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THREE CYCLOHEXENE OXIDES FROM *UVARIA GRANDIFLORA*

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Key Word Index—*Uvaria grandiflora*; Annonaceaes; cyclohexene oxides; zeylenone; grandiflorone; grandifloracin.

Abstract—Three new cyclohexene oxides, zeylenone, grandiflorone and grandifloracin, were isolated from the stem and leaves of *Uvaria grandiflora* and their structures established on the basis of spectral data. Zeylenone was found to be a highly active nucleoside transport inhibitor. ©1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Cyclohexene oxides are mostly found in the genera *Uvaria* and *Piper* [1, 2]. Previously, we reported on the isolation of two known cyclohexene oxides, zeylenol (**1**) and chlorohydrin pipoxide (**2**) from [3] *U. grandiflora*. Further investigation of the CH_2Cl_2 part of the extract has led to the isolation of compounds **3**–**5**. Compound **3** was shown to inhibit thymidine and uridine transport markedly in Ehrlich carcinoma cells. This paper describes the characterization of **3**–**5**.

RESULTS AND DISCUSSION

Compound **3** showed three ion peaks at *m/z* 383 $[M+H]^+$, 405 $[M+Na]^+$ and 421 $[M+K]^+$ in its positive-ion FAB mass spectrometry and 21 resonance signals in its ^{13}C-^1H COLOC. From this data, its molecular formula was determined to be $C_{21}H_{18}O_7$. The EIMS of **3** was very similar to that of **1** in that it showed the same fragments as **1** at *m/z* 163, 122, 105 and 77, but the fragments at *m/z* 260, 201, 188, 138 and 97 were 2 amu lower than those of **1**. Thus **3** was presumed to be an analogue of **1**. By comparison of the ^1H and ^{13}C NMR spectra of **1** and **3**, it was confirmed that a hydroxyl group (δ_{CH} 4.33; δ_C 70.9) in **1** was replaced by a ketone group (δ_C 196.6) in **3**. The ketone group was located on C-6 as indicated by its mass spectral fragments and ^1H-^1H COSY.

The relative stereochemistry of **3** was established from the coupling constants 4.2 Hz ($^3J_{3,4}$), which showed H-4 was equatorial to H-3 (allylic couplings have equatorial values of about 4 Hz or axial values of 1.9–2.6 Hz [4, 5]), and 1.2 Hz $^4J_{2,4}$, which showed H-4 was equatorial to H-2 (*W*-coupling), also **3** formed ketal **3a** with acetone and $CuSO_4$ indicating that 2-OH and 1-OH were on the same side. Thus, the structure of **3** was established as 6-ketone zeylenol, named as zeylenone.

Compound **4**, named as grandiflorone, showed three ion peaks at *m/z* 383 $[M+H]^+$, 405 $[M+Na]^+$ and 421 $[M+K]^+$ in its positive-ion FAB-mass spectrum, and 21 resonance signals in its ^{13}C-^1H COLOC. Thus, its molecular formula was determined to be $C_{21}H_{18}O_7$. Analysis of the ^1H NMR, DEPT and ^{13}C-^1H COLOC spectra indicated that **4** contained two benzoyl groups, one ketone group ((δ_C 206.8) and five oxycarbons (δ_C 76.2, 75.9, 75.2, 70.0 and 67.1). There were only seven oxygen atoms in the molecule, so the structure had to contain one oxo-bridge. The HMBC experiment (Fig. 1) indicated that H-7 (δ_H 4.22, 3.89) coupled with the oxymethylene at δ_C 70.0 (C-4) and the oxo-bridge located between C-4 and C-7. The ^3J coupling of H-2 (δ_H 5.65) to the ester carbonyl at δ_C 165.0 and H-3 (δ_H 5.29) to the ester carbonyl at δ_C 164.2, indicated that the two benzoyl groups were located at C-2 and C-3.

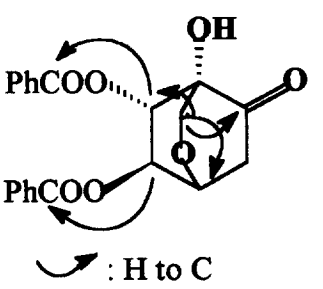
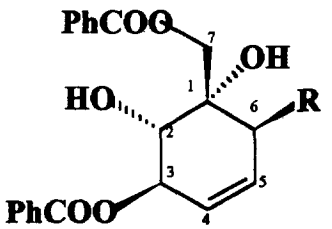
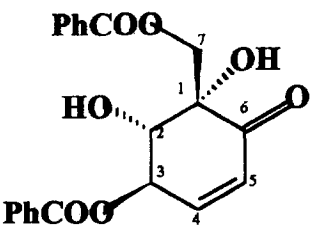


Fig. 1. Key HMBC correlations of **4**.



