Aromatic Constituents of *Uvaria grandiflora*

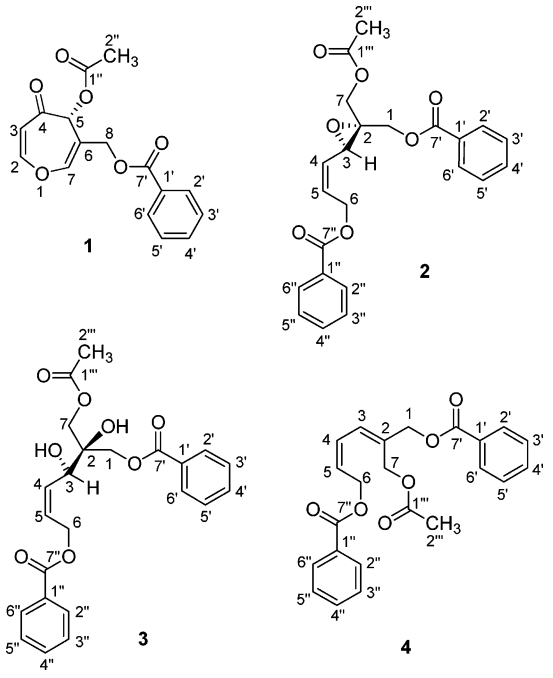
Sridevi Ankisetty,[†,‡] Hala N. ElSohly,*[,†] Xing-Cong Li,[†] Shabana I. Khan,[†] Babu L. Tekwani,[†] Troy Smillie,[†] and Larry Walker[†,§]

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, and Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi 38677

Received November 30, 2005

Grandiuvarone A (**1**) and grandiuvarins A−C (**2**−**4**) were isolated from the bark of *Uvaria grandiflora*. The structures of these new aromatic compounds were elucidated on the basis of spectroscopic analyses, especially 2D NMR techniques. Only compound **1** exhibited antileishmanial activity, with IC_{50}/IC_{90} values of 0.7/1.5 μg/mL. The positive controls pentamidine and amphotericin B had IC_{50}/IC_{90} values of 1.6/6.6 and 0.17/0.34 μg/mL, respectively.

Plants belonging to the familyAnnonaceae are woody trees, shrubs, and vines comprising about 130 genera and 2300 species.[1] The genus *Uvaria* is a member of the plant family Annonaceae and has been a rich source of varied secondary metabolites with unusual chemical structures. Several dihydrochalcones,[2,3] flavanones,[4,5] and polyoxygenated cyclohexenes[6] have been reported from the genus *Uvaria*. Previous work on *Uvaria grandiflora* resulted in the isolation of several polyoxygenated cyclohexenes.[7−9] Some of the polyoxygenated cyclohexenes have shown interesting antitumor[10] and antimalarial[6] activities. We describe in this study the isolation, structural elucidation, and biological activities of four new compounds (**1**−**4**) from the bark of *U. grandiflora*.



Compound **1** was isolated as a viscous oil. The molecular formula was deduced as $C_{16}H_{14}O_6$ by HRESIMS, which indicated the presence of 10 double-bond equivalents. The ^{13}C NMR and DEPT-135 spectra showed 16 carbon signals for 1 × CH_3, 1 × CH_2, 9 × CH, and 5 × C. The IR spectrum showed the presence of two ester carbonyls (1748 and 1719 cm^{-1}) and a cyclic six- or seven-membered α,β-unsaturated ketone (1688 cm^{-1}). The ^1H NMR spectrum (Table 1) contained signals corresponding to an acetoxy group at δ 2.01 (3H, s), self-coupled oxymethylene protons at δ 4.94, 4.98 (2H, d, J = 12.4 Hz), an acetoxy methine at δ 5.80 (1H, s), two olefinic protons of an α,β-unsaturated ketone at δ 7.10 (1H, d, J = 7.2 Hz) and 5.70 (1H, d, J = 7.2 Hz), benzoyloxy group protons resonating between δ 7.90 and 7.40 (5H), and an olefinic methine at δ 7.02 (1H, s).

