CHEMICAL TRANSFORMATION OF EUGENOL ISOLATED FROM CLOVE OIL TO 4-ALLYL-2-METHOXY-6-SULFONICPHENOL AND 4-ALLYL-2-METHOXY-6-AMINOPHENOL

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ABSTRACT

The overall objective of this research was to develop new compounds with potential biological activity from readily accessed natural products, in particular eugenol. Eugenol has been reported to posses antioxidant and anticancer properties. 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 isolate eugenol from clove oil and made its sulfonic and amine derivatives through chemical transformation. Clove oil was extracted from clove buds with dichloromethane and followed by isolation of eugenol using column chromatography to afford eugenol (73%). Eugenol has been reported to posses antioxidant and anticancer properties. In an attempt to enhance intrinsic activity of this natural compound, some derivatives were possible to synthesize. Eugenol was transformed to its sulfonic derivative in moderate yield by treatment with chlorosulfonic acid and to its amine by reduction of its nitro derivative. This transformation was rapidly confirmed by GC-MS analysis which showed significance molecular ion at m/z 244 corresponding to molecular formula C_{10}H_{12}SO_3. and at m/z 179 corresponding to molecular formula C_{10}H_{11}O_2NH_2.

Keywords: Chemical Transformation, Eugenol, 4-Allyl-2-methoxy-6-sulfonicphenol and 4-Allyl-2-methoxy-6-aminophenol

INTRODUCTION

Clove are harvested primarily in Indonesia, Madagascar, and Zanzibar; it is also grown in India called Lavang, Pakistan, and Sri Lanka. According to FAO, Indonesia produced almost 80% of the world's clove output in 2005 followed at a distance by Madagascar and Tanzania. Cloves can be used in cooking either whole or in a ground form, but as they are extremely strong, they are used sparingly. Clove is sometimes added to tobacco in cigarettes, and clove cigarettes ("kreteks") typically contain 60% tobacco and 40% ground cloves [1].

Eugenol (4- Allyl-2-methoxyphenol), a constituent of clove, has been used for antibacterial [2], acaricidal [3], anti-Helicobacter [4], and antiproliferative [5]. 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 [6].

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 [7]. In view of these evidences on biological properties of eugenol, to enhance intrinsic activity of this natural compound. In the present preliminary work, two derivatives were successfully transformed from eugenol (1) in moderate yields.

EXPERIMENTAL SECTION

General

Unless otherwise stated, all chemical reagents were purchased with the highest commercially available purity (Merck and Sigma) and were used without previous purification. GC-MS were recorded on GC-MS QP-5050A, BC-17A and MS 5050A Merk 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.

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 hours. 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 ratios : 4/1, 3/2, 1/1, and 0/100). Twenty-five fractions were collected from these elution. Fractions shown to be identical by thin layer chromatography were combined and evaporated in vacuo. Fractions 1, 2, and 3 were combined affording an oil (50 mg). Fractions 6 to 12 were combined affording an yellowish oil (1.46 g) (73%).

Synthesis of sulfonic derivative

To stirred solution of eugenol (1) (100 mg, 0.6 mmol) in dichloromethane (20 mL) was added chlorosulfonic 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% yield). Compound (2), GC-MS: M+ 244, cal for C10H12SO3, Major fragments: 200, 183, 165, 151, 136 (base peak).

