

Isolation, characterization and modification of zerumbone from *Zingiber zerumbet*

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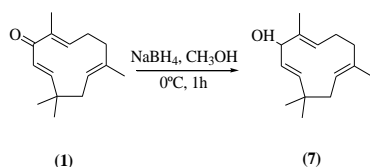
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GRAPHICAL ABSTRACT**ABSTRACT**

Zingiber zerumbet Smith belongs to Zingiberaceae family. The most important part of this plant is its rhizomes which have been associated with variety of medicinal uses. Rhizome of the *Zingiber Zerumbet* Smith is pale yellow with pungent smell and used as traditional medicine in relieving stomach ache, as a diuretic, and when macerated in alcohols are regarded as a tonic and depurative. Hydrodistillation of the rhizome of *Zingiber zerumbet* yield essential oil with five major compounds which are zerumbone (79.79%), α -humulene (6.85%), camphene (1.04%), caryophyllene oxide (1.36%), and (1*R*,3*E*,7*E*,11*R*)-1,5,5,8-tetramethyl-12-oxabicyclo-9,1,0-dodeca-3,7-diene (3.24%). The soxhlet extraction method of the rhizome using *n*-hexane as solvent yielded pale yellow extract crude (8.45 g, 1.69%). Recrystallization of the crude yielded zerumbone as white crystal solid (0.191 g, 2.26%). The zerumbone undergo reactions with *m*-CPBA in dichloromethane to give zerumbone epoxide (152 mg, 89.91%). Both zerumbone and zerumbone epoxide were characterized using ¹H and ¹³C NMR spectroscopy.

Keywords: *Zingiber zerumbet*, essential oil, zerumbone, zerumbone epoxide

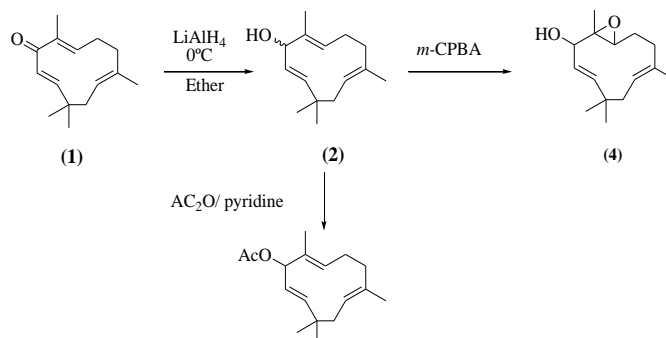
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1. INTRODUCTION

Zingiberaceae is one of the largest families of the plant kingdom which have perennial rhizomatous herbs, simple and distichous leaves, inflorescence terminal of leafy shoot, flower delicate, ephemeral and highly modified and its fruit is a capsule (Siriruga, 1999). Species of Zingiberaceae are used for treatment of various diseases or ailments, including post-partum protective treatment, cures for diarrhea, stomach-aches, indigestion, rheumatism, fever, coughs, asthma, swellings and vermifuge. Common species of this family are 'bonglai' (*Zingiber cassumuna*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), 'lempoyang' (*Zingiber zerumbet*), greater galangal (*Alpinia galanga*), and 'temu kunci' (*Boesenbergia rotunda*) (Zanariah *et al.*, 1997).

Zingiber zerumbet (Zingiberaceae families) also known as 'lempoyang', have high content and large variety of terpenoids in the essential oil of its rhizome (Yu *et al.*, 2008). The main constituents in the essential oil of the rhizome are zerumbone (69.9%), α -humulene (12.9%), humulene epoxide II (2.5%), caryophyllene oxide (1.1%) and camphene (1.9%). Kitayama *et al.* (2003) reported that the major compound in the essential oil of its rhizome is sesquiterpenoid ketones, zerumbone (C₁₅H₂₂O) that has its own unique structure, consist of cross conjugated ketone in an 11 membered ring. This sesquiterpenoid is biologically active photochemical that shows various biological properties such as anti-tumor and anti HIV. Nakamura *et al.* (2004) stated that zerumbone (1) is one of the compromising agents against colon and skin cancer. It was also found to be a tumor suppressor (Murakami *et al.*, 2002).

