Potent CYP3A4 Inhibitory Constituents of *Piper cubeba*

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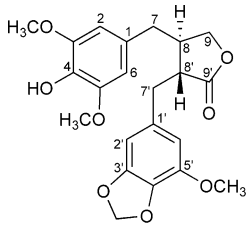
The EtOAc-soluble fraction of the water extract of *Piper cubeba*, having shown potent inhibitory activity on the metabolism mediated by CYP3A4, was subjected to activity-guided isolation to yield two new lignans, (8*R*,8′*R*)-4-hydroxycubebinone (**1**) and (8*R*,8′*R*,9′*S*)-5-methoxyclusin (**2**), and two new sesquiterpenes, (5α,8α)-2-oxo-1(10),3,7(11)-guaiatrien-12,8-olide (**3**) and (1α,2*β*,5α,8α10α)-1,10-epoxy-2-hydroxy-3,7(11)-guaiadien-12,8-olide (**4**), along with 16 known compounds (**5**−**20**). The structures of the isolated compounds were elucidated on the basis of spectroscopic and chemical analyses. The isolated compounds were tested for their inhibitory activity on the metabolism mediated by CYP3A4 or CYP2D6 using [*N*-methyl-^{14}C]erythromycin or [*O*-methyl-^{14}C]dextromethorphan as a substrate, respectively. The compounds (8*R*,8′*R*,9′*S*)-5-methoxyclusin (**2**), (−)-clusin (**10**), (−)-yatein (**13**), ethoxyclusin (**15**), and (−)-dihydroclusin (**17**), having one methylenedioxyphenyl moiety in their structures, showed very potent and selective inhibitory activity against CYP3A4 with IC_{50} values (0.44−1.0 μM) identical to that of the positive control, ketoconazole (IC_{50}, 0.72 μM).

The genus *Piper* belongs to the Piperaceae family, widely distributed in the tropical and subtropical regions of the world, and is used medicinally in various ways. Economically, Piperaceae plants are important for pepper production in the spice markets. Recently, several reports have demonstrated that on simultaneous administration, some spices, herbs, black teas, and soybean products may cause pharmacokinetic interaction with Western drugs.[1−3] The inhibition of cytochrome P450 (CYP) can lead to serious clinical drug interactions when concomitant drugs are metabolized by the same CYP. In our investigation of Indonesian medicinal plants for their inhibitory activity on the metabolism mediated by CYP3A4 and CYP2D6, we observed that EtOAc-soluble fractions of *Piper nigrum*, *Piper cubeba*, and *Zingiber aromaticum* possessed potent inhibitory activity against CYP3A4-mediated metabolism and *Piper nigrum* against CYP2D6-mediated metabolism.[4] In addition, we previously reported the CYP3A4 and CYP2D6 inhibitory constituents of *Z. aromaticum*.[5] On the other hand, some bisalkaloids from *P. nigrum* were reported to have inhibitory activity on CYP3A4.[6,7]

Piper cubeba L. is one of the popular medicinal plants extensively used in Indonesia. The fruits are used as a spice and for the treatment of gonorrhea, dysentery, syphilis, abdominal pain, diarrhea, enteritis, and asthma.[8,9] There are several reports on the constituents of this plant,[10−12] but no report has been found on inhibitory activity against CYP. Thus, we examined the constituents of this plant and isolated two new lignans (**1**, **2**) and two new sesquiterpenes (**3**, **4**), together with 16 known compounds (**5**−**20**). Here, we report the isolation and structure elucidation of the four new compounds and inhibitory activity of the isolated compounds on the metabolism mediated by CYP3A4 and CYP2D6.



