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Flavonoids and Aromatic Compounds from the Rhizomes of *Zingiber zerumbet*

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Repeated column chromatography of the $CHCl_3$-soluble fraction of *Zingiber zerumbet* led to the isolation and identification of two aromatic compounds, *p*-hydroxybenzaldehyde (**1**) and vanillin (**2**), and six kaempferol derivatives, kaempferol-3,4',7-*O*-trimethylether (**3**), kaempferol-3-*O*-methylether (**4**), kaempferol-3,4'-*O*-dimethylether (**5**), 4"-*O*-acetylafzelin (**6**), kaempferol-3-*O*-(4-*O*-acetyl-α-L-rhamnopyranoside)], 2",4"-*O*-diacetylafzelin (**7**), kaempferol-3-*O*-(2,4-*O*-diacetyl-α-L-rhamnopyranoside)], and 3",4"-*O*-diacetylafzelin (**8**), kaempferol-3-*O*-(3,4-*O*-diacetyl-α-L-rhamnopyranoside)]. The structures of **1-8** were identified by analysis of spectroscopic data as well as by comparison with published values. This is the first report on the isolation of compounds **1-3** from this plant.

Key words: *Zingiber zerumbet*, Zingiberaceae, *p*-Hydroxybenzaldehyde, Vanillin, kaempferol-3,4',7-*O*-trimethylether

INTRODUCTION

Zingiber zerumbet Smith (Zingiberaceae), a wild ginger, grows in wide ranges around Southeast Asia. The rhizomes of the plant are employed as a traditional medicine for anti-inflammation and the like in some restrict (Farnsworth and Bunyapraphatsara, 1992). Zerumbone, a predominant sesquiterpene from the *Z. zerumbet*, has been studied intensively as a food phytochemical that has distinct potentials for use in anti-inflammation, chemoprevention, and chemotherapy strategies (Dai *et al.*, 1997; Kitayama *et al.*, 2001; Murakami *et al.*, 1999; Murakami *et al.*, 2002; Tanaka *et al.*, 2001). Previous phytochemical investigations on this plant have resulted in the isolation of several sesquiterpenoids and flavonoids (Dai *et al.*, 1997; Matthes *et al.*, 1980; Masuda *et al.*, 1991).

In our ongoing project directed toward the discovery of novel naturally occurring bioactive compounds from Indonesian medicinal plants (Han *et al.*, 2003; Park *et al.*, 2002), the rhizomes of *Z. zerumbet* were chosen for more detailed investigation on its chemical constituents. Repeated column chromatography of the $CHCl_3$-soluble fraction of *Z. zerumbet* led to the isolation and identification of two aromatic compounds (**1** and **2**) and six kaempferol derivatives (**3-8**).

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MATERIALS AND METHODS

Plant material

The rhizomes of *Zingiber zerumbet* Smith were collected in Surabaya, Indonesia, in June 2001 and were identified by Professor Tri Windono (University of Surabaya, Indonesia). A voucher specimen (no. 21/DT/VI/2001) has been deposited at University of Surabaya, JL. Raya Kalirungkut, Surabaya 60293, Indonesia.

General experimental procedures

Melting points were measured on a J-923 (Jisico, Korea) and are uncorrected. LREIMS were recorded on an Autospec M393 mass spectrometer (Micromass, U.K.) operated at 70 eV. NMR experiments were conducted on a Unity INOVA 400 MHz FT-NMR (Varian, CA), and TMS was used as an internal standard.

TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) followed by charring at 110°C for 5-10 min. Silica gel (Merck 60A, 200-400 mesh ASTM) and Sephadex LH-20 (Amersham Pharmacia Biotech)

were used for column chromatography. All solvents used for chromatographic separations were distilled before use.

Extraction and isolation

The dried and milled plant material (500 g) was extracted with MeOH (3×3 L) for 7 days at 25°C. The extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract (69.6 g) was suspended in distilled water (0.5 L) and then partitioned with *n*-hexane (3×0.5 L) to afford an *n*-hexane-soluble syrup on drying (16.6 g). Next, the aqueous extract was partitioned again with $CHCl_3$ (3×0.5 L) to give a $CHCl_3$-soluble extract and an aqueous residue. The $CHCl_3$-soluble extract (12.0 g) was chromatographed over silica gel as stationary phase using a CH_2Cl_2-MeOH gradient (from 1:0 to 0:1 v/v) as mobile phase to afford 11 pooled fractions (F01-F011). Compounds **4** (80 mg) and **5** (62 mg) were obtained as yellowish needles by recrystallization in MeOH from fractions F06 (0.55 g) and F05 (2.3 g), respectively. Fraction F04 [eluted with CH_2Cl_2-MeOH (99:1 v/v); 0.60 g] was chromatographed over silica gel as stationary phase using *n*-hexane-EtOAc gradient (from 15:1 to 1:1 v/v) as mobile phase to afford eight subfractions (F0401-F0408). Compound **3** (26.5 mg) was purified from fraction F0404 by recrystallization (in acetone). Fraction F0405 was subjected to a further Sephadex LH-20 column chromatography, with 100% MeOH as mobile phase followed by prep. TLC (CH_2Cl_2-EtOAc, 9:1 as developing solvent), to give compounds **1** (8.5 mg, R_f = 0.50) and **2** (12 mg, R_f = 0.62).