There are numerous methods reported the preparation of zerumbone analogues. Among them are reduction of zerumbone (1) with LiAlH₄ at 0°C afforded zerumbol (2), acetylation of (2) gave (3) using AC₂O/ pyridine, and epoxidation of (2) with *m*-CPBA yielded (4) (Scheme 1). Therefore in this study, isolation of zerumbone from rhizome of *Zingiber zerumbet* and the modification of zerumbone to form its derivatives were investigated.



Scheme 1 Preparation of zerumbone analogues.

2. EXPERIMENTAL

Thin layer chromatography (TLC) was performed on 0.20 mm precoated silica gel aluminum sheets (Merck Kieselgel 60 F254). Spots were visualized under UV light (254 and 365 nm) and sprayed with vanillin sulphuric acid reagent. Melting points were measured using melting point apparatus equipped with microscope, Leica Gallen III. The ^1H and ^{13}C NMR (400 MHz) spectra were recorded on Bruker Advance 400 Spectrometer. Infrared spectra were recorded using FTIR-ATR, Pelkin Elmer series 1600 Spectrometer.

2.1 Plant material

The fresh rhizomes of *Zingiber zerumbet* were purchased from wet market in Larkin, Johor.

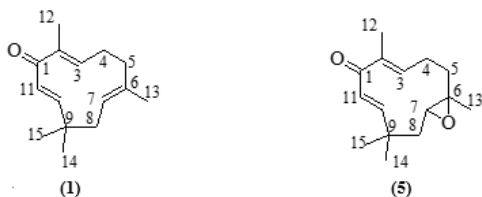
2.2. Extraction and isolation

Fresh rhizomes (500 g) were chopped into small dices. The rhizomes then subjected to hydrodistillation using Clevenger-type glass apparatus for 6 hours for isolation of oil. The oil was extracted using ether (3 x 5 mL) followed by drying over MgSO_4 . The solution was then filtered using filter paper and concentrated using rotatory vapour to yield pale yellow essential oil (5.58 g, 1.12%).

The rhizome (500 g) were chopped and dried at room temperature. The rhizomes then subjected to hot extraction using Soxhlet apparatus for 18 hours using *n*-hexane as the solvent. After the extraction was accomplished, the solvent was evaporated using rotatory vapour to yield crude extracts (8.45 g, 1.69%). The crude extract was recrystallized using hot *n*-hexane and crystallized overnight to produce white crystalline solid, zerumbone (**1**) (0.191 g, 2.26%) with R_f value 0.58 in petroleum: diethyl ether (3:1): melting point 61-63°C, Ref: 65.3°C and IR spectra: $\nu_{\text{max}} \text{ cm}^{-1}$ 3026 (=CH sp²), 2963 and 2920 (CH sp³), 1653 (C=O conjugated), 1638 (C=C). ^1H NMR δ (CDCl_3) 1.09 (3H, s, H15), 1.23 (3H, s, H14), 1.56 (3H, s, H13), 1.82 (3H, s, H12), 1.90 (2H, m, H8), 2.28 (2H, m, H4), 2.59 (2H, m, H5), 5.29 (1H, m, H7), 5.87 (1H, d, $J=16.4$ Hz, H10), 5.91 (1H, d, $J=16.4$ Hz, H11), 6.02 (1H, m, H3). ^{13}C NMR δ (CDCl_3) 11.8 (CH₃, C12), 15.2 (CH₃, C13), 24.2 (CH₃, C15), 24.4 (CH₃, C14), 29.4 (CH₂, C4), 37.8 (C, C9), 39.4 (CH₂, C5), 42.3 (CH₂, C8), 124.9 (=CH, C7), 127.1 (=CH, C11), 136.3 (C=C, C6), 137.9 (C=C, C2), 148.9 (C=C, C3), 160.8 (C=C, C10), 204.4 (C=O, C1).

