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Photostability of 2-hydroxymethyl-4,8-dibenzo [1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (NSC 656240), a potential anticancer drug

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Abstract

Stability studies of 2-hydroxymethyl-4,8-dibenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (NSC 656240, dithiophene), a poorly water-soluble (∼5 μg/ml) potential anticancer drug are reported. Dithiophene stability turned out to be very sensitive to laboratory fluorescent lighting. The rate of photodegradation of dithiophene was studied in aqueous solutions at room temperature (∼25 °C) at various pH values, in MeOH, CH_3CN, DMF, DMA, and in mixed nonbuffered aqueous/organic solutions. The aqueous pH-rate profile indicated no sensitivity to changing pH values. ^1H NMR and LC/MS methods were used to characterize the degradation products. Dithiophene photodegradation in the presence of air followed an apparent autoxidation pathway with dithiophene-2-aldehyde and dithiophene-2-carboxylic acid as the major degradants. The structures were confirmed against authentic samples. Dithiophene photodegradation under anaerobic conditions followed an apparent disproportionation pathway with only one identified major product, dithiophene-2-aldehyde.

Keywords: Dithiophene; Photo-oxidation; Photodegradation; Stability

1. Introduction

The purpose of this study was to determine some physical properties and the chemical stability of dithiophene, a potent anticancer drug candidate. Specifically, this paper describes some chemical stability studies performed on dithiophene, a drug whose degradation was found to be very sensitive to laboratory fluorescent lighting exposure.

Naturally occurring substituted anthraquinones (Chang and Lee, 1984; Zee-Cheng et al., 1979), naphthoquinones (Hyashi et al., 1987), furanonaphthoquinones (Rao and Kingston, 1982) and their synthetic analogues (Hyashi et al., 1987) were shown to possess cytotoxic antileukemic activities. The synthetic unsubstituted dithiophene derivatives of naphthoquinone (Goncalves and Brown, 1952; Tagawa and Ueno, 1978) and benzoquionone (Chao et al., 1998) also were found to be cytotoxic against epithelial cancer KB cells. Among all synthetic dithiophene derivatives of benzoquinone, 2-hydroxymethyl-4,8-dibenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (NSC 656240, dithiophene) displayed high

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potency in the melanoma cell panel with a mean log GI_{50} value less than −6.99 (Chao et al., 1999).

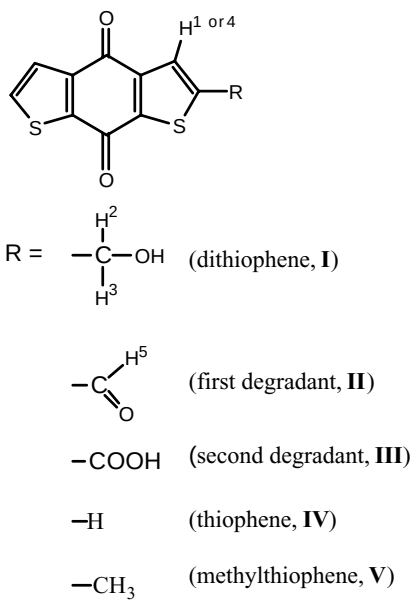
2. Materials and methods

2.1. Materials

NSC 656240 (**I**, dithiophene, 2-hydroxymethyl-4,8,-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene), NSC 656239 (**II**, dithiophene-2-aldehyde), NSC 656243 (**III**, dithiophene-2-carboxylic acid), NSC 149708 (**IV**, thiophene), and NSC 656238 (**V**, methyl thiophene) were supplied by the National Cancer Institute (see Scheme 1). All organic solvents were HPLC grade. The aqueous experiments were carried out in deionized, glass-distilled water. All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) and were of analytical grade.

2.2. HPLC assay

High-performance liquid chromatography (HPLC) was performed on a system consisting of a Shimadzu LC-6A pump, a general data processor Shimadzu C-R3A, and a Kratos Spectroflow 757 variable-wavelength detector operating at 340 nm. The HPLC method employed an Altech C_{18} reversed-phase analytical column (150 mm × 4.6 mm, 5 μm particle size). The column was operated at room temperature. All the analyses were performed under isocratic conditions with mobile phase consisting of an acetonitrile:H_2O mixture, 60:40 (v/v), with the flow rate 1 ml/min. Sample injection volume was 20 μl, and run time was 15 min. Calibration curves were linear ($r^2 > 0.99$). A typical chromatogram of dithiophene degradation when exposed to laboratory fluorescent lighting in the presence of air reveals three peaks at retention times of 3.3, 5.3, and 0.9 min, corresponding to dithiophene and two major degradation products **II** and **III**, respectively (see later discussion). Authentic samples of NSC 656239 and NSC 656243 had identical retention times to the two degradants and on co-injection the peaks coeluted.