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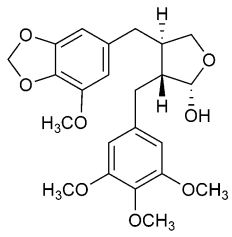

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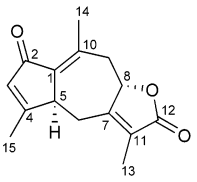

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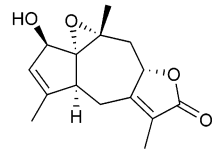

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Results and Discussion

The fruits of *P. cubeba* were extracted with H_2O, and the H_2O extract was fractionated with EtOAc and MeOH to yield EtOAc-soluble and MeOH-soluble fractions. The EtOAc-soluble fraction showed inhibitory activity (inhibition: 75% against CYP3A4, 30% against CYP2D6) similar to the MeOH-soluble fraction (inhibition: 84% against CYP3A4, 43% against CYP2D6). Thus, the EtOAc-soluble fraction was subjected to further chemical analysis to give two new lignans, (8*R*,8′*R*)-4-hydroxycubebinone (**1**) and (8*R*,8′*R*,9′*S*)-5-methoxyclusin (**2**), and two new sesquiterpenes, (5α,8α)-2-oxo-1(10),3,7(11)-guaiatrien-12,8-olide (**3**) and (1α,2*β*,5α,8α10α)-1,10-epoxy-2-hydroxy-3,7(11)-guaiadien-12,8-olide (**4**), together with 16 known compounds: α-asarone (**5**),[13] α-methylcubebin (**6**),[10] magnosalin (**7**),[14,15] (−)-hinokinin (**8**),[16] (−)-cubebin (**9**),[17] (−)-clusin (**10**),[17] (−)-cubebininolide (**11**),[18] (−)-dihydrocubebin (**12**),[17] (−)-yatein (**13**),[19] 2,4,5-trimethoxyphenylacetone (**14**),[20] ethoxyclusin (**15**),[18] (−)-cubebinin (**16**),[12] (-)-dihydroclusin (**17**),[12] 1-(2,4,5-trimethoxyphenyl)-1,2-propanedione (**18**),[21] (−)-thujaplicatin trimethyl ether (**19**),[22] and medioresinol (**20**).[23]

Compound **1** was isolated as a pale yellow oil, having $[\alpha]^{25}_{D}$ −30.0° (*c* 0.08, $CHCl_3$) and a molecular formula of $C_{22}H_{24}O_8$ by HRFABMS. Its IR spectrum showed absorption bands corresponding to hydroxyl (3580 cm^{-1}), carbonyl (1770 cm^{-1}), and methylenedioxy (930 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** showed the presence of four

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** (in $CDCl_3$)[a]

	1			**2**		
position	δ_H	δ_C	HMBC[b]	δ_H	δ_C	HMBC[b]
1		129.0	2, 6, 7		134.9	2, 6, 7
1′		132.1	2′, 6′, 7′		133.7	2′, 6′, 7′
2	6.22 s	105.2	6	6.23 s	102.5	6
2′	6.31 s	103.2	6′	6.34 s	105.8	6′
3		147.1	2, 3-OCH_3, 4-OH		149.2	2, OCH_2O
3′		149.0	2′, OCH_2O		153.1	2′, 3′-OCH_3
4		133.6	2, 6, 4-OH		135.2	2, 6, OCH_2O
4′		134.1	2′, 6′, OCH_2O		136.4	2′, 6′, 4′-OCH_3
5		147.1	6, 5-OCH_3, 4-OH		143.8	6, 5-OCH_3
5′		143.6	6′, 5′-OCH_3		153.1	6′, 5′-OCH_3
6	6.22 s	105.2	2	6.22 s	108.8	2
6′	6.30 s	108.7	2′	6.34 s	105.8	2′
7	2.60 m	38.9	2, 6, 8, 9	2.63 m	39.2	2, 6, 8, 9
7′	2.94 dd (14.0, 4.9)	35.0	2′, 6′, 8′	2.77 m	34.2	2′, 6′, 8′
	2.85 dd (14.0, 6.6)			2.47 m		
8	2.47 m	41.3	7, 9, 8′	2.16 m	46.1	7, 9, 8′, 9′
8′	2.59 m	46.5	8, 9, 7′	2.23 m	53.0	8, 9, 7′, 9′
9	3.88 dd (8.5, 8.5)	71.3	8, 8′	3.80 m	72.3	8, 8′, 9′
	4.19 dd (8.5, 7.5)			3.92 m		
9′		178.4	8, 9, 7′, 8′	5.26 br s	103.4	8, 9, 7′, 8′
3-OCH_3	3.85 s	56.3				
4-OH	5.42 s					
5-OCH_3	3.85 s	56.3		3.86 s	56.6	
3′-OCH_3				3.82 s	56.1	
4′-OCH_3				3.85 s	60.9	
5′-OCH_3	3.86 s	56.7		3.82 s	56.1	
OCH_2O	5.94 s	101.4		5.93 s	101.3	

