Cytotoxicity of Curcuminoids and Some Novel Compounds from *Curcuma zedoaria*

Wan-Jr Syu,[†] Chien-Chang Shen,[‡] Ming-Jaw Don,[‡] Jun-Chih Ou,[‡] Gene-Hsiang Lee,[§] and Chang-Ming Sun*[,‡]

Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, 112 Taiwan, National Research Institute of Chinese Medicine, Taipei, 112 Taiwan, and Instrumentation Center, College of Science, National Taiwan University, Taipei, 107 Taiwan

Received June 19, 1998

Bioassay-directed fractionation of an EtOH extract of *Curcuma zedoaria* led to isolation of an active curcuminoid, which was identified as demethoxycurcumin (**2**) by comparison of its ^{1}H and ^{13}C NMR spectra with literature data and by direct comparison with synthetic material. Curcumin (**1**) and bisdemethoxycurcumin (**3**) were also obtained. Curcuminoids (**1**−**3**) were synthesized and demonstrated to be cytotoxic against human ovarian cancer OVCAR-3 cells. The observed CD_{50} values of **1**, **2**, and **3** were 4.4, 3.8, and 3.1 μg/mL, respectively. Three additional novel compounds, 3,7-dimethylindan-5-carboxylic acid (**4**), curcolonol (**5**), and guaidiol (**6**), were also isolated from the EtOH extract. The structures and relative stereochemistry of **4**−**6** were determined by spectroscopic methods and X-ray crystallographic analysis.

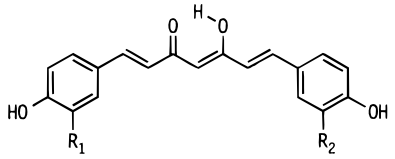
The Chinese traditional medicine *Curcuma zedoaria* (Berg.) Rosc. (Zingiberaceae) has been clinically used for the treatment of cervical cancer.[1−4] The essential oil of *C. zedoaria* exhibits antimicrobial activity against *Staphylococcus aureus*, *Vibrio comma*, and *Escherichia coli*.[5] A water extract of *C. zedoaria* demonstrated antimutagenic activity against benzo[α]pyrene-induced mutations in the Salmonella/microsomal system.[6] Polysaccharides and the protein-bound polysaccharides of *C. zedoaria* showed inhibition of sarcoma-180 and Echrlich ascites tumor in mice, respectively.[7,8] Furthermore, two sesquiterpene derivatives (curcumol and curdione) of *C. zedoaria* showed cytotoxicity against sarcoma-37, Echrlich ascites tumor, and cervical carcinoma-U14 in mice.[9]

More than twenty sesquiterpenes,[10] curcuminoids (**1**−**3**),[11] and ethyl *p*-methoxycinnamate[12] have been reported as chemical constituents of *C. zedoaria*. In our continuing search for antitumor agents, we found that a crude ethanolic extract of *C. zedoaria* showed inhibitory activity against OVCAR-3 cells (a human ovarian cancer cell line). We herein report the bioassay-directed fractionation of an ethanolic extract of *C. zedoaria* and the isolation of the bioactive compound, demethoxycurcumin (**2**). Three novel components, 3,7-dimethylindan-5-carboxylic acid (**4**), curcolonol (**5**), and guaidiol (**6**), were isolated from the nonbioactive fractions, and their structures and relative stereochemistry were determined by spectroscopic methods and X-ray crystallographic analysis.

Results and Discussion

The concentrated ethanolic extract of *C. zedoaria* was dissolved in H_2O and partitioned with EtOAc. Only the EtOAc layer showed cytotoxicity against OVCAR-3 cells. The EtOAc extract was then fractionated by column chromatography, and the fractions were assayed for cytotoxicity. The active fractions were combined and rechromatographed resulting in an active mixture which contained the major component (**2**). To investigate the activity of pure **2** and its derivatives, compounds **1**−**3** were prepared by reaction of vanillin, 4-hydroxybenzaldehyde, and acetylacetone.[13] The relative cytotoxicities of these curcuminoids against OVCAR-3 were then compared.

