SHORT COMMUNICATION

MARKOVNIKOV ADDITION OF CHLOROSULFURIC ACID TO EUGENOL ISOLATED FROM CLOVE OIL

I Made Sudarma*, Emmy Yuanita, and I Wayan Suana

Chemistry Laboratory, Faculty of Science, University of Mataram,
Jl. Majapahit 62 Mataram, West Nusa Tenggara, 83125 Indonesia

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ABSTRACT

The objective of this research was to synthesize new compounds with potential biological activity from readily accessed natural products. Eugenol has been reported to posses antioxidant and anticancer properties and was prepared by extracting from clove buds with dichloromethane and followed by isolation using column chromatography to afford pure eugenol (73%). In an attempt to enhance intrinsic activity of this natural compound, some derivatives were possible to synthesize. The main aim of this preliminary research was to transform eugenol to become sulfonic derivative. Eugenol was transformed to its sulfonic derivative in moderate yield (64%) by treatment with chlorosulfuric acid which undergoes Markovnikov addition. This product was rapidly confirmed by GC-MS and NMR analyses. Selective inhibition was performed by cyclic sulfonic ester derivative which could inhibit Eschericia coli and Staphylococcus aureus but not for Bacillus cereus.

Keywords: *Markovnikov addition; chlorosulfuric acid; eugenol*

ABSTRAK

Tujuan umum dari penelitian ini adalah mengembangkan senyawa baru yang berpotensi mempunyai aktifitas biologi dari senyawa bahan alam yang mudah diperoleh khususnya eugenol. Eugenol telah dilaporkan mempunyai sifat sebagai anti oksidan dan antikanker. Dalam upaya meningkatkan kemampuan aktifitas biologi dari senyawa bahan alam ini, beberapa turunannya telah berhasil disintesa. Tujuan utama dari penelitian adalah mentransformasikan eugenol menjadi turunan sulfonik. Minyak cengkeh diekstrak dari bunga cengkeh dengan dichloromethana dan selanjutnya eugenol diisolasi dengan kromatografi kolom dan diperoleh eugenol (73%). Eugenol ditransformasikan menjadi turunan sulfonik melalui reaksi addisi Markovnikov dengan hasil moderat setelah diperlakukan dengan asam klorosulfurik. Transformasi ini secara cepat dapat dikonfirmasi dengan analisis GC-MS dan NMR. Cyclic sulfonic ester dapat menghambat dengan selektif terhadap Eschericia coli and Staphylococcus aureus namun tidak untuk Bacillus cereus.

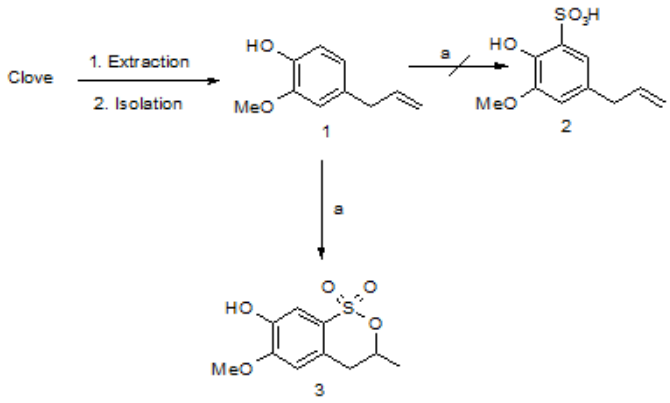
Kata Kunci: *addisi Markovnikov; asam chlorosulfurik; eugenol*

INTRODUCTION

Natural products particularly eugenol has been investigated for further chemical transformation [1-2], [3], chemical transformation of this compound to the new cyclic sulfonic ester derivative was reported. Eugenol (4-Allyl-2-methoxyphenol), a constituent of clove, has been used for antibacterial [4], acaricidal [5], anti-Helicobacter [6], and antiproliferative [7]. It is used in the form of a paste or mixture as dental cement, filler, and restorative material. Plant oils, including clove, may be used in livestock to inhibit microbial fermentation in waste products. Clove oil may be found in high concentration licorice (glycyrrhizin) products to prevent gel formation in an aqueous solution [8].

The therapeutic benefits of eugenol are well known. In recent times, it has been studied for a variety of promising biological properties. It has been reported to participate in photochemical reactions and to possess insecticidal, antioxidant, anti-inflammatory, anticancer activities [9]. In view of these evidences on biological properties of eugenol, to enhance intrinsic activity of this natural compound. In the present preliminary work, new cyclic derivative was successfully transformed from eugenol in moderate yield.

* Corresponding author. Tel/Fax : +62-370 646506
Email address : sud_arma@yahoo.co.id



Conditions: a. $CH_2Cl_2/ClSO_3H$ reflux 15 min, 64%

Scheme 1. Isolation and transformation of eugenol

EXPERIMENTAL SECTION

Materials

Unless otherwise stated, all chemical reagents were purchased with the highest commercially available purity (Merck and Sigma) and were used without previous purification. The material used included: clove buds, dichloromethane, hexane, chlorosulfuric acid, sodium hydroxide, methanol, analytical thin layer chromatography.

