Bioactive Dibenzylbutyrolactone and Dibenzylbutanediol Lignans from *Peperomia duclouxii*

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Six new dibenzylbutyrolactone (**6**−**11**) and two new dibenzylbutanediol lignans (**12**, **13**) were obtained from *Peperomia duclouxii*. The structures were elucidated mainly by the analysis of NMR and MS data. The anticancer activity against a normal (WI-38) and a simian virus 40-transformed human lung fibroblast cell (VA-13) and a hepatoma G2 cell (HepG2) and the MDR reversal activity of the isolated compounds were examined. Compound **7** showed moderate inhibitory activity against VA-13 and HepG2 with IC_{50} values of 23.2 and 26.4 μM, respectively. Compound **2** inhibited the growth of HepG2 cells with an IC_{50} of 42.8 μM. Compounds **2** and **13** exhibited stronger MDR reversal activity than verapamil, at 25 and 2.5 μg/mL, respectively, and **4**, **5**, and **7** showed comparable activity with verapamil, at 25, 25, and 2.5 μg/mL, respectively.

Peperomia duclouxii C. DC. in Lecomte (Piperaceae) is a folk anticancer herb in the People's Republic of China.[1] Five dibenzylbutyrolactone (**1**−**5**) and four dibenzylbutanediol lignans had been obtained from the EtOAc extract in our previous investigation.[2] Further work on its chemical constituents resulted in the isolation of six new dibenzylbutyrolactone (**6**−**11**) and two new dibenzylbutanediol lignans (**12**, **13**). The structures were elucidated by the analysis of NMR and MS, and the absolute configurations were established by the optical rotations. The anticancer activity of the isolated compounds was evaluated on a human normal lung fibroblast cell (WI-38), a malignant lung tumor cell induced from WI-38 (VA-13), and a liver tumor cell (HepG2), and the multidrug-resistant (MDR) reversal activity was examined on a MDR human ovarian cancer cell line (2780AD).

Results and Discussion

Compound **6** has the molecular formula $C_{21}H_{20}O_7$, as determined from the high-resolution EIMS. The UV spectrum showed maximum absorbance peaks at 242 and 286 nm, and the IR spectrum indicated the presence of a γ-lactone at 1770 cm^{-1} and a methylenedioxy group at 974 cm^{-1}. Similar to compound **1**,[2] the ^1H NMR spectrum exhibited the characteristic signals of the butyrolactone moiety at δ 2.53 (1H, m, H-2), 2.46 (1H, m, H-3), 4.15 (1H, dd, J = 7.1, 9.3 Hz, H-4a), 3.87 (1H, dd, J = 7.1, 9.5 Hz, H-4b), 2.56 (1H, m, H-5a), 2.44 (1H, m, H-5b), 2.99 (1H, dd, J = 5.1, 14.0 Hz, H-6a), and 2.83 (1H, dd, J = 7.6, 14.0 Hz, H-6b) (Table 1). Harmatha et al.[3] studied the chemical shifts of the known *cis*- and *trans*-dibenzylbutyrolactones and concluded that the *trans*-derivatives tended to show a poorly resolved spectrum with a four-proton multiplet (H-2, 3, 5a, 5b) at δ 2.5−2.6, a two-proton multiplet (H-6a, 6b) at δ 2.9, with a very small nonequivalence of the protons of each of the two benzyl groups, and the distinct nonequivalence of the C-4-methylene protons (δ 3.9 and 4.2). In contrast, in the *cis*-derivatives, the benzylic methylenes and H-2 and H-3 were relatively well resolved within a broad range (δ 2.3−3.3), while the hydrogens in each of the benzyl groups were distinctly nonequivalent, although the hydrogens of the C-4-methylene group were almost equivalent in the δ 4.0−4.1 range. The chemical shifts of compound **6** were similar to those of *trans*-derivatives. The absence of an NOE effect between H-2 and H-3 confirmed the 2,3-*trans* configuration. The deshielded protons at δ 6.17 (1H, d, J = 1.5 Hz, H-2′) and 6.13 (1H, d, J = 1.5 Hz, H-6′), and δ 6.62 (1H, d, J = 1.7 Hz, H-2″), 6.72 (1H, d, J = 7.8 Hz, H-5″), and 6.59 (1H, dd, J = 1.7, 7.8 Hz, H-6″) indicated the presence of tri- and tetrasubstituted aromatic rings, respectively. Moreover, the tetrasubstituted aromatic ring was a 5-methoxy-3,4-methylenedioxyphenyl group from the proton and carbon signals similar to those of compound **1** (Tables 1 and 2) and the EIMS fragment at m/z 165.[2] The remaining proton and carbon signals were attributed to the 3,4-methylenedioxyphenyl group, which was confirmed by the EIMS fragment at m/z 135. The $^1H-^1H$ COSY correlations between H-5 of the butyrolactone moiety and H-2, H-6 of the 5-methoxy-3,4-methylenedioxyphenyl group indicated their linkage, which was confirmed by the HMBC correlations. Similarly, the 3,4-methylenedioxyphenyl group resided at C-6 of the butyrolactone moiety. Harmatha et al.[3] also investigated the relationship between the specific rotation and the absolute configuration of the known lignans and concluded that the (2*R*,3*R*)-isomer was levorotatory and the (2*S*,3*S*)-enantiomer was dextrorotatory. The positive specific rotation of compound **6** suggested the absolute configuration as 2*S*,3*S*. Thus, it was named (2*S*,3*S*)-2-(3,4-methylenedioxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone.

