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ORIGINAL ARTICLE

Thermal, photosynthesis and antibacterial studies of bioactive safrole derivative as precursor for natural flavor and fragrance

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Escherichia coli;
Staphylococcus aureus

Abstract Safrole [5-allylbenzo[d][1,3]dioxole] was subjected to photochemical oxidation reaction with hydrogen peroxide in the presence of sodium lamp to give the corresponding epoxy derivative [5-oxiranylmethylbenzo[1,3]dioxole. The thermal oxidation of safrole with 3-chloroperoxybenzoic acid at room temperature gave the same epoxide derivative in quantitative yield. Antibacterial studies were carried out on safrole and its photoproducts (safrole epoxide and safrole hydroperoxide). The results revealed that safrole hydroperoxide was the most effective than safrole epoxide than safrole against Gram-positive bacteria *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATC C25923, and Gram negative bacteria *Escherichia coli* ATCC25422. This result proved that safrole derivatives are beneficial to human health, having the potential to be used for medical purposes.

1. Introduction

Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005).

Propenyl benzene are common aromatic compounds, these are widely used as starting compounds for the production of various flavor and fragrances (Xu et al., 2007). Flavors and fragrances are widely used in the food beverage and cosmetic industries (Priefert et al., 2001). Most of them in the world market today are obtained by chemical synthesis. Less than 5% is extracted from plants and can therefore be classified as natural (Xu et al., 2007).

In recent years, according to the Food and Drug Administration (FDA) and European legislation products obtained by photo and biotechnological methods can also be considered natural, if the substrate for the process is of natural origin (Serra et al., 2005).

Medicinal and aromatic plants have demonstrated its contribution to the treatment of diseases such as HIV/AIDS, malaria, diabetes, sickle-cell anemia, mental disorders (Elujoba et al., 2005; Okigbo et al., 2005) and microbial infections (Okigbo and Mmeka, 2006).

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Natural phenyl terpenoides undergo oxidation on exposure to air. The oxidation process is enhanced thermally or by irradiation to form epoxide derivative, which acts as the precursor of flavors and fragrance (Elgendy and Khayyat, 2008a). This is the first step in the biosynthesis of important products (Hua et al., 2007; Yamada et al., 2007; Zang et al., 2006).

Venturello (1992) reported that oxidation of safrole (**1**) with tungsten phosphoammonium chloride complex $(R_4N)_3PW_4O_{24}$ gives epoxy derivative 2.

Taking into account important activities of plant phenylpropenides, in the present work, we believed it to be relevant to examine thermal and photochemical oxidation of safrole, and study the biological activity of the oxidation product.

2. Materials and methods

2.1. Safrole [5-allylbenzo[d][1,3]dioxole] (1)

Safrole [5-allylbenzo[d][1,3]dioxole] (**1**) is the major component (80%) of the essential oil of sassafras (*Piper hispidinervum*) (*Piperaceae*) in its leaves. IR spectra were performed on a Perkin-Elmer 16 FPC FT-IR spectrophotometer as thin films. ^{1}H-NMR and ^{13}C-NMR spectra were obtained in $CDCl_3$ solution with a Brucker AVANCE D.P.X. 600 MHz apparatus. GCMS were determined by Joel JMS 600H, GC Hewlett Packard, HP 6890 Series, with capillary column (30 m × 0.32 mm × 0.25 μm) HP-5 cross linked 5% dimethyl polysiloxane. A sodium lamp (Phillips G/5812 SON) was used for photoirradiation reactions. Thin layer chromatography (TLC) and preparative layer chromatography (PLC): Polygram SIL G/W 254, Mecherey-Nagel. A rotatory evaporator (at 20 °C 15 torr) was used to remove the solvents.

