Lignans from the Roots of *Saururus chinensis*

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Four new lignans, saucerneol F (**1**), saucerneol G (**2**), saucerneol H (**3**), and saucerneol I (**4**), were isolated from the EtOAc extract of the roots of *Saururus chinensis*, together with one known compound, saucerneol D (**5**). The structures of compounds **1**−**4** were elucidated by spectroscopic analysis. These compounds showed cytotoxic activities against HT-29, MCF-7, and HepG-2 cell lines.

Saururus chinensis (Saururaceae) is a perennial herbaceous plant that has been used in the treatment of various diseases such as edema, jaundice, gonorrhea, fever, and inflammation in Korean folk medicine.[1] Studies of the genus *Saururus* have shown the presence of lignans,[2−5] aristolactams, flavonoids, anthraquinones, and fruanoditerpenes,[10−13] some of which exhibited neuroleptic,[6] hepatoprotective,[7] antifeedant,[8] and antioxidant activities.[9] Previously, we reported the isolation of protective agents against sepsis in the animal model from this plant.[14] In this paper, we report the isolation and structural determination of five lignans, as well as their cytotoxic activity against human colon adenocarcinoma (HT-29), human breast adenocarcinoma (MCF-7), and human liver hepatoblastoma (HepG-2) cell lines.

The MeOH extract of the roots of *S. chinensis* was partitioned by *n*-hexane, EtOAc, BuOH, and H_2O successively. The EtOAc extract was chromatographed on silica gel, Sephadex LH-20, and reversed-phase columns to afford five lignans (**1**−**5**). Compound **5** was identified as the known compound saucerneol D by comparison of the ^1H and ^{13}C NMR data and the specific rotation value.[15] Compound **1** was obtained as an amorphous, brown powder, with a molecular formula of $C_{30}H_{32}O_8$ determined by HRFABMS (*m*/*z* found 543.2000 $[M + Na]^+$; calcd 543.1995). The UV and IR spectra of **1** revealed the presence of hydroxy (3468 cm^{-1}) and oxygenated phenyl groups (234 and 284 nm, 1505 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** were similar to those of **5**, but lacked the signals of two methoxy groups of **5** and, instead, showed the signal of one additional methylenedioxy group (δ_H 5.964, 2H, δ_C 101.2). Slight differences of chemical shifts at C-1″, C-2″, C-3″, C-4″, C-5″, and C-6″ were found in the 0.05−3 ppm range, respectively, in the ^1H and ^{13}C NMR spectra of **1** from those of **5**. DEPT, HMQC, HMBC, and NOESY spectra of **1** established the one-dimensional structure (Figure 1). The specific rotation of **1** {$[\alpha]^{25}_D$ −60.6 (*c* 0.2, $CHCl_3$)} exhibited the same sign as that of **5** {$[\alpha]^{25}_D$ −88.1 (*c* 1.2, $CHCl_3$)}. The relative configuration of **1** could be deduced by comparison with literature data for related lignans (Supporting Information).[15−29] From the above evidence, **1** was determineded as *threo*-1-(benzo[*d*][1,3]dioxol-5-yl)-2-[4-{(2α,3α,4*β*,5*β*)-5-(benzo[*d*][1,3]dioxol-5-yl)-3,4-dimethyltetrahydrofuran-2-yl}-2-methoxyphenoxy]propan-1-ol and named saucerneol F.

The molecular formula of **2** was found to be $C_{20}H_{20}O_6$ by HRFABMS (*m*/*z* found 357.1335 $[M + H]^+$; calcd 357.1338). The

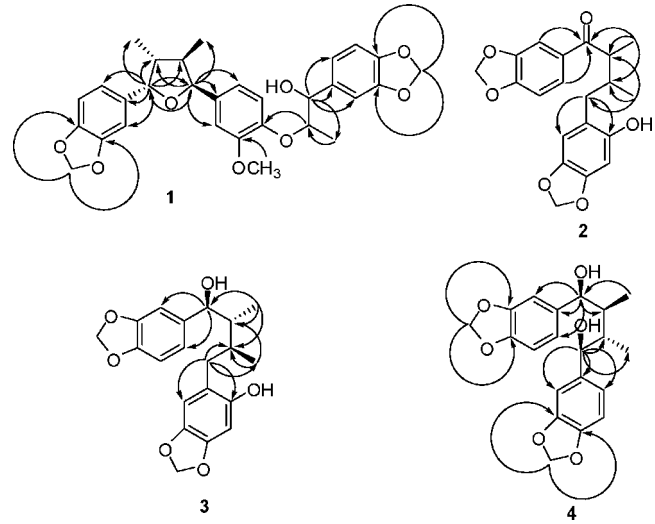

Figure 1. Key HMBC correlations of compounds **1**−**4**.