Synthesis of nitro derivative

Eugenol 1.0 g (6.10 mmol) was dissolved in dichloromethane (25 mL) and was added to a mixture which contained 4.5 g (33 mmol) of potassium hydrogen sulphate, 3.0 g (35.3 mmol) of sodium nitrate and 3.5 g of wet silica to 50% P/P, the mixture was left with constant stirring at room temperature for 4 hours. The complete disappearance of the starting product was confirmed by thin layer chromatography (TLC) (Dichloromethane:n-hexane 1:3). The reacted mixture was filtered through silica and the solid was washed with dichloromethane, and the solvent evaporated in vacuum to give a reddish oil. Pure product was obtained after chromatographic column (5:1 – 3:1 dichloromethane in hexane), which gave 750 mg of the desired compound (3) (75% yield). Compound (3) (oil): M+ 209, cal for C10H12NO3, Major fragments: 195 (M+ – CH2), 178, 163, 147, 131, 119, 103, 91 (base peak), IR (film) νmax/cm⁻¹: 3232 (O-H), 3084 (C=CH-Ar), 3014 (CH=CH2), 2936, 2829, 1634 (C=C), 1547 (NO2), 1399, 1327, 1260 (C-O), 1127 (C-O), 1066, 999, 912, 764. 1H NMR (400.1 MHz, CDCl3): δ 3.35 (2H, d, H3'); 5.13 (2H, m, H3'); 5.91 (1H, m, H3'); 6.96 (1H, s, H3); 7.50 (1H, d, J 0.9 Hz, H5); 10.67 (1H, s, OH).

Synthesis of amine derivative

Nitro-eugenol (2) (400 mg, 1.91 mmol) was dissolved in a mixture of ethanol (10 mL) and concentrated hydrochloric acid (10 mL), and the solution refluxed with tin powder (1 g) for 2 hours. Further quantity of tin (1 g) and hydrochloric acid (5 mL) were then added, and the mixture refluxed for another 2 hours. The ethanol was removed and the acid liquor cooled and diluted with water (25 mL), the solution was basified with concentrated aqueous ammonia until a white precipitate formed. The solution was filtered and dichloromethane was added (2 x 50 mL) to afford the desired compound (4) (56% yield). GC-MS: M+ 179, cal for C10H13NO3, Major fragments: 164, 152, 136, 118, 106, 91 (base peak).

RESULT AND DISCUSSION

Preliminary preparation of this work was extraction and isolation of eugenol from clove buds. Extraction of clove buds with dichloromethane gave yellowish oil, and GC-MS analyses of this oil gave five major components, namely eugenol, beta-caryophyllene, alpha-humulene, eugenyl acetate, and caryophyllene oxide [8]. Medium Performance Liquid Chromatography was employed to isolate eugenol (1) from clove oil. 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 chlorosulfonic acid mainly produced 4-Allyl-2-methoxy-6-sulfonicphenol (2) (Figure 1).

A major component of this crude product (72%) was peak no. 7 which represented 4-Allyl-2-methoxy-6-sulfonicphenol (2). This compound was confirmed by its mass spectrum which showed the molecular ion at m/z 244 corresponding to molecular formula C10H12SO3 (Figure 2).

Nitrification of eugenol with nitric acid and acetic acid glacial gave two products namely eugenyl acetate...

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and 4-Allyl-2-methoxy-6-nitrophenol (3) [8]. The presence of acetic acid gave opportunity to form ester of eugenol acetate and this compound was not easily to separate from eugenol due to similarly in polarity [8], and a new method nitrification reported by Carrasco [3] was employed to afford a moderate yield of 4-Allyl-2-methoxy-6-nitrophenol (3) (Figure 3).

Nitrification of eugenol (1) was a substitution reaction and theoretically would produce 4-Allyl-2-methoxy-6-nitrophenol (2) due to the presence of hydroxyl group in eugenol. The hydroxyl group directed the –NO$_2$ substituent to ortho position [5]. The presence of this nitro compound was identified by GC-MS (Figure 4) which gave molecular ion at m/z 209 corresponding to molecular formula C$_{10}$H$_{11}$NO$_4$.

Reduction of crude product of nitrification with Sn/HCl in ethanol produced four major components (Figure 5). Component or peak no. 11 was identified as 4-Allyl-2-methoxy-6-aminophenol (4). This compound was confirmed by its mass spectrum which showed the molecular ion at m/z 179 corresponding to molecular formula C$_{10}$H$_{11}$O$_2$NH$_2$ (Figure 6).

Further purification of crude product was needed to confirm the results of these transformation reactions.

CONCLUSION

Eugenol Isolated from Clove Oil has been transformed to 4-Allyl-2-methoxy-6-sulfonicphenol and 4-Allyl-2-methoxy-6-aminophenol in moderate yields.

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