the CH_3HgCH_2 radical may decompose on the wall of the reaction vessel.

Finally it should be mentioned that a mixture of CH_3HgCH_3 and CD_3HgCD_3 does exchange to form CH_3HgCD_3 when left standing in a Pyrex vessel at room temperature in the dark. However, contrary to a recent suggestion,[5] there is no exchange within the mass spectrometer itself. Mass spectrometric analysis, carried out on a Consolidated Model 21-103, of an equimolar mixture of CH_3HgCH_3 and CD_3HgCD_3 which was analyzed on the mass spectrometer immediately after mixing gave less than 0.7% CH_3HgCD_3. It must thus be concluded that the buildup of CH_3HgCD_3 reported for this compound in the liquid phase experiments occurred mainly during the manipulations of the sample. This, however, does not affect any of the conclusions reached concerning the lack of a hot-radical effect in the liquid nor the cage recombination of the methyl radicals. This latter conclusion was based on the small amount of CH_3CD_3 formed from a freshly prepared mixture of CH_3HgCH_3–CD_3HgCD_3–2,3-dimethylbutane. Likewise it does not affect the conclusion that reaction 1 occurs in the liquid phase since this was based mainly on the azomethane experiments.

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NATIONAL BUREAU OF STANDARDS R. E. REBBERT
WASHINGTON, D. C. 20234 P. AUSLOOS

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Free-Radical Addition to Azobenzene in Cumene Solution. Electron Paramagnetic Resonance Spectra of Some Long-Lived Radical Intermediates

Sir:

One aspect of the photochemistry of aromatic azo compounds, the *cis–trans* isomerization, has been the subject of many investigations. However, relatively little attention has been given to the possibility of free-radical attack at the azo bridge.[1] Free-radical addition to the azo bridge has been suggested by Kharasch, *et al.*,[2] and Blaisdell[3] has demonstrated the ability of the azo bridge to abstract hydrogen atoms in the photoreduction of azobenzene in isopropyl alcohol solution.

We wish to report the results of a preliminary investigation in which we have obtained evidence for free-radical attack at the azo site. A stable free radical was photochemically produced and observed spectroscopically by irradiation of an azobenzene–cumene solution in an e.p.r. cavity. Product analysis coupled with a theoretical calculation to aid in interpretation of the e.p.r. spectrum served to identify the long-lived radical intermediate.

A degassed solution of azobenzene in cumene was irradiated at room temperature in the microwave cavity of a Varian V4500 e.p.r. spectrometer with 100-kc. field modulation. The light source was a PEK mercury high pressure lamp equipped with filters which absorbed wave lengths shorter than 4000 Å.

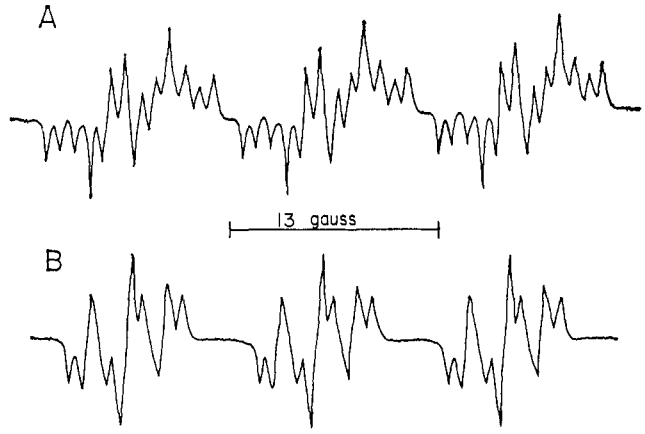
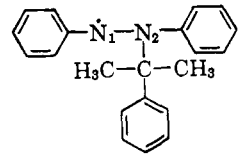


Fig. 1.—First derivative of e.p.r. absorption spectra of irradiated azobenzene–cumene solution (A), and irradiated 4,4′-dimethoxyazobenzene–cumene solution (B).

