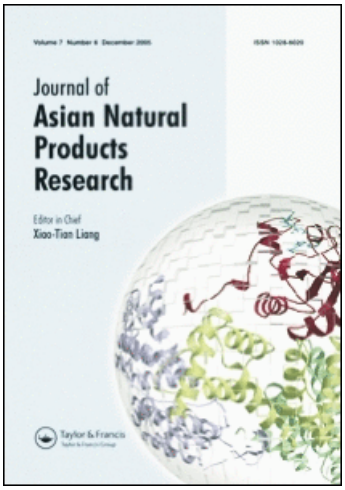

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Uvamalols D-G: Novel polyoxygenated seco-cyclohexenes from the roots of *Uvaria macrophylla*

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UVAMALOLS D–G: NOVEL POLYOXYGENATED SECO-CYCLOHEXENES FROM THE ROOTS OF *UVARIA MACROPHYLLA*

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Uvamalols D–F (**1**–**4**), novel polyoxygenated seco-cyclohexenes, were isolated from the roots of *Uvaria macrophylla,* and their structures were elucidated by interpretation of spectral data.

Keywords: *Uvaria macrophylla*; Annonaceae; Uvamalols D, E, F and G

INTRODUCTION

Uvaria macrophylla belongs to the genus *Uvaria* of the family Annonaceae, widely distributed in Hainan, Guangdong, and Guangxi provinces of southern China [1]. Acetogenins, alkaloids, terpenoids, and flavonoids have been reported in the literature, among which acetogenins exhibited strongly inhibitory activities toward a number of human cancer cell lines [2–6]. In the course of our ongoing screening for antitumour agents from annonaceous plants, we discovered that an EtOH extract from the roots of the title plant showed significant cytotoxicities against mouse lymphocytic leukaemia cells in a preliminary biological screening procedure. Bioassay-directed fractionation of the EtOH extract led to the isolation of thirty-two compounds. In the present paper, we continue to report new compounds isolated from this plant, uvamalols D–G (**1**–**4**), a new type of polyoxygenated seco-cyclohexenes [2,6].

RESULTS AND DISCUSSION

Uvamalol D (**1**) was isolated as white needles, mp 101–103°C, $[\alpha]_D^{23}$ +68 (*c* 0.15, $CHCl_3$), Its ESIMS gave a protonated molecular ion peak at *m/z* 447 and its isotopic peak at *m/z* 449

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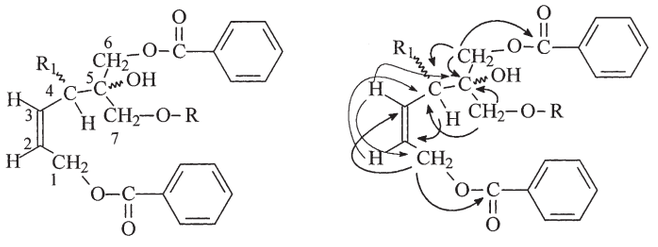
allowing a molecular formula of $C_{23}H_{23}O_7Cl$ to be determined in combination with elemental analysis.