The cytotoxicity of synthetic **2** was slightly higher than that isolated from the plant. Compounds **1** and **3** were also cytotoxic to OVCAR-3 cells. Representative growth inhibition activities are shown in Figure 1. Of these three effective curcuminoids, compound **3** with the CD_{50} value of 3.1 μg/mL was the most active followed by **2** (3.8 μg/mL) and **1** (4.4 μg/mL). These three compounds have been previously found to have antipromotor activity against carcinogenesis,[14] and **1**, **2**, and **3** are in the same order of increasing activity as our results of the cytotoxicity against OVCAR-3. Compound **1** has been extensively studied as a chemopreventive agent against cancers in various models. It was found to be cytotoxic to human HCT-15[15] and HT-29[15,16] colon cancer cells, erb B2 oncogene-transformed NIH 3T3 cells,[16] mouse sarcoma cells,[16] human 293 kidney cancer cells,[16] and human heptacellular carcinoma Hep G2 cells,[16] but not to several primary fibroblast cells.[16] Here we further demonstrated that **1**, as well as **2** and **3**, had a cytotoxicity effect against ovarian cancer OVCAR-3 cells.

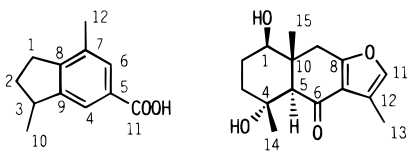


1 $R_1 = R_2 = OCH_3$

2 $R_1 = H$, $R_2 = OCH_3$

3 $R_1 = R_2 = H$

From the nonbioactive fractions of the EtOAc extract, three novel compounds **4**−**6** along with a number of known sesquiterpenes were isolated. Compounds **4**−**6** exhibited no apparent cytotoxicity to OVCAR-3 cells at concentration up to 20 μg/mL.



4 **5** **6**

* To whom correspondence should be addressed. Phone: +886-2-28201999, ext. 6641. Fax: +886-2-28264276. E-mail: cmsun@cma23.nricm.edu.tw.
[†] National Yang-Ming University.
[‡] National Research Institute of Chinese Medicine.
[§] National Taiwan University.

10.1021/np980269k CCC: $15.00
Published on Web 10/17/1998

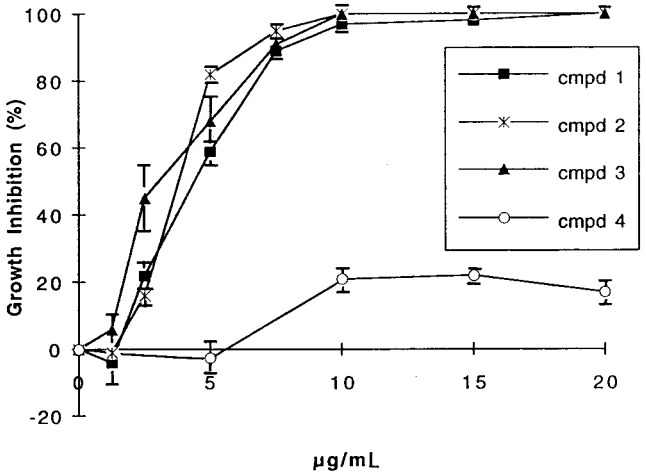


Figure 1. Effects of compounds **1**−**4** on growth of OVCAR-3 cells after 48 h incubation. Increasing concentrations of compounds **1**−**3** dose-dependently inhibited the growth of OVCAR-3 cells whereas compound **4** did not show a similar effect.

