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A SELF-SENSITIZED PHOTOREACTION OF RHODACYANINE DYE, MKT 077

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Abstract : A rhodacyanine dye, 1-ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]-methyl] pyridinium chloride (MKT 077, **1**) was oxidized by self-sensitized singlet oxygen to produce two carbonyl compounds under photo-radiation, one of which was further rearranged via Norrish Type I like cleavage to give new types of merocyanine dyes (**5** and **8**). The merocyanine dyes were also synthesized by the treatment of thioamide (**6**) with oxalyl chloride.

MKT 077 (1-ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]-methyl] pyridinium chloride, **1** in Figure 1), which is structurally categorized in rhodacyanine dye, is an antitumor agent which selectively inhibits mitochondrial function and it has been in the phase I clinical trial. We demonstrated that compound **1** exhibited a selective antitumor activity against several human carcinoma cell lines, in spite of low toxicity against normal epithelial cell line CV-1, and it was efficacious in tumor-bearing nude mice models[1]. Mechanistic studies also revealed that the inhibition of respiratory activity in mitochondrial membrane fragments in a dose-dependent manner was the cause of this selective antitumor activity of compound **1**[2].

The putative decomposition products within a period of storage and administration must be identified and their pharmacological properties also must be evaluated in order to qualify for clinical use. Compound **1** might undergo a self-sensitized photoreaction resulting in the formation of free radical species or singlet oxygen that might induce its decomposition. This is due to the fact that the rhodacyanine dyes were originally developed as sensitizers for the photographic systems in which the electron-transfer from their triplet excited state to the ground state of silver halides took place. In this paper, we have focused on the analytical study of a novel self-sensitized photoreaction of rhodacyanine dye **1** and the synthesis of its photoreaction products.

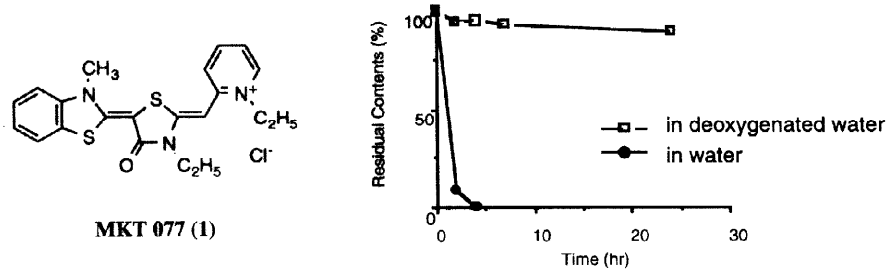

Figure 1. Chemical structure of MKT 077 (**1**) and influence of oxygen on stability of **1** against light.

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Firstly, we examined the stability of compound **1** in an aqueous solution which was exposed to fluorescent light, and the effect of oxygen on it, as well. The aqueous solution (0.1 mg/mL) was exposed to fluorescent light (20,000 lux) at room temperature, and the reaction products were analyzed by using high performance liquid chromatography (HPLC). Compound **1** decomposed in 4 hours (Figure 1) producing three major decomposition products which were detected by HPLC (Figure 2). In contrast, only 10 % of compound **1** in deoxygenated aqueous solution (O_2 <10 ppm) decomposed in 24 hours (Figure 1). This suggested that the self-sensitized singlet oxygen produced by the energy transfer from triplet exited state of **1** was involved in the process of decomposition. Singlet oxygen supposedly adds to either or both conjugational carbon-carbon bonds of **1** to produce dioxetanes, which are unstable intermediates that undergo further fragmentation to two carbonyl compounds.

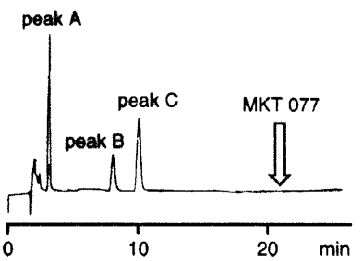


Figure 2. HPLC chromatogram of the decomposition products[3].

The two putative decomposition products, 3-methylbenzothiazol-2-one (**2**)[4] and 3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-thiazolin-2,4-dione (**3**)[5] (Figure 3), were synthesized and then compared with the decomposition products in Figure 2. HPLC analysis and spectroscopic studies (MS, UV) indicated that peak C was compound **2**[6], while compound **3** did not correspond to any peak. This result suggested that the 1,2-addition of singlet oxygen occurred on the carbon-carbon double bond between the benzothiazole and 4-oxothiazolidine, and that compound **4** to be the counterpart product of the fragmentation (Figure 3).

