Bioactive Lignans from *Peperomia duclouxii*

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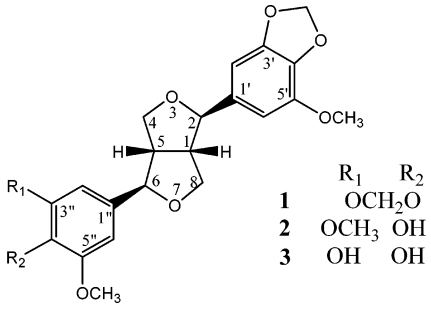
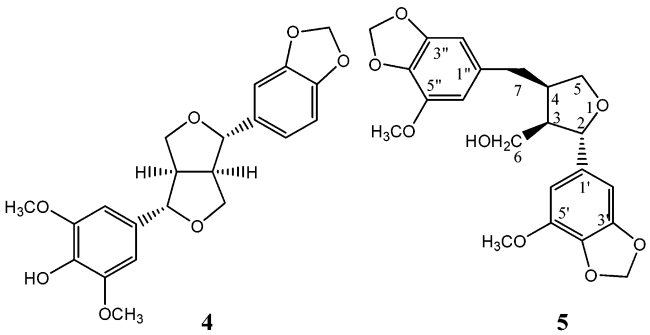
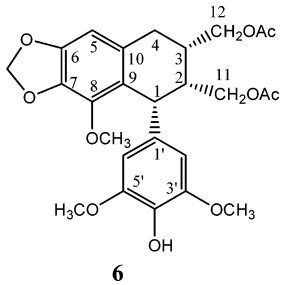
Six new lignans (**1**−**6**), along with 14 known compounds, were obtained from *Peperomia duclouxii*. The new structures were elucidated mainly by the analysis of their NMR and MS data. The absolute configurations of **1**−**6** were determined by comparing their optical rotations or CD spectra with those of known compounds. In cytotoxic and MDR reversal cell activity assays, compound **3** showed cancer cell growth inhibitory activity against VA-13 and HepG2 cells, with IC_{50} values of 5.3 and 13.2 μg/mL, and more potent effects on calcein accumulation in MDR 2780AD cells than verapamil, a positive control. Compound **6** showed anti-inflammatory activity using an ICAM-1 assay (induction of the intercellular adhesion molecule-1), stimulated by IL-1α and TNF-α.

Peperomia duclouxii C. DC. in Lecomte (Piperaceae) has been used traditionally as an anticancer agent in mainland China.[1] Seventeen lignans have been obtained from this species, and their cytotoxic and MDR (multidrug resistance) reversal activities were evaluated.[2,3] Further investigation has resulted in the isolation of six new lignans (**1**−**6**), three known lignans, and 11 other types of known compounds. The structural elucidation and bioactivity of these new compounds are reported in this paper.





4 **5**



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Results and Discussion

Compound **1** was assigned the molecular formula $C_{22}H_{22}O_8$ from the ion peak at *m*/*z* 414.1291 in the high-resolution EIMS. Signals of two protons of an AB system at δ 6.51 (1H, brs, H-2′) and 6.53 (1H, brs, H-6′), a methylenedioxy at δ 5.95 (2H, s), and a methoxy at δ 3.90 (3H, s) were attributed to a 5-methoxy-3,4-methylenedioxyphenyl group from the HMBC spectrum. Signals of an oxymethine [δ 4.69 (1H, d, J = 4.2 Hz, H-2) and 85.8 (C-2)], an oxymethylene [δ 4.25 (1H, dd, J = 9.3, 6.8 Hz, H-8a), 3.87 (1H, dd, J = 9.3, 3.4 Hz, H-8b), and 71.8 (C-8)], and a methine [δ 3.04 (1H, m, H-1) and 54.3 (C-1)] were also apparent in the ^{1}H and ^{13}C NMR spectra. The ^{1}H−^{1}H COSY spectrum indicated the linkage phenyl−^{2}CH(O)−^{1}CH−^{8}CH$_2$O, which was confirmed from the HMBC spectrum. The number of carbon atoms of the above moiety was 11, which is half of the molecular formula of **1**. A HMBC cross-peak between the methine proton and carbon was observed, so compound **1** could be assigned with a symmetrical structure and these two residues were connected by a CH−CH bond. The degrees of unsaturation suggested the presence of two fused tetrahydrofuran rings. Moreover, the HMBC cross-peaks between the oxymethine proton and the oxymethylene carbon and the absence of any HMBC correlations between the oxymethine proton and carbon and between the oxymethylene proton and carbon suggested the linkage CH−O−CH$_2$, rather than CH−O−CH or CH$_2$−O−CH$_2$. Accordingly, compound **1** was assigned as 2,6-bis(5-methoxy-3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane. The NOE cross-peaks between H-1 and H-2′ and H-6′ of the phenyl group suggested the *trans*-orientation of H-1 and H-2. The two tetrahydrofuran rings were fused in the *cis*-form by considering the ring tension, and thus the relative configuration could be proposed as 1,2-*trans*-1,5-*cis*-5,6-*trans*. Although compound **1** exhibited the same ^{1}H NMR data as (+)-excelsin,[4] which has the absolute configuration

