Dibenzylbutyrolactone and Dibenzylbutanediol Lignans from *Peperomia duclouxii*

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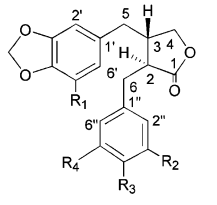
Nine new lignans (**1**−**9**), including five dibenzylbutyrolactones and four dibenzylbutanediols, were obtained from an ethyl acetate extract of the whole plants of *Peperomia duclouxii*. The structures of **1**−**9** were determined by spectroscopic methods (mainly extensive 1D and 2D NMR experiments and by mass spectral measurements). The absolute structures were elucidated as 2*S*,3*S* from their optical rotations and by chemical transformations.

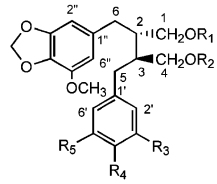
Lignans are widely distributed in nature and exhibit a variety of structural types. Various lignans are known to have anticancer, antimitotic, and antiviral activity and to specifically inhibit certain enzymes.[1] There are several reviews on lignan structures and biological activity.[2−5]

Peperomia duclouxii C. DC. in Lecomte (Piperaceae) (named "Duan sui cao hu jiao" in China) mainly grows in Yunnan, Guangxi, Guangdong, and Fujian Provinces of the People's Republic of China and is used traditionally for the treatment of various types of cancer.[6,7] There have been no reports on the constituents of *P. duclouxii*. Secolignans were isolated from *P. japonica*,[8] *P. glabella*,[9] and *P. dindigulensis*,[10] and other compounds of the polyketide,[11] chromone,[12] benzopyran,[13] quinone,[14] and phenolic[15] types were also obtained from other plants in this genus.

In the present study, we have investigated the lignan constituents of *P. duclouxii*. Nine new lignans (**1**−**9**), with three known simple phenolic derivatives, were obtained from the ethyl acetate extract with silica gel column chromatography and normal-phase and reversed-phase HPLC. The structures of **1**−**9** were established by spectroscopic methods, and their absolute configurations were determined as 2*S*,3*S* by optical rotations and by chemical correlations.



Compound	R_1	R_2	R_3	R_4
1	OCH_3	OCH_2O		OCH_3
2	OCH_3	OCH_3	OCH_3	OCH_3
3	OCH_3	OCH_3	OH	H
4	OCH_3	OCH_3	OH	OCH_3
5	H	OCH_3	OH	OCH_3



Compound	R_1	R_2	R_3	R_4	R_5
6	Ac	Ac	OCH_3	OCH_3	OCH_3
7	Ac	H	OCH_2O		OCH_3
8	Ac	Ac	OCH_3	OH	OCH_3
9	H	H	OCH_2O		H

Results and Discussion

Compound **1** exhibited an ion peak at *m*/*z* 414.1310 in the high-resolution EIMS, consistent with the molecular formula $C_{22}H_{22}O_8$. The IR spectrum exhibited the presence of γ-lactone (1772 cm^{-1}) and methylenedioxy groups (972 cm^{-1}). The ^1H NMR spectrum indicated two sets of tetrasubstituted aromatic ring signals, at δ 6.17 (1H, d, J = 1.5 Hz, H-2′) and 6.15 (1H, d, J = 1.5 Hz, H-6′), and at δ 6.31 (2H, s, H-2″, 6″), and the characteristic signals of two methylenedioxy groups attached to the aromatic rings at δ 5.95 (4H, m) and two methoxy groups at δ 3.86 (6H, s) were also apparent. Moreover, signals at δ 2.54 (1H, ddd, J = 5.0, 7.2, 7.5 Hz, H-2), 2.47 (1H, m, H-3), 4.18 (1H, dd, J = 7.0, 9.5 Hz, H-4a), 3.88 (1H, dd, J = 7.3, 9.5 Hz, H-4b), 2.57 (1H, dd, J = 5.0, 11.9 Hz, H-5a), 2.49 (1H, dd, J = 7.8, 11.9 Hz, H-5b), 2.95 (1H, dd, J = 5.0, 14.0 Hz, H-6a), and 2.82 (1H, dd, J = 7.2, 14.0 Hz, H-6b) were observed in the ^1H NMR spectrum. The HMQC spectrum showed corresponding carbon resonances at δ 46.5 (C-2), 41.2 (C-3), 71.2 (C-4), 38.8 (C-5), and 35.2 (C-6), respectively. On the basis of the $^1H-^1H$ COSY and HMBC spectra (Table 1), these signals were assigned to a dibenzylbutyrolactone lignan. Thus, the planar structure of compound **1** was deduced to be 2,3-bis(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone, which was consistent with $C_{22}H_{22}O_8$. The mass spectral fragment peak at *m*/*z* 165 (88%) confirmed the presence of a 5-methoxy-3,4-methylenedioxybenzyl group. Harmatha et al.[16] discussed the chemical shifts of the *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 protons of the C-4-methylene group (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 ^1H NMR spectrum of compound **1** exhibited the characteristic signals of a *trans*-2,3-dibenzylbutyrolactone lignan, so its relative configuration was *trans*. In the same report,[16] the authors studied the optical rotations of the known lignans and stereoselective synthetic lignans and then concluded that the (2*R*,3*R*)-isomer was levorotatory and the (2*S*,3*S*)-isomer was dextrorotatory. Since compound **1** showed a positive optical rotation (+29.0°, *c* 0.547), the absolute configuration was assigned as 2*S*,3*S*.

