[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE SPRAGUE ELECTRIC CO.]

Molecular Compounds. VII. Interactions between 1,3,5-Trinitrobenzene and Pyridine-N-Oxides in Chloroform Solution

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The color formation observed in chloroform solutions of 1,3,5-trinitrobenzene and pyridine-N-oxide or methyl substituted pyridine-N-oxides is discussed in terms of complex formation and physical perturbation of the trinitrobenzene spectrum.

Pyridine-N-oxide is a strong electron donor, and 1,3,5-trinitrobenzene is an electron acceptor. It might, therefore, be expected that these two molecules would form a charge-transfer complex in solution, and, in fact, coloration is observed in chloroform solutions of 1,3,5-trinitrobenzene with pyridine-N-oxide, 2-picoline-N-oxide, 4-picoline-N-oxide and 2,6-lutidine-N-oxide. However, when the spectroscopic data were treated in the usual manner[1] to determine equilibrium constants for complex formation, good linear plots were obtained, but these intersected the Y-axis slightly below the origin. The plots shown in Fig. 1 for solutions of pyridine-N-oxide and 1,3,5-trinitrobenzene in chloroform at 24.8 ± 0.1° are typical of those obtained with all of the amine oxides. Moreover, attempts to prepare a solid complex from these reactants were unsuccessful. This suggests the possibility that something other than 1:1 complex formation may be responsible for the color formation observed in these solutions.

This possibility is strengthened by the fact that chloroform solutions of pyridine-N-oxide and 1,3,5-trinitrobenzene do not show a new absorption maximum in the spectral region from 250–450 mμ, although from 320–480 mμ, the measured total optical density is greater than the sum of the optical densities due to the two individual components. This absence of a characteristic charge-transfer maximum raises the question of whether a molecular complex is present in these solutions.

It becomes pertinent, therefore, in this instance, to consider whether or not the observed intensification of the absorption is due to a purely physical perturbation. This question of differentiating between weak complex formation and physical perturbation arises in other cases where the molecular complex is not isolable as a definite chemical entity.[2-4] It is our present purpose to consider two types of physical perturbation and to consider their applicability to the present data.

Experimental

Solvents and Reagents.—Baker and Adamson reagent grade chloroform containing 0.75% ethanol as stabilizer was used without further purification. All of the chloroform was of the same lot number. 1,3,5-Trinitrobenzene, Eastman Kodak White Label, was crystallized from chloroform as almost colorless crystals, m.p. 120.8–122°. The amine oxides were all obtained from the Reilly Tar and Chemical Corp. and purified as follows. Pyridine-N-oxide was either sublimed at 60° and 0.1 mm., and dried over sulfuric acid in an evacuated desiccator, or distilled *in vacuo*, not allowing the temperature to rise above 130°[5]; b.p. 82° at 0.1 mm. The distillate solidified and was crystallized from a benzene–hexane mixture in a dry-box and dried in an evacuated desiccator; m.p. 65–66° in a sealed capillary. 4-Picoline-N-oxide was crystallized from benzene–chloroform and vacuum dried; m.p. 181–182°. 2-Picoline-N-oxide was distilled; b.p. 89–90° at 0.8–0.9 mm. The distillate crystallized on seeding; the solid was dissolved in boiling benzene; addition of hexane to the solution in a dry-box gave small waxy crystals which were filtered and vacuum dried.[6] 2,6-Lutidine-N-oxide was distilled; b.p. 83–84° at 1–1.5 mm. It was not obtained as a crystalline solid.

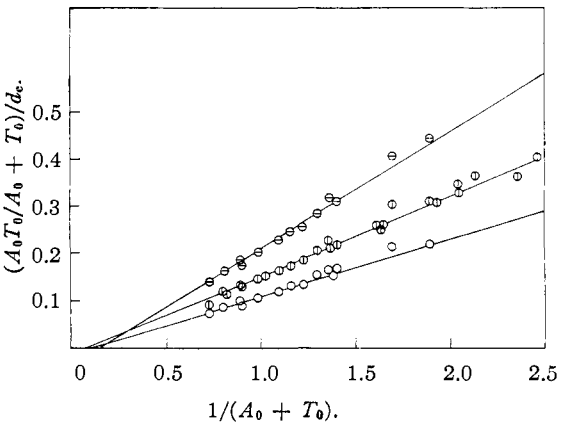

Fig. 1.—Plot of $\frac{A_0T_0}{A_0 + T_0}/d_c$ *vs.* $\frac{1}{A_0 + T_0}$: upper line, 424 mμ; middle line, 420 mμ; lower line, 416 mμ.

