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單一狀態 酸素의 트랩劑*

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A Singlet Oxygen Trapping Agent

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요 약. 빌리루빈과 옥소디피로메텐들의 광산소화 분해 반응속도를 측정하던중 옥소디피로메텐들이 디페닐이소벤조퓨란보다 좋은 단일상태 산소의 트랩제라는 사실을 알게 되었다. 빌리루빈과 그 모델물질인 옥소디피로메텐들은 아주 빠른 속도로 단일상태 산소와 반응하거나 퀜칭하였다. 한 새로운 옥소디피로메텐이 간단한 방법으로 합성되었다.

ABSTRACT. Measuring the reaction rate of bilirubin and oxodipyrromethenes with signlet oxygen, we have found oxodipyrromethenes to be better singlet oxygen trapping agents than diphenylisobenzofuran, the best such agent known so far. The photooxygenation rates of bilirubin and the model compounds, oxodipyrromethenes approached the diffusion control threshold. A new oxodipyrromethene is synthesized.

1. INTRODUCTION

In the related study of phototherapy of neonatal jaundice, we recently reported the reaction rates of bilirubin IX-α (BR, or **1**) and the related tetrapyrroles[1] with singlet oxygen generated by a sensitizer, rose benagel, and light. While measuring photooxygenation rates of bilirubin and oxodipyrromethenes in methanol, we have found oxodipyrromethenes to be better singlet oxygen trapping agents than diphenylisobenzofuran, the best such agent known so far.

Matheson and collaborators[2] reported that diphenylisobenzofuran (DPBF, **2**) was the best singlet oxygen trapping agent.

In this report the reaction rates of bilirubin IX-α (BR), oxodipyrromethenes (ODPM or **3**, **4**) and DPBF with singlet oxygen ($^1O_2$) generated thermally and photochemically are compared.

The simple synthetic method of oxodipyrromethene is included.

2. RESULTS AND DISCUSSION

Synthesis of 5′-oxo-3′-ethyl-4′, 3, 5-trimethyl-1′, 5′-dihydro (2. 2′)-dipyrromethene (3). The key step was the condensation of 2-bromomethylene-3-ethyl-4-methyl-3-pyrrolin-5-one

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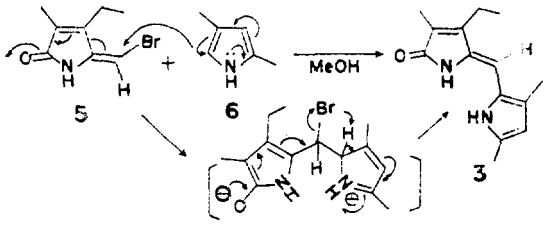
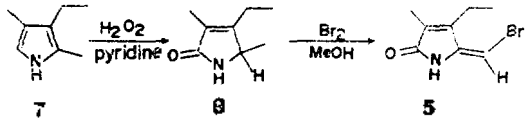
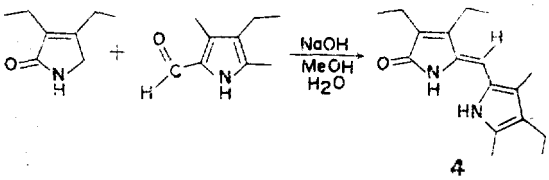
(5) with 2,4-dimethylpyrrole(6) to give 3 (60 %). This is probably a substitution reaction as shown below (see experimental section for identification).



The two-step synthesis[3] of 5 involved the oxidation of kryptopyrrole (7) with hydrogen peroxide and bromination of the product (8).



Synthesis of 5′-oxo-3′,4,4′-triethyl-3,5-dimethyl-1′, 5′-dihydro-(2.2′)-dipyrromethene(4).[4] The oxodipyrromethene 4 was prepared by base-catalyzed condensation of kryptopyrrole aldehyde with 3,4-diethyl-3-pyrrolin-2-one in 52 % yield.



