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A sesquiterpene acid and flavonoids from *Polygonum viscosum*

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Abstract

4-Isobutyl-6-methyl-5-oxo-3a,4,5,7a-tetrahydro-1*H*-inden-13-oic acid (named viscosumic acid) and quercetin 3-*O*-(6″-feruloyl)-β-D-galactopyranoside, and the known 3′,5-dihydroxy-3,4′,5′,7-tetramethoxyflavone have been isolated from *Polygonum viscosum*. The structures of these isolates were determined primarily on the basis of extensive 1D and 2D NMR spectral analyses, notably, ^{13}C PENDANT, COSY45, TOCSY, GOESY, NOESY, HMQC and HMBC.

Keywords: *Polygonum viscosum*; Polygonaceae; Viscosumic acid; Sesquiterpene; Flavonoid; Quercetin 3-*O*-(6″-feruloyl)-β-D-galactopyranoside; 3′,5-Dihydroxy-3,4′,5′,7-tetramethoxyflavone

1. Introduction

Polygonum viscosum Buch.-Ham. Ex D. Don (family: Polygonaceae), Bengali name — "Bishkatali", is an erect, annual Nepalese herb naturalised in Bangladesh, north-east India, China and Japan. A flavonoid glycoside was reported previously (Datta et al., 2000), and an ethanolic extract of young shoots of this species was found to possess antibacterial activity (Hoque et al., 1989). We now report on the isolation and characterisation of two novel secondary metabolites, a sesquiterpene acid and a flavonoid glycoside, and a known flavone from this plant.

2. Results and discussion

A combination of column chromatography (CC) and preparative thin layer chromatography (PTLC) of the EtOAc-extract of whole plants of *P. viscosum* yielded the known flavone, 3′,5-dihydroxy-3,4′,5′,7-tetramethoxyflavone (**1**), and the RP-HPLC analysis of the MeOH-extract provided two novel metabolites, quercetin 3-*O*-(6″-feruloyl)-β-D-galactopyranoside (**2**), and 4-isobutyl-6-methyl-5-oxo-3a,4,5,7a-tetrahydro-1*H*-inden-13-oic acid (named viscosumic acid, **3**). While compound **1** was readily identified by direct comparison of its spectroscopic data with literature data (Martos et al., 1997), comprehensive ^{13}C-NMR data (confirmed from HSQC and HMBC experiments) for this compound are presented for the first time. Structures of **2** and **3** were conclusively determined by extensive spectroscopic analysis.

The UV absorption maxima of **2** at 255, 270 sh, 300 sh and 358 nm were characteristic for quercetin derivatives with a galactosyl moiety at C-3 (Mabry et al., 1970). The HR–FABMS experiment revealed the [M

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+ H]$^+$ ion at m/z 641.1505 confirming the molecular formula $C_{31}H_{28}O_{15}$. The ^{1}H and ^{13}C PENDANT spectra (Table 1), together with ^{1}H–^{1}H COSY and ^{1}H–^{1}H TOCSY established the presence of a quercetin skeleton, a galactose unit, and a feruloyl moiety in the molecule. All the ^{1}H and ^{13}C signals were almost identical with those published for quercetin 3-*O*-(6″-caffeoyl)-β-D-galactopyranoside (**4**) (Datta et al., in press; Shigematsu et al., 1982) with the exception that, for **2**, there was an extra signal for a methoxy group (δ_H 3.94, δ_C 54.3). The attachment of this methoxy group at C-3‴ was confirmed from a ^3J correlation from the methoxy protons to C-3‴, observed in the HMBC spectrum (Table 1). This was further supported from the nOe interactions, 3‴- OMe ↔ H-2‴, obtained from GOESY experiments (Kessler et al., 1986; Stonehouse et al., 1994; Stott et al., 1995). Thus, instead of a caffeoyl moiety (as in **4**), a feruloyl moiety was present in **2**. A ^3J correlation from H-1″ to C-3 confirmed that the galactose unit was attached to C-3. Similarly, another ^3J correlation from H_2-6″ to C-9‴ established the attachment of the feruloyl moiety at C-6″. Thus, this flavonoid glycoside was identified as **2,** and to our knowledge, is a new discovery.