UV spectrum showed maxima at 229, 276, and 306 nm, indicating an aromatic phenolic ketone moiety in **2**. The IR spectrum of **2** revealed the presence of hydroxy (3424 cm^{-1}) and conjugated ketone groups (1658 cm^{-1}). The ^1H NMR spectrum exhibited two distinct *sec*-methyls (H-9′ and H-9), two methines (H-8′ and H-8), benzylic methylene signals (H-7b′ and H-7a′), two methylenedioxy groups, and five aromatic protons (H-2, H-5, H-6, H-3′, and H-6′). The ^1H and ^{13}C NMR spectra showed two separate sets of aromatic carbon atoms, one due to a 3,4-methylenedioxy moiety, the other due to a 2′-hydroxy-4′,5′-methylenedioxyphenyl unit. In the HMBC spectrum of **2**, long-range correlations of C-7 with H-2, H-6, H-8, and H-9 were observed (Figure 1). In the NOESY spectrum of **2**, H-9′ showed a correlation with 6′-H but not with H-3′. On the basis of these data, **2** was determined as 1-(benzo[*d*][1,3]dioxol-5-yl)-4-(6-hydroxybenzo[*d*][1,3]dioxol-5-yl)-2,3-dimethylbutan-1-one and named saucerneol G.

The molecular formula of **3** was found to be $C_{20}H_{22}O_6$ by HREIMS (*m*/*z* found 340.1316 $[M - H_2O]^+$; calcd 340.1311). The IR and UV spectra of **3** revealed the presence of hydroxy (3424 cm^{-1}) and phenolic groups (1504 cm^{-1}, 231 and 290 nm). The ^1H NMR spectrum showed the presence of two methyl doublets (H-9 and H-9′), two methine groups (H-8′ and H-8), one benzylic methylene (H-7b′ and H-7a′), one benzylic methine group substituted by oxygen (H-7), two methylenedioxy groups, and five aromatic protons (H-2, H-5, H-6, H-3′, and H-6′). The 2D $^1H-^1H$ COSY spectrum indicated coupling among H-7, H-8, H-9, H-7′, H-8′, and H-9′. The ^1H and ^{13}C NMR spectra showed two separate

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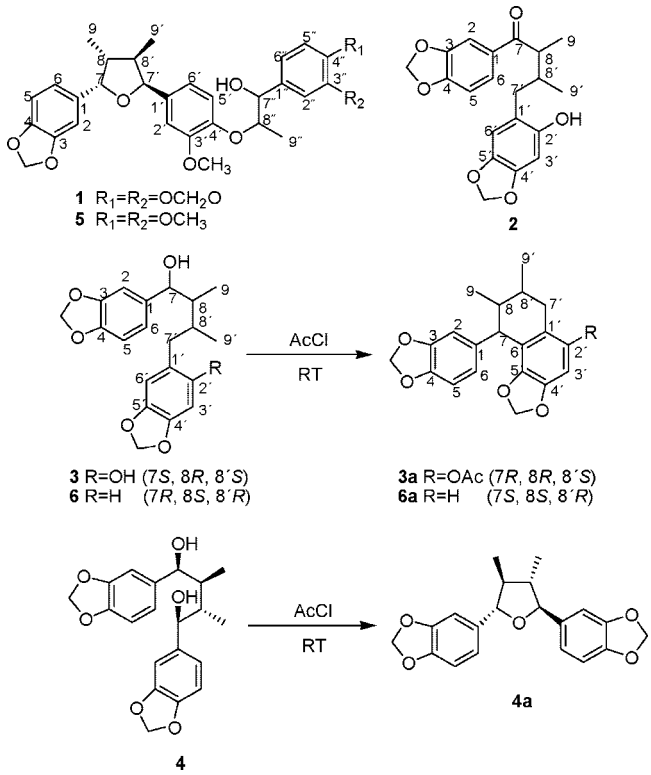

