Photoreduction of Methylene Blue by Visible Light in the Aqueous Solution Containing Certain Kinds of Inorganic Salts. II. Photobleached Product

By Hiroshi OBATA

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Koizumi, Usui and the present author have discovered[1] that the deaerated aqueous solution of methylene blue undergoes photobleaching by visible light, and they have clarified the general features of the phenomenon. The bleached solution has a well-defined absorption spectra with a new peak at 256 mμ and the color is recovered when air is introduced into the solution. It was further established that the absorption peak of the remaining oxidized form gradually shifts accompanied with the bleaching reaction. Also the recovered dye has its maximum at about 640 mμ, which is essentially the same as the ultimate position of the remaining oxidized form before aeration.

As regards the photobleaching it seems most probable to attribute it to the formation of leuco dye, but if so, one has perhaps to assume the ultimate fission of water by visible light, which occurs with great difficulty for the energetic reason. If the photobleached product be some substance other than the leuco dye, this difficulty may be avoided. In connection with such a possibility it seems necessary to pay some attention to Lewis' suggestion[2] that a certain colorless form of methylene blue with the structure $(Mb^{+}\cdots OH^{-})H^{+}$ (OH^{-} is attached to ring nitrogen) exists in the aqueous solution. In case the photobleached product be such a substance or some others related with it instead of the leuco dye, then its chemical behavior would be expected to be quite different from that of the chemically prepared leuco dye which is believed to be MbH.

Secondly, as to the cause for the shift of the absorption maximum, Yoshida's work seems to be noteworthy[3]. He concludes that in the alcoholic solution methylene blue is demethylated by exposure to the visible light accompanied with the photoreduction. It seems natural to expect that the same reaction occurs in the aqueous solution, but as another possibility, the photo-hydrolysis of the dimethylamino group cannot be neglected a priori since it is well-known that in the alkaline aqueous solution, methylene blue is converted into methylene violet[4].

The present paper concerns the elucidation of the above two problems, and the investigation consists mainly of the comparison between the chemically prepared leuco dye and the photobleached product from the following three standpoints.

1) Comparison of the absorption spectra of the photobleached product with those of leucomethylene blue, benzoylleucomethylene blue and acetylleucomethylene blue. The latter two substances are considered to have a structure related with MbOH.

2) Comparison of the oxidative and reductive behavior of the photobleached product with that of leucomethylene blue.

3) Comparison of the pH dependence of the absorption spectrum of the photobleached product and that of leucomethylene blue.

The results lead to the conclusion that the photobleached product is most certainly a leuco dye partially demethylated.

Experimental

Materials.—*Methylene Blue.*—Grübler's reagent was recrystallized twice from water and then once more from butanol. The molar extinction coefficient was 62000. Most experiments were done with the solution of 2×10^{-5} mol./l.

Leucomethylene Blue.—The deaerated solution of methylene blue was reduced to the leuco form by warming the solution to about 60°C with a small amount of zinc powder. The maximum of the absorption spectrum of this solution is 256 mμ and the value agrees with that of the solution reduced by sodium hydrosulfite or hydrogen with palladium asbestos. This result ensures that the solution reduced by adding zinc powder is leucomethylene blue. The solution reduced by passing hydrogen in the presence of palladium asbestos was used for a few experiments.

Benzoylleucomethylene Blue and Acetylleucomethylene Blue[5].—*Leucomethylene Blue.*—A solution of 25 g. of methylene blue in 500 ml. of water was heated almost to boiling, 25 g. of zinc powder was

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[1] Y. Usui, H. Obata and M. Koizumi, This Bulletin, **34**, 1049 (1961).

[2] G. N. Lewis, O. Goldschmid, T. T. Magel and J. Bigeleisen, *J. Am. Chem. Soc.*, **65**, 1150 (1943).

[3] Z. Yoshida and S. Kazama, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **59**, 1418 (1956).

[4] A. Bernthsen, *Ann.* **230**, 211 (1885).

