# Calamistrins A and B, Two New Cytotoxic Monotetrahydrofuran Annonaceous Acetogenins from *Uvaria calamistrata*

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*Received July 31, 1998*

Two new bioactive monotetrahydrofuran acetogenins, calamistrins A (**1**) and B (**2**), and two known compounds, uvarigrin (**3**) and uvarigranin (**4**), have been isolated from the roots of *Uvaria calamistrata*. The structures of the new compounds were elucidated by spectroscopic and chemical methods. The absolute stereochemistry of the stereogenic centers was established by Mosher ester methodology.

Plants of the family Annonaceae have been investigated as potential sources of cytotoxic acetogenins in recent years. *Uvaria calamistrata* Hance (Annonaceae) is a climbing shrub distributed in Hainan, Guangdong, and Guangxi Provinces of the People's Republic of China, and in Vietnam.[1] The leaves and roots of this plant are used by local residents to treat such diseases as lumbago and backache.

In the present investigation we describe the isolation, structure elucidation, and cytotoxic activity of two new monotetrahydrofuran acetogenins, calamistrins A (**1**) and B (**2**). Compounds **1** and **2** have a free hydroxyl located at the $\beta$ position of the flanking carbinol of the tetrahydrofuran ring in the lactone side, as found in three other plants.[2−4] The absolute configurations of compounds **1** and **2** have been established by the $^1$H NMR analysis of their Mosher esters.

## Results and Discussion

Calamistrin A (**1**) was isolated as colorless plate crystals. The molecular formula was established as $C_{37}H_{68}O_6$ by FABMS ($[MH]^+$ at $m/z$ 609) and elemental analysis. A prominent IR carbonyl absorption at 1755 cm$^{-1}$ suggested the presence of an $\alpha$,$\beta$-unsaturated $\gamma$-lactone in **1**, which was confirmed from the NMR spectra, showing diagnostic signals at $\delta_H$ 6.98 (1H, H-35), 4.99 (1H, H-36), and 1.40 (3H, H-37) and $\delta_C$ 173.8 (C-1), 148.8 (C-35), 134.4 (C-2), 77.3 (C-36), and 19.3 (C-37) (see Table 1). The signal for two methylene protons at $\delta$ 2.27 (2H, t, H-3) and the corresponding carbon signal at $\delta$ 25.2 suggested the absence of any oxygen-bearing group at C-4 and C-5.[5] The fragment ions at $m/z$ 590, 572, and 554 in the EIMS and at $m/z$ 591, 573, and 555 in the FABMS showed the successive loss of three $H_2O$ from the $[M]^+$ and $[MH]^+$, respectively, indicating the presence of three OH groups in **1**. This was confirmed by the $^1$H NMR data ($\delta_H$ 2.09, 2.07, 2.05, each 3H, s) of the triacetyl derivative (**1a**), the EIMS of the tri-TMSi derivative (**1b**) (see Figure 1), and the strong OH absorption band (3362 cm$^{-1}$) in the IR spectrum of **1**. The presence of a mono-THF ring with two OH groups at adjacent carbons of the ring was deduced by the $^1$H NMR signals at $\delta_H$ 3.90 (H-18 and H-21), 3.72 (H-17), and 3.81 (H-22) and the corresponding $^{13}$C NMR signals at $\delta_C$ 83.6 (C-21), 82.3 (C-18), 71.7 (C-17), and 71.4 (C-22), which are characteristic for mono-THF acetogenins with two flanking OH groups.[5]

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**Table 1.** $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR Data of Compounds **1** and **2** ($CDCl_3$, $J$ in Hz)

