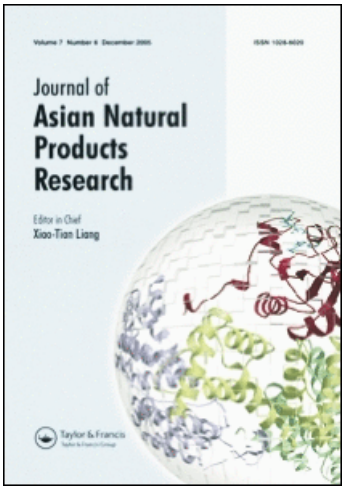

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New Polyoxygenated Cyclohexenes from *Uvaria Calamistrata*

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NEW POLYOXYGENATED CYCLOHEXENES FROM *UVARIA CALAMISTRATA*

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Five new polyoxygenated cyclohexenes, named uvacalol A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**) were isolated from the roots of *Uvaria calamistrata*. On the basis of spectral analysis and chemical derivatization, including the preparation of Mosher esters, the structures of compound **1–5** were established as (2R,3S,4R,5S)-2-acetoxyl-5-ethoxyl-1-benzoyloxymethylcyclohex-1(6)-ene-3,4-diol-3-benzoate, (2R,3S,4R,5S)-2-acetoxyl-5-ethoxyl-1-benzoyloxymethylcyclohex-1(6)-ene-3,4-diol-4-benzoate, (2R,3S,4R,5S)-5-ethoxyl-1-benzoyloxymethylcyclohex-1(6)-ene-2,3,4-triol-3-benzoate, (2R,3S,4R,5S)-3-methoxyl-1-benzoyloxymethylcyclohex-1(6)-ene-2,3,5-triol and (2R,3S,4R,5S)-2-acetoxyl-1-benzoyloxymethylcyclohex-1(6)-ene-3,4,5-triol-5-benzoate, respectively.

Keywords: Annonaceae; *Uvaria calamistrata*; Cyclohexene; Uvacalols

INTRODUCTION

In earlier work on the phytochemical investigation of the Annonaceous plants, we have reported the existence of a group of polyoxygenated cyclohexenes from the plants *Uvaria grandiflora*, *U. boniana* and *Annona muricata*, in which some cyclohexenes and their derivatives, especially some α,β-unsaturated cyclohexanones have significant activities against human cancer cell lines [1–4]. In our continued investigation for tumor inhibitory constituents from the ethanolic extract of the roots from *Uvaria calamistrata* Hance., five new polyoxygenated cyclohexenes, named uvacalol A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**) were isolated. These compounds belong to a novel

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class of polyoxygenated cyclohexenes, which possess a 1-methylcyclohex-1(6)-ene skeleton. In this paper we report the isolation and structural elucidation of the new polyoxygenated cyclohexenes.

RESULTS AND DISCUSSION

Compound **1** was isolated as white solid, a molecular formula $C_{25}H_{26}O_8$ was determined from comprehensive examination of the spectral data (EIMS and ^{1}H NMR) and elemental analysis. In its ^{1}H NMR spectrum, the proton signals of an olefinic methine, four oxygenated methines and two methylenes between δ 4.0 and 6.5 suggested the presence of the basic skeleton of a 1-hydroxymethyl cyclohexene, which was confirmed by the ^{13}C NMR spectrum, having a pair of olefinic carbon signals at δ 132.2 and 129.0, four oxygenated methine signals and two methylenoxy signals from δ 60 to 80. The existence of a single olefinic proton in ^{1}H NMR suggested that the double bond was situated at C-1/C-6, this was supported by its C–H COSY which showed correlation between the signal at δ 129.0 (C-6) with the ^{1}H signal at δ 6.19 (1H, d, J = 2.5 Hz H-6) while no correlation was observed between the ^{13}C signal at δ 132.2 (C-1) with any ^{1}H signal. The ^{1}H NMR spectrum of **1** showed the presence of two benzoyloxyl groups (δ 7.2–8.2, 10H, m), which was in agreement with EIMS m/z 105 ($ArCO^+$) and the carbon signals at δ 165.0 and 165.9 in the ^{13}C NMR spectrum. The proton signals at δ 2.03 (s, 3H) in the ^{1}H NMR and the carbon signal at δ 169.9 in the ^{13}C NMR spectrum revealed the existence of an acetoxyl group. The proton signals at δ 3.72 (1H, dq, J = 15.6, 6.9 Hz), 3.75 (1H, dq, J = 15.6, 6.9 Hz) and 1.26 (3H, t, J = 6.9 Hz) indicated the presence of an ethoxyl group, while the two allylic methylene protons appeared as a singlet at δ 4.83. On the basis of their coupling constants and ^{1}H–^{1}H COSY, the protons at δ 5.88, 5.52, 4.28 and 4.09 were located at C-2, 3, 4 and 5 positions in the cyclohexene ring, respectively. Based on the fact that there were cross-peaks between δ_C 165.0 and δ_H 4.83, 169.9 and 5.88, 165.9 and 5.52 in the HMBC spectrum, the two benzoyloxyls must be attached to C-7 and C-3, acetoxyl to C-2. Therefore, the remaining ethoxyl group must be placed at C-5 (δ_C 76.2, δ_H 4.09, dd, J = 5.7, 2.5 Hz) due to the correlation between the signals at δ_C 76.2 and δ_H 3.72, 3.75. The IR of **1** showed the presence of a hydroxyl (ν 3485.2 cm^{-1}), genimal to the proton at δ 4.28 (1H, dd, J = 5.7, 2.2 Hz), which was shifted to δ 5.68 after acetylation.