[5] G. Cohn, *Ber.*, **33**, 1567 (1900); L. Chalkley, *J. Am. Chem. Soc.*, **47**, 2057 (1925).

added and then, during vigorous stirring, 70 ml. of pure concentrated hydrochloric acid was added as quickly as possible. After a few minutes, the color of this solution changed to pale greenish yellow. The filtrate was allowed to stand in the nitrogen atmosphere overnight; it was then cooled in an ice-bath and filtered. The precipitate obtained was well dried in a vacum desiccator. This precipitate was greenish and stable in the air for several days; yield 30 g.

Benzoylation.—The 4.5 g. of dry zinc salt obtained was dissolved in 9 ml. of dry pyridine, previously deoxygenated by flashing with nitrogen gas, and the solution was treated with 5 ml. of benzoyl chloride, heated for one hour in the nitrogen gas, and then allowed to stand overnight. The reaction mixture was poured into a large volume of cold water, the tarry substance that separated off was washed with 10% sodium carbonate solution and with water until it became a white powder, and then it was extracted with 10% hydrochloric acid. The acidic solution was made alkaline with aqueous ammonia and the white precipitate was collected and dried. The dried substance was recrystallized twice from alcohol. The crystalline substance obtained was pale yellow and melted at 190.5～192°C. The molar extinction coefficient was 55000.

Acetylation.—Acetylation was done with acetic anhydride by the same method as benzoylation. The melting point was 180.3～180.5°C, pale green. The molar extinction coefficient was 51000.

$K_3Fe(CN)_6$, $K_4Fe(CN)_6$ — G. R. reagents were recrystalized from water three times.

$SnCl_4 \cdot 5H_2O$, $SnCl_2 \cdot 2H_2O$ — G. R. reagents were used without further purification.

Apparatus for Photobleaching and Procedures.—Figure 1 shows the main part of the apparatus for bleaching the aqueous solution of methylene blue. As a light source, two tungsten lamps for spot light (500 W) were employed and the range of wavelength was chosen by means of a filter (Mazda VR2) and any shorter wavelength than 600 mμ was cut off completely. The reaction cell was a glass rectangular type for Beckman spectrophotometer and could be degassed. Absorption spectra were measured by a Kotaki or Hitachi spectrophotometer according to the visible or ultraviolet region. The photobleaching reaction of methylene blue was carried out in the aqueous solution without any buffered reagents at 30°C.

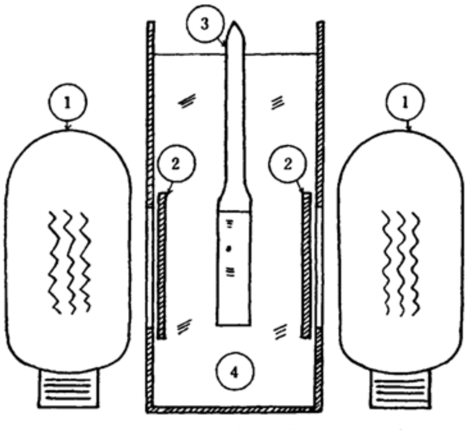

Fig. 1. Apparatus for photobleaching.
① Tungstene lamp for spot light (500 W)
② Filter
③ Reaction cell
④ Thermostatt

Results

Photobleaching of Aqueous Methylene Blue Solution in Vacuo.—It required more than ten hours for the aqueous methylene blue solution to be bleached in vacuo. After the irradiation of 10 hr., there remained about ten per cent of the oxidized form and the wavelength of absorption maximum (667 mμ) moved to about 640～650 mμ. Further, when the bleached solution was exposed to the air, the solution regained the blue color and the position of the absorption peak was practically the same as in the case of the above value. The optical density of the recolorized solution was about 70 per cent that of the original methylene blue solution. Thus the general features of the spectral change is quite similar to that in the case of the buffered solution[1].

Comparison of the Absorption Spectra of the Photobleached Product with Those of the Related Compounds.—If there should exist any difference in the absorption spectra of leucomethylene blue (DH) and those of DOH, the photobleached product would easily be identified spectroscopically. But the fact is that nothing has been reported about DOH, except Lewis' proposal so far as the author knows and still less about its absorption spectra. For the purpose of getting knowledge about the absorption spectra of DOH, those of the synthesized benzoylleucomethylene blue and acetylleucomethylene blue were measured.

