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ANOMALIES OF VIOLOGENS IN BASES AND WATER

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SUMMARY

The use of viologens as oxidation–reduction indicators in the biochemical field has greatly increased recently.

In the presence of organic and inorganic bases the viologens undergo an auto-reduction which casts doubts on their general reliability as oxidation–reduction indicators.

The inorganic bases act on the viologens only at high concentrations of the latter; however, some nitrogen bases, like pyrrolidine and piperidine, cause the blue coloration even at the low concentrations used in biochemical work.

KOH, NaOH and Na_2CO_3 (not $NaHCO_3$) gave the characteristic blue coloration of the reduced form when added to aqueous solutions of the viologens. Piperidine, pyrrolidine and pyridine affect the viologens in a similar manner.

Benzyl viologen was reacted with NaOH, $NaOCH_3$ and pyrrolidine and the products of each reaction were isolated.

On the basis of these results a mechanism is proposed for this disproportionation.

Although reduced viologens do not follow Beer's law in water, we have found that they do follow it in certain organic solvents.

The conclusions show that reductions of both benzyl and methyl viologen carried out at high pH values or in the presence of a nitrogen base may be partially or totally the result of the interaction of the base with the viologen.

INTRODUCTION

Viologens have been widely used as photochemical indicators in studies on photosynthesis[1–3]. This is due to the fact that the change from colorless to blue makes it possible to visualize a process that results in a marked increase in reducing power.

Early work with viologens performed by MICHAELIS AND HILL[4] demonstrated that the blue coloration which appears upon reduction of the viologens results from a one-electron reduction. When the two-electron reduction takes place, the compound turns almost colorless and can be reoxidized to the blue form.

Recently, the use of viologens as oxidation–reduction indicators in both the chemical[5,6] and biochemical[7,8] fields has greatly increased. We have discovered an auto-reduction of the viologens in the presence of organic and inorganic bases which casts doubt on the general reliability of observations made using them as indicators.

KOSOWER AND COTTER[9,10] observed that the spectrum of reduced methyl viologen in water varies with concentration, indicating that Beer's law is not valid. We have observed that in solutions containing a small amount of water and organic solvents such as dimethylformamide or pyrrolidine buffered with acetic acid, the extinction coefficient does not vary with concentration.

MATERIALS AND METHODS

All spectra and extinction coefficients were recorded under vacuum in a Beckman DK-2 spectrophotometer equipped with a constant temperature cell holder. All spectra were run at 30°.

A quartz cell was sealed to a pyrex sidearm in which solutions were frozen and degassed. The cell and side arm were equipped with a vacuum stop-cock which could be closed when the cell was to be removed from the vacuum system.

In all experiments the solutions were frozen with liquid N_2 and evacuated under a pressure of 10^{-5} to 10^{-6} mm. They were then melted and the process repeated four or five times. In this way essentially all dissolved O_2 was removed.

NMR spectra were run on a Varian A-60 Spectrometer at about 35°.

RESULTS

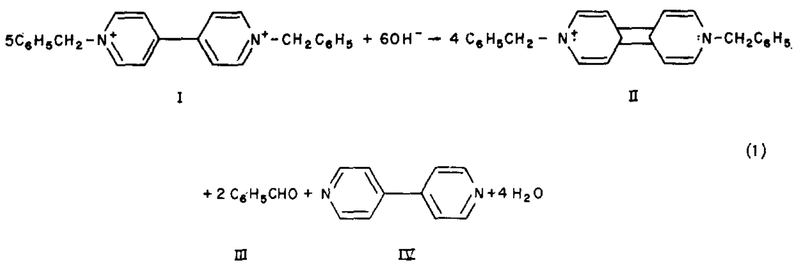
Disproportionation of the viologens

In the course of photoreduction work being conducted in these laboratories with chlorophyll and nitrogen bases, using viologens as indicators, it was observed that bases such as pyrrolidine, piperidine, and pyridine reduced the viologens on contact, even in the absence of chlorophyll.