Determination of Reaction rate (Photochemical Method). Since the generation of singlet oxygen by a photosensitizer was known already, the mechanism for the reaction of singlet oxygen can be drawn as follows[5].

$$S+{}^1O_2 \xrightarrow{k_R} \text{product (reacticn)}$$

$$S+{}^1O_2 \xrightarrow{k_Q} S+{}^3O_2 \text{ (quenching)}$$

$${}^1O_2 \xrightarrow{k_d} {}^3O_2 \text{ (decomposition)}$$

where S is substrate (*e.g.* BR. 3 or 4)

The substrate disappearance rate for the mechanism can be formulated as shown below.

$$-\frac{d[S]}{dt}=K\left(\frac{[S]k_R}{(k_R+k_Q)[S]+k_d}\right) \quad (1)$$

$$-[\Delta S]^{-1}=(K\Delta t)^{-1}\left(\frac{k_R+k_Q}{k_R}+\frac{k_d}{k_R}[S]^{-1}\right) \quad (2)$$

where K is the rate of ${}^1O_2$ formation. Since $K=I_a\Phi_{isc}f_{^1O_2}$, substiting for K and rearranging gives equation 3.

$$-\left(\frac{[\Delta S]}{I_a\Delta t}\right)^{-1}=(\Phi_{isc})^{-1}\left(\frac{k_R+k_Q}{k_R}+\frac{k_d}{k_R}[S]^{-1}\right) \quad (3)$$

where I_a=rate of absorption of light by the sensitizer in mole quanta/sec; Φ_{isc}=triplet quantum yield of the sensitizer, $f_{^1O_2}$=yield of ${}^1O_2$ from triplet sensitizer=1[6~8]. A plot of $([\Delta S]/I_a\Delta t)^{-1}$ $VS\cdot[S]^{-1}$ will give a linear plot if $[\Delta S] \ll [S]$. The ratio of slope to intercept is k_d/k_R+k_Q and the reciprocal of the intercept is Φ_{isc} k_R/k_R+k_Q. k_d value for ${}^1O_2$ is known for methanol solvent (1.4×10^5 S^{-1})[6] and this k_d in methanol was used for k_R+k_Q determination. I_a could be measured by Rinecke's salt actinometry[9]. The Φ_{isc} of rose bengal in methanol (0.76)[7] was used for separation of k_R and k_Q.

The physical and chemical reaction rates of bilirubin (BR) and oxodipyrromethenes(ODPM) with ${}^1O_2$ are reported in *Table* 1.

Table 1. Physical and chemical rate constants for BR and ODPM with ${}^1O_2$.

Substrate	$\frac{k_R}{10^9\times k_R, M^{-1}S^{-1}}$	$\frac{k_Q}{10^9\times k_Q, M^{-1}S^{-1}}$
Bilirubin IX-α	0.28	1.8
	0.43*	1.8*
3	0.64	0.50
4	1.4	1.3
DPBF	0.5**	

*Foote's value in chloroform[5]
**Foote's value in methanol[5,11]

For comparison, several known k_R and k_Q values of bilirubin are reported in the table. Our k_R and k_Q values of bilirubin are essentially the same as Foote and Ching's values[5]. Bilirubin IX-α is reactive to $^1O_2$ but quenches $^1O_2$ somewhat more effectively. This efficiency of the chemical reaction and physical quenching of bilirubin may explain the fact that the phototherapy of neonatal jaundics is effective and that untoward side effect are rare. It may be noted that the oxodipyrromethenes are reactive toward $^1O_2$.

The k_R values ($1.4\times10^9 M^{-1}S^{-1}$) of th dipyrroles are very significent because they are the best singlet oxygen trapping agents. The k_R value of DPBF, which was previously known as the best singlet oxygen acceptor, is $0.5\sim0.7\times10^9 M^{-1}S^{-1}$.[10]

Rio[11] reported that reaction of DPBF with $^1O_2$ gave the photooxide of 1, 3-diphenylisobenzofuran, which decomposed to several products in various conditions. However photooxygenation products of oxodipyrromethenes are not completely known[12].