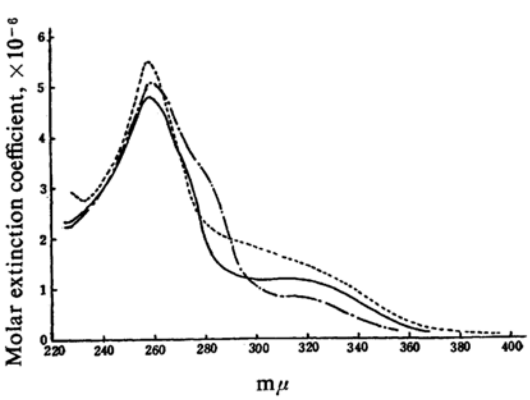

Fig. 2. Absorption spectra of leucomethylene blue and its derivatives.

—— Leucomethylene blue
----- Benzoylleucomethylene blue
—·— Acetylleucomethylene blue

These were fairly stable in the aerated condition and were expected to have the absorption band similar to that of DOH, because they are closely related with DOH in structure. The absorption spectra of benzoylleucomethylene blue and acetylleucomethylene blue were compared with that of leucomethylene blue in alcoholic solution. Figure 2 shows the absorption spectra of these three substances. They are similar in shape and the wavelengths of the maxima agree fairly completely. These results suggest that the absorption bands of DH and DOH resemble each other, hence one can not decide spectroscopically whether the photobleached product be DH or DOH.

Comparison of the Oxidative and Reductive Behavior between Photobleached Product and Leucomethylene Blue.—The reactivity of the photobleached product was compared with that of leucomethylene blue. The outline of the principle for this comparison is as follows. Two solutions containing respectively about equal amounts of leucomethylene blue and the photobleached product are added in vacuo, each to the same amount of solution containing a definite quantity of a suitable oxidation reduction system. Then the quantity of dye which has been oxidized is determined spectroscopically. The comparison of the ratio of the colored and colorless species in the equilibrium state for the two cases would be useful for the identification of the photobleached product. The reagents chosen are (1) $K_3Fe(CN)_6$-$K_4Fe(CN)_6$ system, (2) $SnCl_4$-$SnCl_2$ system and (3) thionine-leucothionine system.

(1) *The Reaction with* $Fe(CN)_6^{3-}$—$Fe(CN)_6^{4-}$ *System.*—The mixture of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, each 1.6×10^{-5} mol., was added in vacuo to 7 ml. of the solution containing the dye system (leucomethylene blue or the photobleached product). The calculated concentration of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in the solution thus prepared, being 2.3×10^{-3} mol./l., was about 100 times that of the dye-system. As expected from the values of E_0' at pH 7 (0.43 V. for $Fe(CN)_6^{3-}$-$Fe(CN)_6^{4-}$, 0.011 V. for methylene blue-leucomethylene blue)[6] the addition of $K_3Fe(CN)_6$-$K_4Fe(CN)_6$ system to the solution of methylene blue rapidly produced a blue color of the oxidized dye; but the solution gradually changed its color and turned to a green tint after a few minutes. As to the photobleached solution, a change quite similar to that above was observed.

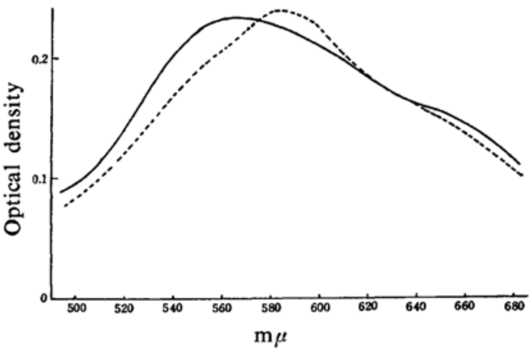

Fig. 3. Absorption spectra of the complex with $K_3Fe(CN)_6$.
—— Oxidized form of the photobleached product
---- Oxidized form of leucomethylene blue

In Fig. 3 the absorption spectra of these two solutions are shown and in addition, that of the aqueous methylene blue solution containing $K_3Fe(CN)_6$. A large shift of λ_{max} of methylene blue suggests the formation of a certain complex between the dye and $K_3Fe(CN)_6$. A further shift for the photobleached product, ca. 15 mμ corresponds, without doubt, to that of the component dye.