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Table 1. NNR Data for Compound **1** in $CDCl_3$

position	δ_C	δ_H	$^1H-^1H$ COSY	HMBC
1	178.4			H-2, H-4, H-6
2	46.5	2.54 (1H, ddd, 5.0, 7.2, 7.5)	H-6	H-4, H-5, H-6
3	41.2	2.47 (1H, m)	H-4	H-2, H-4, H-5, H-6
4	71.2	4.18 (1H, dd, 7.0, 9.5)	H-3, H-5	H-5
		3.88 (1H, dd, 7.3, 9.5)		
5	38.8	2.57 (1H, dd, 5.0, 11.9)	H-4, H-2′, H-6′	H-4, H-2′, H-6′
		2.49 (1H, dd, 7.8, 11.9)		
6	35.2	2.95 (1H, dd, 5.0, 14.0)	H-2, H-2″, H-6″	H-2″, H-6″
		2.82 (1H, dd, 7.2, 14.0)		
1′	132.3			H-5
2′	102.5	6.17 (1H, d, 1.5)	H-5, H-6′	H-5, H-6′
3′	149.1			H-2′
4′	134.0			H-2′, H-6′, $-OCH_2O-$
5′	143.5			H-6′, OCH_3-5′
6′	108.1	6.15 (1H, d, 1.5)	H-5, H-2′, OCH_3-5′	H-5, H-2′
1″	132.0			H-6
2″	103.2	6.31 (1H, s)	H-6	H-6, H-6″
3″	149.0			H-2″, $-OCH_2O-$
4″	134.1			H-2″, H-6″
5″	143.6			H-6″, OCH_3-5″
6″	108.5	6.31 (1H, s)	H-6, OCH_3-5″	H-6, H-2″
$-OCH_2O-$	101.4	5.95 (4H, m)		
OCH_3-5′,5″	56.6	3.86 (6H, s)	H-6′, H-6″	

Table 2. ^1H NMR Data for Compounds **1**−**5** (500 MHz, $CDCl_3$)[a]

proton	**1**	**2**	**3**	**4**	**5**
2	2.54 (1H, ddd, 5.0, 7.2, 7.5)	2.58 (1H, m)	2.55 (1H, ddd, 5.1, 7.0, 7.2)	2.57 (1H, m)	2.55 (1H, m)
3	2.47 (1H, m)	2.50 (1H, m)	2.46 (1H, m)	2.47 (1H, m)	2.47 (1H, m)
4	4.18 (1H, dd, 7.0, 9.5)	4.18 (1H, dd, 7.3, 9.0)	4.14 (1H, dd, 7.1, 9.2)	4.17 (1H, dd, 7.3, 9.0)	4.15 (1H, dd, 7.3, 9.0)
	3.88 (1H, dd, 7.3, 9.5)	3.88 (1H, dd, 7.3, 9.0)	3.86 (1H, dd, 7.3, 9.2)	3.87 (1H, dd, 7.6, 9.0)	3.86 (1H, m)
5	2.57 (1H, dd, 5.0, 11.9)	2.60 (1H, dd, 6.1, 13.4)	2.58 (1H, dd, 9.8, 17.1)	2.58 (1H, dd, 5.8, 16.6)	2.61 (1H, dd, 6.1, 13.2)
	2.49 (1H, dd, 7.8, 11.9)	2.52 (1H, dd, 8.1, 13.4)	2.47 (1H, m)	2.50 (1H, dd, 8.0, 16.6)	2.50 (1H, m)
6	2.95 (1H, dd, 5.0, 14.0)	2.94 (1H, dd, 5.4, 14.0)	2.96 (1H, dd, 5.1, 14.2)	2.93 (1H, dd, 5.5, 14.0)	2.90 (1H, dd, 5.1, 14.2)
	2.82 (1H, dd, 7.2, 14.0)	2.90 (1H, dd, 6.6, 14.0)	2.88 (1H, dd, 7.0, 14.2)	2.88 (1H, dd, 6.7, 14.0)	2.90 (1H, dd, 6.8, 14.2)
2′	6.17 (1H, d, 1.5)	6.17 (1H, brs)	6.16 (1H, d, 1.2)	6.17 (1H, d, 1.5)	6.45 (1H, d, 1.5)
5′					6.69 (1H, d, 7.6)
6′	6.15 (1H, d, 1.5)	6.16 (1H, brs)	6.12 (1H, d, 1.2)	6.14 (1H, d, 1.5)	6.47 (1H, dd, 1.5, 7.6)
2″	6.31 (1H, s)	6.37 (1H, s)	6.66 (1H, d, 1.7)	6.36 (1H, s)	6.36 (1H, s)
5″			6.83 (1H, d, 7.8)		
6″	6.31 (1H, s)	6.37 (1H, s)	6.62 (1H, dd, 1.7, 7.8)	6.36 (1H, s)	6.36 (1H, s)
$-OCH_2O-$	5.95 (4H, m)	5.93 (2H, m)	5.94 (2H, s)	5.94 (2H, m)	5.94 (1H, d, 1.5)
					5.93 (1H, d, 1.5)
OCH_3-5′	3.86 (3H, s)	3.86 (3H, s)	3.85 (3H, s)	3.85 (3H, s)	
OCH_3-3″		3.83 (3H, s)	3.84 (3H, s)	3.86 (3H, s)	3.86 (3H, s)
OCH_3-4″		3.82 (3H, s)			
OCH_3-5″	3.86 (3H, s)	3.83 (3H, s)		3.86 (3H, s)	3.86 (3H, s)