The mass fragmentation pattern of **1** was typical of substituted cyclohexene. The expected Retro-Diels–Alder (RDA) process was in competition

with aromatization. A pair of complementary fragment ions (*m/z* 290, 164) due to RDA process was useful to determine the molecular composition and the place of substituents. The fragmentation pathway of aromatization resulted by loss of substituents from molecular ion ($[M\text{-}H_2O\text{-}HOAc]^+$, $[M\text{-}HOAc\text{-}HOBz]^+$) confirmed the presence of substituents (see Fig. 1).

The relative configuration of **1** was elucidated on the basis of careful analysis of its coupling constants and NOE DIFF experiment. The coupling constant of $J_{5,6}$ (2.5 Hz) suggested that the relative stereochemistry of H-5 was pseudo-axial, as the 2.5 Hz coupling constant between allylic proton and adjacent olefinic proton in cyclohexene ring requires an axial allylic proton [5]. In addition, H-4 sharing 5.7 Hz coupling with H-5 suggested that H-4 and H-5 were trans pseudo-axial, while $J_{3,4}$ (2.2 Hz) and $J_{2,3}$ (5.3 Hz) indicated that H-2 and H-3 were pseudo-equatorial. The absence of mutual NOEs between H-2 and H-4 established that the two protons were situated on opposite faces of the six-membered ring and further supported the elucidation above. In comparison with that of a similar known compound

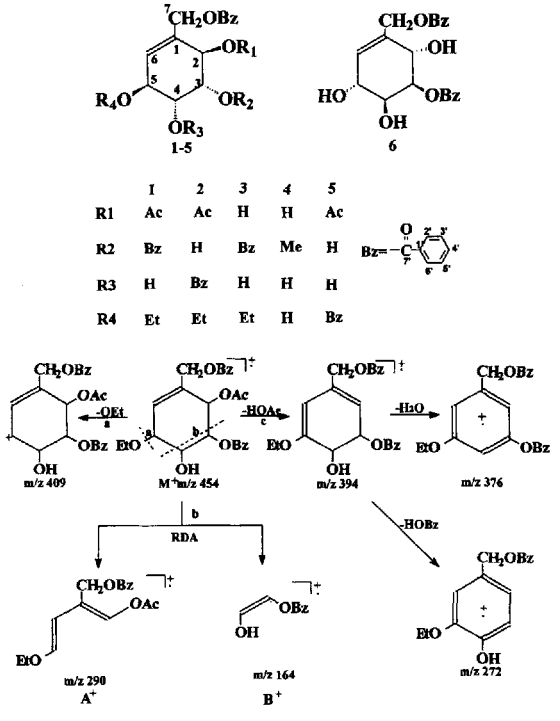

FIGURE 1 Major fragments of **1**.

piperenol A (**6**) [6], the coupling relationship from H-2 to H-4 was similar. So compound **1** had the same relative configuration as **6** (the absolute configuration proposed in Ref. [6] is shown here). It is seen that the substituents from C-2 to C-5 have the aaee arrangement. The alternative conformer with eeaa arrangement is suppressed, if not completely inhibited, by much more pronounced steric congestion between C-2 and C-1 groups when the C-2 group is equatorial. Although compound **1** had two benzoyloxyls located at C-3 and C-7 which looked worthwhile to elucidate its absolute configurations by its CD spectrum, there was a difficulty to predict the absolute configurations of **1** due to the presence of the primary alcoholic group. To establish the absolute configuration of **1**, the (S−) and (R−)-methoxytrifluoromethyl-phenylacetic acid esters (Mosher esters) of **1** were prepared. The proton chemical shifts along with the $\Delta\delta_H$ ($\delta_S - \delta_R$) were summarized in Table I. According to Mosher's assumptions [7], only the R configuration of C-4 could have a greater shielding of both H-2 and H-3 and less shielding of both H-5 and H-6 in the (S)-MTPA derivative (**1s**), and conversely in the (R)-MTPA ester (**1r**). Thus, the R configuration was assigned for the chiral center at C-4, which was in agreement with its CD curve, exhibiting negative Cotton extrema centered at 235 and 226 nm resulted from the interaction of two benzoyloxyls. The absolute configurations of the other chiral centers could then be assigned according to the relative stereochemistry elucidated above. Thus, the structure of **1** was established as (2R,3S,4R,5S)-2-acetoxyl-5-ethoxyl-1-benzoyloxymethyl-cyclohex-1(6)-ene-3,4-diol-3-benzoate.