In order to eliminate any possible artifacts from our photoreduction experiments it was considered necessary to study the mechanism of this base-induced reaction. At first it was thought that the nitrogen bases were reducing the viologens. It was then discovered that inorganic bases such as KOH, NaOH and Na_2CO_3 (but not $NaHCO_3$) would give the characteristic blue coloration of the reduced form when added to aqueous solutions of a viologen.

We next proceeded to investigate the mechanism of this reaction. Benzyl viologen in aqueous solution was allowed to react at room temperature with an excess of NaOH. The blue coloration formed immediately and stirring was continued for 4 h. The vacuum was broken and the reaction products were immediately extracted three times with ethyl ether. The ether was evaporated at room temperature and the residue vacuum distilled at 10^{-5} mm and 50°. A colorless liquid smelling like benzaldehyde was collected. When the temperature was raised to 140°, white crystals collected on the side of the distilling tube. The ultraviolet and NMR spectra of the liquid were found to be identical with those of benzaldehyde. The spectrum of the white crystals was the same as that of 4,4'-dipyridyl. Treatment with zinc in dilute acetic acid produced the characteristic blue coloration of reduced 4,4'-dipyridyl. Benzyl viologen is stable when heated to 140° *in vacuo*.

These results can be explained by the following equation:



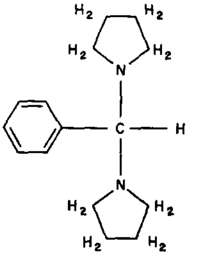
(1)

To confirm this cleavage, benzyl viologen dissolved in dry methanol was allowed to react with an excess of sodium methoxide for 3 h at room temperature. The excess methanol was removed by vacuum distillation at room temperature. On raising the temperature to 60° a colorless oil was collected and identified as benzaldehyde dimethyl acetal by comparing its NMR spectrum with that of a sample synthesized by the method of ADKINS AND NISSEN[11]. White crystals which appeared in the condensing area when the flask was heated to 140° were identified as 4,4'-dipyridyl by NMR.

Methyl viologen was similarly reacted with an excess of NaOH and 4,4'-dipyridyl was isolated. The presence of formaldehyde was confirmed with the phenylhydrazine–nitroprusside test.

Although the preceding experiments left little doubt that the reaction formulated above was the one taking place, it did not necessarily follow that the action of the nitrogen bases on the viologens was identical to the action of hydroxide and methoxide.

With this in mind, pyrrolidine was vacuum distilled into an aqueous solution of benzyl viologen and stirred for 3 h at room temperature. The contents were distilled under vacuum at 50° while the collecting flask was immersed in solid CO_2–acetone. White crystals in the water–pyrrolidine mixture were isolated. The NMR spectrum of the crystals indicated that they were benzylidine dipyrrolidine (V) (pyrrolidine β-protons, 1.64 δ; pyrrolidine α-protons, 2.48 δ; methine proton, 3.90 δ; benzene protons 7.31 δ). A carbon–hydrogen analysis was consistent with this structure (calculated for $C_{15}H_{22}N_2$: C, 77.60 %; H, 9.55 %. Found: C, 77.62 %; H, 9.70 %); m.p. 37.6–40.0°; b.p. 168° d. Benzylidine dipyrrolidine (V) can also be formed by refluxing benzaldehyde and pyrrolidine over drierite.

