Photosensitized Oxidation of Leuco-uranine. I. Qualitative Studies of the Nature of the Reaction in the Deaerated Solution

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In a previous paper(1), the present authors joined in studying some properties of leucouranine prepared by reducing uranine with the use of sodium amalgam. It was found that the aqueous solution of it is rather stable in the presence of dissolved oxygen but that it is easily oxidized to the colored form by adding some suitable sensitizer to the solution and by then irradiating with the light appropriate to the sensitizer. A remarkable result then found was that the restoration of the color also takes place in the absence of oxygen and, moreover, faster and more completely. Besides, it has been established that the photoproduct obtained by the irradiation of the deaerated alcoholic solution of xanthene dyes(2) shows similar behavior; this affords definite evidence that it consists mostly of the reduced form of the dye.

The purpose of the present paper is to

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1) K. Uchida, S. Kato and M. Koizumi, This Bulletin, **35**, 16 (1962).

2) M. Imamura and M. Koizumi, ibid., **28**, 117 (1955); **29**, 899 (1956); M. Imamura, ibid., **30**, 249 (1957).

clarify the nature of this sensitized oxidation in the deaerated condition; this oxidation is peculiar because the oxidation of the leuco dye may take place accompanied by no eventual reduction of the sensitizer. Thus no even slight change in the spectrum of acridine, used as a sensitizer, was perceived; a more definite fact was that uranine itself is efficient as a sensitizer. For the present research, acridine and uranine were chosen as sensitizers; the former, which absorbs light in the 400~330 mμ region where neither leuco-uranine nor uranine has any absorption, can afford an unequivocal interpretation of the results, while the latter has no possibility of promoting the reaction by being itself reduced to the leuco form. The nature of the reaction is studied rather qualitatively, the quantitative treatment being postponed to the next report.

Experimental

Materials.—*Leuco-uranine.*—Uranine was reduced by sodium amalgam and, after repeated recrystallization from an ethanol solution, was desiccated in a vacuum. The details of the procedure were the same as reported on in the previous paper. The solution is rather stable in the dark, but it gradually gets colored due to the formation of the oxidized form of uranine. For example, in several months the oxidation proceeds to the extent of more than half in the case of a 10^{-2} M solution. The coloration is less in the dilute solution, the magnitude being less than 20% for a 10^{-4} M solution.

Acridine.—Tokyo Kasei special-grade acridine was recrystallized twice from the water-ethanol mixture.

Uranine. — Commercial material was repeatedly recrystallized from ethanol.

Sodium Hydroxide.—The saturated solution was prepared from the G. R. reagent of the Wakojunyaku Co. The supernatant liquid was diluted to the desired concentration.

Disodium Hydrogen Phosphate and Potassium Dihydrogen Phosphate. — The G. R. Wakojunyaku reagents were recrystallized three times from the aqueous solution.

Sodium Carbonate, Barium Hydroxide, Ethanol and Sodium Thiosulfate.—Special grade reagents were used without further purification.

Procedure.—The stock solution of leuco-uranine 9.5×10^{-5} M was ordinarily diluted ten times. The evacuation of the solution was usually performed five to six times by the use of a mercury diffusion pump, melting and freezing being repeated each time.

When acridine was used as a sensitizer, 365 mμ light from a mercury lamp was used for exciting the acridine, which has an absorption maximum at 355 mμ. Neither uranine nor leuco-uranine has any absorption in this region. The concentration of acridine is usually 1.3×10^{-4} M. The apparatus specially designed for kinetic study was used; details will be described in the next paper. A high-pressure mercury lamp (Matsuda SHL-100UV) was used as the light source.

When uranine was used as a sensitizer, 1×10^{-6} M of uranine was added in some experiments, but in most experiments, especially in later ones, uranine was not added purposely, since the solution of leuco-uranine already had a small quantity of uranine produced during its storage. The apparatus for the photochemical reaction was similar to those used for other experiments in our laboratory. The reaction cell was the one for a spectrophotometer, 1×1×4 cm., especially designed for evacuation. As a light source, a 75 V. 500 W. tungsten lamp was used at 55 V. The ultraviolet part was removed by a Waltz yellow filter Y1 (> 430 mμ).

Results and Discussion

I. Possibility of the Evolution of Hydrogen Gas. — On the basis of the results already reported, it seemed most natural to infer that molecular hydrogen is evolved accompanying the production of the oxidized dye; first this was examined.

One-hundred milliliters of the aqueous solution of leuco-uranine 6×10^{-5} M was irradiated using uranine or acridine as a sensitizer. When the oxidation of leuco dye had almost completely taken place, the water was distilled to another trap in vacuum and, at the same time, the gas non-condensable by liquid nitrogen was gathered by the use of a small mercury diffusion pump and a Toepler-pump. The quantity of the gas obtained was about 9.8 ml. at 1 mmHg, this quantity being about one-tenth of that expected stoichiometrically. The gas was examined spectroscopically by the use of a small Geissler tube. Faint hydrogen lines were detected in one experiment (sensitizer: acridine), but in the other two experiments no trace of hydrogen could be found.