Compound **7** has the same molecular formula and very close UV, IR, and ^{13}C NMR spectra to those of compound **6**. The ^1H NMR spectrum was also similar to that of compound **6**, except for some of the aromatic protons (Table 1). The $^1H-^1H$ COSY correlations and the EIMS fragment peaks at m/z 165 and 135 confirmed the presence of the 5-methoxy-3,4-methylenedioxybenzyl group and the 3,4-methylenedioxybenzyl group. Different from compound **6**, the $^1H-^1H$ COSY indicated the former was attached to C-2 and the latter was attached to C-3 of the butyrolactone moiety. Compound **7** is thus the regioisomer of compound **6**. Koul et al. previously obtained a dibenzylbutyrolactone with the same

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Table 1. ^{1}H NMR Data for Compounds **1** and **6**−**11** in $CDCl_3$ (500 MHz)[a]

proton	**1**	**6**	**7**	**8**	**9**	**10**	**11**
2	2.54 (1H, ddd, 5.0, 7.2, 7.5)	2.53 (1H, m)	2.53 (1H, m)	2.53 (1H, m)		2.62 (1H, dd, 2.7, 6.1)	4.23 (1H, d, 6.4)
3	2.47 (1H, m)	2.46 (1H, m)	2.48 (1H, m)	2.48 (1H, m)	3.83 (1H, m)	2.79 (1H, m)	3.42 (1H, m)
4	4.18 (1H, dd, 7.0, 9.5)	4.15 (1H, dd, 7.1, 9.3)	4.16 (1H, dd, 7.1, 9.3)	4.16 (1H, dd, 6.8, 9.1)	4.30 (1H, dd, 6.7, 9.2)	4.38 (1H, dd, 8.1, 8.8)	4.55 (1H, dd, 7.3, 9.0)
	3.88 (1H, dd, 7.3, 9.5)	3.87 (1H, dd, 7.1, 9.5)	3.87 (1H, dd, 7.3, 9.3)	3.87 (1H, m)	4.26 (1H, dd, 1.7, 9.2)	3.97 (1H, dd, 5.5, 8.8)	4.13 (1H, dd, 5.9, 9.0)
5	2.57 (1H, dd, 5.0, 11.9)	2.56 (1H, m)	2.60 (1H, dd, 5.7, 12.9)	2.58 (1H, dd, 9.8, 17.1)	3.03 (1H, dd, 4.6, 14.4)	2.47 (1H, dd, 7.6, 13.7)	2.80 (1H, dd, 8.1, 13.9)
	2.49 (1H, dd, 7.8, 11.9)	2.44 (1H, m)	2.50 (1H, m)	2.47 (1H, m)	2.63 (1H, dd, 10.0, 14.4)	2.25 (1H, dd, 8.1, 13.7)	2.73 (1H, dd, 8.1, 13.9)
6	2.95 (1H, dd, 5.0, 14.0)	2.99 (1H, dd, 5.1, 14.0)	2.94 (1H, dd, 5.0, 13.9)	2.94 (1H, dd, 5.0, 13.9)	7.50 (1H, d, 1.6)	5.27 (1H, d, 2.7)	
	2.82 (1H, dd, 7.2, 14.0)	2.83 (1H, dd, 7.6, 14.0)	2.82 (1H, dd, 7.2, 13.9)	2.82 (1H, dd, 7.0, 13.9)			
2′	6.17 (1H, d, 1.5)	6.17 (1H, d, 1.5)	6.46 (1H, br.s)	6.18 (1H, d, 1.5)	6.34 (1H, d, 1.2)	5.95 (1H, d, 1.5)	6.32 (1H, brs)
5′			6.70 (1H, d, 7.8)				
6′	6.15 (1H, d, 1.5)	6.13 (1H, d, 1.5)	6.47 (1H, dd, 1.7, 7.8)	6.15 (1H, d, 1.5)	6.29 (1H, d, 1.2)	5.99 (1H, d, 1.5)	6.27 (1H, brs)
2″	6.31 (1H, s)	6.62 (1H, d, 1.7)	6.31 (1H, d, 1.2)	6.38 (1H, d, 1.7)	6.80 (1H, s)	6.48 (1H, s)	7.21 (1H, s)
5″		6.72 (1H, d, 7.8)					
6″	6.31 (1H, s)	6.59 (1H, dd, 1.7, 7.8)	6.30 (1H, d, 1.2)	6.28 (1H, d, 1.7)	6.80 (1H, s)	6.48 (1H, s)	7.21 (1H, s)
−OCH_2O−	5.95 (4H, m)	5.94 (4H, m)	5.94 (4H, m)	5.94 (2H, s)	5.93 (2H, m)	5.94 (1H, d, 1.5)	5.92 (1H, d, 1.2)
						5.91 (1H, d, 1.5)	5.91 (1H, d, 1.2)
OCH_3-5′	3.86 (3H, s)	3.86 (3H, s)		3.86 (3H, s)	3.87 (3H, s)	3.82 (3H, s)	3.82 (3H, s)
OCH_3-3″					3.92 (3H, s)	3.86 (3H, s)	3.94 (3H, s)
OCH_3-5″	3.86 (3H, s)		3.86 (3H, s)	3.83 (3H, s)	3.92 (3H, s)	3.86 (3H, s)	3.94 (3H, s)