Compound **2** was isolated as colorless solid. Its molecular formula $C_{25}H_{26}O_8$ was determined from spectral examination and elemental analysis. The IR, ^{13}C NMR and ^1H NMR spectra of **2** were very similar to those of **1**. The proton signals of two benzoyloxyls, an ethoxyl and an acetoxyl appeared at the same regions as those of **1** in ^1H NMR spectrum. The ^1H NMR difference of **2** from **1** was that the H-4 signal at δ 4.28 (1H, dd, $J = 5.7$, 2.2 Hz) in **1** was shifted to δ 5.46 (1H, dd, $J = 2.2$, 4.4 Hz) in **2** while the H-3 signal at δ 5.52 (1H, dd, $J = 5.3$, 2.2 Hz) in **1** was replaced by the signal at δ 4.30 (1H, dd, $J = 6.2$, 2.2 Hz) in **2**. The cross peak between the carbon signal at δ 165.9 and proton signal at δ 5.46 in HMBC showed the attachment of benzoyloxyl to C-4. HMBC also revealed the correlation of acetyl carbonyl (δ 171.3) with methine proton (δ 5.73, d, $J = 6.1$ Hz) at C-2. The two allylic methylene protons of **2** exhibited an AB coupling pattern (δ 4.84, d, $J = 13.0$ Hz and 4.90, d, $J = 13.0$ Hz). EIMS exhibited nearly same fragment ions as those of **1** and confirmed the existence of OH, AcO, EtO and BzO groups and the identity of the positions attached by AcO, BzO and EtO in fragment A. The result from EIMS was in agreement to

TABLE I ^{1}H NMR data (500 MHz, $CDCl_3$) of compound **1–6**

Proton	**1**	**2**	**3**	**4**	**5**	**6***
2	5.88 (d, 5.3)	5.73 (d, 6.1)	4.49 (d, 4.6)	4.32 (d, 5.3)	5.86 (d, 7.1)	4.28 (d, 5.2)
3	5.52 (dd, 5.3, 2.2)	4.30 (dd, 6.2, 2.2)	5.47 (dd, 4.6, 2.4)	3.96 (dd, 5.3, 2.4)	3.97 (dd, 7.1, 2.2)	5.22 (dd, 2.4, 5.2)
4	4.28 (dd, 2.2, 5.7)	5.46 (dd, 2.2, 4.4)	4.29 (dd, 2.4, 6.3)	4.04 (dd, 2.4, 5.6)	4.48 (dd, 2.2, 3.6)	3.91 (dd, 2.4, 6.0)
5	4.09 (dd, 5.7, 2.5)	4.22 (dd, 4.4, 1.8)	4.13 (dd, 6.3, 2.6)	4.60 (dd, 5.6, 2.9)	5.78 (dd, 3.6, 2.2)	4.16 (dd, 2.9, 6.0)
6	6.18 (d, 2.5)	6.11 (d, 1.8)	6.06 (d, 2.6)	5.85 (d, 2.9)	5.78 (d, 2.2)	5.85 (d, 2.9)
7	4.83 (s)	4.84 (d, 13.0)	5.06 (d, 13.9)	4.97 (d, 13.0)	4.94 (d, 13.4)	4.81 (d, 13.2)
		4.90 (d, 13.0)	4.84 (d, 13.9)	4.90 (d, 13.0)	4.82 (d, 13.4)	4.89 (d, 13.2)
Ar	8.0–7.40 (m)	8.10–7.35 (m)	8.0–7.35 (m)	8.10–7.50 (m)	8.10–7.40 (m)	7.94–7.50 (m)
OAc	2.03 (s)	2.12 (s)			2.07 (s)	
OCH_2	3.72 (dq, 15.6, 6.9)	3.78 (dq, 16.2, 6.9)	3.74 (dq, 18.2, 6.9)			
	3.75 (dq, 15.6, 6.9)	3.71 (dq, 16.2, 6.9)	3.69 (dq, 18.2, 6.9)			
CH3	1.26 (t, 6.9)	1.26 (t, 6.9)	1.26 (t, 6.9)			
OCH_3				3.27 (s)		

*Literature data, measured in DMSO-d_6.

NMR data. On the basis of J values of the methine protons between δ 3.90 and 6.20, **2** must be suggested to have the same relative configuration as **1**. (R) and (S)-Mosher esters of **2** also allowed us to establish the absolute configuration of chiral carbinol center (C-3) and further determined the absolute configurations of other chiral centers. The ^{1}H NMR data of **2r** and **2s** were listed in Table II. The larger upfield shift of methylene protons and methyl protons of AcO attaching to C-2 in **2s** as well as the less upfield shift of methine protons at C-4, and C-5 elucidated the chiral center (C-3) as S configuration. The conclusion was in agreement with the negative Cotton effects at 238, 225 nm in the CD curve. Thus, The structure of **2** was established as (2R, 3S, 4R, 5S)-2-acetoxy-5-ethyloxy-1-benzoyloxymethylcyclohex-1(6)-ene-3,4-diol-4-benzoate.