Table 1. ^1H and ^{13}C NMR, HMBC, and COSY Data of Compound **1** ($CDCl_3$)[a]

position	δ_H	δ_C	HMBC	COSY
1				
2	7.1 (d, 7.2)	153.6	C-3, 4, 7	H-3
3	5.7 (d, 7.6)	108.7	C-2, 4, 5	H-2
4		182.5		
5	5.8 (s)	75.8	C-4, 6, 7, 1″	
6		115.1		
7	7.02 (s)	144.9	C-2, 5, 6, 8	
8	4.94, 4.98 (d, 12.4)	62.0	C-5, 6, 7, 7′	
1′		129.5		
2′,6′	7.9 (d, 8.0)	129.6 (2)	C-4′, 2′, 7′	H-5
3′,5′	7.4 (t, 7.6)	128.5 (2)	C-2′	H-6′, H-4′
4′	7.58 (m)	133.3	C-3′	H-3′
7′		166.0		
1″		169.4		
2″	2.10 (s)	20.5	C-1″	

[a] The assignment was based upon DEPT, COSY, HMQC, and HMBC experiments.

The presence of these functionalities, coupled with its molecular formula, 10 degrees of unsaturation, and detailed analysis of the ^1H NMR and ^{13}C NMR data (Table 1), indicated that compound **1** was a monocyclic α,β-unsaturated ketone with one acetoxy and one benzoyl ester group. The positions of the acetoxy and benzoyloxy groups were fixed at C-5 and C-6, respectively, by the HMBC correlations observed between H-5 and C-4, C-6, C-7, C-1'' and between H-7 and C-2, C-6, C-8, and C-5. The absence of a COSY correlation between H-2 and H-7 and the absence of HMBC correlations between H-7 and C-3 and between H-2 and C-6 and also the downfield shifts of C-2 and C-7 (δ 153.6 and 144.9, respectively) indicated the presence of an oxygen atom in the cyclic system. The absolute configuration at C-5 was determined as *R* by a CD experiment, which exhibited a negative Cotton effect at 316 nm ($\Delta\epsilon = -14.23$) due to n−π* transition of the conjugated ketone, which allowed the assignment of the 5*R* configuration[11] for **1**. Thus, the structure of **1** was elucidated as a new natural product, named grandiuvarone (5*R*-acetoxy-6-benzoyloxymethyl-5*H*-oxepin-4-one).

Compound **2** was obtained as a viscous liquid. The HRESIMS displayed a peak at *m*/*z* 428.1671 $[M + NH_4]^+$, supporting a

* To whom correspondence should be addressed. Tel: 662-915-7610. Fax: 662-915-7989. E-mail: helsohly@olemiss.edu.
† National Center for Natural Products Research, University of Mississippi.
§ Department of Pharmacology, University of Mississippi.
‡ Current address: Department of Pharmacognosy, School of Pharmacy, University of Mississippi.

10.1021/np050500b CCC: $33.50
Published on Web 02/15/2006

Table 2. ^{1}H and ^{13}C NMR and Selected HMBC Data for Compounds **2**−**4** ($CDCl_3$)[a]