Instrumentation

GC-MS were recorded on GC-MS QP-5050A, BC-17A and MS 5050A Shimadzu. GC Parameters were setup as follows, Oven Temp (°C) = 60.0, Oven Equil. Time (min) = 0.50; Injection Temp (°C) = 280.0; Interface Temp (°C) = 300.0; Column Length (m) = 30; Column Diameter (mm) = 0.25; Column Pressure (kPa) = 100; Column Flow (mL/min) = 1.6; Linear velocity = 46.4; Split Ratio = 22; Total Flow (mL/min) = 40.2; Program Time (min) = 27.00. MS parameter, Start M/Z = 33.00 End M/Z = 550.00; Scan interval (Sec.) = 0.50; Scan Speed (amu/sec) = 1000.

The original ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra are directly reproduced throughout. They were generally recorded in $CDCl_3$ on a Bruker spectrometer at 400 MHz.

Procedure

Extraction and GC-MS Analysis

Dried clove buds (100 g) was grounded to fine particles and percolated with dichloromethane (200 mL) and kept for 24 h and then the liquid extract was filtered and evaporated to afford yellowish oil (12 g). This oil was analyzed by GC-MS to confirm the presence of eugenol.

Isolation of eugenol

Medium pressure liquid chromatography was employed to separate eugenol (**1**) from the clove oil (2 g). Gradient elution starting with 100% hexane and increased by the following hexane/dichloromethane ratio : 4/1, 3/2, 1/1, and 0/100). Twenty-five fractions were collected from this elution. Fractions shown to be identical by thin layer chromatography were combined and evaporated in *vacuo*. Fractions 1, 2, and 3 were combined affording oil (50 mg). Fractions 6 to 12 were combined affording yellowish oil (1.46 g) (73%). This oil was identified as eugenol (**1**) by GC-MS and NMR analyses. M^+. 164, cal for $C_{10}H_{12}O_2$ Major fragments: 49 (M^+. $-CH_3$), 131, 121, 103, 91, 77 (C_6H_6, base peak).

Synthesis of cyclic sulfonic ester derivative

To stirred solution of eugenol (**1**) (100 mg, 0.5 mmol) in dichloromethane (20 mL) was added chlorosulfuric acid (2 mL) drop by drop. The solution was stirred at room temperature for 30 min then refluxed for 15 min. The solution was evaporated and water (10 mL) then added, basified to pH 8 with 1M sodium hydroxide, then extracted with dichloromethane. The organic phase was dried and evaporated to dryness to give an amorphous gray solid, 72% of (**3**) from GC-MS analyses and recrystalized from methanol to afford gray-whiteneedles (78 mg, 0.32 mmol, 64%). Compound (**3**), GC-MS: M^+. 244, cal for $C_{10}H_{12}SO_5$ Major fragments: 200, 183, 165, 151, 136 (base peak). IR (film) nmax/cm^{-1}: 3232 (O-H), 3084 (C=CH-Ar), 2936, 2829, 1399, 1327, 1260 (C-O), 1127 (C-O), 1066, 999, 912, 764. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 1.62 (3H, d, J 6.6 Hz, $-CH_3$); 2.16 (1H, s, OH); 2.85 – 2.31 (2H, m,$-CH_2$-); 3.93 (3H, s, $-OCH_3$); 5.20 (1H, m, -CH-); 6.60 (1H, s, ArH); 7.33(1H, s, ArH). ^{13}C-NMR (400.1 MHz, $CDCl_3$): δ 21.3 ($-CH_3$); 35.6 ($-CH_2$); 56.4 (-CH-); 110.1 (ArCH); 110.6 (ArCH); 126.8 (ArC); 127.2 (ArC); 145.4 (ArC); 150.1 (ArC).

RESULT AND DISCUSSION

The addition of chlorosulfuric acid to asymmetrically substituted alkenes or benzene such as eugenol leads to single product (**3**). The single product (**3**) is predicted by the Markovnikov rule, which states that when a chlorosulfuric acid is added to an asymmetrically substituted alkene, the major product results from the addition of the hydrogen atom to the double-bonded carbon that is attached to more hydrogen atoms, while chlorosulfuric ion adds to the other double-bonded carbon. This arrangement creates a more stable carbocation intermediate then cyclised to the benzene ring by eliminating of HCl. Product (**2**) was