sets of aromatic carbon atoms, one due to a 3,4-methylenedioxy moiety, the other due to a 2′-hydroxy-4′,5′-methylenedioxyphenyl unit. In the NOESY spectrum of **3**, H-9′ showed a correlation with 6′-H but not with H-3′. From the HMBC spectrum of **3**, the one-dimensional structure of **3** was determined to be the 2′-hydroxy derivative of the reported compound **6** (Figure 1).[30] The absolute configuration at C-7 of **3** was established by Mosher ester methodology.[31−33] The differences of chemical shift values obtained by subtracting (*R*)-MTPA ester from (*S*)-MTPA ester [$\Delta\delta_H$ ($\delta_S - \delta_R$)] are shown in Table 1, and the negative values of $\Delta\delta_H$ ($\delta_S - \delta_R$) at H-8, 9, 8′, and 9′ suggested a 7*S* configuration in compound **3**. To determine the configurations at C-8 and C-8′, **3** was converted to an aryltetralin type compound (**3a**) with acetyl chloride by the reported reaction, in which inversion of the configuration at C-7 of **6** to that of **6a** was shown.[30] The pattern of cyclization of **3a** was confirmed by a 1D-NOE experiment, which demonstrated correlations of acetyl protons to both 3′-H and 7′-H. The observed coupling constants, $J_{7,8} = 9.2$ Hz and $J_{7',8'} = 10.9$ Hz, for **3a** indicated all-axial orientations of H-7, H-8, H-7′, and H-8′ and confirmed the all-*trans* arrangement of the two methyl groups and the pendant phenyl group with all pseudo-equatorial positions. On the basis of this evidence, the structure of **3** was proposed to be 6-{(2*S*,3*R*,4*S*)-4-(benzo[*d*][1,3]dioxol-5-yl)-4-hydroxy-2,3-dimethylbutyl}benzo[*d*][1,3]dioxol-5-ol, and **3** was named saucerneol H.

The ^{1}H and ^{13}C NMR spectra of **4** showed signals of only 10 protons and 10 carbons, and the high-resolution mass spectrum confirmed its molecular formula as $C_{20}H_{20}O_6$, which indicated the symmetric feature of this compound. The ^{1}H and ^{13}C NMR spectra of **4** suggested the presence of two 3,4-methylenedioxy phenyl units and an 8,8′-dimethyl-7,7′-diol-type skeleton. To determine the absolute configuration of C-7, Mosher ester derivatives ($\mathbf{4}_R$ and $\mathbf{4}_S$) of **4** were prepared, and ^{1}H NMR data of $\mathbf{4}_R$ and $\mathbf{4}_S$ were also assigned on the basis of the ^{1}H−^{1}H-COSY spectra (Table 2). The negative values of $\Delta\delta_H$ ($\delta_S - \delta_R$) at H-9, 7′, 8′, and 9′ suggested a 7*S* configuration for compound **4**. To determine the configurations at C-8 and C-8′, **4** was converted to a tetrahydrofuran-type compound (**4a**) by treatment with acetyl chloride.[30] ^{1}H and ^{13}C NMR data of **4a** were in excellent accordance with those of (−)-galbacin, which possesses a 7*S*,8*S*,8′*S*,7′*S* configuration, and the specific rotation value of **4a** showed the same sign as (−)-galbacin, $[\alpha]^{22}_D$ −41.5 (*c* 0.026, $CHCl_3$) {$[\alpha]_D$ −11.7}.[4,28,34] On the bais of this evidence, **4** was suggested to be (1*S*,2*S*,3*S*,4*S*)-1,4-di(benzo[*d*][1,3]dioxol-5-yl)-2,3-dimethylbutane-1,4-diol and named saucerneol I.