Scheme 1. Chemical structure of dithiophene, its analogs and degradants.

2.3. Equilibrated solubility

Equilibrated solubility of dithiophene was determined by adding an excess of drug to various solvents, vortexing for 30 s and agitating in a water bath maintained at 25 °C for 24 h. Any suspension was then centrifuged. The aliquot of supernatant was diluted with HPLC mobile phase and analyzed for the drug content by HPLC.

2.4. Identification of major photodegradation products

The degradation of dithiophene in various deuterated solvents (MeOH-d_4, DMSO-d_6, *i*-PrOH-d_8) was followed by ^{1}H NMR and HPLC. Solutions of dithiophene in deuterated solvents with substrate concentration ∼0.5 mg/ml in NMR tubes were exposed to laboratory fluorescent lighting for several days. Periodically, 20 μl aliquots of solution were extracted from the NMR tube and subjected to HPLC analysis. The whole sample was analyzed by ^{1}H NMR spectroscopy carried out at ambient temperature using a Bruker AM-500 spectrometer.

The first and the second degradants were isolated using a preparative C_{18} column and an acetonitrile:H_2O (60:40, v/v) mobile phase. LC/MS spectra of the degradants were obtained on a Micromass Quattro Micro instrument using electrospray ionization (ESI).

The HPLC analysis was carried out with an Altech column, 1 ml/min flow rate and a CH_3CN:H_2O (60:40, v/v) mobile phase on a Waters 2690 system. The flow was split postcolumn with 200 μl/min going into mass spectrometer.

2.5. Kinetics of dithiophene degradation

The degradation of dithiophene in the presence and absence of laboratory fluorescent lighting at 25 °C was followed by HPLC and UV-Vis spectrophotometry. Solutions of dithiophene were prepared in duplicate at a concentration ~3 μg/ml in methanol, methanol–water, acetonitrile, acetonitrile–water and 3 mg/ml in DMF, DMF–water, DMA, DMA–water in sample vials. One set of vials was kept exposed to laboratory fluorescent lighting while the other was kept in the dark. The samples were not agitated. The laboratory fluorescent lighting intensity was not controlled during the experiment. The light intensity was checked, however, with ferrioxalate chemical actinometry from time to time. The results of actinometry measurements showed light intensity stability with time. Degradation of dithiophene at 25 °C was determined at various water contents in organic solvents ranging from 0.5 to 67%. HPLC analysis was accomplished by withdrawal of 30 μl samples from the sample vials exposed to light and protected from light at various predetermined times. UV-Vis spectra of samples exposed to light and protected from light between runs were recorded on a Perkin-Elmer spectrophotometer Lambda 6.

The reaction order with respect to dithiophene concentration was determined by running the degradation experiments at different initial concentrations of dithiophene (14, 27, 40 μg/ml in CH_3CN:H_2O, 60:40, v/v mixture). Apparent first-order rate constants for dithiophene degradation (3 μg/ml, 1% acetonitrile) at 25 °C were determined in aqueous buffer solutions at various pH values (3.0–8.3), concentrations (10, 25, and 50 mM) at the same ionic strength ($I = 0.15$ M NaCl). Citrate buffer was used in pH range 3.0–4.0, acetate buffer in pH range 4.0–5.0, phosphate buffer in 6–7.4 pH range, and TRIS buffer at pH 8.3. All solutions were incubated at 25 °C. The pH values were measured with an Accumet AR15 pH meter (Fisher Scientific). Reaction mixture aliquots were removed periodically and analyzed by HPLC.