| position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 173.8 | | 173.8 | |
| 2 | 134.4 | | 134.4 | |
| 3 | 25.2 | 2.27 (t, 6.8) | 25.2 | 2.27 (t, 6.8) |
| 4−14 | 22.6−39.1 | 1.10−1.60 (m) | 22.6−38.7 | 1.10−1.60 (m) |
| 15 | 68.8 | 3.90 (m) | 70.4 | 3.38 (m) |
| 16 | 25.1 | 1.53 (m) | 25.1 | 1.54 (m) |
| 17 | 71.7 | 3.72 (m) | 71.5 | 5.10 (m) |
| 18 | 82.3 | 3.90 (m) | 82.5 | 3.84 (m) |
| 19−20 | 22.6−39.1 | 1.92, 1.64 | 22.6−38.7 | 1.90, 1.62 (m) |
| 21 | 83.6 | 3.90 (m) | 82.8 | 3.81 (m) |
| 22 | 71.4 | 3.81 (m) | 71.7 | 3.90 (m) |
| 34−33 | 22.7−39.1 | 1.10−1.60 (m) | 22.6−38.7 | 1.10−1.60 (m) |
| 34 | 14.1 | 0.87 (t, 6.8) | 14.1 | 0.87 (t, 6.8) |
| 35 | 148.8 | 6.98 (d, 1.5) | 146.8 | 6.95 (d, 1.5) |
| 36 | 77.3 | 4.99 (dq, 1.5, 6.8) | 77.3 | 4.99 (dq,1.5, 6.8) |
| 37 | 19.3 | 1.40 (d, 6.8) | 19.2 | 1.40 (d, 6.8) |
| Ac | | | 171.6, 21.2 | 2.07 (s) |

The placement of the mono-THF ring flanked by OH groups and the isolated OH group were determined by careful analysis of the EIMS fragment ions of **1** and its TMSi derivative (**1b**). The assignment of these fragment ions is presented in Figure 1. The EIMS of **1** exhibited fragment ions at $m/z$ 295, 339, and 409 and the corresponding dehydrated fragments at $m/z$ 277, 321, and 391, which placed the THF ring clearly at C-18 along the aliphatic chain and allowed the assignment of three hydroxyl groups at C-15, C-17, and C-22, respectively. This finding was supported by the presence of the corresponding fragment ions in the EIMS of **1b**. In fact, the EIMS of **1** and **1b** were nearly identical to those of uvarigrin[2] (which has the same molecular composition and the same locations of the mono-THF ring and the three hydroxyls as **1**) and its tri-TMSi derivative. The formation of an acetonide derivative (**1c**) and a formaldehyde acetal derivative (**1d**) also supported the placement of the isolated hydroxyl in **1**.

Comparison with the diagnostic NMR chemical shifts of a pair of model mono-THF compounds with adjacent hydroxyl groups in the *threo* and *erythro* configuration enabled us to interpret the $^1$H and $^{13}$C NMR spectra of **1** and led to the assignment of the relative stereochemistry in the mono-THF portion of the molecule.[5] The relative stereochemistry between C-21 and C-22 was determined as *erythro* by comparing the $^{13}$C NMR signal for C-22 ($\delta$ 71.4) and the $^1$H NMR signals for H-21 ($\delta$ 3.90) and H-22 ($\delta$ 3.81) with those of model compounds of known relative

10.1021/np980334q CCC: $18.00 © 1999 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/21/1999

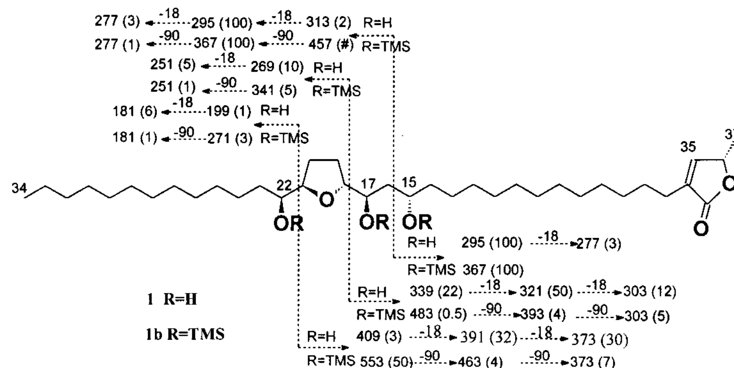



**Figure 1.** Diagnostic EIMS fragment ions (*m*/*z*) of **1** and **1b** (# peak not observed).

**Table 2.** $^1$H NMR Data (500 MHz, $CDCl_3$) of the Diagnostic Protons from the (*S*)- and (*R*)-per-MTPA Mosher Derivatives of Acetonide Calamistrin A (**1c**) and Calamistrin B (**2**)

