Mechanistic Studies on the Photooxidation of Chlorpromazine in Water and Ethanol

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It has been established that chlorpromazine undergoes photooxidation *via* its cation radical, yielding chlorpromazine 5-sulfoxide as an ultimate photoproduct. Molecular oxygen has been found indispensable not only for the formation of chlorpromazine cation radical but also for the formation of the sulfoxide from the radical. Oxygen atom in the sulfoxide originates from the atmospheric oxygen and not from water, which was ascertained by both tracer experiment and kinetic analysis. It was concluded that phenazathionium ion is not involved in the reaction. The possibility of singlet oxygen participation in the photooxidation was also ruled out. An intermediate, which is formed from the cation radical, was found as a precursor of the sulfoxide and was tentatively assigned as a peroxyl type radical.

Many photochemical and photobiological studies have been carried out on the photooxidation of chlorpromazine, but there are some discrepancies and ambiguities especially in the behavior of paramagnetic species and their assignment.

Lagercrants[1] showed that ultraviolet irradiation of an aqueous solution of chlorpromazine yielded a radical, the ESR signal of which decayed within about one minute in the dark, the resulting solution turning yellow. The ESR absorption could then be reproduced by blue light(435 nm) illumination inactive to initial chlorpromazine. Degassing of the solution had very little influence on the dynamical behavior of the ESR signal caused by the irradiation. The effect of ultraviolet irradiation of an aqueous solution of chlorpromazine was examined also by Boag and Cotzias.[2] They reported a pale pink(λ_{max}; 530 nm) paramagnetic species which was assigned to the semiquinone cation radical identical with the one produced by the action of trace metal ion(Ce^{4+}) or by potentiometric electrolysis. In spite of the fact that the same photochemical technique was employed, Piette and Forrest[3] reported another radical species, differing in color and in the pattern of ESR signal from those observed by Boag and Cotzias.[2] They assigned this paramagnetic species to the same radical as that detected in urinary metabolites from patients dosed with chlorpromazine drug.

Billon[4] carried out the first systematic investigation on the electrochemical oxidation of phenothiazine and its *N*-substituted derivatives, and showed that the two-electron oxidation yielded a very reactive dication (phenazathionium ion) which underwent hydrolysis to form sulfoxide even with a trace of water. Dismutative formation of the phenazathionium ion of chlorpromazine, suggested from the second-order decay of the cation radical of chlorpromazine formed in the enzymic oxidation systems of peroxidase–H_2O_2 was reported by Cavanaugh[5] and by Piette *et al.*[6] Dismutative formation and subsequent hydrolysis of this phenazathionium ion of chlorpromazine was also proposed by Merkle *et al.*,[7] based on the fact that a mixture of chlorpromazine and its sulfoxide was obtained by dissolving solid free radical in water, prepared with perchloric acid as oxidizing reagent. The formation of the sulfoxide through the phenazathionium ion derivatives was proposed for many oxidation systems, the oxidants being bromine,[8] manganic ion under aerobic conditions,[9] lead tetraacetate,[10] and sulfuric acid.[11] Thus, the phenazathionium ions have been assigned to the precursor of the sulfoxide. The indispensable hydrolysis causes the incorporation of oxygen atom into the sulfoxide from water. Though there have been many reports on sulfoxide formation through the phenazathionium ion, no direct evidence for its participation has been obtained except for the case of electrochemical oxidation.[4]

Photosensitized oxidation of chlorpromazine in the biological system is very interesting as regards photoallergic effects.[12] Blois[13] reported that the visible light irradiation of the melanine polymer into which chlorpromazine was incorporated gave a red pigmentation in the spot exposed to light. It may be worthwhile to mention the oxidation of dimethyl sulfide into the sulfoxide by photosensitization with the use of several dyes.[14]

We have investigated the photooxidation of chlorpromazine in water and in ethanol, especially on the dynamic characteristics of the radical cation and other transients formed as the intermediates by means of steady light and flash photolytic techniques. The possibility of the participation of singlet oxygen as an oxidizing agent was also examined. Strong evidence was obtained from tracer experiments for the view that molecular oxygen in air is incorporated as the oxygen atom in the sulfoxide, and the mechanism involving the hydrolysis of the phenazathionium ion was eliminated at least in the photochemical reaction. A peroxyl type radical was tentatively proposed as a favorable intermediate.