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

Table 2. ^{13}C NMR Data for Compounds **1** and **6**−**11** in $CDCl_3$ (125 MHz)[a]

carbon	**1**	**6**	**7**	**8**	**9**	**10**	**11**
1	178.4	178.4	178.4	178.6	172.5	178.3	172.7
2	46.5	46.4	46.5	46.4	125.5	52.8	53.6
3	41.2	41.3	41.1	41.2	39.6	36.4	41.4
4	71.2	71.1	71.2	71.2	69.7	72.8	71.9
5	38.8	38.7	38.4	38.7	38.0	39.7	38.3
6	35.2	34.9	35.1	35.0	138.0	72.2	191.1
1′	132.3	132.3	131.5	132.4	132.1	132.1	131.9
2′	102.5	102.5	108.8	102.5	102.5	102.2	102.9
3′	149.1	149.0	147.9	149.0	149.2	149.0	149.2
4′	134.0	133.9	146.4	133.9	134.2	133.8	134.2
5′	143.5	143.5	108.3	143.6	143.6	143.2	143.7
6′	108.1	108.0	121.6	108.0	108.8	108.2	108.4
1″	132.0	131.3	132.0	129.5	125.4	132.0	127.2
2″	103.2	109.4	103.2	109.6	107.2	101.8	106.8
3″	149.0	147.9	149.0	143.7	147.2	147.1	146.8
4″	134.1	146.5	134.1	131.2	136.9	134.1	140.7
5″	143.6	108.2	143.6	147.1	147.2	147.1	146.8
6″	108.5	122.2	108.3	103.9	107.2	101.8	106.8
−OCH_2O−	101.4	101.4	101.4	101.4	101.5	101.5	101.5
		101.0	101.0				
OCH_3-5′	56.6	56.6		56.6	56.8	56.6	56.6
OCH_3-3″					56.4	56.3	56.5
OCH_3-5″	56.6		56.5	56.1	56.4	56.3	56.5

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

structure from *Piper trichostachyon* and reported its absolute configuration as 2*S*,3*S* by comparison of the negative Cotton effect in the CD spectrum and the negative optical rotation with that of hinokinin.[4] However, the absolute configuration of hinokinin had been established earlier as 2*R*,3*R*,[5] so the absolute configuration of the compound obtained by Koul should be revised to 2*R*,3*R*. Since compound **7** possesses a positive specific rotation, its absolute configuration should be 2*S*,3*S*.[2,3]

The molecular formula of compound **8** was established as $C_{21}H_{22}O_8$ from the high-resolution EIMS. The IR spectrum showed the hydroxyl and γ-lactone moieties at 3572 and 1770 cm^{-1}, respectively. Similar to the above compounds, the ^{1}H NMR spectrum of **8** showed the characteristic proton signals of the *trans*-dibenzylbutyrolactone lignan (Table 1). The two benzyl groups were the 5-methoxy-3,4-methylenedioxybenzyl and 3,4-dihydroxy-5-methoxybenzyl group from the ^{1}H NMR, ^{13}C NMR, and HMBC spectra. The EIMS fragments at *m*/*z* 165 and 153 further confirmed the presence of the above two benzyl groups. Moreover, the former group resided at C-3 and the latter at C-2 of the butyrolactone moiety from the HMBC spectrum. The positive specific rotation indicated the absolute configuration of **8** as 2*S*,3*S*.[2,3] Thus, compound **8** was identified as (2*S*,3*S*)-2-(3,4-dihydroxy-5-methoxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone.

The high-resolution EIMS of compound **9** gave the molecular formula $C_{22}H_{22}O_8$. The IR spectrum indicated the existence of hydroxyl (3552 cm^{-1}), conjugated γ-lactone (1748 cm^{-1}), and methylenedioxy (930 cm^{-1}) groups. The ^{1}H NMR data of the butyrolactone moiety exhibited significant differences with those of the above compounds, especially with an olefinic proton [δ 7.50 (1H, d, J = 1.6 Hz, H-6)] replacing the benzyl methylene and methine protons in the above compounds (Table 1). This indicated that a double bond exists at C2(C6) or C3(C5). The HMBC cross-peak between the carbonyl carbon (C-1) and the olefinic proton suggested the double band at C2(C6). Moreover, the downfield olefinic proton should be located at the deshielding region of the carbonyl group, which meant an *E*-double bond.[6] Other protons of the butyrolactone moiety [δ 3.83 (1H, m, H-3), 4.30 (1H, dd, J = 6.7, 9.2 Hz, H-4a), 4.26 (1H, dd, J = 1.7, 9.2 Hz, H-4b), 3.03 (1H, dd, J = 4.6, 14.4 Hz, H-5a), and 2.63 (1H, dd, J = 10.0, 14.4 Hz, H-5b)] were relatively downfield due to the existence of the double bond. At the same time, the ^{1}H NMR spectrum also showed two sets of tetrasubstituted aromatic rings with *m*-oriented protons at δ 6.34 (1H, d, J = 1.2 Hz, H-2′) and 6.29 (1H, d, J = 1.2 Hz, H-6′) and δ 6.80 (2H, s, H-2″,6″). Combination of the ^{1}H−^{1}H COSY and HMBC spectra indicated that the aromatic rings at C-5 and