| pro- | 1c | | | 2 | | |
|---|---|---|---|---|---|---|
| ton(s) | $\delta_S$ (**1S**) | $\delta_R$ (**1R**) | $\Delta\delta_H(\delta_S - \delta_R)$ | $\delta_S$ (**2S**) | $\delta_R$ (**2R**) | $\Delta\delta_H(\delta_S - \delta_R)$ |
| 3 | 2.2710 | 2.2632 | +0.0078 | 2.2604 | 2.2587 | +0.0017 |
| 15 | 3.9130 | 3.8760 | +0.0347 | 4.8315 | 4.6258 | +0.2057(*R*) |
| 17 | 4.0645 | 4.0030 | +0.0615 | 5.1305 | 5.2493 | −0.0594 |
| 18 | 3.7891 | 3.6351 | +0.1540 | 3.9043 | 3.9917 | −0.0874 |
| 21 | 3.7685 | 3.6062 | +0.1023 | 3.9407 | 3.7346 | +0.2061 |
| 22 | 5.3121 | 5.2768 | +0.0353(*S*) | 5.2097 | 5.0530 | +0.1568(*S*) |
| 23 | 1.5990 | 1.6203 | −0.0203 | 1.5281 | 1.5940 | −0.0659 |

stereochemistry. According to Born's rule,[6] the $^{13}$C NMR chemical shift of **1** for C-17 at $\delta_C$ 71.7 suggested an *erythro* relationship between C-17 and C-18. However, considering the $\gamma$-gauche effect due to the presence of a hydroxyl substituent at C-15, the signal of C-17 would be expected to shift upfield.[3] Therefore, a *threo* relationship between C-17/C-18 was assigned. This was supported by the publishing of X-ray crystallographic data for uvarigrin.[2] In addition, the C-15 upfield shift ($\delta_C$ 68.8) also supported the presence of a $\gamma$-gauche effect due to a hydroxyl group at the $\beta$-position, as the carbons having a single isolated hydroxyl group are typically displayed at $\delta_C$ 70−71 in other acetogenins. The small difference value ($\Delta\delta$ <1.5 ppm) of $^{13}$C NMR signals between C-18 and C-21 in **1** indicated the trans relationship of the THF ring.[6] The formal protons of the formaldehyde acetal derivative (**1d**) resonated at $\delta_H$ 4.96 and 4.91, indicating their similar magnetic environments and allowing the assignment of a trans configuration for the acetal ring.[7] The similarity of diagnostic NMR data in the mono-THF part of **1** with those of tonkinin C[3] also supported the assignment of a *threo*/*trans*/*erythro* relationship from C-17 to C-22.

The absolute stereochemistry of the carbinol chiral centers in **1** could be established by the $^1$H NMR data analysis of its *R*- and *S*- Mosher esters.[7,8] Considering the similar environment between the OH groups at C-15 and C-17, the tri-Mosher ester of **1** could result in an interference between the upfield and downfield shifts, which should affect the determination of the absolute configuration of the carbinol centers. Thus, the mono *R*- and *S*-Mosher esters at C-22 were prepared after the formation of an acetonide derivative between the OH groups at C-15 and C-17. The $^1$H NMR data for the *R*- and *S*-Mosher esters (**1*R*** and **1*S***) of the acetonide of **1** are summarized in Table 2. According to the Mosher assumptions,[9] the absolute configuration at C-22 was assigned as *S*, because the signals of $\Delta\delta_H$ ($\delta_S - \delta_R$) were positive for H-15, H-17, H-18, and H-21, showing relatively less shielding for this side of the molecule in the (*S*) MTPA ester. With the *threo*/*trans*/*erythro* relative configuration from C-17 to C-22 in **1** determined above, the absolute sterochemistry of C-17, C-18, C-21, and C-22 was determined as *R*, *R*, *R*, and *S*, respectively. Based on the assignment of trans configuration for the acetal ring in **1d**, the absolute configuration at C-15 was established as having a *S* configuration. The configuration at C-36 was assumed as *S* due to the fact that the configuration of this chiral center has been determined to be *S* in most of the acetogenins whose absolute stereochemistry has been solved.[10] Thus, the absolute configuration of calamistrin A (**1**) was proposed as 15*S*, 17*R*, 18*R*, 21*R*, 22*S*, and 36*S*, as illustrated in Figure 1. Obviously, **1** was the stereoisomer of uvarigrin (**3**).[2]