[a] The ^{1}H and ^{13}CNMR spectra were measured at 400 and 100 MHz, respectively, and coupling constants (in parentheses) are in Hz. [b] ^{1}H correlating with the ^{13}C resonance.

aromatic protons, an oxygen-substituted methylene, and two methines, together with three methoxyls, a methylenedioxy, and a hydroxyl group (Table 1). The ^{13}C NMR spectrum of **1** showed 22 carbon signals including a carbonyl carbon (δ 178.4). These data were similar to those of (−)-yatein (**13**) isolated from the same extract, except for the presence of a hydroxyl signal. The COSY spectrum confirmed the connectivity of four saturated carbon atoms in the butyrolactone ring and also confirmed the connectivities of C-7 and C-8 and of C-7′ and C-8′, while the COSY, HMQC, and HMBC spectra (Table 1) indicated the presence of 3,5-dimethoxy-4-hydroxyphenyl and 5′-methoxy-3′,4′-methylenedioxyphenyl groups. In addition, the bonding of the 3,5-dimethoxy-4-hydroxyphenyl group to C-7 and the 5′-methoxy-3′,4′-methylenedioxyphenyl group to C-7′ was also established on the basis of the strong HMBC correlations between H-7 and C-1 and between H-7′ and C-1′. The relative configuration at C-8 and C-8′ was determined as *trans* by comparing their carbon chemical shifts with those of known *trans*-lignans **8**, **11**, **13**, and **19**, while the absolute configuration was concluded to be 8*R*,8′*R* on the basis of the negative Cotton effect at 238 nm.[22] Thus, compound **1** was assigned as (8*R*,8′*R*)-4-hydroxycubebinone.

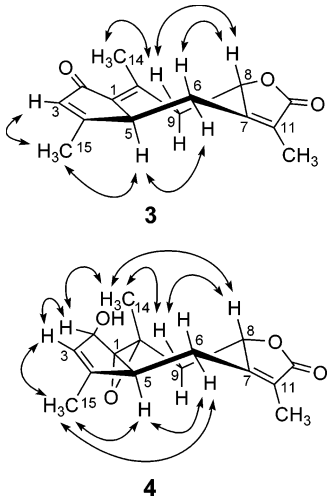
Compound **2** was obtained as a pale yellow oil with $[\alpha]^{25}_D$ −53.4° (*c* 0.3, $CHCl_3$). Its molecular formula was deduced as $C_{23}H_{28}O_8$ by HRFABMS. The IR spectrum suggested the presence of hydroxyl (3470 cm^{-1}) and methylenedioxy (940 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** (Table 1) displayed signals of four aromatic protons, an oxygen-substituted methylene, an oxygen-substituted methine, four methoxyls, and a methylenedioxy group, while the ^{13}C NMR spectrum showed 23 carbon signals. These data were similar to those of (−)-clusin (**10**) isolated from the same extract, except for the presence of one more methoxyl group. The connectivity of five saturated carbons in the butyrolactone ring was confirmed by the COSY, HMQC, and HMBC spectra (Table 1). They also confirmed the presence of 5-methoxy-3,4-methylenedioxyphenyl and 3′,4′,5′-trimethoxyphenyl groups and established their bonding to C-7 and C-7′, respectively. The relative configuration at C-8 and C-8′ was deduced as *trans* by comparing their carbon chemical shifts with those of known *trans*-lignans **9**, **10**, and **16**, while the α-OH orientation at C-9′ was established from the splitting pattern of H-9′ (broad singlet). The absolute configuration was concluded to be 8*R*,8′*R*,9′*S* on the basis of the negative Cotton effect at 240 nm.[22] Thus, compound **2** was assigned as (8*R*,8′*R*,9′*S*)-5-methoxyclusin.