Compound **3** gave UV absorption peaks at 219 and 240 (sh) nm attributable to the α,β-unsaturated carbonyl chromophores. The HR–FABMS showed the [M + H]$^+$ ion at m/z 249.1487 solving for the molecular formula $C_{15}H_{20}O_3$. The ^{1}H-NMR spectrum (Table 2) showed signals for two deshielded olefinic methines (H-2 and H-7), isobutyl moiety (H-9, H_2-8, Me-10 and Me-11), three other methines (H-3a, H-4, H-7a), a methylene (H_2-1), and a deshielded methyl (Me-12). ^{13}C (broad band decoupled) NMR, together with a ^{13}C

Table 1
^{1}H (coupling constant J = Hz in parentheses), ^{13}C, HMQC and HMBC NMR data of **2**

Carbon no.	δ^1H	δ^{13}C	^{1}H–^{13}C correlation[a]		
			^1J	^2J	^3J
2	–	157.8			
3	–	134.3			
4	–	180.1			
5	–	161.9			
6	6.13 *d* (2.0)	98.8	C-6	C-5, C-7	C-8, C-10
7	–	164.7			
8	6.31 *d* (2.0)	93.6	C-8	C-7, C-9	C-6, C-10
9	–	157.8			
10	–	104.2			
1′	–	121.7			
2′	7.80 *d* (2.0)	116.4	C-2′	C-1′, C-3′	C-2, C-4′, C-6′
3′	–	144.6			
4′	–	149.1			
5′	6.87 *d* (8.5)	114.9	C-5′	C-4′, C-6′	C-1′, C-3′
6′	7.58 *dd* (8.5, 2.0)	121.5	C-6′		C-2, C-2′, C-4′
Sugar					
1″	5.17 *d* (7.6)	104.0	C-1″		C-3′
2″	3.80 *m*[b]	71.8	C-2″	C-1′	
3″	3.62 *dd* (9.8, 3.6)	73.9	C-3′		C-1′, C-5′
4″	3.84 *m*[b]	69.1	C-4″		
5″	3.77 *m*[b]	73.7	C-5″	C-6′	
6″	4.19 *dd* (11.5, 4.0)	63.2	C-6″	C-5′	C-9‴
	4.39 *dd* (11.5, 8.0)				
Feruloyl					
1‴	–	126.6			
2‴	7.05 *d* (1.6)	110.6	C-2‴	C-1‴, C-3‴	C-4‴, C-6‴, C-7‴
3‴	–	148.1			
4‴	–	149.4			
5‴	6.80 *d* (8.5)	115.1	C-5‴		C-1‴, C-3‴
6‴	6.91 *dd* (8.5, 1.6)	123.6	C-6‴		C-2‴, C-4‴, C-7‴
7‴	7.38 *d* (16.0)	146.2	C-7‴	C-1″, C-8″	C-2‴, C-6‴, C-9‴
8‴	6.11 *d* (16.0)	113.8	C-8‴	C-9‴	C-1‴
9‴	–	167.6			
3‴-OMe	3.90 *s*	54.9	3‴-OMe		C-3‴

[a] Spectra in CD_3OD referenced to CH_3OH at δ 3.31 (^{1}H, 500 MHz,) and δ 49.15 (^{13}C, 125 MHz)[a] ^{1}H–^{13}C correlation, ^1J from HMQC, ^2J and ^3J from HMBC experiments.

[b] Overlapped peaks, assigned from ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC.

Table 2
^{1}H (coupling constant J = Hz in parentheses), ^{13}C, HMQC and HMBC NMR data of **3**

Carbon no.	δ^1H	δ^{13}C	^{1}H–^{13}C correlation[a]		
			^1J	^2J	^3J
1	2.16 *m*	27.2	C-1	C-2, C-7a	C-3a, C-3, C-7
	2.32 *m*		C-1	C-2, C-7a	C-3a, C-3, C-7
2	7.11 *bt* (4.5)	142.5	C-2	C-1	C-13, C-7a, C-3a
3	–	133.9			
3a (β)	2.43 *m*	38.9	C-3a	C-3	C-7, C-1
4	3.20 *m*	36.5	C-4		
5	–	201.4			
6	–	136.5			
7	7.05 *bd* (5.5)	151.1	C-7	C-7a, C-6	C-12, C-5, C-3a
7a (α)	2.00 *m*	40.4	C-7a		
8	2.74 *m*	41.7	C-8		C-3a, C-5, C-10, C-11
	2.40 *m*		C-8		C-3a, C-5, C-10, C-11
9	2.05 *m*	29.1	C-9		
Me-10	0.98 *d* (7.0)	16.3	C-10	C-9	C-11
Me-11	1.01 *d* (7.0)	21.8	C-11	C-9	C-10
Me-12	1.82 *s*	16.4	C-12	C-6	C-5, C-7
13	–	169.9			

[a] Spectra in CD_3OD referenced to CH_3OH at δ 3.31 (^{1}H, 500 MHz,) and δ 49.15 (^{13}C, 125 MHz)[a] ^{1}H–^{13}C correlation, ^1J from HMQC, ^2J and ^3J from HMBC experiments.

