Constituents of *Zingiber aromaticum* and Their CYP3A4 and CYP2D6 Inhibitory Activity

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A new sesquiterpene 2,9-humuladien-6-ol-8-one (1) was isolated from methanol extract of *Zingiber aromaticum*, along with 15 known compounds. The structures of the isolated compounds were elucidated on the basis of spectroscopic analyses. The isolated compounds were tested for their inhibitory activity on the metabolism mediated by cytochrome P450 3A4 (CYP3A4) and CYP2D6.

Key words *Zingiber aromaticum*; Indonesian medicinal plant; cytochrome P450 3A4 (CYP3A4); cytochrome P450 2D6 (CYP2D6); human liver microsome; drug–herb interaction

Human microsomal cytochrome P450s (CYPs) participate in drug metabolism and detoxification with respect to their properties and catalytic activities toward a number of endogenous and xenobiotic chemicals. About 70% of CYP enzymes in the human liver belong to the families which participate in drug metabolism. Among them, CYP3A4 represents about 30% of the total CYP enzymes.[1)] Recent investigation has shown that more than 50% of clinically used drugs are oxidized by CYP3A4 and about 30% metabolized by CYP2D6.[2,3)] Inhibition of drug-metabolizing enzyme, thus, results in elevated level of parent drug, prolonged pharmacological effects, and an increased incidence of drug-induced toxicity.[4)] Herbs are often administered in combination with therapeutic drugs, raising the potential of drug–herb interactions through inhibition of CYP.[5)] In our investigation of Indonesian medicinal plants for their inhibitory activity on the metabolism mediated by CYP3A4 and CYP2D6, we observed that *Zingiber aromaticum* possessed potent inhibitory activity against CYP3A4-mediated metabolism.[6)]

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.[7)] From the water extract of the rhizomes which is used for traditional treatment, we isolated 18 compounds including five new ones and reported their CYP3A4 and CYP2D6 inhibitory activities.[8)] Because there is only little reports on the constituents of this plant,[8,9)] as a continuation, we have carried out the phytochemical investigation of its MeOH extract and isolated a new sesquiterpene **1** together with 15 known compounds. In this paper, we report the isolation and structure elucidation of the new sesquiterpene and inhibitory activity of the isolated compounds on the metabolism mediated by CYP3A4 and CYP2D6.

Results and Discussion

The air-dried rhizomes of *Z. aromaticum* were extracted with H_2O, H_2O–MeOH (1 : 1), and MeOH. The MeOH extract, having the strongest inhibitory activity on CYP3A4 (90.6%) and CYP2D6 (98.4%) at a concentration of 1.6 mg/ml, was subjected to silica gel column chromatography with a $CHCl_3$–MeOH solvent system to yield 11 fractions. Each fraction was separated by repeated column chromatography and preparative TLC to give 16 compounds. Their structures were elucidated on the basis of spectroscopic analyses, to be a new sesquiterpene [2,9-humuladien-6-ol-8-one (**1**)], three known humulane-type sesquiterpenes [zerumbone,[8,10)] zerumbone epoxide,[10)] tricyclohumuladiol[11)]], three gingerols [(*S*)-6-gingerol,[12,13)] (*S*)-8-gingerol,[13)] (*S*)-10-gingerol[13)]], two shogaols [*trans*-6-shogaol,[14)] *trans*-10-shogaol[15)]], two flavonoids [kaempferol-3-*O*-methyl ether,[16)] kaempferol-3,4′-di-*O*-methyl ether[16)]], two acetylated flavonol glycosides [kaempferol-3-*O*-(3-*O*-acetyl-α-L-rhamnopyranoside),[17)] kaempferol-3-*O*-(4-*O*-acetyl-α-L-rhamnopyranoside)[17)]], kaempferol-3-*O*-α-L-rhamnopyranoside,[17)] β-sitosterol,[18)] and β-sitosterol glucoside.[19)] Among the known compounds, trycyclohumuladiol (**2**), (*S*)-8-gingerol (**3**), (*S*)-10-gingerol (**4**), *trans*-10-shogaol (**5**), and β-sitosterol glucoside (**6**) were found for the first time in this plant.