C-6 of the butyrolactone were the 5-methoxy-3,4-methylenedioxyphenyl and 4-hydroxy-3,5-dimethoxyphenyl groups, respectively. The base peak at *m*/*z* 165, not *m*/*z* 167, in the EIMS also confirmed that the 5-methoxy-3,4-methylenedioxyphenyl group was substituted at C-5. The positive specific rotation was opposite of that of helianthoidin, guamarol, and isoguamarol, which were established as 3*R*; thus the absolute configuration of compound **9** was deduced to be 3*S*.[5−7] Compound **9** is thus (2*E*,3*S*)-2-(4-hydroxy-3,5-dimethoxybenzylidene)-3-(5-methoxy-3,4-methylenedioxybenzyl)-butyrolactone.

Compound **10** has the molecular formula $C_{22}H_{24}O_9$ from the high-resolution EIMS spectrum. The IR spectrum showed the bands of a hydroxyl group at 3624 and 3560 cm^{-1} and hydrogen-bonded γ-lactone group at 1734 cm^{-1}. The proton, carbon, and DEPT NMR showed the presence of four aromatic methines, one oxymethylene, one oxymethine, one methylene, two methines, one methylenedioxy group, three methoxy groups, and eight aromatic quaternary carbons. These signals indicated two tetrasubstituted phenyl groups, and the EIMS gave the characteristic fragments of the 5-methoxy-3,4-methylenedioxybenzyl group at *m*/*z* 165 and the hydroxy(4-hydroxy-3,5-dimethoxyphenyl)methyl group at *m*/*z* 183. The remaining signals were ascribed to the γ-lactone group from the HMBC spectrum. The 2,3-*trans* configuration of the butyrolactone was determined from the absence of NOE correlation between H-2 and H-3. Harmatha et al. reported that the introduction of a hydroxyl group at C-6 did not change the direction of the optical rotation, so the negative optical rotation suggested the absolute configuration as 2*S*,3*R*.[3] The small coupling constant (2.7 Hz) between H-6 and H-2 indicated their *gauche*-staggered orientation,[8,9] so the absolute configuration at C-6 was 6*S*. Thus, compound **10** was defined as (2*S*,3*R*,6*S*)-2-[hydroxy(4-hydroxy-3,5-dimethoxyphenyl)methyl]-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone.

Compound **11** has the molecular formula $C_{22}H_{22}O_9$ from the ion peak *m*/*z* 430.1253 in the high-resolution EIMS. The IR spectrum showed the presence of hydroxyl (3548 cm^{-1}), γ-lactone (1772 cm^{-1}), and conjugated carbonyl groups (1668 cm^{-1}). The $^1H-^1H$ COSY and HMBC spectra showed the presence of a 5-methoxy-3,4-methylenedioxybenzyl group, a 4-hydroxy-3,5-dimethoxybenzoyl group, and a γ-butyrolactone moiety. The EIMS fragments at *m*/*z* 165 and 181 further confirmed the presence of the former two groups. At the same time, the 5-methoxy-3,4-methylenedioxybenzyl group resides at C-3 of the γ-butyrolactone from the $^1H-^1H$ COSY correlation between the benzyl proton (H-5) and H-3 and the HMBC correlations of C-5 with H-2 and H-4. Similarly, the 4-hydroxy-3,5-dimethoxybenzoyl group resides at C-2 of the γ-butyrolactone from the HMBC correlations of the benzoyl carbon with H-2 and H-3. The absence of NOE correlation between H-2 and H-3 suggested their *trans* orientation. Its negative specific rotation, similar to that of (−)-podorhizone, showed its absolute configuration as 2*R*,3*S*[3] and defined compound **11** as (2*R*,3*S*)-2-(4-hydroxy-3,5-dimethoxybenzoyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone.