Table 1. ^{1}H and ^{13}C NMR Data of 3,7-Dimethylindan-5-carboxylic Acid (**4**) in Acetone-d_6[a]

positions	δ_C	DEPT	δ_H	J (Hz)	HMBC (H → C)
1	30.5	CH_2	a 2.91 ddd	4.0, 8.5, 16.5	C-2, C-3, C-8, C-9
			b 2.75 dt	16.5, 8.4	C-2, C-3, C-7, C-8, C-9
2	34.9	CH_2	a 2.35 ddt	4.0, 12.5, 8.0	C-1, C-3, C-8, C-9, C-10
			b 1.60 dq	12.5, 8.5	C-1, C-3, C-8, C-10
3	40.1	CH	3.20 m		C-2, C-4, C-9, C-10
4	122.7	CH	7.67		C-3, C-6, C-8, C-11
5	129.8	C			
6	129.5	CH	7.66		C-4, C-8, C-11, C-12
7	134.4	C			
8	149.1	C			
9	149.6	C			
10	20.3	CH_3	1.23 d	7.0	C-2, C-3, C-9
11	168.1	C			
12	18.9	CH_3	2.28 s		C-6, C-7, C-8

[a] ^{1}H NMR (500 MHz) and HMBC (J = 8 Hz) data were recorded on a Bruker DMX-500 instrument; ^{13}C NMR (50 MHz) and DEPT spectra were performed on a Varian Gemini-200 spectrometer.

Compound **4** was obtained as colorless needles from hexane, mp 153−155 °C (dec). The IR spectrum showed absorption bands for hydroxyl (2500−3000 cm^{-1}) and carbonyl groups (1674 cm^{-1}) of carboxylic acid. The EIMS of **4** gave a molecular ion peak at m/z 190 ($C_{12}H_{14}O_2$), and major fragment ions at m/z 175 and 145 revealed $[M - CH_3]^+$ and $[M - COOH]^+$, respectively. The ^{13}C NMR and DEPT spectra of **4** (Table 1) showed five quaternary (four aromatic carbons and one carboxylic acid carbon), three methine (two aromatic carbons), two methylene, and two methyl carbons. The ^{1}H NMR data (Table 1) showed a methylene group (δ 2.35 and 2.16) adjacent to a benzylic methylene group (δ 2.91 and 2.75). The methyl group at δ 1.23 (d, J = 7.0 Hz) was suggested to be pendent on the benzylic position. In addition, two aromatic proton signals (δ 7.67 and 7.66) suggested the benzene ring to be *m*- or *p*-unsubstituted. Therefore, compound **4** was deduced to have an indan skeleton. In turn, when the aromatic protons (δ 7.67 and 7.66) were irradiated, the aliphatic and aromatic methyl groups showed 2.2% and 4.2% NOE enhancements, respectively, but no NOE enhancement was observed on the benzylic methylene group (δ 2.91 and 2.75). Furthermore, the locations of aromatic methyl group and carboxylic acid group on benzene ring were established by the HMBC correlation (Table 1) H-12/C-6, C-8 and C-10/H-4, H-6, respectively. Thus, compound **4** was shown to be 3,7-dimethylindan-5-carboxylic acid. Since compound **4** appeared optically active as revealed by its specific rotation $[\alpha]_D$ +18° (*c* 1.0, benzene), the absolute configuration at C-3 was assigned as (*R*) by comparison with (+)-(*R*)-1-methylindan ($[\alpha]_D$ +10.0° (*c* 1.4, benzene)).[17]