Compound **3** was obtained as colorless crystals, with molecular formula $C_{23}H_{24}O_7$ which was in agreement with the molecular ion at m/z 412 in EIMS and elemental analysis. The IR, UV and ^{1}H NMR spectra revealed that **3** might be an analogue of **1**. The ^{1}H NMR spectrum of **3** showed the lack of an acetyl signal around δ 2.03 and an obviously upfield shift of H-2 from δ 5.88 to 4.49. The J values of **3** were similar to those of **1**, which suggested that **3** had the same relative stereochemistry as **1**. The fact that the acetylated product of **3** (**3a**) had the same ^{1}H NMR data as the acetyl derivative of **1** supported the structural elucidation. The fragment ions $[M\text{-}H_2O]^+$, $[M\text{-}EtOH]^+$ and $[M\text{-}BzOH]^+$ as well as the RDA fragments also showed the presence and location of OH, EtO and BzO groups. A similar CD curve of **3** to that of **1** indicated that they processed the same absolute configuration. Thus, the structure of **3** was established as (2R,3S,4R,5S)-5-ethoxy-1-benzoyloxymethy-cyclohex-1(6)-ene-2,3,4-triol-3-benzoate.

Compound **4** was obtained as colorless crystals. The molecular formula $C_{15}H_{18}O_6$ was deduced from the EIMS and elemental analysis as well as ^{1}H NMR data. A benzoyl group was indicated by comprehensive analysis of

TABLE II ^{1}H NMR data of compounds **1r**, **1s**, **2r**, and **2s** (300 MHz, $CDCl_3$ δ ppm)

Proton	**1s**	**1r**	$\Delta\delta_H$ ($\delta_S - \delta_R$)	**2s**	**2r**	$\Delta\delta_H$ ($\delta_S - \delta_R$)
2	5.90	5.60	−0.10	5.93	5.90	+0.03
3	5.68	5.72	−0.04	5.66	5.68	−0.02
4	5.65	5.68	−0.03	5.67	5.61	+0.06
5	4.22	4.11	+0.11	4.19	4.15	+0.04
6	6.19	6.11	+0.08	6.14	6.14	0.00
7	4.83	4.81	+0.02	4.80	4.82	−0.02
Ac	2.05	2.03	+0.02	1.98	2.10	−0.02
OCH_2	3.75	3.68	+0.07	3.82	3.80	+0.02
CH_3	1.27	1.23	+0.04	3.81	3.77	−0.04

the IR (1715, 1450, 1280, 712 cm^{-1}), MS (*m/z* 105) and ^1H NMR data (7.4–8.2, 5H, m). The IR absorption band at 3480 cm^{-1} suggested the presence of hydroxyl groups which was confirmed by the formation of a triacetyl derivative of **4**. Comparison between ^1H NMR data of **4** and those of **1** indicated the absence of acetoxyl and ethoxyl groups, and the existence of a methoxyl group (δ 3.27, 3H, s) in **4**. Further investigation on the ^1H NMR and ^1H–^1H COSY of **4** revealed that the benzoyloxyl was attached to C-7 and the methoxyl group to C-3 due to the upfield shift of H-3 (δ 3.96, dd, $J = 5.3$, 2.3 Hz). The signal at δ 5.85 (1H, d, $J = 2.9$ Hz) was assigned to the olefinic proton at C-6. The proton signals at δ 4.32 (1H, d, $J = 5.3$ Hz), 4.04 (1H, dd, $J = 5.6$, 2.4 Hz) and 4.60 (1H, dd, $J = 5.6$, 2.9 Hz) belonged to H-2, 4, 5, respectively. The *J* values from H-2 to H-6 was similar to those in **1**. So, compound **4** was identical in relative stereochemistry to **1**. On consideration of biogenetic pathways of **1**–**4**, **4** may have the same absolute configuration as the others. Thus, the structure of **4** would be suggested as (2R,3S,4R,5S)-3-methyloxy-1-benzoyloxymethyl-cyclohex-1(6)-ene-2,4,5-triol.

Compound **5** was isolated as colorless needles. The molecular formula was suggested as $C_{23}H_{22}O_8$ by its mass spectrum and elemental analysis. The significant difference of **5** from **1** was the lack of the signals of ethoxyl in ^1H NMR spectrum. The combinative analysis of ^1H NMR and ^1H–^1H COSY allowed the assignment of one olefinic proton (δ 5.97, d, $J = 2.2$ Hz) at C-6, two methine protons (δ 5.86, d, $J = 7.1$ Hz and 5.78, dd, $J = 3.6$, 2.2 Hz) at C-2 and C-5 which were attached by ester groups, and two methine protons (δ 4.48, dd, $J = 2.2$, 3.6 Hz and 3.97, dd, $J = 7.1$, 2.2 Hz) at the hydroxyl-bearing C-3 and C-4. The existence of two hydroxyls was confirmed by the di-acetyl and ketal derivatives of **5** (**5a** and **5b**). Careful comparison of ^1H NMR data with those of compound **1**–**3** suggested that the acetoxyl was placed at C-2 and the two benzoyloxyls at C-5 and C-7. This allocation was supported by the mass fragment at *m/z* 366 from the typical RDA fragmentation for **5**. The fragments derived from the successive losses of the usual substituents (AcO, BzO and OH) were observed in the EIMS. The *J* values of protons from H-2 to H-6 showed the similarity of the relative configuration of **5**–**1**. The formation of isopropylidene derivative (**5b**) confirmed the presence and relative configuration of two adjacent hydroxyls in cis orientation at C-3 and C-4. The negative Cotton effect at 228 nm in CD curve established the S configuration at C-5. So, compound **5** must have an absolute configuration identical to that of the former compounds. Compound **5** was established as (2R.3S,4R,5S)-2-acetoxyl-1-benzoyloxylmethyl-cyclohex-1(6)-ene-3,4,5-triol-5-benzoate.