	2			**3**			**4**		
position	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	4.46 (d, 12.4) 4.53 (d, 12.4)	64.6	C-2, 3, 7′, 7	4.36 (d, 12.4) 4.536 (d, 12.4)	64.6	C-2, 3, 7′, 7	4.97 (s)[b]	67.0	C-2′, 7′, 7
2		60.6			74.8			132.8	
3	3.98 (d, 6.4)	57.5	C-2, 4, 5	5.20 (d, 10.8)	58.2	C-1, 5, 7	6.79 (d, 12.0)	127.5	C-1, 2, 5, 7
4	5.60 (dd, 11.2, 6.4)	126.8	C-6	6.04 (t, 10.8)	129.2	C-6	6.59 (t, 11.6)	126.4	C-2, 3, 6
5	6.00 (m)	131.6	C-3	5.90 (m)	128.8	C-3	5.90 (m)	128.8	C-3
6	5.01 (brd, 5.6)	60.8	C-4, 5,7″	4.91 (brd,6.4)	60.0	C-4, 5, 7″	5.03 (d, 7.8)	60.6	C-4, 5, 7″
7	4.29 (s)	62.5	C-3,2,1,1‴	4.36 (d, 12.0) 4.5 1 (d, 12.0)	65.09	C-3, 2, 1, 1‴	4.88 (s)	59.8	C-1, 2, 3, 1‴
1-OBz									
1′		130.0[c]			129.9[c]			130.2[c]	
2′, 6′	8.01 (d, 8.0)	129.2[d]		8.01 (d, 8.0)	128.4[d]		8.07 (d, 7.2)	129.9[d]	
3′, 5′	7.38−7.46 (m)	128.7[e]		7.38−7.46 (m)	128.5[e]		7.4−7.48 (m)	128.7[e]	
4′	7.53 (t, 7.0)	133.3[f]		7.53 (t, 7.0)	133.2[f]		7.53 (t, 7.0)	133.3[f]	
7′		166.2			166.1			166.3	
6-OBz									
1″		129.6[c]			129.2[c]			130.2[c]	
2″, 6″	8.01 (d, 8.0)	129.8[d]		8.01 (d, 8.0)	129.9[d]		8.07 (d,7.2)	129.9[d]	
3″, 5″	7.38−7.46 (m)	128.6[e]		7.38−7.46 (m)	128.6[e]		7.38−7.46 (m)	128.6[e]	
4″	7.53 (t, 7.0)	133.5[f]		7.53 (t, 7.0)	133.4[f]		7.53 (t, 7.0)	134.4[f]	
7″		166.3			166.3			166.6	
7-OAc									
1‴		170.5			170.5			170.9	
2‴	2.03 (s)	20.8	C-1‴	2.09 (s)	20.8	C-1‴	2.09 (s)	21.0	C-1‴

[a] The assignment was based upon DEPT, COSY, HMQC, and HMBC experiments. [b] Multiplicity and coupling constant (*J*, Hz). [c] −*f*The data with the same superscript in each column are interchangeable.

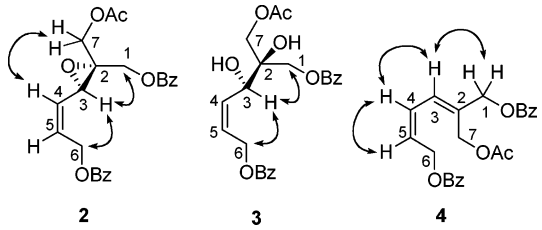


Figure 1. Key NOE correlations of compounds **2**−**4**.

molecular formula of $C_{23}H_{22}O_7$. The ^{13}C NMR and DEPT-135 spectra of **2** showed 23 carbon signals, including six quaternaries (three ester carbonyls at δ 170.5, C-1‴, 166.3, C-7′, 166.2, C-7″; δ 60.6, C-2, 130.1, C-1′; and δ 129.6, C-1″), a double bond (δ 126.8, C-4 and 131.6, C-5), one oxymethine (δ 57.5, C-3), three oxymethylenes (δ 62.5, C-7, 60.8, C-6, and 64.6, C-1), one acetyl methyl (δ 20.8, C-2‴), and 10 aromatic methines resonating between δ 129 and 134. The ^{1}H NMR spectrum gave rise to signals corresponding to aromatic protons resonating at δ 8.01−7.38 (10 H), two olefinic protons at δ 6.00 (1H, m, H-5) and 5.60 (1H, dd, J = 11.2, 6.4 Hz, H-4), three oxymethylenes protons at δ 4.46, 4.53 (2 H, d, J = 12.4 Hz, H-1), 4.29 (2H, s, H-7), and 5.01 (2H, brd, J = 5.6 Hz, H-6), an epoxy methine at δ 3.98 (1H, d, J = 6.4 Hz, H-3), and an acetyl methyl at δ 2.03 (3H, s, H-2‴). COSY correlations were observed from H-3 → H-4 → H-5 → H-6, thus allowing the epoxy group to be placed between C-3 and the quaternary C-2. The positions of the acetoxy and two benzoyloxy groups were fixed at C-7, C-1, and C-6, respectively, on the basis of key HMBC correlations observed between H-7 and C-1‴, C-2; H-1 and C-2, C-7′; and H-6 and C-5, C-7″.

