

Photosynthesis of Safrole Types Potential Intercalative and Oxidative Agent of DNA Through Photooxidation Reaction

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Abstract: Safrole [5-Allyl-benzo [d][1,3] dioxole] was isolated from essential oil of *sassafras* (*piper hispidinervum*) (*piperaceae*). It was subjected to photooxidation reaction in the presence of hematoporphrin, tetraphenylporphrin or rose Bengal as singlet oxygen sensitizer under irradiation of sodium light led to (E)-5-(3-hydroperoxyprop-1-1-enyl) benzo [d][1,3] dioxol (II). Probably, novel hydroperoxide is generated insitu upon irradiation of safrole in the presence of DNA, and they can be responsible for some adverse effects in living cells. Therefore, it seems to be relevant to elucidate biological consequences of hydroperoxide with DNA and other cell components.

Key words: safrole, photooxidation, hydroperoxide, singlet oxygen, hematoporphrin, tetraphenylporphrin and rose bengal

INTRODUCTON

Propenylbenzene are common aromatic compounds, these have been identified as components of numerous plants or their essential oils, which widely used as starting compounds for the production of various flavoring and fragrance chemicals Kim *et al.*, (1999) Plant phenylpropenoids undergo oxidation on exposure to air. The oxidation process is enhanced by irradiation to form hydroperoxide derivatives Andreoni *et al.*, (1995).

Such hydroperoxides are generated in situ upon irradiation of them in the presence of DNA and they can be responsible for some adverse effects in the living cells Elgendy and Khayyat, (2008).

Piper hispidinervum (C.DC.), (*piperaceae*) is promising source of sassafras oils, the source of safrole, currently derived from endangered plants of the *Lauraceae* such as *Ocotea pretios* Ness (Mez), *Cinamoum petrophilum*, *C. mollissimum*, and *Sassafras albidum* Nutt Xu *et al.*, (2007).

Safrole is an important raw material for the chemical industry. It has many fragrance applications in household products such as floor waxes, polishes, soaps, detergents and cleaning agents Siviero and Pimentel, (1997).

On the other hand, the chemistry of singlet molecular oxygen (1O_2), (which formed via photooxidation reaction) has received remarkable attention by chemists not only because of its interesting mechanistic and synthetic aspects but also because of its environmental and biomedical significance Stratakis and Orfanopoulos, (2000).

Taking into account important activities of plant phenylpropenoides especially safrole and the important application of it and its hydroperoxide derivative, in the present work, we believed it to be relevant to examine photooxygenation reaction of safrole.

Experimental:

Safrole [5-Allyl-benzo [d][1,3] dioxole](1) is the major component (80%) of the essential oil of *sassafras* (*piper hispidinervum*) (*piperaceae*) in its leaves. It can be easily extracted by hydrodistillation Carlson and Thompson, (1997) IR spectra were performed on a Perkin-Elmer 16 FPC FT-IR spectrophotometer as thin films. 1H - 1H Cosy NMR, 1H -NMR and ^{13}C -NMR spectra were obtained in $CDCl_3$ solution with a Bruker AVANCE D.P.X. 600 MHz apparatus. GCMS were determined by Joel JMS 600H, GC Hewlett Packerd, HP 6890 Series, with capillary column (30m x 0.32 mm x 0.25 mm) HP-5 cross linked 5% dimethyl polysiloxane. A sodium lamp (Phillips G/5812 SON) was used for photo-irradiation 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. Electrophoresis unit (HU10 with UV Transilluminator Model. M-15).

5-Allyl-benzo [d][1,3] dioxole Safrole (I):

Colorless liquid, bp: 232-234°C, C₁₀H₁₀O₂ (M 162.18). IR spectrum, ν cm⁻¹: 3075, 2975, 2893, 1607, 1121. ¹H -¹H Cosy NMR, ¹H-NMR spectra(CDCl₃), δ , ppm: 2.23 s (2H, C²H₂), 3.24 s (2H, C¹H₂), 5.06d (2H, 3[\]-H, J=6 Hz), 5.88 comp.pat. (1H, 2[\]-H), 6.63d (1H, 6-H, J=8 Hz), 6.67 s (1H, 4-H), 6.69d (1H, 7-H, J=8 Hz). ¹³C-NMR spectrum, δ ppm: 77.3 (C¹), 100.8 (C²), 108.3 (C⁴), 109 (C³), 115.7 (C⁷), 121.3 (C⁶), 133.7 (C⁵), 137.5 (C²), 146.3 (C³), 147.9 (C¹),