Experimental

Materials. Commercial chlorpromazine hydrochloride was used. Triethylamine(Iwaikagaku G. R. grade), ascorbic acid (G. R. grade, Tokyo Daiichikagaku) and ethanol of G. R. grade (Osaka Yukikagaku Kogyo) were used without further purification. Acetic acid-sodium acetate(pH 3.2—6.3) and hydrochloric acid-sodium acetate(pH 0.6—1.7) were used for the buffer systems. H_2O^{18} (40% isotopic content, Miles Laboratory Inc.) was used for the tracer experiment.

Method. The change in absorbance of chlorpromazine was measured by a monitoring light, perpendicular to the exciting light at 253.7 nm. Combinations of a hydrogen arc lamp, grating monochromator(Shimazu GF-16) and photomultiplier (HTV-109) were used. Contamination of the

scattered light from the source in the monitoring light was about 0.5% at the exciting wavelength. The light intensity was measured with a ferric oxalate actinometer. Flash photolytic experiments were carried out with a Ushio UFP-105(500 J) and a hand-made instrument[15] specially designed in our laboratory. Specially designed flash cell and apparatus were used for the bubbling of several gases (Ar, O_2, air) into the solution. The technique of freeze and thaw was applied for complete deaeration. The electrical power of 100 J for one flashing was used unless otherwise stated.

Results

General Features of the Steady Light Photooxidation.

Figure 1 shows the spectral change of chlorpromazine when its aerated solution in water(pH 4.7[16]) is irradiated by 253.7 nm light from a low pressure mercury lamp. Isosbestic points were clearly seen at 247, 263 nm, similar spectral changes with irradiation being observed in ethanol, methanol, 2-propanol, dioxane–water (20 : 1), and mixed solvent systems of water and ethanol. It was found for the sample bubbled with oxygen that these isosbestic points are observable till the later stage of the reaction and that the yield of the photoproduct exceeds the value in the aerated conditions. Lowering the pH of the aqueous solution of chlorpromazine from 4.7 to 0.7 did not alter the characteristics of the spectral change so much but the bleaching rates became smaller.

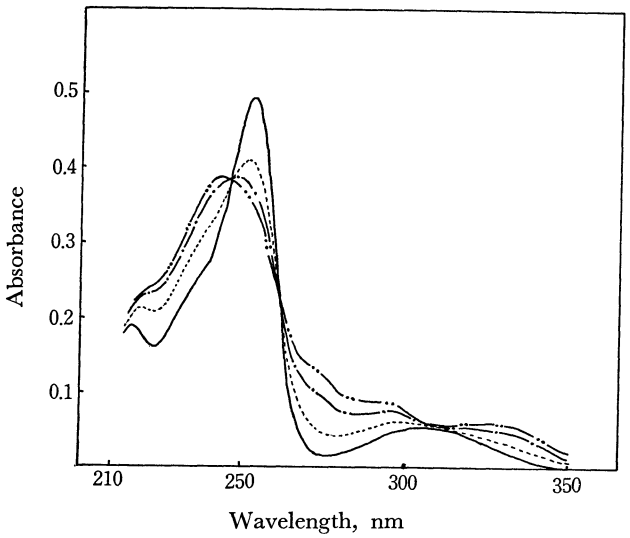


Fig. 1. Spectral change of chlorpromazine in the aerated aqueous solution of pH 4.7.
[CPZ]$=1.4\times10^{-5}$ M. —— 0 s, ---- 8 s, —·— 23 s, —··— 40 s.

The ultraviolet spectrum obtained after the irradiation of 40 seconds in water could be ascribed to chlorpromazine sulfoxide(λ_{max}[17]: 240, 275, 298, and 342 nm) from a comparison with the spectra reported by several workers,[7,17,18] though a considerable amount of the starting chlorpromazine still remained. The mass spectra of the photoproducts in all the systems stated above gave a molecular peak assignable to chlorpromazine sulfoxide. It was established from a comparison of the thin layer chromatogram of the photoproduct with that of chlorpromazine that only one product was formed. Long irradiation which brought about a considerable deviation from the isosbestic point gave the sulfoxide in extremely low yield, the mass spectrum being composed of peaks of low mass number presumably as a consequence of the photochemical decomposition of the photoproduct.