Compound **5** was obtained as colorless prisms from acetone, mp 183−184 °C. The HREIMS revealed a molecular formula of $C_{15}H_{20}O_4$. The IR spectra exhibited strong absorptions at 3420 and 1653 cm^{-1}, indicating the presence of hydroxyl group(s) and conjugated ketone group, respectively. The ^{13}C NMR and DEPT spectra (Table 2) exhibited 15 carbons, including four quaternary carbons at δ 119.6, 119.8, 167.7, and 198.4 and one methine carbon at δ 140.6, which suggested a furan ring was attached by an alkyl group at the α-position and a carbonyl group at the adjacent β-position, which shifted the chemical shift of the α carbon downfield to δ 167.7. In addition, two quaternary (one oxygenated at δ 71.5), two methine (one oxygenated at δ 77.9), three methylene, and three methyl carbons were also observed. The ^{1}H NMR spectrum had a broad singlet at δ 7.29 and a doublet at δ 2.14 (J = 1.3 Hz), which accounted for the α′ proton and β' methyl protons of the furan ring. An AB-type pattern for H-9 was observed at δ 3.03 and 2.84. The ^{1}H NMR spectrum also showed two methyl singlets at δ 1.40 and 0.97, one methine proton at δ 2.61, and one oxymethine proton at δ 3.69. One methylene proton appeared at δ 1.58, and two geminal protons were evident at δ 1.73 and 1.63. The ^{1}H−^{1}H COSY spectrum showed that the geminal protons (δ 1.73 and 1.63) were correlated with an oxymethine proton (δ 3.69) and methylene protons (δ 1.58) to reveal the partial structure: $RCH(OH)CH_2CH_2R$ (R = nonprotonated carbon atom). The above data suggested that **5** was an eudesmane-type sesquiterpene. To confirm the structure, an HMBC experiment was performed and the data are given in Table 2. The connectivities of C-10 with C-1, C-5, and C-9 were established by the HMBC correlation of H-15 with C-1, C-5, and C-9, respectively. The linkages of C-4 with C-3 and C-5 were confirmed by the correlation of H-14 with C-3 and C-5. The correlation H-5 and C-6 confirmed the linkage of C-5 and C-6. The stereochemistry of **5** was established by NOE difference experiments. When the protons at δ 0.97 (H-15) were irradiated, the protons at δ 1.40 (H-14), 1.63 (H-2ax), and 3.03 (H-9eq) showed NOE enhancements, but no enhancement between H-15 and H-5 was observed. Besides, when the protons at δ 2.61 (H-5) were irradiated, the signals at δ 3.69 (H-1), 1.58 (H-3), and 2.84 (H-9ax) showed NOE enhancements, whereas no NOE effect was observed at signals of H-14 and H-15. These NOE data indicated that H-5 and the methyl group at C-10 were in trans relationship and both hydroxyl groups were in an equatorial position. Additionally, a 2D NOESY spectrum gave results consistent with the data of NOE experiments (Table 2).

Compound **6** was obtained as colorless prisms from benzene/hexane, mp 134−136 °C. The EIMS spectrum showed the molecular ion at m/z 238 ($C_{15}H_{26}O_2$) and the fragment peaks at m/z 220 $[M - H_2O]^+$ and 202 $[M - 2H_2O]^+$. The IR spectrum exhibited strong absorption for hydroxyl groups (3363 cm^{-1}) and medium absorption for the double bond (1643 cm^{-1}). The ^{13}C NMR and DEPT spectra (Table 3) showed the presence of 15 carbons, which were assigned to three methyl, six methylene (one olefinic at δ 108.7), three methine, and three quaternary carbons (one olefinic at δ 153.0 and two oxygenated at δ 83.4 and 74.9). The ^{1}H NMR spectrum (Table 3) exhibited three methyl signals at δ 1.63, 1.16, and 1.11 as well as two terminal olefinic proton signals at δ 4.63 and 4.53. Re-

Table 2. ^{1}H and ^{13}C NMR Assignments of Curcolonol (5) by DEPT, HMQC, HMBC, and NOESY Experiments in Acetone-d_6[a]

positions	δ_C	DEPT	δ_H (J)[b]	HMBC (H → C)	NOESY
1	77.9	CH	3.69 m	C-9, C-10, C-15	H-2eq, H-3, H-5, H-9ax
2	28.8	CH_2	eq 1.73 m	C-1, C-3, C-4, C-10	H-1, H-3
			ax 1.63 m	C-1, C-3, C-4	H-3, H-14, H-15
3	39.3	CH_2	1.58 m	C-2, C-5, C-14	H-1, H-5
4	71.5	C			
5	62.8	CH	2.61 s	C-1, C-4, C-6, C-9, C-10, C-14, C-15	H-1, H-3, H-9ax
6	198.4	C			
7	119.8[c]	C			
8	167.7	C			
9	40.3	CH_2	eq 3.03 d (17)	C-5, C-8, C-10, C-15	H-9ax, H-15
			ax 2.84 d (17)	C-1, C-8, C-10, C-15	H-1, H-5, H-9eq
10	45.4	C			
11	140.6	CH	7.29 bs	C-8	H-13
12	119.6[c]	C			
13	9.1	CH_3	2.14 d (1.3)	C-11	H-11
14	25.0	CH_3	1.40 s	C-3, C-4, C-5	H-2ax, H-15
15	15.0	CH_3	0.97 s	C-1, C-5, C-9, C-10	H-2ax, H-9eq, H-14

