

Potentially Bioactive Two New Natural Sesquiterpenoids from the Rhizomes of *Zingiber zerumbet*

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Repeated chromatography of the *n*-hexane-soluble fraction of the MeOH extract of the rhizomes of *Zingiber zerumbet* led to the isolation of two isomers of 6-methoxy-2*E*,9*E*-humuladien-8-one (**1** and **2**) and stigmast-4-en-3-one. The structures of **1** and **2** were determined by spectroscopic methods including 1D and 2D-NMR elucidated by analysis of spectroscopic data as well as by comparison with published values. This is the first report on the isolation of compounds **1** and **2** from the nature. Stigmast-4-en-3-one was first isolated from this plant.

Key words: *Zingiber zerumbet*, Zingiberaceae, Sesquiterpenoids, 6-Methoxy-2*E*,9*E*-humuladien-8-one, Stigmast-4-en-3-one

INTRODUCTION

Zingiber zerumbet (L.) J.E. Smith (Zingiberaceae), a wild ginger, grows widely in Southeast Asia. The rhizomes of this plant are used as an anti-inflammatory in traditional medicine (Farnsworth and Bunyapraphatsara, 1992). In particular, a monocyclic sesquiterpene, zerumbone (2*E*, 6*E*,10*E*-humulatrien-1-one), which was found as a major component of the essential oil of *Z. zerumbet*, has been studied intensively for potential use in anti-inflammatory, chemopreventive, and chemotherapeutic strategies (Dai *et al.*, 1997; Kitayama *et al.*, 2001; Murakami *et al.*, 2002; Tanaka *et al.*, 2001). Previous phytochemical investigations on this plant resulted in the isolation of several sesquiterpenoids and flavonoids (Dai *et al.*, 1997; Masuda *et al.*, 1991; Matthes *et al.*, 1980). Recently, we reported on the isolation of aromatic compounds and kaempferol derivatives from the CHCl₃-soluble fraction of the MeOH extract of the rhizomes of *Z. zerumbet* (Jang *et al.*, 2004).

In our continuous study on the chemical constituents of this plant, we further isolated two new natural sesquiterpenoids (**1** and **2**) and a known steroid, stigmast-4-en-3-one from a *n*-hexane-soluble fraction of the MeOH extract of the rhizomes of *Z. zerumbet*. Here, we describe

the structural elucidations of compounds **1** and **2**.

MATERIALS AND METHODS

General procedure

The melting points were measured on a J-923 melting point apparatus (Jisico, Korea) and are uncorrected. Optical rotations were obtained using a DIP-1000 digital polarimeter (Jasco, Japan) at 25 °C. UV and IR spectra were recorded with a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. LR and HREIMS 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 F₂₅₄ (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H₂SO₄ 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.

Plant materials

The rhizomes of *Zingiber zerumbet* (L.) J.E. Smith were collected in Surabaya, Indonesia, in June 2001 and were identified by Professor Tri Windono (University of Surabaya,

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Indonesia). A voucher specimen (no. 21/DT/VI/2001) has been deposited at University of Surabaya, JL. Raya Kalirungkut, Surabaya 60293, Indonesia.

Extraction and isolation

The dried and milled plant material (5 kg) was extracted with 10 L of MeOH three times by maceration. The extracts were combined and concentrated *in vacuo* at 40 °C. The concentrated extract was suspended in H₂O (2 L) and then partitioned with 2 L of *n*-hexane three times to afford a *n*-hexane-soluble syrup on drying. The *n*-hexane-soluble extract (165 g) was chromatographed over silica gel (12 × 42 cm, 230-400 mesh) as stationary phase using a solvent system [cyclohexane (20 L), cyclohexane-CH₂Cl₂ (1:1 v/v, 10 L), CH₂Cl₂ (6 L), CH₂Cl₂-MeOH (19:1 v/v, 6 L), MeOH (6 L)] to afford 10 pooled fractions (F01-F010). Fraction F09 [eluted with CH₂Cl₂-MeOH (19:1 v/v); 32 g] was chromatographed over silica gel (7 × 30 cm, 230-400 mesh; *n*-hexane-EtOAc gradient from 19:1 to 1:2 v/v, final 100% MeOH) resulting in eighteen subfractions (F0901-F0918). Fractions F0903 and F0904 [eluted with *n*-hexane-EtOAc (19:1 v/v); 2.2 g] were combined, and then was further chromatographed over silica gel (3.2 × 25 cm, 230-400 mesh; *n*-hexane-EtOAc = 19:1 v/v) to give, in turn, compound **1** (193 mg, 0.00386%), compound **2** (242 mg, 0.00484%), and stigmast-4-en-3-one (15 mg, 0.0003%).