PENDANT (Homer and Perry, 1994) experiment revealed the presence of 15 carbons, including a ketonic carbonyl (C-5, δ 201.4), an acid carbonyl (C-13, δ 169.9), two olefinic quaternary carbons (C-3, δ 136.5 and C-6, δ 133.9), two olefinic methines (C-7, δ 151.1 and C-2, δ 142.5), four methines (C-3a, C-4, C-7a and C-9, respectively at δ 38.9, 36.5, 40.4 and 29.1), two methylenes (C-8, δ 41.7 and C-1, δ 27.2) and three methyls (Me-10, Me-11 and Me-12, respectively at δ 16.3, 21.8 and 16.4) in the molecule, and thus indicated this compound to be a sesquiterpene acid. A COSY45 spectrum showed ^{1}H–^{1}H correlations, Me-10 and Me-11 ↔ H-9 ↔ H_2-8 ↔ H-4 ↔ H-3a ↔ H-7a ↔ H_2-1 ↔ H-2, and also H-7a ↔ H-7, and thus established the part-structure **3a** (Fig. 1) which was also supported from the ^{1}H–^{1}H correlations observed in a TOCSY experiment. The HMQC and HMBC spectra (Table 2) showed, respectively, ^{1}H–^{13}C direct ^{1}J, and ^{1}H–^{13}C long-range ^2J and ^3J correlations. In HMBC spectrum (Table 2), the following ^{1}H–^{13}C long-range correlations helped in building the extended part-structure **3b** (Fig. 2): H_2-8 showed ^3J correlation to C-3a, C-5, C-10 and C-11; Me-12 showed correlation, ^2J to C-6 and ^3J to C-5 and C-7; H-7 to C-7a (^2J) and ^3J to C-3a, C-5 and C-12; H-2 to C-13 (^3J). As C-3 and C-3a are, respectively, quaternary and methine carbons, they must be connected to each other to fulfil the molecular formula $C_{15}H_{20}O_3$, and thus to form the structure **3**. This was further confirmed from ^{1}H–^{13}C long-range correlations: ^2J from H-3a to C-3, and ^3J from H-2 to C-3a. However, absence of ^3J correlation either from H-7a to C-3 or H-4 to C-3 might be because the dihedral angle approaches 90° (Marshall, 1983). The relative stereochemistry of the chiral centres in **3** was determined by nOe interactions obtained from a series of GOESY and a ^{1}H–^{1}H NOESY experiments (Table 3).

As flavonoids and their glycosides are of widespread occurrence in the genus *Polygonum* (Isobe and

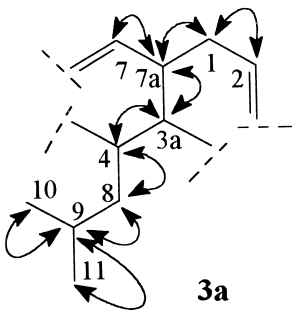


Fig. 1. Part-structure (**3a**) based on ^{1}H–^{1}H COSY45 and ^{1}H–^{1}H TOCSY NMR experiments.

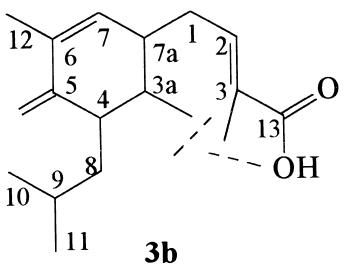
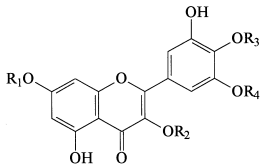
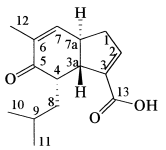


Fig. 2. Extended part-structure (**3b**) based on ^{1}H–^{13}C HMQC and HMBC correlations.

Noda, 1987), they have been used as chemotaxonomic markers within this genus (Isobe and Noda, 1987; Park, 1987; Mun and Park, 1995). Among these *Polygonum* flavonoids, glycosylation at C-3 of the quercetin nucleus has been found to be the most common trend, and present in all species of this genus (Park, 1987). The chemotaxonomic significance of the flavonoids **1** and **2** reported here deserves a consideration. Sesquiterpenes having a substituted indene ring system is not uncommon in the plant kingdom (Dictionary of Natural Products, 1999). However, to our knowledge, an isobutyl group at C-4 together with a –COOH at C-3 on an indene skeleton, as in compound **3**, has never been found before and may also be of chemotaxonomic interest.



Compound	R_1	R_2	R_3	R_4
1	Me	Me	Me	Me
2	H	6-feruloyl-β-D-galactopyranosyl	H	H
4	H	6-caffeoyl-β-D-galactopyranosyl	H	H



3

3. Experimental

3.1. General

UV spectrum was in MeOH. NMR spectra of **1** were recorded on a Varian VXR-500S, and those for **2** and **3** were obtained on a Bruker AVANCE DRX500 Ultrashield instrument. The chemical shifts are expressed in ppm. HR–FABMS was obtained with a JEOL SX 102 mass spectrometer (resolving power = 10,000; polyethyleneglycol was used as the reference substance). HPLC separation was performed in the Waters Prep-LC System coupled with a Waters 486 UV–visible detector. RP stands for reversed-phase C_{18} column. RP-separations were monitored at 254 nm. Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) was used for pre-HPLC fractionation. Silica gel 60-PF254 (Merck 7749) and silica gel (Merck 7734), respectively, for PTLC and CC were used.