Compound **1** was isolated as a colorless amorphous solid, having $[\alpha]_D^{24}$ −11.8° (c=0.10, $CHCl_3$) and a molecular formula of $C_{15}H_{24}O_2$. Its IR spectrum showed absorption bands corresponding to hydroxyl (3700 cm^{-1}) and carbonyl (1700 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** displayed signals of a *trans*-olefin (J=16 Hz), a trisubstituted double bond, an oxygen-substituted methine, a secondary methyl,

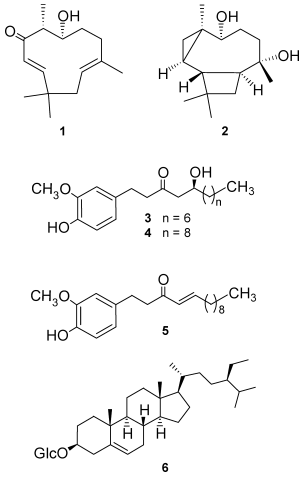

Fig. 1

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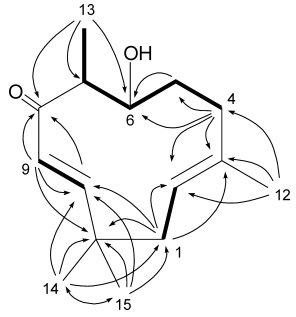

Fig. 2. COSY (Bold Lines) and HMBC (Arrows) Correlations of **1**

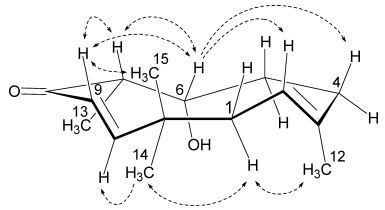

Fig. 3. NOEs Detected in the Difference NOE Experiment of **1**

and three tertiary methyls. The ^{13}C-NMR spectrum of **1** showed 15 carbon signals including a carbonyl carbon (δ 201.0). These data were similar to those of zerumbone, but they were characterized by the presence of a CH_3–CH–CH–OH system instead of the CH_3–C=CH system in zerumbone. The location of the CH_3–CH–CH–OH system was determined to be C(13)H_3–C(7)H–C(6)H–OH from the COSY, HMQC, and HMBC spectra (Fig. 2). The HMBC spectra also confirmed the location of the carbonyl and methyl groups. The relative stereochemistry of **1** was determined on the basis of the results of difference NOE experiments (Fig. 3). In the difference NOE experiment, NOE enhancements were observed from H-6 to H-2, H-7, and H-9, from H_3-15 to H-9, and from H_3-14 to H-10 and H-1. Thus, compound **1** was assigned as 2,9-humuladien-6-ol-8-one. Due to the meager quantity of **1**, the absolute configuration at C-6 and C-7 could not be determined.

The isolated compounds were tested for their inhibitory activity on the metabolism mediated by CYP3A4 or CYP2D6 according to the previously reported method (Table 1).[8] The CYP inhibitory activity of the MeOH extract may be due to zerumbone, a major constituent (20%) in the extract with an IC_{50} value of 21.8 μM.[8] Among gingerol derivatives, (*S*)-10-gingerol (**4**) having a longer side chain showed stronger inhibitory activity against CYP3A4 than (*S*)-8-gingerol (**3**) and (*S*)-6-gingerol, suggesting that the length of the side chain may be important for the inhibitory activity on CYP3A4.

Experimental

General Experimental Procedures High resolution electron impact mass spectrometry (HR-EI-MS) measurements were carried out on a JEOL JMS-700T spectrometer at an ionization voltage of 40 eV. The other instruments and plant material were the same as those described in the previous paper.[8]

Extraction and Isolation The air-dried rhizomes of *Z. aromaticum* were crushed into powder form. The powder (3.0 kg) was extracted with H_2O (24 l, 2 h, 80 °C, ×2), followed by MeOH–H_2O (1 : 1) and MeOH, to give H_2O extract (669 g), MeOH–H_2O extract (89 g), and MeOH extract (27.5 g).