6-Methoxy-2E,9E-humuladien-8-one (1 and 2)

Colorless oil; [α]_D²⁰ 0.0° (c 0.20, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 203 (3.88), 228 (3.72) nm; IR ν_{\max} (KBr) 2938, 1695, 1627, 1451, 1368, 1091 cm⁻¹; EIMS *m/z* (rel. int.) 250 ([M]⁺, 15), 218 (7), 125 (33), 96 (100), 93 (94); HREIMS *m/z* 250.1928 ([M]⁺, calcd 250.1933 for C₁₆H₂₆O₂); ¹H-NMR and ¹³C-NMR, see Table I. **2** was obtained as a colorless oil; [α]_D²⁰ 0.0° (c 0.21, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 202 (3.86), 226 (3.71) nm; IR ν_{\max} (KBr) 2938, 1691, 1621, 1457, 1363, 1093 cm⁻¹; EIMS *m/z* (rel. int.) 250 ([M]⁺, 12), 218 (11), 125 (35), 96(100), 93 (99); HREIMS *m/z* 250.1931 ([M]⁺, calcd 250.1933 for C₁₆H₂₆O₂); ¹H-NMR and ¹³C-NMR, see Table I.

Stigmast-4-en-3-one

Colorless crystals: mp 95-96 °C [(lit. mp 94.0-96.5 °C) (Seca *et al.*, 2000)]; ¹H- and ¹³C-NMR data were in agreement with the literature values (Seca *et al.*, 2000).

RESULTS AND DISCUSSION

Repeated chromatography of the *n*-hexane-soluble fraction of the MeOH extract of the rhizomes of *Zingiber zerumbet* led to the isolation of two isomers of 6-methoxy-2E,9E-humuladien-8-one (**1** and **2**) and stigmast-4-en-3-one. These compounds **1** and **2** have not been previously

Table I. ¹H- and ¹³C-NMR Data for compounds **1** and **2** (in CDCl₃)^a

position	1		2	
	δ_{H} [mult., <i>J</i> (Hz)]	δ_{C}	δ_{H} [mult., <i>J</i> (Hz)]	δ_{C}
1	1.89 t (12.4) 2.22 t (12.4)	41.3t	1.90 dd (12.4, 4.2) 2.26 t (12.4)	41.4t
2	5.09 dd (11.6, 4.8)	121.9d	5.16 dd (12.4, 4.2)	123.0d
3		138.1s		136.9s
4	1.86 (overlap) 2.08 dd (12.8, 6.8)	38.5t	2.02 dt (12.4, 2.4) 2.23 (overlap)	40.0t
5	1.04 m 1.39 m	28.2t	1.55 m 1.70 m	31.9t
6	3.69 brdd (5.4, 3.2)	82.3d	3.36 dt (6.8, 3.4)	82.5d
7	3.08 dq (6.4, 3.2)	49.2d	2.56 q (6.8)	53.6d
8		201.1s		204.2s
9	6.08 d (16.0)	128.0d	6.94 d (16.4)	125.4d
10	6.18 d (16.0)	151.6d	6.18 d (16.4)	153.8d
11		40.0s		39.1s
12	1.36 s	16.2q	1.50 s	16.0q
13	0.97 d (6.4)	6.2q	1.15 d (6.8)	10.5q
14	1.15 s	22.9q	1.17 s	23.4q
15	1.18 s	29.0q	1.17 s	30.0q
6-OCH ₃	3.42 s	57.0q	3.30 s	57.2q

^a Run at 400 MHz for ¹H and 125 MHz for ¹³C. Assignments are based on DEPT, COSY, HSQC, and HMBC experiments.

isolated from a natural source although they have been reported as synthetic products (Kitayama *et al.*, 1999). The known steroid, stigmast-5-ene-3-one was identified by physical and spectroscopic data (mp, MS, ¹H- and ¹³C-NMR) measurement and by comparison with published values (Gupta *et al.*, 1979; Seca *et al.*, 2000). This compound was isolated for the first time from *Z. zerumbet*. Compounds **1** and **2** were elucidated as follows.