The bio-assay of compounds **1–5** exhibited no significant inhibitory activities for cancer cell lines in cell culture with MTT method.

EXPERIMENTAL SECTION

General Experimental Procedure ^1H and ^{13}C NMR spectra were measured on a Brucker AM-500 spectrometer at 500 and 125 MHz, respectively in $CDCl_3$ with TMS as internal standard. IR spectra were recorded on a Perkin-Elmer 683 spectrometer. UV spectra were obtained in MeOH using a Shimadzu UV-240 spectrometer. Mass spectra were performed on VG ZAB-2F spectrometer. The elemental analysis used a MOD.1106 elemental analyzer. The optical rotations were measured on Perkin-Elmer-241 polarimeter. CD was recorded on Jasco-200. M.p. was measured on a micromelting point apparatus and uncorrected.

Plant Material Plant material was collected from Jian-Liang Peak on Hainan Island in July 1996 and identified as *U. calamistrata* by Wan-Zi Song, Department of Botany, Institute of Materia Medica. A voucher specimen was deposited in the Department.

Extraction and Isolation The dried and pulverized roots (10 kg) of *U. calamistrata* were exhaustively extracted with 3×30 l EtOH under refluex. The extract was concentrated *in vacuo* to yield 1,980 g residue (F001) which was partitioned between H_2O and $CHCl_3$ (1 : 1), giving $CHCl_3$ soluble extract (575 g) (F002), and the insoluble interface fraction (120 g) (F003) and the H_2O soluble fraction (1.10 kg) (F004). The $CHCl_3$ extract (F002) was partitioned between 10% H_2O–MeOH and petroleum ether (1 : 1). The methanol part (F005) supplied 350 g solid which was chromatographed over Si gel column (160–200 mesh, 3.5 kg) eluting with petroleum ether–acetone gradient solvent. The eluted fractions were collected in 500 ml each. The fractions 35–40, 42–52, 53–55 eluted with petroleum ether–acetone (4 : 1) were rechromatographed respectively and purified on Si column repeatedly, eluting with gradient petroleum ether–EtOAc (5 : 1–2 : 1). The fractions 35–40 gave compounds **1** (2.52 g) and **2** (1.14 g). The fractions 42–52 yielded compounds **3** (150 mg) and **5** (35 mg). Compound **4** (28 mg) was obtained from fractions 53–55.

General Procedures for the Preparation of MTPA Esters (R) or (S)-α-methoxyl-α-(trifluoromethyl)-phenylactic acid (MTPA), N,N-dicyclohexylcarbodiimide (DCC) and compounds for esterification in the molar ratio of 5 : 7 : 1 were added into the solvent CH_2Cl_2 with catalytic amount of dimethylamino pyridine (DMAP). The mixture was stirred with magnetic

stirrer at room temperature for 8 h. After removing CH_2Cl_2 under vacuum, the residue was dissolved with acetone and filtered. The filtrate was concentrated for the purification of Mosher ester. Purified Mosher ester was obtained by preparative TLC with EtOAc–petroleum ether (1 : 3)

Compound **1** Colorless crystals; m.p. 36–38°C, $[\alpha]_D^{18}$ −53.9 (c 0.13, MeOH); IR (film) ν_{max} 3480 (OH), 2976, 1722 (C=O), 1605, 1595, 1520, 1450, 1370, 1300, 1271, 1150, 1097, 1030, 712 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.30), 228 (4.44), 273 (3.45) nm; CD (MeOH, c 0.13) λ_{max} ($\Delta\varepsilon$): 235 (−0.94), 226 (−1.03) nm; EIMS *m/z* (rel. int.) 409 $[M\text{-}OEt]^+$ (1), 394 $[M\text{-}HOAc]^+$ (1), 376 $[M\text{-}HOAc\text{-}H_2O]^+$ (1), 290 A^+(RDA) (1), 272 $[M\text{-}HOAc\text{-}HOBz]^+$ (15), 244 (3), 167 (5), 164 B^+(RDA) (2), 151 (4), 139 (12), 126 (18), 122 $[BzOH]^+$ (6), 105 $[ArCO]^+$ (100, base peak), 94 (3), 77 (25), 69 (2), 51 (6), 43 Ac^+ (16); ^1H NMR data, see Table I; ^{13}C NMR (125 MHz) δ ($CDCl_3$) 169.9 (s, Ac–CO), 166.0 (s, Bz–C-7′), 165.0 (s, Bz–C-7″), 133.4 (s, C-4′), 133.1 (s, C-4″), 132.2 (s, C-1), 129.8 (d, 2′,6′), 129.7 (d, 2″, 6″), 129.5 (d, 1′,1″), 129.0, (d, C-6), 128.5 (d, 3′, 5′), 128.4 (d, 3″, 5″), 76.2 (d, C-5), 73.4 (d, C-3), 69.5 (d, C-4), 67.8 (d, C-2), 65.7 (t, $-OCH_2-$), 64.4 (t, C-7), 20.7 (q, Ac–Me), 15.5 (q, CH_3); anal. C 66.13% H 5.70%, calcd. for $C_{25}H_{26}O_8$, C 66.05% H5.73%.

