dienone and benzophenone.[3] The triplet energy of **1**, corresponding to the 0–0 band in the emission spectrum, is 67.2 kcal/mole, and the well-resolved band separation is 1680 $cm^{-1}$.

The chemical and spectroscopic properties determined for the excited state of **1** which leads to *p*-cresol (by abstraction–fragmentation) and lumiproduct **5** (by rearrangement) are those of a classic $n,\pi^*$ triplet state. The results strongly indicate that these reactions are competitive processes proceeding from a single diradical-like $^3(n,\pi^*)$ excited state.

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## Electrolytic Generation of Solvated Electrons and Reduction of the Benzene Ring in Ethanol Containing Hexamethylphosphoramide

*Sir:*

Until now, electrolytic generation of solvated electrons could be achieved only in solvents of low proton donor capability such as liquid ammonia[1,2] or certain amines,[3-5] a fact which imposed severe restrictions on the choice of solvent and reaction conditions. Attempts, described below, to generate solvated electrons electrolytically in a solvent of relatively high proton donor capability such as ethanol were doomed to failure due to hydrogen evolution at a potential far below that required for release of electrons into the solvent. The recently published discovery[6] that hexamethylphosphoramide (HMPA), $[(CH_3)_2N]_3PO$, is capable of dissolving alkali metals prompted us to investigate whether electrolytic generation of solvated electrons in a solution of ethanol containing HMPA was possible.

We found that release of electrons into ethanol–HMPA solutions could be achieved and that the solvated electrons can add to the benzene ring. When a solution of HMPA, 0.3 *M* in LiCl, is electrolyzed, dark blue globules, characteristic of solvated lithium, form at the cathode surface at a potential of −2.3 v (*vs.* Ag wire) and the solution in the cathode compartment becomes deep blue, visual evidence that the reaction $Li_s^+ + e^- \rightarrow Li_s^+ \cdots e_s^-$ is taking place.[7] In the presence of ethanol the intensity of the color is lower, depending on the amount of alcohol present. However, in the presence of both alcohol and benzene the solution remains colorless during electrolysis, with only a small amount of blue color visible at the cathode surface. Proof that electrolytic reduction of the benzene ring in ethanol–HMPA solution is possible was obtained by electrolysis of tetralin in a solution composed of 67 mole % ethanol and 33 mole % HMPA, 0.3 *M* in LiCl. The electrolysis was carried out at a cathode potential of −2.5 v (*vs.* Ag wire) in an apparatus described previously.[4] A carbon electrode served as an anode and an aluminum electrode as the cathode. After completion of the electrolysis, analysis of the recovered product by mass spectrometric and glpc methods showed that it consisted of (volume %) tetralin (80), hexalin (2), octalin (1), and decalin (17), and that tetralin had been hydrogenated at a current efficiency of 54%. When the electrolysis of tetralin is carried out under the same conditions but in the absence of HMPA, copious hydrogen evolution takes place and the cathode potential during electrolysis becomes now −1.5 v (*vs.* Ag wire), *i.e.*, 0.8 v more anodic than that at which release of electrons was observed into HMPA or ethanol–HMPA solution. Under these conditions not even traces of reduced tetralin could be detected in the recovered material. It is remarkable that the strong hydrogen evolution that occurs during electrolysis of ethanol at −1.5 v is drastically reduced in the presence of as little as 33 mole % HMPA. This suggests adsorption of the aprotic but highly polar[8] HMPA at the electrode surface to the near exclusion of ethanol. In the presence of HMPA, the charge-transfer process is release of electrons into the solvent and not hydrogen evolution. The high percentage of decalin in the reaction product is probably due to the high proton availability in the solution containing 67 mole % ethanol, since electrolytic reduction of tetralin in ethanol–HMPA containing only 25 mole % ethanol gave hexalin as the main product. Tetralin is not reduced when the electrolysis is carried out in HMPA in the absence of ethanol. Under these conditions, the catholyte becomes dark green during electrolysis. Mass spectrometric analysis of the recovered reaction product indicates the presence of considerable amounts of mono- and dimethyltetralin and small amounts of dimers of these methyltetralins, in addition to unchanged starting material, tetralin.