In spite of the similarity in feature of the spectral change and the identity of photoproduct, the quantum yields for the disappearance of chlorpromazine at 253.7 nm are quite different for aqueous solution (Φ_{H_2O}; 5.6×10^{-2}) and ethanol solution($\Phi_{C_2H_5OH}$; 2.7×10^{-3}), suggesting that the solvent effect causes a drastic change in the efficiency in some elementary processes of the photooxidation, or that the formation of the photoproduct by hydrolysis of the reactive transients is involved.

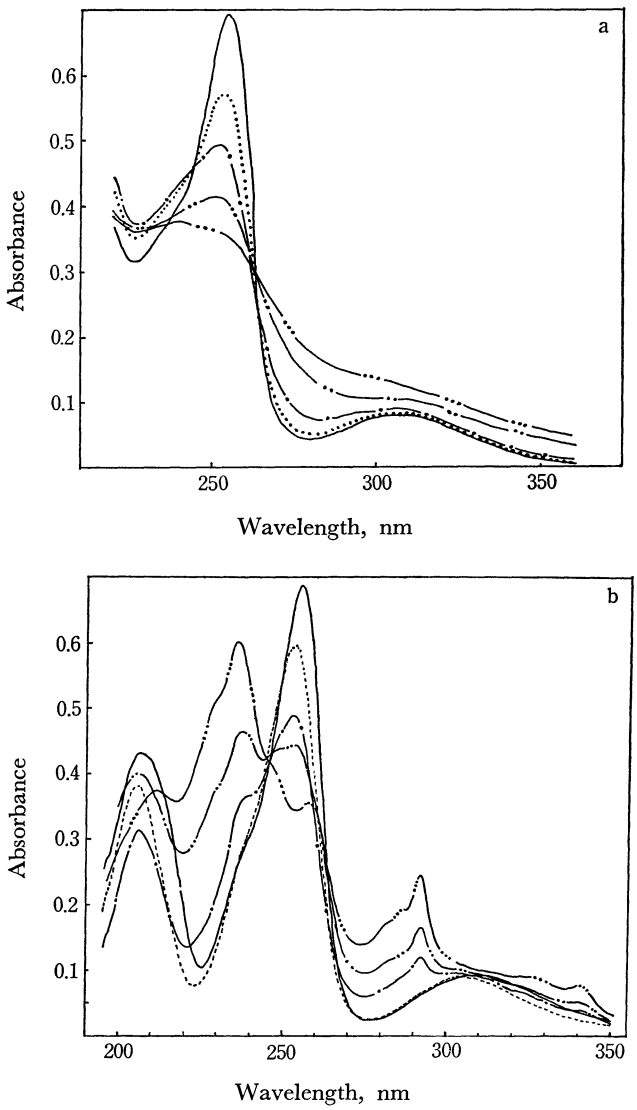


Fig. 2. Spectral change of chlorpromazine in the deaerated solutions.
a : Water (pH 4.7) [CPZ]$=2.0\times10^{-5}$ M. —— 0 s, ---- 6 s, —·— 30 s, —··— 295 s, —····— 22 min.
b : Ethanol [CPZ]$=2.0\times10^{-4}$ M. —— 0 s, ---- 15 s, —·— 19 min, —··— 31 min, —····— 71 min.

Effect of Degassing and H_2O^{18} Tracer Experiment. Spectral changes upon UV illumination of the completely degassed solutions of chlorpromazine in water (pH 4.7) and ethanol are shown in Fig. 2. The bleaching rate of chlorpromazine at 253.7 nm in the completely degassed aqueous solution was approximately the same as the one in the aerated solution, but in the absorption spectrum no peaks ascribable to the sulfoxide were observed. After prolonged irradiation the absorption spectrum showed absorption increase independent of wavelength but no characteristic peaks.

In the case of the degassed ethanol solution, a different photochemical reaction(Fig. 2(b)) occurs with a negligibly small rate ($\Phi_{\text{degassed } C_2H_5OH}$; *ca.* 8×10^{-5}) as compared with the aerated case. We may say that the photochemical reaction leading to the sulfoxide formation practically does not occur.