The above tests do not seem conclusive, however, because the discharge rapidly declined in strength; it is suspected that a small quantity of hydrogen might be fixed in that metallic film which had been spattered from the molybden electrode on to the wall of the tube.

II. Scrutiny of Any Impurity which Might Act as an Oxidizing Agent.—Since the above results were not conclusive, all possibilities of the contamination of a small quantity of the oxidizing agent were examined.

a) Utmost Exclusion of Molecular Oxygen.—The following procedure was adopted for this purpose. First, nitrogen gas which had been purified by bubbling it through the aqueous solution of vanadous sulfate[3] was passed through for more than 30 min. After extensive evacuation, the water was sublimed into another ampoule and it was further evacuated, melting and freezing being repeated for five times. Finally, the water was distilled back to the

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[3] L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948).

TABLE I. THE EFFECT OF THE ADDITION OF SODIUM CARBONATE

Concn. of Na_2CO_3	0	4.0×10^{-3} M	0	4.7×10^{-3} M
Initial rate (M. min^{-1})	0.55×10^{-5}	0.46×10^{-5}	0.63×10^{-5}	0.49×10^{-5}

reaction cell. The experiment using this sample showed that the reaction proceeded and with a rate not so much lower than that of the sample evacuated in the ordinary way.

b) The Effect of the Addition of Sodium Thiosulfate.—The results described above strongly establish the impossibility of molecular oxygen participating in the reaction.

However, oxygen might be loosely attached to leuco dye or some other compound in such a way that it can not be eliminated by mere evacuation. Furthermore, there might exist a trace of some other unknown oxidant which induces the reaction in question. In order to examine such a possibility, the effect of the addition of sodium thiosulfate was studied. If the reaction were due to some unknown oxidant, it would not proceed at all in the presence of sodium thiosulfate, or it would proceed at a much lower rate.

An amount of sodium thiosulfate about equal to that of leuco dye was added; the result was that the reaction took place at a rate about 75% of that for the solution to which no sodium thiosulfate was added.

III. Scrutiny of the Contamination of Any Small Quantity of a Reducible Substance.—From the results in a) and b), it can be said with confidence that no conceivable oxidant takes part in the present reaction.

Another possibility to be examined is that the reaction might be induced by some easily reducible substance contaminating the solution. Since the oxidation-reduction potential of uranine is quite low, the reduction of the carbonate ion may be possible, at least energetically, if the light energy absorbed by a sensitizer is utilized for the reaction. If this be the case, the effect of the addition to the solution or of the elimination of sodium carbonate from it ought to be carefully studied, since there is no doubt that a sample contains more or less sodium carbonate.

c) The Effect of the Addition or of the Elimination of Sodium Carbonate.—The addition of the salt up to 4×10^{-3} M did not essentially affect the rate, although the rate decreased somewhat, as may be seen from Table I.

There is, however, another possibility, that the original solution already contains an appreciable quantity of carbonate ions and, so, further addition of them scarcely affects the rate. Hence, the effect of an extensive elimination of carbonate ions was next examined. Barium hydroxide was added to the concentration of 0.1 M; this should decrease the concentration of carbonate ions down to one-hundredth that of leuco dye. The precipitate of barium carbonate was eliminated by passing the solution through a glass-filter and by repeated decantation, all procedures being done in vacuo. The experiment with the solution thus treated showed about the same rate as that of the solution not so treated.

If the carbonate ion were to be reduced, the formation of the formaldehyde would be expected. Hence, the solution was, after a long irradiation, tested by Tollens' reagent, by Schiff's reagent and by a method based on the catalytic action of aldehyde on the oxidation of *p*-phenylenediamine by means of hydrogen peroxide[4]. All the results proved to be negative. From the above results, it can be concluded that carbonate ions are indifferent to the reaction.

d) The Effect of Ethanol on the Rate.—The contamination of a small quantity of ethanol is inevitable, since it was used for repeated crystallization. Hence, the effect of the elimination or addition of ethanol was tested, though its reduction seems rather improbable. Leuco-uranine was prepared by treating an alkaline (0.5 N sodium hydroxide) solution of uranine 1×10^{-5} M with sodium amalgam. When the experiment was made utilizing the above solution, it was found that a similar reaction occurs and that the addition of 10 vol. % ethanol does not affect the rate at all.

Thus it is clear that ethanol does not participate in the reaction.

IV. Reaction in the Presence of Palladium Asbestos.—All these results, a)—d), led once more to the view that hydrogen is to be evolved as a reaction product in spite of a rather negative result of part I above. To test this from a different view-point, the experiment was undertaken in the presence of palladium asbestos suspended in the solution. If the reaction were to proceed under such conditions, the hydrogen which might be evolved would be arrested by the palladium. The oxidized dye, when kept in the dark, would be expected to be reduced, and this cycle would be repeated again and again. The above test would, in addition, completely eliminate the possibility of any oxidizing agent taking part in the reaction.