The ^{1}H and ^{13}C NMR assignments for compound **2** (Table 2) were made using HMQC and HMBC correlations. The coupling constant of 11.2 Hz between H-4 and H-5, together with NOESY correlations between these two protons, indicated a *Z* configuration for the olefinic bond. The relative configurations of C-2 and C-3 in compound **2** were determined as 2*R**, 3*R** by the key NOE correlations between H-3 and H-6/H-1 and between H-4 and H-7 (Figure 1). Therefore, compound **2** was established to be a new natural product (1,6-dibenzoyloxy-2-acetoxymethyl-2*R**,3*R**-epoxyhex-4(*Z*)-ene) named grandiuvarin A.

Compound **3** was isolated as a white solid. ESIMS and ^{13}C NMR data established its molecular formula as $C_{23}H_{24}O_8$, which is 18 mass units higher than **2**. The IR spectrum gave an absorption peak at 3479 cm^{-1} indicative of a hydroxyl functionality. Analysis of the ^{1}H and ^{13}C NMR data indicated that **3** was related to **2** (Table 2). In the ^{13}C NMR spectrum of **2**, the epoxy signals for C-2 (δ 60.6) and C-3 (δ 57.5) were replaced in **3** by signals at δ 74.8 and 58.2, respectively, clearly indicating the location of hydroxyl groups at C-2 and C-3. In the NOESY spectrum, strong NOE correlations between H-3 and H-6 and between H-3 and H-1 at δ 4.37 were observed, the same as in compound **2**. However, no NOE correlation between H-7 and H-4 was observed. This indicated that the relative configuration of C-3 in compound **3** was the same as in **2**, while the relative configuration of C-2 should be reversed (Figure 1). Thus, the structure of **3** was established as a new natural product (1,6-dibenzoyloxy-2-acetoxymethyl-2*S**,3*R**-dihydroxyhex-4-(*Z*)-ene) named grandiuvarin B.

Compound **4** was obtained as a viscous liquid. The molecular formula was established as $C_{23}H_{22}O_6$ by HRESIMS, which is 34 mass units less than **3**. The IR spectrum was devoid of the hydroxyl absorption peak observed in **3**. However, the ^{1}H NMR and ^{13}C NMR spectra were similar to those of **3**. In the ^{1}H NMR spectrum of **4**, signals for three olefinic protons at δ 6.79 (1H, d, J = 12.0 Hz, H-3), 6.59 (1H, t, J = 11.6 Hz, H-4), and 5.90 (1H, m, H-5), an acetyl moiety at δ 2.09 (3H, s), and aromatic protons for two benzoyloxy groups resonating at δ 7.30−8.1 (10H) were observed. In the ^{13}C NMR spectrum of **3**, the signals for C-2 and C-3 at δ 74.8 and 58.2 were replaced in **4** by signals at δ 132.8 and 127.5, respectively, indicating the presence of a double bond at C-2 and C-3. COSY correlations were observed between H-3 and H-4, H-4 and H-5, and H-5 and H-6. The positions of the acetoxy (at C-7) and benzoyloxy groups (at C-1 and C-6) were established by key HMBC correlations observed between H-7 and C-1‴, H-1 and C-7′, and H-6 and C-7″ (Table 2). NOESY correlations were observed between H-3 and H-4/H-1 and between H-4 and H-5, which gave rise to *E* and *Z* configurations around C_2-C_3 and C_4-C_5 double bonds, respectively. Thus, the structure of **4** was established as a new natural product (1,6-dibenzoyloxy-2-acetoxymethylhex-2(*E*),4-(*Z*)-diene) named grandiuvarin C.