The effects of addition of oxygen on the quantum yield for the disappearance of chlorpromazine in water and ethanol are shown in Fig. 3. Even at such a small oxygen concentration as 10^{-5} M, the spectral changes of chlorpromazine in water are essentially the same as those in the air saturated solution as well as in the oxygen saturated one. This supports the view that a small amount of oxygen inhibits the reaction predominating under completely degassed conditions. This was further established by the following observation; the quantum yield for formation of the sulfoxide estimated from the absorption rise at 275 nm of the sulfoxide increases as oxygen concentration is increased, while the quantum yield for the disappearance of chlorpromazine is almost independent of oxygen concentration. The quantum yields for the two reactions, one *in vacuo* and the other in aerated condition, are equal. Dependence of the quantum yields upon oxygen concentration in ethanol differs from the case in the aqueous solution, suggesting a different reaction scheme, especially on the behavior of the dissolved oxygen.

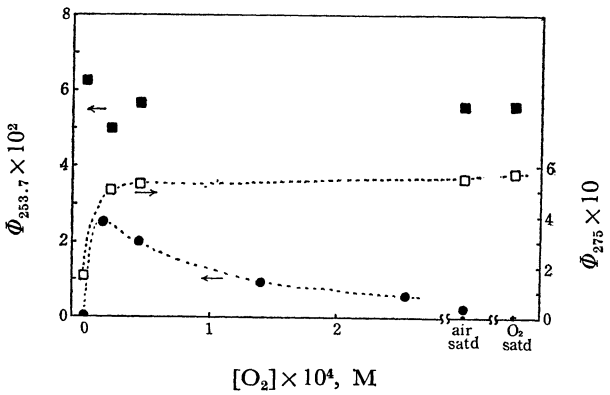


Fig. 3. Dependence of the quantum yields on the oxygen concentration in water and in ethanol. $[CPZ(H_2O)]=2.0\times10^{-5}$ M. $[CPZ(C_2H_5OH)]=1.7\times10^{-5}$ M.

■: Quantum yield for the disappearance of CPZ at 253.7 nm in water (pH 4.7). Inner filter effect is not corrected.

□: Quantum yield for the formation of the sulfoxide in water (pH 4.7) estimated from the absorption at 275 nm.

●: Quantum yield for the disappearance of CPZ at 253.7 nm in ethanol. Inner filter effect is not corrected.

Table 1. Relative intensity of the mass peaks of chlorpromazine sulfoxide obtained by photooxidation in various solvents

Medium		334	335	336	337
normal	H_2O	100	26	48	9
calcn[a]	H_2O^{18}	100	26	91	
exptl[b]	C_2H_5OH-H_2O^{18}	100	26	48	
exptl	H_2O^{18}	100	24	53	

a) The relative intensities are calculated from the results in normal H_2O on the assumption of 30% in O^{18} concentration. b) C_2H_5OH: $H_2O^{18}=100:1$ in volume ratio.

Tracer experiments using H_2O^{18} instead of normal H_2O were carried out in order to confirm the mechanism proposed by several workers,[4,5,7] according to which the sulfoxide is formed by the hydrolysis of the phenazathionium ion. Photochemical reactions in H_2O^{18} and in ethanol containing a small amount of H_2O^{18} occur with the same rates and with the same spectral changes as in the experiments with normal H_2O. Mass spectrometric measurement, details of which are given in Table 1, definitely excludes the possibility of oxygen incorporation into sulfoxide from H_2O^{18}. It may be concluded that the formation of sulfoxide by way of hydrolysis of the phenazathionium ion[7-11] is not involved in the photochemical oxidation of chlorpromazine and that the oxygen atom in the sulfoxide should originate from molecular oxygen in air, in line with the occurrence of quite different reactions under the degassed conditions.

$$CPZ + O_2(air) \xrightarrow{h\nu} CPZ{\rightarrow}O \qquad (1)$$

Effects of Light Intensity and Ionic Strength. Further experiments concerning the possible formation of the phenazathionium ion were carried out. In the photochemical reaction where no strong oxidant is involved, formation of the phenazathionium ion, if it occurs at all, should be due to the disproportionation of the cation radical[5,6] or with less probability to the direct photoionization of the cation radical by the absorption of the exciting light. The mechanisms require that the intensity of exciting light considerably affect the quantum yield. However, it was found that the quantum yield does not depend upon the light intensity for the aqueous, ethanol, and acetic acid–water (1 : 1) solutions (Fig. 4). A mechanism involving the quantitative disproportionation of the cation radical, by which no light intensity effect is expected on the quantum yield, was rejected by the dominant first-order decay of the cation radical in our flash photolytic experiments.

