

## Steroids and polyketides from *Uvaria hamiltonii* stem bark

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Two known steroids, stigmasterol and 6 $\beta$ -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 $\beta$ -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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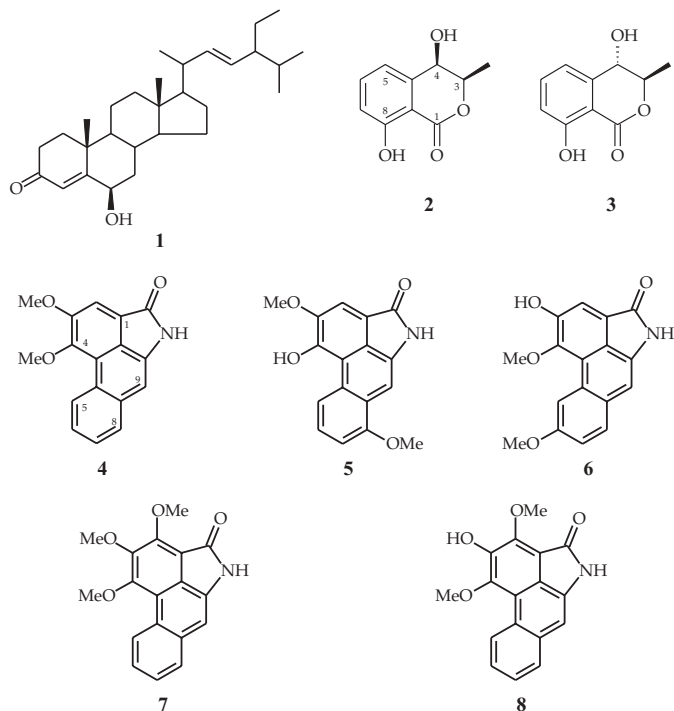


Fig. 1. Compounds 1-8 isolated from Bangladeshi *Uvaria hamiltonii* (*Annonaceae*).

## EXPERIMENTAL

### General spectrochemical procedures

The  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (125 MHz) NMR spectra were recorded in  $\text{CDCl}_3$  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 PF<sub>254</sub> 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 PF<sub>254</sub> 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 <sup>13</sup>C NMR spectrum showed 29 carbon resonances. These suggested the molecular formula C<sub>29</sub>H<sub>46</sub>O<sub>2</sub> for **1** and by direct comparison of its spectral data (Table I) with literature values (4–6), compound **1** was identified as 6 $\beta$ -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 <sup>1</sup>H NMR spectrum (500 MHz, CDCl<sub>3</sub>) of the isomeric mixture of *cis*- and *trans*-4-hydroxymelleins (**2** and **3**) exhibited six down-field ( $\delta$  6.90, 6.97, 7.00, 7.01, 7.47 and 7.52) and six up-field signals ( $\delta$  1.49, 1.56, 4.55, 4.58, 4.60 and 4.67) as well as two highly deshielded singlets ( $\delta$  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 <sup>1</sup>H NMR, which appeared as one-proton doublets at  $\delta$  6.90 ( $J = 7.0$  Hz) and 7.01 ( $J = 7.0$  Hz), a one-proton triplet at  $\delta$  7.47 ( $J = 7.0$  Hz), a three-proton doublet ( $J = 6.5$  Hz) at  $\delta$  1.56 for a methyl group, a one-proton doublet of a quartet at  $\delta$  4.67 ( $J = 1.5, 6.5$  Hz) as well as a one-proton broad singlet at  $\delta$  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  $\delta$  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 <sup>1</sup>H and <sup>13</sup>C NMR spec-

Table I. NMR spectral assignments for 6 $\beta$ -hydroxystigmasta-4,22-dien-3-one (1) in CDCl<sub>3</sub>

