

Steroids and polyketides from *Uvaria hamiltonii* stem bark

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Two known steroids, stigmasterol and 6 β -hydroxystigmasta-4,22-dien-3-one (1) and two unusual polyketides, *cis*-4-hydroxymellein (2) and *trans*-4-hydroxymellein (3) were isolated from the stem bark of *Uvaria hamiltonii*. The structures of the compounds were elucidated independently by high-resolution 2D-NMR techniques and confirmed by comparison with previously reported values.

Keywords: *Uvaria hamiltonii* (Annonaceae), polyketides

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Uvaria hamiltonii Hook f. and Thorns. is a shrub found in the forests of Bangladesh and Sikkim, Bihar and Asam of India (1). Although *U. hamiltonii* growing in Bangladesh has not been studied extensively, two flavanones, an aurone, a chalcone and a tetrahydroxanthene have been reported from this plant (2). We have studied *U. hamiltonii* and reported five aristolactam alkaloids identified as aristolactam B-II (4), griffithinam (5), aristolactam A-III (6), piperolactam C (7) and goniopedaline (8) (3). This paper deals with the isolation and structure elucidation of two known steroids, *viz.* stigmasterol and 6 β -hydroxystigmasta-4,22-dien-3-one (1) (4–6), reported for the first time from any *Uvaria* species, and two polyketides, *cis*-4-hydroxymellein (2) and *trans*-4-hydroxymellein (3) (Fig. 1). This is the first and second report of polyketides 2 and 3, respectively, from any plant source. The polyketide 2 was previously isolated from several fungi, including *Lasiodiplodia theobromae* (7), *Cercospora taiwanensis* (8), *Aspergillus ochraceus* (9) and *Aspergillus melleus* (10–11) while its *trans* isomer was reported from *Apiospora camptospora* (7), *Septoria nodorum* (12) and *Moringa oleifera* (13).

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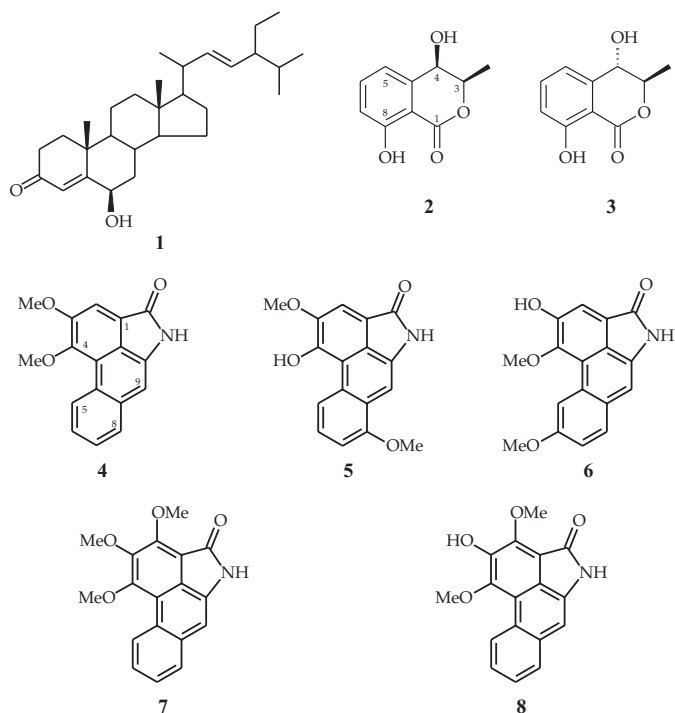


Fig. 1. Compounds 1-8 isolated from Bangladeshi *Uvaria hamiltonii* (*Annonaceae*).

EXPERIMENTAL

General spectrochemical procedures

The ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded in CDCl_3 on a Varian VXR 500S spectrometer (Varian, USA) and the chemical shifts are reported in ppm relative to the residual non-deuterated solvent signals. Inverse detected heteronuclear correlations were measured using the HSQC (optimized for $^1J_{\text{CH}} = 140$ Hz) and HMBC (optimized for $^nJ_{\text{CH}} = 8.3$ Hz) pulse sequences with a pulsed-field gradient. COSY-45 spectra were used to determine the proton-proton connectivities. FABMS were recorded on a VG Micromass ZAB mass spectrometer (Walters Corp., USA) using *m*-nitrobenzyl alcohol (NBA) or polyethylene glycol as a matrix.

Plant material

The stem bark of *U. hamiltonii* (*Annonaceae*) was collected from the hilly areas of Chokoria, Cox's Bazar (Bangladesh) in October 1998 and was identified by Prof. M. Salar Khan, Bangladesh National Herbarium (Dhaka, Bangladesh) where a voucher specimen had been maintained under the accession number DACB-27943.

