Sesquiterpenes and Flavonol Glycosides from *Zingiber aromaticum* and Their CYP3A4 and CYP2D6 Inhibitory Activities

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Three new sesquiterpenes, (2*R*,3*S*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-one (**1**), (2*R*,3*R*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-one (**2**), and (5*R*)-2,6,9-humulatrien-5-ol-8-one (**3**), and two new flavonol glycosides, kaempferol-3-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside) (**4**) and kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (**5**), were isolated from the EtOAc-soluble fraction of the water extract of *Zingiber aromaticum*, along with 13 known compounds (**6**−**18**). 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. Kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (**5**) showed the most potent inhibitory activity (IC_{50}, 14.4 μM) on the metabolism mediated by CYP3A4, and kaempferol-3-*O*-methyl ether (**14**) inhibited CYP2D6 most potently (IC_{50}, 4.63 μM).

Cytochrome P450 (CYP) comprises a superfamily of hemoproteins that plays an important role in the metabolism of a wide variety of xenobiotics and endogenous compounds. In human liver microsomes, CYP3A4 is the most abundant enzyme, and recent investigations revealed that more than 50% of clinically used drugs are oxidized by CYP3A4[1] and about 30% of drugs are metabolized by CYP2D6.[2] Inhibition of CYP-mediated metabolism often causes drug−drug interactions and limits the use of a drug because of adverse clinical effects. In our investigation of Indonesian medicinal plants for their inhibitory activity on the metabolism mediated by CYP3A4 and CYP2D6, we observed that an EtOAc-soluble fraction of *Zingiber aromaticum* possessed potent inhibitory activity against CYP3A4-mediated metabolism.[3]

Z. aromaticum Vahl (Zingiberaceae) is one of the popular traditional medicines extensively used in Indonesia. The rhizomes are used by the name of "Lempuyang wangi" for the treatment of cholescystopathy, whooping cough, jaundice, arthritis, anorexia, cold, cholera, anemia, malaria, rheumatism, and abdominalgia.[4,5] There is only one report on the constituents of this plant,[6] and no report has been found on inhibitory activity against CYP. Thus, we examined the constituents of this plant and isolated three new sesquiterpenes (**1**−**3**) and two new flavonol glycosides (**4**, **5**), together with 13 known compounds (**6**−**18**). In this paper, we report the isolation and structure elucidation of the five new compounds and inhibitory activity of the isolated compounds on the metabolism mediated by CYP3A4 and CYP2D6.

Results and Discussion

The rhizomes of *Z. aromaticum* were extracted with H_2O, and the H_2O extract was fractionated with EtOAc and

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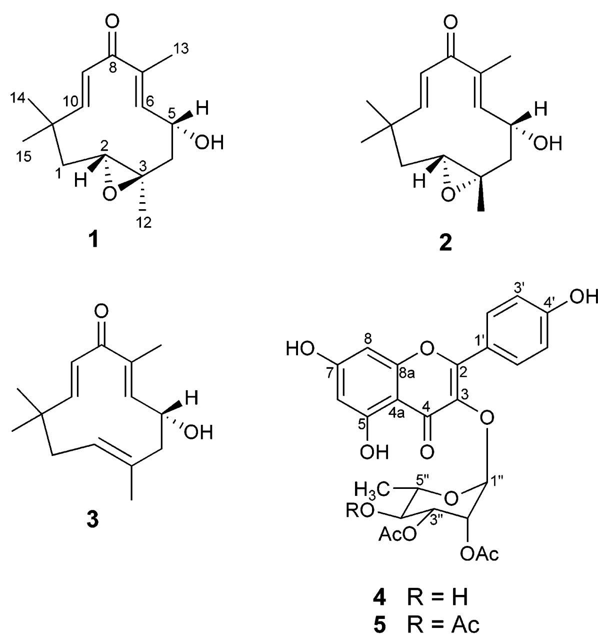
Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**3** (in $CDCl_3$)[a]