2.6. Photo-oxidation measurements

Six milliliters of dithiophene solutions (~10 μg/ml) in oxygenated and deoxygenated water containing 10% (v/v) of CH_3CN were prepared and exposed to laboratory fluorescent lighting. A deoxygenated water:CH_3CN mixture was prepared by bubbling argon through the mixture for 15 min and argon was bubbled through one of the solutions during the whole experiment. Thirty-microliter aliquots were extracted from both vials periodically and were subjected to HPLC analysis.

2.7. Determination of reaction quantum yield

Actinometry was performed with the ferrioxalate chemical actinometer (Murov et al., 1993). The actinometric solution, 2 ml (0.2 M) aqueous $K_3Fe(C_2O_4)_3$, was pipetted into a 5 ml glass vial. Two milliliters of fresh dithiophene solution in 50% aqueous acetonitrile were pipetted into another vial. Both samples were irradiated with a laboratory fluorescent lighting for 40 min and were subjected to quantitative analysis. The amount of dithiophene degraded was calculated using HPLC data. To calculate the light intensity, 0.5 ml of irradiated aqueous $K_3Fe(C_2O_4)_3$ were mixed with 2 ml of the 0.2% 1,10-phenanthroline and 0.25 ml of acetate buffer in a 10 ml volumetric flask. The solution was diluted to the mark with water and the absorbance at 510 nm was measured. Using the absorbance measured and corrected for a blank non-irradiated solution, the light intensity was calculated (Murov et al., 1993). The dithiophene photodegradation quantum yield was calculated as follows:

$$\phi = \frac{\text{The no. of moles of dithiophene which decomposed}}{\text{The no. of Einsteins absorbed}}.$$

3. Results and discussion

3.1. Stability in different solvents

When dithiophene was maintained in a methanol solution exposed to ordinary laboratory fluorescent lighting, HPLC analysis of dithiophene showed that it had fully degraded within 24 h. The major degradant

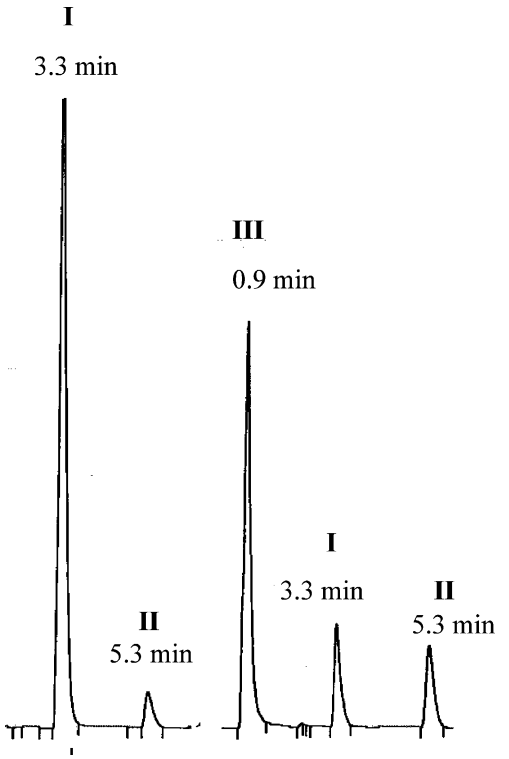


Fig. 1. HPLC chromatogram of dithiophene in aqueous CH_3CN (40% H_2O) in the presence of air while exposed to light (310–600 nm) for an hour (**1**) and 20 h (**2**). Peak numbers I–III refers to compounds **I–III** (Scheme 1).

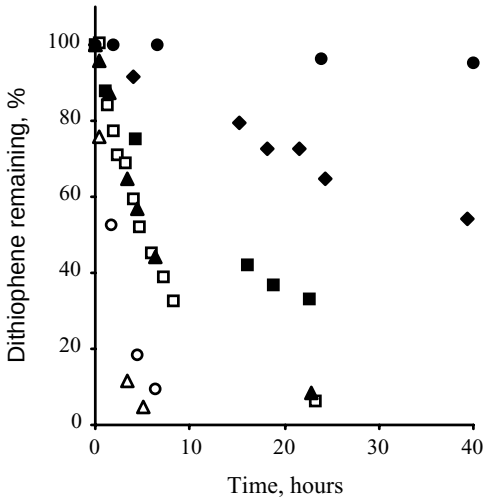


Fig. 2. Plot of dithiophene remaining (%) in various solvents as a function of light exposure time. Exposed to laboratory fluorescent lighting in the presence of air: (◆) in pure CH_3CN; (■) in 10% H_2O–CH_3CN; (□) in 67% H_2O–CH_3CN; (▲) in pure H_2O; (△) in 5% H_2O–MeOH; (○) in 40% H_2O–MeOH. Protected from light: (●) in 40% H_2O–MeOH.