2.3. Zerumbone epoxide

A mixture of *m*-CPBA 70% (165.6 mg, 0.960 mmol) with zerumbone (152 mg, 0.696 mmol) in CH_2Cl_2 (5 mL) were stirred at room temperature for 18 hours. Anhydrous potassium carbonate (320 mg, 2.31 mmol) was added to the mixture and stirred for 5 minutes. The mixture was filtered and the solvent was evaporated under vacuum to afford zerumbone epoxide (**5**) ((152 mg, 81.91%) with R_f value 0.12 in petroleum ether: diethyl ether (3:1): melting point 76-78°C. ^1H NMR δ (CDCl_3) 1.11 (CH₃, s, H15), 1.25 (CH₃, s, H14), 1.32 (CH₃, s, H13), 1.88 (CH₃, s, H12), 1.95 (2H, m, H8), 2.30 (2H, m, H4), 2.49 (2H, m, H5), 2.75 (1H, d, $J=15.6$ Hz, H7), 5.98 (1H, m, H10), 6.125 (1H, m, H11), 6.136 (1H, m, H3). ^{13}C NMR δ 12.1 (CH₃, C12), 15.6 (CH₃, C13), 24.0 (CH₃, C15), 24.7 (CH₃, C14), 29.8 (CH₂, C4), 36.0 (C, C9), 38.2 (CH₂, C5), 42.7 (CH₂, C8), 61.4 (CH-O, C7), 62.8 (C-O, C6), 128.3 (CH, C11), 139.7 (C=C, C2), 147.7 (C=C, C3), 159.4 (C=C, C10), 202.9 (C=O, C1).

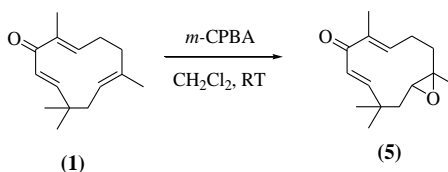


3. RESULTS AND DISCUSSION

Hydrodistillation of the rhizome of *Zingiber zerumbet* gave pale-yellow colour of essential oil (5.58 g, 1.12%). The essential oil were analysed using GC and GCMS with Ultra 1 GC capillary column. The analyzed data shows that the essential oil consists of five major compounds, zerumbone(**1**), (1*R*,3*E*,7*E*,11*R*)-1,5,5,8-tetramethyl-12-oxabicyclo-9,1,0-dodeca-3,7-diene, carophylene oxide, α -humulene and camphene. Hot extraction of powdered rhizome at room temperature by using *n*-hexane as a solvent for 18 hours afforded dark yellow sticky hexane crude extracts (8.45 g, 1.69%). Recrystallization of the hexane crude extract yielded zerumbone (**1**) as white crystal (0.191 g, 2.26%). The R_f value of zerumbone is 0.58 in petroleum ether: diethyl ether (3:1). The TLC analysis with vanillin sulphuric acid spraying reagent revealed purple spot and change to yellow indicates the presence of terpenoids.

The IR spectrum of zerumbone (**1**) showed the characteristic band for $=CH_2$ sp^2 at 3026 cm^{-1} , two peak of CH sp^3 at 2963 and 2920 cm^{-1} , and strong band of $C=O$ conjugated at 1635 cm^{-1} for carbonyl group of α,β -unsaturated ketone. There was also peak at 1638 cm^{-1} corresponds to the presence of $C=C$ band. The analysis of 1H NMR data for zerumbone (**1**) showed the presence of singlet signal indicated the presence of geminal dimethyl group at C14 and C15 with chemical shift δ 1.23 and 1.09 respectively. A singlet signal at δ 1.56 was attributed to methyl group on a trisubstituted double bond at C13, while a singlet peak at δ 1.82 revealed the presence of methyl group at C12. A methylene group at δ 1.90 with multiplet signal was observed for H8. Two methylene protons were observed at δ 2.28 and 2.59 indicating H4 and H5 respectively with both having multiplet signals. A doublet signal at δ 5.29 showed the presence of vinyl proton at H7. Olefinic proton was observed with doublet signal. 5.87 for C10 ($J = 16.4\text{ Hz}$) and δ 5.91 at C11 ($J = 16.4\text{ Hz}$). Other olefinic proton with multiplet signal was observed at 6.02 which assigned to H3. The analysis of ^{13}C NMR data for zerumbone (**1**) showed 15 peaks which correspond to 15 carbon atoms in the molecule. Analysis of the spectrum showed four methyl group C12 (11.8), C13 (15.2), C14 (24.4) and C15 (24.2), four olefinic carbon C3 (148.9), C7 (124.9), C10 (160.8) and C11 (127.1), three quaternary carbon C6 (136.3), C2 (137.9) and C9 (37.8) and also one carbonyl group C1 (204.4).