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**4** in $CDCl_3$ (500 MHz)[a]

proton	excelsin[b]	**1**	**2**	**3**	**4**
1	3.0 (1H, m)	3.04 (1H, m)	3.07 (1H, m)	3.05 (1H, m)	3.07 (1H, m)
2	4.67 (1H, d, 5)	4.69 (1H, d, 4.2)	4.72 (1H, d, 3.9)	4.69 (1H, d, 4.9)	4.74 (1H, d, 4.2)
4	4.10−4.36 (1H, m)	4.25 (1H, dd, 9.3, 6.8)	4.25 (1H, dd, 9.3, 7.0)	4.26 (1H, dd, 8.9, 7.1)	4.24 (1H, dd, 9.3, 6.8)
	3.72−3.97 (1H, m)	3.87 (1H, dd, 9.3, 3.4)	3.89 (1H, m)	3.86 (1H, m)	3.89 (1H, m)
5	3.0 (1H, m)	3.04 (1H, m)	3.07 (1H, m)	3.05 (1H, m)	3.07 (1H, m)
6	4.67 (1H, d, 5)	4.69 (1H, d, 4.2)	4.70 (1H, d, 4.4)	4.69 (1H, d, 4.9)	4.70 (1H, d, 4.2)
8	4.10−4.36 (1H, m)	4.25 (1H, dd, 9.3, 6.8)	4.28 (1H, dd, 9.3, 7.0)	4.24 (1H, dd, 9.0, 7.1)	4.27 (1H, dd, 9.0, 7.1)
	3.72−3.97 (1H, m)	3.87 (1H, dd, 9.3, 3.4)	3.88 (1H, m)	3.86 (1H, m)	3.87 (1H, m)
2′	6.50 (1H, s)	6.51 (1H, brs)	6.53 (1H, brs)	6.52 (1H, brs)	6.85 (1H, brs)
5′					6.78 (1H, d, 8.0)
6′	6.50 (1H, s)	6.53 (1H, brs)	6.55 (1H, brs)	6.54 (1H, brs)	6.81 (1H, dd, 8.0, 1.2)
2″	6.50 (1H, s)	6.51 (1H, brs)	6.58 (1H, s)	6.56 (1H, d, 1.5)	6.58 (1H, s)
6″	6.50 (1H, s)	6.53 (1H, brs)	6.58 (1H, s)	6.51 (1H, d, 1.5)	6.58 (1H, s)
−OCH_2O−	5.94 (4H, s)	5.95 (4H, s)	5.96 (2H, s)	5.96 (2H, s)	5.95 (2H, s)
OCH_3-5′	3.90 (3H, s)	3.90 (3H, s)	3.92 (3H, s)	3.91 (3H, s)	
OCH_3-3″			3.90 (3H, s)		3.90 (3H, s)
OCH_3-5″	3.90 (3H, s)	3.90 (3H, s)	3.90 (3H, s)	3.89 (3H, s)	3.90 (3H, s)

[a] Signals were assigned from ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. [b] Measured at 60 MHz.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**4** in $CDCl_3$ (125 MHz)[a]

carbon	**1**	**2**	**3**	**4**
1	54.3	54.4	54.4	54.4
2	85.8	85.8	85.8	85.8
4	71.8	71.9	71.9	71.9
5	54.3	54.3	54.1	54.3
6	85.8	86.1	85.8	86.1
8	71.8	71.7	71.7	71.6
1′	135.7	135.8	135.8	135.1
2′	100.0	100.0	100.1	106.5
3′	149.0	149.1	149.0	147.1
4′	134.6	134.6	134.6	148.0
5′	143.6	143.6	143.6	108.2
6′	105.4	105.6	105.5	119.3
1″	135.7	132.0	131.7	132.1
2″	100.0	102.7	106.4	102.7
3″	149.0	147.1	143.8	147.1
4″	134.6	134.3	134.0	134.3
5″	143.6	147.1	147.0	147.1
6″	105.4	102.7	100.8	102.7
−OCH_2O−	101.5	101.5	101.5	101.1
OCH_3-5′	56.6	56.7	56.7	
OCH_3-3″		56.4		56.4
OCH_3-5″	56.6	56.4	56.2	56.4

[a] Signals were assigned from ^{1}H−^{1}H COSY, HMQC, and HMBC spectra.