Compound **3** was isolated as a yellow amorphous solid, $[\alpha]^{25}_D$ +127.5° (*c* 0.4, $CHCl_3$), with a molecular formula of $C_{15}H_{16}O_3$ by HRFABMS. The absorption band at 1755 cm^{-1} in its IR spectrum indicated the presence of a carbonyl group. The ^{1}H NMR spectrum of **3** (Table 2) displayed signals corresponding to three tertiary methyls, an oxygen-substituted methine, an aliphatic methine, and an olefinic methine. The ^{13}C NMR spectrum of **3** showed 15 carbon signals including a conjugated ketone (δ 195.6) and a lactone carbonyl (δ 173.5), six olefinic carbons (δ 134.3, 168.9, 160.6, 122.9, 142.9, 137.5), and an oxygen-substituted carbon (δ 77.9). The COSY and HMQC spectra revealed only the connectivities between C-5 and C-6 and between C-8 and C-9. The planar structure was deduced on the basis of the long-range correlations observed in the HMBC spectrum (Table 2). In the HMBC spectrum of **3**, long-range correlations were observed between H_3-13 and C-7, C-11, and C-12, between H_3-14 and C-9, C-10, and C-1, and between H_3-15 and C-3, C-4, and C-5. Likewise, H-5 showed long-range correlations with C-2, C-3, and C-7, and H-9 with C-1, C-7, and C-14. These data indicated that **3** should be a guaiane-type sesquiterpene. The relative configuration of **3** was assigned on the basis of the results of NOE experiments (Figure 1). NOEs were observed from H-5 to H-6 (δ 3.20) and H_3-15. Because H-5 of the guaiane-type sesquiterpenes is defined in the α-orientation,[24] H-5, H-6 (δ 3.20), and H_3-15 should be in α-orientation. Then, the NOEs from H-8 to H-6 (δ 1.91) and H-9 (δ 2.80)

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **3** and **4** (in $CDCl_3$)[a]

	3			**4**		
position	δ_H	δ_C	HMBC[b]	δ_H	δ_C	HMBC[b]
1		137.5	3, 5, 6, 9, 14		74.5	2, 3, 5, 6, 9, 14
2		195.6	3, 5	4.40 br s	79.9	3, 5
3	6.16 s	134.3	5, 15	5.79 br s	127.4	2, 5, 15
4		168.9	3, 5, 6, 15		149.5	2, 3, 5, 6, 15
5	3.05 d (12.0)	46.1	3, 6, 15	2.15 dd (13.0, 2.2)	50.3	2, 3, 6, 15
6	3.20 d (13.0)	30.2	5, 8	3.08 dd (14.0, 2.2)	30.8	5, 8
	1.91 m			2.31 dd (14.0, 13.0)		
7		160.6	5, 6, 8, 9, 13		161.0	5, 6, 8, 9, 13
8	4.65 d (12.0)	77.9	6, 9	4.86 d (13.0)	79.1	6, 9
9	2.48 m	41.9	8, 14	1.58 dd (13.0, 13.0)	42.2	8, 14
	2.80 dd (13.0, 3.0)			2.73 dd (13.0, 3.0)		
10		142.9	9, 14		59.6	9, 14
11		122.9	6, 13		122.3	6, 13
12		173.5	13		173.9	13
13	1.91 s	8.4		1.84 s	8.3	
14	2.47 s	20.6	9	1.63 s	20.7	9
15	2.17 s	16.6	3	1.93 s	15.6	3

[a] The ^{1}H and ^{13}CNMR spectra were measured at 400 and 100 MHz, respectively, and coupling constants (in parentheses) are in Hz. [b] ^{1}H correlating with the ^{13}C resonance.



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4

Figure 1. NOEs observed in the NOE difference experiments of **3** and **4**.

suggested the β-orientation of H-8 and H-9 (δ 2.80). From these data, compound **3** was determined as (5α,8α)-2-oxo-1(10),3,7(11)-guaiatrien-12,8-olide.