During irradiation at room temperature an e.p.r. spectrum was readily observed with an intensity dependent upon the initial concentration of azobenzene. The spectrum exhibited 36 lines (Fig. 1A) and persisted for several hours after irradiation was terminated. The spectrum is a triplet, each component of which contains four groups of lines of which the intensities follow a 1:3:3:1 binominal distribution. Each of these groups is further split into three lines of approximately equal intensity.

The radical structure consistent with the observed spectrum is



The primary triplet of the spectrum is ascribed to the strong interaction of nitrogen atom N_1 with the unpaired electron. The four groups of lines within the triplet are ascribed to isotropic hyperfine interactions of the three equivalent *ortho* and *para* protons of the phenyl group attached to N_1. The three lines within each group may be due to weak interaction with the second nitrogen atom.

With this assignment, and use of the McConnell[4] relation, $A_H = -22.5\rho_C$, and the equation[5] $A_N = Q_N\rho_N - \Sigma Q_i\rho_i$ where $Q_N = 24$ and $Q_i = 49$, the experimental coupling constants and spin densities shown in Table I were obtained. The theoretical spin densities given in Table I were calculated on the monophenylaminyl fragment of the radical using the Hückel method.[6] These values serve to substantiate the above interpretation of the observed e.p.r. spectrum.

Separate experiments were carried out on a larger scale in which the reaction mixture was subjected to liquid chromatography techniques after irradiation. N,N′-Diphenyl-N-(1-methyl-1-phenylethyl)hydrazine was eluted from a 3-ft. column which was packed

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TABLE I

COUPLING CONSTANTS, A, AND SPIN DENSITIES, ρ, FOR THE PHOTOPARAMAGNETIC AZOBENZENE–CUMENE SYSTEM

Nucleus	A, gauss	ρ (exptl.)	ρ (theor.)
N_1	11.8	0.553	0.606
N_2	.88	.09	...
ortho-H	2.59	.115	0.115
meta-H	...	...	.0034
para-H	2.59	0.115	.128

with activated alumina and washed with a mixture of benzene and ethyl alcohol (9:1). Its identity was confirmed by infrared and n.m.r. analysis. The n.m.r. spectrum exhibited a simple peak and two complex multiplets centered on τ-values of 8.5 (A), 2.7 (B), and 2.2 (C), with the integrated areas of A and (B + C) being in the ratio of 1 to 2.7. Peak A is ascribed to the methyl protons, and peaks B and C may be assigned to the protons on the azophenyl group and the isopropylphenyl group, respectively. The weak interaction from the proton on the nitrogen, however, was not observed. We are now improving our experimental technique for quantitative separation of products so that quantum yields may be obtained.

These results suggest that when azobenzene is excited in its first absorption band, it can abstract a hydrogen atom from cumene. The resulting cumene radical may then add to the azo bridge of azobenzene forming a very stable free radical. This long-lived radical is subsequently reduced by cumene to give N,N′-diphenyl-N-(1 methyl-1-phenylethyl)hydrazine.

Further studies are in progress concerning complete product analysis, temperature variation, and the effects on the hyperfine splittings of substitution with various groups into the *ortho*, *para*, and *meta* positions of the benzene rings in azobenzene. Thus, the 4,4′-dimethoxyazobenzene–cumene system gives a triplet spectrum consisting of 21 lines (Fig. 1B). Interpretation of this and other spectra is in progress and details of kinetic and theoretical studies of a series of such systems will be forthcoming.