1*R*,2*S*,5*R*,6*S*, its optical rotation was opposite. Accordingly, the absolute configuration was assigned as 1*S*,2*R*,5*S*,6*R* in **1**.

Compound **2** gave the molecular formula $C_{22}H_{24}O_8$ from the HREIMS. It had proton and carbon NMR signals similar to compound **1**, including the presence of a 5-methoxy-3,4-methylenedioxyphenyl ring (Tables 1 and 2). The evident differences were the appearance of two aromatic protons of an A_2 system and two additional equivalent methoxy signals in the ^{1}H NMR spectrum of compound **2**. Thus, another aromatic ring was assigned as a 4-hydroxy-3,5-dimethoxyphenyl, which was confirmed from the HMBC spectrum. The difference of the two aromatic groups induced the small difference of chemical shifts of H-2 and H-6, H-4 and H-8, C-2 and C-6, and C-4 and C-8 in **2**. This compound is levorotatory; thus it was determined as (1*S*,2*R*,5*S*,6*R*)-2-(5-methoxy-3,4-methylenedioxyphenyl)-6-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

The molecular formula of compound **3** was $C_{21}H_{22}O_8$ from the HREIMS. Its proton and carbon NMR spectroscopic data were similar to those of compound **2**, with a significant difference being the disappearance of one *O*-methyl group. Four nonequivalent aromatic protons [δ 6.52 (1H, brs, H-2′), 6.54 (1H, brs, H-6′), 6.56 (1H, d, J = 1.5 Hz, H-2″), and 6.51 (1H, d, J = 1.5 Hz, H-6″)] and the HMBC correlations indicated that the 4-hydroxy-3,5-dimethoxyphenyl group in **2** is replaced by a 3,4-dihydroxy-5-methoxyphenyl group in **3**. Compound **3** was established as (1*S*,2*R*,5*S*,6*R*)-2-(5-methoxy-3,4-methylenedioxyphenyl)-6-(3,4-dihydroxy-5-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane from its negative optical rotation.

The molecular formula, $C_{21}H_{22}O_7$, was ascribed to compound **4** from the molecular ion peak at *m*/*z* 386.1369 in the HREIMS. The ^{1}H NMR spectrum of **4** was similar to that of **2** (Table 1), except for the disappearance of one methoxy group and three aromatic protons of an ABC system replacing two aromatic protons of an AB system. The two other aromatic protons [δ 6.58 (2H, s, H-2″,6″)] and two methoxy groups were equivalent, and they exhibited ^{1}H−^{1}H COSY correlations to one another. Thus, the two phenyl groups were assigned as a 3,4-methylenedioxyphenyl unit and a 4-hydroxy-3,5-dimethoxyphenyl unit and were confirmed from the EIMS fragments at *m*/*z* 149 (3,4-methylenedioxybenzoyl), *m*/*z* 135 (3,4-methylenedioxybenzyl), *m*/*z* 181 (4-hydroxy-3,5-dimethoxybenzoyl), and *m*/*z* 167 (4-hydroxy-3,5-dimethoxybenzyl). Compound **4** is dextrorotatory and thus was determined as (1*R*,2*S*,5*R*,6*S*)-2-(3,4-methylenedioxyphenyl)-6-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

Compound **5** was assigned as $C_{22}H_{24}O_8$ from the HREIMS. The two 5-methoxy-3,4-methylenedioxyphenyl groups were established from the ^{1}H and ^{13}C NMR spectra. The remaining one oxymethine, two oxymethylenes, two methines, and one methylene were connected as a $O^5CH_2-{}^4CH(^7CH_2)-{}^3CH(^6CH_2OH)-{}^2CHO$ unit from the ^{1}H−^{1}H COSY spectrum, and this was confirmed from the HMBC spectrum. Moreover, the HMBC cross-peaks between H-2 and C-5 and between H-5 and C-2 suggested the presence of a tetrahydrofuran ring. The two 5-methoxy-3,4-methylenedioxyphenyl groups could be located at C-2 and C-7 from the HMBC spectrum. Thus, compound **5** was assigned as 2-(5-methoxy-3,4-methylenedioxyphenyl)-4-(5-methoxy-3,4-methylenedioxybenzyl)-3-furanmethanol. NOE effects for H-2′, H-6′, and H-4 were observed by irradiating H-3, which indicated the *trans*-form of H-2 and H-3 and the *cis*-form of H-3 and H-4 were present. The positive optical rotation ($[\alpha]_D^{25}$ +1.6) was similar to (2*S*,3*R*,4*R*)-2-(3,4-methylenedioxyphenyl)-4-(3,4-dimethoxybenzyl)-3-furanmethanol;[5] thus the absolute configuration of **5** was established as 2*S*,3*R*,4*R*.