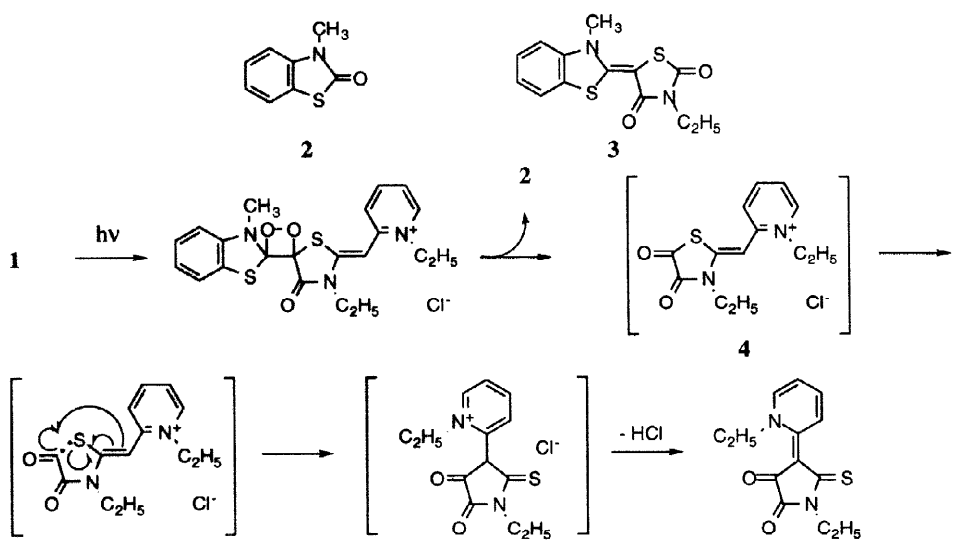
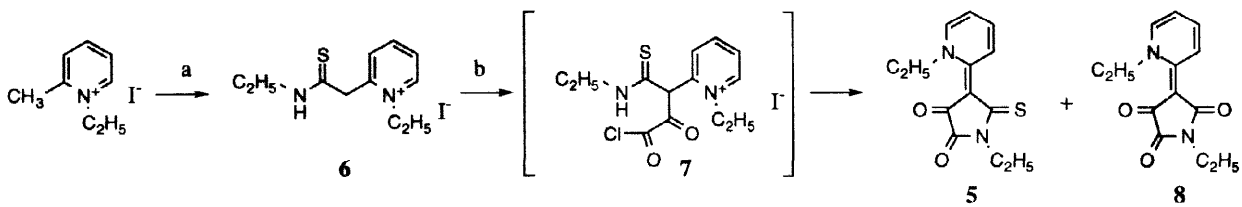


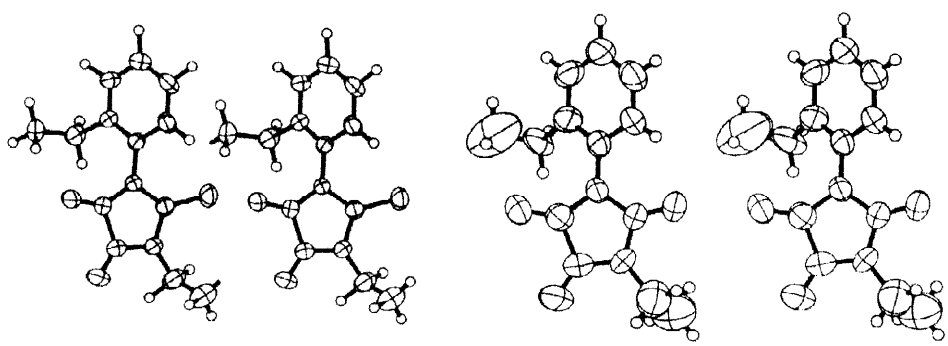
Figure 3. Chemical Structure of the putative decomposition products (**2** and **3**), and proposed mechanism of photochemical transformation from **1** to **5**.

^{1}H-NMR spectra of peak A and B did not exhibit any characteristic methine proton of compound **4** (5-6 ppm), after being purified by using fractional HPLC. This suggested that the putative photoreaction product **4** was unstable under this condition and that it was further fragmented. The plausible mechanism for the photochemical transformation for **4** is presented in Figure 3 because the putative unstable intermediate **4** has thiolactone moiety. The mechanism involves the radical pair intermediate; acyl and thiyl radicals formed by the Norrish Type I like cleavage of the C-S bond upon irradiation of light[7], which undergoes the rearrangement to produce the product **5**.