2.2. Test organisms

Gram-positive, e.g. *Bacillus subtilis* ATCC6633 and Gram-negative, e.g. *Escherichia coli* ATCC25422, were obtained from the library of military Hospital in Riyadh. *Staphylococcus aureus* ATCC25923 was obtained from the laboratory of Jeddah King Fahad Hospital in Saudi Arabia. It was cultured on Mueller Hinton media (Oxioid CM 41) at 37 °C.

2.3. The methods

2.3.1. Photochemical oxidation of safrole (1) with hydrogen peroxide

A solution of 30% hydrogen peroxide H_2O_2 (2.5 ml) was carefully added in a dropwise manner over a period of 5 min to a solution of 5 mmol compound (**1**) in 25 ml of ethanol C_2H_5OH under stirring at 0 °C. The mixture was irradiated for 55 h using a sodium lamp in a nitrogen atmosphere. The mixture was then evaporated under reduced pressure at room temperature to give a resinous material. The residue was treated with 25 ml of chloroform. The extract was dried over anhydrous sodium sulfate Na_2SO_4 and evaporated under reduced pressure to give the crude product which was purified by column chromatography on silica gel adsorbent using petroleum ether (60–80 °C) and diethyl acetate (9:2) to isolate compound **2**.

2.3.2. Oxidation of safrole (1) using m-chloroperoxybenzoic acid

A solution of 10 mmol of 80% *m*-chloroperoxybenzoic acid was added cautiously dropwise over a period of 15 min to a solution of 5 mmol of compound **1** in 25 ml of chloroform under stirring at 0 °C. The mixture was then stirred at room temperature under nitrogen atmosphere. The progress of the reaction being monitored by thin layer chromatography (TLC) and peroxide test (using a 10% solution of KI). The mixture was carefully washed with a saturated aqueous solution of $NaHCO_3$ (3 × 10 ml) and distilled water (3 × 10 ml). The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure at room temperature. The residue product was purified by column chromatography on silica gel adsorbent using petroleum ether (60–80 °C) and diethyl acetate (9:2) to isolate compound **2** as a viscous oily substance.

2.3.2.1. 5-Oxiranyl methylbenzo[1,3]dioxole (2). Colorless oil, $C_{10}H_{10}O_3$ (*M* 178.16). IR spectrum, υ, cm^{-1}: 3018, 2896, 1606.3, 1099. ^{1}H-NMR spectrum, δ, ppm: 2.53 d (1H, 1′-H, J = 5 Hz), 2.75 dd (1H, 3′-H, J = 4.9 Hz)), 2.77 d (1H, 1′-H, J = 5 Hz), 2.8 dd (1H, 3′-H, J = 4.9 Hz), 3.10 comp. pat. (1H, 2′-H), 5.91 s (2H, C^2H_2), 6.63 d (1H, 6-H, J = 8 Hz), 6.73 s (1H, 4′-H), 6.75 d (1H, 7-H, J = 8 Hz)). ^{13}C-NMR spectrum, δ, ppm: 38.4 ($C^{1'}$), 46.9 ($C^{3'}$), 52.9 ($C^{2'}$), 100.8 (C^2H_2), 108.1 (C^4), 109.8 (C^7), 121.7(C^6) 130.1 (C^5), 146.5 (C^1), 147.7(C^3). GC–MS data:retention time 12.83 min; m/z (I_{rel} %): 178.16(98)[M^+], 162 (5) $[M–O]^+$, 148 (30) $(M–CH_2O]^+$, 132(5)$[C_9H_8O]^+$, 105 (15) $[C_8H_9]^+$, 75 (7) $[C_6H_3]^+$.

2.4. Antimicrobial activity of safrole, safrole epoxide and safrol hydroperoxide

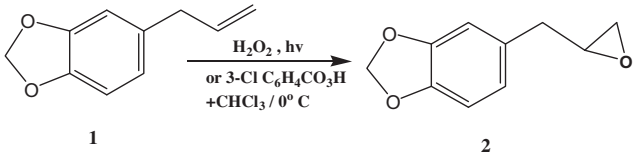
The antimicrobial activity of the above mentioned compounds was separately determined using the disk diffusion method in plates containing 15 ml of Muller–Hinton agar medium (Oxioid (CM 41), Hampshire, England) were seeded with a 24 h culture of the bacterial strains in nutrient broth, the turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 McFarland standard, resulting in a suspension containing approximately 1–2 × 108 CFU/ml. Mueller–Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface.