Compound **6**, $C_{26}H_{30}O_{10}$, was assigned the presence of a 4-hydroxy-3,5-dimethoxyphenyl moiety and a pentasubstituted phenyl group from its NMR spectra. Two oxymethylenes, three methines, and one methylene were evident in the ^{1}H NMR spectrum, and the ^{1}H−^{1}H COSY spectrum revealed their linkage as

Table 3. Cell Growth Inhibitory Effects of Compounds **2**−**6**, Medioresinol, and Zhepiresinol against the WI-38, VA-13, and HepG2 Cell Lines (IC_{50} μg/mL)[a]

compound	WI-38	VA-13	HepG2
2	96.6	>100	>100
3	49.8	5.3	13.2
4	>100	>100	>100
5	74.3	99.1	72.0
6	41.3	62.4	74.4
medioresinol	76.9	>100	84.7
zhepiresinol	57.7	50.7	60.2
taxol	0.02	0.01	0.05
adriamycin	0.76	0.08	0.48

[a] Cell growth inhibitory effects on three cells were determined, and IC_{50} is defined as the compound concentration causing 50% growth inhibition.

$^1CH-^2CH(^{11}CH_2O)-^3CH(^{12}CH_2O)-^4CH_2$, which was confirmed from the HMBC spectrum. The HMBC cross-peaks between H-1 and C-8, C-9, C-10, and between H-4 and C-5, C-9, C-10 were used to establish the presence of a tetrahydronaphthalene ring. The 4-hydroxy-3,5-dimethoxyphenyl group was placed at C-1 from the HMBC correlations between H-2′ and H-6′ of the phenyl group and C-1. The two hydroxymethyl groups were deduced to be acetylated from their downfield proton NMR data and the relative HMBC cross-peaks. Thus, the structure of compound **6** was assigned as 1-(4-hydroxy-3,5-dimethoxyphenyl)-6,7-methylenedioxy-8-methoxy-1,2,3,4-tetrahydronaphthalene-2,3-dimethanol diacetate. The NOE enhancements of H-1 irradiating at H-2 and H-3 indicated their mutual *cis*-configuration. It has been reported that the absolute configurations of aryltetralin lignans may be determined from their CD spectra, in which the Cotton effects at ca. 289 and 273 nm reflect the configuration at C-1.[6] Compound **6** showed a positive Cotton effect at 289 nm and a negative Cotton effect at 276 nm, which were similar to those of 1α-aryl compounds. Thus, its absolute configuration was established as 1*R*,2*R*,3*S*.

In addition to the above six new lignans, three known lignans, (−)-syringaresinol,[7] (−)-medioresinol,[8] and zhepiresinol,[9] and 11 other known compounds, (+)-isololiolide,[10] (+)-blumenol A,[11] *trans*-*N*-(*p*-coumaroyl)tyramine, *cis*-*N*-(*p*-coumaroyl)tyramine, *trans*-*N*-feruloyltyramine, *cis*-*N*-feruloyltyramine, *trans*-*p*-coumaric acid, *cis*-*p*-coumaric acid, *trans*-ferulic acid, 3-hydroxytyrosol, and vanillic acid, were also isolated from *P. duclouxii*. Their structures were determined by comparison of NMR data with the corresponding literature or by comparison with the authentic standard compounds.

The cytotoxic activities of compounds **2**−**6**, (−)-medioresinol, and zhepiresinol were evaluated against human normal lung (WI-38), malignant lung (VA-13), and hepatoma (HepG2) cells. Compound **3** exhibited growth inhibitory activities against VA-13 and HepG2, with IC_{50} values of 5.3 and 13.2 μg/mL, respectively (Table 3). Its cytotoxicity to the normal cell line (WI-38) was less potent (IC_{50} 49.8 μg/mL). The other compounds showed weak or no activity, and their IC_{50} values ranged from 41.3 to 100 μg/mL.

One mechanism underlying MDR in mammalian tumor cells relates to enhanced removal of drugs due to overexpression of efflux transporter proteins, such as P-glycoprotein (Pgp) and multidrug resistance protein (MRP).[12] Thus, agents that inhibit these proteins may be able to overcome the MDR effect. The calcein derived from calcein AM by endogenous esterase is used as an easily operated functional fluorescent probe for this drug efflux protein.[13−15] The effects of compounds **2**−**6**, (−)-medioresinol, and zhepiresinol on the accumulation of calcein were evaluated using MDR 2780AD cells, with a known MDR reversal agent, verapamil, as positive control. Compound **3** showed more potent activity than verapamil at 25 μg/mL (Table 4).