Compound **4** was obtained as a yellow oil, $[\alpha]^{25}_D$ +61.0° (*c* 0.4, $CHCl_3$). Its molecular formula was deduced as $C_{15}H_{18}O_4$ by HRFABMS. The IR spectrum showed absorptions due to hydroxyl (3500 cm^{-1}) and carbonyl (1780 cm^{-1}) groups. The ^{1}H NMR spectrum of **4** revealed signals due to three tertiary methyls, two oxygen-substituted methines, an aliphatic methine, and an olefinic methine (Table 2). Moreover, the ^{13}C NMR spectrum of **4** showed signals of 15 carbons including that of a carbonyl carbon (δ 173.9). These data were similar to those of **3**, except for the presence of a hydroxyl and an epoxy group and disappearance of the carbonyl group. The hydroxyl group was located at C-2 on the basis of the low-field shift of H-2 (δ 4.40) and C-2 (δ 79.9) assigned by the COSY, HMQC, and HMBC spectra. The HMBC spectrum also confirmed the location of the epoxide ring and the carbonyl group (Table 2). The relative configuration of **4** was assigned from the results of NOE experiments, in which associations were observed from H_3-14 to H-9β, H-2, and H-8, from H-2 to H-3, and from H_3-15 to H-3, H-5, and H-6α. Thus, **4** should have the configuration shown in Figure 1. From these data, compound **4** was determined as (1α,2β,5α,8α10α)-1,10-epoxy-2-hydroxy-3,7(11)-guaiadien-12,8-olide.

Table 3. IC_{50} Values[a] of the Isolated Compounds on the Metabolism Mediated by CYP3A4 and CYP2D6

compound	CYP3A4	CYP2D6
1	7.4	>100
2	0.83	>100
3	8.8	>100
4	98.2	>100
5	92.3	>100
6	7.7	>100
7	85.4	>100
8	8.0	26.5
9	9.1	35.5
10	0.83	>100
11	14.9	>100
12	9.5	17.5
13	1.0	95.7
14	28.7	>100
15	0.44	87.9
16	15.0	>100
17	0.80	>100
18	74.0	>100
19	1.1	>100
20	13.7	>100
ketoconazole	0.72	
quinidine		0.082

[a] IC_{50} values in μM.

The isolated compounds were tested for their inhibitory activity on the metabolism mediated by CYP3A4 or CYP2D6 using [*N*-methyl-^{14}C]erythromycin or [*O*-methyl-^{14}C]dextromethorphan as a substrate, respectively (Table 3). (8*R*,8′*R*,9′*S*)-5-Methoxyclusin (**2**, IC_{50}, 0.83 μM), (−)-clusin (**10**, IC_{50}, 0.83 μM), (−)-yatein (**13**, IC_{50}, 1.0 μM), ethoxyclusin (**15**, IC_{50}, 0.44 μM), and (−)-dihydroclusin (**17**, IC_{50}, 0.80 μM), having one methylenedioxyphenyl moiety in their structures, showed very potent inhibitory activity against CYP3A4 with IC_{50} values identical to that of the positive control, ketoconazole (IC_{50}, 0.72 μM). (8*R*,8′*R*)-4-Hydroxycubebinone (**1**), (5α,8α)-2-oxo-1(10),3,7(11)-guaiatrien-12,8-olide (**3**), α-methylcubebin (**6**), (−)-hinokinin (**8**), (−)-cubebin (**9**), (−)-dihydrocubebin (**12**), and (−)-thujaplicatin trimethyl ether (**19**) also showed potent inhibitory activity on the metabolism mediated by CYP3A4 with IC_{50} values less than 10 μM (Table 3), while other compounds possessed moderate or weak inhibitory activities. On the other hand, all of the isolated compounds showed only weak or no inhibitory activity against CYP2D6, indicating the selectivity of the compounds isolated against CYP3A4. It was reported that compounds with a methylenedioxyphenyl

