

While there are a number of aromatic hydrocarbon charge-transfer complexes, the tropylium ion-aromatic complexes appear to be the first directly observed in which the acceptor is a carbonium ion. On the basis of the observed K value for mesitylene-tropylium ion complex formation, tropylium ion as an acceptor compares favorably with other π -acids. The present charge-transfer complexes of the stable tropylium ion are instructive models for possible reaction intermediates containing π, π -interacting aromatic hydrocarbon and carbonium ion species.¹¹

In principle, olefins should conceivably be able to replace aromatic hydrocarbons as donors toward tropylium ion. While such complexing of tropylium ion with olefins has not yet been examined thoroughly, visible colors are produced on addition of tropylium salt to a solution of *trans*-stilbene, isoprene or tropilidene.¹²

Regarding charge-transfer complexes of tropylium ion with simpler donors such as halide ion, Doering¹ has suggested that the sequence of colors of the crystalline tropylium halides indicates a charge-transfer process. Also, Harmon¹³ has ascribed a band for tropylium bromide in methylene chloride at 401 $m\mu$ to a charge-transfer transition. However, with halide ion donors charge-transfer complex formation tends to be followed or accompanied by more deep-seated reactions. For example, Dauben² has reported that tropylium iodide decomposes on standing to tropylium triiodide and other products. Also, we have observed that addition of tetrabutylammonium bromide to tropylium salt in ethylene dichloride gives rise to rapid formation of tribromide ion. We have similar indications that thiocyanate ion is oxidized by tropylium ion.

(11) Of related interest is the demonstration by A. K. Colter and S. S. Wang that acetolysis of 2,4,7-trinitro-9-fluorenyl *p*-toluenesulfonate is accelerated by small concentrations of aromatic hydrocarbons [page 22-O of Abstracts, 139th Meeting of the American Chemical Society, St. Louis, Mo., March 21-30, 1961].

(12) Immediate color formation from tropylium ion and 7-methyl-tropilidene already has been noted by Kenneth Conrow during tropylium ion-catalyzed isomerization of 7-methyltropilidene [*J. Am. Chem. Soc.*, **83**, 2343 (1961)].

(13) K. M. Harmon and A. B. Harmon, *ibid.*, **83**, 865 (1961).

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EVIDENCE FOR COÖRDINATION OF MONOPHENYL DIIMIDE WITH HEME PROTEINS

Sir:

It has been reported that the reaction of ferrihemoglobin (Hb^+) and phenylhydrazine results in reduction of Hb^+ to ferrohemoglobin (Hb) and

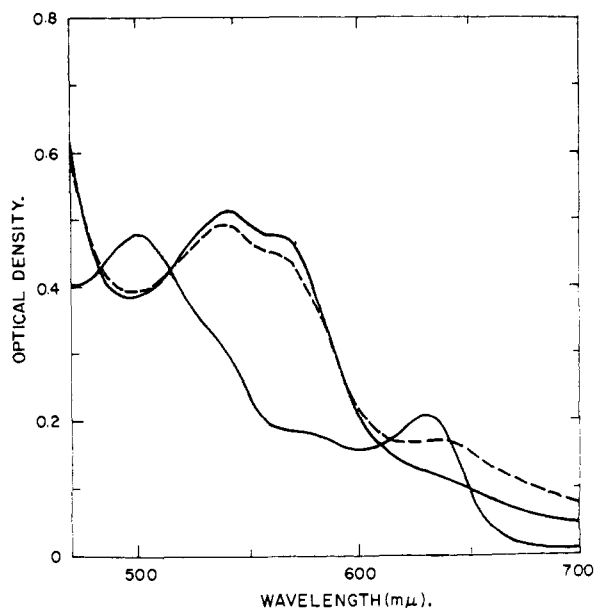


Fig. 1.—Absorption spectra of solutions 0.05 mM in heme at pH 6.8. The Hb^+ spectrum has maxima at 631 and 500 $m\mu$. The other spectra are those of the compounds of oxidized phenylhydrazine with Hb (broken line) and Hb^+ (solid line).

oxidation of phenylhydrazine to benzene and nitrogen.¹ We observed, however, that addition of excess phenylhydrazine to a solution of Hb^+ resulted in a brownish solution, the absorption spectrum of which differed from those of Hb , Hb^+ , or of a mixture of only these compounds. This observation led to experiments reported below, which indicate that an oxidation product of phenylhydrazine coordinates with both Hb and Hb^+ to form compounds having characteristic absorption spectra.

Bovine hemoglobin in phosphate buffer of pH 6.8, $\Gamma/2$ 0.1, was used in these experiments. Solutions of Hb , Hb^+ , and $HbCO$ were prepared as previously described.² Absorption spectra of samples 0.05 mM in heme were recorded with the Cary Model 14M spectrophotometer. Oxidized phenylhydrazine was prepared by mixing solutions of phenylhydrazine and $K_3Fe(CN)_6$ at concentrations up to 10 mM and 20 mM , respectively. At higher concentrations the mixed solutions became turbid. It was found that two moles of ferricyanide are reduced per mole of phenylhydrazine oxidized. In order to prevent interconversion between the ferrous and ferric compounds of hemoglobin, excess of phenylhydrazine and ferricyanide were added to solutions for experiments with Hb and Hb^+ , respectively.

Addition of oxidized phenylhydrazine to Hb^+ in presence of oxygen resulted in a compound with an absorption maximum at 541 $m\mu$. This compound and Hb^+ have isosbestic points at 609 and 516 $m\mu$. The same compound was the major

(1) H. H. Rostorffer and J. R. Totter, *J. Biol. Chem.*, **221**, 1047 (1956).