Compound **12**, $C_{26}H_{30}O_{10}$, exhibited an acetyl group at 1732 cm^{-1} and a methylenedioxy group at 974 cm^{-1} in the IR spectrum. The ^1H and ^{13}C NMR spectra showed signals similar to those of 2,3-bis(5-methoxy-3,4-methylenedioxybenzyl)butane-1,4-diol monoacetate isolated from this plant,[2] except for one additional acetyl group and the downfield shift of H-3, H-4, and C-4 and the upfield shifts of H-2 and C-3. Compound **12** is thus defined as 2,3-bis(5-methoxy-3,4-methylenedioxybenzyl)butane-1,4-diol diacetate. Similar to the monoacetate derivative,[2] the positive specific rotation suggested the absolute configuration as 2*S*,3*S*.[3]

Compound **13** has the molecular formula $C_{22}H_{26}O_8$ from the HREIMS. Similar to compound **12**, it showed only half of the signals of dibenzylbutanediol derivatives in the ^1H and ^{13}C NMR spectra. The significant difference was the absence of the signals of the acetyl groups in compound **13**, which suggested it was a diol derivative. The upfield shifts of C-1(4) and H-1(4) and H-2(3) and the downfield shifts of C-2(3) in compound **13** were in accordance with the above deduction. Different from other dibenzylbutanediol lignans obtained from *P. duclouxii*,[2] it showed a negative specific rotation, which indicated the absolute configuration as 2*R*,3*R*.[3] Compound **13** is thus (2*R*,3*R*)-2,3-bis(5-methoxy-3,4-methylenedioxybenzyl)butane-1,4-diol.

Table 3. Cell Growth Inhibitory Activity of Compounds **1**−**13** against WI-38, VA-13, and HepG2 Cell Lines (IC_{50} μM)[a]

compound	WI-38	VA-13	HepG2
1	>241	130.3	207.9
2	>232	117.7	42.8
3	>259	209.6	200.5
4	195.3	130.4	204.5
5	>259	183.3	>260
6	>260	>260	>260
7	>260	23.2	26.4
8	141.4	181.4	162.4
9	240.6	156.6	197.5
10	>231.4	228.6	>231.4
11	172.1	126.9	185.4
12	>199	>199	>199
13	184.7	218.9	151.4
Taxol	0.034	0.0043	6.9
ADM	0.38	0.22	0.69

[a] IC_{50} was calculated as the concentration of compound required to give 50% inhibition of cell growth. Values represented are means of three independent experiments. Taxol and ADM are positive controls.

Three cell lines (WI-38, VA-13, HepG2) were used to evaluate the anticancer activity of compounds **1**−**13** (Table 3). Compound **7** showed moderate cell growth inhibitory activity against a malignant lung tumor model (VA-13) and a hepatoma model (HepG2), with IC_{50} values of 23.2 and 26.4 μM, and the effect was stronger than that against a human normal lung cell model (WI-38), with an IC_{50} of more than 260 (Figure 2). Compound **2** inhibited the growth of HepG2 cells with an IC_{50} of 42.8 μM.

One mechanism underlying MDR in mammalian tumor cells has been assigned to enhanced removal of drugs due to overexpression of efflux transporter proteins, such as P-glycoprotein (Pgp), and the multidrug resistance proteins (MRP).[10] Thus, agents that inhibit the function of this protein could overcome the MDR effect. Calcein AM is used as an easily operated functional fluorescent probe for this drug efflux protein.[11−13] The effects of compounds **1**−**10** and **13** on the cellular accumulation of calcein in MDR human ovarian cancer 2780AD cells (MDR reversal activity) were examined by comparison with that of verapamil, a known MDR reversal agent (Tables 4 and 5). All compounds except for compounds **3**, **6**, and **10** exhibited accumulation of calcein in MDR 2780AD cells, especially compounds **2** and **13**, exhibiting stronger activity than verapamil at 25 and 2.5 μg/mL, respectively. Compounds **4**, **5**, and **7** showed comparable activity with verapamil, at 25, 25, and 2.5 μg/mL, respectively. The above bioassay results suggested that the weak cell growth inhibitory activity of certain compounds in this herb could be enhanced by the MDR reversal agents that coexist in the same plant.

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 a JEOL JMS DX-303 and a JEOL Mstation JMS-700 mass spectrometer. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10 × 250 mm stainless steel column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