Each pure compound was checked for antibacterial activity; disk of filter paper (3 mm in diameter) was soaked with 2.6×10^{-5}mol of the compound and placed on the inoculated plate into duplicate plates of each pure compound and chloroform (solvent) as test control. The plates were allowed to stand at refrigerator temperature for 2 h for the compound to diffuse into the agar and then the cultures were incubated at 35 °C for 24 h. Antibacterial activities were determined by measuring the diameter of the inhibition zone formed around the disk for each compound.

3. Result and discussion

The essential oil of *P. hispidinervum* (C.DC.), (*Piperaceae*) contains high levels (83–93%) of safrole in leaves which can be easily extracted by hydrodistillation (Khayyat, 2011). Photochemical epoxidation of safrole (**1**) with hydrogen

peroxide (H_2O_2, 30% by volume) in ethanolic medium under irradiation with sodium light (irradiation time 55 h) to 35% of 5-oxiranylmethylbenzo(1,3)dioxole (**2**).



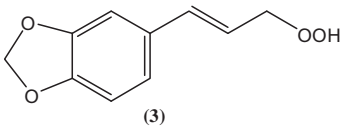
equation 1 (1)

The structure of epoxide derivative **2** was established by spectral measurements. The ^{1}H-NMR spectrum of **2** showed two doublet signals at δ 2.75 and δ 2.80 ppm for two protons 2H-3′ in position 3′ , two doublet at δ 2.53 ppm and δ 2.77 ppm for the two methylene protons CH_2-1′ and complex pattern at δ 3.1 ppm for proton H-2′. In the ^{13}C-NMR spectrum of **2**, signals from the oxiran carbon atoms were presented at δ_C 46.9 ppm (($C^{3'}$) and δ_C 52.6 ppm (($C^{2'}$). The mass spectrum of **2** contained the molecular ion peak at m/z 178.

Thermal oxidation of safrole (**1**) with 3-chloroperoxybenzoic acid in chloroform at room temperature gave the same epoxide derivative **2** as the only product in almost quantitative yield (Eq. (1)).

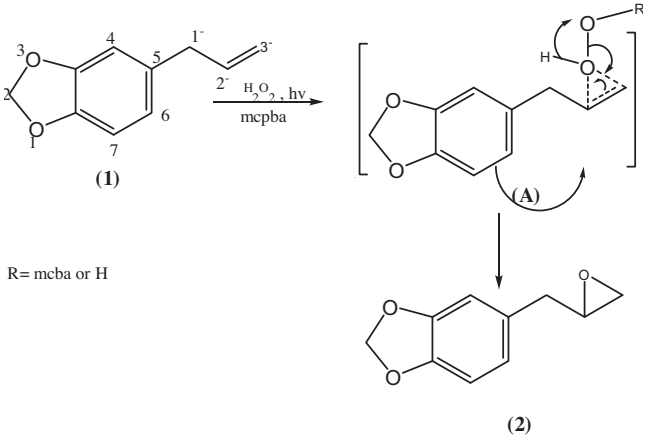
A probable mechanism for the formation of epoxy derivative **2** in the thermal and photochemical oxidation of safrole (**1**) is shown in Scheme 1. Attack by *m*-chloroperoxybenzoic acid or hydrogen peroxide on the side-chain double bond in molecule **1** gives oxiran intermediate A and elimination of *m*-chlorobenzoic acid or water molecule depending on the oxidant used from the latter yield oxiran **2** (Elgendy and Khayyat, 2008b) Scheme 2.