[a] ^{1}H NMR (500 MHz), HMBC (J = 8 Hz) and NOESY spectra were recorded on a Bruker DMX-500 instrument; ^{13}C NMR (50 MHz), DEPT, and HMQC (J = 150 Hz) spectra were performed on a Varian Gemini-200 spectrometer. [b] J = coupling constant in hertz. [c] Assignments may be interchangeable.

Table 3. ^{1}H and ^{13}C NMR Assignments of Guaidiol (6) by DEPT, HMQC, and HMBC Experiments in Acetone-d_6[a]

positions	δ_C	DEPT[b]	δ_H (J)[c]	HMBC (H → C)
1	50.3	CH	2.00 m	C5, C6, C10, C15
2	30.8	CH_2	a 1.80 m	C1, C3, C5, C10
			b 1.36 m	C3, C4, C5, C10
3	37.7	CH_2	a 1.69 m	C2,
			b 1.51 m	C2, C4,
4	74.9	C		
5	53.1	CH	2.73 m	C1, C3, C4, C6, C14
6	27.4	CH_2	a 1.80 m	
			b 1.40 m	
7	44.7	CH	2.05 m	C6, C9
8	33.4	CH_2	a 1.86 m	C7
			b 1.55 m	C10
9	40.6	CH_2	1.60 m	
10	83.4	C		
11	153.0	C		
12	108.7	CH_2	a 4.63 m	C7, C12
			b 4.53 m	C7, C12
13	21.2	CH_3	1.63 dd (0.8, 1.4)	C7, C12
14	33.3	CH_3	1.11 s	C3, C4, C5
15	26.1	CH_3	1.16 s	C1, C9, C10

[a] ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), HMQC (J = 150 Hz), and HMBC (J = 8 Hz) experiments were measured with a Varian Unity Inova 600 instrument. [b] DEPT spectra were determined on a Varian Gemini-200 spectrometer. [c] J = coupling constant in hertz.

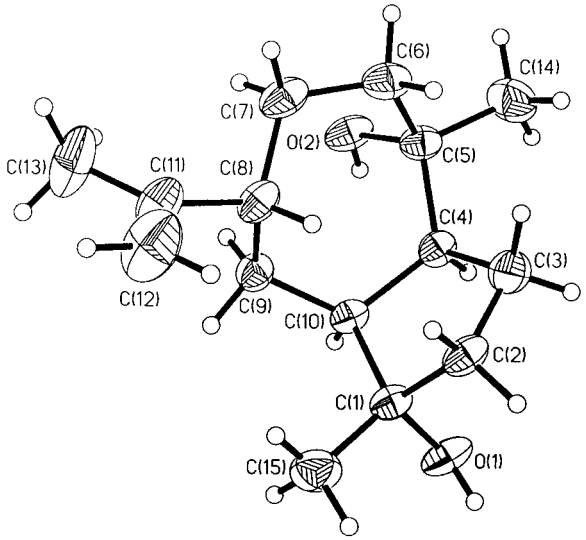


Figure 2. Perspective structure of guaidiol (**6**) (atom numbering of this figure does not correspond to the line drawing of the structure).

crystallization of **6** gave suitable crystals for X-ray diffraction analysis, which established the molecular structure and relative stereochemistry as shown in Figure 2. The molecular structure contained two fused five- and seven-membered rings which exhibited a cis connection. In Figure 2, the two methyl groups at C-4 and C-10 are in the α orientation while the isopropenyl group at C-7 is in the β orientation. The assignment of all protons and carbons for **6** was subsequently made by HMQC and HMBC data (Table 3).