[a] Signals were assigned from the $^1H-^1H$ COSY, HMQC, and HMBC spectra.

The high-resolution EIMS of compound **2** suggested a molecular formula of $C_{23}H_{26}O_8$. The peaks at 1772 and 972 cm^{-1} in the IR spectrum were attributed to γ-lactone and methylenedioxy groups. The ^1H NMR spectrum of compound **2** displayed the characteristic proton signals of a *trans*-dibenzylbutyrolactone lignan.[16] Moreover, the proton and carbon resonances of this compound were in accordance with those of 5′-methoxyyatein,[17] but its optical rotation (+24.8°, *c* 0.393) and the Cotton effects at 287 and 240 nm ($[\theta]_{287}$ +3036, $[\theta]_{240}$ +19866) in the CD spectrum were opposite of those of 5′-methoxyyatein, for which the optical rotation was −21° (*c* 1) and the Cotton effects were negative ($[\theta]_{278}$ −2970, $[\theta]_{239}$ −11550). Therefore, the absolute configuration of compound **2** was opposite of that of 5′-methoxyyatein and should be 2*S*,3*S*, and it was assigned as (+)-5′-methoxyyatein.

The molecular formula of compound **3** was $C_{21}H_{22}O_7$ from the high-resolution EIMS. The IR spectrum suggested the presence of hydroxyl (3560 cm^{-1}), γ-lactone (1768 cm^{-1}), and methylenedioxy groups (972 cm^{-1}). This was also shown to be a *trans*-dibenzylbutyrolactone lignan from the ^1H NMR spectrum with diagnostic aliphatic proton signals (Table 2). The remaining five aromatic proton signals revealed the existence of tetrasubstituted and trisubstituted benzene rings. From the HMBC spectrum, the benzene ring located at C-5 of the butyrolactone was found to be a 5-methoxy-3,4-methylenedioxyphenyl group and the substituent at C-6 was a 4-hydroxy-3-methoxyphenyl group. The fragment peaks at *m*/*z* 165 (48%) and 137 (75%) in EIMS confirmed the presence of the above-mentioned benzyl groups. Further, the positive optical rotation and the positive Cotton effects at 278 and 235 nm in the CD

Table 3. ^{13}C NMR Data for Compounds **1**−**5** (125 MHz, $CDCl_3$)[a]

carbon	1	2	3	4	5
1	178.4	178.5	178.6	178.6	178.6
2	46.5	46.5	46.6	46.6	46.6
3	41.2	41.1	41.0	40.9	40.9
4	71.2	71.2	71.2	71.2	71.2
5	38.8	38.7	38.7	38.7	38.4
6	35.2	35.3	34.6	35.1	35.1
1′	132.3	132.3	132.4	132.3	131.6
2′	102.5	102.4	102.5	102.4	108.7
3′	149.1	149.2	149.0	149.1	147.9
4′	134.0	134.0	134.0	134.0	146.4
5′	143.5	143.5	143.5	143.5	108.3
6′	108.1	108.3	108.1	108.2	121.5
1″	132.0	133.3	129.4	128.6	128.6
2″	103.2	106.3	111.5	105.9	105.9
3″	149.0	153.3	146.7	147.1	147.0
4″	134.1	137.0	144.5	133.6	133.6
5″	143.6	153.3	114.2	147.1	147.0
6″	108.5	106.3	122.0	105.9	105.9
$-OCH_2O-$	101.4	101.5	101.4	101.4	101.1
OCH_3-5′	56.6	56.7	56.6	56.7	
OCH_3-3″		56.1	55.9	56.3	56.3
OCH_3-4″		60.9			
OCH_3-5″	56.6	56.1		56.3	56.3

[a] Signals were assigned from the $^1H-^1H$ COSY, HMQC, and HMBC spectra.

spectrum, which were similar to those of compound **1** and **2**, supported the structure, which was assigned as (2*S*,3*S*)-2-(4-hydroxy-3-methoxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone.