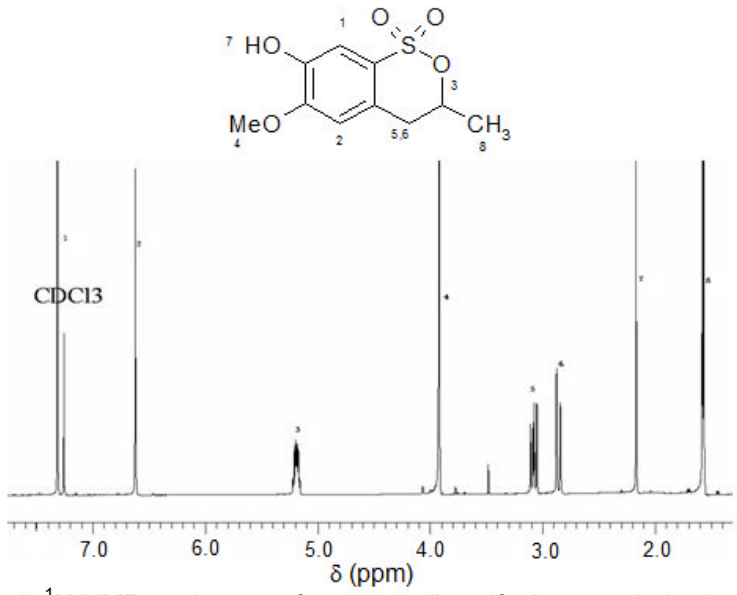
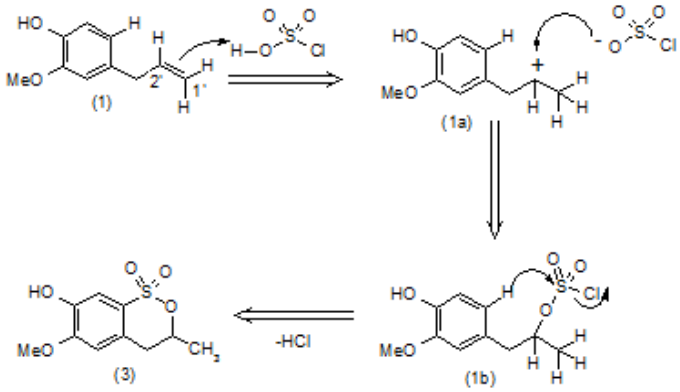


Fig 1. ^{1}H-NMR assignment for new cyclic sulfonic ester derivative (3)



Scheme 2. Proposed mechanism for the formation of (**3**)

not observed from the reaction due to competitive reaction between asymmetric alkene and benzene ring.

Chemical transformations of eugenol to its derivatives were analyzed by GC-MS. GC-MS is a rapid instrument to analyze crude products of chemical transformation before further works such isolation and purification. GC-MS analysis of the reaction of eugenol (**1**) with chlorosulfuric acid was proposed mainly produced 4-Allyl-2-methoxy-6-sulfuricphenol (**2**) (Scheme 1) but the ^{1}H-NMR analyses suggested that compound (**2**) was not present. A major component of this crude product which represented new cyclic sulfonic ester derivative (**3**). This compound was recrystallized in Methanol to give white needles (64%) and confirmed by its mass spectrum which showed the molecular ion at m/z 244 corresponding to molecular formula $C_{10}H_{12}SO_5$

On the basis of spectroscopic evidence and mechanistic considerations, the cyclic form of sulfonic ester derivative structure was proposed for (**3**). ^{1}H and ^{13}C-NMR analyses confirmed the cyclic form of sulfonic ester derivative (**3**) (Fig. 1) and excluded the 4-Allyl-2-methoxy-6-sulfuricphenol (**2**) as proposed in Scheme 1. Fig. 1 showed additional methyl group as a doublet at δ 1.58 which gave appropriate information about cyclic sulfonic ester derivative (3) compare to 4-Allyl-2-methoxy-6-sulfuricphenol (2). This analysis was supported by ^{13}C-NMR and DEPT which gave 4 quaternary carbons, 3 methine carbons, 1 methylene carbon, and 2 methyl carbons. Generally, the ^{1}H-NMR spectrum supported the proposed structure (**3**).

Proposed mechanism for the formation of the new cyclic product (3) was shown by Scheme 2.

In the first stage, the chemical basis for Markovnikov's Rule is the transformation of the most stable carbocation during the addition process [10-11]. The addition of the hydrogen from H-OSO_2Cl to one carbon atom in the double bond of allyl group of Eugenol (1) creates a positive charge on the other carbon, forming a carbocation intermediate (1a). The more substituted the carbocation the more stable it is, due to induction and hyperconjugation effects [11]. The carbocation C2' is more stable than carbocation C1' and $^{-}OSO_2Cl$ will attack the carbocation C2' leads to the formation of intermediate (1b). However, the other less substituted C1' or less stable carbocation C1' will still be formed, and will proceed to form the minor product with the opposite attachment of $^{-}OSO_2Cl$ but such intermediate is not detected from the GC-MS and NMR analyses.

In the second stage of the reaction, a base donates electrons to the hydrogen atom and generated the losses of HCl to afford cyclic product (3).

Preliminary bioassay of eugenol and its derivatives showed biological activities against *Eschericia coli, Bacillus cereus,* and *Staphylococcus aureus*. Highest inhibition was shown by eugenol which gave wide inhibition spectrums compared its derivatives. Selective inhibition was performed by cyclic sulfonic ester derivative (**3**) which could inhibited *Eschericia coli* and *Staphylococcus aureus* but not for *Bacillus cereus* [3].

CONCLUSION

Eugenol (**1**) Isolated from Clove Oil has been transformed through Markovnikov intermediate to new cyclic sulfonic ester derivative (**3**) in moderate yield.

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