Safrole hydroperoxide (**3**) have been prepared photochemically in the previous work (Khayyat, 2011).



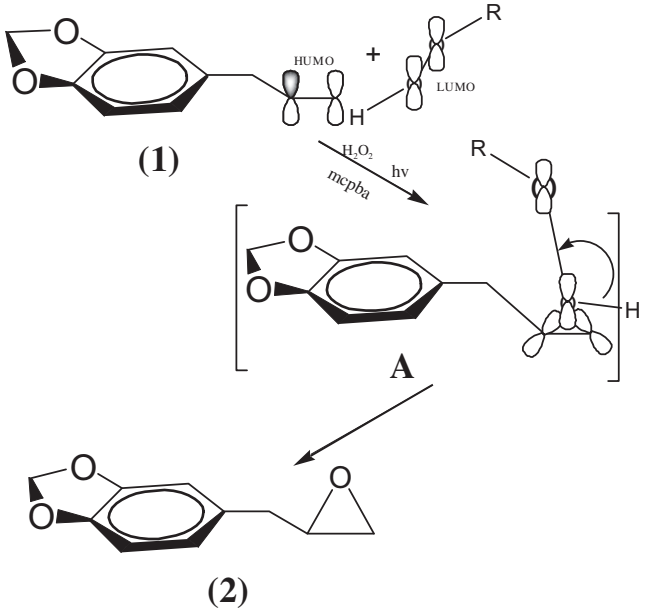
(3)

Some of the oils have been reported to be good source of antifungal and antibacterial compounds (Mastura et al., 1999; Nor Azah et al., 2002). These contain valuable chemical compounds which proved to be active against many pathogenic bacteria (Lien-Yu et al., 2009), fungi (Budavaris, 1989) and cancer (Dinkov-Kostova, 2002).



Scheme 1 Epoxidation of safrole.

Impairment in drug diffusion is a major limitation in the evaluation of the antimicrobial effects of plant extracts using the agar diffusion method (Esimone et al., 2006). The antibacterial activities of safrole (**1**), safrole epoxide (**2**) and safrol hydroperoxide (**3**) showed that the growth of *B. subtilis* ATCC6633 was not inhibited by safrole and safrole epoxide, but safrole hydroperoxide inhibited the growth of *B. subtilis* ATCC6633, the diameter of inhibition zone was 20 mm (Fig. 1, Table 1). If the inhibition zone measures 2 and 3 mm, then the epoxide has a good antibacterial action. If the inhibition zone measures more than 3 mm across, then



Scheme 2 Mechanism of epoxidation reaction of safrole.

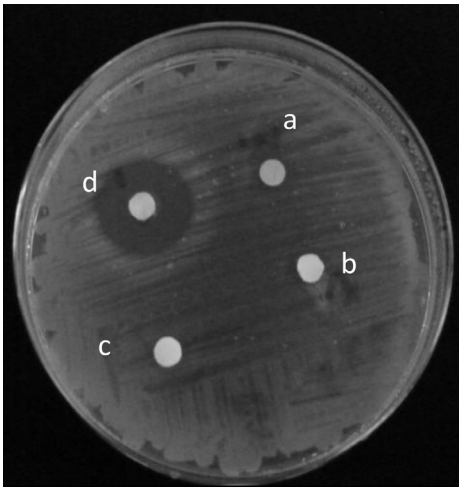


Figure 1 Effect of a – chloroform (control), b – safrole, c – safrole epoxide and d – safrole hydroperoxide on *B. subtilis* ATCC6633.

Table 1 Antibacterial activity of safrole, epoxide and safrole hydroperoxide.

