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POLYOXYGENATED CYCLOHEXENES FROM *UVARIA GRANDIFLORA*

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Key Word Index—*Uvaria grandiflora*; Annonacea; polyoxygenated cyclohexenes.

Abstract—Three new polyoxygenated cyclohexenes, named uvarigranol G–I, along with two known compounds, 1-epizeylenol and zeylenyl 2,6-diacetate, were isolated from the roots of *Uvaria grandiflora*. Based on spectral analyses and chemical derivitization, their absolute stereostructures were established. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Most Polyoxygenated cyclohexenes have the rather simple molecular skeleton of 1-methylcyclohex-4-ene. However, they contain multiple oxygenic substituents, such as benzoxy, hydroxyl, alkoxyl, epoxyl and acetoxyl groups, and have varied stereochemistries. They once aroused widespread interest owing to their unusual structures, biogenesis and biological activity. So far about 30 of these compounds have been isolated mainly from the genera *Uvaria* (Annonaceae), *Piper* (Piperaceae) and *Croton* (Euphorbiaceae) [1, 2]. Our previous studies have resulted in the isolation and structural elucidation of six new polyoxygenated cyclohexenes, uvarigranol A–F, and the known zeylenol (**6**) from the ethanol extract of the roots of *Uvaria grandiflora* Roxb [3–5]. As a part of our continuing research into the same plant material, we now wish to report the isolation and structural elucidation of three new polyoxygenated cyclohexenes, named uvarigranol G(**1**), H(**2**) and I(**3**), and two known compounds, 1-epizeylenol (**4**) and zeylenyl 2,6-diacetate (**5**). Compound **3** has a methylcyclohexene skeleton too, but the double bond is located at C-5/C-6 instead of the usual C-4/C-5.

RESULTS AND DISCUSSION

Uvarigranol G (**1**) gave rise to $[M+H]^+$ ion at *m/z* 385 in its FABMS spectrum. The molecular formula $C_{21}H_{20}O_7$ was established by elemental analysis. The spectral data for **1** suggested that **1** was a stereoisomer of **6**, which differed from **6** only in its configuration at C-6 and/or C-1. Since **1** and **6** gave the same cyclohexenone derivative (**1a**) on oxidation with pyridinium chlorochromate, the configuration at C-1 of **1** had to be identical to that of **6**. Based on the already known absolute stereochemistry of **6**, the absolute configuration of **1** was established as 1*R*,2*S*,3*R*,6*R*.

Uvarigranol H (**2**) had similar IR and UV spectra to those of **1** and **6**. Its ^1H NMR spectrum contained the typical signals for seven protons on a methylcyclohexene skeleton (δ 4.3–6.1). In addition, the spectrum contained the signals of two benzoxy groups in (δ 7.3–8.1), one hydroxyl group (δ 3.02) and two acetoxy groups (δ 2.15 and 1.93). Since all of the oxymethine and oxymethylene protons resonated at the lower-field range due to acylation, the hydroxyl group was assigned to be attached to the quaternary C-1 as found in all compounds in this series.

The EI mass spectrum of **2** provided conclusive evidence for the location of the four acyl groups (Fig. 1). The fragment ion $(AcOCH_2CO)^+$ at 101, in place of the more commonly encountered $[BzOCH_2CO]^+$ at *m/z* 163, placed the acetoxy group at C-7 [3, 6]. The diene ion at *m/z* 232 arising from RDA cleavage, and the fragment ion at *m/z* 335 established the location of the two benzoxy groups at C-2 and C-3, respectively. and the second acetoxy group at C-6.

The coupling constants of $J_{2,3}$ and $J_{5,6}$ were similar to those of **6** and suggested the *trans* diaxial relationship between H-2/H-3, and the pseudo-equatorial orientation of H-6 in the preferred half-chair conformation of **2**. NOE difference spectroscopy was used to determine the configuration at C-1. Irradiation of

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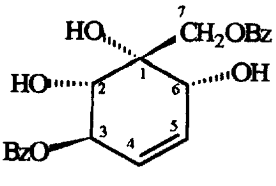

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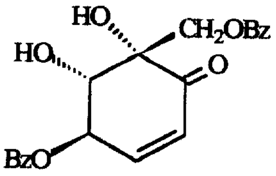

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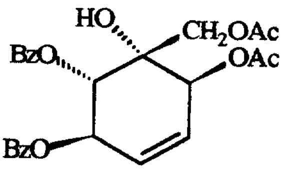

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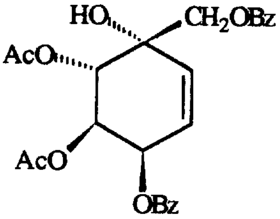

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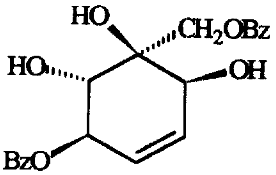