Table 1. Characteristic ^{1}H NMR Data of Mosher Esters of **3** for Determination of Absolute Configuration

	position				
	7	8	9	8′	9′
$\mathbf{3}_S$ (δ_S)	5.49	1.82	0.30	1.68	0.55
$\mathbf{3}_R$ (δ_R)	5.46	1.91	0.42	2.02	0.63
$\Delta\delta$ ($\delta_S - \delta_R$)	*S*	−0.09	−0.12	−0.34	−0.08

Table 2. Characteristic ^{1}H NMR Data of Mosher Esters of **4** for Determination of Absolute Configuration

	position				
	7	9	7′	8′	9′
$\mathbf{4}_S$ (δ_S)	5.57	0.68	4.26	2.14	0.57
$\mathbf{4}_R$ (δ_R)	5.60	0.63	4.15	1.76	0.46
$\Delta\delta$ ($\delta_S - \delta_R$)	*S*	−0.05	−0.11	−0.38	−0.11

Compounds **1**−**5** and the positive control, camptothecin, exhibited cytotoxic activities against the HT-29 cell line (IC_{50} values of 10, 55, 53, 21, 13, and 2 μM, respectively), the hepG-2 cell line (IC_{50} values of 11, 62, 61, >100, 16, and 0.3 μM, respectively), and the MCF-7 cell line (IC_{50} values of >100, 64, 72, >100, >100, and 10 μM, respectively).

Experimental Section

General Experimental Procedures. Melting points were measured using the capillary melting point apparatus, Electrothermal 9100 (Essex, UK), and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. FT-IR spectra were recorded on a JASCO FT-IR 300E (Tokyo, Japan) spectrophotometer and UV spectra on a JASCO V-550 (Tokyo, Japan) spectrophotometer. ^{1}H NMR (250, 600, and 900 MHz) and ^{13}C NMR (62.9 and 150 MHz) were recorded on a Bruker AMX250, DMX600, and Bruker Biospin Avancell 900 spectrometer (Karlsruhe, Germany). Samples were dissolved in $CDCl_3$ and reported in ppm downfield from TMS. HIFABMS and HIEIMS were obtained on a JEOL JMS700 spectrometer (JEOL, Japan). The stationary phases used for column chromatography (silica gel 60, 70−230 and 230−400 mesh, and Lichroprep RP-18 gel, 40−63 μm, Merck) and TLC plates (silica gel 60 F_{254} and RP-18 F_{254s}, 0.25 mm, Merck) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating. (*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA-Cl] and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl] were purchased from Aldrich (St. Louis, MO; purity 99.0%).

Plant Material. The roots of *S. chinensis* were purchased in February 2003 from a folk medicine market, "Yak-ryong-si", in Daegu, Republic of Korea. These materials were confirmed taxonomically by Professor Gi-Hwan Bae, Chungnam National University, Daejeon, Korea. A voucher specimen (YNSC2004) has been deposited at the College of Pharmacy, Yeungnam University.

Extraction and Isolation. The dried roots of *S. chinensis* (9.7 kg) were extracted with 70% MeOH (×3) by refluxing for 24 h, and the MeOH solution was then evaporated to dryness (1.0 kg). The MeOH extract was suspended in H_2O (1.4 L), and the resulting H_2O layer was successively partitioned with *n*-hexane, EtOAc, and BuOH (each 1.4 L × 3). The EtOAc extracts (130 g) were loaded onto a silica gel column (12 × 100 cm, 70−230 mesh) and eluted by a stepwise gradient of *n*-hexane−EtOAc (100:0 → 0:100) and then EtOAc−MeOH (100:0 → 0:100). The eluates (500 mL in each flask) were combined into 39 fractions (SCE1−SCE39) on the basis of silica gel TLC. Fractions 25 (1.3 g) and 28 (1.4 g) were chromatographed on a reversed-phase column (4 × 50 cm, LiChroprep RP-18), using MeOH−H_2O (gradient elution, from 50:50 to 100% MeOH), to give **1** (230 mg) and **4** (140 mg), respectively. Fractions 20 (1.3 g) and 26 (1.0 g) were subjected

to reversed-phase column chromatography (4 × 50 cm, LiChroprep RP-18), using MeOH−H_2O (gradient elution, from 40:60 to 100% MeOH), to give **2** (70 mg) and **3** (40 mg), respectively. Fraction 29 (500 mg) was chromatographed on a Sephadex LH-20 column (4.5 × 80 cm, Sephadex LH-20) eluted with MeOH (3.0 L) to give **5** (400 mg).