Uranine 1.0×10^{-5} M in the alkaline solution

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[4] F. Feigl, "Spot Tests", Vol. II, Elsevier, Amsterdam (1956), p. 208.

of 0.5 N sodium hydroxide was reduced by hydrogen in the presence of a small quantity of palladium asbestos to the extent that a small quantity of uranine was left behind to take the role of sensitizer. The sample was then evacuated and irradiated as usual. The recovery of color went on at about the same as in the case of the leuco dye prepared by the sodium amalgam method.

The sample when kept in the dark (in the presence of palladium asbestos) became almost colorless, but when irradiated once more it regained its color. This could be repeated more than three times.

An analogous experiment could be performed in the presence of sodium amalgam.

The above experiment definitely demonstrates that no impurity takes part in the oxidation of leuco-uranine and that the uranine ⇌ leuco-uranine process is accompanied by no other side reaction of an appreciable magnitude.

Thus, the oxidation of leuco-uranine is most probably due to the detachment of hydrogen, although this had not yet been proved successfully by the experiment described in part I.

The evolution of the hydrogen gas is to be examined by a different method in the near future.

V. Semiquinone as an Intermediate of the Reaction.—During the course of the reaction in which uranine was used as a sensitizer, the existence of semiquinone was clearly observed spectroscopically. A typical result, which was obtained by a Hitachi recording spectrophotometer, is shown in Fig. 1.

The concentration of leuco-uranine in this case is 1×10^{-5} M, while that of sodium hydroxide is 0.10 N. About 1×10^{-6} M uranine was existent in the original solution. It is seen that, after 3 min. of irradiation, the absorption with a maximum ca. 393 mμ clearly appears. This maximum declines gradually, as is shown in the figure, when kept in the dark. There is no doubt, judging from the findings of some previous workers[5] that the absorption in the 350∼420 mμ region is due to semiquinone. The molar extinction coefficient at the maximum was reported to be 6.5×10^4. Although the details of the reaction mechanism must await further examination, it seems certain that semiquinone is formed in the early stage of the reaction and that this changes into an oxidized form of uranine either by dismutation or by evolving hydrogen through the intermolecular reaction. If only the dismutation were to occur in the dark, the rise of the maximum at 490 mμ ought to be much smaller.

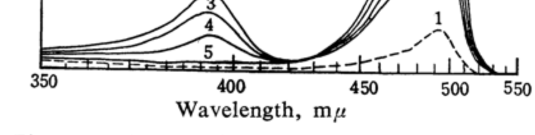

Fig. 1. Behavior of the intermediate which is produced by the irradiation of an alkaline solution of leuco-uranine (uranine used as a sensitizer).

1 : Initial
2 : 1.5 min. after irradiation (3 min.)
3 : 6 min. after irradiation
4 : 12 min. after irradiation
5 : About 2.5 hr. after irradiation

VI. Reexamination of the Spectral Change of Acridine During the Reaction. — In view of the results described in part V, there is the distinct possibility of the formation of semi-hydrogenated acridine when acridine is used as a sensitizer. If this were the case, more or less acridan would be produced according to the experimental conditions. From such a viewpoint, the spectra of acridine during the course of the reaction was reexamined more carefully under various conditions*. No definite evidence for the formation of either acridan or semi-hydrogenated acridine was obtained, but in some cases, especially at a low concentration of acridine, a clear drop of the maximum at 355 mμ was perceived. Its magnitude fluctuates from one experiment to another; the reason for this is not clear.

All the above facts suggest that, to some extent, acridine is partially hydrogenated at an earlier stage of the reaction and that some of it changes into acridine with the formation of molecular hydrogen while the remaining part dismutes into acridine and acridan.

Summary

The nature of the photosensitized oxidation of leuco-uranine (in the deaerated condition), using uranine or acridine as a sensitizer, was investigated from various viewpoints. The possibility of either an oxidizing or a reducing impurity taking part in the reaction was definitely ruled out. The uranine $\underset{\text{dark}}{\overset{\text{light}}{\rightleftharpoons}}$ leuco-

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[5] M. Imamura, This Bulletin, 31, 962 (1958); L. Lindqvist, *Arkiv. Kemi.*, 16, 79 (1960).

* Thus, the concentration of leuco was varied from 1×10^{-5} to 2×10^{-5}M and that of acridine from 1.8×10^{-5} to 13×10^{-5}M. The concentration of sodium hydroxide was always 0.122 N.

uranine reaction could be repeatedly carried out in the presence of palladium asbestos. When uranine was used as a sensitizer (alkaline solution), an appreciable quantity of semiquinone could be detected as an intermediate. Reexamination of the spectra of acridine (when acridine was used as a sensitizer) showed that in some cases acridine takes part in the eventual reaction.

It is concluded from various pieces of indirect evidence that the overall reaction is the evolution of hydrogen gas from the leuco dye, although the direct detection of hydrogen formation has not yet been successful.

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