C/H	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H <sup>1</sup> H COSY	HSQC	Major HMBC correlations	
	$\delta_{\text{H}}$ mult <i>J</i> (Hz)	$\delta_{\text{C}}$ mult			<sup>2</sup> <i>J</i>	<sup>3</sup> <i>J</i>
1	1.69, 2.02	37.1 (t)	H-2 $\alpha$ , H-2 $\beta$	37.1		C-3, C-5
2 $\alpha$	2.35, <i>t</i> , 6.5	34.3 (t)	H-2 $\beta$	34.3	C-3	
2 $\beta$	2.50, <i>t</i> , 6.5		H-2 $\alpha$		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  $^1\text{H}$   $^{13}\text{C}$  direct correlations and thus confirmed the assignment of all carbons with attached protons, while the HMBC experiment established the key  $^2J$  and  $^3J$   $^1\text{H}$   $^{13}\text{C}$  connectivities (Table II). The hydroxyl group at  $\delta$  11.01 in *cis*-isomer showed HMBC correlations to carbons at  $\delta_{\text{C}}$  118.4, 162.02 and 106.9 while in the *trans*-isomer, the hydroxyl at  $\delta$  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  $\text{CDCl}_3$

C/H	<b>2</b>				<b>3</b>					
	$^{13}\text{C}$	$^1\text{H}$	mult	$J$ (Hz)	HMBC <sup>a</sup>	$^{13}\text{C}$	$^1\text{H}$	mult	$J$ (Hz)	HMBC <sup>a</sup>
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

<sup>a</sup> 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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## REFERENCES

1. J. D. Hooker, *The Flora of British India*, L. Reeve & Co., London 1872, Vol. 1, p. 48.
2. L. Huang, M. E. Wall, M. C. Wani, H. Navarro, T. Santisuk, V. Reutrakul, E. K. Seo, N. R. Farnsworth and A. D. Kinghorn, New compounds with DNA strand-scission activity from the combined leaf and stem of *Uvaria hamiltonii*, *J. Nat. Prod.* **61** (1998) 446–450.
3. C. M. Hasan, K. N. Asha and M. A. Rashid, Aristolactams from *Uvaria hamiltonii*, *Biochem. Syst. Ecol.* **29** (2001) 207–208.
4. N. Katsui, H. Matsue, T. Hirata and T. Masamune, Phytosterols and triterpenes in the roots of the kidney bean (*Phaseolus vulgaris*), *Bull. Chem. Soc. Japan* **45** (1972) 223–226.
5. M. della Greca, P. Monaco and L. Previtera, Stigmasterols from *Typha latifolia*, *J. Nat. Prod.* **53** (1990) 1430–1435.
6. M. I. Fernandez, J. R. Pedro and E. Seoane, Constituents of a hexane extract of *Phoenix dactylifera*, *Phytochemistry* **22** (1983) 2087–2088.
7. D. C. Aldridge, S. Galt, D. Giles and W. B. Turner, Metabolites of *Lasiodiplodia theobromae*, *J. Chem. Soc. C* **1971**, 1623–1627.
8. L. Camarda, L. Merlini and G. Nasini, Metabolites of *Cercospora*. Taiwapyrone, an  $\alpha$ -pyrone of unusual structure from *Cercospora taiwanensis*, *Phytochemistry* **15** (1976) 537–539.
9. R. J. Cole, J. H. Moore, N. D. Davis, J. W. Kirksey and U. L. Diener, 4-Hydroxymellein. New metabolite of *Aspergillus ochraceus*, *J. Agr. Food Chem.* **19** (1971) 909–911.
10. M. J. Garson and J. Staunton, New polyketide metabolites from *Aspergillus melleus*: structural and stereochemical studies, *J. Chem. Soc. Perkin Trans. I* **1984** 1021–1026.
11. J. S. E. Holker and T. J. Simpson, Studies on fungal metabolites. Part 2. Carbon-13 nuclear magnetic resonance biosynthetic studies on pentaketide metabolites of *Aspergillus melleus*: 3-(1,2-epoxypropyl)-5,6-dihydro-5-hydroxy-6-methyl-pyran-2-one and mellein, *J. Chem. Soc. Perkin I* **1981** 1397–1400.
12. M. Devys, M. Barbier, J. F. Bousquet and A. Kollmann, Isolation of the new (–)-(3R,4S)-4-hydroxymellein from the fungus *Septoria nodorum* bark, *Z. Naturforsch. C: Biosci.* **47** (1992) 779–781.
13. M. P. Saluja, R. S. Kapil and S. P. Popil, Chemical constituents of *Moringa oleifera* Lamk. (hybrid variety) and isolation of 4-hydroxymellein, *Indian J. Chem.* **16B** (1978) 1044–1045.

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 $\beta$ -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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