A molecular formula of $C_{22}H_{24}O_8$ was confirmed for compound **4** by high-resolution EIMS. The presence of hydroxyl, γ-lactone, and methylenedioxy groups was supported by the bands at 3560, 1770, and 974 cm^{-1} in the IR spectrum. The ^1H NMR spectrum indicated the characteristic signals of a *trans*-dibenzylbutyrolactone lignan,[16] and the proton signals closely resembled those of compound **2** (Table 2), although a significant difference was that there was one less methyl signal in compound **4** compared with compound **2**. Comparison of the ^{13}C NMR chemical shifts of **2** and **4** indicated the methoxy group at C-4″ in **2** was substituted by a hydroxyl group in **4** and that the change of substituent at C-4″ induced the upfield shifts of C-1″, 3″, 4″, 5″ of **4** when compared with **2** (Table 3). The presence of a 4-hydroxy-3,5-dimethoxybenzyl group was confirmed by the fragment peak at *m*/*z* 167 (74%) in the EIMS and by HMBC correlations. Accordingly, the structure of **4** was assigned as 2-(4-hydroxy-3,5-dimethoxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone. This compound had the same absolute configuration 2*S*,3*S* as that of compound **2** from their similar positive optical rotations and the positive Cotton effects in the CD spectra.

Compound **5** was assigned the molecular formula $C_{21}H_{22}O_7$ from its high-resolution EIMS. The absorptions at 3560, 1770, and 974 cm^{-1} in the IR spectrum were ascribed to the hydroxyl, γ-lactone, and methylenedioxy group, respectively. Compound **5** exhibited characteristic resonances for a *trans*-dibenzylbutyrolactone lignan in its ^1H NMR and ^{13}C NMR spectra (Tables 2 and 3). The ^1H NMR spectrum showed that the two aromatic rings were trisubstituted and tetrasubstituted, respectively. Further comparison of the ^1H and ^{13}C NMR of compounds **4** and **5** (Tables 2 and 3) indicated that there was one less methoxy group in compound **5** than in compound **4** and the methoxy group at C-5′ of compound **4** was absent in compound **5**. Moreover, the fragment peak at *m*/*z* 135 (30%) in the EIMS confirmed the existence of the 3,4-methylenedioxybenzyl group. Thus, compound **5** was established as 2-(4-hydroxy-3,5-dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone. The same 2*S*,3*S* configuration was elucidated from the positive optical rotation value and the positive Cotton effects in the CD spectrum similar to those in the case of compound **4**. This is the first report of this compound in nature, while a racemic mixture was synthesized[18] and the (−)-isomer was obtained from *Thuja occidentalis*.[19]

Compound **6**, $C_{27}H_{34}O_{10}$, showed the presence of a carbonyl group (1736 cm^{-1}) in the IR spectrum. The ^1H NMR spectrum indicated the presence of a dibenzylbutanediol diacetate. The four downfield methyleneoxy protons at δ 4.19 (1H, dd, J = 5.9, 11.2 Hz), 4.02 (1H, dd, J = 5.9, 11.2 Hz), 4.23 (1H, dd, J = 5.9, 11.2 Hz), and 4.00 (1H, dd, J = 5.9, 11.2 Hz) were ascribed to the methylene protons at C-1 and C-4, respectively. Moreover, the HMBC correlations between H-1, -4, CH_3 [δ 2.07 (6H, s)] and the carbonyl groups at δ 170.9 indicated that the methyleneoxy groups were acetylated. In addition, multiplets of the two methine protons at δ 2.13 (1H, m) and 2.09 (1H, m) were assigned to H-2 and H-3 from their correlations with the proton and the carbon signals of the two methyleneoxy groups in the $^1H-^1H$ COSY and HMBC spectra. The four methylene protons between δ 2.60 and 2.65, which correlated with the carbon signals of the methyleneoxy groups in the HMBC spectrum, were attributed to H_2-5 and H_2-6. The two aromatic rings were established, therefore, as 5-methoxy-3,4-methylenedioxyphenyl and 3,4,5-trimethoxyphenyl groups, according to the HMBC experiment. In the EIMS, corresponding benzyl fragments at *m*/*z* 165 (52%) and 181 (74%) were observed. From the evidence mentioned above, compound **6** was characterized as 2-(5-methoxy-3,4-methylenedioxybenzyl)-3-(3,4,5-trimethoxybenzyl)butane-1,4-diol diacetate. The 2*S*,3*S* configuration of **6** was confirmed by the evidence that the lithium aluminum reduction product of compound **2** had physical constants and spectroscopic data identical with compound **6**. Its enantiomer has been synthesized from the corresponding dibenzylbutanediol isolated from *Piper clusii*.[21]