We tried to synthesize the putative compound **5** in attempt to prove the validity of the photoreaction mechanism illustrated in Figure 3. As shown in Scheme 1, the treatment of compound **6**, which was obtained by the reaction of 1-ethylpyridinium iodide and ethyl isothiocyanate, with oxalyl chloride gave two products by HPLC analysis. These two products were found to be chromatographically (HPLC) and spectroscopically (MS, ^{1}H-NMR, UV–Vis) identical to peaks A and B shown in Figure 2. Although the spectroscopic data did not elucidate on the structure of the two products completely, the X-ray crystallography studies revealed that peaks A and B were compounds **8** and **5**, respectively (Figure 4). The activated methylene group was supposed to react with oxyalyl chloride to produce the intermediate **7**, which spontaneously underwent cyclization to produce compounds **5**[8] (31.5 % from **6**) in the reaction of the thioamide **6** with oxyalyl chloride under basic condition. Compounds **8**[9] was a by-product (3.5 %) which was supposed to be produced by in situ hydrolysis of compound **5**.



Scheme 1. a. $C_2H_5NCS/NaH/THF$; b. $(COCl)_2/NEt_3/CH_3CN$



Compound **5** Compound **8**

Figure 4. X-ray determined structure of compounds **5** (left) and **8** (right).

In conclusion, the results of our study confirmed the proposed photochemical transformation mechanism of rhodacyanine dye **1**, as illustrated in Figure 3. Such a photochemical reaction involves the formation and the cleavage of oxetane intermediate, Norrish Type I like C-S bond cleavage and rearrangement, and that it gives new types of merocyanine dyes involving the conjugation system from the nitrogen atom to the carbonyl group, which were independently synthesized by ring-closure with thioamide **6** and oxyalyl chloride. The photoreaction products compounds; **2**, **5**, and **8**, are now being evaluated for their pharmacological properties in detail.

Acknowledgement

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References and Notes

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2 a) Modica-Napolitano, J. S.; Koya, K.; Weisberg, E.; Brunelli, B. T.; Li, Y.; Chen, L. B. *Cancer Res.* **1996**, *56*, 544-550. b) Weisberg, E.; Koya, K.; Modica-Napolitano, J.; Li, Y.; Chen, L. B. *Cancer Res.* **1996**, *56*, 551-555.

3 HPLC condition: column; Tosoh Tsk-Gel ODS-80 Tm (4.7 × 150 mm), eluent; methanol/water = 2/3 containing 0.2 % acetic acid/triethylamine (flow rate; 1.0 mL/min).

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5 Compound **3** was synthesized by alkaline hydrolysis of 3-Ethyl-5-(3-methylbenzothiaolin-2-yliden)-2-methylthio-4-oxothiazolinium *p*-toluenesulfonate, which was the intermediate of MKT 077 (**1**) (see reference 1.b).

6. The mass number and the absorption spectrum of peak C were obtained by using LC-MC (Thermoquest Co., TSQ7000) and a multichannel HPLC (Shimadzu Co., SPD-10AV), respectively.

7 a) Sakamoto, M.; Takahashi, M.; Moriizumi, S.; Yamaguchi, K.; Fujita, T.; Watanabe, S. *J. Am. Chem. Soc.* **1996**, *118*, 8138-8139. b) Penn, J. H.; Liu, F. *J. Org. Chem.* **1994**, *59*, 2608-2612.

8 ^{1}H-NMR data for **5** (300 MHz, $CDCl_3$); 1.39 ppm (t, J = 7.9 Hz, 3H), 1.60 ppm (t, J = 7.9 Hz, 3H), 4.12 ppm (q, J = 7.9 Hz, 2H), 4.71 ppm (q, J = 7.9 Hz, 2H), 7.78 ppm (dd, J = 7.6, 7.6 Hz, 1H), 8.18 ppm (dd, J = 7.6, 7.6 Hz, 1H), 8.33 ppm (d, J = 7.6, 7.6 Hz, 1H), 8.60 ppm (d, J = 7.6 Hz, 1H). HRMS (EI) for $C_{13}H_{14}N_2O_2S$ calcd, 262.0776; found 262.0771.

9 ^{1}H-NMR data for **8** (300 MHz, $CDCl_3$); 1.30 ppm (t, J = 7.9 Hz, 3H), 1.52 ppm (t, J = 7.9 Hz, 3H), 3.70 ppm (q, J = 7.9 Hz, 2H), 4.70 ppm (q, J = 7.9 Hz, 2H), 7.31 ppm (dd, J = 7.6, 7.6 Hz, 1H), 7.91 ppm (dd, J = 7.6, 7.6 Hz, 1H), 8.09 ppm (d, J = 7.6, 7.6 Hz, 1H), 8.21 ppm (d, J = 7.6 Hz, 1H). HRMS (EI) for $C_{13}H_{14}N_2O_3$ calcd, 246.1004; found 246.1013.