Expression of excess amounts of ICAM-1 on the surface of endothelial cells of a blood vessel plays an important role in the progress of the inflammatory reaction.[16−18] The inhibitory effects on the induction of ICAM-1 of compounds **1**, **2**, **4**−**6**, (−)-syringaresinol, (−)-medioresinol, and zhepiresinol were evaluated in the presence of IL-1α or TNF-α, using human A549 cells (human lung carcinoma), and the cell viability was measured by an MTT assay. Compound **6**, an aryltetralin lignan, inhibited induction of ICAM-1 induced by IL-1α and TNF-α with IC_{50} values of 107 and 13.4 μM, respectively, and without cytotoxicity to A549 cells (IC_{50} > 316 μM).

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Horiba SEPA-200 polarimeter. UV and IR spectra were recorded on a JASCO V-550 UV/vis spectrophotometer in $CHCl_3$ and a Hitachi 270-30 spectrometer in $CHCl_3$, respectively. ^1H and ^{13}C NMR spectra were run on a Varian UNITY-PS 500 spectrometer using $CDCl_3$ as solvent. HREIMS were recorded on JEOL JMS DX-303 and JEOL Mstation JMS-700 mass spectrometers. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10 × 250 mm stainless steel column or an Inertsil Prep-ODS GL 10 × 250 mm column, monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

Plant Material. The whole plants of *P. duclouxii* were collected from Lvchun, Yunnan Province, People's Republic of China, in February 2002. The plant was identified by Mr. Kaijiao Jiang, Kunming Institute of Botany. A voucher specimen (2002-2) has been deposited at the Faculty of Engineering, Niigata University, Japan.

Extraction and Isolation. The dried plant material (1.65 kg) was powdered and extracted four times (7.5 L/each) with MeOH at room temperature, and about 100 g of a residue was obtained after evaporating the solvents in vacuo. The residue was suspended in H_2O and partitioned with hexane, EtOAc, and *n*-BuOH, respectively, to afford a hexane extract (17.3 g), an EtOAc extract (29.0 g), and a *n*-BuOH extract (15.0 g). The hexane extract was divided into four fractions (FH_1−FH_4) by silica gel column chromatography (CC) using gradient of hexane and EtOAc as solvents. Fraction FH_4 (2.8 g) was subjected to further silica gel CC to afford nine subfractions (FH_{4-1}−FH_{4-9}). Compound **1** (99.8 mg) was obtained from FH_{4-5} with repeated normal-phase HPLC separations [hexane−EtOAc (75:25 and 8:2)]. The EtOAc extract was chromatographed over a silica gel column eluted with hexane and EtOAc to give five fractions (F_1−F_5). Fraction F_2 (3.18 g) was divided into five subfractions (F_{2-1}−F_{2-5}) using silica gel CC eluting with hexane and gradient mixtures of hexane and EtOAc of increasing polarity. Fraction F_{2-3} gave compounds **4** (3 mg) and **6** (2 mg) with repeated normal-phase HPLC [hexane−EtOAc (7:3, 75:25, and 8:2)] and reversed-phase HPLC [MeOH−H_2O (7:3) and MeOH−MeCN−H_2O (1:1:2)]. Fraction F_{2-4} gave compounds **2** (12 mg) and **5** (3 mg), (+)-isololiolide (3 mg), and (+)-blumenol A (9 mg) with repeated normal-phase HPLC [hexane−EtOAc (65:35, 7:3, and 75:25)]. Fraction F_3 (2.92 g) was divided into five subfractions over silica gel CC (F_{3-1}−F_{3-5}). Compound **3** (0.7 mg), (−)-syringaresinol (3.6 mg), (−)-medioresinol (2.1 mg), zhepiresinol (0.8 mg), *trans*-*N*-(*p*-coumaroyl)tyramine (2.2 mg), *cis*-*N*-(*p*-coumaroyl)tyramine (2.2 mg), *trans*-ferulic acid (1.1 mg), *trans*-*N*-feruloyltyramine (29.9 mg), *cis*-*N*-feruloyltyramine (17.1 mg), *trans*-*p*-coumaric acid (7 mg), *cis*-*p*-coumaric acid (2.2 mg), vanillic acid (1.6 mg), and 3-hydroxytyrosol (21.5 mg) were obtained from F_{3-4} using normal-phase HPLC [hexane−EtOAc (7:3, 65:35, 6:4, or 55:45)].

(1*S*,2*R*,5*S*,6*R*)-2,6-Bis(5-methoxy-3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (1): colorless gum; $[\alpha]_D^{25}$ −28.5 (*c* 0.30, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244 (2.90), 276 (3.91) nm; IR ($CHCl_3$) ν_{max} 2948, 2892, 1638, 1510, 1456, 1432, 1362, 1324, 1224, 1216, 1136, 1094, 1044, 972, 932 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; EIMS *m*/*z* 415 $[M + H]^+$ (23), 414 $[M]^+$ (92), 191 (67), 180 (46), 179 (100), 165 (82); HREIMS *m*/*z* 414.1291 ($C_{22}H_{22}O_8$ requires 414.1315).