moiety frequently give rise to inhibition of CYP-dependent drug metabolism. The complexity arises from the ability of their methylenic carbon to undergo oxidation to a carbene, which then interacts with the heme iron of CYP to produce a stable heme-adduct termed a CYP metabolic-intermediate complex.[25] Interestingly, the compounds possessing one methylenedioxyphenyl moiety showed more potent inhibition than those with two methylenedioxyphenyls. These results suggest the possibility of potential drug−lignan or drug−sesquiterpene interactions in individuals who consume *P. cubeba* in conjunction with drugs being metabolized by CYP3A4.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solution. NMR spectra were recorded on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer using glycerol as a matrix. CD spectra were measured in a JASCO J-805 spectropolarimeter. Column chromatography was performed with silica gel 60 (Nacalai tesque, Inc., Kyoto, Japan), and analytical and preparative TLC were conducted on precoated Merck Kieselgel 60F_{254} plates (0.25 or 0.50 mm thickness).

Biological Material. Fruits of *P. cubeba* were obtained at GORO traditional market, Jakarta, Indonesia, in May 2002. A voucher sample (TMPW 22277) is preserved in the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. The dried fruits of *P. cubeba* were crushed into a powder form. The powder (4.0 kg) was extracted with H_2O (20 L, reflux, 2 h, ×3), and the insoluble portion was separated by filtration. The filtrate was evaporated under reduced pressure and then lyophilized to give a H_2O extract (581 g). The H_2O extract (250 g) was further fractionated into EtOAc-soluble (8.5 g) and MeOH-soluble fractions (24.5 g).

The EtOAc-soluble fraction (8.0 g) was subjected to silica gel column chromatography with hexane−EtOAc and MeOH to afford 12 fractions: fraction 1, 5% EtOAc−hexane eluate, 28.7 mg; fraction 2, 5% EtOAc−hexane eluate, 473 mg; fraction 3, 10% EtOAc−hexane eluate, 154 mg; fraction 4, 10% EtOAc−hexane eluate, 135 mg; fraction 5, 15% EtOAc−hexane eluate, 206 mg; fraction 6, 20% EtOAc−hexane eluate, 766 mg; fraction 7, 20% EtOAc−hexane eluate, 728 mg; fraction 8, 30% EtOAc−hexane eluate, 1.5 g; fraction 9, 40% EtOAc−hexane eluate, 104 mg; fraction 10, 50% EtOAc−hexane eluate, 643 mg; fraction 11, EtOAc eluate, 728 mg; fraction 12, MeOH eluate, 2.1 g. Further silica gel column chromatography and preparative TLC of these fractions yielded the following compounds: fraction 2, **5** (10.6 mg); fraction 3, **6** (4.7 mg); fraction 4, **7** (4.2 mg); fraction 5, **8** (31.2 mg); fraction 6, **9** (28.0 mg); fraction 7, **13** (6.4 mg); fraction 8, **1** (4.0 mg), **2** (5.0 mg), **3** (8.8 mg), **4** (8.7 mg), **10** (103.6 mg), **11** (27.8 mg), **12** (39.1 mg), **14** (3.3 mg), **15** (1.9 mg), **18** (0.7 mg), and **19** (2.6 mg); fraction 9, **14** (2.0 mg) and **20** (2.1 mg); fraction 10, **14** (2.2 mg) and **16** (7.7 mg); fraction 11, **17** (6.3 mg).

(8*R*,8′*R*)-4-Hydroxycubebinone (1): pale yellow oil; $[\alpha]^{25}_D$ −30.0° (*c* 0.08, $CHCl_3$); CD λ_{max} (2.40 × 10^{-4} M, EtOH) nm 238 ([θ] −6851); IR ($CHCl_3$) ν_{max} 3580, 1770, 1600, 930 cm^{-1}; HRFABMS *m/z* 417.4812 (calcd for $C_{22}H_{25}O_8$ $[M + H]^+$, 417.4818); ^1H and ^{13}C NMR, Table 1.

(8*R*,8′*R*,9′*S*)-5-Methoxyclusin (2): pale yellow oil; $[\alpha]^{25}_D$ −53.4° (*c* 0.3, $CHCl_3$); CD λ_{max} (2.31 × 10^{-4} M, EtOH) nm 240 ([θ] −3500); IR ($CHCl_3$) ν_{max} 3470, 1590, 1480, 940 cm^{-1}; HRFABMS *m/z* 433.1754 (calcd for $C_{23}H_{29}O_8$ $[M + H]^+$, 433.1759); ^1H and ^{13}C NMR, Table 1.