On the basis of these results and previous work on the reduction of the benzene ring in ethylenediamine,[4] we believe that electrochemical reduction of the benzene ring in ethanol–HMPA involves addition of the solvated electron, $e_s^-$, to the solvated benzene ring, $B_s$,

$$S + e^- \longrightarrow e_s^-$$
$$B_s + e_s^- \longrightarrow S + B_s^-$$

where S indicates the solvent molecules required to solvate an electron. Subsequent protonation of the benzene anion, $B_s^-$, and further addition of electron and proton complete the hydrogenation of a double bond, as has been pointed out previously.[9] Apparently, the cathode in ethanol–HMPA solution containing alkali halide functions in the same way as in liquid ammonia[2] and amines,[4] *i.e.*, as an electron electrode.

The present results demonstrate for the first time that electrolytic generation of solvated electrons in a solvent of relatively high proton donor capability is

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feasible and that these electrons are available for addition to organic compounds.

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## Pepsin as an Esterase

*Sir:*

Current interest in the mechanism of pepsin action prompts us to report the rapid cleavage, by crystalline swine pepsin, of the depsipeptide benzyloxycarbonyl-L-histidyl-*p*-nitro-L-phenylalanyl-β-phenyl-L-lactic acid methyl ester (Z-His-Phe($NO_2$)-Pla-OMe) at the ester bond linking the Phe($NO_2$) and Pla residues. At pH 4, the rate of this hydrolysis exceeds the rate of cleavage of the peptide bond linking the Phe($NO_2$) and Phe residues in the corresponding peptide benzyloxycarbonyl-L-histidyl-(*p*-nitro)L-phenylalanyl-L-phenylalanine methyl ester (Z-His-Phe($NO_2$)-Phe-OMe). Both the depsipeptide and the peptide are cleaved by pepsin more rapidly at pH 4 than at pH 2, thus exhibiting a pH dependence similar to that observed previously for the action of pepsin on synthetic substrates of the type Z-His-Phe-Phe-OEt.[1]

Z-His-Phe($NO_2$)-Pla-OMe (mp 134–136°) was prepared in 64% yield by the azide method, with Z-Phe($NO_2$)-Pla-OMe (mp 135–136°) as an intermediate, the latter having been made (65% yield) by the benzenesulfonyl chloride method.[2] The peptide Z-His-Phe($NO_2$)-Phe-OMe (mp 217–218° dec) was obtained in 85% yield by the azide method, with Z-Phe($NO_2$)-Phe-OMe (mp 167–168°) as an intermediate, the latter having been made (93% yield) by the N,N′-dicyclohexylcarbodiimide method. The final products and all intermediates gave satisfactory elemental analyses and were homogeneous by thin layer chromatography.

Because these substrates, like those recently developed in this laboratory,[1] have a site of protonation at the imidazolyl group, they are moderately soluble in aqueous buffered media in the pH range 2–5, and the addition of an organic solvent is not required; such solvents have been shown to inhibit the action of pepsin on synthetic substrates.[3] In contrast to widely used pepsin substrates (*e.g.*, Ac-Phe-Tyr), the compounds used in the present work do not contain a free carboxylate group; the presence of such a group adjacent to the sensitive bond has been shown to inhibit pepsin action.[1] The replacement of the central L-phenylalanyl residue of Z-His-Phe-Phe-OMe by a *p*-nitro-L-phenylalanyl residue permits spectrophotometric measurement at favorable wavelengths of the kinetics of enzymic cleavage, but does not affect markedly the rate of pepsin action at the sensitive peptide bond. The results obtained by the spectrophotometric method and the ninhydrin method[1] for the cleavage of Z-His-Phe($NO_2$)-Phe-OMe were identical. For measurement of the rate of cleavage of the ester linkage in Z-His-Phe($NO_2$)-Pla-OMe the ninhydrin procedure is not applicable, and spectrophotometry is the method of choice.