peak (**II**) eluted around 5.3 min (Fig. 1). This solution, upon further exposure to light, resulted in further degradation to a more polar degradant (**III**) that eluted at or near the solvent front. Dithiophene appeared to be photo-reactive since the presence of light accelerated its degradation. The instability of the drug to light was observed in all solutions: in methanol, methanol–water, acetonitrile, acetonitrile–water, DMF, and DMA. Typical plots of the loss of dithiophene from irradiated solutions are presented in Fig. 2. Dithiophene degradation was solvent-dependent. It was faster in methanol than in acetonitrile. Presence of water in acetonitrile accelerated the degradation reaction and slowed down the degradation in methanol. The photodegradation was supported by changes in the UV-Vis spectra of the drug in 40% H_2O:MeOH solution. The rate of dithiophene degradation was faster in MeOH ($k_{obs} = 0.6\,h^{-1}$) than in H_2O ($k_{obs} = 0.1\,h^{-1}$) and in CH_3CN ($k_{obs} = 0.02\,h^{-1}$). The effect of protic solvents, methanol, and water, on the energy of excited states could be one of the reasons in the degradation rate difference in acetonitrile and in methanol and water. When a sample was protected from light, the observed UV spectra did not show any evidence of drug degradation for 3 days (Fig. 3A), while exposure to laboratory fluorescent lighting, the spectra changed significantly over 1 day (Fig. 3B). The 236 and 291 nm peaks decreased during exposure, the 332 broad peak remained almost unchanged while a new peak developed at 280 nm. No clear isosbestic points were detected, indicating that more than one product was formed. The dithiophene degradation was easily stopped by the removal from a light source. That is, the degradation was light catalyzed and not just light initiated.

The short-term stability of 3 mg/ml solutions in DMA, DMF, and 70% DMA/DMF 30% H_2O in the presence and absence of light was monitored by HPLC. The light-protected solutions have been analyzed for intact drug by HPLC after 1 and 10 days of storage. In all formulations, protected from light, dithiophene was quite stable, i.e. after 10 days all

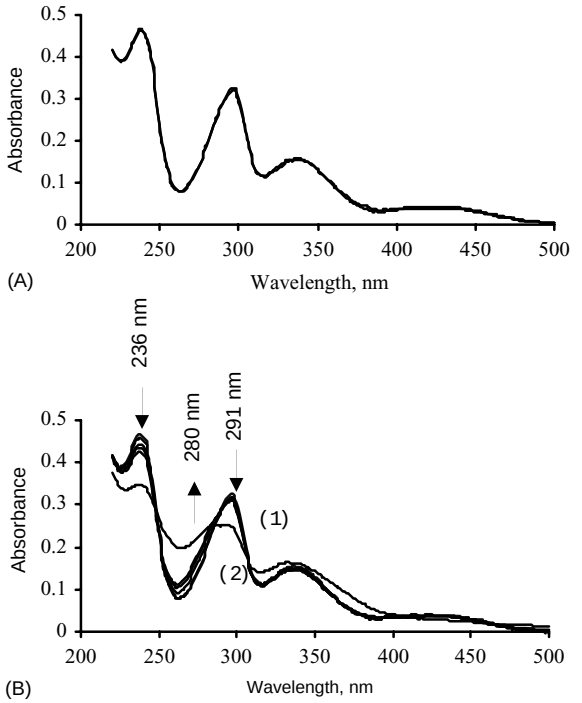


Fig. 3. (A and B) UV-Vis spectra of dithiophene in the presence of air in 40% H_2O–MeOH solution protected from light as a function of time (0–3 days) (A), and exposed to light as a function of time (0–10 h) (B).

formulations contained 100% intact drug. The samples stored at ambient temperature but exposed to light almost totally degraded within 24 h. Stability data are presented in Table 1. The presence of water accelerated the degradation reaction.