Acetylation of **1** Compound **1** (15 mg) was treated with Ac_2O (0.5 ml) and dry C_5H_5N (0.5 ml) at room temperature overnight. After the usual work-up, the mixture was chromatographed on Si gel column (petroleum ether–Me_2CO 3 : 1) to furnish a monoacetate (**1a**) of **1** (10 mg) as a white gum. ^1H NMR data (300 MHz) δ ($CDCl_3$) 8.10–7.20 (10H, m, Ar–H), 6.15 (1H, $J = 3.6$ Hz, H-6), 5.92 (1H, d, $J = 6.3$ Hz, H-2), 5.61 (1H, dd, $J = 5.4$, 3.6 Hz, H-5), 5.48 (1H, dd, $J = 5.4$, 2.7 Hz, H-4), 4.12 (1H, dd, $J = 5.4$, 3.6 Hz, H-5), 3.79 (1H, dq, $J = 14.6$, 7.2 Hz, $-OCH_2-H_a$), 3.67 (1H, dq, $J = 14.6$, 7.2 Hz, $-OCH_2-H_b$), 2.08 (3H, s, Ac), 2.05 (3H, s, Ac), 1.26 (3H, t, Me).

R-MTPA-1 (**1r**) 10 mg of sample, 32 mg of DCC and 26 mg of R-MTPA were added into 1.5 ml of CH_2Cl_2 with a few crystals of DMAP and treated according to the general procedure. Purification by preparative TLC (petroleum ether–AcOEt 3 : 1), yielded **1r** (7 mg). ^1H NMR (300 MHz) δ ($CDCl_3$) 8.10–7.20 (14H, m, Ar–H), 6.11 (1H, d, $J = 3.6$ Hz, H-6), 6.00 (1H, d, $J = 6.3$ Hz, H-2), 5.72 (1H, dd, $J = 6.3$, 2.7 Hz, H-3), 5.69 (1H, dd, $J = 2.7$, 7.1 Hz, H-4), 4.86 (1H, d, $J = 12.9$ Hz, H_a-7), 4.76 (1H, d, $J = 12.9$ Hz, H_b-7), 4.11 (1H, dd, $J = 7.1$, 3.6 Hz, H-5), 3.69 (2H, q, $J = 7.2$ Hz, $-OCH_2-$), 3.46 (3H, s, mtpa-OMe), 2.03 (3H, mtpa-Me), 1.23 (3H, t, $J = 7.2$ Hz, Me).

S-MTPA-1 (**1s**) The preparative procedure was similar to that of (R)-MTPA-**1**. ^1H NMR (300 MHz) δ ($CDCl_3$) 8.00–7.40 (14H, m, Ar–H), 6.19

(1H, d, $J=3.3$ Hz, H-6), 5.90 (1H, d, $J=6.3$ Hz, H-2), 5.68 (1H, dd, $J=6.3$, 2.4 Hz, H-3), 5.66 (1H, dd, $J=5.4$, 1.8 Hz, H-4), 4.85 (1H, d, $J=14.4$ Hz, H_a-7), 4.81 (1H, d, $J=14.4$ Hz, H_b-7), 4.11 (1H, dd, $J=3.3$, 5.4 Hz, H-5), 3.76 (2H, q, $J=6.9$ Hz, $-OCH_2-$), 3.50 (3H, s, mtpa-OMe), 2.05 (3H, s, mtpa-Me), 1.29 (3H, t, $J=6.9$ Hz, Me).

Compound **2** Amorphous solid; m.p. 38–40°C, $[\alpha]_D^{22}$ −11.0 (c 0.13, MeOH); IR (film) ν_{max} 3481 (OH), 3100, 2976, 1724 (C=O), 1600, 1580, 1440, 1360, 1320, 1273, 1230, 1170, 1113, 1070, 1026, 712 cm^{-1}; UV (MeOH) λ_{max} (log ε): 205 (4.38), 228 (4.52), 273 (3.36) nm; CD (MeOH, c 0.04) $\lambda_{max}(\Delta\varepsilon)$ 238 (−2.10), 225 (−2.11) nm; EIMS *m/z* (rel. int.) 409 $[M\text{-}OEt]^+$ (1), 394 $[M\text{-}HOAc]^+$ (1), 290 A^+ (RDA) (2), 272 $[M\text{-}HOAc\text{-}H_2O]^+$ (7), 167 (7), 164 B^+ (RDA) (1.5), 151 (4), 139 (5), 126 (15), 122 $[BzOH]^+$ (8), 105 $[ArCO]^+$ (100, base peak), 94 (4), 77 (24), 51 (5), 43 Ac^+ (15); ^1H NMR data, see Table I; ^{13}C NMR (125 MHz) δ ($CDCl_3$) 171.3 (s, Ac–CO), 166.0 (s, Bz–C-7′), 165.9 (s, Bz–C-7″), 133.4 (s, C-1), 133.2 (s, C-4′), 133.1 (d, C-4″), 129.8 (s, C-1′), 129.7 (s, C-1″), 129.6 (d, C-6), 128.5 (d, C-2′, 5′, 2″, 5″), 128.2 (d, C-3′, 5′, 3″, 5″), 73.4 (d, C-3), 73.3 (d, C-5), 71.3 (d, C-2), 69.5 (d, C-4), 66.1 (t, $-OCH_2-$), 64.2 (t, C-7), 20.9 (q, Ac–Me), 15.5 (q, Me); anal. C 66.01% H 5.75%, calcd. for $C_{25}H_{26}O_8$, C 66.05% H 5.73%.