Compounds **1**−**4** were evaluated for antimicrobial, antiprotozoal, and cytotoxic activities. Only compound **1** exhibited antileishmanial activity against *Leishmania donovani* with IC_{50}/IC_{90} values of 0.7/1.5 μg/mL. The positive controls pentamidine and amphotericin B had IC_{50}/IC_{90} values of 1.6/6.6 and 0.17/0.34 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were determined on an Auto Pol IV automatic polarimeter. CD spectra were recorded on a Jasco J 715 spectropolarimeter. UV spectra were measured on a Hewlett-Packard 8453 spectrophotometer. IR spectra were recorded in KBr, with an ATI Mattson Genesis Series FTIR spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-400 (400 MHZ) NMR spectrometer. Chemical shifts are expressed in δ values relative to the internal standard TMS or the deuterated solvent. Multiplicity determination (DEPT) and 2D NMR spectra (COSY, HMQC, HMBC) were performed with standard pulse programs. HRESIMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. TLC and PTLC were performed on Kieselgel $60F_{254}$ (Merck) and Uniplate silica gel GF_{254} (Analtech) (1.5 mm, 20 × 20 cm glass plates), respectively. Column chromatography was run using silica gel (40 μm, J.T. Baker) and Sephadex LH-20 (Supelco). The isolated compounds were visualized under UV light (254 nm) followed by spraying with 20% sulfuric acid in MeOH.

Plant Material. *Uvaria grandiflora* (bark) was collected from New Guinea (summer of 2000) and identified by Topul Rali. A voucher specimen is deposited at The National Center for Natural Products Research (voucher NWG 208).

Extraction and Isolation. The powdered plant material (300.0 g) was percolated with 96% EtOH (3 × 2.5 L). The ethanol extracts were combined and evaporated to dryness under vacuum at 25 °C to yield a residue (30.4 g). The extract was dissolved in 80% aqueous MeOH and then extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated to dryness to afford a brown residue (3.2 g). The $CHCl_3$ fraction was subjected to column chromatography using normal-phase silica gel and $CHCl_3$/MeOH mixtures. Fractions each of 500 mL were collected and pooled into seven fractions (A−G) on the basis of TLC. Compound **1** was obtained from fraction C (120 mg) by column chromatography using Sephadex LH-20 and eluting with $CHCl_3$/MeOH (1:1) (500 mL). Fraction B (180 mg) containing compound **2** was applied on a silica gel column (15 g), eluting with *n*-hexane/$CHCl_3$ mixtures, wherein compound **2** was eluted by *n*-hexane/$CHCl_3$ (4:6, 120 mL). Compounds **3** and **4** were obtained from fraction D (300 mg) by preparative TLC using silica gel GF_{254} and eluting with 7% ethyl acetate in benzene (R_f values of 0.45 and 0.60, respectively).

Grandiuvarone (1): colorless viscous oil (79.0 mg); $[\alpha]_D$ +34.9 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (3.93), 274 (3.20) nm; IR (KBr) ν_{max} 2922, 2359, 1748, 1719, 1688, 1604, 1266, 1223 cm^{-1}; CD (MeOH) λ ($\Delta\epsilon$) 253 (56.2), 316 (−14.2) nm; ^1H and ^{13}C NMR ($CDCl_3$) (Table 1); HRESIMS *m*/*z* 325.0691 $[M + Na]^+$ (calcd for $C_{16}H_{14}O_6Na$, 325.0682).

Gandiuvarin A (2): yellow liquid (40.8 mg); $[\alpha]_D$ +34.8 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 236 (4.05), 274 (3.33) nm; IR (KBr) ν_{max} 2360, 1721, 1451, 1270, 1112 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) (Table 2); HRESIMS *m*/*z* 428.1671 $[M + NH_4]^+$ (calcd for $C_{23}H_{22}O_7NH_4$, 428.1703), 433.1247 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_7Na$, 433.1257).