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-I3 micro-melting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Nicolet Magna 560 FT-IR spectrometer. CIMS was measured with the direct insertion probe on a Finnigan GCQ GC/MS spectrometer using NH_3 as the reactant gas. EIMS spectra were obtained from a Finnigan Mat 95 spectrometer at 70 eV. HREIMS data were taken on a JEOL JMS-HX 110 mass spectrometer. TLC analysis was carried out on precoated Si gel plates (Kieselgel 60 F_{254}, Merck Art. 5554). For column chromatography, Si gel (MN Kieselgel 60, 70−230 mesh) was employed.

Biological Assays. The procedures and conditions for the cytotoxicity assay are the same as previously described.[18]

Plant Material. The root of *C. zedoaria*, imported from the People's Republic of China, was purchased from a Chinese medicine shop in Taipei in March, 1996. The material was identified by J.-C. Ou. A voucher specimen is retained in the National Research Institute of Chinese Medicine, Taipei.

Extraction and Isolation. Whole specimens of the air-dried plant (10 kg) were extracted with 95% ethanol (50 L) three times at 60 °C for 24 h. The ethanolic extracts were combined and concentrated in vacuo to 2 L. The concentrated extract was dissolved in H_2O (10 L) and then extracted with EtOAc (3 × 10 L). After evaporation of EtOAc, the concentrated mixture was mixed with 800 g of Si gel (230−400 mesh). The air-dried mixture was subjected to a chromatographic column (10 × 100 cm) and then eluted with 25%, 30%, and 40% EtOAc/hexane (16 L each) followed by 10% MeOH/acetone (20 L). Fractions (1 L each) were collected, and like fractions were combined. The combined fractions 27−37 showed cytotoxicity against OVCAR-3 cells and was rechromatographed (5 × 100 cm) with 22% and 25% EtOAc/hexane (32 L each) as

the eluent. Cytotoxic activity was observed in subfractions 51−55 (900 mL each), which was further chromatographed (2.5 × 50 cm) with 20% (500 mL) and 25% (4 L) acetone/hexane as eluent. Tubes 146−180 (20 mL each) showed cytotoxicity and were purified by preparative TLC using 45% acetone/hexane as the developing solvent to yield **2** (11 mg). Nonbioactive fractions were rechromatographed using gradient solvents of hexane and EtOAc to afford compounds **4** (7 mg), **5** (16 mg), and **6** (5 mg).

Demethoxycurcumin (2) was an orange solid and identified by comparison of its ^{1}H and ^{13}C NMR (acetone-d_6) spectra with literature data.[13,19,20] CIMS: *m/z* 339 $[M + 1]^+$.

3,7-Dimethylindan-5-carboxylic acid (4): colorless needles (hexane); mp 153−155 °C (dec); $[\alpha]^{25}_D$ +18° (*c* 1.0, benzene); TLC R_f 0.67 (50% hexane/EtOAc); IR (film) ν_{max} 3300−2500, 1674, 1422, 1302, 1284, 1244, 959, 774 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* (rel int) 190 $[M]^+$ (64), 175 (100), 145 (59), 131 (73), 129 (29), 128 (29), 115 (33), 105 (10), 91 (34), 77 (15); HREIMS *m/z* 190.0992 (calcd for $C_{12}H_{14}O_2$, 190.0994).

Curcolonol (5): colorless prisms (acetone); mp 183−184 °C; $[\alpha]^{25}_D$ 0° (*c* 2.0, EtOH); TLC R_f 0.35 (15% acetone/CH_2Cl_2); IR (film) ν_{max} 3420, 2934, 2872, 1723, 1653, 1562, 1426, 1381, 1275, 1126, 1067, 1040, 922, 742 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m/z* (rel int) 264 $[M]^+$ (13), 249 (29), 246 (15), 231 (5), 228 (5), 213 (12), 163 (100), 135 (35), 122 (37), 107 (31), 94 (14); HREIMS *m/z* 264.1354 (calcd for $C_{15}H_{20}O_4$, 264.1362).