Compound **7**, $C_{24}H_{28}O_9$, showed in the IR spectrum hydroxyl group (3636 cm^{-1}), acetyl group (1734 cm^{-1}), and methylenedioxy group (966 cm^{-1}) absorptions. Comparison of the ^1H NMR signals with those of **6** (Table 4) indicated that it was a dibenzylbutanediol monoacetate due to the observation of one upfield methyleneoxy signal δ 3.64 (2H, d, J = 5.4 Hz) and the appearance of a signal for only one acetate group. The ^1H NMR spectrum also displayed the presence of two methylenedioxy groups, two methoxy groups, and four aromatic protons, which were attributed to two 5-methoxy-3,4-methylenedioxyphenyl groups. The positive optical rotation[16] supported the same 2*S*,3*S* configuration as that of **6**. Thus, **7** was assigned as (2*S*,3*S*)-2,3-bis(5-methoxy-3,4-methylenedioxybenzyl)butane-1,4-diol monoacetate.

Compound **8**, $C_{26}H_{32}O_{10}$, exhibited in the IR spectrum absorptions for a hydroxyl group at 3560 cm^{-1}, an acetyl group at 1736 cm^{-1}, and a methylenedioxy group at 974 cm^{-1}. The ^1H NMR spectrum showed signals similar to those of compound **6** (Table 4). Although there were only three methoxy group signals in its ^1H NMR spectrum, the upfield shifts of the ^{13}C NMR resonances for C-1′, 3′, 4′, 5′ and the symmetry of the chemical shifts of the aryl ring adjacent to position C-5 indicated that the substituent should be a 4-hydroxy-3,5-dimethoxyphenyl group. This was confirmed by HMBC correlations and the fragment peak at *m*/*z* 167 (51%) in the EIMS. According to the positive optical rotation,[16] the structure of compound **8** was

Table 4. ^{1}H NMR Data for Compounds **6**−**9** in $CDCl_3$ (500 MHz)[a]

proton	6	7	8	9
1	4.19 (1H, dd, 5.9, 11.2) 4.02 (1H, dd, 5.9, 11.2)	4.17 (1H, dd, 6.0, 11.2) 4.05 (1H, dd, 5.6, 11.2)	4.19 (1H, dd, 6.0, 11.4) 4.02 (1H, dd, 5.6, 11.4)	3.80 (1H, dd, 4.1, 11.4) 3.53 (1H, dd, 4.1, 11.4)
2	2.13 (1H, m)	2.17 (1H, m)	2.06 (1H, m)	1.85 (1H, m)
3	2.09 (1H, m)	1.93 (1H, m)	2.06 (1H, m)	1.85 (1H, m)
4	4.23 (1H, dd, 5.9, 11.2) 4.00 (1H, dd, 5.9, 11.2)	3.64 (2H, d, 5.4)	4.23 (1H, dd, 5.6, 11.2) 3.99 (1H, dd, 6.1, 11.2)	3.80 (1H, dd, 4.1, 11.4) 3.53 (1H, dd, 4.1, 11.4)
5	2.62 (1H, m) 2.61 (1H, m)	2.61 (2H, d, 7.6)	2.61 (2H, d, 7.3)	2.75 (1H, dd, 8.3, 13.4) 2.62 (1H, dd, 5.6, 13.4)
6	2.65 (1H, dd, 8.1, 14.0) 2.60 (1H, dd, 7.8, 14.0)	2.68 (1H,dd, 7.1, 13.9) 2.56 (1H, dd, 8.1, 13.9)	2.61 (2H, d, 7.3)	2.75 (1H, dd, 8.3, 13.4) 2.62 (1H, dd, 5.6, 13.4)
2′	6.27 (1H, s)	6.30 (1H, d, 1.5)	6.26 (1H, s)	6.64 (1H, d, 1.2)
5′				6.72 (1H, d, 7.8)
6′	6.27 (1H, s)	6.27 (1H, d, 1.5)	6.26 (1H, s)	6.61 (1H, dd, 1.2, 7.8)
2″	6.23 (1H, brs)	6.28 (1H, d, 1.5)	6.23 (1H, d, 1.5)	6.34 (1H, brs)
6″	6.22 (1H, brs)	6.24 (1H, d, 1.5)	6.19 (1H, d, 1.5)	6.31 (1H, brs)
$-OCH_2O-$	5.93 (2H, s)	5.93 (4H, m)	5.93 (2H, m)	5.93 (4H, m)
OCH_3-5″	3.85 (3H, s)	3.86 (3H, s)	3.83 (3H, s)	3.87 (3H, s)
OCH_3-3′	3.80 (3H, s)		3.83 (3H, s)	
OCH_3-4′	3.82 (3H, s)			
OCH_3-5′	3.80 (3H, s)	3.86 (3H, s)	3.83 (3H, s)	
$CH_3(CO)$	2.07 (6H, s)	2.07 (3H, s)	2.08 (3H, s) 2.07 (3H, s)	

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

deduced as (2*S*,3*S*)-2-(5-methoxy-3,4-methylenedioxybenzyl)-3-(4-hydroxy-3,5-dimethoxybenzyl)butane-1,4-diol diacetate.