Calamistrin B (**2**) was isolated as waxy solid. The molecular formula $C_{39}H_{70}O_7$ was determined by FABMS (*m*/*z* 651) and elemental analysis. The $^1$H and $^{13}$C NMR spectra (see Table 1) exhibited characteristic signals of a mono-THF acetogenin having the same $\alpha,\beta$-unsaturated $\gamma$-lactone moiety and a similar THF ring to **1**. Both the IR carbonyl absorption band at 1720 cm$^{-1}$ and the NMR data ($\delta_C$ 171.6, 21.2 and $\delta_H$ 2.07, 3H, s) suggested the presence of an acetoxyl group, which was supported by the fragment ion at *m*/*z* 591 ($[MH - HOAc]^+$) in the FABMS. The presence of two hydroxyl groups was indicated by successive losses of two $H_2O$ unit from the $[M - HOAc]^+$ fragment in the EIMS and $[MH - HOAc]^+$ fragment in the FABMS. This was confirmed by the $^1$H NMR data ($\delta_H$ 2.08, 2.06, 2.03, each 3H, s) of the diacetyl derivative (**2a**), the EIMS spectrum of di-TMSi derivative (**2b**) and the formation of a bis-Mosher ester.

The locations of the THF ring, the two hydroxyl groups, and an acetoxyl group along the aliphatic chain in **2** were determined by the analysis of the EIMS of **2** and **2b** (see Figure 2). The EIMS of **2**/**2b** gave fragment ions at *m*/*z* 295/367, 381/453, and 451/523, which clearly showed the position of the THF ring between C-18 and C-21, the two OH groups at C-15 and C-22, and the acetoxyl group at C-17. This was in agreement with the corresponding fragment ions derived from the losses of $H_2O$, HOAc, and HOTMSi in the EIMS of **2** and **2b**. The correlation of H-15 ($\delta$ 3.38, m) with H-17 ($\delta$ 5.10, m) through H-16 ($\delta$ 1.54, m) in the $^1$H−$^1$H COSY NMR spectrum substantiated the location of the isolated hydroxyl group at C-15.

A thorough comparison of its diagnostic NMR data with those of similar compounds such as tonkinin A[3] allowed the determination of the relative stereochemistry in the analogous THF region of **2**. The small difference value ($\Delta\delta$ <1.5 ppm) in the $^{13}$C NMR resonances between C-18 ($\delta_C$ 82.5) and C-21 ($\delta_C$ 82.8) indicated the trans relationship of the THF ring.[5] The $^{13}$C NMR signal of C-22 at $\delta_C$ 71.7 was consistent with an *erythro* relative configuration between C-21 and C-22. With the consideration of $\gamma$-gauche

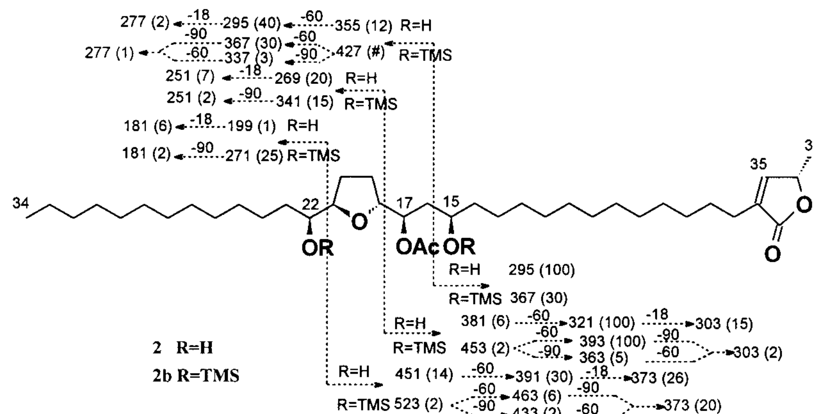



**Figure 2.** Diagnostic EIMS fragment ions ($m/z$) of **2** and **2b** (# peak not observed).

effects between the substituents at C-15 and C-17,[3] the $^{13}C$ NMR resonance for C-17 at $\delta_C$ 71.5 led to the assignment of the relative stereochemistry between C-17 and C-18 as *threo*. According to Hoye's rule, the $^1H$ NMR data of **2** supported the same determination of a *threo*/*trans*/*erythro* relationship from C-17 to C-22.[7]