The increase of ionic strength which accelerates the reaction between the charged molecules with the same sign[19] should bring about an increase of the quantum yield, provided that the dismutative formation of the reactive phenazathionium ion from the cation radical occurs. However, a decreasing tendency in quantum yield was found with increasing ionic strength.

All these results strongly rejected the possibility of

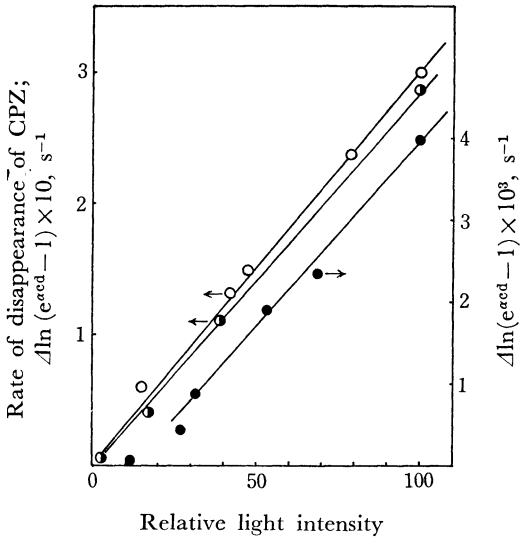


Fig. 4. Dependence of the rate of disappearance of chlorpromazine on the light intensity.
●: Ethanol, ○: Water (pH 4.7), ◑: Water-acetic acid (1:1)

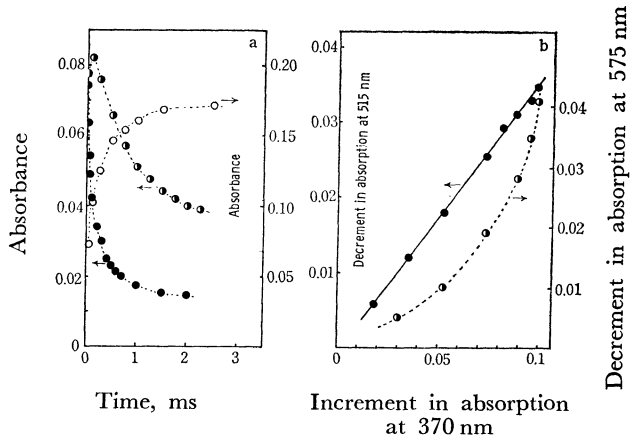


Fig. 6. Changes of the transient absorption with time at 515, 575 and 370 nm.
a: Plots of the absorbance *vs.* time. ●: 515 nm, ◑: 575 nm, ○: 370 nm.
b: Plots of the decrease in absorptions at 515(●) and 575(◑) nm against the increment in absorption at 370 nm.

the dismutative formation of phenazathionium ion from the cation radical. An alternative route for the sulfoxide formation through the cation radical should be considered.

Transient Intermediates by Flash Photolysis. The transient absorption spectra of chlorpromazine flashed in the aerated aqueous solution of pH 4.7 are shown in Fig. 5. A very short-lived intermediate easily discriminated as the absorption at about 515 nm is assigned to the chlorpromazine cation radical. A satisfactory agreement of this spectrum with that in concentrated H_2SO_4 solution of chlorpromazine and also with other spectra reported[2,5,7,9,17] as the chlorpromazine cation radical, supports the assignment. The transient absorption around 575 nm decayed with a little slower rate than that of the cation radical and a realtively slow rise of absorption around 370 nm was observed, acompanied with the decline of the cation radical. Thus three transient intermediates were clearly differentiated. The time characteristics of absorptions at 515, 575 and 370 nm after flashing are shown in Fig. 6(a). From the proportionality between the absorption increment at 370 nm and the decrease at 515 nm (Fig. 6(b)), it is very likely that the absorption at 515 nm due to the cation radical is replaced by the one at 370 nm with one to one correspondence. It should be noted that a small increase in absorption at 575 nm is equivocally observed at an early stage, till about 100 μs after flashing. Deaeration caused the extreme decrease in the yield of the cation radical. This suggests the involvement of oxygen in the reaction yielding the cation radical. No such considerable effect of deaeration on the yield of the 575 nm absorbing intermediate was observed.