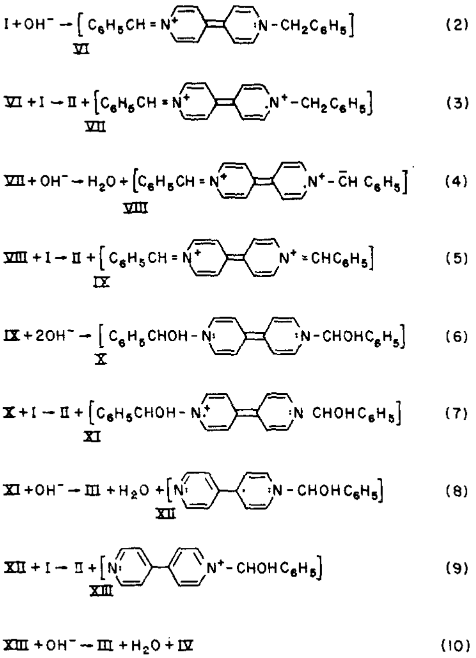


V

The distillate was extracted with ethyl ether. The oil remaining when the ether was evaporated was identified as benzaldehyde. Raising the temperature of the distilling flask to 140° produced the usual white crystals of 4,4'-dipyridyl. With prolonged heating at 160° a new type of crystal, slightly yellow in color, was collected in the distilling arm. NMR indicated these to be pyrrolidine hydrochloride.

The yield of 4,4'-dipyridyl obtained in the preceding reaction was 91.4 % of the theoretical based on the proposed stoichiometry. The benzaldehyde recovered, both as benzaldehyde and benzylidene dipyrrolidine, was 78.7 % of the theoretical.

A reaction sequence which would account for all of these observations is given in Eqns. 2–10.



Reaction 2 to form compound VI is reasonable because of the electron withdrawing property of the pyridinium ion, which would greatly enhance the acidity of the methylene hydrogen of the benzyl group. Compound VI would be expected to be a powerful reducing agent, capable of donating an electron to benzyl viologen with the formation of the characteristic colored radical ion II. The resulting dication, VII, would again have enhanced acidity of the methylene group from the pyridinium ion. The intermediate ion VIII, stabilized by twenty canonical structures, would react, on collision with benzyl viologen, to form the more stable dication IX. Two successive collisions of IX with OH^- would produce X. This, like VI, should be a powerful reducing agent for benzyl viologen. Compound XI, with a positive charge next to a carbinol function, should be activated towards attack and cleavage by OH^- to produce benzaldehyde. Repetition of the processes of Eqns. 7 and 8 gives Reactions 9 and 10 to complete the sequence.

Two consequences of this mechanism are that the success of the cleavage be dependent upon the concentration of the viologen and that it be dependent upon the concentration of the OH^-. Experimentally, both of these conditions were found to hold. Whereas a 10 M solution of pyridine in water brought about no coloration in a solution 0.1 mM in benzyl viologen, the blue color was observed when the viologen concentration was increased to 0.01 M. 0.1 M KOH solutions gave the blue color when the benzyl viologen concentration was 0.01 M, but not when the viologen concentration was lowered to 0.38 mM. As the dilution of the base was increased, the reaction failed.

Pyrrolidine was observed to be more effective in promoting the reaction than either pyridine or KOH. In an aqueous medium disproportionation was observed with a pyrrolidine concentration as low as 9.0 mM and a benzyl viologen concentration as low as 0.1 mM. The fact that pyrrolidine will perform the cleavage at a greater dilution than KOH indicates that the cleavage reaction in this case involves direct attack by pyrrolidine in Steps 6, 8 and 10 rather than cleavage to benzaldehyde with subsequent condensation to benzylidine dipyrrolidine. In the earlier experiment in which both benzaldehyde and benzylidine dipyrrolidine were isolated, pyrrolidine was present in sufficient excess to react with all the benzaldehyde. The fact that it did not is also evidence for the direct cleavage by pyrrolidine.

Applicability of Beer's law

There is controversy in the literature as to whether the viologens obey Beer's law. MICHAELIS AND HILL[4] claim that they do within wide limits: 'the absorption of a highly concentrated solution in a thin layer matched that of a solution twenty times weaker in a correspondingly thicker layer'. However, they report at the same time that the color of the reduced viologen solutions varies with concentration. They also observed that the absorption spectrum of benzyl viologen in water is temperature dependent. These two observations would indicate the presence of some kind of equilibrium.

KOSOWER AND COTTER[9] report that the spectrum of methyl viologen in water varies with concentration and they postulate that a monomer–dimer equilibrium of the reduced viologen is involved.