Compound **9**, $C_{21}H_{24}O_7$, exhibited absorptions for one or more hydroxyl groups (3636 cm^{-1}) and methylenedioxy groups (932 cm^{-1}) in the IR spectrum. Analysis of the ^{1}H NMR (Table 4) and HMBC spectra showed the presence of a 3,4-methylenedioxybenzyl group and a 5-methoxy-3,4-methylenedioxybenzyl group. The presence of these two benzyl groups was also indicated by the prominent fragment peaks at *m*/*z* 135 (56%) and 165 (70%) in the EIMS. Except for the proton signals of the two benzyl groups, two methine protons and four methyleneoxy protons were observed, which were ascribed to a 1,4-butanediol unit, based on the HMBC spectrum. Thus, this compound was also a dibenzylbutanediol derivative. Koul et al.[22] previously obtained a dibenzylbutanediol with the same planar structure and reported its absolute configuration as 2*S*,3*S* by chemical correlation with 2-(5-methoxy-3,4-methylenedioxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone, which was indicated as 2*S*,3*S* based on the comparison of the negative CD and the negative optical rotation data with hinokinin. However, the absolute configuration of hinokinin[23] had been established earlier as 2*R*,3*R* and not 2*S*,3*S*, so (2*S*,3*S*)-2-(5-methoxy-3,4-methylenedioxybenzyl)-3-(3,4-methlenedioxybenzyl)butyrolactone should be revised to 2*R*,3*R*, and the dibenzylbutanediol should be revised to 2*R*,3*R*. Compound **9** was optically dextrorotatory and was established as (2*S*,3*S*)-2-(5-methoxy-3,4-methylenedioxybenzyl)-3-(3,4-methylenedioxybenzyl)butane-1,4-diol.

In addition, three known phenolic derivatives, *p*-tyrosol, pyrogallol, and *p*-hydroxybenzoic acid, were isolated from *P. duclouxii*. Their ^{1}H and ^{13}C NMR data were consistent with those in the SDBS database.[24]

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Horiba SEPA-200 polarimeter, and CD spectra were recorded on a JASCO J-720W spectrometer. IR and UV spectra were recorded on a Hitachi 270-30 spectrometer in $CHCl_3$ and a JASCO V-550 UV/vis spectrophotometer in $CHCl_3$, respectively. ^{1}H and ^{13}C NMR spectra were run on a Varian UNITY-PS 500 spectrometer using $CDCl_3$ as solvent. HREIMS were taken 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 an Inertsil Prep-ODS GL 10 × 250 mm stainless steel column and 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 methanol at room temperature with the aid of a supersonic machine, and about 100 g of a residue was obtained after evaporating the methanol. The residue was suspended in water and partitioned with hexane, ethyl acetate, and *n*-butanol, respectively, and afforded a hexane extract (17.3 g), an ethyl acetate extract (29.0 g), and an *n*-butanol extract (15.0 g). The ethyl acetate extract was chromatographed over a silica gel column eluted with hexane and ethyl acetate and gave five fractions (F_1−F_5). Three compounds, **1** (11 mg), **2** (91 mg), and **6** (6 mg), were obtained from F_1 (2.7 g) using silica gel column chromatography followed by normal-phase [hexane−EtOAc (7:3)] and reversed-phase HPLC [MeOH−H_2O (7:3)]. Fraction F_2 (3.18 g) was subjected to silica gel column chromatography and yielded five subfractions (F_{2-1}−F_{2-5}). Fraction F_{2-2} (0.28 g) gave compound **1** (12 mg), *p*-tyrosol (53 mg), and pyrogallol (1 mg), using normal-phase HPLC eluted with hexane−EtOAc (7:3 and 8:2). Workup of fraction F_{2-3} (0.517 g) gave compounds **2** (42 mg), **3** (5 mg), **5** (4 mg), and *p*-hydroxybenzoic acid (9 mg), using the same HPLC conditions as those used to purify compound **1**. Fraction F_{2-4} (0.543 g) gave compounds **4** (6 mg) and **8** (4 mg) using repeated HPLC eluted with hexane−EtOAc (65:35 and 7:3) and compound **7** (2 mg) using repeated HPLC eluted with hexane−EtOAc (65:35, 7:3, and 78:22). Finally, fraction F_3 (2.92 g) was divided into five subfractions over silica gel column chromatography eluting with hexane and gradient mixtures of hexane and ethyl acetate of increasing polarity, and F_{3-3} afforded compound **9** (2.7 mg) with repeated normal-phase HPLC using hexane−EtOAc (65:35) as solvent.

