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 mμ. This compound and Hb have isosbestic points at 596 and 537 mμ. 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 Miklukhin[3] oxidation of phenylhydrazine by ferricyanide to benzene and nitrogen involves intermediate formation of the unstable compound, monophenyl diimide ($C_6H_5N{=}NH$). Beaven and White[4] suggested the same compound as a possible intermediate product in the oxidation of phenylhydrazine in the presence of HbO_2 and noted the analogy between phenylhydrazine and phenylhydroxylamine. Phenylhydroxylamine is oxidized by oxygen or Hb^+ to nitrosobenzene, which coördinates with Hb.[5,6] Pauling[7] has postulated that coördination 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 $Fe{=}\overset{\cdot\cdot}{\underset{\cdot\cdot}{O}}{-}\overset{+}{\underset{\cdot\cdot}{N}}{-}C_6H_5$ and $Fe{=}\overset{+}{\underset{\cdot\cdot}{N}}H{-}\overset{\cdot\cdot}{\underset{\cdot\cdot}{N}}{-}C_6H_5$, 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 $Fe{-}\ddot{N}{=}\ddot{N}{-}C_6H_5$ 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, coördinates with Hb or Hb^+. We therefore propose that the absorption spectra of Fig. 1 result from the coördination of monophenyl diimide with Hb and Hb^+.

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(8) E. A. Robinson and H. A. Itano, unpublished studies.

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

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RECEIVED JUNE 15, 1961

CORRELATION OF INDICATOR RATIOS OF AZULENES WITH RATES OF ACID CATALYZED HYDROGEN EXCHANGE[1]

Sir:

In a recent paper, Kresge and Chiang[2] reported that in aqueous perchloric acid the indicator ratio, $I = C_{B_H^+}/C_B$, 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 azulene[4] 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*-phenylenedianine, which diprotonates.

Indicator ratios for aqueous solutions are shown in Fig. 1. These were measured at 350 and 274 mμ for azulene, at 279 mμ for 1-methylazulene and at 370 mμ for 1-nitroazulene. For all three $d(\log I)/dC_{H^+}$ 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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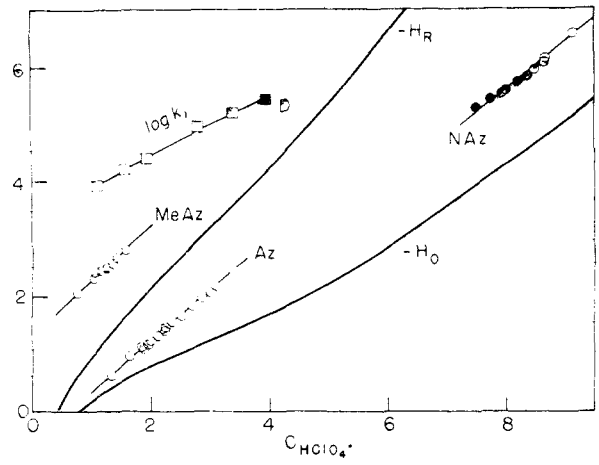

Fig. 1.—Curve MeAz is log I + 2.5 for 1-methylazulene; curve Az is log I + 1.5 for azulene; curve NAz is log I + 6 for 1-nitroazulene; curve D is log k_1 + 9 (in sec.$^{-1}$) for detritiation of 1-nitroazulene-t_1(3) All data are for 25°.

$(\log I)/dH_0$ are: 1.6 for 1-methylazulene; 1.9 for azulene; 1.0_5 for 1-nitroazulene. As an indication of relative basicities, the H_0 values at which I is unity are −0.36, −0.92 and −4.68 for the above three compounds respectively, values which are in reasonable accord with the expected effects of the substituents involved.

In a limited sense these equilibrium results confirm and extend those of Kresge and Chiang in that for two of the azulenes, for which monoprotonation on carbon is assured, log I is more nearly proportional to $-H_R$ than to $-H_0$. However, for nitroazulene this is not true, even though the evidence from ultraviolet spectra, infrared spectra and proton exchange are all consistent with the proposal that protonation is on the 3-carbon for this molecule also. Figure 1 suggests that perhaps the most significant feature of azulene and methylazulene is their greater basicity a consequence being that their protonation reaction occurs in an acidity region where $-H_0$ is only a slowly increasing function of C_{H^+}. Clearly one cannot yet conclude that correlation of log I with H_R is a general feature of protonation on the carbon of aromatics.

The rate coefficients in sec.$^{-1}$ for detritiation of azulene in aqueous solution by solvated protons are given by $k_1 = 0.19C_{H^+}$.[4,7] Since these data result from measurements in the pH range of from two to four, a region where the functions C_{H^+}, h_0 and h_R are virtually identical, no significant comparisons of acidity function dependence are possible. The situation with nitroazulene (Fig. 1) is, however, more favorable. For this substrate the rate of aqueous detritiation is almost linear in h_0, the observed first order rate coefficients being well fitted by the equation $k_1 = 5.3 \times 10^{-6} h_0^{1.05}$ sec.$^{-1}$. The acidity dependence for forming the transition state for hydrogen exchange is thus within experimental error the same as the dependence for forming the equilibrium conjugate acid. This different behavior from that reported for trimethoxybenzene cannot be rationalized by considerations of base strength since nitroazulene is slightly the weaker base. We conclude that there is considerable specificity in the interactions of these systems with acid, a not surprising conclusion in view of the high electrolyte concentrations of the media involved and the consequent large and doubtless somewhat specific medium effects on the activity coefficients of the various species.

(7) B. Challis and J. Schulze, unpublished work.

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RECEIVED JUNE 23, 1961

DEHYDROBUFOTENINE, A NOVEL TYPE OF TRICYCLIC SEROTONIN METABOLITE FROM *Bufo Marinus*

Sir:

Dehydrobufotenine,[1] the principal indole constituent isolable from the parotid glands of the South American toad (*Bufo marinus*), has now become easily available by a simplified ion exchange column procedure.[2] From 100 toads 600 mg. of the crystalline hydrochloride has been obtained. The original assignment of structure I to dehydrobufotenine has been in doubt since the close similarity of its ultraviolet spectrum with that of serotonin was noticed.[3] The novel tricyclic structure III now has been found to be the correct expression.

Dehydrobufotenine (III) with sodium in liquid ammonia undergoes the Emde-type fission typical of quaternary anilinium bases[4] and almost quantitatively opens up to bufotenine (II). The ultraviolet spectrum of dehydrobufotenine (λ_{max} 293 mμ) on oxidation in aqueous solution with NBS[5] changed to λ_{max} 265 mμ characteristic of an oxindole.[3] Final confirmation of the tricyclic structure III, rather than the isomer IV,[1] came from a detailed study of the n.m.r. spectra[6] of III and II

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(6) We thank Mr. Robert Bradley for taking the n.m.r. spectra on a Varian DP-60 instrument.