(1*S*,2*R*,5*S*,6*R*)-2-(5-Methoxy-3,4-methylenedioxyphenyl)-6-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (2): colorless gum; $[\alpha]_D^{25}$ −32.8 (*c* 0.09, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 243 (3.40), 272 (3.90) nm; IR ($CHCl_3$) ν_{max} 3560, 3012, 2944, 1636, 1620, 1514, 1466, 1432, 1362, 1324, 1222, 1118, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; EIMS *m*/*z* 417 $[M + H]^+$ (27), 416 $[M]^+$ (100), 193 (13), 191

Table 4. Effects of Compounds **3** on the Accumulation of Calcein in MDR 2780AD Cells[a]

compound	concentration, μg/mL	average of fluorescence/well ± SD[b]	% of control[c]	verapamil %[d]
control	0	4649 ± 1017		
verapamil	0.25	4592 ± 145	99	100
	2.5	5321 ± 250	114	100
	25	6728 ± 1294	145	100
3	0.25	4719 ± 362	101	103
	2.5	4891 ± 609	105	92
	25	7862 ± 642	169	117

[a] The amount of calcein accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.25, 2.5, and 25 μg/mL of each test compound. [b] Values represent the mean of triplicate determinations. [c] Values are the relative amount of calcein accumulated in the cell compared with the control experiment. [d] Values are expressed as the relative amount of calcein accumulation in the cell as compared with that of verapamil.

(22), 182 (19), 181 (32), 180 (19), 179 (29), 167 (18), 165 (27); HREIMS *m*/*z* 416.1445 ($C_{22}H_{24}O_8$ requires 416.1472).

(1*S*,2*R*,5*S*,6*R*)-2-(5-Methoxy-3,4-methylenedioxyphenyl)-6-(3,4-dihydroxy-5-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (3): colorless gum; $[\alpha]_D^{25}$ −32.8 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 246 (4.20), 275 (3.93) nm; IR ($CHCl_3$) ν_{max} 3572, 2952, 1610, 1500, 1456, 1432, 1362, 1306, 1212, 1136, 1096, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; EIMS *m*/*z* 403 $[M + H]^+$ (5), 402 $[M]^+$ (23), 191 (26), 180 (48), 179 (60), 168 (26), 167 (52), 165 (100), 153 (26); HREIMS *m*/*z* 402.1288 ($C_{21}H_{22}O_8$ requires 402.1315).

(1*R*,2*S*,5*R*,6*S*)-2-(3,4-Methylenedioxyphenyl)-6-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (4): colorless gum; $[\alpha]_D^{25}$ +62.6 (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244 (3.46), 284 (3.42) nm; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; EIMS *m*/*z* 387 $[M + H]^+$ (25), 386 $[M]^+$ (100), 193 (17), 182 (15), 181 (26), 167 (25), 161 (27), 150 (18), 149 (57), 135 (26); HREIMS *m*/*z* 386.1369 ($C_{21}H_{22}O_7$ requires 386.1366).

(2*S*,3*R*,4*R*)-2-(5-Methoxy-3,4-methylenedioxyphenyl)-4-(5-methoxy-3,4-methylenedioxybenzyl)-3-furanmethanol (5): colorless gum; $[\alpha]_D^{25}$ +1.6 (*c* 0.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244 (2.90), 276 (3.90) nm; IR ($CHCl_3$) ν_{max} 3628, 3032, 2944, 1636, 1500, 1456, 1432, 1326, 1226, 1134, 1094, 1046, 964 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.52 (1H, brs, H-2′), 6.51 (1H, brs, H-6′), 6.38 (1H, brs, H-2″), 6.34 (1H, brs, H-6″), 5.95 (2H, s, OCH_2O), 5.94 (2H, s, OCH_2O), 4.78 (1H, d, *J* = 6.1 Hz, H-2), 4.05 (1H, dd, *J* = 6.6, 8.4 Hz, H-5a), 3.91 (1H, m, H-6a), 3.90 (3H, s, OCH_3-5′), 3.89 (3H, s, OCH_3-5″), 3.78 (1H, dd, *J* = 6.6, 10.7 Hz, H-6b), 3.73 (1H, dd, *J* = 6.6, 8.4 Hz, H-5b), 2.88 (1H, dd, *J* = 5.2, 13.6 Hz, H-7a), 2.69 (1H, m, H-4), 2.52 (1H, dd, *J* = 10.7, 13.6 Hz, H-7b), 2.37 (1H, m, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.0 (C, C-3′,3″), 143.5 (C, C-5′,5″), 137.8 (C, C-1′), 134.9 (C, C-1″), 134.4 (C, C-4′), 133.6 (C, C-4″), 107.9 (CH, C-6″), 105.2 (CH, C-6′), 102.5 (CH, C-2″), 101.4 (CH_2, OCH_2O), 101.3 (CH_2, OCH_2O), 99.9 (CH, C-2′), 82.9 (CH, C-2), 72.9 (CH_2, C-5), 60.9 (CH_2, C-6), 56.7 (CH_3, OCH_3-5′,5″), 52.6 (CH, C-3), 42.3 (CH, C-4), 33.6 (CH_2, C-7); EIMS *m*/*z* 417 $[M + H]^+$ (29), 416 $[M]^+$ (100), 192 (14), 179 (26), 166 (51), 165 (59); HREIMS *m*/*z* 416.1464 ($C_{22}H_{24}O_8$ requires 416.1472).