1 R=OH
2 R=Cl



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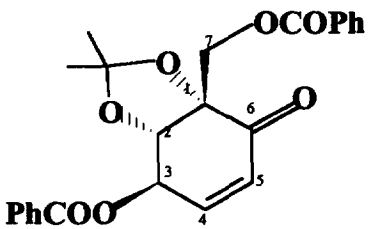
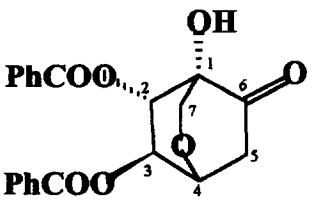
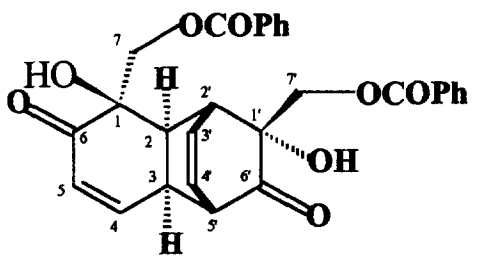


3a



4

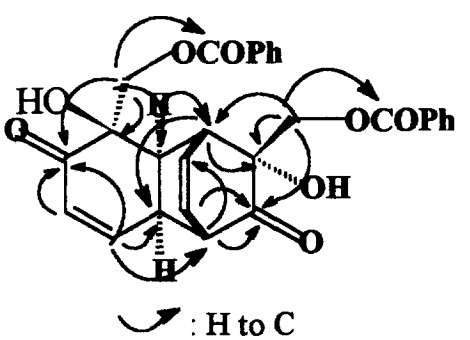


5

The relative stereochemistry of **4** was established from a NOESY spectrum. Interactions between H-2 (δ_H 5.65) and H-7 (δ_H 4.22) indicated that they were on the same side of the molecule, while those between H-3 (δ_H 5.29) and H-5 (δ_H 2.76) indicated H-3 was on the other side.

Compound **5**, named as grandifloracin, showed two ion peaks at *m/z* 489 $[M+H]^+$ and 511 $[M+Na]^+$ in its positive-ion FAB-mass spectrum, and 28 resonance signals in its ^{13}C-^1H COLOC. Thus its molecular formula was determined to be $C_{28}H_{24}O_8$. The ^1H NMR spectrum of **5** showed the presence of two oxymethylenes (C-7, δ_C 71.8 and C-7′, δ_C 68.2), two benzoyl groups, two ketone groups (C-6, δ_C 198.1 and C-6′, δ_C 208.0), two double bonds (C-4, δ_C 146.6/C-5, δ_C 128.1 and C-3′, δ_C 135.2/C-4′, 128.5), two oxygen-bearing aliphatic carbons (C-1, δ_C 75.4 and C-1′, δ_C 74.4) and four methylenes (C-2, δ_C 37.4, C-3, δ_C 40.0, C-2′, δ_C 41.1 and C-5′, δ_C 52.2). Analysis of the ^1H-^1H COSY and HMBC spectra showed that **5** continued two cyclohexene moieties (one moiety included C-1 to C-7, the other included C-1′ to C-7′) (Fig. 2). In addition, by means of the cross-peaks in the ^1H-^1H COSY between H-2/H-2′ and H-3/H-5′ and TOCSY (which showed that H-2, H3, H-2′, H-3′, H-4′ and H-5′ were in one spin coupling system) spectra, it was established that the two cyclohexene moieties were linked by two carbon–carbon bonds and two bonds located on C2-C2′ and C3-C5′. The stereo relationships were obtained by NOESY cross-peaks of between H-7 and H-2, H-7 and H-3 (which indicated that C-7, H-2 and H-3 were on the same side) and H-7′ and H-3′ (which showed these same sides of C-3′ and C-7′). On the basis of the above findings, grandifloracin, a bicyclohexene oxide, was suggested to have structure **5**.

Compound **3** showed remarkable inhibition of nucleoside transport in Ehrlich carcinoma cells. IC_{50} values for thymidine transport and uridine transport



: H to C

Fig. 2. Key HMBC correlations of **5**.