position	**1** δ_H	δ_C	HMBC[b]	**2** δ_H	δ_C	HMBC[b]	**3** δ_H	δ_C	HMBC[b]
1	1.96 d (13) 1.43 t (13)	39.8	2, 10, 14, 15	1.96 d (13) 1.44 t (13)	42.6	2, 10, 14, 15	2.36 t (12) 1.90 m	42.5	2, 10, 14, 15
2	2.68 d (13)	58.9	1, 4, 12	2.76 d (13)	62.7	1, 4, 12	5.29 br d (11)	126.7	1, 4, 12
3		58.2	2, 4, 12		59.3	2, 4, 12		133.1	1, 2, 4, 12
4	2.11 dd (13, 10) 1.69 dd (13, 5)	46.2	2, 5, 6, 12	2.66 dd (13, 5) 1.26 d (13)	47.7	2, 5, 6, 12	2.75 m 2.16 m	49.2	2, 5, 6, 12
5	4.40 td (10, 5)	67.1	4, 6	4.60 m	64.9	4, 6	4.64 m	64.9	4, 6
6	5.63 d (10)	133.8	4, 5, 13	5.94 d (10)	144.5	4, 5, 13	5.82 d (10)	145.6	4, 5, 13
7		139.9	5, 6, 9, 13		142.5	5, 6, 9, 13		140.6	5, 6, 9, 13
8		200.9	6, 9, 10, 13		202.7	6, 9, 10, 13		204.2	6, 9, 10, 13
9	6.13 d (16)	129.3	10	6.16 d (16)	128.7	10	6.00 d (16)	127.3	10
10	6.44 d (16)	160.0	1, 9, 14, 15	6.36 d (16)	161.2	1, 9, 14, 15	5.91 d (16)	162.4	1, 9, 14, 15
11		36.4	1, 9, 10, 14, 15		35.9	1, 9, 10, 14, 15		38.5	1, 9, 10, 14, 15
12	1.38 s	19.5	2, 4	1.23 s	16.8	2, 4	1.57 s	16.5	2, 4
13	2.01 s	20.9	6	2.00 s	12.6	6	1.92 s	12.2	6
14	1.30 s	24.0	1, 10, 15	1.31 s	24.1	1, 10, 15	1.08 s	29.3	1, 10, 15
15	1.14 s	29.6	1, 10, 14	1.09 s	29.7	1, 10, 14	1.21 s	24.2	1, 10, 14

[a] The ^{1}H and ^{13}C NMR 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.



MeOH to yield EtOAc-soluble and MeOH-soluble fractions. The EtOAc-soluble fraction showed inhibitory activity (inhibition: 76% and 16% against CYP3A4 and CYP2D6, respectively) similar to the MeOH-soluble fraction (inhibition: 80% and 35% against CYP3A4 and CYP2D6, respectively), and thus the EtOAc-soluble fraction was subjected to further chemical analysis to give three new sesquiterpenes, (2*R*,3*S*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-one (**1**), (2*R*,3*R*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-one (**2**), and (5*R*)-2,6,9-humulatrien-5-ol-8-one (**3**), and two new flavonol glycosides, kaempferol-3-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside) (**4**) and kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (**5**), together with 13 known compounds: zerumbone (**6**),[6,7] zerumbone epoxide (**7**),[7] kaempferol-3-*O*-(2,4-di-*O*-acetyl-α-L-rhamnopyranoside) (**8**),[7] kaempferol-3-*O*-(3,4-di-*O*-acetyl-α-L-rhamnopyranoside) (**9**),[6,7] kaempferol-3-*O*-(2-*O*-acetyl-α-L-rhamnopyranoside) (**10**),[8] kaempferol-3-*O*-(3-*O*-acetyl-α-L-rhamnopyranoside) (**11**),[8] kaempferol-3-*O*-(4-*O*-acetyl-α-L-rhamnopyranoside) (**12**),[8] kaempferol-3-*O*-α-L-rhamnopyranoside (**13**),[8] kaempferol-3-*O*-methyl ether (**14**),[7] kaempferol-3,4′-di-*O*-methyl ether (**15**),[7] (*S*)-6-gingerol (**16**),[9] *β*-sitosterol (**17**),[10] and *trans*-6-shogaol (**18**).[11] The MeOH-soluble fraction gave compounds **1**, **2**, **4**, and **6**−**14**. Except for compounds **6** and **9**, all of these compounds were found for the first time in *Z. aromaticum*.