(2) *The Reaction with* Sn^{4+}—Sn^{2+} *System.*—In this case a more quantitative comparison can be done. Thus when a large quantity of the solution containing $SnCl_4$ and $SnCl_2$, 1 : 1 in mole ratio, is added to the solution of the reduced dye, the latter will be oxidized to such an extent that its potential attains the value of Sn^{4+}-Sn^{2+} system, which on account of its great excess, maintains practically its original value. The concentration of oxidized form (X_o) and that of reduced form (X_r) of dye in the equilibrium state can easily be obtained spectroscopically by measuring the optical density of the above solutions in both deaerated and aerated conditions, the former providing the value of X_o and the latter X_o+X_r. In this way the value of X_o/X_r in two cases, leucomethylene blue and the photobleached product can be compared at a constant oxidation reduction potential determined by Sn^{4+}/Sn^{2+} ratio.

In practice the experiment was done as follows. The stock solutions of stannic chloride and stannous chloride both in 1 N hydro chloric acid were neutralized by sodium acetate to adjust the pH value to 5. Two milliliters of 1 : 1 stannic chloride-stannous chloride solution was added to 5 ml. of reduced dye solution, the concentration of stannic chloride and stannous chloride having been suitably selected to make their ultimate concentration 0.02 mol./l. Upon mixing, the blue color immediately

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[6] M. Clark, "Oxidation Reduction Potential in Organic System", Williams & Wilkins Co., Balimore (1960), p. 132; L. F. Hewitt, "Oxidation-Reduction Potential in Bacteriology and Biochemistry", 6th Ed., Livingstone Ltd., Edinburgh (1950), p. 21.

appeared and its optical density was measured at 660 and 645 mμ respectively for the case of leucomethylene blue and the photobleached dye. Aerating the system the optical density was measured once more at the above wavelengths. Typical results are shown in Table I.

TABLE I. OPTICAL DENSITY OF LEUCOMETHYLENE BLUE AND THE PHOTOBLEACHED PRODUCT AFTER ADDING $SnCl_4$-$SuCl_2$ SYSTEM

	Leucomethylene blue	Photobleached product
Deaerated state	0.201	0.286
Aerated state	0.352	0.380
X_o/X_r	1.331	3.04

E_0' value of the photobleached product may be evaluated in the following way. The oxidation-reduction potential of the solution which is practically decided by the Sn^{4+}/Sn^{2+} ratio is expressed as follows.

$$E_h = E'_{0Sn} + \frac{RT}{2F}\ln\frac{[Sn^{4+}]}{[Sn^{2+}]}$$

$$= E'_{0Mb} + \frac{RT}{2F}\ln\frac{X_{oMb}}{X_{rMb}}$$

$$E'_{0ph} + \frac{RT}{2F}\ln\frac{X_{oph}}{X_{rph}}$$

where E'_{0Sn}, E'_{0Mb} and E'_{0ph} are respectively the midpoint potential (the potential when the concentration of oxidant and reductant is the same) of Sn^{4+}/Sn^{2+}, methylene blue, and the photobleached system at pH 5. Using the value of E'_{0Mb} (0.101 V.)$^{6)}$ in the literature and the value of X_{oMb}/X_{rMb} in Table I, one can determine the E_h value, which was found to be 0.1048 V. Since E_h has the same value for the photobleached system, one can easily get E'_{0ph} from X_{oph}/X_{rph}. The value obtained is 0.090 V. which is close to the value of methylene blue.