Grandiuvarin B (3): white solid (12.2 mg); mp 139−141 °C; $[\alpha]_D$ +59.5 (*c* 0.88, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (3.49), 230 (3.77) nm; IR (KBr) ν_{max} 3479, 2923, 1721, 1452, 1112 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) (Table 2); ESIMS *m*/*z* 429 $[M + H]^+$.

Grandiuvarin C (4): viscous liquid (8.7 mg); UV (hexane) λ_{max} (log ϵ) 210 (3.85), 230 (3.90); IR (ν_{max}) 2924, 2359, 1721, 1270, 772; ^1H and ^{13}C NMR ($CHCl_3$) (Table 2); HRESIMS *m*/*z* 417.1258 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_6Na$, 417.1314).

Antileishmanial Assay. Compounds **1**−**4** were tested in vitro against a culture of *Leishmania donovani* promastigotes, grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS, Gibco Chemical Co.) at 26 °C. A 3-day-old culture was diluted to 5×10^5 promastigotes/mL. Drug dilutions were prepared directly in cell suspension in 96-well plates. Plates were incubated at 26 °C for 48 h, and growth of leishmania promastigotes was determined by Alamar Blue assay.[12] Standard fluorescence was measured on a Fluostar Galaxy plate reader (BMG Lab Technologies) at an excitation wavelength of 544 nm and emission wavelength of 590 nm. Pentamidine and amphotericin B were used as the standard antileishmanial agents. Percent growth was calculated and plotted versus test concentration for computing the IC_{50} and IC_{90} values.

Acknowledgment. The authors thank Dr. C. Dunbar for HRESIMS analyses and Mr. F. Wiggers for running HMQC and HMBC NMR experiments. This work was supported in part by the United States Department of Agriculture, Agricultural Research Service Specific Agreement no. 58-6408-2-0009, and by NCDDG Grant no. RO1-CA-88456.

References and Notes

(1) Schultes, R. E.; Raffauf, R. F. *The Healing Forest. Medicinal and Toxic Plants of the Northwest Amazonia*; Dioscorides Press: Portland, OR, 1992; pp 56−64.
(2) Okorie, D. A. *Phytochemistry* **1977**, *16*, 1591−1594.
(3) Hufford, C. D.; Oguntimein, B. O. *J. Nat. Prod.* **1982**, *45*, 337−342.
(4) ElSohly, H. N.; Lasswell, W. L., Jr.; Hufford, C. D. *J. Nat. Prod.* **1979**, *42*, 264−270.
(5) Nkunya, M. H. H.; Waibel, R.; Achenbach, H. *Phytochemistry* **1993**, *34*, 853−856.
(6) Takeuchi, Y.; Shi, Q.-W.; Sugiyama, T.; Oritani, T. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 537−542.
(7) Pan, X.-P.; Yu, D. Q. *Phytochemistry* **1995**, *40*, 1709−1711.
(8) Liao, Y. H.; Xu, L. Z.; Yang, S. L.; Dai, J.; Zhen, Y. S.; Zhu, M.; Sun, N. J. *Phytochemistry* **1997**, *45*, 729−732.
(9) Pan, X.-P.; Chen, R. Y.; Yu, D. Q. *Phytochemistry* **1998**, *47*, 1063−1066.
(10) Kupchan, S. M.; Hemingway, R. J.; Smith, R. M. *J. Org. Chem.* **1969**, *34*, 3898−3902.
(11) Liu, J. Y.; Liu, C. H.; Zou, W. X.; Tian, X.; Tan, R. X. *Helv. Chem. Acta* **2002**, *85*, 2664−2667.
(12) Mikus, J.; Steverding, D. A. *Parasitol. Int.* **2000**, *48*, 265−269.

NP050500B