Determination of Reaction Rates (by Thermal Method). Singlet oxygen was generated with reaction of sodium hypochlorite and hydrogen peroxide in methanolic solution[13]. Relative decomposition rates of substrate in methanolic solution of sodium hypochlorite and hydrogen peroxide are shown in *Table* 2.

Oxodipyrromethene **4** is also more reactive to $^1O_2$ than DPBF or BR in this condition.

Table 2. Change of substrate *vs.* reaction time at 25 °C.

Subst.	Change (%)		
	1 min	3 min	5 min
BR	3.8	10.4	16.5
DPBF	5.2	16.3	21.6
4	18.8	51.4	67.2

3. EXPERIMENTAL

General. The dipyrroles used for photooxidation were prepared in this laboratory. The bilirubin and DPBF used for kinetic studies were purchased from Matheson. Solvents were reagent grade unless otherwise specified. Melting points were determined on a Thomas-Hoover unimelt capillary apparatus and were uncorrected. Nuclear Magnetic Resonance (nmr) spectra were measured in deuteriochloroform on a Varian A-60, perkin-Elmer R-24B spectrometer. Chemical shifts were reported in parts per million (δ) downfield from TMS as an internal standard. Mass spectra were determined on a Jeol JMS-07 instrument at 70 eV. Visible and UV spectra were recorded on a Cary-14 spectrophotometer. Infrared spectra were obtained from sample in chloroform with a Beckman IR-8 spectrophotometer. Kinetic photooxygenation studies were accomplished in uv cell (1 cm path, 3 m*l*) using 10 nm bandpass monochromatic light from a Bausch and Lomb monochromator (Model 33-86-07) equipped with a 15 W tungsten lamp.

Preparation of 2, 4-Dimethylpyrrole (6).[16] 2, 4-dimethylpyrrole was obtained from base-catalyzed hydrolysis and decarboxylation of 2, 4-dimethyl-3, 5-dicarboethoxypyrrole in 33% yield, b. p 61~67 °C/11mmHg (*lit.*[16] 58°C/9 mmHg)

Preparation of Kryptopyrrole (7).[4] Kryptopyrrole (**7**) was prepared by the Wolff-Kishner reduction of 3-acetyl-5-carboethoxy-2, 4-dimethylpyrrole in 67 % yield, b. p 77~82 °C/7 mmHg (*lit.*[14] 86 °C/11 mmHg, 61~66 %).

Preparation of 4-Ethyl-3, 5-dimethyl-3-pyrrolin-2-one (8). 4-Ethyl-3, 5-dimethyl-3-pyrrolin-2-one (**8**) was obtained by method of Fisher, *et al.*[15] in 54 % yield, m. p 83 °C (*lit.*[17]

m. p 83 °C).

Preparation of 2-Bromomethylene-3-pyrrolin-5-one (5). 2-Bromomethylene-3-pyrrolin-5-one was prepared by bromination[3] of pyrrolinone **8** in 58 % yield, m. p 137~141 °C (*lit.*[3] 139~141 °C, 54 %); nmr ($CDCl_3$) 1.11 (*t*, 3H, J=7.5Hz, CH_3), 1.83 (*s*, 3H, CH_3), 2.40 (*q*, 2H, J=7.5Hz, CH_2), 5.90 (*s*, H, =CH), 7.40 (*br. s.* 1H, NH); uv (95 % EtOH), λ_{max} =282nm, $\varepsilon_{282}=1.9\times10^4$ (*lit.*[3] $\lambda_{max}=282$nm, $\varepsilon_{282}=1.8\times10^4$); and ir ($cm^{-1}$, $CHCl_3$), 3475 (ν_{NH}), 3125 ($\nu_{=CH}$), 1710 ($\nu_{C=O}$), 1650 ($\nu_{C=C}$).