Compounds **1**, $[\alpha]^{25}_{D}$ +5.3° (*c* 0.4, $CHCl_3$), and **2**, $[\alpha]^{25}_{D}$ −10.9° (*c* 0.2, $CHCl_3$), were obtained as pale yellow oils. They showed the same molecular formula of $C_{15}H_{22}O_3$, and their IR spectra displayed absorptions corresponding to hydroxyl (3300 cm^{-1}) and carbonyl (1700 cm^{-1}) groups. Their ^{1}H NMR spectra (Table 1) were very similar and showed the presence of a trisubstituted olefin, a *trans*-olefin (J = 16 Hz), two oxygen-substituted methines, and four methyls, while their ^{13}C NMR spectra showed 15 carbon signals including a carbonyl carbon (Table 1). These data were similar to those of zerumbone epoxide (**7**) isolated from the same extract, except for the presence of a hydroxyl. The location of the hydroxyl group was determined to be at C-5, based on the deshielded H-5 (**1**, δ 4.40; **2**, δ 4.60; **7**, δ 2.27−2.46) and C-5 (**1**, 67.1; **2**, 64.9; **7**, 24.6) assigned by the COSY, HMQC, and HMBC spectra. The HMBC spectra also confirmed the location of the epoxide ring and the carbonyl group (Table 1). On the other hand, nuclear Overhauser effects (NOEs) observed in the difference NOE experiments of **1** and **2** (Figure 1) indicated that they were stereoisomeric as far as the epoxide ring was concerned. In the case of **1**, NOEs were observed from H_3-14 to H-2 and H-9, from H-5 to H-2 and H_3-13, from H_3-12 to H-6 and H-10, and from H_3-15 to H-10, while in the case of **2**, NOEs were observed from H_3-14 to H-9 and H-2, from H_3-12 to H-2 and H-5, from H_3-13 to H-5, and from H_3-15 to H-10. Thus, **1** and **2** should have the conformation shown in Figure 1. Their absolute configuration at C-5 was determined by the advanced Mosher's method.[12] In the ^{1}H NMR spectra of the MTPA esters, H-2 and H-4 of (*R*)-MTPA esters **1a** and **2a** appeared at higher field than those of (*S*)-MTPA esters **1b** and **2b**, whereas H-6 and H_3-13 of **1a** and **2a** were at lower field than those of **1b** and **2b** (Figure 2). Thus, the configuration at C-5 in **1** and **2** should be *R*. From these data, compounds **1** and **2** were determined as (2*R*,3*S*,5*R*)- and (2*R*,3*R*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-ones, respectively.

Compound **3** was isolated as a pale yellow oil, having $[\alpha]^{25}_{D}$ −3.5° (*c* 0.3, $CHCl_3$) and a molecular formula of $C_{15}H_{22}O_2$. Its IR spectrum showed absorption bands corresponding to hydroxyl (3330 cm^{-1}) and carbonyl (1709 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** (Table 1)

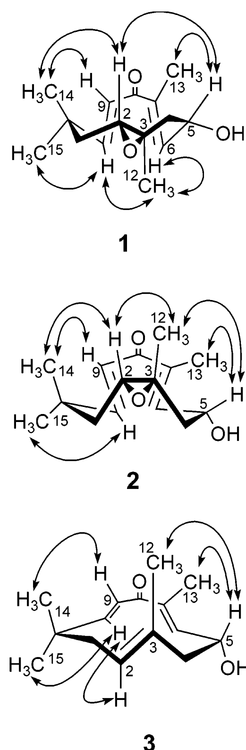


Figure 1. NOEs observed in the NOE difference experiments of **1**, **2**, and **3**.

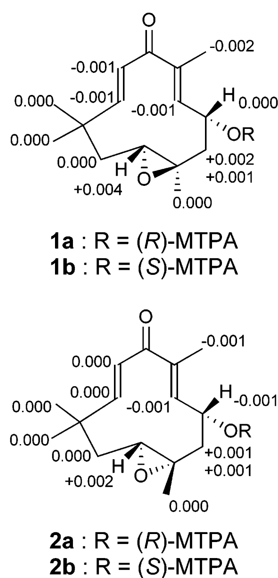


Figure 2. $\Delta\delta = (\delta_S - \delta_R)$ values obtained from the MTPA esters of **1** and **2** in $CDCl_3$ at 27 °C.

displayed signals of two trisubstituted olefins, a *trans*-olefin (J = 16 Hz), an oxygen-substituted methine, and four methyls. The ^{13}C NMR spectrum of **3** showed 15 carbon signals including a carbonyl carbon (δ 204.2) (Table 1). These data were similar to those of zerumbone (**6**), except for the presence of a hydroxyl group. The location of the hydroxyl group was determined to be at C-5, on the basis of the low-field shift of H-5 (**3**, δ 4.64; **6**, δ 2.18−2.47) and C-5 (**3**, 64.9; **6**, 24.4), which were assigned by the COSY, HMQC, and HMBC spectra. The conformation of **3** was determined by the NOE experiments (Figure 1). The absolute configuration at C-5 could not be determined because of the meager quantity obtained, but it was assumed to be *R*, the same as **1** and **2**, because they were isolated from the same extract. Thus, compound **3** was assigned as (5*R*)-2,6,9-humulatrien-5-ol-8-one.