3.2. Identification of degradation products

Dithiophene degradation in different deuterated solvents was followed by ^{1}H NMR. Two new signals at 10 ppm (H^5 proton, **II** in Scheme 1) and 8.3 ppm (H^4 proton, **II** in Scheme 1) appeared and increased while a decrease of the signal at 4.7 ppm (protons $H^{2,3}$, **I** in Scheme 1) was assigned to the oxidation of the dithiophene alcohol group to the corresponding aldehyde (**II**) (Scheme 1). In fact, the ^{1}H NMR spectra of degradation product **II** and ^{1}H NMR spectra of dithiophene-2-aldehyde, described in the literature (Chao et al., 1999), were identical. Further oxidation of aldehyde produces dithiophene-2-carboxylic acid (**III**), which explains the presence of the hydrophilic second degradant, eluting at the solvent front. ^{1}H NMR data were confirmed by LC/MS. Mass spectrum of the first degradant **II**, *m/z* and relative intensities, 248 (M^-, 50), 247 (100), 219 (10) correspond to the mass spectrum of dithiophene-2-aldehyde (Chao et al., 1999). The mass spectrum of the second degradant, 264 (M^-), and 220 (M^-, 44), corresponds to mass spectrum of dithiophene-2-carboxylic acid (**III**). Both NMR and LC/MS results were confirmed by running authentic samples of **II** and **III** obtained from NCI.

3.3. Kinetics of dithiophene degradation

The kinetics of dithiophene degradation was studied in 25 mM aqueous buffer solutions as a function of pH. The degradation followed apparent first-order kinetics for at least three half-lives in the pH range 3–8. The pH-rate profile in 25 mM buffers in the 6–7.5 pH region showed some apparent sensitivity to pH where

Table 1
Stability of dithiophene in pure DMF, DMA and 70% aqueous DMA/DMF solutions at 25 °C in the presence and absence of light

Formulation	Conditions	Intact drug, 0 day of storage (%)	Intact drug after storage (%)
DMF	Light protected	100	100[a]
DMA	Light protected	100	100[a]
70% aqueous DMF	Light protected	100	100[a]
70% aqueous DMA	Light protected	100	100[a]
DMF	In the presence of light	100	1[b]
DMA	In the presence of light	100	30[b]
70% aqueous DMF	In the presence of light	100	1[b]
70% aqueous DMA	In the presence of light	100	9[b]

[a] The storage time was 10 days.
[b] The storage time was 24 h.

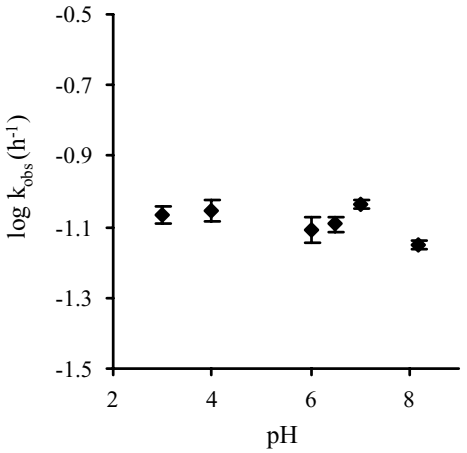


Fig. 4. pH-rate profile for dithiophene degradation in 25 mM buffers ($I = 0.15$ M NaCl) at 25 °C in the presence of light and air.

phosphate buffer was used. The effect of phosphate buffer concentration was studied at pH values 6, 6.5, and 7 and data were extrapolated to zero phosphate buffer concentration. The pH-rate profile is shown in Fig. 4. The pH-rate profile indicates no specific acid and base catalysis during the dithiophene degradation but there appeared to be an effect of phosphate buffer species HPO_4^{2-} on the dithiophene light stability. The cause of this sensitivity was not pursued further.

3.4. Mechanistic studies

Various approaches were used in an attempt to understand the mechanism of dithiophene degradation. These included stability studies of two analogous compounds, thiophene (**IV**) and methylthiophene (**V**), and the effect of oxygen on dithiophene degradation. The stability of **IV** and **V** was followed by HPLC and ^{1}H NMR methods under identical lighting conditions to determining the stability of **I**. Neither thiophene nor methylthiophene showed any evidence of degradation over 5 days when exposed to laboratory fluorescent lighting. Therefore, degradation of dithiophene, as indicated above, is consistent with changes occurring in the $–CH_2OH$ substituent of this molecule.