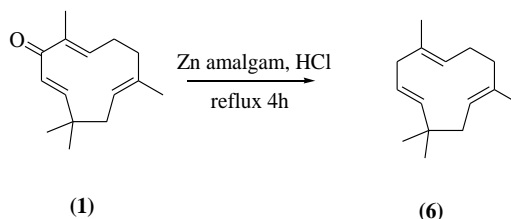
Epoxidation of zerumbone (**1**) with *m*-CPBA in dichloromethane afforded zerumbone epoxide (**5**) (152 mg, 81.91%) as white crystals with R_f value is 0.12 in petroleum ether: diethyl ether (3:1) as an eluent. (**Scheme 2**). The reaction does not occur at C2 and C10 because these two olefinic carbons are more stable due to conjugation of the carbonyl group. This reaction also yield side product which is *m*-chlorobenzoic acid. Hence, addition of anhydrous potassium carbonate into the reaction neutralized the acid and separated the zerumbone epoxide as a salt. The salt can be easily separated via filtration. The 1H NMR spectrum of zerumbone epoxide displayed multiplet signal at δ 5.98-6.14 correspond to only three olefinic protons H3, H10 and H11. Doublet signal at 2.75 ($J=15.6\text{ Hz}$) was observed for H7 which have been shifted from δ 5.29 because olefinic proton at H7 of the zerumbone (**1**) was oxidized to form epoxide. ^{13}C NMR spectrum for zerumbone epoxide (**5**) shows 15 peaks correspond to 15 carbon atoms in the molecule. Analysis of the spectrum shows carbon 6 (62.8) and carbon 7 (61.4) were become more shielded and move to the upfield compared to zerumbone (**1**) due to epoxidation process.



Scheme 2 Reaction of zerumbone with *m*-CPBA to form zerumbone epoxide

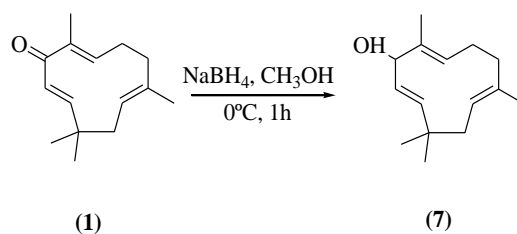
3.1. Attempted synthesis of humulene (6)

Zerumbone (**1**) (100 mg, 0.106 mmol) was added to a mixture of water (1.15 mL), zinc amalgam (133 mg, 0.50 mmol) and hydrochloric acid 32% (5 mL). The mixture was reflux for 4 hours. The mixture was then added with water (10 mL) and extracted with diethyl ether (10 mL x 3). After that, the mixture was then washed with sodium bicarbonate (5 mL x 3). The organic layer was separated and concentrated using rotatory evaporator. It was then purified using column chromatography (petroleum ether: ether, 6:1) to yield unchanged starting material with $R_f=0.58$ similar to zerumbone. The experiment was repeated few times by increasing the reaction time but there is still unchanged zerumbone.



3.2. Attempted reaction for zerumbol (7)

Zerumbone (**1**) (100 mg, 0.45 mmol), taken in methanol (3.33 mL) and cooled to 0°C , sodium borohydride (17.32 mg, 0.31 mmol) was added slowly and the mixture was stirred at the same temperature for 1 hour. Progress of the reaction was monitored by TLC analyses using petroleum ether: diethyl ether (3:1). After the completion of the reaction (1.5 h), mixture was concentrated. Ethyl acetate (5 mL) was added to the resulting syrupy liquid. The organic layer was separated and washed with water (10 mL x3). It was then dried over anhydrous sodium sulphate and the liquid was concentrated to afford viscous oil. Unfortunately, no reaction occur even 1.5 h. The reaction is remains unchanged even when the stirring time was increased.



4. CONCLUSION

The essential oil of the rhizome of *Zingiber zerumbet* by hydrodistillation was obtained with zerumbone (**1**) (79.79%) as its major compound. The minor compounds existed in the rhizome oil are (1*R*,3*E*,7*E*,11*R*)-1,5,5,8-tetramethyl-12-oxabicyclo-9,1,0-dodeca-3,7-diene (3.24%), caryophyllene oxide (1.36%), α -humulene (6.85%), camphene (1.04%). The isolation of zerumbone (**1**) from the rhizome of *Zingiber zerumbet* afforded white crystal solid (0.191 g, 2.26%). The reaction of zerumbone with *m*-CPBA afforded zerumbone epoxide (**5**) in (152 mg, 81.91%).

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