Compound **4** was obtained as a pale yellow amorphous solid of $[\alpha]^{25}_D$ −60.6° (*c* 0.5, MeOH). Its molecular formula was deduced as $C_{25}H_{24}O_{12}$ by HRFABMS. The IR spectrum suggested the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1740 cm^{-1}) groups. The ^1H NMR spectrum of **4** displayed signals for *ortho*-coupled aromatic protons (J = 8.8 Hz), *meta*-coupled aromatic protons (J = 2.2 Hz), a methyl group, and oxygen-substituted methines together with two acetyl methyls (Table 2), while the ^{13}C NMR spectrum showed 25 carbon signals including three carbonyl carbons at δ 179.2, 172.2, and 171.4 (Table 2). These data were similar to those of kaempferol-3-*O*-α-L-rhamnopyranoside (**13**), except for the presence of two acetyl groups. The locations of the acetyl groups were determined to be at C-2 and C-3 of the rhamnose moiety, on the basis of the low-field shifts of H-2″ and H-3″ (**4**, δ 5.51, 4.98; **13**, δ 4.22, 3.69), which were assigned by the COSY, HMQC, and HMBC spectra. Thus, compound **4** was determined to be kaempferol-3-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside).

Compound **5** was isolated as a pale yellow amorphous solid, having $[\alpha]^{25}_D$ −9.8° (*c* 0.15, MeOH) and a molecular formula of $C_{27}H_{26}O_{13}$. Its IR spectrum showed absorptions of hydroxyl (3350 cm^{-1}) and carbonyl (1700 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **5** were similar to those of **4**, except for the presence of one more acetyl group in **5** (Table 2). The locations of the acetyl groups were determined to be at C-2, C-3, and C-4 of the rhamnose moiety by the low-field shifts of H-2″, H-3″, and H-4″ compared with those of **13**, which was confirmed by the analyses of the COSY, HMQC, and HMBC spectra. Thus, compound **5** was determined as kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside).

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). Kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (**5**) showed potent inhibitory activity against CYP3A4 with an IC_{50} value of 14.4 μM, similar to that of dillapiol (IC_{50}, 16.7 μM), a known plant-derived inhibitor with the most potent inhibition.[13] Zerumbone (**6**), kaempferol-3-*O*-(3,4-di-*O*-acetyl-α-L-rhamnopyranoside) (**9**), and kaempferol-3,4′-di-*O*-methyl ether (**15**) showed moderate inhibitory activity on the metabolism mediated by CYP3A4 with IC_{50} values less than 30 μM. Among the kaempferol derivatives, the presence of an acetyl group on the rhamnose moiety may enhance the inhibitory activity; **5** with three acetyl groups was more potent than **4**, **8**, and **9** with two acetyl groups, and the latter three compounds were more potent than monoacetylated derivatives **10**−**12**, which were more potent than **13** without an acetyl group. Among the sesquiterpene derivatives, zerumbone (**6**) was the most potent inhibitor of CYP3A4, and the presence of a hydroxyl group at C-5 decreased the activity. On CYP2D6 inhibition, kaempferol-3-*O*-methyl ether (**14**) was the most potent inhibitor, with an IC_{50} value of 4.6 μM, while other compounds possessed only weak or no inhibitory activities. These results suggest the possibility of potential drug−flavonol or drug−sesquiterpene interactions in individuals who consume *Z. aromaticum* in conjunction with drugs being metabolized by CYP3A4 or CYP2D6.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectropho-

