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TWO POLYOXYGENATED CYCLOHEXENES FROM *UVARIA GRANDIFLORA*

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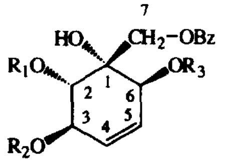
Key Word Index—*Uvaria grandiflora*; Annonaceae; roots; cyclohexenes; uvarigranols C and D.

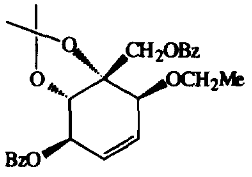
Abstract—Two new polyoxygenated cyclohexenes with a rare ethoxy group, named uvarigranols C and D, were isolated from the roots of *Uvaria grandiflora*. Their structures were established by spectroscopic and chemical methods.

INTRODUCTION

In the course of our phytochemical studies on *Uvaria grandiflora*, we have recently isolated two new compounds, uvarigranol A(**2**) and B(**3**), and a known compound zeylenol(**1**) [1,2], which belong to a new class of polyoxygenated cyclohexenes mostly found in the genus *Uvaria* and *Piper* [3,4]. In this report, we describe the isolation and structural elucidation of two other novel compounds of this type, named uvarigranols C(**4**) and D(**5**) from an ethanol extract of the roots of *U. grandiflora*. Both new compounds have the rare ethoxyl substituent on the cyclohexene ring.



	R₁	R₂	R₃
1	H	Bz	H
2	H	H	Bz
3	Ac	Bz	H
4	H	Bz	Et
5	Ac	Bz	Et



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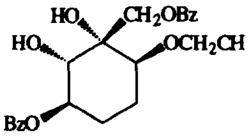

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RESULTS AND DISCUSSION

Compound **4** was obtained as white needles. The FAB-mass spectrum gave a $[M + H]^+$ at m/z 413, indicating a M_r of 412; the molecular formula was established as $C_{23}H_{24}O_7$ by elemental analysis. The IR spectrum of **4** suggested the presence of hydroxyl group(s) (3575, 3472, 1111 and 1090 cm^{-1}), ester group(s) (strong absorption at 1698 cm^{-1}) and a monosubstituted phenyl ring (1601, 1449 and 714 cm^{-1}). The UV spectrum gave absorption maxima at λ230, 274 and 280 nm, indicating the presence of one or two benzoyl groups.

The most informative evidence about the structure of **4** was derived from its ^{1}H NMR spectral data, which, together with those of other related compounds, is summarized in Table 1. The presence of two benzoyl groups was confirmed by the aromatic proton signals between δ7.3–8.1 (10H, *m*). The presence of an ethoxy group was suggested by the signals at δ3.76 (1H, *dq*, $J = 9.1, 7.0$ Hz), 3.63 (1H, *dq*, $J = 9.1, 7.0$ Hz) and 1.22 (3H, *t*, $J = 7.0$ Hz), which consisted of a typical ABX_3 coupling system; this was supported by the EI-mass fragment ion at m/z 367 due to the direct loss of an ethoxyl radical from the

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Table 1. ^{1}H NMR spectral data of compounds **1** and **4**–**7** (500 MHz, $CDCl_3$)

Proton	**1**	**4**	**5**	**6**	**7**
H-2	4.32 *d* (6.1)*	4.22 *d* (5.6)	5.68 *d* (6.8)	4.54 *d* (3.1)	3.95 *d* (9.0)
H-3	5.70 *dddd* (6.1, 2.6, 1.6, 1.1)	5.68 *ddd* (5.6, 2.8, 1.5)	5.81 *ddd* (6.8, 2.6, 1.7)	5.73 *ddd* (3.1, 2.5, 1.5)	5.25 *td* (9.0, 4.6)
H-4	5.88 *ddd* (10.1, 2.6, 0.7)	5.88 *dd* (10.1, 2.8)	5.93 *dd* (10.1, 2.6)	5.82 *ddd* (10.1, 2.5, 1.0)	1.90 *m*
H-5	5.99 *ddd* (10.1, 4.0, 1.6)	6.07 *ddd* (10.1, 3.5, 1.5)	6.07 *ddd* (10.1, 3.9, 1.7)	5.97 *ddd* (10.1, 2.1, 1.5)	1.90 *m*
H-6	4.32 *ddd* (4.0, 1.1, 0.7)	4.05 *d* (3.5)	4.01 *d* (3.9)	4.17 *dd* (2.1, 1.0)	3.68 *br s*
H-7A, B	4.75, 4.89 each *d* (12.3)	4.78, 4.74 each *d* (12.1)	4.64, 4.60 each *d* (12.1)	4.78, 4.44 each *d* (12.2)	4.81, 4.60 each *d* (11.6)
Ar-H	7.3–8.1 *m*	7.3–8.1 *m*	7.3–8.1 *m*	7.3–8.1 *m*	7.4–8.1 *m*
OH	3.18, 2.96, 3.32	3.10, 3.00 *br s*	2.8 *br s*	—	3.0 *br s*
OEt	—	3.73 *dq* (9.1, 7.0) 3.63 *dq* (9.1, 7.0) 1.22 *t* (7.0)	3.76 *dq* (9.1, 7.0) 3.64 *dq* (9.1, 7.0) 1.22 *t* (7.0)	3.89 *dq* (9.1, 7.0) 3.71 *dq* (9.1, 7.0) 1.26 *t* (7.0)	3.63 *dq* (9.1, 7.0) 3.39 *dq* (9.1, 7.0) 1.19 *t* (7.0)
OAc	—	—	2.06 *s*	—	—