Saucerneol F (1): amorphous, brown powder (EtOAc−MeOH); mp 59−61 °C; $[\alpha]^{25}_D$ −60.6 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 234 (4.36), 284 (4.12) nm; IR (KBr) ν_{max} 3468, 2963, 2891, 1505, 1443, 1249, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, 900 MHz) δ 6.98 (1H, d, J = 8.1 Hz, H-5′), 6.92 (1H, br s, H-2″), 6.89 (1H, br s, H-2′), 6.86 (1H, d, J = 7.2 Hz, H-6″), 6.82 (1H, br s, H-2), 6.82 (1H, br d, J = 6.7 Hz, H-6′), 6.80 (1H, d, J = 7.9 Hz, H-5), 6.78 (1H, d, J = 8.0 Hz, H-5″), 6.76 (1H, br d, J = 7.8 Hz, H-6), 5.964 (2H, s, OC*H*$_2$O-3,4), 5.955 (2H, s, OC*H*$_2$O-3″,4″), 5.43 (1H, d, J = 6.8 Hz, H-7′), 5.42 (1H, d, J = 6.9 Hz, H-7), 4.62 (1H, d, J = 8.4 Hz, H-7″), 4.10 (1H, dq, J = 8.1, 6.3 Hz, H-8″), 3.93 (3H, s, OC*H*$_3$), 2.28 (1H, ddq, J = 13.6, 6.8, 6.8 Hz, H-8′), 2.26 (1H, ddq, J = 13.6, 6.8, 6.8 Hz, H-8), 1.16 (3H, d, J = 6.2 Hz, H-9″), 0.71 (3H, d, J = 6.8 Hz, H-9), 0.70 (3H, d, J = 6.8 Hz, H-9′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 150.8 (C, C-3′), 147.9 (C, C-3″), 147.7 (C, C-4″), 147.6 (C, C-3), 146.6 (C, C-4), 146.5 (C, C-4′), 136.9 (C, C-1′), 135.6 (C, C-1), 134.2 (C, C-1″), 121.3 (CH, C-6″), 119.5 (CH, C-6), 119.1 (CH, C-5′), 118.9 (CH, C-6′), 110.3 (CH, C-2′), 108.3 (CH, C-5″), 108.0 (CH, C-5), 107.8 (CH, C-2″), 107.1 (CH, C-2), 101.2 (CH_2, OCH_2O-3,4), 101.1 (CH_2, OCH_2O-3″,4″), 84.2 (CH, C-8″, 83.9 (CH, C-7), 83.7 (CH, C-7′), 78.6 (CH, C-7″), 56.0 (CH_3, OCH_3), 44.1 (CH, C-8), 44.0 (CH, C-8′), 17.1 (CH_3, C-9″), 14.9 (CH_3, C-9, C-9′); HRFABMS *m*/*z* 543.2000 $[M + Na]^+$ (calcd for $C_{30}H_{32}O_8Na$, 543.1995).

Saucerneol G (2): amorphous, brown powder (MeOH−H_2O); mp 41−43 °C; $[\alpha]^{22}_D$ +13 (*c* 0.59, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (4.14), 276 (3.69), 306 (3.88) nm; IR (KBr) ν_{max} 3424, 2954, 2903, 1658, 1504, 1442, 1251, 1173, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.59 (1H, dd, J = 8.2 1.5 Hz, H-6), 7.45 (1H, d, J = 1.5 Hz, H-2), 6.86 (1H, d, J = 8.2 Hz, H-5), 6.49 (1H, s, H-3′), 6.48 (1H, s, H-6′), 6.04 (2H, s, OC*H*$_2$O), 5.84 (2H, s, OC*H*$_2$O), 3.17 (1H, m, H-8), 2.60 (1H, d, J = 13.5 Hz, H-7a′), 2.15 (1H, m, H-8′), 2.00 (1H, dd, J = 13.5, 10.1 Hz, H-7b′), 1.21 (3H, d, J = 7.2 Hz, H-9), 0.97 (3H, d, J = 6.4 Hz, H-9′); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 204.7 (C, C-7), 152.2 (C, C-3), 149.9 (CH, C-5′), 148.3 (C, C-4), 146.7 (C, C-4′), 140.3 (C, C-1′), 130.6 (C, C-1), 125.1 (CH, C-6), 117.4 (C, C-2′), 110.1 (CH, C-6′), 108.5 (CH, C-2), 108.0 (CH, C-5), 102.0 (CH_2, OCH_2O), 100.8 (CH_2, OCH_2O), 98.6 (CH, C-3′), 46.3 (CH, C-8), 37.7 (CH_2, C-7′), 35.6 (CH, C-8′), 16.5 (CH_3, C-9), 16.2 (CH_3, C-9′); HRFABMS *m*/*z* 357.1335 $[M + H]^+$ (calcd for $C_{20}H_{21}O_6$, 357.1338).