For the determination of absolute stereochemistry at the carbinol chiral center in the THF part and of the isolated carbinol chiral center of **2**, Mosher's methodology was used.[7,8] The di-(*S*)- and di-(*R*)-methoxylfluoromethylphenylacetic acid (MTPA) esters (Mosher esters) of **2** (**2*S*** and **2*R***) were prepared. Their diagnostic proton chemical shifts were summarized in Table 2. The negative $\Delta\delta_H$ ($\delta_S - \delta_R$) values at C-17 and C-18 gave probably the *R* configuration at C-15 based on Mosher's assumption,[9] while the positive $\Delta\delta_H$ ($\delta_S - \delta_R$) value at C-21 and the negative $\Delta\delta_H$ ($\delta_S - \delta_R$) value at C-23 led to the *S* configuration at the C-22 chiral center. On the basis of the relationship elucidated above, the absolute configuration of **2** was determined as 15*R*, 17*R*, 18*R*, 21*R*, and 22*S*. The absolute configuration at C-36 was assumed to *S* due to the same rationale as described for **1**. So, calamistrin B (**2**) is the stereoisomer of uvarigranin (**4**).[2,4]

Compounds **1** and **2** exhibited significant inhibitory activities for three human tumor cell lines as shown in Table 3 with the MTT method. No significant activity was observed in **1** and **2** against a MCF human colon adenocarcinoma cell line. The $IC_{50}$ values of **1** and **2** for $A_{2780}$, KB, and HCT-8 are listed in Table 3.

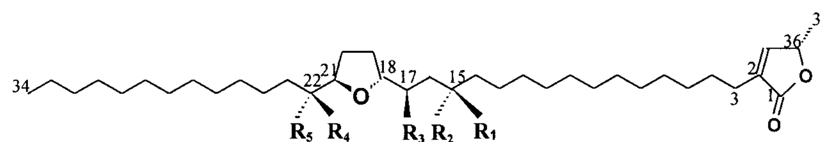
**Table 3.** Cytotoxicity ($IC_{50}$ $\mu$g/mL) of Compounds **1** and **2** for Three Human Cancer Cell Lines

| compound | KB | $A_{2780}$ | HCT-8 |
|---|---|---|---|
| **1** | 2.0 | 1.4 | $3.7 \times 10^{-1}$ |
| **2** | 6.1 | 3.1 | $2.2 \times 10^{-2}$ |

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Reichert Nr 229 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarmeter. IR spectra were recorded on a Perkin–Elmer 683 infrared spectrometer. NMR spectra were run on a Bruker AM 500 spectrometer with TMSi as internal standard. EIMS were obtained on a ZAB-2F mass spectrometer. FABMS were performed on ZabspecE mass spectrometer. Elemental analyses were determined on a MOD.1106 elemental analyzer.

**Plant Material.** The roots of *U. calamistrata* were collected at Jian-liang Peak on Hainan Island, People's Republic of China, in July 1996, and identified by Professor Wan-Zi Song. A voucher specimen (no. 46) was deposited in the herbarium of Department of Botany, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | H | OH | OH | OH | H |
| **1a** | H | OAc | OAc | OAc | H |
| **1b** | H | OTMSi | OTMSi | OTMSi | H |
| **1c** | H | $OC(CH_3)_2O$ | | OH | H |
| **1d** | H | $OCH_2O$ | | OH | H |
| **1S** | H | $OC(CH_3)_2O$ | | *S*-MTPA | H |
| **1R** | H | $OC(CH_3)_2O$ | | *R*-MTPA | H |
| **2** | OH | H | OAc | OH | H |
| **2a** | OAc | H | OAc | OAc | H |
| **2b** | OTMSi | H | OAc | OTMSi | H |
| **2S** | *S*-MTPA | H | OAc | *S*-MTPA | H |
| **2R** | *R*-MTPA | H | OAc | *R*-MTPA | H |
| **3** | H | OH | OH | H | OH |
| **4** | H | OH | OAc | H | OH |