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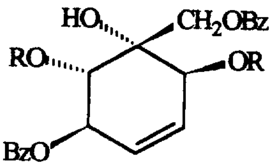


5 R=Ac
6 R=H

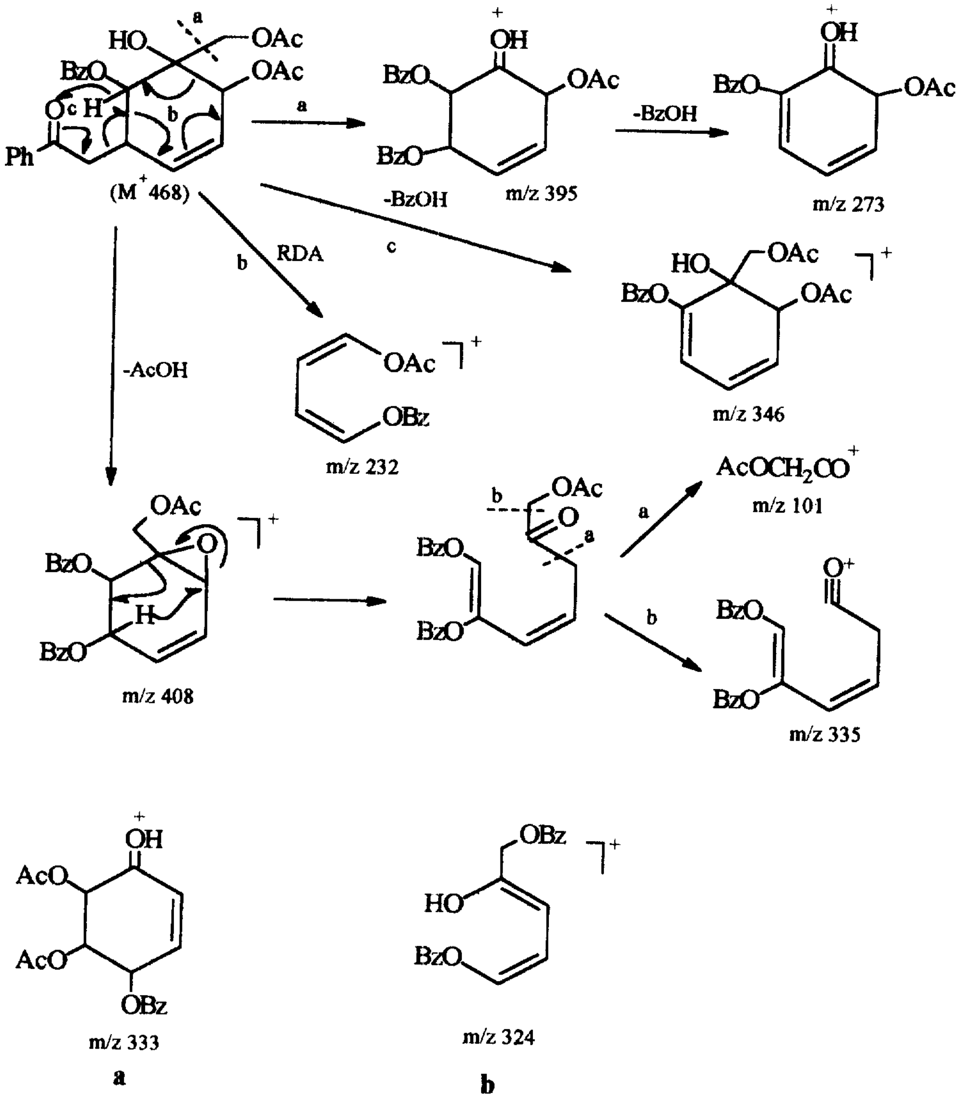
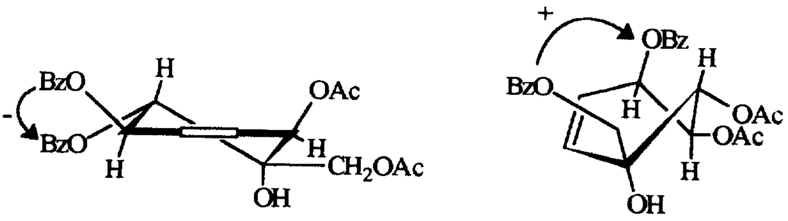


Fig 1. Major fragment ions in EI mass spectrum of uvarigranol H (**2**)



2 **3**

Fig 2. The chirality of the dibenzoate of uvarigranol H (**2**) and I (**3**).

H-7 caused a NOE shift of H-2 and *vice versa*. Hence, the *cis* relationship between H-2 and the methylene at C-1 was established.