The MeOH extract (10 g) was subjected to silica gel column chromatography with a $CHCl_3$–MeOH solvent system to afford 11 fractions: fr. 1, $CHCl_3$ eluate, 201 mg; fr. 2, $CHCl_3$ eluate, 1.7 g; fr. 3, $CHCl_3$ eluate, 853 mg; fr. 4, $CHCl_3$ eluate, 317 mg; fr. 5, 1% MeOH–$CHCl_3$ eluate, 1.2 g; fr. 6, 5% MeOH–$CHCl_3$ eluate, 2.1 g; fr. 7, 10% MeOH–$CHCl_3$ eluate, 316 mg; fr. 8, 15% MeOH–$CHCl_3$ eluate, 223 mg; fr. 9, 20% MeOH–$CHCl_3$ eluate, 316 mg; fr. 10, 30% MeOH–$CHCl_3$ eluate, 227 mg; fr. 11, MeOH eluate, 988 mg. Fraction 2 gave zerumbone (1.7 g). Fraction 4 was further separated by reversed-phase preparative TLC (CH_3CN : MeOH : H_2O = 1 : 1 : 1) to give *trans*-6-shogaol (8.3 mg) and *trans*-10-shogaol (**5**, 1.3 mg), while fraction 5 gave **1** (2.2 mg), zerumbone epoxide (22 mg), (*S*)-6-gingerol (7.6 mg), (*S*)-8-gingerol (**3**, 3.4 mg), (*S*)-10-gingerol (**4**, 6.7 mg), and β-sitosterol (6.8 mg) with the same method. Fraction 6 was separated by reversed-phase preparative TLC (MeOH : H_2O = 7 : 3) to yield tricyclohumuladiol (**2**, 4.5 mg), kaempferol-3-*O*-methyl ether (15 mg), and kaempferol-3,4′-di-*O*-methyl ether (5.0 mg). Fractions 7 and 8 were subjected to reversed-phase preparative TLC (CH_3CN : MeOH : H_2O = 1 : 1 : 1) to yield β-sitosterol glucoside (**6**, 12 mg) and kaempferol-3-*O*-(4-*O*-acetyl-α-L-rhamnopyranoside) (12 mg), respectively. Fraction 9 was separated by normal-phase preparative TLC ($CHCl_3$: MeOH = 8 : 2) to yield kaempferol-3-*O*-(3-*O*-acetyl-α-L-rhamnopyranoside) (1.5 mg) and kaempferol-3-*O*-α-L-rhamnopyranoside (11 mg).

2,9-Humuladien-6-ol-8-one (**1**): Colorless amorphous solid; $[\alpha]_D^{24}$ −11.8° (c=0.10, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3700, 1700, 1460, 1070. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.04 (3H, d, J=6.8 Hz, H_3-13), 1.14 (3H, s, H_3-15), 1.16 (1H, m, H-5α), 1.17 (3H, s, H_3-14), 1.31 (1H, m, H-5β), 1.38 (3H, s, H_3-12), 1.88 (1H, dd, J=4.6, 12.6 Hz, H-1β), 1.96 (1H, m, H-4β), 2.10 (1H, q, J=6.3 Hz, H-4α), 2.22 (1H, t, J=12.6 Hz, H-1α), 2.71 (1H, qd, J=6.8, 3.4 Hz, H-7), 4.24 (1H, m, H-6), 5.11 (1H, dd, J=12.6, 4.6 Hz, H-2), 6.11 (1H, d, J=16.1 Hz, H-9), 6.17 (1H, d, J=16.1 Hz, H-10). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 6.1 (C-13), 16.1 (C-12), 23.0 (C-14), 28.9 (C-15), 30.7 (C-5), 37.7 (C-4), 39.9 (C-11), 41.3 (C-1), 54.3 (C-7), 73.1 (C-6), 122.2 (C-2), 128.1 (C-9), 137.8 (C-3), 152.0 (C-10), 201.0 (C-8). HR-EI-MS *m/z*: 236.1771 $[M]^+$ (Calcd for $C_{15}H_{24}O_2$: 236.1776).

CYP Inhibitory Assay Procedure was the same as that described in the previous paper.[8]

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Table 1. IC_{50} Values of the Isolated Compounds on the Metabolism Mediated by CYP3A4 and CYP2D6

Compound	CYP3A4	CYP2D6
1	27.2	>100
2	>100	>100
3	81.6	68.7
4	41.3	>100
5	90.2	45.7
6	>100	>100
Ketoconazole	0.24	
Quinidine		0.068

IC_{50} values in μM. IC_{50} values of other isolated compounds were reported in the previous paper.[8]

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