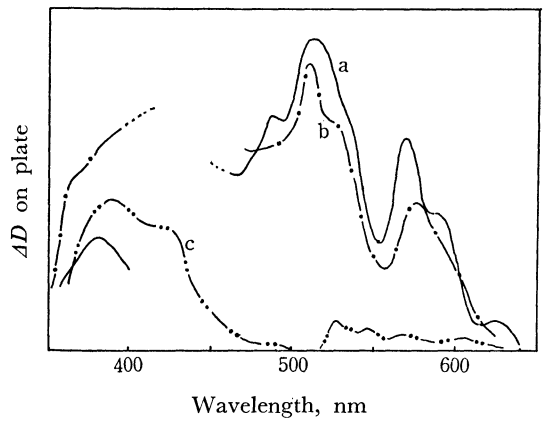


Fig. 5. Transient absorption spectra obtained by the flash photolysis of the aerated aqueous solution of chlorpromazine (pH 4.7).
a: 30 μs, b: 300 μs, c: 1 ms,

Flashing of the degassed ethanol solution of chlorpromazine gave the 575 nm light absorbing species as well as the chlorpromazine triplet state with an absorption around 480 nm, the assignment of which was made from a comparison of its shape and peak position with those of the phenothiazine triplet state[20] and also from the behavior of this species toward oxygen. The absorption around 370 nm was hardly detected in this case. Aerated ethanol solution gave only the cation radical in an extremely small amount upon flashing.

Influence of pH *and Oxygen Concentration on the Kinetics of the Transients.* The spectral change by ultraviolet irradiation is indifferent to the change of pH from 4.7 to 0.7 except for the bleaching rates. This makes it possible to determine the reactive intermediates by kinetical analysis of the reaction mechanism. The effect of pH on the yields and lifetimes of the transient intermediates are summarized in Table 2. The pH effect on the quantum yields for the steady light irradiation is shown in Fig. 7. The 370 nm light absorbing species(370-Int.) has smaller yields at low pH values, while the yield of 575 nm light absorbing species(575-Int.) remains almost constant over the whole pH range examined. The lifetimes of the above two

Table 2. pH effect on the yields of 575-Int. and 370-Int. in the aerated aqueous solution
[CPZ] = 1.1×10^{-4} M

pH	370-Int.		575-Int.		CPZ^{+}	
	Yield	Rate const.	Yield	Rate const.	Yield	Rate const.
0.65	0.03_2	—	0.05_3	7.8×10^2	0.07_8	5.1×10^3
1.7	0.01_8	—	0.04_4	8.5×10^2	0.03_4	—
3.2	0.06_6	1.2×10^2	0.05_0	1.1×10^3	0.04_6	6.1×10^3
4.7	0.10_1	1.4×10^2	0.03_9	1.2×10^3	0.03_6	6.1×10^3
6.3	0.12_0	1.2×10^2	0.03_5	1.1×10^3	0.03_2	5.8×10^3
8.0	0.11_6	—	0.03_1	1.3×10^3	0.03_1	5.1×10^3

Table 3. Yields of intermediates by flash photolysis of the aqueous solutions of CPZ, deaerated, and bubbled with argon, air, and oxygen
[CPZ] = 1.4×10^{-4} M

	370-Int.	575-Int.	CPZ^{+}	*d* O. D. (275)
Deaerated	0.06_1	0.04_7	0.03_1	
Argon	0.06_6	0.04_0	0.03_6	0.02_6
Air	$0.12_4(2.2)$	$0.08_8(1.6)$	0.05_6	0.03_3
Oxygen	$0.19_4(2.9)$	$0.11_0(1.5)$	0.06_6	0.05_2

Note) Values in parentheses are absorptions of the transient divided by the absorption of CPZ^{+}.

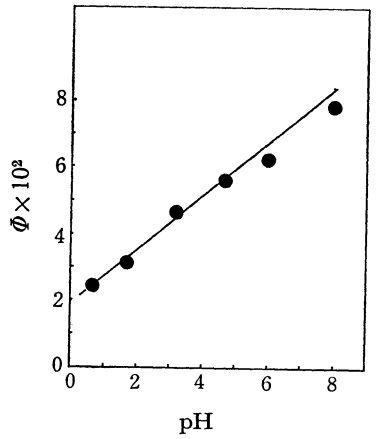


Fig. 7. Dependence of the quantum yield of chlorpromazine upon the value of pH in the aerated aqueous solution. [CPZ] = 1.5×10^{-5} M.

transients remain almost unchanged over the pH range tested. It is obvious that 370-Int. is a reactive intermediate judging from a similar tendency of decrease of the yield to that of the final product with lowering pH of the medium.