**Extraction and Isolation.** The dried and pulverized roots (10 kg) of *U. calamistrata* were extracted exhaustively with 95% EtOH, and the solvent was removed in vacuo to yield 1.98 kg of an extract that was partitioned between $H_2O$ and $CHCl_3$ (1:1), giving 575 g of $CHCl_3$-soluble fraction. The $CHCl_3$ fraction was partitioned between petroleum ether and 90% MeOH to yield a MeOH fraction (350 g) that was subjected to a Si gel column for chromatography eluting with a gradient petroleum ether−$Me_2CO$ system and collected the eluate in 500 mL fractions. Fractions 31−38 and 121−135 showed the presence of acetogenins detected by TLC with 10% $H_2SO_4$ alcohol solution containing 0.5% vanillin as spray agent. Fractions 31−38 and 121−135 were combined and evaporated in vacuo, respectively. The residue (22.1 g) of fractions 31−38 was subjected to chromatography on a 400 g of Si gel column (Φ 3.5 cm × 80 cm) with gradient petroleum ether−EtOAc (6:1 → 3:1) in 250-mL fractions. Eluted fractions 25−30 containing compound **2** were evaporated in vacuo to give a residue (1.1 g) that was repeatedly chromatographed on Si gel column alternately with petroleum ether−$Me_2CO$ (8:1) and petroleum ether−EtOAc (5:1) to yield **2** (223 mg) and **4** (121.3 mg). The residue (12.6 g) of fractions 121−135 was dissolved with $Me_2$-CO and cooled in refrigerator. Pale crystals (230 mg) were obtained from the cooled solution, which were recrystalized in $Me_2CO$ to yield 175-mg plates of **1**. The mother liquor was evaporated and chromatographed on a 40-g Si gel column eluting with $CHCl_3$−$Me_2CO$ (10:1) to give **3** (25 mg).

**Calamistrin A (1):** colorless plate crystals; mp 96−98 °C; $[\alpha]^{20}_D$ +35°} (*c* 0.60, MeOH); IR (KBr) $\nu_{max}$ 3362 (OH), 2918, 2849, 1755 (lactone C=O), 1746, 1470, 1400, 1100, 1072 $cm^{-1}$; $^1H$ NMR data (500 MHz, $CDCl_3$) and $^{13}C$ NMR data (125 MHz, $CDCl_3$) see Table 1; EIMS (70 eV) see Figure 1; FABMS *m/z* 609 ($[MH]^+$), 591 ($[MH - H_2O]^+$), 573 ($[MH - 2H_2O]^+$), 555 ($[MH - 3H_2O]^+$), 295; *anal.* C 72.89%, H 11.15%, calcd for $C_{37}H_{68}O_6$, C 73.02%, H 11.18%.

**Calamistrin B (2):** waxy solid; $[\alpha]^{28}_D$ +24.8° (*c* 0.40, MeOH); IR (KBr) $\nu_{max}$ 3504 (OH), 2918, 2850, 1755 (lactone >C=O), 1738, 1720 (MeCO), 1469, 1375, 1320, 1200, 1150, 1100, 1072, 1026, 975, 720 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) and $^{13}C$ NMR data (125 MHz, $CDCl_3$), see Table 1: EIMS (70 eV) see Figure 2; FABMS *m/z* 651 ($[MH]^+$) (40), 591 ($[MH - HOAc]^+$) (100), 573 ($[MH - HOAc - H_2O]^+$) (70), 555 ($[MH - HOAc - 2H_2O]^+$) (75), 373 (20), 321 (35), 295 (50); *anal.* C 72.00%, H 10.77%, calcd for $C_{39}H_{70}O_7$, C 71.88%, H 10.74%.

**Acetyl Derivative (1a).** A small amount (3 mg) of **1** was treated with $Ac_2O$−pyridine at room temperature for 12 h. Preparative TLC of the reacted solution yielded the purified tri-acetyl derivative **1a** (gum, 1.8 mg): $^1H$ NMR data (300 MHz, $CDCl_3$) $\delta$ 6.99 (1H, d, $J$ = 1.6 Hz, H-35), 5.03−4.86 (4H, m, H-15, 17, 22, 36), 3.96−3.90 (2H, m, H-18, 21), 2.27 (2H, t, $J$ = 6.7 Hz, H-3), 2.09 (3H, s, Ac), 2.07 (3H, s, Ac), 2.05 (3H, s, Ac), 1.41−2.00 (m), 1.40 (3H, d, H-37), 1.44−1.10 (br), 0.87 (3H, t, $J$ = 6.6 Hz, H-34).

**TMSi Derivative (1b).** A small amount (0.5 mg) of **1** was treated with *N,O*-bis (trimethylsilyl)-acetamide−pyridine and heated at 70 °C for 30 min to yield a tri-TMSi derivative (**1b**). The mixture was used directly to measure EIMS; for EIMS fragments of **1b**, see Figure 1.