The IR spectrum (KBr, cm^{-1}) displayed absorption bands for hydroxyl (3421), carbonyl (1732 and 1705), and aromatic moieties (1601, 1583 and 1493). The presence of 10 aromatic proton signals at δ 7.30–8.10 (10H, m) in the ^1H NMR spectrum, together with the signals for 10 carbons at δ 166.4, 166.3, 133.4, 133.1, 129.6, 129.5, 129.2, 129.1, 128.5 and 128.1 in the ^{13}C NMR spectrum, as well as the basic ion peak at *m/z* 105 in the EIMS, indicated the presence of two benzoyls in the molecule [3]. Similarly, the signals for the protons at δ 2.01 (3H, s) and the carbons at δ 170.8 and 20.7 suggested that there is an acetyl; in addition, the signals for the two coupling olefinic protons at δ 6.04 (1H, t, $J = 10.0$ Hz) and 5.95 (1H, dt, $J = 10.0$, 6.6 Hz) indicated that there is a double bond in the structure. Analysis of the data of the ^{13}C and DEPT NMR for **1** showed that the signals at δ 64.9, 64.8 and 60.0 were attributed to secondary carbons, the signal at δ 57.9 to a tertiary carbon and the signal at δ 74.5 to a quaternary carbon, respectively. In the ^1H–^1H COSY spectrum, the protons at δ 6.04 (1H, t, $J = 10.0$ Hz) and 5.95 (1H, dt, $J = 10.0, 6.6$ Hz) were correlated with the protons at δ 5.21 (1H, t, $J = 10.0$ Hz) and 4.97 (2H, m), respectively, while these protons were respectively assigned as being connected with the carbons at δ 129.1, 129.3, 57.9 and 60.0 in the HMQC spectrum. These findings allowed establishment of the connectivities of $-O-CH_2-CH=CH-CH<$. Observation of the HMBC correlations of the protons at δ 4.56 (1H, d, $J = 11.7$ Hz) and 4.36 (1H, d, $J = 11.7$ Hz) with the carbon at δ 166.3 suggested that a benzoyl was attached to 6-OH. Further studies on the long-range correlations displayed in the HMBC spectrum showed that another benzoyl and acetyl were linked to 1-OH and 7-OH, respectively. The remaining quaternary carbon at δ 74.5 should be connected with C-4, C-6, and C-7. In the ^{13}C NMR spectrum, the chemical shift of C-5 was deshielded compared to that of C-6, so the only chlorine residue was logically located at C-4. The *J* value between H-2 and H-3 was 10.0 Hz indicating the geometry of the double bond at C-2 to be the *Z*-configuration, and the correlation of the proton at δ 4.97 (H-1) with the proton at δ 5.21 (H-4) in the NOESY spectrum also supported this conclusion. However, the relative and absolute configurations at C-4 and C-5 could not be assigned.

Uvamalol E (**2**) was isolated as oil, $[\alpha]_D^{23}$ +59 (*c* 0.17, MeOH), and its molecular formula was determined to be $C_{21}H_{22}O_7$ by HRFABMS *m/z* 409.1245 $[M + Na]^+$.

A preliminary examination of the spectral data suggested that **2** has a similar structure to **1** (Fig. 1), both containing the same skeleton and two benzoyls [3], apart from the absence of acetyl signals at δ 2.01 (3H, s) and δ 170.8 and 20.7 in **1**. C-4 was shifted downfield 11.9 ppm compared to the analogous signal for **1**, which was caused by the hydroxyl instead of chlorine. Likewise, the locations of two benzoyloxys at C-1 and C-6 were verified by



1 R=AcO R_1=Cl

2 R=H R_1=OH

FIGURE 1 Structures and key HMBC correlations for **1** and **2**.

the HMBC correlations of the protons at δ 5.01 (1H, dd, $J = 13.0, 6.5$ Hz) and 4.81 (1H, dd, $J = 13.0, 8.0$ Hz) as well as the protons at δ 4.59 (1H, d, $J = 10.5$ Hz) and 4.32 (1H, d, $J = 10.5$ Hz) with the carbons at δ 166.8 and 166.7, respectively, and the configuration of the double bond at C-2 was also determined to be the Z form.

Uvamalol F (**3**) was obtained as a wax, and the molecular formula was assigned as $C_{21}H_{20}O_5$ by FABMS ($[M + H]^+$ at *m/z* 353) and elemental analysis.

The absorption bands at 3469, 1718, 1601 and 1583 cm^{-1} were attributable to the absorptions of hydroxyl, carbonyl, and benzene ring. The ^1H NMR spectrum of **3** showed the signals for 10 aromatic protons at δ 7.40 $-$ 8.10, four olefinic protons at δ 5.60–6.60, four oxygenated methylene protons at δ 4.30–5.01 and one oxygenated methine proton at δ 5.01, which revealed the presence of two benzoyls and two double bonds [3], in combination with the signals as showed in the ^{13}C and DEPT NMR spectra for 12 aromatic and olefinic carbons at δ 125.0–135.0, two carbonyl carbons at δ 166.6 and 166.4 as well as a strong ion peak *m/z* 105 in the EIMS. Analysis of the HMQC data for **3** exhibited that the protons at δ 5.66 (1H, m), 6.55 (1H, m), 6.57 (1H, m), and 5.82 (1H, m) corresponded to the carbons at δ 131.0, 126.7, 126.1, and 127.4, respectively, and the protons at δ 5.01 (3H, m) to carbons at δ 60.4 and 64.7, so the connectivities of $-OCH_2-CH=CH-CH=CH-CH(OH)-$ were further deduced by the correlations of the proton at δ 5.66 with the protons at δ 6.55 and 5.01 and the proton at δ 5.01 with the protons at δ 5.82 and 4.32 (1H, q, $J = 11.0$ Hz) in the $^1H-^1H$ COSY spectrum. The linkages of two benzoyl substituents to 1-OH and 7-OH were demonstrated by the long-range correlations of the protons at δ 5.01 (H-1), 4.32 (H-7), and 4.42 (H-7) with the carbons at δ 166.6 (C-1′) and 166.4 (C $-$ 1‴) in the HMBC spectrum. Correlations of the proton at δ 6.55 with those at δ 5.66 and 5.01 (2H, m) as well as the proton at δ 6.57 with those at δ 5.82 and 5.01 (1H, m) in the NOESY spectrum indicated that the double bonds at C-2 and C-4 were both in the *E* form, but the stereochemistry of C-6 remains to be determined.