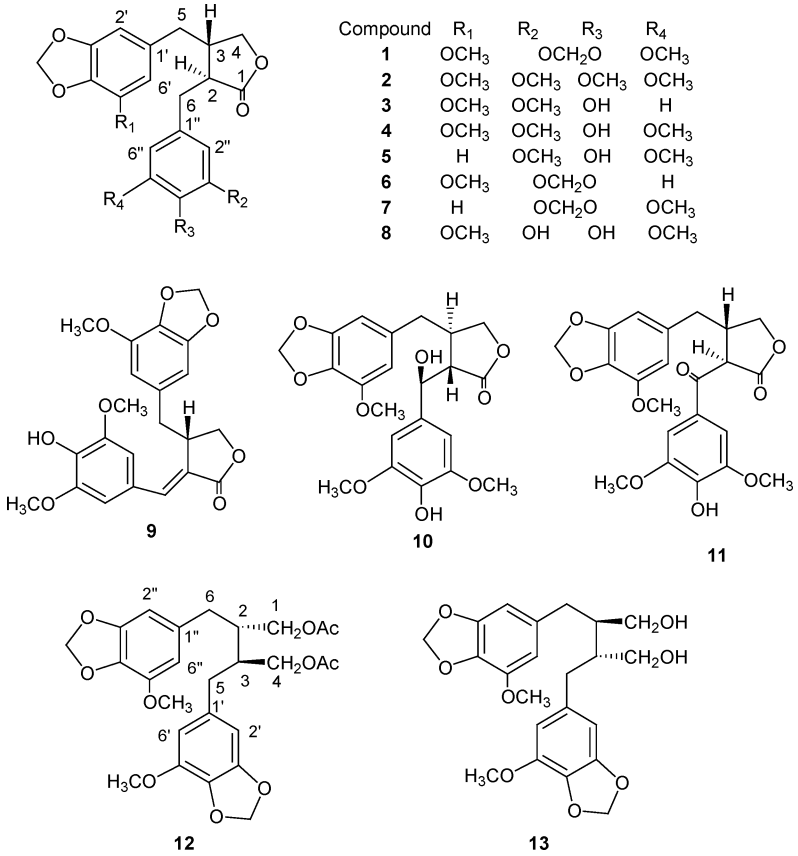

Figure 1. Structures for compounds **1**−**13**.

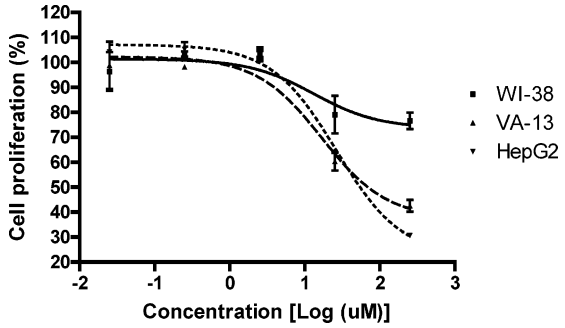

Figure 2. Cell growth inhibitory effects of compound **7** against WI-38, VA-13, and HepG2 cell lines. Data plotted are representatives of three independent experiments (mean ± SD): WI-38 (solid line), VA-13 (dash line), HepG2 (dotted line).

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 with the aid of a supersonic machine, and about 100 g of 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 an *n*-BuOH extract (15.0 g). The hexane extract was divided into four fractions (FH_1−FH_4) with silica gel column chromatography using a gradient of hexane and EtOAc as solvents. FH_4 (2.8 g) was subjected to further silica gel column chromatography to afford nine subfractions (FH_{4-1}−FH_{4-9}). Compounds **6** (10.9 mg) and **7** (10.2 mg) were obtained from FH_{4-4} with repeated normal-phase HPLC separations [hexane−EtOAc (85:15, 82:18, and 75:25)]. Compound **12** (2.5 mg) was isolated from FH_{4-5} by normal-phase HPLC [hexane−EtOAc (75:25 and 82:18)]. The EtOAc extract was chromatographed over a silica gel column eluted with hexane and EtOAc to give five fractions (F_1−F_5). F_3 (2.92 g) was divided into five subfractions (F_{3-1}−F_{3-5}) over silica gel column chromatography eluting with hexane and gradient mixtures of hexane and EtOAc of increasing polarity. F_{3-3} gave compounds **8** (1.4 mg), **9** (40 mg), and **10** (6.3 mg) with repeated normal-phase HPLC [hexane−EtOAc (65:35 and 75:25)]. F_{3-4} gave compounds **11** (1.0 mg) and **13** (38 mg) with normal-phase HPLC [hexane−EtOAc (50:50 and 65:35)].

Table 4. Effects of Compounds **1**, **6**, **7**, and **8** 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	2502 ± 220		
verapamil	0.25	2228 ± 151	89	100
	2.5	2645 ± 291	106	100
	25	3599 ± 349	144	100
1	0.25	2318 ± 25	93	104
	2.5	2275 ± 319	91	86
	25	2909 ± 206	116	81
6	0.25	2281 ± 16	91	102
	2.5	1844 ± 196	74	70
	25	2405 ± 271	96	67
7	0.25	2009 ± 73	80	90
	2.5	2720 ± 169	109	103
	25	3104 ± 384	124	86
8	0.25	2448 ± 72	98	110
	2.5	2280 ± 145	91	86
	25	3006 ± 160	120	84

[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 test compounds. [b] The values represent the mean of triplicate determinations. [c] The values are the relative amount of calcein accumulated in the cell compared with the control experiment. [d] The values are expressed as the relative amount of calcein accumulation in the cell as compared with that of verapamil.

(2*S*,3*S*)-2-(3,4-Methylenedioxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (6): colorless gum; $[\alpha]_D^{25}$ +29.9 (*c*

Table 5. Effects of Compounds **2**, **3**, **4**, **5**, **9**, **10**, and **13** 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	1951 ± 122		
verapamil	0.25	2024 ± 85	104	100
	2.5	1883 ± 217	97	100
	25	2597 ± 127	133	100
2	0.25	1863 ± 199	96	92
	2.5	1927 ± 70	99	102
	25	3014 ± 130	155	116
3	0.25	1505 ± 60	77	74
	2.5	1669 ± 185	86	89
	25	1649 ± 94	85	64
4	0.25	1673 ± 270	86	83
	2.5	1777 ± 199	91	94
	25	2514 ± 185	129	97
5	0.25	1813 ± 164	93	90
	2.5	1825 ± 104	94	97
	25	2487 ± 159	127	96
9	0.25	1761 ± 254	90	87
	2.5	1753 ± 22	90	93
	25	2224 ± 109	114	86
10	0.25	1772 ± 90	91	88
	2.5	1810 ± 277	93	96
	25	2023 ± 187	104	78
13	0.25	1885 ± 142	97	93
	2.5	2010 ± 215	103	107
	25	2361 ± 228	121	91

[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 test compounds. [b] The values represent the mean of triplicate determinations. [c] The values are the relative amount of calcein accumulated in the cell compared with the control experiment. [d] The values are expressed as the relative amount of calcein accumulation in the cell as compared with that of verapamil.