Table 3
^1H–^1H nOe interactions in **3**, obtained from GOESY and NOESY experiments[a]

From	To
H-1 (δ 2.32)	H-2, H-7a
H-1 (δ 2.16)	H-2
H-2	H-1 (δ 2.32)
H-3a	H-4
H-4	H-8 (δ 2.74, 2.40), H-3a (w)
H-7	H-8 (δ 2.40) (w), H-9 (w), Me-10, Me-11, Me-12
H-7a	H-1 (δ 2.32), H-7
H-8 (δ 2.74)	H-8 (δ 2.40), H-4
H-8 (δ 2.43)	H-4, H-7 (w), H-8 (δ 2.74), Me-10, Me-11
H-9	Me-10, Me-11, H-7 (w)
Me-12	H-7
Me-11	H-9, H-8 (δ 2.40), H-7
Me-10	H-9, H-8 (δ 2.40), H-7

[a] Spectra obtained in CD_3OD. W = weak nOe.

3.2. Plant material

The whole plants were collected from Panchari, Chittagong, Bangladesh, and a voucher specimen (voucher no. 764) representing this collection has been retained in the Herbarium of the Department of Botany, University of Dhaka, Bangladesh.

3.3. Extraction

Ground dried whole plant parts (2.3 kg) of *P. viscosum* were extracted, successively, with *n*-hexane, EtOAc and MeOH. The EtOAc- and MeOH-extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45°C to yield, 9.31 g and 12.8 g of dried extracts, respectively.

3.4. Isolation of compounds

The EtOAc-extract was fractionated by CC eluting with a step-gradient of $CHCl_3$-MeOH mixture of increasing polarity. Similar fractions (100% $CHCl_3$ to 25% MeOH in $CHCl_3$) were combined and subjected to further CC purification (step-gradient of n-hexane-EtOAc mixture of increasing polarity) yielding the fraction containing **1**. PTLC (solvent system– hexane:EtOAc = 7:3, visualised under UV lights) of this fraction produced **1** (5.4 mg). A portion of the MeOH extract (3.8 g) was fractionated on a Sep-Pak, using 20%, 40%, 60%, 80% and 100% MeOH–water mixture (200 ml each) as eluent. Preparative RP-HPLC (gradient elution, 10–100% acetonitrile in water in 50 min, 55 ml/min.) of the Sep-Pak fraction (60% MeOH in water) yielded **3** (3.2 mg) and impure **2** which was, then, further purified by semi-preparative RP–HPLC (isocratic elution, 20% acetonitrile in water, 25 ml/min) to obtain pure **2** (3.0 mg).

3.5. 3′,5-Dihydroxy-3,4′,5′,7-tetramethoxyflavone *1*

Amorphous. UV, ^1H-NMR (as published data: Mar-

tos et al., 1997). and ^{13}C-NMR ($CDCl_3$): δ 178.8 (C-4), 165.6 (C-7), 162.0 (C-5), 156.8 (C-9), 155.3 (C-2), 152.0 (C-5′), 149.2 (C-3′), 139.7 (C-3), 137.8 (C-4′), 126.0 (C-1′), 108.6 (C-2′), 106.1 (C-10), 105.1 (C-6′), 98.0 (C-6), 92.2 (C-8), 61.1 (4′-OMe), 60.3 (3-OMe), 56.1 (7-OMe), 55.8 (5′-OMe). HR–FABMS *m/z*: 375.1073 $[M + H]^+$ $C_{19}H_{19}O_8$, calcd. 375.1073.

3.6. Quercetin 3-O-(6″-feruloyl)-β-D-galactopyranoside **2**

Gum. UV λ_{max} nm: 255, 270 sh, 300 sh and 358. ^{1}H and ^{13}C-NMR (Table 1). HR–FABMS *m/z*: 641.1505 $[M + H]^+$ $C_{31}H_{29}O_{15}$, calcd. 641.1506.

3.7. Viscosumic acid (4-isobutyl-6-methyl-5-oxo-3a,4,5,7a-tetrahydro-1H-inden-13-oic acid) **3**

Amorphous. UV λ_{max} nm: 219, 240 sh. ^{1}H and ^{13}C NMR (Table 2). HR–FABMS *m/z*: 249.1487 $[M + H]^+$ $C_{15}H_{21}O_3$, calcd. 249.1491.

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