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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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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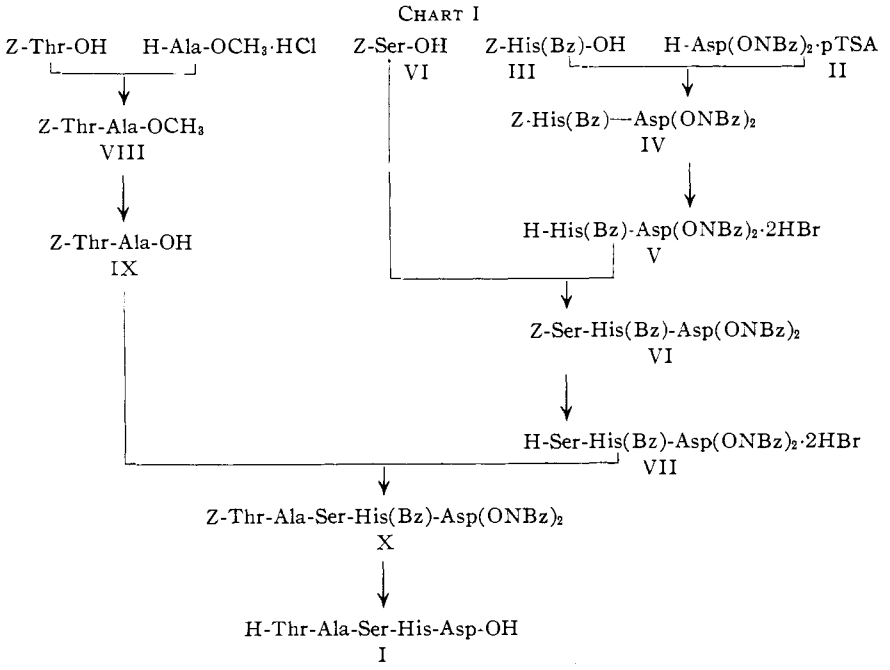
CHART I



C, 59.29; H, 4.94; N, 11.53. Found: C, 59.21; H, 4.93; N, 11.89). Hydrogen bromide in trifluoroacetic acid was used to cleave the carbobenzyloxy group of VI, affording bis-*p*-nitrobenzyl-L-seryl-*im*-benzyl-L-histidyl-L-aspartate dihydrobromide (VIII), m.p. 93–103° dec., $[\alpha]^{21}D$ −4.1° (*c* 2.2, dimethylformamide) (*Anal.* Calcd. for $C_{34}H_{37}N_7O_{11}Br_2$: C, 46.43; H, 4.24; N, 11.15; Br, 20.01. Found: C, 45.95; H, 4.27; N, 10.59; Br, 19.50).

Condensation of carbobenzyloxy-L-threonine and methyl-L-alaninate hydrochloride in methylene chloride containing 1 equiv. of triethylamine afforded the dipeptide ester methyl carbobenzyloxy-L-threonyl-L-alaninate (VIII), m.p. 132.5–134°, $[\alpha]^{25}D$ −34.1° (*c* 2.0, CH_3OH) (*Anal.* Calcd. for $C_{16}H_{22}N_2O_6$: C, 56.8; H, 6.55; N, 8.28. Found: C, 56.6; H, 6.51; N, 8.19). Saponification of VIII with barium hydroxide afforded carbobenzyloxy-L-threonyl-L-alanine (IX), m.p. 129–130.5°, $[\alpha]^{25}D$ −18.5° (*c* 2.0, CH_3OH) (*Anal.* Calcd. for $C_{15}H_{20}N_2O_6$: C, 55.5; H, 6.22; N, 8.64. Found: C, 55.3; H, 6.32; N, 8.48). Condensation of IX and VII was effected in methylene chloride containing 2 equiv. of triethylamine. The pentapeptide bis-*p*-nitrobenzyl carbobenzyloxy-L-threonyl-L-alanyl-L-seryl-*im*-benzyl-L-histidyl-L-aspartate (X), m.p. 145° dec., $[\alpha]^{21}D$ −24.3° (*c* 1.9, CH_3OH) (*Anal.* Calcd. for $C_{49}H_{53}N_9O_{16}$: C, 57.47; H, 5.22; N, 12.37. Found: C, 57.24, 57.34; H, 5.54, 5.18; N, 12.42), was purified by chromatography over silica gel using a 9:1 chloroform–methanol eluent.

Removal of all protective groups from X was accomplished by hydrogenolysis over 10% palladium on charcoal using 80% ethanol as solvent, affording I, m.p. dec. 177°, $[\alpha]^{24.6}D$ −17.4° (*c* 1.95, H_2O) (*Anal.* Calcd. for $C_{20}H_{31}N_7O_{10}\cdot 0.5H_2O$: C, 44.6; H, 5.99; N, 18.2. Found: C, 44.5; H, 6.39; N, 17.8). Quantitative amino acid analysis on acid hydrolysates of each peptide were carried out using a gas chromatographic technique which is to be published. The free pentapeptide I was homogeneous to paper chromatography and paper electrophoresis under a variety of conditions and was completely digested by the enzyme leucine aminopeptidase,[7] indicating that all amino acids were present in the natural configuration.

Studies on the catalytic activity and other chemistry of I and closely related derivatives are continuing.

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Nonclassical Bridged Ion in Acetolysis of *threo*-3-Anisyl-2-butyl *p*-Bromobenzenesulfonate[1]

Sir:

Some results of our further investigation of the acetolysis of *threo*-3-anisyl-2-butyl *p*-bromobenzenesulfonate[2] (I-OBs) are of special interest as regards the competition between bridged and classical ions and that between ion pairs and dissociated ions[2a,d] in solvolysis. In this communication we report on the bridged ion and in the following one we discuss ion pairs and dissociated carbonium ions from I-OBs.

Acetolysis of I-OBs has been studied in a series of acetic acid–acetic anhydride mixtures.[3] The major product is *threo* acetate in all the solvents, even in pure acetic anhydride, although some of the exact details of how I-OAc is produced in the latter solvent are not clear. Reproducible values of the titrimetric rate constant, k_t^0, followed by titration with standard sodium acetate in acetic acid, were obtained in pure acetic anhydride, the k_t^0 values in several different batches of solvent showing no variation due to changes in concentration of trace contaminants (*e.g.*, AcOH).

(1) Research sponsored by the National Science Foundation.

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