The CD spectrum of **2** exhibited a split curve centred at λ 228 nm with a negative Cotton effect at λ 236 nm ($\Delta\varepsilon - 22.14$) and a positive Cotton effect at λ 221 nm ($\Delta\varepsilon + 11.25$), indicating the counterclockwise manner in which the two benzoyl groups at C-2 and C-3 were oriented (Fig 2) [7]. Thus, the absolute configuration was established as 1*R*,2*S*,3*R*,6*S*.

Uvarigranol I (**3**) on FAB mass spectrum gave a $[M+H]^+$ ion at *m/z* 469, indicating a M_r of 468. The molecular formula $C_{25}H_{24}O_9$ was established by elemental analysis. The IR spectrum suggested the presence of a hydroxyl group (3468 cm^{-1}), ester carbonyl groups (1750 and 1724 cm^{-1}) and mono-substituted phenyl rings (1602, 1435 and 710 cm^{-1}). In the ^{1}H NMR spectrum of **3** a ten-proton multiplet (δ 7.3–8.1) and two three-proton singlets revealed the presence of two benzoxy and two acetoxy groups. This was further confirmed by the fragment ions at *m/z* 105$[PhCO]^+$, 409$[MH\text{-}AcOH]^+$ and 349$[MH\text{-}2AcOH]^+$ in the EI mass spectrum of **3**. The EI mass spectrum also gave an ion at *m/z* 451 due to the loss of H_2O from $[M+H]^+$, indicating the presence of a hydroxyl group (C_1-OH).

Besides the above proton signals, the ^{1}H NMR spectrum of **3** showed the signals of another seven protons (δ 4.1–6.2). Among them, an AB quartet at δ 4.20 and 4.48 (11.3 Hz) was attributed to the two methylene protons at C-7 similar to those of known polyoxygenated cyclohexenes. However, the coupling pattern of the other five protons was quite different from those of the above compounds. One of the two olefinic protons resonated at δ 6.06 (*d*, $J = 9.9$ Hz, H-6) as a sharp doublet, indicating that the double bond was located at C-5/C-6 instead of the usual C-4/C-5 position. Based on a careful analysis of the coupling constants, all of the proton signals were assigned (Table 1).

The EI mass spectrum of **3** showed the fragment ion (**a**) at *m/z* 333 due to the loss of benzoyloxymethyl segment from the $[M]^+$, and the diene ion (**b**) at *m/z* 324 due to RDA cleavage, which established the placement of the two benzoxy groups at C-4 and C-7, and the two acetoxy groups at C-2 and C-3, respectively.

The $J_{2,3}$ value of 10.7 Hz revealed the *trans* diaxial relationship between H-2/H-3. The $J_{3,4}$ value of 4.0 Hz revealed the pseudo-equatorial orientation of H-4 [8]. On selective irradiation of H-7, signal enhancement of H-2 was measured, whereas no NOE for H-3 was observed. Accordingly, the benzoyloxymethyl at C-1 was deduced to be in pseudo-equatorial position, or *cis* to H-2.

The CD spectrum of **3** exhibited a positive Cotton effect a λ 236 nm ($\Delta\varepsilon + 9.36$) and a negative Cotton effect at λ 219 nm ($\Delta\varepsilon - 8.12$), indicating that the two benzoyl groups were oriented in a clockwise manner (Fig. 2). Since the overall relative configuration was already known, the absolute configuration of **3** was established as 1*S*,2*S*,3*R*,4*R*.

EXPERIMENTAL

Plant material. Roots of *Uvaria grandiflora* Roxb. were collected in July 1993 in Yongxing county, Hainan province, Southern China. The species was identified by Prof. Wan-Zhi Song of the Institute of Materia Medica, Chinese Academy of Medical Sciences.

Table 1. ^{1}H NMR data of uvarigranol G (**1**), H (**2**) and I (**3**) (500 MHz, $CDCl_3$)

H	**1**	**2**	**3**
2	3.98 *d* (7.6)	5.85 *d* (7.0)	5.90 *d* (10.7)
3	5.82 *m*	6.04 *m*	5.58 *dd* (10.7,4.0)
4	5.82 *m*	6.04 *m*	5.91 *dd* (5.2,4.0)
5	5.77 *ddd* (10.0,1.8,2.0)	5.98 *ddd* (10.1,2.0,4.1)	6.15 *dd* (9.9,5.2)
6	4.39 *br d* (2.0)	5.42 *d* (4.1)	6.06 *d* (9.9)
7	4.56 *d*, 4.63 *d* (11.1)	4.39 *d*, 4.33 *d* (12.0)	4.20 *d*, 4.48 *d* (11.3)
ArH	7.3–8.1 *m*	7.3–8.1 *m*	7.3–8.1 *m*
OAc	—	2.15 *s*, 1.93 *s*	2.12 *s*, 1.97 *s*

* The data in parentheses are *J* values (in Hz).