The enzymic cleavage of the two substrates is restricted to the scission of the ester or amide bond between the Phe($NO_2$) residue and the Pla or Phe residue. This was demonstrated for the depsipeptide by the isolation, from the peptic hydrolysate, of Z-His-Phe($NO_2$) (mp 236° dec) in 78% yield, and thin layer chromatography showed β-phenyllactic acid methyl ester to be the only other product of hydrolysis. In the cleavage of Z-His-Phe($NO_2$)-Phe-OMe, the increase in ninhydrin color stopped after 100% hydrolysis of one peptide bond, Z-His-Phe($NO_2$) was isolated in 86% yield, and thin layer chromatography showed phenylalanine methyl ester to be the only ninhydrin-reactive component of the hydrolysate.

The initial rates of hydrolysis, as determined spectrophotometrically, were linear, and satisfactory Michaelis–Menten kinetics were observed; the values of $K_M$ (app) and $k_{cat}$ are shown in Table I. Comparison of

**Table I.** Kinetics of Pepsin Action on Synthetic Substrates[a]

| Substrate | $K_M \times 10^5$, *M* pH 2.0 | pH 4.0 | $k_{cat} \times 10^2$, $sec^{-1}$ pH 2.0 | pH 4.0 |
|---|---|---|---|---|
| Z-His-Phe($NO_2$)-Pla-OMe[b] | 40 ± 8 | 40 ± 3 | 13 ± 3 | 77 ± 4 |
| Z-His-Phe($NO_2$)-Phe-OMe[c] | 52 ± 8 | 46 ± 3 | 7 ± 2 | 29 ± 3 |

[a] Enzyme preparation, twice-crystallized swine pepsin (Worthington lot PM 708); substrate concentration, 0.05–0.25 m*M* (5 points in plots of $S/v$ against $S$); pH controlled by sodium citrate buffers (0.04 *M*); 37 ± 0.1°; initial rates followed with a Cary Model 15 recording spectrophotometer equipped with automatic sample changer. At pH 4, $\Delta\epsilon_{310} = 1060$ for the cleavage of the Phe($NO_2$)–Pla bond, and 800 for the cleavage of the Phe($NO_2$)–Phe bond; at pH 2, $\Delta\epsilon_{265} = -420$ for both substrates. Control experiments, in which either the enzyme or the substrate was omitted, showed no significant change in absorbance during the time period of the kinetic measurements. [b] Enzyme concentration 0.02 mg/ml ($5.72 \times 10^{-7}$ *M*) at pH 2; 0.005 mg/ml ($1.43 \times 10^{-7}$ *M*) at pH 4. [c] Enzyme concentration 0.04 mg/ml ($1.14 \times 10^{-6}$ *M*) at pH 2; 0.02 mg/ml ($5.72 \times 10^{-7}$ *M*) at pH 4.

the kinetic constants at pH 4 for Z-His-Phe($NO_2$)-Phe-OMe with those for Z-His-Phe-Phe-OEt reported previously[1] ($K_M = 1.8 \times 10^{-4}$ *M*; $k_{cat} = 0.31$ $sec^{-1}$) indicates that the principal effect of the *p*-nitro group is to increase $K_M$ slightly, without marked effect on $k_{cat}$. Of special importance is the finding that the values of $K_M$ for Z-His-Phe($NO_2$)-Phe-OMe and for Z-His-Phe($NO_2$)-Pla-OMe are nearly the same, but that the value of $k_{cat}$ at pH 4 for the ester is nearly three times that for the amide. The slower rate of hydrolysis of both substrates at pH 2 is a reflection of lower $k_{cat}$ values, the $K_M$ values being nearly the same at pH 2 and pH 4.

Benzyloxycarbonyl-L-histidyl-L-phenylalanyl-D-phenylalanine ethyl ester (mp 187–188°) and the corresponding LDL compound (mp 137–139°) are completely resistant to cleavage by pepsin and were found to be competitive inhibitors of the enzymic hydrolysis of both Z-His-Phe($NO_2$)-Phe-OMe and Z-His-Phe($NO_2$)-Pla-OMe. Lineweaver–Burk plots gave the same value of $K_I$ ($2.8 \times 10^{-4}$ *M*) at pH 4.0 and 37° for the LDL compound with the two substrates, and

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