Fractions, F07 and F08 [eluted with CH_2Cl_2-MeOH (49:1 v/v), 2.3 and 0.15 g, respectively], were combined and then chromatographed on silica gel with gradient mixtures of *n*-hexane-EtOAc (from 9:1 to 1:1, final 100% MeOH), yielding, in turn, compounds **7** (591 mg) and **8** (373 mg). Compound **6** (168 mg) was isolated from fraction F09 (0.43 g) by chromatography over Sephadex LH-20 using mixture of $CHCl_3$-MeOH (1:3 v/v) as the solvent system.

p-Hydroxybenzaldehyde (1)

Pale yellow crystals, mp: 113-114°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.86 (1H, s, CHO), 7.82 (2H, d, *J* = 8.6 Hz, H-2 and H-6), 6.98 (2H, d, *J* = 8.6 Hz, H-3 and H-5); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 191.2 (CHO), 161.6 (C-4), 132.5 (C-2 and C-6), 129.9 (C-1), 116.0 (C-3 and C-5); LREIMS *m/z* (rel. int.): 122 ([M]$^+$, 85), 121 (100), 93 (38), 65 (38).

Vanillin (2)

White crystals, mp: 79-81°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.83 (1H, s, CHO), 7.43 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 7.42 (1H, d, *J* = 1.8 Hz, H-2), 7.05 (1H, d, *J* = 8.2 Hz, H-5), 3.97 (1H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 191.2 (CHO), 152.0 (C-3), 147.4 (C-4), 130.1 (C-1), 127.8 (C-6), 114.6 (C-5), 109.0 (C-2); LREIMS *m/z* (rel. int.): 152 ([M]$^+$, 63), 151 (37), 121 (100), 93 (22), 65 (23).

kaempferol-3,4',7-*O*-trimethylether (3)

Yellowish crystals, mp: 139-140°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 12.7 (1H, s, 5-OH), 8.07 (2H, d, *J* = 9.0 Hz, H-2' and H-6'), 7.01 (2H, d, *J* = 9.0 Hz, H-3' and H-5'), 6.43 (1H, d, *J* = 2.0 Hz, H-8), 6.34 (1H, d, *J* = 2.0 Hz, H-6), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.86 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 179.0 (C-4), 165.6 (C-7), 162.2 (C-4'), 161.9 (C-5), 156.9 (C-8a), 156.1 (C-2), 139.0 (C-3), 130.4 (C-2' and C-6'), 123.0 (C-1'), 114.3 (C-3' and C-5'), 106.2 (C-4a), 98.0 (C-6), 92.3 (C-8), 60.3 (3-OCH_3), 56.0, 55.9 (4',7-OCH_3); LREIMS *m/z* (rel. int.): 328 ([M]$^+$, 75), 327 (70), 285 (48), 135 (32).

RESULTS AND DISCUSSION

Repeated column chromatography of the $CHCl_3$-soluble fraction of *Zingiber zerumbet* led to the isolation of *p*-hydroxybenzaldehyde (**1**) (Kwon and Kim, 2003), vanillin (**2**) (Sun *et al.*, 2001), kaempferol-3,4',7-*O*-trimethylether (**3**) (Rossi *et al.*, 1997), kaempferol-3-*O*-methylether (**4**) (Nakatani *et al.*, 1991), kaempferol-3,4'-*O*-dimethylether (**5**) (Nakatani *et al.*, 1991), 4"-*O*-acetylafzelin (**6**), kaempferol-3-*O*-(4-*O*-acetyl-α-L-rhamnopyranoside)] (Masuda *et al.*, 1991), 2",4"-*O*-diacetylafzelin (**7**), kaempferol-3-*O*-(2,4-*O*-diacetyl-α-L-rhamnopyranoside)] (Masuda *et al.*, 1991), and 3",4"-*O*-diacetylafzelin (**8**), kaempferol-3-*O*-(3,4-*O*-diacetyl-α-L-rhamnopyranoside)] (Masuda *et al.*, 1991). The structures of the compounds (**1-8**) were identified by physical and spectroscopic data (mp, $[\alpha]_D$, MS, ^{1}H- and ^{13}C-NMR) mea-