**Acetonide Derivative (1c).** A 10.0-mg quantity of **1**, 0.1 mL of 2,2-dimethoxyl propane, and 1 mL of $CH_2Cl_2$ were mixed well. The mixture was stirred with a magnetic stirrer at room temperature for 8 h and was used for preparative TLC on a Si gel $GF_{254}$ plate. The purified acetonide derivative (**1c**) (7.1 mg) was used for the preparation of MTPA esters.

**(*R*)- and (*S*)-MTPA Derivatives of 1c (1*R*, 1*S*).** (*R*)- or (*S*)-α-Methoxyl-α-(trifluoromethyl)phenylacetic acid (MTPA), *N,N*-dicyclohexyl carbodiimide (DCC), and **1c** in the molar ratio of 5:7:1 were added into 1 mL anhydrous $CH_2Cl_2$ with a few crystals of 4-(dimethylamino)-pyridine (DMAP). The mixture was stirred at room temperature for 10 h. Comparative TLC with **1c** was used to check that the reaction was complete. The reacted solution was used for the purification of MTPA esters by preparative TLC. The purified (*R*)- and (*S*)-MTPA esters of **1c** (**1*R*** and **1*S***) was used for a $^1H$ NMR determination (see Table 2).

**Formaldehyde Acetal Derivatives (1d).** A 5-mg portion of **1**, 1 mL of $CH_2Cl_2$, 20 mg of paraformaldehyde, and a few crystals of *p*-toluenesulfonic acid were mixed well and stirred at room temperature for 8 h. The reaction mixture was purified by preparative TLC to yield **1d**: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.98 (1H, d, $J$ = 1.5 Hz, H-35), 4.99 (1H, m, H-36), 4.96 (1H, d, $J$ = 9.8 Hz, $-OCH_2O-H_a$), 4.91 (1H, d, $J$ = 9.8 Hz, $-OCH_2O-H_b$), 4.08 (1H, m, H-21), 3.93 (3H, m, H-15, 18, 22), 3.72 (1H, m, H-17), 2.28 (2H, t, $J$ = 6.9 Hz, H-3), 1,10−2.10 (m), 1.40 (3H, d, $J$ = 6.9 Hz, H-37), 0.88 (3H, $J$ = 6.3 Hz, H-34).

**Acetyl Derivative (2a).** The preparative procedure of **2a** was same as that for **1a**: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.98 (1H, q, $J$ = 1.8 Hz, H-35), 5.01−4.87 (4H, m, H-15, 17, 22, 36), 3.99−3.91 (2H, m, H-18, 21), 2.27 (2H, t, $J$ = 6.6 Hz, H-3), 2.08 (3H, s, Ac), 2.06 (3H, s, Ac), 2.03 (3H, s, Ac), 1.41−2.00 (m), 1.40 (3H, d, H-37), 1.40−1.10 (br), 0.88 (3H, t, $J$ = 6.6 Hz, H-34).

**TMSi Derivative (2b).** The preparation of **2b** was similar to that for **1b**. For EIMS fragments of **2b**, see Figure 2.

**(*R*)- and (*S*)-MTPA Derivatives of 2 (2*R* and 2*S*).** The preparation of **2*R*** and **2*S*** was analogous to that for **1*R*** and **1*S***. For the $^1H$ NMR data for diagnostic protons of **2*R*** and **2*S***, see Table 2.

**Bioassays.** Cytotoxicity against human tumor cell lines for **1** and **2** was measured in 5-day MTT test at the Department of Parmacology, Institute of Materia Medica, Chinese Academy of Medical Science, using KB human epidermoid cancer cells, HCT-8 human ileocecal carcinoma cells, A2780 human epithelial tumor cells, and MCF-7 human mammary adenocarcinoma cells.[11,12] Cells were seeded in 96-well plates (1000 cells/well per 0.2 mL of growth medium). All cell-growth assays were carried out in RPMI medium 1640 containing 10% NCS (newborn calf serum), and fresh medium with or without compound was added 24 h later. After culturing for 5 days, 0.2 mL of MTT (0.2 mg/mL in medium) was then aspirated. The cells were dissolved in 0.2 mL of DMSO, and the absorbance at 540 nm was measured in a microplate reader.

**Acknowledgments.** This work was supported by the Science Foundation of the Chinese Academy of Medical Sciences. We would like to thank Professor Wan-Zhi Song, Department of Botany, for the plant identification.

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NP980334Q