Uvamalol G (**4**) was isolated as an oil, and its HRFABMS gave a quasi-molecular ion peak at *m/z* $[M + Na]^+$ 375.1237 in agreement with $C_{21}H_{20}O_5$, which indicated it was an isomer of **3**.

Similarities in the IR, UV, and ^1H and ^{13}C NMR spectra suggested that **4** had a skeleton analogous to that of **3** with two benzoyls in the structure (Figs. 2 and 3). However, comparison of the ^{13}C NMR data of **4** with those of **3** revealed some significant differences: **4** had one additional quaternary carbon at δ 137.5 and one secondary carbon at δ 58.3, and lacked two tertiary carbons at δ 131.0 and 68.1 compared to **3**, which indicated that **4** has a branched structure containing the fragment of $(-OCH_2-)_2C=CH-CH=CH-CH_2O-$. The HMBC spectrum, in which both of the two ester carbonyl carbons at δ 166.4 and 166.8 were correlated to the protons at δ 5.01 (4H, m), revealed that the two carbonyls were linked to 1-OH and 6-OH. The *Z* form of the double bond at C-2 was deduced from the coupling

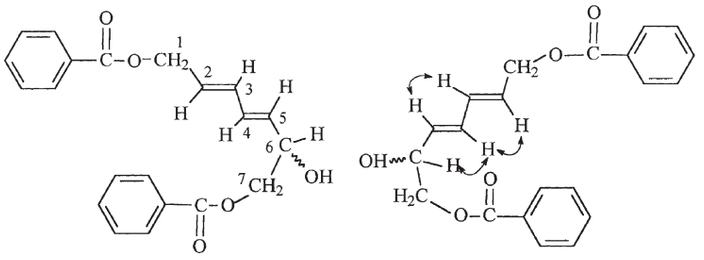


FIGURE 2 Structure and key NOE correlations for **3**.

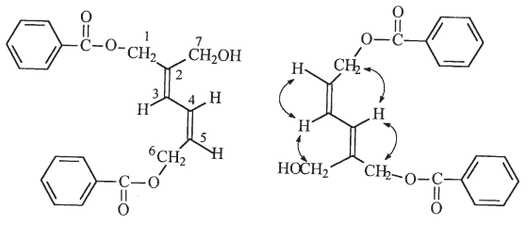
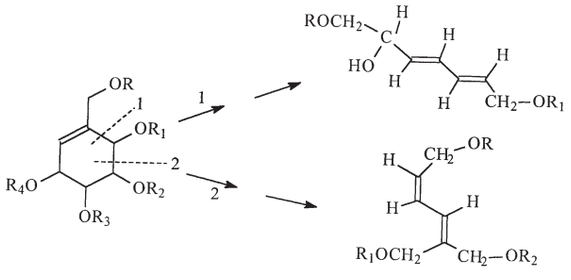


FIGURE 3 Structure and key NOE correlations for **4**.

constant of 10.0 Hz between H-4 and H-5, while a significant cross peak between H-4 (δ 6.57) and H-7 (δ 4.32) in the NOESY spectrum indicated that the double bond at C-2 was in the *E* form.

Because the seco-cyclohexenes and polyoxygenated cyclohexenes have the same carbon number, suggesting that they have close relationship in the biogenetic pathway, the seco-cyclohexenes are tentatively proposed to be precursors of polyoxygenated cyclohexenes or to stem from cleavage of polyoxygenated cyclohexenes (see Scheme 1).