The initial yields of the intermediates upon flashing in our four different samples are shown in Table 3. One sample was degassed completely by the freeze-thaw technique, the others being saturated with argon, air, and oxygen. The absorption at 370 nm is moderately increased by the saturation of oxygen, while that at 575 nm is not much affected. The 370-Int. owes its origin to the cation radical which has been identified as the precursor of the sulfoxide as evidenced by several workers.[7,9] The significant increase of the 370-Int. with oxygen concentration supports the formation of 370-Int. through the reaction of the cation radical with oxygen.

Thus it may be concluded from the effect of pH and oxygen concentration on the reaction that the photoproduct, chlorpromazine sulfoxide, is formed from 370-Int. which comes from the cation radical.

$$CPZ^{+} + O_2 \longrightarrow \text{370-Int.} \longrightarrow CPZ{\rightarrow}O \qquad (2)$$

Effect of the Intensity of Flashing Light. The decay curve of the cation radical could be reproduced by the first-order decay formulation, even if a high flashing power was supplied for the large concentration of the intermediates. Figure 8 shows the dependence on the flashing light intensity of the amounts of the transient intermediates in terms of their maxima in absorbance. It is evident that no biphotonic process contributes to the cation radical production nor to the formation of the other two intermediates. This is in line with the results of the light intensity effect in steady

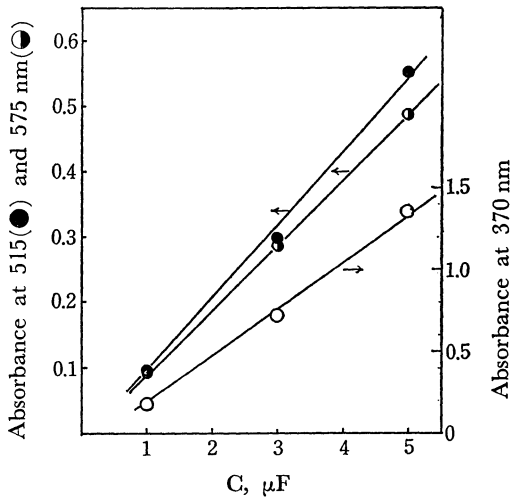


Fig. 8. Dependence of the yields of the transients on the intensity of flashing light in the aerated aqueous solution of pH 4.7. : $CPZ^{\cdot+}$, : 575-Int., ○: 370-Int.

light experiments. The phenazathionium ion, a two electron oxidized form of chlorpromazine, was not detected even in the photolysis of a high flashing light intensity.

Effect of the Addition of Radical Quenchers and of Singlet Oxygen Quencher. The effect of the addition of triethylamine(TEA) on the quantum yield in ethanol is shown in Fig. 9(a). TEA has a considerable affinity to the cation radical on account of its low ionization potential or high reducing power. The result suggests that the two reaction intermediates are involved in the reaction. The plot of $1/\Phi$ against [TEA] gives a curve consisting of two straight lines, which shows unequivocally the existence of two intermediates (Fig. 9(b)). One has a lifetime far longer than that of a typical triplet state in air saturated ethanol and might be assigned to some radical species. Assignment of the other species is unimportant, since the spectral change occuring in such a high concentration of TEA is very small, reflecting the occurrence of different reactions.

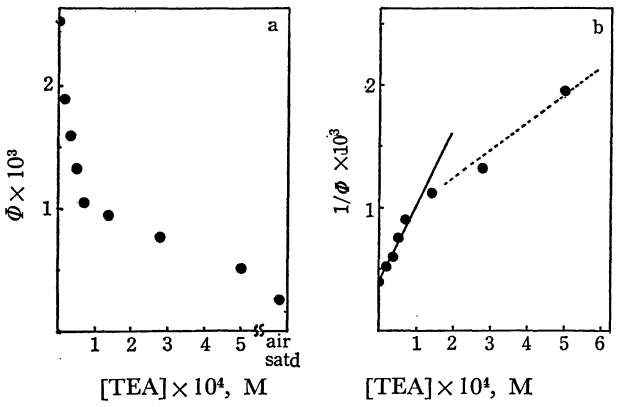


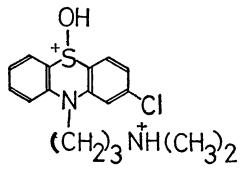
Fig. 9. Dependence of the quantum yield on the triethylamine (TEA) concentration in the aerated ethanol solution.
a: Plots of Φ *vs.*[TEA]. b: Plots of $1/\Phi$ *vs.*[TEA]