(2*S*,3*S*)-2,3-Bis(5-Methoxy-3,4-methylenedioxybenzyl)-butyrolactone (1): pale yellow gum; $[\alpha]_D^{25}$ +29.0° (*c* 0.547, $CHCl_3$); UV ($CHCl_3$) λ_{max} 243, 278 nm; CD (*c* 1 mM, MeOH) $[\theta]_{284}$ +2980, $[\theta]_{278}$ +2970, $[\theta]_{244}$ +10200; IR ($CHCl_3$) ν_{max} 3012, 2940, 1772, 1636, 1508, 1456, 1434, 1364, 1316, 1214, 1136, 1096, 1046, 972 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C

NMR ($CDCl_3$, 125 MHz) data, see Table 1; EIMS *m/z* 415 $[M + H]^+$ (26), 414 $[M]^+$ (96), 166 (100), 165 (88); HREIMS *m/z* 414.1310 (calcd for $C_{22}H_{22}O_8$, 414.1315).

(2*S*,3*S*)-(+)-5′-Methoxyyatein (2): pale yellow gum; $[\alpha]_D^{25}$ +24.8° (*c* 0.393, $CHCl_3$); UV ($CHCl_3$) λ_{max} 241, 275 nm; CD (*c* 1 mM, MeOH) $[\theta]_{287}$ +3036, $[\theta]_{240}$ +19866; IR ($CHCl_3$) ν_{max} 3012, 2944, 1772, 1636, 1594, 1508, 1458, 1428, 1228, 1216, 1134, 1046, 972 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 3, respectively; EIMS *m/z* 431 $[M + H]^+$ (27), 430 $[M]^+$ (100), 182 (34), 181 (55), 166 (29), 165 (23); HREIMS *m/z* 430.1601 (calcd for $C_{23}H_{26}O_8$, 430.1628).

(2*S*,3*S*)-2-(4-Hydroxy-3-methoxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (3): colorless gum; $[\alpha]_D^{25}$ +26.4° (*c* 0.293, $CHCl_3$); UV ($CHCl_3$) λ_{max} 241, 281 nm; CD (*c* 1 mM, MeOH) $[\theta]_{278}$ +1875, $[\theta]_{235}$ +15000; IR ($CHCl_3$) ν_{max} 3560, 3028, 2944, 1768, 1636, 1616, 1510, 1456, 1434, 1374, 1320, 1270, 1236, 1222, 1136, 1096, 1038, 972, 928 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 3, respectively; EIMS *m/z* 387 $[M + H]^+$ (26), 386 $[M]^+$ (100), 166 (83), 165 (48), 138 (12), 137 (75); HREIMS *m/z* 386.1383 (calcd for $C_{21}H_{22}O_7$, 386.1366).

(2*S*,3*S*)-2-(4-Hydroxy-3,5-dimethoxybenzyl)-3-(5-methoxy-3,4-methylenedioxybenzyl)butyrolactone (4): pale yellow gum; $[\alpha]_D^{25}$ +30.5° (*c* 0.380, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 276 nm; CD (*c* 1 mM, MeOH) $[\theta]_{285}$ +2386, $[\theta]_{239}$ +12750; IR ($CHCl_3$) ν_{max} 3560, 3012, 2944, 1770, 1622, 1510, 1466, 1432, 1370, 1318, 1224, 1134, 1118, 1046, 1022, 974 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 3, respectively; EIMS *m/z* 417 $[M + H]^+$ (28), 416 $[M]^+$ (100), 168 (36), 167 (74), 166 (47), 165 (31); HREIMS *m/z* 416.1484 (calcd for $C_{22}H_{24}O_8$, 416.1472).

(2*S*,3*S*)-2-(4-Hydroxy-3,5-dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone (5): pale yellow gum; $[\alpha]_D^{25}$ +34.8° (*c* 0.253, $CHCl_3$); UV ($CHCl_3$) λ_{max} 241, 285 nm; CD (*c* 1 mM, MeOH) $[\theta]_{288}$ +2240, $[\theta]_{239}$ +11790; IR ($CHCl_3$) ν_{max} 3560, 3032, 2948, 1770, 1622, 1506, 1494, 1466, 1448, 1368, 1342, 1230, 1214, 1148, 1118, 1042, 974, 940 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 3, respectively; EIMS *m/z* 387 $[M + H]^+$ (26), 386 $[M]^+$ (100), 168 (41), 167 (89), 136 (13), 135 (30); HREIMS *m/z* 386.1370 (calcd for $C_{21}H_{22}O_7$, 386.1366).

(2*S*,3*S*)-2-(5-Methoxy-3,4-methylenedioxybenzyl)-3-(3,4,5-trimethoxybenzyl)butane-1,4-diol diacetate (6): colorless gum; $[\alpha]_D^{25}$ +19.8° (*c* 0.313, $CHCl_3$); UV ($CHCl_3$) λ_{max} 241, 275 nm; IR ($CHCl_3$) ν_{max} 3012, 2944, 1736, 1636, 1594, 1506, 1458, 1424, 1372, 1336, 1222, 1132, 1094, 1042 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 4 and 5, respectively; EIMS *m/z* 519 $[M + H]^+$ (51), 518 $[M]^+$ (100), 182 (60), 181 (74), 166 (47), 165 (52); HREIMS *m/z* 518.2125 (calcd for $C_{27}H_{34}O_{10}$, 518.2153).