SCHEME 1 Proposed biosynthesis for cyclohexenes and seco-cyclohexenes.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Perkin–Elmer 683 FT infrared spectrometer. UV spectra were obtained on a Shimadzu UV-240 instrument. NMR spectra were run on a Varian INOVA-500 NMR spectrometer with TMS as internal standard. EIMS were obtained on a VG ZAB-2F mass spectrometer and ESIMS, FABMS, HREIMS and HRFABMS were performed on an Autospec-Utima ETOF Spec mass spectrometer.

Plant Material

The roots of *Uvaria macrophylla* were collected in Jianfengling, Hainan Province, China, in December 2000 and identified by Professor Shi-Man Huang. A voucher specimen (No. 62161) is deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation

Dried roots (10.0 kg) were ground into a crude powder and extracted with 95% EtOH to afford 1.4 kg of residue on removal of the solvent under reduced pressure. The EtOH extract was partitioned between H_2O and $CHCl_3$ giving a water-soluble fraction I (294 g) and a chloroform-soluble fraction II (230 g), as well as an insoluble fraction III (800 g). Fraction II was first dissolved in 90% MeOH and then defatted with petroleum ether to give a methanol-soluble fraction IV (175 g). Fraction IV was subjected to a Si gel column for chromatography eluting with a gradient of petroleum ether–acetone and collected in 1000 ml fractions (fraction 1–110), fractions 36–42 and 68–74 showed the presence of polyoxygenated cyclohexenes detected by TLC with 10% H_2SO_4 alcohol solution containing 0.5% vanillin as spray agent. Fractions 36–42 and 68–74 were pooled and evaporated *in vacuo*, yielding 15.2 g of residue. The residue was further subjected to chromatography on a 600 g Si gel column with petroleum ether–EtOAc (6:4–3:7) in 250 ml fractions (fractions 1–36). Fractions 27 and 31 contained compounds **1** and **4** as well as compounds **2** and **3**, respectively. These fractions were further purified by preparative TLC with petroleum ether–$CHCl_3$–EtOAc (2:1:2) to give 102 mg of **1** ($R_f = 0.65$), 25 mg of **4** ($R_f = 0.62$), 3.5 mg of **3** ($R_f = 0.57$), and 4.2 mg of **2** ($R_f = 0.51$).

Uvamalol D (1)

White needles; mp 102–103°C; $[\alpha]_D^{23}$ +68 (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 204 (4.32), 230 (3.02) nm; IR (KBr) ν_{max}: 3421 (OH), 3070, 2985, 2958, 1732 (C=O), 1705 (C=O),1601, 1583, 1493, 1450, 1387, 1356, 1228, 1124, 1051, 976, 816, 796, 715, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) data, see Table I; ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table II; ESIMS *m/z*: 449 ($[M + H + 2]^+$, 13), 447 ($[M + H]^+$, 40), 429 ($[M-H_2O]$, 20), 411 ($[M-Cl]^+$, 100); EIMS *m/z*: 411 ($[M-Cl]^+$, 10), 289 (5), 250 (5), 237 (8), 215 (4), 189 (7), 176 (4), 153 (6), 129 (4), 105 (100), 88 (35); elemental analysis: C 61.65%, H, 5.07%, Cl 8.05% (calcd. for $C_{23}H_{23}O_7Cl$, C 61.74%, H, 5.14%, Cl 7.98%).

TABLE I ^1H (500 MHz) NMR spectral data for uvamalols D–G (**1**–**4**) in $CDCl_3$*