(1*R*,2*R*,3*S*)-1-(4-Hydroxy-3,5-dimethoxyphenyl)-6,7-methylenedioxy-8-methoxy-1,2,3,4-tetrahydronaphthalene-2,3-dimethanol diacetate (6): colorless gum; $[\alpha]_D^{25}$ +28.7 (*c* 0.03, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 242 (3.46), 281 (2.98) nm; CD (*c* 0.644 mM, $CHCl_3$) $[\theta]_{289}$ +5355, $[\theta]_{276}$ −11485; IR ($CHCl_3$) ν_{max} 3560, 2948, 1736, 1624, 1516, 1482, 1466, 1428, 1370, 1328, 1238, 1224, 1210, 1116, 1048, 974, 934 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.41 (1H, s, H-5), 6.29 (2H, s, H-2′,6′), 5.89 (2H, s, OCH_2O), 4.18 (1H, dd, *J* = 4.2, 11.5 Hz, H-11a), 4.13 (1H, dd, *J* = 4.6, 11.5 Hz, H-12a), 4.12 (1H, d, *J* = 5.4 Hz, H-1), 4.02 (1H, dd, *J* = 6.6, 11.5 Hz, H-12b), 4.00 (1H, dd, *J* = 6.6, 11.5 Hz, H-11b), 3.81 (6H, s, OCH_3-3′,5′), 3.47 (3H, s, OCH_3-8), 2.69 (1H, dd, *J* = 4.5, 15.5 Hz, H-4a), 2.62 (1H, dd, *J* = 11.0, 15.5 Hz, H-4b), 2.11 (1H, m, H-2), 2.09 (3H, s, CH_3CO), 2.02 (3H, s, CH_3-CO), 1.96 (1H, m, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1 (C, *C*OCH_3), 171.0 (C, *C*OCH_3), 147.8 (C, C-6), 146.8 (C, C-3′,5′), 141.8 (C, C-8), 137.4 (C, C-1′), 135.7 (C, C-7), 132.9 (C, C-4′), 130.8 (C, C-10), 123.8 (C, C-9), 104.9 (CH, C-2′,6′), 102.8 (CH, C-5), 100.8 (CH_2, OCH_2O), 66.8 (CH_2, C-12), 64.5 (CH_2, C-11), 59.0 (CH_3, OCH_3-8), 56.4 (CH_3, OCH_3-3′,5′), 44.6 (CH, C-3), 42.2 (CH, C-1), 35.9 (CH, C-2), 33.0 (CH_2, C-4), 21.0 (CH_3, *C*H_3CO); EIMS *m*/*z* 503 $[M + H]^+$ (32), 502 $[M]^+$ (100), 442 (19), 411 (38), 382 (25); HREIMS *m*/*z* 502.1843 ($C_{26}H_{30}O_{10}$ requires 502.1840).

Growth Inhibitory Activity to WI-38, VA-13, and HepG2 Cells in Vitro. The cell lines were obtained from the Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki, Japan. WI-38 and VA-13 cells were maintained in Eagle's MEM medium (Nissui Pharmaceutical Co., Tokyo, Japan) and RITC 80-7 medium (Asahi Technoglass Co., Chiba, Japan), respectively, both supplemented with 10% (v/v) fetal bovine serum (FBS) (Filtron PTY Ltd., Australia) with 80 μg/mL of kanamycin. HepG2 cells were maintained in D-MEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) FBS (Filtron) with 80 μg/mL kanamycin. The activity was measured as previously described.[19]

Cellular Accumulation of Calcein. MDR ovarian cancer 2780AD cells (AD10) were maintained in PRMI-1640 medium (Invitrogen) supplemented with 10% (v/v) FBS (Filtron PTY Ltd.) with 80 μg/mL kanamycin. The activity was measured as previously described.[3]