(3) *The Reaction with Thionine-Leucothionine System.*—E_0' of thionine is only a little higher than that of methylene blue and the light absorption by thionine is almost negligible in the concentration $10^{-5}\sim10^{-6}$ mol./l. at a wavelength of the absorption maximum of methylene blue. But, if the concentration of the thionine solution was chosen above 10^{-3} mol./l. in order to keep the value of E_h approximately constant after mixing, then the absorption of thionine at 665 mμ would become so great that it would prevent a simple spectroscopic analysis of the methylene blue system. Moreover it is troublesome to prepare a solution containing a definite amount of thionine and leucothionine and to add it to the solution to be tested in vacuo. For this reason the analogous experiment as in the case of Sn^{4+}-Sn^{2+} system was not undertaken in this case and the following procedure was adopted. A suitable amount of thionine solution $\sim10^{-6}$ mol./l. in concentration is added to the solution to be examined in order to oxidize about a half of the existent leuco form. In case of leucomethylene blue, the concentration of oxidized form produced by the addition of thionine can easily be obtained spectroscopically. The total sum of oxidized and reduced form is also determined by measuring the optical density of the solution after aeration. From these two values the concentration of the oxidized and reduced forms of methylene blue as well as thionine in the equilibrium can easily be calculated. The use of E_0' value of thionine$^{6)}$ (0.068 V. at pH 6.8) enables one to decide the E_0' value of methylene blue and vice versa. After having checked the above method by leucomethylene blue, it was applied to the photobleached product in order to estimate its E_0' value. But in this case the exact value for the concentration of oxidized and reduced form can not be decided spectroscopically because of the lack of the molar absorbancy. As an approximation the value of the molar extinction coefficient of methylene blue at 665 mμ was provisionally used.

To illustrate by an example, the absorption spectra when 3 ml. of thionine solution was added to the solution of 5 ml. of photobleached product are shown in Fig. 4. Curves ② and ③ refer respectively to the deaerated and aerated solution and ① is the absorption spectrum due to the remaining oxidized form in the original photobleached solution (after having been corrected for dilution). From these data the concentration of each species in the equilibrium state was calculated and shown in Table II.

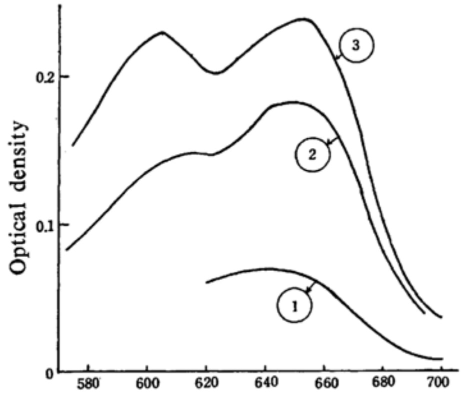


Fig. 4. Absorption spectra of the photobleached product when thionine solution added.

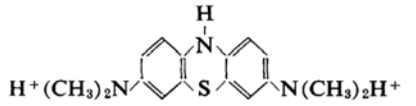
TABLE II. CONCENTRATION IN THE EQUILIBRIUM STATE BETWEEN THIONINE AND THE PHOTOBLEACHED PRODUCT

	Oxidized form	Reduced form	E_h
Thionine	0.08×10^{-6}	2.04×10^{-6}	0.0257
Photobleached product	3.64×10^{-6}	1.10×10^{-6}	

(The oxidized form remaining in the original photobleached solution: 1.6×10^{-6} mol./l.)

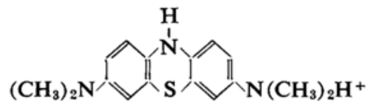
From the data in Table II E_0' of the photobleached product was calculated to be 0.0101 V. Two other similar experiments gave the values, 0.0090 and -0.0122 V. The average is 0.0023 V. It is to be added that when 10% less value is used for the extinction coefficient of the photobleached product, E_0' value in the above case varies from 0.0101 to 0.028 V.; thus the value itself is not so exact, but it is certain that it is close to that of methylene blue.

Comparison of the pH Dependence of the Absorption Spectra between Methylene Blue and the Photobleached Product.—Leucomethylene blue ionizes in two steps at pH 4.5 and 5.9, that is, there are three species of leucomethylene blue in respective pH regions as shown below.



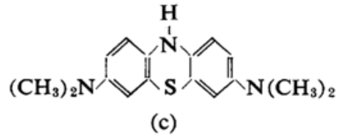
(a)

pH<4.5



(b)

4.5<pH<5.9



(c)

pH>5.9

Accordingly, the absorption spectra of leucomethylene blue in three pH regions may be different from each other. This was examined and the analogous experiment was done also for the photobleached product. Five milliliters of the solution of leucomethylene blue or the photobleached product was poured into 2 ml. of the acetate buffer solution in vacuo. The pH values chosen were 3.54, 4.02, 5.00, 6.62 and 8.25 each successively corresponding to the region where the species a, a, b, c, and c are predominant.