Position	**1**	**2**	**3**	**4**
1	4.97, m	5.01,dd, (13.0, 6.5) 4.81, dd, (13.0, 8.5)	5.01, m	5.01, s
2	5.95, dt, (10.0, 6.6)	5.84, m	5.82, m	
3	6.04, t, (10.0)	5.95, t, (10.0)	6.55, m	6.69, t, (11.5)
4	5.21, t, (10.0)	4.85, d, (10.0)	6.57, m	6.57, t, (11.5)
5			5.66, m	5.82, dt, (11.5, 6.5)
6	4.56, d, (11.7) 4.36, d, (11.7)	4.59, d, (10.5) 4.32, d, (10.5)	5.01, m	5.01, m
7	4.91, d, (11.7) 4.34, d, (11.7)	3.85, d, (11.5) 3.81, d, (11.5)	4.32, q, (11.0) 4.42, q, (11.0)	4.32, s
$2''$ ($6''$)	8.01, m	8.01, m	8.05, m	8.01, m
$3''$ ($5''$)	7.39, m	7.42, m	7.45, m	7.42, m
$4''$	7.55, m	7.54, m	7.57, m	7.54, m
$2'''$ ($6'''$)	8.01, m	8.00, m	8.05, m	8.01, m
$3'''$ ($5'''$)	7.39, m	7.40, m	7.45, m	7.42, m
$4'''$	7.55, m	7.53, m	7.57, m	7.54, m
AcO	2.01, s			

Assignments were confirmed by ^1H–^1H COSY, HMQC, and HMBC experiments.
*Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz.

TABLE II ^{13}C (125 MHz) NMR spectral data for uvamalols D–G (**1**–**4**) in $CDCl_3$*

Position	**1**	**2**	**3**	**4**
1	60.0	60.9	60.4	67.0
2	129.3	131.2	127.4	137.5
3	129.1	128.0	126.1	126.4
4	57.9	69.4	126.7	125.4
5	74.5	74.9	131.0	127.8
6	64.8	65.0	64.7	60.5
7	64.9	64.0	68.1	58.3
1′	166.3	166.8	166.6	166.4
1″	129.5	129.6	129.6	129.6
2″ (6″)	129.5	129.6	130.0	129.6
3″ (5″)	128.1	128.3	128.4	128.4
4″	133.1	133.3	133.2	133.1
1‴	166.4	166.7	166.4	166.8
1⁗	129.6	129.5	129.6	129.6
2⁗ (6⁗)	129.6	129.5	129.7	129.7
3⁗ (5⁗)	128.5	128.1	128.3	128.5
4⁗	133.4	133.1	133.0	133.2
AcO	20.7, 170.8			

*Chemical shift values are given in ppm, and assignments were confirmed by ^1H–^1H COSY, HMQC, and HMBC experiments.

Uvamalol E (2)

Oil, $[\alpha]_D^{23}$ +59 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ): 204 (4.27), 229 (4.07), 273 (2.98) nm; IR (KBr) ν_{max}: 3448 (OH), 3064, 2954, 1718 (C=O), 1601, 1583, 1452, 1361, 1315, 1275, 1176, 1115, 1026, 756, 710, 687 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) data, see Table I; ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table II; EIMS *m/z*: 387 ($[M + H]^+$, 10), 359 (10), 289 (5), 265 (5), 195 (2), 154 (40), 123 (2), 105 (95), 81(65), 69 (100); HRFABMS *m/z*: 409.1245 ($[M + Na]^+$, calcd. for $C_{21}H_{22}O_7Na$, 409.1263).

Uvamalol F (3)

Wax, $[\alpha]_D^{23}$ + 31 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ): 204 (4.21), 230 (4.10), 292 (2.96) nm; IR (film) ν_{max}: 3469(OH), 3062, 2922, 2850, 1718 (C=O), 1601, 1583, 1491, 1452, 1315, 1273, 1176, 1113, 1070, 1026, 806, 710, 687 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) data, see Table I; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table II; FABMS *m/z*: 353 ($[M + H]^+$, 4), 282 (5), 231 (10), 175 (5), 159 (7), 133 (10), 105 (100), 77 (34), 69 (56); elemental analysis: C 71.51%, H, 5.59% (calcd. for $C_{21}H_{20}O_5$, C 71.58%, H, 5.67%).

Uvamalol G (4)

Oil, UV (MeOH) λ_{max} (log ϵ): 204 (4.30), 229 (4.01), 281 (2.96) nm; IR (film) ν_{max}: 3467 (OH), 3060, 2924, 2852, 1720 (C=O), 1601, 1496, 1450, 1315, 1273, 1250, 1176, 1111, 1070, 1026, 908, 806, 710, 687 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) data, see Table I; ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table II; FABMS *m/z*: 375 ($[M + Na]^+$, 1), 133 (10), 105 (100), 81 (50), 69 (70); HRFABMS *m/z*: 375.1237 ($[M + Na]^+$, calcd. for $C_{21}H_{20}O_5Na$, 375.1208).

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