The Absorption Spectra Measurements.—A Beckman model DU spectrophotometer was used throughout. Stoppered Corex absorption cells were used, and the cell housing was maintained at constant temperature by means of two Beckman thermospacers, through which water from a constant temperature bath was circulated. Measurements from 410–480 mμ were made at at least seven different sets of concentrations of the two molecular species. For the pyridine-N-oxide–1,3,5-trinitrobenzene measurements, the amine oxide concentrations were varied from 1.4–0.15 *M*, and the trinitrobenzene concentrations were varied from 0.01–0.20 *M*. The concentrations of 2-picoline-N-oxide were varied from 1.5–0.5 *M* as the trinitrobenzene concentrations varied from 0.20–0.02 *M*. The 4-picoline-N-oxide concentrations varied from 0.8–0.2 *M* as the trinitrobenzene concentrations were varied from 0.02–0.10 *M*. In the experiments with 2,6-lutidine-N-oxide, the amine oxide concentrations were varied from 1.3–0.6 *M* and the trinitrobenzene concentrations from 0.02–0.10 *M*.

Results and Discussion

Chloroform solutions of 1,3,5-trinitrobenzene and pyridine-N-oxide or methyl substituted pyridine-N-oxides show absorption, in the region from

(1) R. M. Keefer and L. J. Andrews, THIS JOURNAL, **74**, 1891 (1952); S. D. Ross, M. Bassin, M. Finkelstein and W. A. Leach, *ibid.*, **76**, 69 (1954).

(2) W. Haller, G. Jura and G. C. Pimentel, *J. Chem. Phys.*, **22**, 720 (1954).

(3) D. E. Schuler and R. H. Schuler, THIS JOURNAL, **76**, 3092 (1954).

(4) N. S. Bayliss and C. J. Brackenridge, *ibid.*, **77**, 3959 (1955).

(5) *Org. Syntheses*, **33**, 79 (1953).

(6) E. Matsumura, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **74**, 363 (1953); *C. A.*, **48**, 6442[e] (1954).

TABLE I

PERTURBATION OF THE SPECTRUM OF 1,3,5-TRINITROBENZENE BY PYRIDINE-N-OXIDE AND METHYL SUBSTITUTED PYRIDINE-N-OXIDES

Amine N-oxide	Temp., °C.	$\frac{\Delta d_c}{\Delta[\text{Amine oxide}]_0[\text{Trinitrobenzene}]_0}$				
		416 mμ		420 mμ	424 mμ	
Pyridine-	0	7.95 ± 0.30		5.57 ± 0.19	3.68 ± 0.09	
	24.8	9.16 ± .54		6.53 ± .35	4.70 ± .28	
	30.4	9.35 ± .32		6.75 ± .22	4.82 ± .24	
	40.3	9.86 ± .27		7.30 ± .18	5.12 ± .14	
		430 mμ	434 mμ	440 mμ	444 mμ	450 mμ
2-Picoline-	24.8	10.3 ± 0.3	7.79 ± 0.17	4.91 ± 0.14	3.63 ± 0.08	2.32 ± 0.06
		420 mμ	424 mμ	430 mμ	434 mμ	
4-Picoline-	24.8	35.7 ± 0.7	27.6 ± 0.7	18.5 ± 0.4	14.1 ± 0.4	
		440 mμ	444 mμ	450 mμ	454 mμ	460 mμ
2,6-Lutidine-	24.8	10.7 ± 0.1	8.33 ± 0.11	5.55 ± 0.05	4.21 ± 0.05	2.72 ± 0.05

320–480 mμ, in addition to that ascribable to the two solutes individually; *i.e.*, where d_T is the total

$$d_T > \epsilon_A A_0 + \epsilon_T T_0$$

measured optical density, A_0 is the initial amine oxide concentration and T_0 is the initial 1,3,5-trinitrobenzene concentration. This additional absorption, d_c, may be defined as

$$d_c = d_T - \epsilon_A A_0 - \epsilon_T T_0$$

As can be seen from Fig. 1, when the spectroscopic data are plotted in the usual manner for 1:1 complex formation, the resultant straight lines cannot be used to evaluate the equilibrium constant, since they intersect the Y-axis below the origin. It is, of course, possible that the intersection points on the Y-axis for these plots are, within experimental error, indistinguishable from zero and that this corresponds to a case of weak complexing with the extinction coefficient for the complex very large. Nevertheless, in this particular case, since it was not possible to isolate or prepare the complex, since a characteristic charge-transfer maximum could not be identified in the spectrum from 250–450 mμ and since, as will be shown, the additional absorption, d_c, increases with increasing temperature, it seems appropriate to consider whether some explanation other than 1:1 complex formation may not account more satisfactorily for the observed d_c's.