Guaidiol (6): colorless prisms (benzene/hexane); mp 134−136 °C; $[\alpha]^{25}_D$ +30° (*c* 1.0, EtOH); TLC R_f 0.26 (15% acetone/CH_2Cl_2); IR (film) ν_{max} 3363, 3069, 2960, 2913, 2873, 1643, 1456, 1372, 1274, 1125, 1075, 916, 885, 743 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS *m/z* (rel int) 238 $[M]^+$ (1), 223 (8), 220 (20), 205 (69), 202 (30), 187 (28), 177 (34), 162 (100), 159 (45), 149 (45), 147 (36), 135 (22), 133 (17); HREIMS *m/z* 238.1923 (calcd for $C_{15}H_{26}O_2$, 238.1933).

Synthesis of Curcuminoids. Compounds **1**−**3** were prepared by the modified method reported in the literature.[13] 2,4-Pentanedione (5.0 g, 0.050 mol) and boric oxide (2.5 g, 0.035 mol) were stirred in dry EtOAc (50 mL) for 0.5 h at 40 °C. A solution of vanillin (7.6 g, 0.050 mol), 4-hydroxybenzaldehyde (6.1 g, 0.050 mol), and tributyl borate (46 g, 0.20 mol) in dry EtOAc (50 mL) was added, and the mixture was stirred at 40 °C for 0.5 h. *n*-Butylamine (1 mL) in dry EtOAc (10 mL) was added dropwise during 10 min. The reaction mixture was kept stirring overnight. A hydrochloric acid solution (0.4 N, 75 mL) was then added, and the mixture was stirred at 60 °C for 1 h. The EtOAc layer was washed with water and dried, and the solvent was evaporated. The extract was chromatographed (5 × 100 cm) using gradient solvents of $CHCl_3$ and MeOH to afford compounds **1** (2.1 g), **2** (2.6 g), and **3** (0.7 g) as orange solid. **Curcumin (1):** mp 178−180 °C (lit.[19] mp 182−183 °C); ^{13}C NMR (acetone-d_6, 50 MHz) δ 184.5, 150.0, 148.8, 141.4, 128.2, 123.8, 122.3, 116.2, 111.6, 101.6, 56.3; CIMS *m/z* 369 $[M + 1]^+$. **Demethoxycurcumin (2):** mp 172−174 °C (lit.[19] mp 172−173 °C); ^{13}C NMR (acetone-d_6, 50 MHz) δ 184.5, 184.4, 160.4, 149.9, 148.7, 141.4, 141.0, 130.9, 128.1, 127.7, 123.8, 122.2, 122.0, 116.8, 116.2, 111.5, 101.7, 56.3; CIMS *m/z* 339 $[M + 1]^+$. **Bisdemethoxycurcumin (3):** mp 221−223 °C (lit.[19] mp 223−224 °C); ^{13}C NMR (acetone-d_6, 50 MHz) δ 184.5, 160.5, 141.0, 130.9, 127.7, 122.0, 116.8, 101.7; CIMS *m/z* 309 $[M + 1]^+$.

X-ray Crystal Structure Analysis of Guaidiol (6).[21] A colorless crystal of **6** with dimensions 0.50 × 0.20 × 0.20 mm was selected for X-ray analysis. The crystallographic data were collected on a Siemens Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. Structure analysis was made by using the SHELXTL program on PC.[22] The compound crystallized in the space group $P2_12_12_1$, a = 7.8949(2) Å, b = 11.1943(2) Å, c = 16.4296(10) Å, orthorhombic, V = 1452.01(5) Å^3, Z = 4, D_{calc} = 1.090 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.70 cm^{-1}, F(000) = 528, and T = 295 K. A total of 8775 reflections were collected in the range of $2.20^o \le \theta \le 27.35°$, of which only 1582 unique reflections with $I > 2\sigma(I)$ were corrected for the analysis. The structure was solved using direct methods and refined by full-matrix least-squares on F^2 values. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0448, R_w = 0.0607 with goodness-of-fit = 1.035. Scattering factors were taken from *International Tables for X-ray Crystallography*.[23]

Acknowledgment. We gratefully acknowledge financial support through the National Science Council (NSC 87-2315-B-010-007-MH to W.J.S. and NSC 87-2113-M-077-001 to C.M.S.) and NRICM for this work.