Table 1. NMR spectral data of compounds **3** and **4** ($CDCl_3$)*

	3		**4**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	77.2 (C)		75.2 (C)	
2	71.7 (CH)	4.36 (*ddd*, *J* = 4.2, 2.7, 1.2)	75.9 (CH)	5.65 (*t*, *J* = 1.1)
3	69.4 (CH)	5.94 (*td*, *J* = 4.2, 1.0)	76.2 (CH)	5.29 (*dd*, *J* = 2.2, 1.1)
4	142.7 (CH)	6.93 (*ddd*, *J* = 10.0, 4.2, 1.2)	70.0 (CH)	4.59 (*m*)
5	128.6 (CH)	6.30 (*dd*, *J* = 10.0, 1.0)	40.5 (CH_2)	3.07 (*dd*, *J* = 19.4, 4.5)
				2.76 (*d*, *J* = 19.4)
6	196.6 (C)		206.8 (C)	
7	65.3 (CH_2)	4.82 (*d*, *J* = 11.6)	67.1 (CH_2)	4.22 (*d*, *J* = 9.6)
		4.58 (*d*, *J* = 11.6)		
1-OH†		4.14 (*s*)		
2-OH†		3.29 (*d*, *J* = 2.7)		

* Assignment based on DEPT, COSY, COLOC and HMBC.
† Disappeared after addition of D_2O.

were 8.2 μM and 10.1 μM, respectively. As reported, new nucleoside transport inhibitors have been found in plants and microbes and these substances may be active as antitumour agents or as biochemical modulators potentiating the effect of other drugs [6]. Compound **3** displayed cytotoxicity to cultured cancer cells and showed equal potency against sensitive and multidrug resistant cell lines. As determined by MTT assay, the IC_{50} values of **3** for mammary carcinoma MCF-7 cell line and its resistant MCF-7/ADM subline were 2.2 μM and 2.6 μM and those for oral epidermoid carcinoma KB cell line and the resistant KB/VCR subline were 0.48 μM and 0.56 μM, respectively. When used in combination, **3** potentiated the cytotoxicity of 5-fluorouracil and methotrexate against MCF/ADM cells. Compounds **4** and **5** were inactive.

EXPERIMENTAL

^1H, ^{13}C NMR, DEPT and 2D NMR spectra were recorded on a Bruker AM 500 spectrometer in $CDCl_3$ using TMS as int. standard. EIMS (70 eV) and FABMS were determined on a MAT-711 mass spectrometer. Mps are uncorr.

Plant material. The stem and leaves of *Uvaria grandiflora* were collected in the Hainan province of China in July 1988, and identified by Professors Lin Shouqun and Lian Wenyan of this institute.

Extraction and isolation. The dried plant material (8.5 kg) was extracted with EtOH × 3 at 40° and the combined extract was evapd under red. press. at 55° to give 130 g syrup. The syrup was dissolved in dil. HCl and the insoluble fraction was then extracted with CH_2Cl_2, EtOAc and MeOH successively under reflux in a Soxhlet apparatus. The CH_2Cl_2 extract (34 g) was chromatographed over a silica gel column, which was eluted with a petrol–Et_2O gradient to afford 10 combined frs (F1–F10). Purification of F8 by silica gel CC (petrol–Et_2O, 1:1) yielded zeylenone (**3**) (3500 mg). Grandiflorone (**4**) (40 mg) and grandifloracin (**5**) (35 mg) were obtained by silica gel CC [petrol–Et_2O **4**: (7:3), **5**: (3:7)] of F7.

Zeylenone (**3**). Needles, mp 155–156°, $[\alpha]_D^{20}$ −126.5° ($CHCl_3$; *c* 0.747); UV $\lambda_{max}^{CHCl_3}$ nm: 224, 268; IR ν_{max}^{KBr} cm^{-1}: 3405, 1710, 1700, 1690, 1600, 1580, 1495, 1275, 1105, 1100, 710. EIMS *m/z* (rel. int.): 260 (2.6), 219 (1.1), 201 (1.8), 188 (2.6), 163 (1.8), 138 (3.8), 123 (3.3), 122 (3.2), 110 (2.6), 105 (100), 97 (20.8), 77 (20.2); FABMS *m/z* (rel. int.): 383 $[M+H]^+$, 405 $[M+Na]^+$ and 421 $[M+K]^+$; ^1H NMR: two benzoyl groups: δ 8.00 (2H, *m*), 7.91 (2H, *m*), 7.52 (2H, *m*), 7.38 (4H, *m*), other data are given in the Table 1; ^{13}C NMR: two benzoyl groups: 128.4 (2C), 128.6 (2C), 128.8, 129.1, 129.7 (2C), 129.9 (2C), 133.4, 133.7, 165.4, 166.2, other data are given in Table 1.

