 (1H, dd, $J = 15.2, 7.2$ Hz, H-23), 1.19 (3H, s, Me-10), 0.93 (3H, d, $J = 6.8$ Hz, Me-20), 0.86 (3H, d, $J = 6$ Hz, Me-25), 0.82 (3H, t, $J = 6.5$ Hz, Me-28), 0.73 (3H, s, Me-13).

Results and Discussion

The HR-EMIS of the yellow crystalline compound (1) gave a molecular ion peak at m/z 374 appropriate for the molecular formula, $C_{19}H_{18}O_8$. The 1H NMR spectrum showed an aromatic A-ring proton signal for H-6 at δ 6.71, a C-5 hydroxyl at 13.17, four methoxyl resonances between 3.85-4.04 and three B-ring aromatic proton signals typical for a 3',4'-dioxxygenated flavonols (Butterham and Hight, 1964). The *meta* coupled (H-2') and *ortho-meta* coupled (H-6') appeared at δ 8.01-8.07 while the *ortho* coupled H-5' signal was observed at δ 7.37. The ^{13}C spectrum showed 19 carbons including four methoxyls, four methines, one carbonyl and the 10 quaternary carbon signals which further supported the molecular formula.

The correlations between protons with carbons were revealed by HC-COBI and HMBC experiments (Table 1). Among four methoxyls, the deshielded one at δ 4.04 was attached to C-3 as its connectivity was observed to the oxygenated quaternary at δ 139.2, which is the typical resonance for C-3 in 3-methoxy flavonols. The remaining three methoxyls at δ 3.85,

4.03 and 3.91, demonstrated connectivity over three bonds to the oxygenated quaternary carbons at δ 159.4, 129.8 and 149.1, respectively. These carbons were also identified as C-7, C-8 and C-3' by the 2J or 3J correlations from H-6 and H-5, respectively. On the basis of these data, compound **1** was identified as 5,4'-dihydroxy-3,7,8,3'-tetramethoxyflavonol (ternatin), a flavonoid previously isolated from *Begonia glabra* (Ensemeyer and Langhammer, 1982)

Compound **2** was obtained as white needle shaped crystals and the HR-EMIS showed the molecular ion peak at m/z 412 solving the molecular formula as

$C_{29}H_{48}O$. The 1H NMR spectrum revealed a multiplet of one proton intensity at δ 3.55, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The typical signal for H-6 of the steroidal skeleton was evident from a multiplet at δ 5.37 that integrated for one proton. The olefinic proton at H-22 and H-23 appeared as characteristics downfield signals δ 5.16 and 5.03 respectively. The spectrum further revealed signals for six methyl groups. On this basis, compound **2** was characterized as stigmaterol (Ikan, 1991), which was further confirmed by co-TLC with authentic stigmaterol.

Table 1. 1H - ^{13}C correlations for ternatin (**1**) as determined by HC-COBI and HMBC experiments.

Protons	1H - ^{13}C correlation		
	1J	3J	2J
OMe	60.4 (OMe-3)	139.2 (C-3)	
OMe	56.9 (OMe-7)	159.4 (C-7)	
OMe	61.9 (OMe-8)	129.8 (C-8)	
OMe	56.3 (OMe-3')	149.1 (C-3')	
OH-5	-	106.2 (C-10), 96.6 (C-6)	158.3 (C-5)
H-6	96.6 (C-6)	129.8 (C-8), 106.2 (C-10)	159.4 (C-7), 158.3 (C-5)
H-2'	112.9 (C-2')	156.9 (C-2), 152.2 (C-4)	149.1 (C-3')
H-5'	117.4 (C-5')	149.1 (C-3'), 122.4 (C-1')	152.3 (C-4')
H-6'	123.9 (C-6')		

Compound **3** was obtained as white gum. The FABMS displayed the pseudo-molecular ion peak at m/z 411.6971 $[M+H]^+$ establishing its molecular formula as $C_{29}H_{46}O$. The 1H NMR spectrum of **3** revealed a downfield signal for olefinic proton at δ 5.73, typical for H-4 of a steroidal nucleus containing a ketone group at C-3 position. The side chain olefinic protons at H-22 and H-23 appeared as characteristic downfield resonances at δ 5.16 and 5.05, respectively. The spectrum also showed a total of six methyl group signals, including three doublets, a triplet and two singlets. Thus, compound **3** was characterized as stigmasta-4,22-dien-3-one (SDBS, 2000). Its identity was substantiated by co-TLC with authentic sample.

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