Acetyl Derivative (**2a**) *of* **2** The preparative procedure of **2a** was similar to that of **1a**. ^1H NMR data (300 MHz) δ ($CDCl_3$) 8.10–7.20 (10H, m, Ar H), 6.15 (1H, $J=3.9$ Hz, H-6), 5.92 (1H, d, $J=6.6$ Hz, H-2), 5.60 (1H, dd, $J=4.5$, 2.7 Hz, H-5), 5.54 (1H, dd, $J=6.6$, 2.7 Hz, H-3), 4.85 (2H, s, H-7), 4.14 (1H, dd, $J=2.7$, 4.5 Hz, H-4), 3.81 (1H, dq, $J=16.2$, 7.2 Hz, OCH_2–H_a), 3.70 (1H, dq, $J=16.2$, 7.2 Hz, $-OCH_2-H_b$), 2.09 (3H, s, Ac), 2.03 (3H, s, Ac), 1.25 (3H, t, Me).

R-MTPA-2 (**2r**) The preparative procedure of **2r** was similar to that of R-MTPA-**1**. ^1H NMR (300 MHz) δ ($CDCl_3$) 8.10–7.20 (14H, m, Ar–H), 6.14 (1H, d, $J=3.6$ Hz, H-6), 5.90 (1H, d, $J=5.7$ Hz, H-2), 5.69 (1H, dd, $J=5.4$, 2.3 Hz, H-4), 5.61 (1H, dd, $J=2.7$, 6.0 Hz, H-3), 4.77 (2H, s, H-7), 4.13 (1H, dd, $J=5.4$, 3.6 Hz, H-5), 3.78 (1H, dq, $J=15.0$, 7.2 Hz, $-OCH_2-$ H_a), 3.75 (1H, dq, $J=15.0$, 7.2 Hz, $-OCH_2-H_b$), 3.12 (3H, s, mtpa-OMe), 2.10 (3H, s, Ac), 1.22 (3H, t, $J=7.2$ Hz, Me).

S-MTPA-2 (**2s**) The preparative procedure of **2s** was similar to that of R-MTPA-**1**. ^1H NMR (300 MHz) δ ($CDCl_3$) 8.10–7.20 (14H, m, Ar–H), 6.13 (1H, d, $J=3.3$ Hz, H-6), 5.92 (1H, d, $J=6.3$ Hz, H-2), 5.70 (1H, dd, $J=5.1$, 2.3 Hz, H-4), 5.66 (1H, dd, $J=2.4$, 6.0 Hz, H-3), 4.80 (2H, s, H-7), 4.17 (1H, dd, $J=5.1$, 3.3 Hz, H-5), 3.82 (1H, dq, $J=16.8$, 6.9 Hz, $-OCH_2-$ H_a), 3.70 (1H, dq, $J=16.8$, 6.9 Hz, $-OCH_2-H_b$), 3.41 (3H, s, mtpa-OMe), 1.98 (3H, s, Ac), 1.25 (3H, t, $J=6.9$ Hz, Me).

Compound **3** Crystal solid; m.p. 70–72°C, $[\alpha]_D^{18}$ +200 (c 1.4, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.36), 228 (4.54), 273 (3.37), 381 (sh, 3.24) nm; CD (MeOH, 0.14) λ_{max} ($\Delta\varepsilon$) 238 (−1.26), 229 (−1.24) nm; IR (KBr) ν_{max} 3423 (OH), 2974, 2893, 1715 (C=O), 1630, 1600, 1570, 1493, 1452, 1300, 1307, 1273, 1195, 1117, 1070, 1026, 712 cm^{-1}; ^1H NMR data, see Table I; EIMS *m/z* (rel. int.) *m/z* 412 ($[M]^+$, 1), 394 $[M-H_2O]^+$ (7), 366 $[M-HOEt]^+$ (18), 301 (4), 272 $[M-HOBz-H_2O]^+$ (3), 248 A^+ (RDA) (2), 244 (4), 227 (3), 168 (3), 164 (1), B^+ (RDA) 151 (4), 135 (3.5), 123 (100, base peak), 105 $[ArCO]^+$ (75), 91 (3.5), 77 (3), 67 (2.5); anal. C 67.18% H 5.80%, calcd. for $C_{23}H_{24}O_7$, C 66.93%, H 5.82%.