When d_c is plotted against A_0T_0 a straight line passing through the origin is obtained. Some typical data for pyridine-N-oxide and 1,3,5-trinitrobenzene in chloroform at 24.8 ± 0.1° are shown in Fig. 2. The slopes of these lines, $\Delta d_c/\Delta A_0T_0$, are pertinent to the subsequent discussion, and in Table I we have presented some representative data. To indicate the accuracy of the results, the values are given as the average slopes ± the average deviations. In all cases measurements were made from 410–480 mμ, and in the case of pyridine-N-oxide at 24.8°, the wave length range covered was 320–480 mμ. The selected values in Table I are those where the differences between d_c and d_T were of convenient magnitudes.

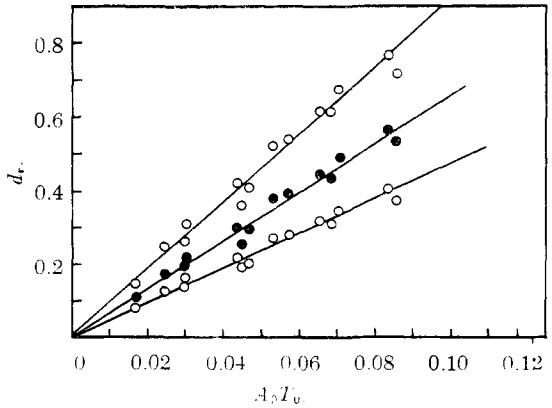

Fig. 2.—Plot of d_c *vs.* A_0T_0: upper line, 416 mμ; middle line, 420 mμ; lower line, 424 mμ.

The plots shown in Fig. 2 are in accord with two possible modes of perturbation. The first type has been discussed in detail by Bayliss and Brackenridge,[5,7] who treat the perturbation as a solvent effect resulting from active solvent molecules in the cage surrounding the perturbed solute. The exact nature of this perturbation is not specified, although it is suggested that it may depend "on favorable mutual orientation between the solute and active solvent molecules."

The second type of perturbation is related to that discussed by Bayliss and Brackenridge but is more specific in that the effect is attributed entirely to collisions between the solute and the perturbing molecules. For either type a plot of d_c *vs.* A_0T_0 would be linear and would pass through the origin as shown in Fig. 2. However, if the temperature is varied, it becomes possible to distinguish between an effect which depends on mutual orientations and an effect which depends on collisions. If we focus our attention on the slopes, $\Delta d_c/\Delta A_0T_0$, we would expect a decrease in the slopes as the temperature is increased, if the effect is an orientational one, but an increase in the slopes if the effect is collisional. Since the slopes increase with increasing temperature (Table I), a purely orientational perturbation of the type described by Bayliss and Brackenridge is eliminated.[8]

All of the observations reported herein are fully in accord with the collisional perturbation hypothesis. In particular, plots of d_c *vs.* A_0T_0 are lin-

(7) See also N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, **58**, 1002, 1006 (1954).

(8) Collision frequencies are proportional to the square root of the absolute temperature. It might, therefore, be expected that the slopes would be proportional to the square root of the absolute temperature, if the observed color formation were due to collisional perturbation. In actual fact, plots of $\Delta d_c/\Delta A_0T_0$ *vs.* $\sqrt{T}$ are linear. However, the variations with temperature are so small that other powers of the temperature give equally good linear plots.

ear and pass through the origin, and the slopes increase with increasing temperature. Nevertheless, the possibility that a charge-transfer complex is present in these solutions is not fully eliminated. Even the increase in absorption with increasing temperature is possible depending upon the extent and energies of solvation of the donor, acceptor and complex.[9]

(9) The reaction in solution is not simply

$$\text{Donor} + \text{Acceptor} \rightleftarrows \text{Complex}$$

but

$$\text{Donor}\cdot S\cdot x + \text{Acceptor}\cdot S\cdot y \rightleftarrows \text{Complex}\cdot S\cdot z + (x + y - z)S$$

where S is the solvent and x, y and z are the numbers of solvent molecules solvating each species, respectively.

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[COMMUNICATION NO. 38 FROM THE DEPARTMENT OF BIOPHYSICS, FLORENCE R. SABIN LABORATORIES, UNIVERSITY OF COLORADO MEDICAL CENTER]

Kinetics of the Antigen–Antibody Reaction. Effect of Salt Concentration and *p*H on the Rate of Neutralization of Bacteriophage by Purified Fractions of Specific Antiserum[1a,b]

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The kinetics of neutralization of bacteriophage T2r$^+$ by purified fractions of specific antiserum indicate an electrostatically controlled diffusion process. The effects of salt concentration and *p*H on the rate of neutralization are interpreted in terms of changes in the electrostatic interactions between oppositely charged antigen and antibody combining sites, modified by changes in collision frequency between virus particles and antibody molecules. It appears that one or more carboxylate groups are involved in the antigen–antibody bond.