Degradation of dithiophene was found to be catalyzed by normal laboratory fluorescent lighting. The degradation was stopped by removal of the irradiation source. The main degradation products were an aldehyde and a carboxylic acid, clearly photo-oxidative products. The reaction quantum yield of the photo-decomposition of dithiophene in 50% aqueous acetonitrile solution under aerobic condition exposed to laboratory fluorescent light was found to be 0.020 ± 0.002. The dithiophene solution was exposed to light for 40 min to satisfy the quantum yield determination requirements that the change in drug concentration should not exceed 15% (Moore, 1987). The proposed mechanism of dithiophene degradation in water in the presence of air is given in Scheme 2. Irradiation of dithiophene with laboratory fluorescent lighting leads to an excited singlet state formation, which can undergo intersystem crossing (ISC) to form the first excited triplet (1). The excited triplet can then further undergo several reactions. The triplet can be quenched by ground state oxygen forming singlet oxygen and returning the excited triplet dithiophene to the ground state. In the excited triplet state, an intramolecular hydrogen transfer from the methylene group to the benzoquinone oxygen can occur, leading to the triplet biradical (2). Dithiophene biradical (2) reacts with ground state oxygen molecule producing a hydroperoxy biradical (3) that decomposes to the corresponding aldehyde (**II**). A further path leading to the dithiophene-2-carboxylic acid formation may involve a hydration reaction of the aldehyde. It is proposed that irradiation of dithiophene-2-aldehyde hydrate (4) leads to an excited singlet state, which undergoes in-

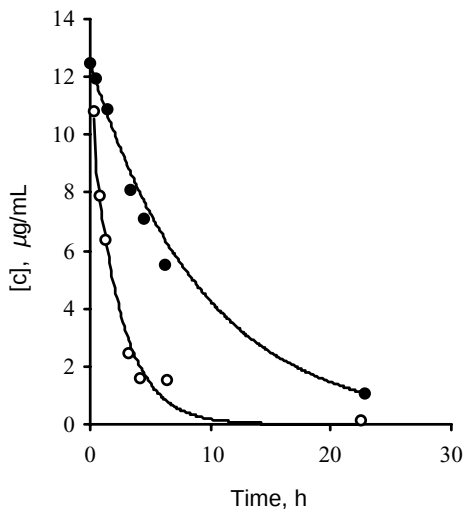
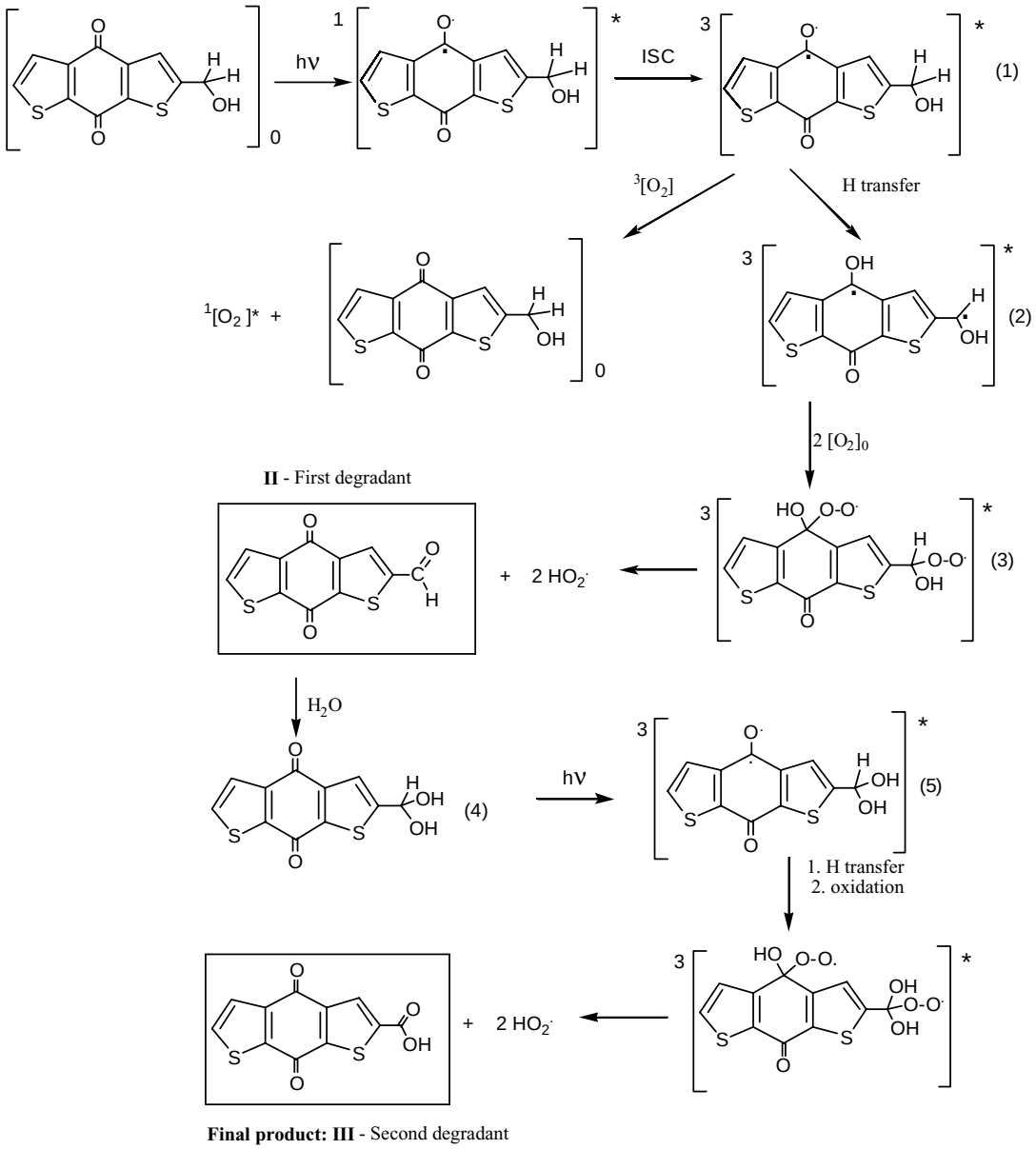