Grandiflorone (**4**). Needles, mp 88–90°, $[\alpha]_D^{21.5}$ −140.0° ($CHCl_3$; *c* 0.497); UV $\lambda_{max}^{CHCl_3}$ nm: 226, 272; IR ν_{max}^{KBr} cm^{-1}: 3410, 1730, 1705, 1700, 1600, 1580, 1495, 1270, 1105, 710; FABMS *m/z* (rel. int.): 383 $[M+H]^+$, 405 $[M+Na]^+$ and 421 $[M+K]^+$; ^1H NMR: two benzoyl groups: δ 8.10 (2H, *m*), 7.90 (2H, *m*), 7.58 (1H, *m*), 7.56 (1H, *m*), 7.47 (2H, *m*), 7.41 (2H, *m*), other data are given in the Table 1; ^{13}C NMR: two benzoyl groups: 128.8 (4C), 128.8, 129.1, 129.8 (2C), 130.0 (2C), 133.6, 133.7, 164.2, 165.0, other data are given in Table 1.

Grandifloracin (**5**). Needles, mp 161–163°, $[\alpha]_D^{21.6}$ −13.6° ($CHCl_3$; *c* 0.728); UV $\lambda_{max}^{CHCl_3}$ nm: 224, 268; IR ν_{max}^{KBr} cm^{-1}: 3460, 3400, 1715, 1705, 1690, 1600, 1580, 1492, 1270, 1100, 710; EIMS *m/z* (rel. int.): 442 (0.5), 415 (0.6), 294 (0.7), 226 (1.7), 198 (8.2), 185 (2.7), 122 (11.2), 105 (100), 77 (29.1); FABMS *m/z*: 511 $[M+Na]^+$, 489 $[M+H]^+$; ^1H NMR: two benzoyl group: δ 8.04 (2H, *m*), 7.92 (2H, *m*), 7.55 (2H, *m*), 7.46 (4H, *m*); other data are given in the Table 2; ^{13}C NMR: two benzoyl groups: δ 128.4, 128.5, 129.3, 129.7, 129.9, 133.3, 133.4, 165.8, 165.9; other data are given in Table 2.

Ketal of **3** (**3a**). Compound **3** (35 mg) was dissolved

Table 2. NMR spectral data of compound **3** ($CDCl_3$)*

C	δ_C	δ_H (*J* in Hz)
1	75.4 (C)	
2	37.4 (CH)	3.34 (*dd*, *J* = 8.0, 1.9)
3	40.0 (CH)	3.42 (*m*)
4	146.6 (CH)	6.58 (*dd*, *J* = 10.0, 4.2)
5	128.1 (CH)	6.21 (*dd*, *J* = 10.0, 1.5)
6	198.1 (C)	
7	71.8 (CH_2)	4.37 (*d*, *J* = 11.0)
		4.40 (*d*, *J* = 11.0)
1′	74.4 (C)	
2′	41.1 (CH)	3.67 (*dt*, *J* = 6.5, 1.9)
3′	135.2 (CH)	6.43 (*ddd*, *J* = 9.5, 6.5, 1.3)
4′	128.5 (CH)	6.01 (*ddd*, *J* = 9.5, 6.5, 1.9)
5′	52.2 (CH)	3.34 (*ddd*, *J* = 6.5, 2.2, 1.3)
6′	208.0 (C)	
7′	68.2 (CH_2)	4.23 (*d*, *J* = 12)
		4.46 (*d*, *J* = 12)

* Assigned based on DEPT, COSY, COLOC and HMBC.

in Me_2CO (4 ml) and $CuSO_4$ (150 mg) added. The reaction mixt. was refluxed at 70° for 4 hr. Removal of solvent and $CuSO_4$ left the crude extract which was purified by silica gel to give **3a** (20 mg) as wax. IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1730, 1700, 1690, 1600, 1580, 1498, 1280, 1100 and 710; ^1H NMR (500 MHz, $CDCl_3$): 7.95 (2H, *dd*, *J* = 8.0, 1.2 Hz), 7.85 (2H, *dd*, *J* = 8.0, 1.2 Hz), 7.50 (2H, *m*), 7.35 (2H, *td*, *J* = 8.0, 1.2 Hz), 7.28 (2H, *td*, *J* = 8.0, 1.2 Hz), 7.04 (1H, *ddd*, *J* = 10.2, 4.4, 1.5 Hz), 6.38 (1H, *d*, *J* = 10.2 Hz), 5.97 (1H, *dd*, *J* = 4.4, 1.5 Hz), 4.70 (1H, *d*, *J* = 12 Hz), 4.68 (1H, *t*, *J* = 1.5 Hz), 4.63 (1H, *d*, *J* = 12 Hz), 1.48 (3H, *s*), 1.40 (3H, *s*).

Bioactivity. Activity of nucleoside transport inhibition was determined by radiolabeled precursor assay. Briefly, Ehrlich carcinoma cells from ascites were pre-incubated with the drug at 37° for 5 min, then incubated with radiolabeled thymidine or uridine for 30 sec. The operation was performed one sample at a time. The radioactivity was measured by a Beckman liquid scintillation system [7].

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