Extraction and isolation

Successive Soxhlet-extraction of the air-dried and ground plant materials (650 g) yielded 6.2 g of petroleum ether extract, 9.4 g of dichloromethane extract and 42.3 g of methanol extract. An aliquot of the petroleum ether extract (3.0 g) was subjected to Sephadex LH-20 chromatography using 1:4, 1:9, 0:10 petroleum ether/chloroform mixtures and finally 5% MeOH in chloroform as mobile phases. The final 400 mL eluent, which was a chlorophyll-free fraction (2.0 g), was further fractionated by column chromatography over Kieselgel 60 (70–230 mesh) and the column was eluted with petroleum ether and petroleum ether/EtOAc mixtures of increasing polarity (95:5, 90:10, 85:15, 80:20, 70:30, 50:50, 40:60, 20:80), with 92 fractions collected (each *ca.* 25 mL). Evaporation of solvents from sub-fractions 62–80, followed by preparative TLC over silica gel PF₂₅₄ using toluene/EtOAc (85:15) gave 10.2 mg of compound **1** as colourless needles. The dichloromethane extract (4.0 g) was subjected to Sephadex LH-20 and the chlorophyll-free fraction (3.0 g) was chromatographed over Kieselgel 60 using petroleum ether/EtOAc mixtures of increasing polarity and EtOAc as mobile phases, yielding 72 fractions (each *ca.* 25 mL). Depending upon the TLC behaviour, fractions 31–43 were subjected to preparative TLC over silica gel PF₂₅₄ using toluene/EtOAc (85:15), which afforded an isomeric mixture of **2** and **3** as amorphous solids (13.2 mg).

RESULTS AND DISCUSSION

Concentrated petroleum ether extract of the stem bark of *U. hamiltonii* furnished stigmasterol and a hydroxy steroidal ketone (**1**), while the dichloromethane extract yielded an isomeric mixture (1:1) of two polyketides, **2** and **3**. The FABMS of **1** displayed the $[M + H]^+$ ion at m/z 427 and its ¹³C NMR spectrum showed 29 carbon resonances. These suggested the molecular formula C₂₉H₄₆O₂ for **1** and by direct comparison of its spectral data (Table I) with literature values (4–6), compound **1** was identified as 6 β -hydroxy-stigmasta-4,22-dien-3-one. Assignments of the protons and carbons in the molecule were unambiguously established by 2D NMR studies. This is the first report on the isolation of compound **1** from the family *Annonaceae*.

The ¹H NMR spectrum (500 MHz, CDCl₃) of the isomeric mixture of *cis*- and *trans*-4-hydroxymelleins (**2** and **3**) exhibited six down-field (δ 6.90, 6.97, 7.00, 7.01, 7.47 and 7.52) and six up-field signals (δ 1.49, 1.56, 4.55, 4.58, 4.60 and 4.67) as well as two highly deshielded singlets (δ 10.97, 11.01). The hydroxymelleins showed a molecular ion at m/z 195 in its FABMS spectrum, consistent with the composition $[C_{10}H_{10}O_4 + H]^+$. The *cis*-isomer was characterized by the presence of three downfield signals for aromatic protons in the ¹H NMR, which appeared as one-proton doublets at δ 6.90 ($J = 7.0$ Hz) and 7.01 ($J = 7.0$ Hz), a one-proton triplet at δ 7.47 ($J = 7.0$ Hz), a three-proton doublet ($J = 6.5$ Hz) at δ 1.56 for a methyl group, a one-proton doublet of a quartet at δ 4.67 ($J = 1.5, 6.5$ Hz) as well as a one-proton broad singlet at δ 4.55. These data were indicative of the system ArCH(OH)CH(Me)OCO. Furthermore, a hydrogen-bonded phenolic proton appeared as a highly deshielded one-proton singlet at δ 11.01. The *cis*-configuration was assigned to H-3 and H-4 on the basis of the small coupling constant (1.5 Hz) whereas the corresponding *trans*-isomer had a higher value – 4.5 Hz (7). The ¹H and ¹³C NMR spec-

Table I. NMR spectral assignments for 6 β -hydroxystigmasta-4,22-dien-3-one (1) in CDCl₃