A voucher specimen is deposited in the Herbarium of the Botany Department of the same institute.

Extraction and isolation. The air-dried and ground roots (10 kg) were extracted with 95% EtOH under reflux. The extract was concd *in vacuo* and fractionated into $CHCl_3$- and H_2O-soluble frs. The $CHCl_3$-soluble fr. was further partitioned between petrol and 90% MeOH. Repeated chromatography of the 90% MeOH fr. over silica gel using petrol–EtOAc (3:1), $CHCl_3$–MeOH (19:1) and petrol–Me_2CO (2:1) as eluents yielded compounds **1** (95 mg), **2** (42 mg), **3** (60 mg), **4** (20 mg) and **5** (50 mg), together with the previously isolated compounds [3–5].

Uvarigranol G (**1**). White powder, mp 67–69°, $[\alpha]_D^{16} -44°$ (c 0.08, $CHCl_3$); Anal. calcd for $C_{21}H_{20}O_7$, C 65.62, H 5.21; found C 64.45, H 5.15; IR ν_{max}^{KBr} cm^{-1}: 3440, 1710, 1608, 1456, 1320, 1275, 1110, 1025, 710; ^1H NMR: Table 1; EIMS m/z (rel. int.): $[M+H]^+$ 385(0.5), $M+H\text{-}H_2O]^+$ 367(1), 231(4), 215(5), 203(5), 190(8), 163(3), 140(2), 127(2), 123(15), 122(12), 110(7), 105(100), 99(20), 94(3), 81(5), 77(50).

Oxidation of **1** *to* **1a**. Uvarigranol G (**1**) (10 mg) and pyridinium chlorochromate (6 mg) were dissolved in dry CH_2Cl_2 (1 ml), and stirred for 1 h, by which time the starting product had disappeared (TLC monitor). The usual work up gave 9 mg of a residue which was purified by prep. TLC to yield 5 mg of **1a**: crystals, mp 157–158°, $[\alpha]_D^{15} -24°$ (c 0.02, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1} 3430, 1710, 1700, 1608, 1455, 1382, 1320, 1274, 1115, 1100, 715; ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.06 (1H, *br s*, OH), 4.00 (1H, *br s*, OH), 4.38 (1H, *dd*, J = 3.4, 1.2 Hz, H-2), 4.59 (1H, *d*, J = 11.5 Hz, H-7A), 4.86 (1H, *d*, J = 11.5 Hz, H-7B), 5.95 (1H, *dd*, J = 3.4, 4.1 Hz, H-3), 6.53 (1H, *d*, J = 10.1 Hz, H-5), 6.98 (1H, *ddd*, J = 10.1, 4.1, 1.2 Hz, H-4), 7.3–8.1 (10 H, *m*, ArH). In the same way, zeylenol (**6**) was also converted into **1a**.

Uvarigranol H (**2**). Amorphous powder: mp 50–52°, $[\alpha_D^{16} -38°$ (c 0.07, $CHCl_3$); Anal. calcd for $C_{25}H_{24}O_9$, C 64.10, H 5.13; found C 63.55, H 5.25 IR ν_{max}^{KBr} cm^{-1} 3470, 1727, 1610, 1450, 1246, 1115, 1026, 712; UV λ_{max}^{EtOH} (log ε): 228(4.3), 273(3.0); ^1H NMR: Table 1; EIMS m/z (rel. int.): $408[M\text{-}AcOH]^+$ (1), 395(0.5), 346(1), 335(1), 273(9), 245(5), 232(2), 226(2), 203(3), 182(4), 164(2), 153(2), 140(1), 122(5), 105(100), 101(3), 94(1), 77(8); CD λ_{max}^{EtOH} ($\Delta\varepsilon$): 221(+11.25), 236(−22.14).

Uvarigranol I (**3**). White powder, mp 132–134°, Anal calcd for $C_{25}H_{24}O_9$, C 64.10, H 5.13: found C 63.62, H 5.24; IR ν_{max}^{KBr} cm^{-1}: 3468, 1750, 1724, 1602, 1435, 710; UV (EtOH) λ_{max}^{EtOH} (log): 229(3.8), 273(3.0), 281(2.9); ^1H NMR: Table 1; EIMS m/z (rel. int.): $451[MH\text{-}H_2O]^+$ (0.5), $409[MH\text{-}AcOH]^+$ (1), $349[MH\text{-}2AcOH]^+$ (2), $347[MH\text{-}BzOH]^+$ (1), 333(4), 324(0.5), 273(8), 244(2), 231(4), 221(3), 193(1), 169(15), 127(7), 122(3), 123(3), 110(8), 109(2), 105(100), 94(2), 77(23); CD (EtOH) λ_{max}^{EtOH} ($\Delta\varepsilon$): 219(−8.12), 236(+9.39).

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