One could find, however, no differences between methylene blue and the photobleached product both in shape and in the position of the maximum wavelength at all pH values, the latter always being at 256 mμ. But some difference was found in the molar extinction coefficient at 256 mμ, with the highest value at pH 5 and decreasing both in larger and smaller pH regions. The results are shown in Table III and Fig. 5. In Table III the optical density of the leuco form at the maximum absorption (256 mμ) (denoted as **B**), that of the oxidized form produced by aeration of the respective solution of leuco form (at 665 mμ for methylene blue and 645 mμ for the photobleached product) (denoted as A), and the ratio B/A are tabulated. The value of B/A or its relative value standardized to that at pH 5 would be a measure of the molar extinction coefficient at 256 mμ of leuco dye, because the absorption spectrum of methylene blue is essentially the same in the above pH region and the same would perhaps apply to the photobleached product.

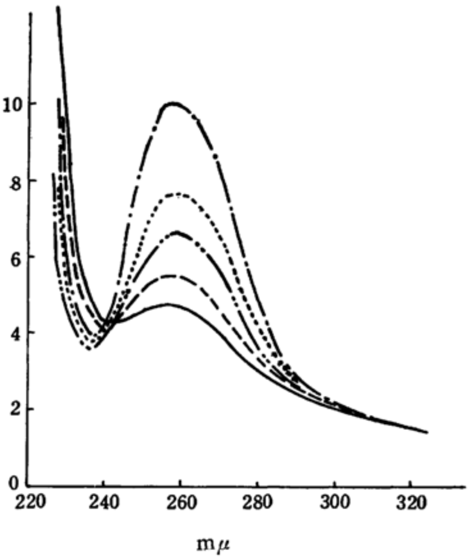


Fig. 5. Absorption spectra of the photobleached product in respective pH value.
(Absorption bands in shorter wavelength than 240 mμ are caused by acetic acid.)

——— pH 3.54
------ pH 4.02
—·— pH 5.50
········ pH 6.62
—··— pH 8.62

In evaluating the data in Table III it must be noted that a certain correction was made for a small amount of coexisting oxidized form in the solution of leuco dye, by making use

TABLE III. OPTICAL DENSITIES IN VARIOUS pH VALUES

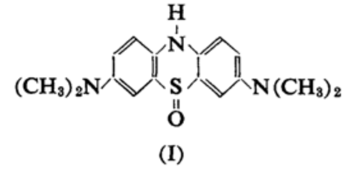
		pH				
		3.54	4.02	5.00	6.62	8.25
Photobleached product	A	0.442	0.335	0.186	0.380	0.372
	B	0.610	0.561	0.405	0.463	0.384
	B/A	1.447	1.675	2.18	1.22	1.033
	Relative value	0.663	0.768	1	0.559	0.474
Leucomethylene blue	A	0.340	0.243	0.230	0.160	0.164
	B	0.284	0.367	0.254	0.168	0.161
	B/A	0.836	0.992	1.045	0.730	0.636
	Relative value	0.798	0.947	1	0.698	0.606

of the fact that the ratio of the optical density at 256 mμ to that of 665 mμ or 645 mμ, as the case may be, in the oxidized form is approximately 1 : 3.

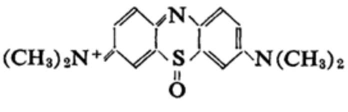
From the above results it is certain that as regards the pH dependence of the absorption spectrum there is no marked difference between leucomethylene blue and the photobleached product.

Discussion

From the results obtained in the present paper which demonstrate clearly the marked resemblance between the photobleached product and leucomethylene blue both in oxidative and reductive behavior as well as acid base character, there scarcely remains any doubt that the photobleached product is a leuco form dye. Lewis' colorless species (DOH)H^+ [2], for which the oxidative and reductive behavior as observed would never be expected, can safely be rejected as a photoproduct. But here another conceivable substance which is speculative but somewhat fascinating will be mentioned in connection with this colorless form. This is a compound supposed to be formed, should OH^- be photochemically split into H^- and O (I). It may happen that this is oxidized to II by aeration. If such a compound as I were formed, there might be a possibility that the photofission becomes more feasible and that such a reaction is accompanied by the product of neither oxygen nor hydrogen peroxide. Although there are no such substances cited in the literature, but some *S*-oxides are really known in the derivatives of phenthiazines, which are hypsochromic than their mother compounds, for example, such compounds as 2, 7 - dinitrophenthiazine - *S* - oxide[7] (yellow against dark brown of 2, 7-dinitrophenthiazine) and benzothiazino tropone-*S*-oxide[8] (380 mμ against 440 mμ of benzothiazino-tropone). Hence the compound I does not contradict the present result so far as the shift of the absorption peak is concerned.