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **4** (in CD_3OD) and **5** (in $CDCl_3$)[a]

position	**4** δ_H	δ_C	HMBC[b]	**5** δ_H	δ_C	HMBC[b]
2		159.0	2′, 6′		157.0	2′, 6′
3		135.3	1″		134.9	1″
4		179.2			177.9	
4a		105.9	6, 8		105.2	6, 8
5		158.5	6		158.1	6
6	6.28 d (2.2)	94.8	8	6.37 br s	94.0	8
7		165.9	6, 8		162.5	6, 8
8	6.10 d (2.2)	99.9	6	6.26 br s	98.2	6
8a		163.2	8		162.3	8
1′		122.3	2′, 3′, 5′, 6′		122.1	2′, 3′, 5′, 6′
2′,6′	7.71 d (8.8)	131.8	3′, 5′	7.80 d (8.4)	130.7	3′, 5′
3′,5′	6.85 d (8.8)	116.7	2′, 6′	7.00 d (8.4)	115.8	2′, 6′
4′		161.7	2′, 3′, 5′, 6′		161.3	2′, 3′, 5′, 6′
1″	5.40 d (1.7)	100.0	2″, 3″, 5″	5.56 d (1.7)	99.3	2″, 3″, 5″
2″	5.51 dd (3.0, 1.7)	72.1	1″, 3″, 4″	5.58 dd (3.0, 1.7)	69.3	1″, 3″, 4″
3″	4.98 dd (10.0, 3.0)	72.9	1″, 2″, 4″, 5″	5.31 dd (10.0, 3.0)	70.5	1″, 2″, 4″, 5″
4″	4.33 dd (10.0, 9.0)	70.5	2″, 3″, 5″, 6″	4.95 dd (10.0, 9.0)	69.0	2″, 3″, 5″, 6″
5″	3.21 m	70.8	1″, 3″, 4″, 6″	3.50 m	68.4	1″, 3″, 4″, 6″
6″	0.88 d (6.0)	17.7	4″, 5″	0.93 d (6.0)	17.1	4″, 5″
2″-*CO*CH$_3$		171.4	2″, 2″-COCH$_3$		171.4	2″, 2″-COCH$_3$
2″-CO*CH$_3$*	1.99 s	20.9		2.12 s	20.9	
3″-*CO*CH$_3$		172.2	3″, 3″-COCH$_3$		170.0	3″, 3″-COCH$_3$
3″-CO*CH$_3$*	1.93 s	20.6		2.01 s	20.8	
4″-*CO*CH$_3$					169.9	4″, 4″-COCH$_3$
4″-CO*CH$_3$*				2.00 s	20.7	

[a] The ^{1}H and ^{13}C NMR 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.

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

compound	CYP3A4	CYP2D6
1	62.5	>100
2	42.6	>100
3	35.5	>100
4	55.8	>100
5	14.4	43.3
6	21.8	>100
7	48.4	>100
8	31.6	>100
9	20.6	50.5
10	59.0	>100
11	98.3	>100
12	90.0	>100
13	>100	>100
14	36.2	4.63
15	21.8	45.5
16	36.4	>100
17	>100	>100
18	77.7	>100
ketoconazole	0.245	
quinidine		0.078

[a] IC_{50} values in μM.

tometer in KBr disk or in $CHCl_3$ solution. HRFABMS measurements were performed on a JEOL JMS-700T spectrometer using glycerol as a matrix. The ^{1}H, ^{13}C, and 2D NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard. 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. Rhizomes of *Z. aromaticum* were obtained at GORO traditional market, Jakarta, Indonesia, in May 2002. A voucher sample (TMPW 22289) 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 air-dried rhizomes of *Z. aromaticum* were crushed into a powder form. The powder (3.0 kg) was extracted with H_2O (24 L, 2 h, 80 °C, ×2), and the insoluble portion was separated by filtration. The filtrate was evaporated under reduced pressure and then lyophilized to give a H_2O extract (669 g). The H_2O extract (300 g) was further fractionated into EtOAc-soluble (3.0 g) and MeOH-soluble fractions (6.8 g).