References and Notes

(1) Jiangsu New Medical College. *Zhong Yao Da Ci Dian* (*Dictionary of Chinese Materia Medica*); Shanghai Scientific and Technological Publishers: Shanghai, 1988; pp 2450−2452.
(2) Lu Da Gynecologic and Obstetric Hospital. *Zhong Cao Yao Tong Xun* (*Chin. Tradition. Herb. Drug.*) **1972**, *2*, 69−72.
(3) Lu Da Gynecologic and Obstetric Hospital. *Xin Yi Yao Xue Za Zhi* (*J. Tradition. Chin. Med.*) **1977**, *3*, 109−111.
(4) Chen Guan Hospital *Xin Yi Yao Xue Za Zhi* (*J. Tradition. Chin. Med.*) **1976**, *3*, 116−117.
(5) Rao, B. G.; Nigam, S. S. *Indian J. Med. Res.* **1970**, *58*, 627−633.
(6) Lee, H.; Lin, J.-Y. *Mutat. Res.* **1988**, *204*, 229−234.
(7) Moon, C. K.; Park, K. S.; Lee, S. H.; Yoon, Y. P. *Arch. Pharm. Res.* **1985**, *8*, 42−44.
(8) Kokan, Takuo Tsumura Juntendo, Inc. *JPN Kokai Tokkyo Koho JP* 60 67,428 (85 67,428), 1985; *Chem. Abstr.* **1985**, *103*, 92834*w*.
(9) Xu, H.-X.; Zheng, S.-C.; Zuo, S.-X.; Sun, F.-Y. *Zhong Cao Yao Tong Xun* (*Chin. Tradition. Herb. Drug.*) **1979**, *10*, 433−437.
(10) Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin*; Springer-Verlag: Berlin, 1992; pp 401−415.
(11) Kuroyanagi, M.; Natori, S. *Yakugaku Zasshi* **1970**, *90*, 1467−1470.
(12) Gupta, S. K.; Banerjee, A. B.; Achari, B. *Lloydia* **1976**, *39*, 218−222.
(13) Roughley, P. J.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2379−2388.
(14) Anto, R. J.; George, J.; Babu, K. V. D.; Rajasekharan, K. N.; Kuttan, R. *Mut. Res.* **1996**, *370*, 127−131.
(15) Hanif, R.; Qiao, L.; Shiff, S. J.; Rigas, B. *J. Lab. Clin. Med.* **1997**, *130*, 576−584.
(16) Jiang, M. C.; Yang-Yen, H. F.; Yen, J. J. Y.; Lin, J. K. *Nutr. Cancer* **1996**, *26*, 111−120.
(17) Hansen, H.-J.; Sliwka, H.-R.; Hug, W. *Helv. Chim. Acta* **1979**, *62*, 1120−1128.
(18) Sun, C.-M.; Syu, W.-J.; Huang, Y.-T.; Chen, C.-C.; Ou, J.-C. *J. Nat. Prod.* **1997**, *60*, 382−384.
(19) Pedersen, U.; Rasmussen, P. B.; Lawesson, S.-O. *Liebigs Ann. Chem.* **1985**, 1557−1569.
(20) Ali, M.; Bagati, A.; Gupta, J. *Indian J. Chem.* **1995**, *34B*, 884−888.
(21) Crystallographic data for compound **6** in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax, +44 1223 336033, or e-mail, deposit@ccdc.cam.ac.uk).
(22) Sheldrick, G. M. *1994 SHELXTL/PC, Version 5.03*, Siemens Analytical X-ray Instruments Inc., Madison, WI.
(23) Ibers, J. A.; Hamilton, W. C., Eds. *International Tables for X-ray Crystallography*; The Kynoch Press: Birmingham, U.K., 1974; Vol. IV.

NP980269K