(5α,8α)-2-Oxo-1(10),3,7(11)-guaiatrien-12,8-olide (3): yellow amorphous solid; $[\alpha]^{25}_D$ +127.5° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1755, 1709, 1630, 1380, 1360 cm^{-1}; HRFABMS *m/z* 245.1254 (calcd for $C_{15}H_{17}O_3$ $[M + H]^+$, 245.1261); ^1H and ^{13}C NMR, Table 2.

(1α,2*β*,5α,8α10α)-1,10-Epoxy-2-hydroxy-3,7(11)-guaiadien-12,8-olide (4): yellow oil; $[\alpha]^{25}_D$ +61.0° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3500, 1780, 1650, 1370, 1350 cm^{-1}; HRFABMS *m/z* 263.1210 (calcd for $C_{15}H_{19}O_4$ $[M + H]^+$, 263.1219); ^1H and ^{13}C NMR, Table 2.

CYP Inhibitory Assay. Human liver microsome (HLM; Xenotech, LLC, Kansas) was stored at −80 °C prior to use. β-Nicotinamide adenine dinucleotide phosphate oxidized form ($NADP^+$), glucose-6-phosphate (G-6-P), and G-6-P dehydrogenase (Oriental Yeast Co., Ltd., Tokyo, Japan) were used as NADPH-generating system.

Inhibitory activity on the metabolism mediated by CYP3A4 or CYP2D6 in vitro was determined using a radiometric measurement of [^{14}C]formaldehyde formed by the reaction with [*N*-methyl-^{14}C]erythromycin or [*O*-methyl-^{14}C]dextromethorphan (American Radiolabeled Chemicals, Inc., St. Louis, MO) as a substrate, respectively.[26,27] Briefly, in disposable culture tubes (13 × 100 mm; Iwaki, Tokyo, Japan) containing phosphate buffer (pH 7.4), [*N*-methyl-^{14}C]erythromycin (0.1 μCi/incubation; 1000 μM in 5% of MeOH), or [*O*-methyl-^{14}C]-dextromethorphan (0.1 μCi/incubation; 100 μM in 5% of MeOH), and 50 μL of HLM (4 mg/mL) were added to varying concentrations of test specimens in 500 μL of total incubation volume. After a preincubation period of 5 min in a shaking water bath at 37 °C, the reaction is initiated by adding 50 μL of NADPH-generating system (4.20 mg/mL of $NADP^+$ in a solution of 100 mM G-6-P, $MgCl_2$, and 10 U/mL G-6-P dehydrogenase), and the incubation was continued for 10 min (CYP3A4) or 20 min (CYP2D6) in a shaking water bath at 37 °C. The reaction was stopped by addition of 125 μL of 10% trichloroacetic acid (Nacalai tesque, Inc., Kyoto, Japan), and the solution was centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was applied to Envi-Carb solid-phase extraction columns (Supelco, UK) and was eluted with 2 volumes of ultrapure water (2 × 500 μL). After adding 10 mL of Clear-sol I (Nacalai tesque, Inc., Kyoto, Japan), the eluted radioactivity was quantified by liquid scintillation counting LS 6500 (Beckman). Ketoconazole or quinidine sulfate dihydrate (Wako Pure Chemicals Industry, Ltd., Osaka, Japan) was used as a positive control for CYP3A4 or CYP2D6, respectively, while MeOH was used as a negative control. Correction was made for radioactivity eluted from control incubations in which HLM- and NADPH-generating systems had been omitted. The assays were performed in duplicate for all test specimens, and remaining activity was analyzed using the software product WinNonlin Ver.3.1 (Pharsight Corporation, Mountain View, CA). IC_{50} values (concentrations of test specimen causing 50% reduction in activity relative to the negative control) were calculated by linear regression analysis of the log test specimen concentration versus percentage control activity plots.

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Supporting Information Available: Structures of known compounds (**5**−**20**) are available free of charge via the Internet at http://pubs.acs.org.

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