General Photooxygenation of safrole (I):

Solutions of **I** (10 mmol) in different solvents according to the type of sensitizers were irradiated externally by means of sodium lamp at -5°C. During the irradiation a continuous stream of dry oxygen gas was allowed to pass through the reaction mixtures at a slow rate to avoid evaporation of solvent. The solvent was evaporated at 20°C/15 Torr. The crude products were purified by column chromatography on silica gel adsorbent by eluting with a mixture of petroleum ether 60 – 80 °C and ether (8:2) Elgendy and Khayyat, (2008).

(E)-5-(3-hydroperoxyprop-1-enyl) benzo [d][1,3] dioxol (II):

pall yellow oil, C₁₀H₁₀O₄ (M 194.16). IR spectrum, ν cm⁻¹ : 3426, 3000, 2927, 1602, 1126. ¹H-NMR spectrum, δ , ppm: 5.95 s (2H, C²H₂), 6.00 br.s (2H, C¹H₂), 6.04 d (1H, 3-H, J=12Hz), 6.8 s (1H, 4[\]-H), 6.86 d (1H, 2-H, J=12Hz), 6.87 d (1H, 7[\]-H, J=8Hz), 6.921d (1H, 6[\]-H, J=8Hz), 10 S (1H, OOH). ¹³C-NMR spectrum, δ ppm: 68.2 (C¹), 90.6 (C²), 108.5 (C⁴), 109.7 (C⁷), 114.7 (C⁶), 125.1 (C²), 128.8 (C³), 129.4 (C⁵), 148.2 (C¹), 149.3 (C³). GC- Mass data; retention time 17.9 min, m/z (I_{rel} %): 194 (5) [M⁺], 177 (10) [M⁺-OH], 162 (15) [M⁺-O₂], 149 (100) [M⁺ - CO₂H].

Study on photochemical DNA damage by hydroperoxide II:

A solution of DNA in saline, 1ml, was added to a solution of 1mg of compound II in 5 ml of ethanol and the mixture was irradiated using a sodium lamp for 18 hours at 0°C. Sample was withdrawn at different times (every 2 hours) to determine the damaging effect by the gel electrophoresis technique Kochevar and Dunm, (1990). The photograph of the gel was taken under UV light (λ 365 nm). The results showed moderate degree of DNA damage in the presence of hydroperoxide II after irradiation for 8 hours, and high degree of DNA damage after irradiation for 18 hours (Fig 1).

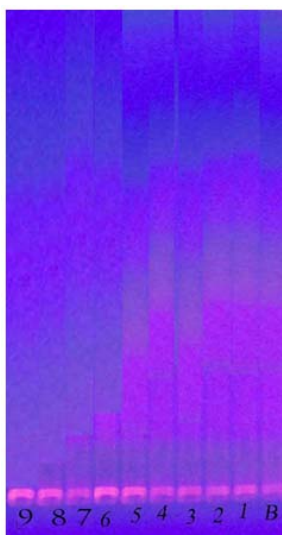


Fig. 1: Effect of safrole hydroperoxide II on DNA fragmentation

RESULT AND DISCUSSION

Safrole [5-Allyl-benzo [d][1,3] dioxole](I) is the major component (80%) of the essential oil of sassafras (*piper hispidinervum*) (*piperaceae*) in its leaves, which can be easily extracted by hydrodistillation Carlson and Thompson, 1997. They are proved to be active against many pathogenic bacteria Lien-Yu, *et al.*, (2009), fungi Budavaris, (1989) and cancer Dinkov-Kostova, (2002).

The chemical structure of **I** was confirmed by spectral measurements. The ¹H-¹H Cosy NMR and ¹H-NMR spectra of **I** showed two singlet at δ 2.23 and at 3.24 from protons in two methylene groups (C²H₂ and C¹H₂) respectively, doublet at δ 5.06 of methylene protons (C³H₂), complex pattern at δ 5.88 of H-2, doublet at δ 6.63 of proton H-6, singlet at δ 6.67 of proton H-4 and doublet at δ 6.69 ppm of proton H-7.

In ¹³C-NMR spectrum of **I**, the C¹, C², C³ and C² were located at δ_c 77.3, 100.8, 109 and 137.5 ppm, respectively.