Similar quenching experiments using ascorbic acid as a radical quencher in the case of aqueous solution were performed in steady light and flash photolytic experiments. The results are summarized in Table 4. The quantum yields of the disappearance of chlorpromazine and the decay rates of the cation radical are affected considerably by the addition of ascorbic acid, which substantiates the mechanism of the sulfoxide formation through the chlorpromazine cation radical.

Tetramethylethylene, a prominent singlet oxygen quencher, showed no inhibitory effect in ethanol against chlorpromazine photooxidation even at a high concentration of 3.4×10^{-1} M. Thus the photooxidation of chlorpromazine *via* singlet oxygen mechanism can be excluded. This is consistent with the independence of quantum yield on the chlorpromazine concentration.

General Discussion

It is impossible to identify the 370-Int. conclusively, but the establishment of oxygen uptake from the atmospheric oxygen into the sulfoxide may reject the possibility of sulfonium ion(I) as the precursor of the sulfoxide. In support of this conclusion, the halosulfonium ion(R_2SX^+), known as a familiar intermediate for sulfoxide formation in the oxidation of the corresponding sulfide by halogen, undergoes hydrolysis and incorporation of the oxygen atom of water is attained.[21] The phenazathionium ion is also excluded definitely for the same reason.



(I)

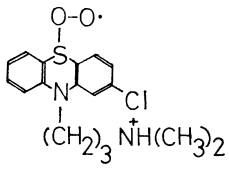
The electrochemical oxidation of phenothiazine leading to the formation of the cation radical requires 270 mV as half-wave potential for one electron oxidation, and 750 mV for the formation of the two electron oxidized form.[4] The reaction of this phenazathionium ion with the initial phenothiazine occurs rapidly and two molecules of the cation radical are obtained.[4] Thus the occurrence of the dismutative reaction of the cation radical of chlorpromazine is not thermodynamically possible. It is to be added that the absorption spectra of the phenazathionium ions have not been reported and the formation of the sulfoxide through the phenazathionium ion of chlorpromazine has been concluded only from the observation of the second-order decay of the cation radical.

Table 4. Effects of addition of ascorbic acid(AA) on the quantum yield and also on the yields of transients in water, and of tetramethyl ethylene(TME) on the quantum yield in ethanol

Solvent	Quencher concn(M)	370-Int.	575-Int.	CPZ^+	Kd(CPZ^+)	$\Phi_{253.7}$
H_2O(pH 4.7)	AA 0	0.18_1	0.07_6	0.09_0	6.1×10^3	5.3×10^{-2}
	1.0×10^{-4}	0.15_0	0.05_6	0.07_2	6.1×10^3	4.9×10^{-2}
	3.0×10^{-4}	0.13_8	0.05_0	0.08_0	8.2×10^3	2.9×10^{-2}
	5.0×10^{-4}	0.08_8	0.04_3	0.05_6	1.6×10^4	$8\text{—}9\times10^{-3}$ a)
C_2H_5OH	TME 0					2.5×10^{-3}
	5.3×10^{-5}					2.5×10^{-3}
	6.7×10^{-2}					3.0×10^{-3}
	3.0×10^{-1}					— b)

a) Error due to the inner filter effect; $\pm1\times10^{-3}$. b) Absorption overlap is very large but reaction occurs.

The assignment of 370-Int. is difficult in the present stage but we tentatively assume it to be a peroxyl radical(II) from its predominant formation with oxygen bubbling. A similar type of radical species was reported in the formation of dimethyl sulfoxide from dimethyl sulfide.[22]



(II)

As to the formation of the cation radical, ionization by the consecutive absorption of two photons and singlet oxygen mechanism are both excluded, so that the remaining possibility is the electron transfer process with molecular oxygen as an acceptor.

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