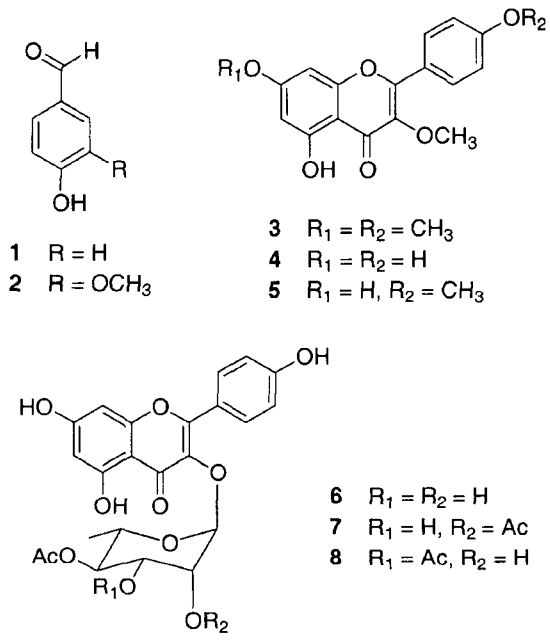

Fig. 1. Structures of compounds **1-8** isolated from *Z. zerumbet*

surement and by comparison with published values.

Compound **1** was obtained as pale yellow crystals and gave a protonated molecular ion at *m/z* 122 $[M]^+$ by LREIMS, suggesting its molecular formula as $C_7H_6O_2$. The ^{1}H-NMR spectrum of **1** showed resonances for a typical aldehyde signal at δ_H 9.86 (1H, s). Two *ortho*-coupled doublets centered at δ_H 6.98 (2H, J = 8.6 Hz) and δ_H 7.82 (2H, J = 8.6 Hz) were observed in the ^{1}H-NMR spectrum of **1**, indicative of the protons of a *para*-disubstituted benzene ring. The ^{13}C-NMR and DEPT experiments with **1** showed the presence of signals for two quaternary aromatic carbons, with one of them (δ_C 161.6) bearing oxygen atom, and three methine groups including an aldehyde carbon (δ_C 191.2). On the basis of the evidence mentioned above, the structure of compound **1** was identified as *p*-hydroxybenzaldehyde.

Compound **2** was obtained as white crystals. The LREIMS spectrum of compound **2** gave M^+ at *m/z* 152, suggesting its molecular formula as $C_8H_8O_3$. The ^{1}H-NMR spectrum of **2** also showed resonances for a typical aldehyde signal at δ_H 9.83 (1H, s). A set of ABX-type signals [δ_H 7.42 (1H, d, J = 1.8 Hz), 7.05 (1H, d, J = 8.2 Hz), and 7.43 (1H, dd, J = 8.2, 1.8 Hz)] and a methoxyl signal at δ_H 3.97 (3H, s) were observed in the ^{1}H-NMR spectrum of **2**, indicating that it has a 1,3,4-trisubstituted aromatic ring. The structure of compound **2** was identified as vanillin by analysis of the ^{1}H-NMR and ^{13}C-NMR spectra and comparison of its MS and ^{1}H-NMR data with those of literature values (Sun *et al.*, 2001).

Compound **3** was obtained as yellowish crystals and gave a protonated molecular ion at *m/z* 328 $[M]^+$ by LREIMS. The ^{1}H-NMR spectrum of **3** showed a downfield resonance at δ_H 12.7, attributed to a chelated hydroxyl proton, while two doublets in the aromatic region (at δ 8.07 and 7.01, each 2H, J = 9.0 Hz) suggested the presence of a *para*-substituted aromatic ring (B ring). The ^{1}H-NMR spectrum of **3** also showed two *meta*-coupled doublets centered at signals at δ_H 6.43 (1H, d, J = 2.0 Hz) and 6.34 (1H, d, J = 2.0 Hz) and three methoxyl groups at δ_H 3.89, 3.87, and 3.86. On the basis of careful analysis of the ^{1}H-NMR and ^{13}C-NMR data and comparison of these results with the values previously reported in the literature (Rossi *et al.*, 1997), compound **3** was identified as kaempferol-3,4',7-*O*-trimethylether. This is the first report on the isolation of compounds **1**-**3** from *Zingiber zerumbet*.

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