*Data in parentheses are *J* values (in Hz).

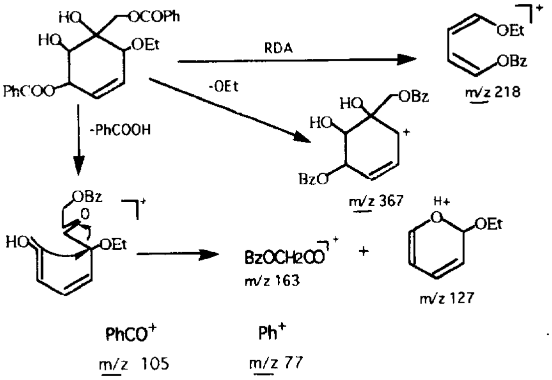

Fig. 1. Major fragment ions in EI–mass spectrum of uvarigranol C (**4**).

$[M]^+$. The ^{1}H NMR spectrum also showed two olefinic protons at δ6.07 and 5.88 with a typical coupling constant of 10.1 Hz, a carbinyl proton (secondary alcohol) at δ4.22 and two hydroxyl protons at δ3.10 and 3.0. The two benzoxy groups were clearly at C-7 and C-3 from the down-field shifts of H-7 at δ4.78 and 4.74 (each 1H, *d*, $J = 12$ Hz) and H-3 at 5.68 (*ddd*, $J = 5.6, 2.8$ and 1.5 Hz), whilst the ethoxyl group was established at C-6 from the upfield shift of H-6 at δ4.05 (*d*, $J = 3.5$ Hz) in comparison with the ^{1}H NMR spectral data of **1**. The coupling constant $J_{2,3}$ of 5.6 Hz suggested that the relative stereochemistry of the two substituents at C-2 and C-3 was favourably *trans*-diequatorial; in addition, $J_{5,6}$ of 3.5 Hz suggested that the ethoxyl group at C-6 was probably pseudo-axial [5].

Treatment of **4** with acetone yielded a ketal derivative (**6**), the isopropylidene moiety in the structure being indicated by the proton signals at δ1.56 (3H, *s*) and 1.45 (3H, *s*). Formation of the cyclic isopropylidene demonstrated the *cis*-vicinal relationship between C_1-OH and C_2-OH in **4**.

The above assignment of the stereochemical structure of **4** was confirmed by the preparation of the dihydro derivative (**7**). The ^{1}H NMR spectrum of **7** showed the H-3 signal at δ5.25 (*td*, $J = 9.0$ and 4.6 Hz) and H-2 at δ3.95 (*d*, $J = 9.0$ Hz); the large $J_{2,3}$ value (9.0 Hz) was in agreement with their *trans*-diaxial stereochemistry. H-6 of **7** appeared as a singlet at δ3.68 ($1/2W < 5$ Hz) corresponding to its equatorial orientation.

With reference to the fragmentation pattern of compound **1** [2], some major fragment ions of **4** are summarized in Fig. 1; these also supported the proposed structure of **4**.

The absolute configuration shown for **4** was based on comparison with compound **1**. The two benzoxyl groups at C-3 and C-7 have the same relative stereochemistry and their CD spectra exhibited a similar strong negative Cotton effect (**1**, λ226 nm, $\Delta\varepsilon - 13.5$; IV, λ229 nm, $\Delta\varepsilon - 15.5$). Thus, **4** must have an absolute configuration identical to that of **1**.

Compound **5** was obtained as a white solid. Its IR spectrum was similar to that of **4**, but exhibited one more ester carbonyl absorption band at 1752 cm^{-1}. The ^{1}H NMR spectrum showed one more three-proton singlet at δ2.06, as well as a down-field shift of H-2 from δ4.22 to 5.68 in comparison with that of **4**. These observations suggested that compound **5** was probably an acetate derivative of **4**. Acetylation of **4** using Ac_2O-pyridine established the correlation between **4** and **5**. Therefore, compound **5** was identified as the 2-acetate of **4**.