(I)



(II)

Apart from such a speculation one may conclude that the photobleached product is a leuco dye of a certain kind.

Next, there will be discussion as to whether the accompanying photoreaction is demethylation or hydrolysis of dimethylamino group. Substances to be taken up as possible photobleached products would be the following four; methylene azur A (*N, N*-dimethylthionine), methylene azur B (trimethylthionine), or thionol (III), methylene violet (*N, N*-dimethylthionolin) (IV). Unfortunately there are few reliable data available either for the absorption spectra or for the oxidation-reduction potential of such substances, and comparison with the present results are not always possible. According to Kehrmann[9] the absorption maximum of methylene azur A and methylene azur B are respectively 638 mμ and 656 mμ and the former is rather close to this observed value. As for the reduction potential on the other hand, Rapkin et al.[10] reported that E_0' of methylene azur B is about the same as that of methylene blue but their data have seemingly

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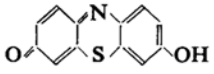
[7] F. Kehrmann, *Ber.*, **46**, 2809 (1913); F. Kehrmann and H. Goldstein, *Helv. Chim. Acta.*, **4**, 27 (1921).

[8] Unpublished result in Prof. Nozoe's Laboratory in our Faculty.

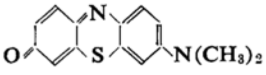
[9] F. Kehrmann, *Ber.*, **46**, 2136 (1913).

[10] L. Rapkin, A. P. Struky and R. Wurmser, *J. Chim. Phys.*, **26**, 340 (1926).

to be reexamined according to a recent investigation[11].



(III)



(IV)

Thionol was reported to have the absorption maximum at 590 or 595 mμ[4,12], which does not agree with the present value, while methylene violet has an absorption maximum at 635 mμ in the aqueous solution[4] (or 597.4 mμ in alcoholic solution[13]). As for the E_0' value of the latter compounds there is evidence that it is much lower than that of methylene blue, although its reliable value is not found in the literature. Thus the E_0' value of thionolin, the demethylated analogue of methylene violet, was reported to be -0.22 V. (at pH 7.11)[14], which is much lower than that of thionine (0.062 V. at pH 7.0), the demethylated analogue of methylene blue (0.011 V. at pH 7.0). From these data it would be natural to expect that the E_0' value of methylene violet is smaller than -0.22 V. at pH 7. Thus the possibilities of photohydrolysis which lead to III or IV, can be denied altogether.

To sum up the above discussion, a photobleached product is most certainly a partially demethylated methylene blue, which is perhaps methylene azur A.

Summary

In order to identify the photobleached substances which are produced when the aqueous solution of methylene blue is irradiated with visible light, the following three kinds of experiments were carried out.

1) Comparison of the absorption spectra of the photobleached product with those of leucomethylene blue, benzoylleucomethylene blue and acetylleucomethylene blue.

2) Comparison of the oxidative and reductive behavior of the photobleached product with that of leucomethylene blue.

3) Comparison of the pH dependence of the absorption spectrum of the photobleached product and that of leucomethylene blue.

It was concluded that the photobleached product is the leuco form dye and its oxidized form is most certainly a partially demethylated methylene blue, perhaps methylene azur A.

The author wishes to express his thanks to Professor Masao Koizumi for his suggestions and criticism throughout this investigation.

Department of Chemistry
Faculty of Science
Tohoku University
Katahira-cho, Sendai

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[11] For example, as regards toluidin blue, compare Rapkin's data with B. P. Nikol'skü and V. V. Pal'chevskü, *Zhur. Fiz. Khim.*, **32**, 1280 (1958).

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