0.727, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 286 nm; IR ($CHCl_3$) ν_{max} 2896, 1770, 1636, 1506, 1494, 1448, 1372, 1322, 1248, 1222, 1212, 1136, 1096, 1044, 974, 934, 812 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* 385 [M + H]$^+$ (95), 384 [M]$^+$ (100), 166 (100), 165 (100), 136 (91), 135 (100); HREIMS *m*/*z* 384.1183 ($C_{21}H_{20}O_7$ requires 384.1209).

(2*S*,3*S*)-2-(5-Methoxy-3,4-methylenedioxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone (7): colorless gum; $[\alpha]_D^{25}$ +24.5 (*c* 0.653, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 286 nm; IR ($CHCl_3$) ν_{max} 3012, 2904, 1772, 1636, 1506, 1494, 1448, 1318, 1246, 1218, 1136, 1096, 1044 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* 385 [M + H]$^+$ (15), 384 [M]$^+$ (55), 166 (60), 165 (100), 136 (25), 135 (42); HREIMS *m*/*z* 384.1202 ($C_{21}H_{20}O_7$ requires 384.1209).

(2*S*,3*S*)-2-(3,4-Dihydroxy-5-methoxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (8): colorless gum; $[\alpha]_D^{25}$ +19.2 (*c* 0.073, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 277 nm; IR ($CHCl_3$) ν_{max} 3572, 1770, 1630, 1498, 1456, 1370, 1306, 1224, 1216, 1136, 1096, 1048, 952 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]$^+$ (10), 402 [M]$^+$ (43), 166 (100), 165 (73), 154 (37), 153 (55); HREIMS *m*/*z* 402.1288 ($C_{21}H_{22}O_8$ requires 402.1315).

(2*E*,3*S*)-2-(4-Hydroxy-3,5-dimethoxybenzylidene)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (9): colorless gum; $[\alpha]_D^{25}$ +65.7 (*c* 0.407, $CHCl_3$); UV ($CHCl_3$) λ_{max} 244, 329 nm; IR ($CHCl_3$) ν_{max} 3552, 3012, 2948, 1748, 1646, 1616, 1514, 1466, 1432, 1358, 1332, 1238, 1210, 1158, 1118, 1098, 1046, 1000, 930, 834 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]$^+$ (10), 414 [M]$^+$ (39), 250 (12), 249 (77), 166 (15), 165 (100); HREIMS *m*/*z* 414.1349 ($C_{22}H_{22}O_8$ requires 414.1315).

(2*S*,3*R*,6*S*)-2-[Hydroxy(4-hydroxy-3,5-dimethoxyphenyl)methyl]-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (10): colorless gum; $[\alpha]_D^{25}$ −38.9 (*c* 0.380, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 275 nm; IR ($CHCl_3$) ν_{max} 3624, 3560, 2996, 1734, 1636, 1514, 1466, 1432, 1378, 1248, 1216, 1136, 1116, 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* 433 [M + H]$^+$ (15), 432 [M]$^+$ (59), 414 (15), 249 (19), 183 (100), 166 (84), 165 (60); HREIMS *m*/*z* 432.1393 ($C_{22}H_{24}O_9$ requires 432.1421).

(2*R*,3*S*)-2-(4-Hydroxy-3,5-dimethoxybenzoyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (11): colorless gum; $[\alpha]_D^{25}$ −58.2 (*c* 0.067, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3548, 2948, 1772, 1668, 1636, 1616, 1458, 1428, 1334, 1286, 1220, 1212, 1138, 1118, 1098, 1046, 864 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* 431 [M + H]$^+$ (3), 430 [M]$^+$ (11), 191 (100), 181 (76), 165 (56), 161 (63), 153 (20); HREIMS *m*/*z* 430.1253 ($C_{22}H_{22}O_9$ requires 430.1264).