Introduction

Jerne[2] and Jerne and Skovsted[3] first reported that the rate of neutralization of bacteriophage by specific antiserum is considerably increased when the salt concentration is lowered. This has been confirmed by others.[4,5] The authors have made a preliminary report[6] of studies dealing with the effect of salt concentration and *p*H on the kinetics of neutralization of T2r$^+$ by purified fractions of specific antiserum, prepared by the method of electrophoresis–convection. These studies are reported in detail in the present communication.

Experimental

Materials.—Bacteriophage T2r$^+$ and *Escherichia coli* B were used in these experiments. Phage lysates were diluted in distilled water and incubated several hours at 37° in order to activate inhibited viruses.[7] Two different rabbit anti-T2 sera were studied: Serum I was a pooled sample from two animals, while Serum II was obtained from a single animal.

Fractionations.—The details of construction and operation of the electrophoresis–convection apparatus have been described previously.[8] Each of the antisera was carried through seven or nine successive stages of fractionation in the cold, the material from the bottom reservoir of the electrophoresis–convection cell at the end of each stage serving as the starting material for the succeeding stage. The successive steps of fractionation, which were carried out in buffers of ionic strength 0.1 and *p*H values shown in Table I, are designated as stage 1,2, etc.; and the fractions removed from the upper reservoir at the end of each stage are designated as top 1, 2, etc. The material taken from the bottom reservoir after the last stage was further separated into a globulin and albumin fraction (fractions BG and BA, respectively) by salting out with $(NH_4)_2SO_4$ at *p*H 7. A portion of each fraction was dried by lyophilization and used for electrophoretic analysis. The remainder was sterile-filtered and stored at 2° for subsequent immunological testing.

Electrophoretic analyses were carried out using the conventional moving boundary method.

Immunological Tests.—In the absence of the complication of serum activation of inhibited virus particles, neutralization of 90–99% of bacteriophage T2 by a large excess of specific antiserum follows the first-order kinetic[3,9] law $\mathrm{dln}\ V/dt = k(1/D)$ with a Q_{10} of 1.4. The symbol V represents the number of viable viruses remaining at time t after mixing with antiserum; D, the antiserum dilution in the reaction mixture; and k, a specific rate constant independent of the serum dilution. Whole antiserum and each of its fractions were tested for their ability to neutralize phage, with the aid of the plaque counting technique as described previously.[4] Routinely the virus–antibody reaction mixtures contained about 10^8 virus particles and 3–25 γ of serum protein per ml., and all tests were carried out under conditions of large excess of antibody. Dilutions of the serum fractions in media of low salt concentration were made immediately before testing for neutralizing activity.[10] Activated phage-lysates and purified viruses gave the same result. Heating the antiserum at 56° for 30 minutes had no effect on the rate at which it neutralized viruses; adsorption of the antiserum with either intact or ultrasonically lysed *E. coli* also had little or no effect upon the rate. Control tubes contained phage without antibody in solutions of the same salt concentration and *p*H as the reaction mixture.

(1) (a) This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institute of Health, Public Health Service; in part by an institutional grant from the Damon Runyon Fund and the American Cancer Society; and in part by research contract No. AT(11-1)-269 with the Division of Biology and Medicine, Atomic Energy Commission. (b) Some of this work was presented at the 126th Meeting of the American Chemical Society, New York, N. Y., September, 1954.

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(3) N. K. Jerne and L. Skovsted, *Ann. Inst. Pasteur*, **84**, 73 (1953).

(4) J. R. Cann and E. W. Clark, *J. Immunol.*, **72**, 463 (1954).

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(7) B. P. Sagik, *J. Bacteriol.*, **68**, 430 (1954).

(8) J. R. Cann and J. G. Kirkwood, *Cold. Spring Harbor Symposia Quant. Biol.*, **14**, 9 (1950); J. R. Cann, D. H. Campbell, R. A. Brown and J. G. Kirkwood, THIS JOURNAL, **73**, 4611 (1951).

(9) In contrast to the case[4] of T4, the Q_{10} for the neutralization of T2 is the same in 3×10^{-3} as in 0.15 *M* NaCl.

(10) Under certain conditions the neutralizing activity of unfractionated antiserum changes in a rather complicated manner during incubation at low salt concentrations.[4,6] While the serum fractions behave in a less complex fashion, prolonged incubation in media of low salt concentration does result in some loss of neutralizing activity.