Diacetyl-3 (**3a**) The preparative procedure of **3a** was the same as that of **1a**. ^1H NMR data of **3a** was identical to those of **1a**.

Compound **4** Colorless crystals; m.p. 80–82°C, $[\alpha]_D^{18}$ +92.3 (c 0.065, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.26), 228 (4.16), 274 (3.13), 281 (3.05) nm; IR (KBr) ν_{max} 3480 (OH), 3406 (OH), 2922, 1715 (C=O), 1703, 1620, 1450, 1317, 1286, 1135, 1100, 1082, 712 cm^{-1}; ^1H NMR data, see Table I. EIMS *m/z* (rel. int.) 294 M^+(1), 263 $[M-OMe]^+$ (18), 220 A^+ (RDA) (1), 140 (8), 122 $[BzOH]^+$ (34), 105 $[ArCO]^+$ (100), 77 (55), 74 B^+ (RDA) (2.5), 60 $[HOAc]^+$ (3), 51 (15); anal. C 61.10% H 6.10%, cacld. for $C_{15}H_{18}O_6$, C 61.22% H 6.13%.

Triacetyl **4** (**4a**) The preparative procedure of **4a** was similar to that of **1a**. ^1H NMR (300 MHz) δ ($CDCl_3$) 8.20–7.40 (5H, m, Ar–H), 6.12 (1H, d, J = 2.7 Hz, H-6), 5.68 (1H, d, J = 5.4 Hz, H-2), 4.61 (1H, dd, J = 5.4, 2.7 Hz, H-3), 5.48 (1H, dd, J = 6.3, 2.7 Hz, H-5), 5.38 (1H, dd, J = 2.7, 6.3 Hz, H-4), 4.84 (2H, br s, H-7), 3.30 (3H, s, –OMe), 2.09 (3H, s, Ac), 2.08 (3H, s, Ac), 2.06 (3H, s, Ac).

Compound **5** Colorless needles; m.p. 88–90°C, $[\alpha]_D^{18}$ −169.6 (c 0.055, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.45), 238 (4.54), 273 (3.42), 380 (sh, 3.35) nm; CD (MeOH, c 0.055) λ_{max} ($\Delta\varepsilon$) 227 (−1.1) nm; IR (KBr) ν_{max} 3454 (OH), 2995, 1720 (C=O), 1602, 1590, 1452, 1390, 1315, 1271, 1190, 1111, 1070, 1028, 712 cm^{-1}; ^1H NMR data, see Table I. EIMS *m/z* (rel. int.): 409 ($[M-H_2O]^+$, 2), 367 $[M-HOAc]^+$ (4), 348 $[M-HOAc-H_2O]^+$ (1), 305 $[M-OBz]^+$ (3), 244 $[M-HOAc-HOBz]^+$ (22), 215 (20), 182 (3), 153 (36), 140 (15), 105 $[OBz]^+$ (100), 94 (28), 77 (72), 60 $[HOAc]^+$ (2), 52 (13), 43 Ac^+ (26); anal. C 64.8%, H 5.16% calcd. for $C_{23}H_{22}O_8$ C 64.66% H 5.18%.

Diacetyl-5 (**5a**) The preparation of **5a** was similar to that of **1a**. ^1H NMR (300 MHz) δ ($CDCl_3$) 8.20–7.40 (10H, m, Ar–H), 6.02 (1H, d, J = 2.4 Hz, H-6), 6.00 (1H, dd, J = 2.4, 7.8 Hz, H-5), 5.94 (1H, d, J = 4.8 Hz, H-2), 5.78 (1H, dd, J = 2.1, 4.8 Hz, H-3), 5.35 (1H, dd, J = 7.8, 2.1 Hz, H-4),

4.89 (1H, d, $J = 10.8$ Hz, H-7_a), 4.87 (1H, d. $J = 10.8$ Hz, H-7_b), 2.13 (3H, s, Ac), 2.07 (3H, s, Ac), 2.06 (3H, s, Ac).

Ketal Derivative of **5** (**5b**) Compound **5** (5 mg) was stirred in anhydrous CH_2Cl_2 (1 ml) with catalytic amount of *p*-toluenesulfonic acid and 2,2-bimethoxypropane (0.2 ml) at room temperature for 8 h. The reaction solution was purified with preparative TLC using petroleum ether–EtOAc (3 : 1). The isopropylidene derivative **5b** was obtained as a colorless gum (3 mg). ^{1}H NMR (300 MHz) δ ($CDCl_3$) 8.20–7.20 (10H, m, Ar–H), 6.23 (1H, br s, H-6), 5.82 (1H, dd, $J = 1.8$, 4.2 Hz, H-5), 5.46 (1H, d, $J = 7.2$ Hz, H-2), 4.96 (2H, br s, H-7), 4.91 (1H, dd, $J = 7.2$, 2.4 Hz, H-3), 4.54 (1H, dd, $J = 4.2$, 2.4 Hz, H-4), 2.06 (3H, s, Ac), 1.32 (3H, s, Me), 1.31 (3H, s, Me).

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