Synthesis of 5′-oxo-3′-ethyl-4′, 3, 5-trimethyl-1′, 5′-dihydro-(2. 2′)-dipyrromethene (3). 2-Bromomethylene-3-ethyl-4-methyl-3-pyrrolin-5-one (4 g, 18.8 mmole) in 83 m*l* methanol was added to 2,4-dimethylpyrrole (2.0 g, 21 mmole) in a 150 m*l* flask. The mixture was heated at reflux for 1.5 hr under nitrogen and then cooled to -5°C.

The fish egg-like solid was filtered and dried (2.6 g, 60 %). The crude product (1.3 g) was dissolved in chloroform (360 m*l*) and washed with 10 m*M* NaOH solution. The chloroform soltion was dried by addition of anhydrous sodium sulfate. After evaporation of the solvent, the residue was crystallized from benzene to give yellow needles, m·p 245~246 °C : nmr ($CDCl_3$) 1.13 (*t*, 3H, J=8Hz, CH_3), 1.93 (*s*, 3H, CH_3-sp^2), 2.15 (*s*, 3H, CH_3-sp^2), 2.43 (*s*, 3H, CH_3-sp^2), 2.45 (*q*, 2H, J=8 Hz, CH_2), 5.77 (*m*, 1H, CH,) 6.05 (*s*, 1H, =CH ; mass spectrum, *m/e* (rel. intens.), 230 (M^+, 100 %), 215 (57 %), 200 (36 %), 187 (21 %); uv (methanol), $\lambda_{max}=407$ nm, $\varepsilon_{407}=3.4\times10^4$; (chloroform), $\lambda_{max}=398$nm, $\varepsilon_{398}=3.3\times10^4$; and ir ($cm^{-1}$, chloroform) 3400 ($\nu_{NH}$), 1670 ($\nu_{C=O}$), 1640 ($\nu_{C=C}$); (in KBr), 3370 ($\nu_{NH}$), 1660 ($\nu_{C=O}$), 1625 ($\nu_{C=C}$).

Anal. Calcd. for $C_{14}H_{18}N_2O$: C, 73.01; H, 7.88; N, 12.16. Found: C, 73.06; H, 7.94; N, 12.36.

Photochemical Kinetics. About 1 mg of substrates (*e. g.* **3**, **4**, bilirubin, *etc.*) was dissolved in methanol in a 25 m*l* volumetric flask.

Aliquots of 1, 1.5~3.5 m*l* of the solution were withdrawn and diluted to 10 m*l* with the rose bengal (RB)/methanol solution. The final concentrations of substrate (*e. g.* **3**, **4** bilirubin, *etc.*) were determined by the substrate extinction coefficient and the absorption spectra of the substrate. The concentration of rose bengal for all solutions was the same (4.0×10^{-5} *M*, fraction of absorption of light at 557nm=1). Exactly 2 m*l* of each solution was placed in a 10 nm path quartz cuvette and the uv or visible spectrum was taken.

The absorbance of the substrate was corrected by subtracting the absorbance of RB, even though the rose bengal absorption was weak at the absorption maximum of all substrates (2.5~5.0 %). Next, the solution in the cuvette was irradiated at 557 nm (monochromatic light, tungsten lamp) in methanol for an appropriate time period (5~10 % substrate concentration change). (The λ_{max} of RB is 557nm in methanol).

The light intensity was measured with potassium Reinecke's salt actinometry before and after the photooxygenation reaction. The potassium Reinecke's salt actinometry solution (2 m*l*, 0.016 *M*, pH 3.5~5.5) was placed in 1 cm path length quartz cuvette (same cell as for the photooxygenation) and the absorbance of the solution at 557 was checked to determine the fraction of light absorption.