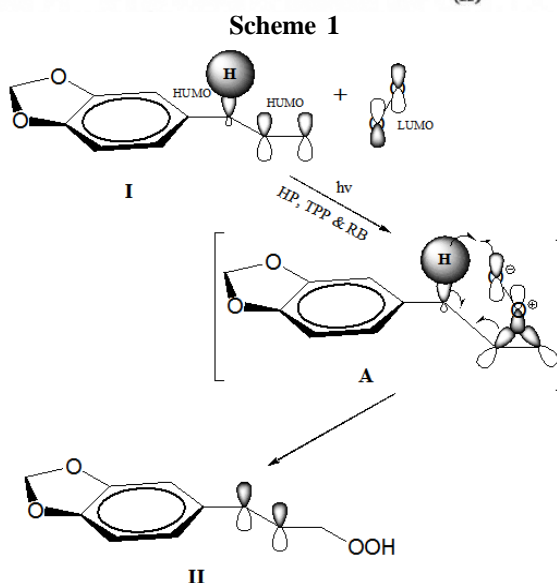
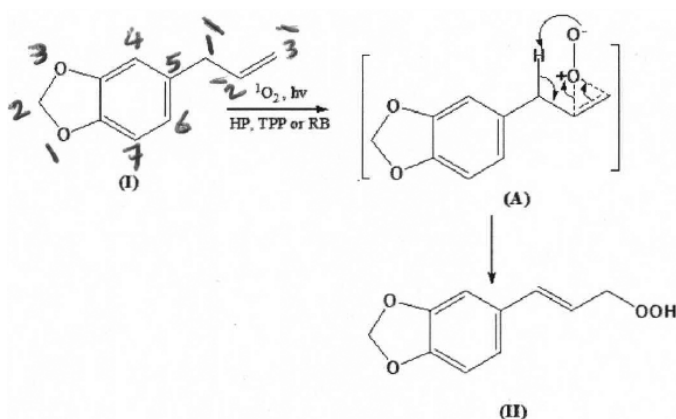
Interestingly, the photo induced oxygenation of safrole (**I**) in the presence of hemato porphin (HP), tetraphenyl porphin (TPP) or rose bengal (RB) as singlet oxygen sensitizers at -5°C led to the formation of (E)-5-(3-hydroperoxyprop-1-enyl) benzo [d][1,3] dioxol (**II**) in different yield as a sole product (scheme1,2) (table1).

The structure of photooxygenation product **II** was established by spectral measurements. IR spectrum of **II** showed signal at 3426.26 cm⁻¹ for OOH- group. Compound **II** displayed in ¹H-NMR spectrum a broad singlet at δ 6.00 for of the side- chain allylic two protons of C¹H₂, doublet at δ 6.04 of H-3, doublet at δ 6.86 of H-2, and a singlet at δ 10.00 ppm due to proton the hydroperoxide group. Signals at δ_c 125.1 and δ_c 128.8 ppm in the ¹³C-NMR spectrum of **II** were assigned to C² and C³. The hydroperoxide carbon atom resonated at δ_c 68.2 ppm.

Compound **II** was characterized by a retention time of 17.9 min (GC-MS), and its mass spectrum contained the molecular ion peak with m/z 194 ppm.

Table 1: Photooxygenation of safrole (**I**) in the presence of hemato porphyrin(HP), tetraphenyl porphyrin (TPP)and Rose Bengal (RB):

Initial Comp. no.	Solvent	Sensitizer	Reaction Time, h	Overall Yield, %
I	CHCl ₃	HP	14	45%
I	CHCl ₃	TPP	16	20%
I	EtOH	RB	19	10%



Scheme 1,2 illustrate a probable mechanism of photosensitized oxygenation of **II** in the presence of Hp,TPP or RB leading to formation of peroxirane intermediate A which undergo further breaking to form **II**.

It is known that some hydroperoxides cause photochemical DNA damage (Epe *et al.*, 1993; Elgendy and Khayyat, 2008). Therefore, compound **II** was tested for DNA-damaging activity.

For this purpose, a sample of DNA in saline was mixed with a solution of hydroperoxide **II** in ethanol and the mixture was irradiated using a sodium lamp. It has been concluded that Novel safrole hydroperoxide can be obtained by photosensitized oxygenation.

The results (see Experimental) clearly indicated high degree of DNA degradation in the presence of compound **II**, when the irradiation time was prolonged to 18 hours.

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