Saucerneol H (3): sticky solid (MeOH−H_2O); $[\alpha]^{22}_D$ −51.9 (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 231 (4.10), 290 (3.93) nm; IR (KBr) ν_{max} 3424, 2954, 2903, 1658, 1504, 1442, 1251, 1173, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 6.80 (1H, s, H-2), 6.72 (2H, s, H-5, H-6), 6.52 (1H, s, H-6′), 6.33 (1H, s, H-3′), 5.93 (2H, s, OC*H*$_2$O), 5.84 (2H, s, OC*H*$_2$O), 4.27 (1H, d, J = 9.7 Hz, H-7), 2.81 (1H, dd, J = 13.3, 3.8, H-7a′), 2.25 (1H, m, H-7b′), 2.18 (1H, m, H-8′), 1.74 (1H, m, H-8), 0.85 (3H, d, J = 6.5 Hz, H-9′), 0.56 (3H, d, J = 7.0 Hz, H-9); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 148.7 (C, C-5′), 147.8 (C, C-3), 147.1 (C, C-4), 146.2 (C, C-4′), 140.7 (C, C-1′), 138.0 (C, C-1), 120.6 (CH, C-6), 119.0 (C, C-2′), 110.3 (CH, C-6′), 108.0 (CH, C-5), 107.0 (CH, C-2), 101.0 (CH_2, OCH_2O), 100.8 (CH_2, OCH_2O), 98.4 (CH, C-3′), 79.5 (CH, C-7), 43.1 (CH, C-8), 38.0 (CH_2, C-7′), 34.0 (CH, C-8′), 14.6 (CH_3, C-9′), 12.0 (CH_3, C-9); HREIMS *m*/*z* 340.1316 $[M - H_2O]^+$ (calcd for $C_{20}H_{21}O_5$, 340.1311).

(7*S*,8*R*,9*R*)-9-(Benzo[*d*][1,3]dioxol-5-yl)-7,8-dimethyl-6,7,8,9-tetrahydronaphtho[2,1-*d*][1,3]dioxol-5-yl acetate (3a): $[\alpha]^{28}_D$ +5.8 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 6.70 (1H, d, J = 7.9 Hz, H-5), 6.61 (1H, dd, J = 8.0, 1.5 Hz, H-6), 6.55 (1H, d, J = 1.4 Hz, H-2), 6.42 (1H, s, H-3′), 5.89 (2H, s, OC*H*$_2$O-3,4), 5.68 and 5.58 (each 1H, d, J = 1.4 Hz, OC*H*$_2$O-4′,5′), 3.44 (1H, d, J = 9.2 Hz, H-7), 2.63 (1H, dd, J = 15.9, 3.3 Hz, H-7a′), 2.30 (3H, s, ArCOOC*H*$_3$), 2.17 (1H, dd, J = 15.9, 10.9 Hz, H-7b′), 1.43 (2H, m, H-8, H-8′), 1.03 (3H, d, J = 6.0 Hz, H-9′), 0.93 (3H, d, J = 6.0 Hz, H-9); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 169.7 (C, CH_3-*C*O_2Ar), 147.2 (C, C-4′), 145.5 (C, C-5′), 145.3 (C, C-3), 143.4 (C, C-4), 141.3 (C, C-1), 139.5 (C, C-6′), 123.2 (C, C-1′), 122.8 (C, C-2′), 122.0 (CH, C-6), 108.9 (CH, C-2), 107.5 (CH, C-5), 101.6 (CH, C-3′), 101.1 (CH_2, OCH_2O-4′,5′), 100.7 (CH_2, OCH_2O-3,4), 50.1 (CH, C-7), 44.8 (CH, C-8), 34.6 (CH, C-8′), 32.9 (CH_2, C-7′), 20.9 (CH_3, ArCOO*C*H_3), 19.9 (CH_3, C-9′), 16.8 (CH_3, C-9).