Compounds **4** and **5** named uvarigranols C and D, respectively, are new compounds. This is the first report of naturally occurring cyclohexenes with a rare ethoxyl group.

Supplementary extraction of the same plant material using $CHCl_3$, instead of 95% EtOH as solvent and subsequent chromatographic separation on a small scale also yielded compounds **4** and **5**. In addition, heating zeylenol (**1**) in 95% EtOH at 80° for 10 hr did not show any chemical changes. These results ruled out the possibility that these two ethoxylated products were artefacts derived from the corresponding alcohols due to the extracting conditions using EtOH under reflux.

EXPERIMENTAL

MPs are uncorr. IR spectra were recorded in KBr. Specific rotations were measured in MeOH.

Plant material. Roots of *U. grandiflora* Roxb. were collected in Hainan Island (southern China) in July 1993. The species was identified by Prof. Song Wan-Zhi of the authors' institute. A voucher specimen is deposited in the Herbarium of the Botany Department (Institute of Materia Medica, Chinese Academy of Medical Sciences).

Extraction and isolation. Air-dried and finely powdered roots (10 kg) were extracted × 3 with 95% EtOH under reflux. The solvent was dist. off, the residue partitioned between $CHCl_3$ and H_2O and the $CHCl_3$ solubles thus further partitioned between petrol and 90% MeOH. Repeated chromatography of the 90% MeOH fr. over silica gel using petrol–EtOAc (3:2), $CHCl_3$–MeOH (19:1) and petrol–Me_2CO (2:1) as eluents yielded the new compounds **4** (1.5 g) and **5** (5.8 g), together with the previously isolated compounds **1**–**3**.

Compound **4**. Recrystallized from $CHCl_3$, white needles, mp 161–163°. Analysis: calcd for $C_{23}H_{24}O_7$: C, 66.98%; H, 5.83%; found: C, 66.39%; H, 5.65%. $[\alpha]_D^{15}$ − 45.56° (*c* 0.124, MeOH). IR ν_{max} cm^{-1}: 3575, 3472, 3100, 2973, 1698, 1601, 1449, 1320, 1280, 1111, 1090, 714. UV λ_{max} nm (log): 230 (4.21), 274 (1.41), 280 (1.37). EIMS *m/z* (rel. int.): 367 $[M - OEt]^+$ (0.3), 231 (2), 218 (10), 215 (1), 163 (2), 150 (1), 140 (3), 127 (38), 123 (3), 122 (5), 105 $[PhCO]^+$ (100), 99 (6), 81 (2), 77 $[Ph]^+$ (23). ^1H NMR: see Table 1. CD: λ_{max} 229 nm, $\Delta\varepsilon$ −15.5 (EtOH).

Compound **5**. White powder, mp 51–53°. Analysis: calcd for $C_{25}H_{26}O_8$: C, 66.07%; H, 5.73; found: C, 66.01%; H, 5.74%. $[\alpha]_D^{24}$ − 47.27° (*c* 0.165, MeOH). IR ν_{max} cm^{-1}: 3450, 2976, 1752, 1719, 1601, 1585, 1451, 1315, 1268, 1225, 1100, 710. UV λ_{max} nm (log): 230 (3.75), 273 (2.10), 282 (1.76). ^1H NMR: see Table 1. CD: λ_{max} 224 nm, $\Delta\varepsilon$ − 18.6 (EtOH).

Acetylation of **4**. Compound **4** (80 mg) was dissolved in pyridine (0.5 ml) and treated with Ac_2O (1 ml) overnight at room temp. Usual work-up gave a white solid (78 mg). Its physical data (IR, mp, $[\alpha]_D$) and TLC R_f values in various solvent systems were identical to **5**.

Preparation of **6**. Compound **4** (50 mg) was stirred in anhydrous Me_2CO (5 ml) with $ZnCl_2$ and 2 drops of H_3PO_4 at room temp. for 20 hr. The reaction soln was evapd in vacuo and isolated by prep. TLC using petrol–EtOAc (4:1). The isopropylidene derivative (**6**) was obtained as a colourless gum (20 mg). ^1H NMR: δ 1.56, 1.45 (each 3H, *s*, $2 \times CH_3$), other spectral data listed in Table 1.

Hydrogenation of **4**. Compound **4** (50 mg) was dissolved in 20 ml EtOH and hydrogenated using 5% Pd/C (5 mg) as catalyst under a H_2 pressure of 2.5 kg/cm^{-2} for 3 hr. The filtrate on concn and chromatography afforded **7** (43 mg). White solid, mp 43–45°. $[\alpha]_D^{16}$ + 23.97° (*c* 0.1189, $CHCl_3$). ^1H NMR: see Table 1.

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