(2*S*,3*S*)-2,3-Bis(5-methoxy-3,4-methylenedioxybenzyl)butane-1,4-diol diacetate (12): colorless gum; $[\alpha]_D^{25}$ +10.9 (*c* 0.133, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 278 nm; IR ($CHCl_3$) ν_{max} 2944, 1732, 1636, 1612, 1498, 1456, 1434, 1372, 1320, 1222, 1212, 1136, 1094, 1046, 974 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.24 (2H, d, J = 1.5 Hz, H-2′, 2″), 6.21 (2H, d, J = 1.5 Hz, H-6″, 6″), 5.94 (2H, d, J = 1.5 Hz, OCH_2O), 5.93 (2H, d, J = 1.5 Hz, OCH_2O), 4.15 (2H, dd, J = 6.0, 11.3 Hz, H-1a, 4a), 4.01 (2H, dd, J = 5.5, 11.3 Hz, H-1b, 4b), 3.85 (6H, s, 5′,5″-OCH_3), 2.62 (2H, dd, J = 7.3, 13.9 Hz, H-5a, 6a), 2.57 (2H, dd, J = 7.6, 13.9 Hz, H-5b, 6b), 2.07 (2H, m, H-2, 3), 2.07 (6H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9 (C, *C*OCH_3), 148.8 (C, C-3′, 3″), 143.4 (C, C-5′, 5″), 134.1 (C, C-1′, 1″), 133.6 (C, C-4′, 4″), 108.1 (CH, C-6′, 6″), 102.8 (CH, C-2′, 2″), 101.3 (CH_2, OCH_2O), 64.2 (CH_2, C-1, 4), 56.5 (CH_3, 5′,5″-OCH_3), 39.8 (CH, C-2, 3), 35.4 (CH_2, C-5, 6), 21.0 (CH_3, *C*H_3CO); EIMS *m*/*z* 503 [M + H]$^+$ (8), 502 [M]$^+$ (25), 166 (99), 165 (100); HREIMS *m*/*z* 502.1819 ($C_{26}H_{30}O_{10}$ requires 502.1840).

(2*R*,3*R*)-2,3-Bis(5-methoxy-3,4-methylenedioxybenzyl)butane-1,4-diol (13): colorless gum; $[\alpha]_D^{25}$ −8.5 (*c* 0.467, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 278 nm; IR ($CHCl_3$) ν_{max} 3008, 2952, 2896, 1634, 1496, 1456, 1432, 1376, 1316, 1292, 1236, 1212, 1188, 1134, 1092, 1046, 972, 930, 830 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.34 (2H, d, J = 1.0 Hz, H-2′, 2″), 6.32 (2H, d, J = 1.0 Hz, H-6′, 6″), 5.93 (4H, s, OCH_2O), 3.87 (6H, s, 5′,5″-OCH_3), 3.82 (2H, t, J = 11.4 Hz, H-1a, 4a), 3.55 (2H, dd, J = 4.2, 11.5 Hz, H-1b, 4b), 2.75 (2H, dd, J = 8.6, 13.7 Hz, H-5a, 6a), 2.62 (2H, dd, J = 6.1, 13.7 Hz, H-5b, 6b), 1.87 (2H, m, H-2, 3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 148.8 (C, C-3′, 3″), 143.4 (C, C-5′, 5″), 134.9 (C, C-1′, 1″), 133.4 (C, C-4′, 4″), 108.1 (CH, C-6′, 6″), 102.9 (CH, C-2′, 2″), 101.2 (CH_2, OCH_2O), 60.3 (CH_2, C-1, 4), 56.5 (CH_3, 5′,5″-OCH_3), 43.9 (CH, C-2, 3), 36.3 (CH_2, C-5, 6); EIMS *m*/*z* 418 [M]$^+$ (1), 400 (10), 166 (100), 165 (50); HREIMS *m*/*z* 418.1640 ($C_{22}H_{26}O_8$ requires 418.1628).

Cell Growth Inhibitory Activity against WI-38, VA-13, and HepG2 in Vitro. The cell lines are available 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) supplemented with 10% (v/v) FBS (Filtron Pty. Ltd., Australia) with 80 μg/mL of kanamycin.

Medium (100 μL) containing ca. 5000 cells (WI-38, VA-13, HepG2) was incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h in a 96-well microplate. Then test samples dissolved in DMSO were added to the medium, and incubation was continued further for 48 h under the same conditions. Coloration substrate, WST-8 [2-(2-methyl-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt], was added to the medium. The resulting formazan concentration was determined by the absorption at 450 nm. Cell viability (%) was calculated as [(experimental absorbance − background absorbance)/(control absorbance − background absorbance)] × 100. Cell viability at different concentrations of the compounds was plotted, and 50% inhibition of growth was calculated as IC_{50}.

Cellular Accumulation of Calcein. MDR ovarian cancer A2780 cells (2780AD) were maintained in PRMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) FBS (Filtron PTY Ltd., Australia) with 80 μg/mL of kanamycin.

Medium (100 μL) containing ca. 1×10^5 cells was incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Test compounds were dissolved in DMSO and diluted with phosphate-buffered saline, PBS(−). Test samples of 50 μL were added to the medium and incubated for 15 min. Then, 50 μL of the fluorogenic dye

calcein AM [1 μM in PBS(−)] was added to the medium, and incubation was continued for 60 min. After removing the supernatant, each microplate was washed with 200 μL of cold PBS(−). The washing step was repeated twice, and 200 μL of cold PBS(−) was added. Retention of the resulting calcein was measured as calcein-specific fluorescence. The absorption maximum for calcein is 494 nm, and the emission maximum is 517 nm.

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