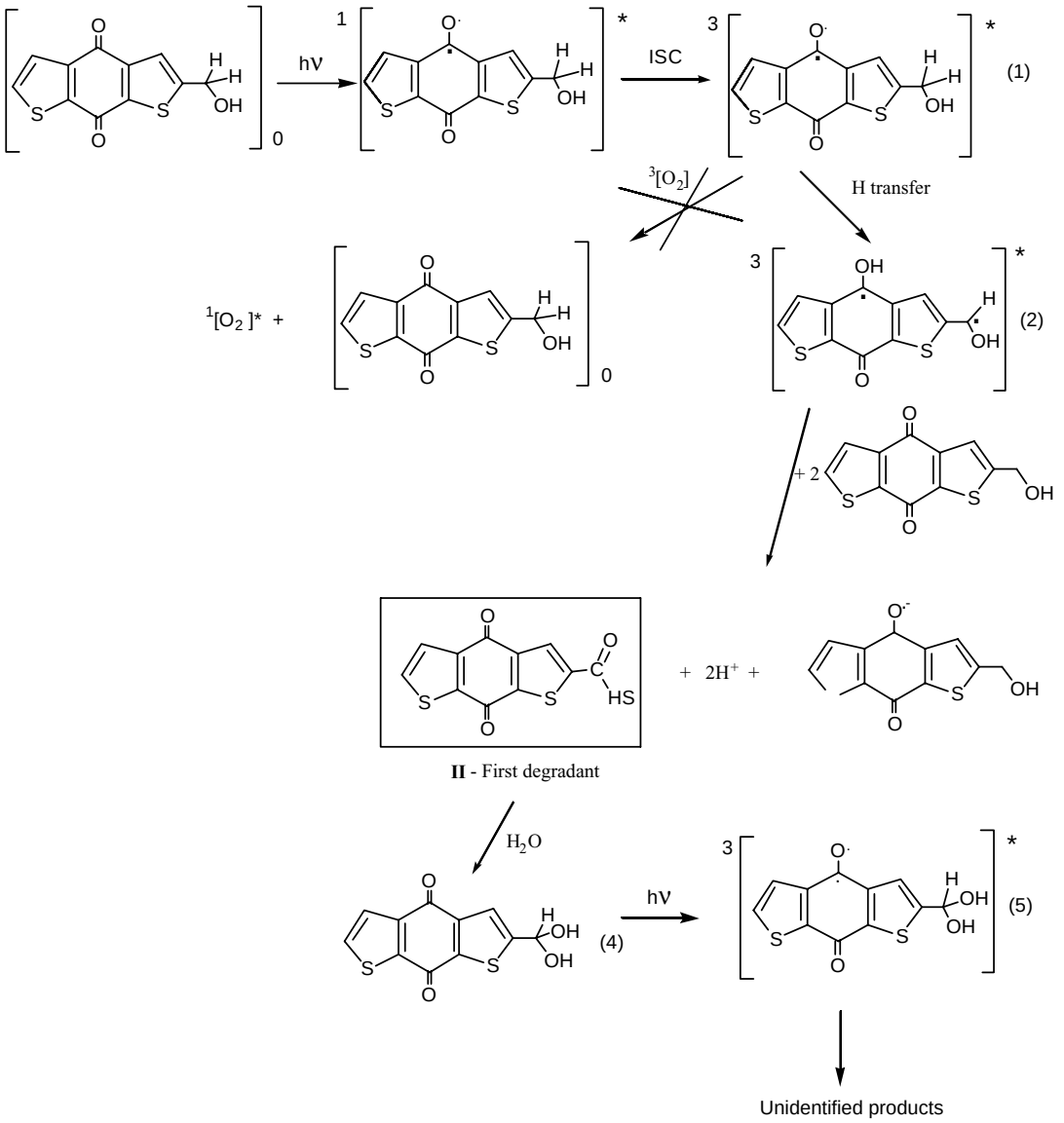
Fig. 5. Dithiophene photodegradation in air-saturated (●) and argon-saturated (○) 40% H_2O–MeOH solutions.