Saucerneol I (4): white powder (MeOH−H_2O); mp 138−142 °C; $[\alpha]^{25}_D$ −70.3 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 235 (4.07), 287 (4.00) nm; IR (KBr) ν_{max} 3333, 2968, 2919, 1503, 1488, 1443, 1248, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 6.86 (2H, s, H-2, 2′), 6.77 (2H, d, J = 8.8 Hz, H-6, H-6′), 6.73 (2H, d, J = 8.1 Hz, H-5, H-5′), 5.92 (4H, s, OC*H*$_2$O × 2), 4.26 (2H, d, J = 9.9 Hz, H-7, H-7′), 2.44 (2H, m, H-8, H-8′), 0.56 (6H, d, J = 6.7 Hz, H-9, H-9′); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 147.8 (C, C-3, C-3′), 147.0 (C, C-4, C-4′), 138.4 (C, C-1, C-1′), 120.5 (CH, C-6, C-6′), 107.9 (CH, C-5, C-5′), 107.0 (CH, C-2, C-2′), 100.9 (CH_2, OCH_2O × 2), 77.1 (CH, C-7, C-7′), 39.1 (CH, C-8, C-8′), 10.4 (CH_3, C-9, C-9′); HREIMS *m*/*z* 358.1414 $[M]^+$ (calcd for $C_{20}H_{21}O_5$, 358.1416).

(−)-Galbacin (4a): $[\alpha]^{25}_D$ −41.5 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 6.89 (2H, s, H-2, H-2′), 6.82 (2H, d, J = 7.9 Hz, H-6, H-6′), 6.76 (2H, d, J = 7.9 Hz, H-5, H-5′), 5.92 (4H, s, OC*H*$_2$O × 2), 4.59 (2H, d, J = 8.9 Hz, H-7, H-7′), 1.72 (2H, m, H-8, H-8′), 1.01 (6H, d, J = 5.7 Hz, H-9, H-9′); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 147.7 (C, C-3, C-3′), 146.9 (C, C-4, C-4′), 136.3 (C, C-1, C-1′), 119.7 (CH, C-6, C-6′), 107.9 (CH, C-5, C-5′), 106.6 (CH, C-2, C-2′), 100.9 (CH_2, OCH_2O × 2), 88.3 (CH, C-7, C-7′), 51.0 (CH, C-8, C-8′), 13.8 (CH_3, C-9, C-9′).

Preparation of (*S*)- and (*R*)-MTPA Esters of 3 and 4. Mosher's esters were prepared according to the reported method.[31−33] To compound **3** (3 mg) in 0.5 mL of CH_2Cl_2 were added sequentially 0.2 mL of anhydrous pyridine, 0.5 mg of 4-(dimethylamino)pyridine, and 12.5 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MPTA-Cl]. The mixture was left at room temperature overnight and checked by TLC to determine if the reaction was completed. After addition of 1 mL of *n*-hexane, the reaction mixture was passed through a column (6 × 0.6 cm, silica gel, 230−400 mesh, 9385) with *n*-hexane−CH_2Cl_2 (1:2). The eluate was dried *in vacuo* to give the (*S*)-MTPA ester of **3**. Using (*S*)-MTPA-Cl, the (*R*)-MTPA ester of **3** was prepared. The same procedure was repeated with **4** (5 mg) to give the (*S*)- and (*R*)-MTPA esters of **4**.

Conversion of 3 and 4 to 3a and 4a. Compounds **3** (6 mg) and **4** (5 mg) were each dissolved in acetyl chloride (3 drops). The solutions were kept at room temperature for 2 h and, after the addition of H_2O, neutralized with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), filtered, and evaporated. The residue that dissolved in CH_2Cl_2 (1−2 mL) was passed through a column (6 × 0.6 cm, silica gel, 230−400 mesh, 9385) with a CH_2Cl_2 mobile phase. The eluates were dried *in vacuo* to give compounds **3a** (3 mg) and **4a** (2 mg).

Cytotoxicity Bioassays. A tetrazolium-based colorimetric assay (MTT assay) was used to determine the cytotoxicities toward human colon adenocarcinoma (HT-29), human breast adenocarcinoma (MCF-7), and human liver hepatoblastoma (HepG-2) cell lines.[35]

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Supporting Information Available: NMR data of **1**, **2**, **3**, **4**, **3a**, and **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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