The EtOAc-soluble fraction (2.1 g) was subjected to silica gel column chromatography with a $CHCl_3$, $CHCl_3$−MeOH, and MeOH solvent system to afford nine fractions: fraction 1, $CHCl_3$ eluate, 48.2 mg; fraction 2, 1% MeOH−$CHCl_3$ eluate, 114 mg; fraction 3, 3% MeOH−$CHCl_3$ eluate, 261 mg, which was identified as **6**; fraction 4, 5% MeOH−$CHCl_3$ eluate, 1.03 g; fraction 5, 5% MeOH−$CHCl_3$ eluate, 175 mg; fraction 6, 10% MeOH−$CHCl_3$ eluate, 181 mg; fraction 7, 20% MeOH−$CHCl_3$ eluate, 155 mg; fraction 8, 30% MeOH−$CHCl_3$ eluate, 92.9 mg; fraction 9, MeOH eluate, 16.8 mg. Further silica gel column chromatography and preparative TLC of these fractions yielded the following compounds: fraction 2, **6** (5.3 mg); fraction 4, **1** (7.5 mg), **3** (9.2 mg), **7** (120 mg), **15** (3.6 mg), **16** (68.3 mg), **17** (7.4 mg), and **18** (1.4 mg); fraction 5, **2** (10.4 mg) and **5** (3.8 mg); fraction 6, **14** (3.2 mg); fraction 7, **4** (15.0 mg), **8** (6.8 mg), and **9** (7.1 mg); fraction 8, **10** (6.1 mg), **11** (4.5 mg), and **12** (4.2 mg); fraction 9, **13** (3.4 mg).

The MeOH-soluble fraction (5.0 g) was also subjected to silica gel column chromatography with a $CHCl_3$, $CHCl_3$−MeOH, and MeOH solvent system to afford 10 fractions: fraction 1, $CHCl_3$ eluate, 239 mg; fraction 2, 1% MeOH−$CHCl_3$ eluate, 457 mg; fraction 3, 5% MeOH−$CHCl_3$ eluate, 108 mg; fraction 4, 5% MeOH−$CHCl_3$ eluate, 345 mg; fraction 5, 10% MeOH−$CHCl_3$ eluate, 138 mg; fraction 6, 10% MeOH−$CHCl_3$ eluate, 431 mg; fraction 7, 20% MeOH−$CHCl_3$ eluate, 292 mg; fraction 8, 30% MeOH−$CHCl_3$ eluate, 178 mg; fraction 9, MeOH eluate, 509 mg; fraction 10, MeOH eluate, 1.4 g. Further silica gel column chromatography and preparative TLC of these fractions yielded the following compounds: fraction 1, **6** (68.3 mg); fraction 2, **7** (28.6 mg); fraction 3, **1** (7.4 mg); fraction 4, **2** (7.7 mg); fraction 5, **14** (3.0 mg); fraction 6, **4** (2.8 mg), **8** (2.6 mg), **9** (1.5 mg), **10** (7.0 mg), **11** (8.6 mg), and **12** (8.7 mg); fraction 7, **13** (16.4 mg).

(2*R*,3*S*,5*R*)-2,3-Epoxy-6,9-humuladien-5-ol-8-one (1): pale yellow oil; $[\alpha]^{25}_D$ +5.3° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3300, 1700, 1650, 1370, 1350, 980 cm^{-1}; HRFABMS *m/z* 251.1641 (calcd for $C_{15}H_{23}O_3$ $[M + H]^+$, 251.1647); ^{1}H and ^{13}C NMR, see Table 1.

(2*R*,3*R*,5*R*)-2,3-Epoxy-6,9-humuladien-5-ol-8-one (2): pale yellow oil; $[\alpha]^{25}_{D}$ −10.9° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3300, 1700, 1630, 1380, 1350, 980 cm^{-1}; HRFABMS *m/z* 251.1643 (calcd for $C_{15}H_{23}O_3$ $[M + H]^+$, 251.1647); ^1H and ^{13}C NMR, see Table 1.

(5*R*)-2,6,9-Humulatrien-5-ol-8-one (3): pale yellow oil; $[\alpha]^{25}_{D}$ −3.5° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3330, 1709, 1660, 1380, 1360, 980 cm^{-1}; HRFABMS *m/z* 235.1692 (calcd for $C_{15}H_{23}O_2$ $[M + H]^+$, 235.1698); ^1H and ^{13}C NMR, see Table 1.

Kaempferol-3-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside) (4): pale yellow amorphous solid; $[\alpha]^{25}_{D}$ −60.6° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3400, 1740, 1650, 1610, 1450, 1360 cm^{-1}; HRFABMS *m/z* 517.1379 (calcd for $C_{25}H_{25}O_{12}$ $[M + H]^+$, 517.1346); ^1H and ^{13}C NMR, see Table 2.

Kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (5): pale yellow amorphous solid; $[\alpha]^{25}_{D}$ −9.8° (*c* 0.15, MeOH); IR (KBr) ν_{max} 3350, 1700, 1630, 1600, 1450, 1350 cm^{-1}; HRFABMS *m/z* 559.1481 (calcd for $C_{27}H_{27}O_{13}$ $[M + H]^+$, 559.1452); ^1H and ^{13}C NMR, see Table 2.

MTPA Esterification of 1 and 2. To a solution of **1** (2 mg) in pyridine (0.5 mL) was added (*S*)-MTPA chloride (10 μL), and the mixture was stirred overnight at room temperature. The reaction mixture was directly subjected to preparative TLC with $CHCl_3$−Et_2O−MeOH (14:6:1) to give (*R*)-MTPA ester **1a** (1.0 mg). By the same procedure, (*S*)-MTPA ester **1b** (1.3 mg) and (*R*)- and (*S*)-MTPA esters **2a** (1.3 mg) and **2b** (1.1 mg) were prepared.

(*R*)-MTPA ester 1a: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.862 (1H, m, H-5), 5.580 (1H, d, J = 10.0 Hz, H-6), 2.040 (3H, s, H_3-13), 6.288 (1H, d, J = 16.0 Hz, H-9), 6.400 (1H, d, J = 16.0 Hz, H-10), 2.350 (1H, dd, J = 13.0, 10.0 Hz, H-4), 1.678 (1H, dd, J = 13.0, 5.0 Hz, H-4), 1.377 (3H, s, H_3-12), 2.797 (1H, d, J = 13.0 Hz, H-2), 1.973 (1H, d, J = 13.0 Hz, H-1), 1.447 (1H, t, J = 13.0 Hz, H-1), 1.355 (3H, s, H_3-14), 1.144 (3H, s, H_3-15); FABMS *m/z* 467 $[M + H]^+$.

(*S*)-MTPA ester 1b: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.862 (1H, m, H-5), 5.579 (1H, d, J = 10.0 Hz, H-6), 2.038 (3H, s, H_3-13), 6.287 (1H, d, J = 16.0 Hz, H-9), 6.399 (1H, d, J = 16.0 Hz, H-10), 2.352 (1H, dd, J = 13.0, 10.0 Hz, H-4), 1.679 (1H, dd, J = 13.0, 5.0 Hz, H-4), 1.377 (3H, s, H_3-12), 2.801 (1H, d, J = 13.0 Hz, H-2), 1.973 (1H, d, J = 13.0 Hz, H-1), 1.447 (1H, t, J = 13.0 Hz, H-1), 1.355 (3H, s, H_3-14), 1.144 (3H, s, H_3-15); FABMS *m/z* 467 $[M + H]^+$.

(*R*)-MTPA ester 2a: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.863 (1H, m, H-5), 5.823 (1H, d, J = 10.0 Hz, H-6), 2.063 (3H, s, H_3-13), 6.146 (1H, d, J = 16.0 Hz, H-9), 6.172 (1H, d, J = 16.0 Hz, H-10), 2.700 (1H, dd, J = 13.0, 5.0 Hz, H-4), 1.255 (1H, d, J = 13.0 Hz, H-4), 1.286 (3H, s, H_3-12), 2.760 (1H, d, J = 13.0 Hz, H-2), 1.970 (1H, d, J = 13.0 Hz, H-1), 1.446 (1H, t, J = 13.0 Hz, H-1), 1.299 (3H, s, H_3-14), 1.091 (3H, s, H_3-15); FABMS *m/z* 467 $[M + H]^+$.

(*S*)-MTPA ester 2b: colorless amorphous solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.862 (1H, m, H-5), 5.822 (1H, d, J = 10.0 Hz, H-6), 2.062 (3H, s, H_3-13), 6.146 (1H, d, J = 16.0 Hz, H-9), 6.172 (1H, d, J = 16.0 Hz, H-10), 2.701 (1H, dd, J = 13.0, 5.0 Hz, H-4), 1.256 (1H, d, J = 13.0 Hz, H-4), 1.286 (3H, s, H_3-12), 2.762 (1H, d, J = 13.0 Hz, H-2), 1.970 (1H, d, J = 13.0 Hz, H-1), 1.446 (1H, t, J = 13.0 Hz, H-1), 1.299 (3H, s, H_3-14), 1.091 (3H, s, H_3-15); FABMS *m/z* 467 $[M + H]^+$.

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.[14,15] 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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