If $\log I_0/I \geqslant 1$, the fraction of light absorption is 1. Then the solution in the cuvette was irradiated at 557 nm with monochromatic light (tungsten lamp) for about two hrs. The solution was shaken every five min. during the irradiation. After the irradiation period, the solution was shaken, then taken to the dark-

room. A 0.5 m*l* aliquot of the irradiated solution was diluted with 1.5 m*l* of 0.1 *M* $Fe(NO_2)_3$. $HClO_4$ solution in a clean cuvette (4.04 g $Fe(NO_3)_3 \cdot 9H_2O$) was dissolved in 100 m*l* volumetric flask with 0.5 *M* $HClO_4$). The reference was prepared with 0.5 m*l* of unirradiated Reinecke's salt actinometry solution (0.016 *M*) and 1.5 m*l* of 0.1 *M* $Fe(NO_3)_3$. $HClO_4$ solution. The photo-released thiocyanate was determined by differential spectrophotometry at 450 nm, using $\varepsilon = 4.3 \times 10^3$.

$$Cr(NH_3)_2(NCS)^-_4 + H_2O \xrightarrow{h\nu} Cr(NH_3)_2(NCS)_3 \cdot H_2O + NCS^-$$

The optical density difference was 0.297 at 450 nm and the known quantum yield of potassium Reinecke's salt is 0.280 at 557 nm.

$$I = \frac{6.023\times10^{20} \times \frac{0.297}{4.3\times10^3} \times 2 \times 2 \times \frac{1}{0.5}}{0.28 \times 7237\text{sec}}$$
$$= 1.64 \times 10^{14}\ \text{q/sec}$$

By way of an example, the k_R and k_Q determination of the substrate **3** in methanol are shown below. Six different concentrations of substrate **3** with constant concentration of rose bengal were prepared as mentioned above. Each solution (2 m*l*) was palced in the cuvette (1 cm path). Then the visible absorption spectra was run to determine the concentration of the substrate **3**. Next, the solution was irradiated with 557 nm, monochromatic light, for 120 sec.

$$I_a \Delta t = 3.29 \times 10^{-5}\ \text{mol} \cdot \text{quanta}$$

The concentration of the substrate was measured by spectrophotometer after irradiation. The results are tabulated in *Table* 3.

$I_a \Delta t = 3.29 \times 10^{-5}$ mol·quanta were used for the calculations. Plot of $\frac{I_a \Delta t}{\Delta S}$ *vs.* $\frac{1}{S}$ gave a slope of 2.87×10^{-4} with an intercept 2.36. *R* (correlation coefficient) was 0.9959.

Table 3. k_R and k_Q determinations for substrate **3** reacting with $^1O_2$.

Concentration (*M*)	$\frac{1}{[S]}$	$[\Delta S]$	$\frac{1}{[\Delta S]}$	$([\Delta S]/I_a \Delta t)^{-1}$
9.9×10^{-6}	1.0×10^{-6}	1.0×10^{-6}	9.7×10^5	31.9
1.5×10^{-5}	6.7	1.6	6.4	21.1
2.1×10^{-5}	4.8	2.2	4.6	15.1
3.6×10^{-5}	2.8	3.2	3.1	10.3
4.2×10^{-5}	2.4	3.5	2.9	9.5
5.3×10^{-5}	1.9	4.0	2.5	8.3

ε of **3** $= 3.4 \times 10_4$

Slope/intercept $= 1.23 \times 10^{-4} M = k_d/(k_R + k_Q)$.

Since $k_d = 1.4 \times 10^5 S^{-1}$, $k_R + k_Q = 1.14 \times 10^9 M^{-1}S^{-1}$.

The reciprocal intercept is 0.424 $\left(= \frac{\Phi_{isc} k_R}{k_R + k_Q}\right)$.

Since $\Phi_{isc} = 0.76$ $\quad k_R = 6.4 \times 10^8 M^{-1}S^{-1}$.

Thermal Kinetics. By way of an example, the determination of decomposition rate of **4** are shown below. Oxodipyrromethene **4** (5 mg) was dissoled in 50 m*l*-volumetric flask with methanol and then 5 m*l* of the solution was diluted to 25 m*l*. After the above solution (1 m*l*), 30 % H_2O_2 (1 m*l*) and sodium hypochlorite (0.2 m*l*) (effective chlorine *ca.* 10 %) were placed in 1 cm path uv cuvette and shaken, the absorbance change was checked every two minutes.

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