(2) H. A. Itano and E. Robinson, *Biochim. et Biophys. Acta*, **29**, 545 (1958).

product when untreated phenylhydrazine was added to Hb⁺ in the presence of oxygen. Hb and phenylhydrazine did not react in the absence of oxygen or ferricyanide. However, addition of oxidized phenylhydrazine to Hb resulted in a compound different from the compound of Hb⁺ and oxidized phenylhydrazine. The compound of Hb and oxidized phenylhydrazine has absorption maxima at 637 and 539 m μ . This compound and Hb have isosbestic points at 596 and 537 m μ . Failure of cyanide ion to alter its spectrum ruled out the presence of Hb⁺. Absorption spectra of the two new compounds are compared with that of Hb⁺ in Fig. 1. The spectrum obtained by the reaction of untreated phenylhydrazine with Hb⁺ under nitrogen appeared to be a mixture of Hb and the compounds of oxidized phenylhydrazine with Hb and Hb⁺. Addition of oxidized phenylhydrazine to HbCO under carbon monoxide resulted in a mixture of HbCO and the Hb compound.

According to Rekasheva and Miklukhin³ oxidation of phenylhydrazine by ferricyanide to benzene and nitrogen involves intermediate formation of the unstable compound, monophenyl diimide (C₆H₅N=NH). Beaven and White⁴ suggested the same compound as a possible intermediate product in the oxidation of phenylhydrazine in the presence of HbO₂ and noted the analogy between phenylhydrazine and phenylhydroxylamine. Phenylhydroxylamine is oxidized by oxygen or Hb⁺ to nitrosobenzene, which coordinates with Hb.^{5,6} Pauling⁷ has postulated that coordination with Hb is restricted to "molecules with such electronic structure that they are able to combine with the electrically neutral (iron) atom of the ferroheme group without changing its electronic charge." Nitrosobenzene and monophenyl diimide have structures that meet this restriction, and the structures $\text{Fe}=\ddot{\text{O}}-\ddot{\text{N}}^+-\ddot{\text{N}}^--\text{C}_6\text{H}_5$ and $\text{Fe}^+-\text{NH}-\ddot{\text{N}}^--\text{C}_6\text{H}_5$, respectively, can be written with use of two unpaired electrons of the iron atom. Hb⁺ forms bonds with molecules in which the electron pair used in the bond is contributed by the attached molecule.⁷ In accordance with this property the structure $\text{Fe}-\ddot{\text{N}}=\ddot{\text{N}}-\text{C}_6\text{H}_5$ can be written for the compound of Hb⁺ and oxidized phenylhydrazine. Dissociation of a hydrogen ion from monophenyl diimide results in a neutral compound. The proposed structures are consistent with the electrophoretic behavior of the compounds of oxidized phenylhydrazine with Hb and Hb.⁸ Both have lower cationic mobilities than Hb⁺.⁸ Neither benzene nor nitrogen, the final products of oxidation of phenylhydrazine by ferricyanide, coordinates with Hb or Hb⁺. We therefore propose that the absorption spectra of Fig. 1 result from the coordination of monophenyl diimide with Hb and Hb⁺.

Preliminary experiments have indicated that oxidized phenylhydrazine coordinates with ferriheme, ferrimyoglobin and ferricytochrome c, and that other derivatives of hydrazine such as naphthylhydrazine, methylhydrazine, and dimethylhydrazine coordinate with Hb⁺ in the presence of ferricyanide.

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CORRELATION OF INDICATOR RATIOS OF AZULENES WITH RATES OF ACID CATALYZED HYDROGEN EXCHANGE¹

Sir:

In a recent paper, Kresge and Chiang² reported that in aqueous perchloric acid the indicator ratio, $I = C_{\text{BH}^+}/C_{\text{B}}$, of 1,3,5-trimethoxybenzene correlates with the H_{R} acidity function or equivalently that a plot of $\log I$ versus $-H_0$ is linear with a slope 2.0. Since the rate of the acid catalyzed detritiation of tritiated trimethoxybenzene follows H_0 with a slope close to unity,^{2,3} Kresge and Chiang drew the conclusion that the transition state for exchange was only part way along toward the conjugate acid.

There are two areas of uncertainty in these indicator ratio studies. One is that protonation may be on oxygen rather than on carbon; a second is that diprotonation may be occurring. We wish to report kinetic and equilibrium studies with a system for which these points can be given specific consideration, the hydrocarbon azulene⁴ and some of its substitution products.

The most basic site of azulene is the 1 (or 3) carbon; the spectrum of the conjugate acid and also its proton exchange properties are consistent with a conjugate acid which involves a tetrahedrally bonded carbon at this site,⁵ *i.e.*, protonation on carbon. Recent studies of the n.m.r. spectrum support this and offer evidence that only monoprotonation occurs.⁶ We have confirmed this last point by making conductivity studies of azulene in anhydrous sulfuric acid. At two concentrations the conductivity (which is due almost entirely to the bisulfate ions that are formed) is very close to that of solutes which monoprotonate, *e.g.*, benzoic acid and *p*-nitroaniline, and is only about half that of a solute, *p*-phenylenediamine, which diprotonates.

Indicator ratios for aqueous solutions are shown in Fig. 1. These were measured at 350 and 274 m μ for azulene, at 279 m μ for 1-methylazulene and at 370 m μ for 1-nitroazulene. For all three $d(\log I)/dC_{\text{H}^+}$ is 0.8 ± 0.1 . However because of their different base strengths and because of the characteristics of the H_0 scale, the values of $-d$

(1) Work supported by a grant from the Atomic Energy Commission.

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(5) For details and references see E. Heilbronner, "Azulenes, in Non-Benzenoid Aromatic Compounds," Interscience Publishers, Inc., New York, N. Y., 1959, Chap. V.

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