Reduction of Compound 2 to Compound 6. A solution of **2** (14.6 mg) in dry Et_2O (5 mL) was added to a suspension of $LiAlH_4$ (16.8 mg) in dry Et_2O (5 mL) with stirring. The mixture was stirred further for 2 h at room temperature and then poured into H_2O at 0 °C. After acidifying the mixture with HCl (3%), it was extracted with ethyl acetate three times. The combined ethyl acetate was washed with H_2O and dried (Na_2SO_4) and then concentrated. The residue (11.8 mg) was purified by normal-phase HPLC [(hexane−EtOAc (7:3)] and gave a butanediol (7.4 mg). The butanediol was acetylated with Ac_2O−pyridine and worked up as usual to give compound **6**, which was consistent with natural compound **6** in all aspects.

(2*S*,3*S*)-2,3-Bis(5-methoxy-3,4-methylenedioxybenzyl)-butane-1,4-diol monoacetate (7): colorless gum; $[\alpha]_D^{25}$ +9.5° (*c* 0.153, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 277 nm; IR ($CHCl_3$) ν_{max} 3636, 3012, 2948, 1734, 1636, 1508, 1456, 1434, 1370, 1318, 1236, 1214, 1134, 1094, 1046, 966, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 4 and 5, respectively; EIMS *m/z* 461 $[M + H]^+$ (23), 460 $[M]^+$ (80), 166 (100), 165 (84); HREIMS *m/z* 460.1765 (calcd for $C_{24}H_{28}O_9$, 460.1734).

(2*S*,3*S*)-2-(5-Methoxy-3,4-methylenedioxybenzyl)-3-(4-hydroxy-3,5-dimethoxybenzyl)butane-1,4-diol diacetate (8): colorless gum; $[\alpha]_D^{25}$ +23.6° (*c* 0.267, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 277 nm; IR ($CHCl_3$) ν_{max} 3560, 3012, 2944, 1736, 1620, 1508, 1466, 1432, 1330, 1240, 1214, 1118, 1096, 1044, 974 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 4 and 5, respectively; EIMS *m/z* 505 $[M + H]^+$ (31), 504 $[M]^+$ (100), 168 (35), 167 (51), 166 (40), 165 (48); HREIMS *m/z* 504.1971 (calcd for $C_{26}H_{32}O_{10}$, 504.1996).

Table 5. ^{13}C NMR Data for Compounds **6**−**9** in $CDCl_3$ (125 MHz)[a]

carbon	6	7	8	9
1	64.3	64.6	64.3	60.5
2	39.5	39.9	39.6	44.1
3	39.8	43.1	39.6	44.1
4	64.2	62.4	64.2	60.5
5	35.7	35.2	35.6	36.3
6	35.4	35.5	35.5	35.9
1′	135.3	134.9	130.6	134.2
2′	105.7	102.9	105.4	109.3
3′	153.1	148.8	146.9	147.6
4′	136.4	133.4	133.0	145.8
5′	153.1	143.4	146.9	108.1
6′	105.7	108.1	105.4	121.8
1″	134.1	134.5	134.2	135.1
2″	102.8	102.9	102.9	103.0
3″	148.9	148.8	148.8	148.8
4″	133.5	133.4	133.5	133.4
5″	143.4	143.4	143.4	143.4
6″	108.2	108.1	108.1	108.2
−OCH_2O−	101.3	101.3	101.3	100.8 101.2
OCH_3-5″	56.6	56.5	56.5	56.6
OCH_3-3′	56.0		56.2	
OCH_3-4′	60.8			
OCH_3-5′	56.0	56.5	56.2	
C═O	170.9	171.0	170.9	
CH_3	21.0	21.0	21.0	

[a] Signals were assigned from the $^1H-^1H$ COSY, HMQC, and HMBC spectra.

(2*S*,3*S*)-2-(5-Methoxy-3,4-methylenedioxybenzyl)-3-(3,4-methylenedioxybenzyl)butane-1,4-diol (9): colorless gum; $[\alpha]_D^{25}$ +19.0° (*c* 0.127, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242, 286 nm; IR ($CHCl_3$) ν_{max} 3636, 3004, 2948, 2892, 2784, 1636, 1616, 1506, 1494, 1454, 1376, 1316, 1220, 1214, 1136, 1094, 1044, 1008, 932 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 4 and 5, respectively; EIMS *m/z* 389 $[M + H]^+$ (2), 388 $[M]^+$ (10), 370 (36), 166 (94), 165 (70), 136 (15), 135 (56). HREIMS *m/z* 388.1518 (calcd for $C_{21}H_{24}O_7$, 388.1523).

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Supporting Information Available: Tables of major COSY and HMBC correlations of compounds **2**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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