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(7) Communications regarding this paper should be addressed to this author.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
RIVERSIDE, CALIFORNIA

J. K. S. WAN
L. D. HESS
J. N. PITTS, JR.[7]

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Synthetic Peptide Models of Enzyme Active Sites. II. L-Threonyl-L-alanyl-L-seryl-L-histidyl-L-aspartic Acid, an Active Esterase Model

Sir:

The pentapeptide L-threonyl-L-alanyl-L-seryl-L-histidyl-L-aspartic acid (I) has been synthesized and investigated as a catalyst for the hydrolysis of *p*-nitrophenyl acetate. This peptide is of particular interest in that three of the amino acids known to be involved in the activity of many enzymes, namely, histidine, serine, and aspartic acid,[1] are present. The catalytic activity observed is more than six times *greater* than that reported previously for any synthetic peptide (including those containing histidine) and suggests that some of the polyfunctional effects associated with enzyme active sites are operating. The amino acid sequence in this peptide has been shown by Milstein and Sanger[2] to be present at the active site of the enzyme phosphoglucomutase.

First-order rate plots for the liberation of *p*-nitrophenol from *p*-nitrophenyl acetate under the influence of the pentapeptide I were linear over that portion of the reaction studied (from about 10% to about 70% completion), which would be the case if I is acting as a true catalyst. Kinetic studies were carried out in 5% (by volume) dioxane in buffer (0.2 M phosphate, pH 7.73) at 25.5°. Data were obtained using 5.52×10^{-5} M *p*-nitrophenyl acetate and pentapeptide I concentrations ranging from 0.94×10^{-5} M to 2.83×10^{-5} M. The catalytic coefficient[3] for I was 92 l. mole^{-1} min.$^{-1}$ compared with 15 l. mole^{-1} min.$^{-1}$ for glycyl-L-histidyl-L-serine,[4] 2 l. mole^{-1} min.$^{-1}$ for imidazole, and 10^4 l. mole^{-1} min.$^{-1}$ for chymotrypsin.

The pentapeptide was synthesized using the scheme outlined in Chart I. All peptide bonds were formed using the water-soluble reagent 1-ethyl-3-(3-dimethylamino)propyl carbodiimide hydrochloride[5] in 30% excess.

Bis-*p*-nitrobenzyl aspartate toluenesulfonate (II), m.p. 165–167°, $[\alpha]^{20}D$ +2.2° (*c* 2.3, CH_3OH) (*Anal.* Calcd. for $C_{25}H_{25}NO_{11}S \cdot 0.5H_2O$; C, 51.45; H, 4.32; N, 7.20. Found: C, 51.71; H, 4.25; N, 7.09), was synthesized by direct esterification of aspartic acid with *p*-nitrobenzyl alcohol using carbon tetrachloride as the azeotroping solvent.[6] Condensation of this amino acid ester with N-carbobenzyloxy-*im*-benzyl-L-histidine (III) in methylene chloride containing 1 equiv. of triethylamine afforded the dipeptide bis-*p*-nitrobenzyl N-carbobenzyloxy-*im*-benzyl-L-histidyl-L-aspartate (IV), m.p. 128–130°, $[\alpha]^{21}D$ −1.4° (*c* 2.1, CH_3OH) (*Anal.* Calcd. for $C_{39}H_{36}N_6O_{11}$: C, 61.25; H, 4.75; N, 10.99. Found: C, 61.57; H, 5.25; N, 10.87). Treatment of IV with hydrogen bromide in glacial acetic acid afforded bis-*p*-nitrobenzyl-*im*-benzyl-L-histidyl-L-aspartate dihydrobromide (V), m.p. 115–117°, $[\alpha]^{16}D$ +6.2° (*c* 2.1, dimethylformamide) (*Anal.* Calcd. for $C_{31}H_{32}N_6O_9Br_2$: C, 46.97; H, 4.07; N, 10.61; Br, 20.17. Found: C, 46.68; H, 3.98; N, 10.5; Br, 20.45). Condensation of N-carbobenzyloxy-L-serine (VI) with V in acetonitrile containing 2 equiv. of triethylamine afforded the tripeptide bis-*p*-nitrobenzyl-N-carbobenzyloxy-L-seryl-*im*-benzyl-L-histidyl-L-aspartate (VI), m.p. 195–196.5°, $[\alpha]^{23}D$ −21.1° (*c* 2.7, dimethylformamide) (*Anal.* Calcd. for $C_{42}H_{41}N_7O_{13}$:

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