Inhibitory Activity on Induction of ICAM-1. A549 cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with heat-inactivated 10% (v/v) FBS (JRH Bioscience, Lenexa, KS), 100 U/mL penicillin G, and 100 μg/mL streptomycin. Mouse anti-human ICAM-1 antibody C167 was purchased from Leinco Technologies, Inc. (Ballwin, MO), and peroxidase-conjugated goat anti-mouse IgG antibody was obtained from Jackson Immuno Research Laboratories, Inc. (West Grove, PA). Recombinant IL-1α and TNF-α were provided by Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Cell surface expression of ICAM-1 and cell viability on the basis of MTT assay were measured as previously described.[19]

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References and Notes

(1) Guizhou Institute of Traditional Chinese Medicine. *Dictionary of Traditional Herbal Medicine of Guizhou*; Guizhou People's Press: Guiyang, People's Republic of China, 1988; p 73.
(2) Li, N.; Wu, J. L.; Sakai, J.; Ando, M. *J. Nat. Prod.* **2003**, *66*, 1421−1426.
(3) Li, N.; Wu, J. L.; Hasegawa, T.; Sakai, J.; Wang, L.; Kakuta, S.; Furuya, Y.; Tomida, A.; Tsuruo, T.; Ando, M. *J. Nat. Prod.* **2006**, *69*, 234−239.
(4) Russell, G. B.; Fenemore, P. G. *Phytochemistry* **1973**, *12*, 1799−1803.
(5) Vidigal, M. C. S.; Cavalheiro, A. J.; Kato, M. J.; Yoshida, M. *Phytochemistry* **1995**, *40*, 1259−1261.
(6) Hulbert, P. B.; Klyne, W.; Scopes, P. M. *J. Chem. Res.* **1981** 401−409.
(7) Buske, A.; Schmidt, J.; Porzei, A.; Adam, G. *Phytochemistry* **1997**, *46*, 1385−1388.
(8) Koshino, H.; Yoshihara, T.; Togiya, S.; Terada, S.; Tsukada, S.; Okuno, M.; Noguchi, A.; Sakamura, S.; Ichihara, A. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1989**, *31*, 244−251.
(9) Jin, X. Q.; Xu, D. M.; Xu, Y. J.; Cui, D. B.; Xiao, Y. W.; Tian, Z. Y.; Lu, Y.; Zheng, Q. T. *Acta Pharm. Sin.* **1993**, *28*, 212−215.
(10) Kimura, J.; Maki, N. *J. Nat. Prod.* **2002**, *65*, 57−58.
(11) González, A. G.; Guiliermo, J. A.; Ravelo, A. G.; Jimenez, I. A. *J. Nat. Prod.* **1994**, *57*, 400−402.
(12) Wortelboer, H. M.; Usta, M.; van Zanden, J. J.; van Bladeren, P. J.; Rietjens, I. M. C. M.; Cnubben, N. H. P. *Biochem. Pharmacol.* **2005**, *69*, 1879−1890.

(13) Eneroth, A.; Åström, E.; Hoogstraate, J.; Schrenk, D.; Conrad, S.; Kauffmann, H. M.; Gjellan, K. *Eur. J. Pharm. Sci.* **2001**, *12*, 205−214.
(14) Tsuruo, T.; Iida-Saito, H.; Kawabata, H.; Oh-hara, T.; Hamada, H.; Utakoji, T. *Jpn. J. Cancer Res. (Gann)* **1986**, *77*, 682−692.
(15) Jonsson, B.; Liminga, G.; Csoka, K.; Fridborg, H.; Dhar, S.; Nygren, P.; Larsson, R. *Eur. J. Cancer* **1996**, *32A*, 883−887.
(16) Kawai, S.; Kataoka, T.; Sugimoto, H.; Nakamura, A.; Kobayashi, T.; Arao, K.; Higuchi, Y.; Ando, M.; Nagai, K. *Immunopharmacology* **2000**, *48*, 129−135.
(17) Yuuya, S.; Hagiwara, H.; Suzuki, T.; Ando, M.; Yamada, A.; Suda, K.; Kataoka, T.; Nagai, K. *J. Nat. Prod.* **1999**, *62*, 22−30.
(18) Higuchi, Y.; Shimoma, F.; Koyanagi, R.; Suda, K.; Mitui, T.; Kataoka, T.; Nagai, K.; Ando, M. *J. Nat. Prod.* **2003**, *66*, 588−594.
(19) Wu, J. L.; Li, N.; Hasegawa, T.; Sakai, J.; Kakuta, S.; Tang, W. X.; Oka, S.; Kiuchi, M.; Ogura, H.; Kataoka, T.; Tomida, A.; Tsuruo, T.; Ando, M. *J. Nat. Prod.* **2005**, *68*, 1656−1660.

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