Scheme 2. Proposed mechanism of dithiophene photodegradation in the presence of air.

tersystem crossing to form the excited triplet (5). The next steps follow the same path as for triplet dithiophene. Hydrogen transfer, biradical and hydroperoxy biradical formation leads to dithiophene-2-carboxylic acid (**III**). Surprisingly, our investigation of dithiophene degradation in the presence of light but under anaerobic conditions shows that dithiophene degrades under argon three times faster than in the presence of air. Rates of dithiophene degradation in the presence and absence of argon are presented in Fig. 5. Under aerobic conditions, oxygen partially plays the role of a quencher for the excited dithiophene molecule. Under anaerobic conditions, in the absence of oxygen, the side reaction with oxygen is absent. The rate

of dithiophene degradation is faster but the pathway is likely different. The main reaction in the presence of oxygen is photo-oxidation. The dithiophene degradation under anaerobic conditions probably follows a disproportionation pathway and has a more complicated character. Comparison of HPLC data for dithiophene degradation in the same time frame in the presence and absence of air show faster accumulation of dithiophene aldehyde and to a larger extent in the absence of air. HPLC chromatogram of dithiophene degradation under anaerobic conditions showed only one major product with a retention time 5.3 min corresponding to aldehyde formation, and several byproducts with shorter retention times. These products were not identified. When samples exposed to anaerobic reaction conditions were then exposed to the air, one major peak with retention time of 0.83 min appeared on the HPLC chromatogram. That peak was



Scheme 3. Proposed mechanism of dithiophene photodegradation in the absence of air.

readily assigned to dithiophene carboxylic acid. The proposed mechanism under anaerobic conditions is shown in Scheme 3.

4. Conclusion

The results of this work show that dithiophene is a photosensitive compound. Irradiation of dithiophene with laboratory fluorescent lighting in the presence of oxygen causes photo-oxidation of the alcohol side chain to an aldehyde followed by further degradation to the carboxylic acid. The aldehyde is the main decomposition product under anaerobic conditions but the photodegradation pathway under these conditions is more likely due to a disproportionation reaction. Although in the presence of laboratory fluorescent lighting dithiophene degrades to the corresponding dithiophene-2-aldehyde and the dithiophene-2-carboxylic acid; it has sufficient short-term stability if protected from light. The dithiophene photodegradation is light catalyzed and not just light induced.

Acknowledgements

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