Compound **1** was obtained as a colorless oil and produced a molecular ion [M]⁺ at *m/z* 250.1928 in its HREIMS, suggesting the molecular formula C₁₆H₂₆O₂. Its ¹H-NMR spectrum showed two proton signals for a *trans*-double bond at δ 6.08 (1H, d, *J* = 16.0 Hz) and 6.18 (1H, d, *J* = 16.0 Hz), an olefinic signals at δ 5.09 (1H, dd, *J* = 11.6, 4.8 Hz), and a vinyl methyl signals at δ 1.36 (3H, s). The ¹³C-NMR spectrum and a DEPT experiment on **1** showed 15 skeletal carbon signals including a carbonyl carbon (δ 201.1). The ¹H- and ¹³C-NMR spectral data of compound **1** (Table I) were similar to those of zerumbone (Kitayama *et al.*, 2001), except for the C-6- and C-7 positions. Signals for the Δ^6 double bond [δ_{H} 6.02 (1H, d, *J* = 11.6 Hz)/ δ_{C} 149.1 and δ_{C} 138.1] and a vinyl methyl group at C-7 [δ_{H} 1.80 (3H, s)/ δ_{C} 12.0] in zerumbone were replaced by two methine signals [δ_{H} 3.08 (1H, dq, *J* = 6.4, 3.2 Hz)/ δ_{C} 49.2 and δ_{H} 3.69 (1H, br dd, *J* = 5.4, 3.2 Hz)/ δ_{C} 82.3], a methyl signal [δ_{H} 0.97 (3H, d, *J* = 6.4 Hz)/ δ_{C}

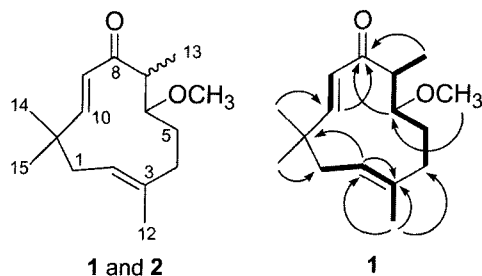


Fig. 1. Structures of compounds **1** and **2** isolated from *Zingiber zerumbet* and selected correlations observed in the COSY (–) and HMBC (–>) Spectra of **1**.

6.2], and a methoxy signal [δ_{H} 3.42 (3H, s)/ δ_{C} 57.0] in **1**. The *2E* configuration of compound **1** was suggested by its ^{13}C -NMR spectrum since the signals at δ 138.1 for C-3 and δ 16.2 for C-12 are in the chemical shift ranges characteristic of these carbon atoms in related compounds with the *E* configuration (Bohlmann and Pauls, 1992). On the basis of this observation and careful analysis of the COSY, HSQC, and HMBC NMR data of **1** (Fig. 1), compound **1** was identified as 6-methoxy-*2E,9E*-humuladien-8-one, which has been previously synthesized (Kitayama *et al.*, 1999).

Compound **2** was also obtained as a colorless oil and produced a molecular ion $[\text{M}]^+$ at m/z 250.1931 in its HREIMS, indicating the same $\text{C}_{16}\text{H}_{26}\text{O}_2$ molecular formula as compound **1**. A comparison of the ^1H - and ^{13}C -NMR spectral data (Table I) of **2** with those of **1** indicated that **2** is a stereoisomer of **1**, which was supported by 2D NMR analysis. Compound **2** has also been previously reported as a synthetic compound (Kitayama *et al.*, 1999). However, the flexible nature of the large ring made it difficult to determine the configurations at C-6 and C-7 of **1** and **2** by NMR (Kitayama *et al.*, 1999).

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