Our experiments agree with the findings of KOSOWER AND COTTER[9] and SCHWARZ[10]. When benzyl viologen at different concentrations was reduced in water, the intensity of the absorption at 605 nm decreased and the absorption at approx. 560 nm increased as the concentration increased. A similar effect is found in the near-ultraviolet region of the spectrum. The peak at approx. 400 nm decreased with increased viologen concentration while the reverse is true of the peak at approx. 370 nm.

We have found that when most of the water is substituted by some organic solvents the viologens do follow Beer's law within the concentration range tested. When benzyl viologen was reduced with dithionite in a solution consisting of 0.8 ml of distilled water made to 5.0 ml with pyrrolidine buffered with acetic acid to pH 6.8 the extinction coefficients given in Table I were obtained.

TABLE I

Concn. benzyl viologen (μM)	*ε at 605 nm*
110	$1.47 \cdot 10^4$
59.5	$1.47 \cdot 10^4$
28.1	$1.48 \cdot 10^4$

DISCUSSION

The basic cleavage of viologens must be taken into consideration by those investigators using the viologens as indicators at high pH values or in the presence of nitrogen bases since the results obtained may be partially or totally the result of the interaction of the viologen with the base.

KRASNOVSKII AND BREEN[6], in studying what they term the dark reaction of methyl viologen with electron donors, found that phenyl hydrazine turns methyl viologen blue in pyridine with small amounts of water. This reduction goes more to completion than when ascorbic acid is used as the reducing agent. They conclude that phenyl hydrazine is a better electron donor than ascorbic acid. However, according to our proposed mechanism, phenyl hydrazine is mainly acting as a base, methyl viologen being both the electron donor and acceptor.

These same investigators report that ascorbic acid reduces methyl viologen in a pyridine–water solution when the amount of water is low (5–10 %) but not when the water is increased above 30 %. They also claim that when the solvent used is aqueous alcohol the reaction between ascorbic acid and methyl viologen is shifted in the direction of the oxidized form of methyl viologen, yet as soon as one or two drops of 1 M NaOH is added the solution turns blue.

Their explanation is that the oxidation–reduction potential of methyl viologen is independent of pH since the reduction does not involve hydrogen. Consequently, the potential of ascorbic acid must be increased at higher pH values. This assumption leaves out of account the possibility that the bases are acting on the viologen to a considerable extent.

In quantitative work, the fact must be kept in mind that reduced viologens do not follow Beer's law in aqueous solutions. Under these conditions, special calibration curves must be prepared to permit reliable results to be obtained. In the basic organic solvents which we have studied, this difficulty does not exist.

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REFERENCES

1 A. K. CHEESBESOV, A. V. KARYAKIN AND V. B. YEFSTEGNEYEV, *Biofizika*, 11 (1966) 983.
2 B. VON RUMBERG AND H. T. WITT, *Z. Naturforsch.*, 196 (1964) 693.
3 B. VON RUMBERG, *Z. Naturforsch.*, 196 (1964) 707.
4 L. MICHAELIS AND E. S. HILL, *J. Gen. Physiol.*, 16 (1933) 859.
5 D. N. KRAMER, G. G. GUILBAULT AND F. M. MILLER, *J. Org. Chem.*, 32 (1967) 1163.
6 A. A. KRASNOVSKII AND G. P. BREEN, *Dokl. Akad. Nauk, SSSR*, 163 (1965) 761.
7 M. S. NAIK AND D. J. D. NICHOLAS, *Biochim. Biophys. Acta*, 131 (1967) 204.
8 M. S. NAIK AND S. J. NICHOLAS, *Biochim. Biophys. Acta*, 118 (1966) 195.
9 E. M. KOSOWER AND J. F. COTTER, *J. Am. Chem. Soc.*, 86 (1964) 5524.
10 W. R. SCHWARZ, JR., Ph.D. Thesis, University of Wisconsin, 1961.
11 H. ADKINS AND B. NISSEN, *J. Am. Chem. Soc.*, 44 (1922) 2749.