Compounds	Diameter of inhibition zones (mm) (Mean ± SD)		
	Bacillus subtilis ATCC6633	*Staphylococcus aureus* ATCC25923	*Escherichia coli* ATCC25422
Safrole	0	0	6 ± 0.35*
Safrole epoxide	0	6 ± 0.70*	8 ± 0.00*
Safrole hydroperoxide	20 ± 0.70**	18 ± 0.70**	16.5 ± 1.41**
LSD	25.42	8.59	3.24

** Significant at 1%.
* Significant at 5%.

it is considered to be very effective, but if there is no inhibition zone then the complex has no activity on the bacterial growth and will not be retained for treatment (Baudoux, 2005). *S. aureus* ATCC25923 growth was not inhibited by **1** but inhibited by **2**, the diameter of inhibition zone was 6 mm, and also inhibited by **3**, the diameter of inhibition zone was 18 mm on Muller–Hinton agar medium (Fig. 2). The growth of *E. coli* ATCC25422 was inhibited by **1**, **2** and **3**, whereas **3** was the most effective against this bacteria, the diameter of inhibition zones were 6, 8 and 16,5 mm by **1**, **2** and **3**, respectively, on Muller–Hinton agar medium (Fig. 3).

The results obtained from this study showed that safrole, safrole epoxide and safrole hydroperoxide have strong antibacterial activities against Gram-negative bacteria *E. coli* ATCC25422, whereas the growth of Gram-positive bacteria *B. subtilis* ATCC6633 was inhibited by safrole hydroperoxide. Also, the growth of *S. aureus* ATCC25923 was inhibited by safrole epoxide, but it is more effective against Gram-negative bacteria *E. coli* ATCC25422, it may refer to the thickness of the bacterial cell wall. The wall of Gram-positive bacteria is thicker than Gram-negative bacteria, this may play a role in the effect of safrole and safrole epoxide on *E. coli* ATCC25422, or the growth of Gram-positive bacteria may be inhibited by more concentrations of safrole and safrole epoxide.

It can be concluded that safrole hydroperoxide was the most effective than safrole and safrole epoxide against Gram-positive bacteria *B. subtilis* ATCC6633, *S. aureus* ATCC25923, and Gram-negative bacteria *E. coli* ATCC25422. This result promoted us to believe that this compound is beneficial to human health, having the potential to be used for medical purposes.

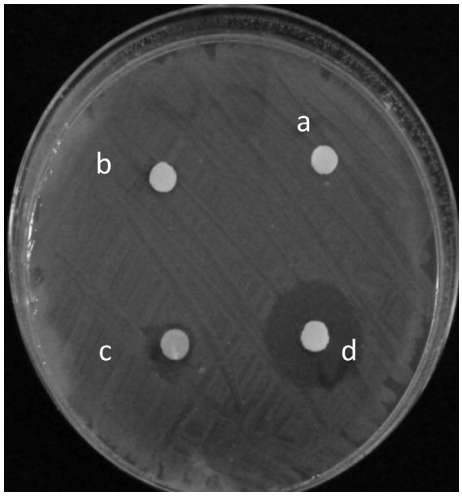


Figure 2 Effect of a – chloroform (control), b – safrole. c – safrole epoxide and d – safrole hydroperoxide on *S. aureus* ATCC25923.

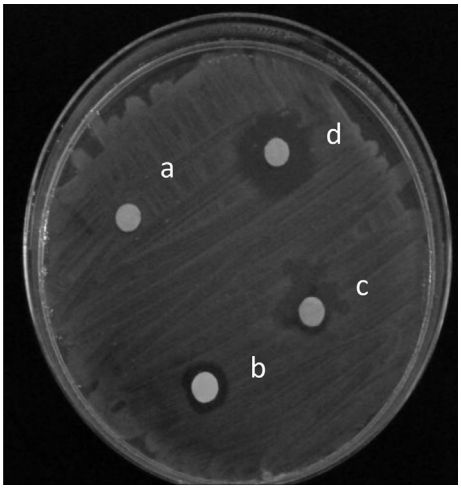


Figure 3 Effect of a – chloroform (control), b – safrole, c – safrole epoxide and d – safrole hydroperoxide on *E. coli* ATCC25422.

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