C/H	¹ H	¹³ C	¹ H ¹ H COSY	HSQC	Major HMBC correlations	
	δ_{H} mult <i>J</i> (Hz)	δ_{C} mult			² <i>J</i>	³ <i>J</i>
1	1.69, 2.02	37.1 (t)	H-2 α , H-2 β	37.1		C-3, C-5
2 α	2.35, <i>t</i> , 6.5	34.3 (t)	H-2 β	34.3	C-3	
2 β	2.50, <i>t</i> , 6.5		H-2 α		C-3	
3	–	200.4 (s)				
4	5.80	126.3 (d)		126.3		C-2, C-6, C-10
5	–	168.5 (s)				
6	4.33, <i>t</i> , 6.5	73.3 (d)	H-7	73.3		C-4, C-8, C-10
7	1.22, 1.96	38.6 (t)	H-8	38.6	C-6, C-8	C-5
8	1.21	29.7 (d)	H-7	29.7		
9	0.88	53.6 (d)	H-11	53.6		C-19
10	–	38.0 (s)				
11	0.81, 1.47	21.0 (t)	H-9, H-12	21.0	C-12	C-8
12	1.13, 2.03	39.6 (t)	H-11	39.6		
13	–	42.5 (s)				
14	0.98	55.9 (d)	H-15	55.9		C-17
15	1.11, 1.58	24.2 (t)	H-14, H-16	24.2		
16	1.27, 1.84	28.2 (t)	H-15, H-17	28.2		C-20
17	1.10	56.1 (d)	H-16	56.1		
18	0.76	12.0 (q)		12.0	C-13	C-12, C-14, C-17
19	1.39	19.5 (q)		19.5	C-10	C-1, C-5, C-9
20	1.33	36.1 (d)	H-17	36.1		
21	0.94, <i>d</i> , 6.5	18.7 (q)	H-20	18.7	C-20	C-17, C-22
22	5.13, <i>dd</i> , 15.0, 8.5	138.1 (d)	H-23	138.1		
23	5.01, <i>dd</i> , 15.0, 8.0	129.5 (d)	H-22	129.5		
24	0.92	45.8 (d)	H-25, H-28	45.8	C-28	C-29
25	1.15	26.1 (d)	H-27	26.1		
26	0.85, <i>d</i> , 6.5	19.8 (q)		19.8		
27	0.82, <i>d</i> , 6.5	19.0 (q)	H-25	19.0		C-24
28	1.03, 1.30	33.9 (t)	H-24	33.9		
29	0.87, <i>t</i> , 8.0	21.2 (q)		21.2		

tral data of compound **3** were almost identical to those of *cis*-4-hydroxymellein (**2**). However, the strong coupling between the protons at C-3 and C-4 established a *trans* relation between these two protons.

The structures of **2** and **3** were established unambiguously by mass and 2D NMR spectral studies, notably COSY-45, HSQC and HMBC experiments. HSQC revealed all ^1H ^{13}C direct correlations and thus confirmed the assignment of all carbons with attached protons, while the HMBC experiment established the key 2J and 3J ^1H ^{13}C connectivities (Table II). The hydroxyl group at δ 11.01 in *cis*-isomer showed HMBC correlations to carbons at δ_{C} 118.4, 162.02 and 106.9 while in the *trans*-isomer, the hydroxyl at δ 10.97 showed connectivities with 117.8, 161.96 and 106.7 and thus established their positions at C-8. The HMBC data also revealed the connectivities of H-4, H-5 and H-7 to C-3, C-4a and C-4, C-7 and C-5, C-8a, respectively. Other important correlations are given in Table II. These spectral data established the planar structures of **2** and **3** which were previously isolated from fungal sources primarily (7, 10).

Table II. NMR spectral assignments for *cis*-4-hydroxymellein (**2**) and *trans*-4-hydroxymellein (**3**) in CDCl_3

C/H	2				3					
	^{13}C	^1H	mult	J (Hz)	HMBC ^a	^{13}C	^1H	mult	J (Hz)	HMBC ^a
1	162.0					161.96				
3	77.9	4.67	dq	(1.5, 6.5)		79.8	4.58	dq	(4.5, 7.0)	
4	67.1	4.55	bs		C-3, C-4a C-5	69.1	4.60	bs		C-3, C-4a C-8a
4a	140.8					141.0				
5	118.1	6.90	d	(7.0)	C-4, C-7	116.1	7.00	d	(8.5)	C-4, C-7
6	137.0	7.47	t	(7.0)	C-4a	136.7	7.52	t	(8.5)	C-4a, C-8
7	118.4	7.01	d	(7.0)	C-5, C-8a	117.8	6.97	d	(8.5)	C-5, C-8, C-8a
8	162.0					161.96				
OH-8		11.01	s		C-7, C-8 C-8a		10.97	s		C-7, C-8, C-8a
8a	106.9					106.7				
Me-3	15.9	1.56	d	(6.5)	C-3, C-4	17.8	1.49	d	(6.5)	C-3, C-4

^a Key correlations

CONCLUSION

The occurrence of chemically diversified classes of compounds including aristolactam alkaloids, polyketides, steroids, flavanones, aurone, chalcone and tetrahydroxanthene in *U. hamiltonii* (*Annonaceae*) may support the traditional use of the plant to treat minor infections.

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S A Ž E T A K

Steroidi i poliketidi iz kore biljke *Uvaria hamiltonii*

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Dva poznata steroida, stigmasterol i 6 β -hidroksistigmasta-4,22-dien-3-on (1) i dva neuobičajena poliketida, *cis*-4-hidroksimelein (2) i *trans*-4-hidroksimelein (3) izolirana su iz kore biljke *Uvaria hamiltonii*. Strukture spojeva određene su neovisno 2D-NMR spektroskopijom visoke rezolucije i usporedbom s literaturnim podacima.

Ključne riječi: *Uvaria hamiltonii* (*Annonaceae*), poliketidi

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