Hypochromism values are more meaningful than those obtained by considering only the effect at the absorption maxima (hypochromicity), since the phenomenon is actually a function of the relative oscillator strengths.

It was determined by difference spectra that no new absorption band was present in Ad-C_n-Nic$^+$ and that there was no appreciable shift in absorption maximum in comparison with Ad-C_3 + Nic$^+$-C_3.[13] The spectra were run in sufficiently dilute solution to obviate intermolecular interactions.

From the results in Table I it will be seen that the hypochromism value for NAD$^+$ is in the same range as the hypochromicity value[4,5] previously ascribed to the coenzyme. At pH 7.0 the hypochromism of Ad-C_2-Nic$^+$ (7.6%), for which the dihedral angle formed by the planes of the heterocycles can be about 39° at the lower limit, is less than that of Ad-C_3-Nic$^+$ (11.4%), in which the extra methylene link provides greater flexibility and permits limiting folded conformations in which the planes of the heterocycles can be parallel. At pH 1, both rings are protonated and the two positively charged rings should repel each other, rendering unfavorable those conformations which are folded. The negligible hypochromism of Ad-C_3-Nic$^+$ at pH 1.0 results from conformations in which the rings are extended in acid media. The contribution of the positive charge in proximity to adenine can be assessed from the hypochromism values obtained for the models Ad-C_2-N$^+$ and Ad-C_3-N$^+$. The diminution in absorption stemming from the presence of the quaternary ammonium ion in the Ad-C_2-N$^+$ system (12.7%) is even greater than that relating to the nicotinamide ring in the analogous coenzyme model, Ad-C_2-Nic$^+$, which contains both heterocycles. The negligible hypochromism of Ad-C_3-N$^+$ relative to that of Ad-C_2-N$^+$ indicates that the effect of the positive charge alone falls off sharply with distance. By contrast, Ad-C_3-Nic$^+$, in which plane-parallel conformations make maximal oscillator interaction possible and bring the positive charge close to the adenine, exhibits ~11% greater absolute hypochromism than its trimethylammonium counterpart.[15]

Table I. Per Cent Hypochromisms, % $H(\nu)$, of NAD$^+$ and Coenzyme Models at the 260-mμ Band

pH (H_2O)	NAD$^+$	Ad-C_2-Nic$^+$	Ad-C_3-Nic$^+$	Ad-C_2-N$^+$	Ad-C_3-N$^+$
7.0	9.0	7.6	11.4	12.7	0.1
1.0	5.5	−2.9	−0.8	1.1	−0.6

Complete assessment of the relative importance of oscillator interaction between adenine and nicotinamide and of perturbation by the positive charge on nicotinamide is not possible, partly because the charge delocalization in the pyridinium system leaves only fractional positive charge on nitrogen and partly because the relative populations of similar conformations of Ad-C_n-Nic$^+$ and Ad-C_n-N$^+$ may not be directly comparable. What can be concluded from this study, however, is that, for NAD$^+$-type systems in aqueous solution, the hypochromism observed in their ultraviolet spectra will be a function of both (a) conformation favorable for interaction between the N-substituted nicotinamide and the adenine and (b) proximity of the positive charge to the adenine ring system. In sequels, additional studies on the ultraviolet and fluorescence spectra of spectroscopic models related to coenzymes and dinucleotide base pairs will be reported.

wish to express our appreciation for the generous assistance of Professor J. Jonas.

(15) F. Hirayama, *J. Chem. Phys.*, **42**, 3163 (1965), has shown in a different kind of experiment with an analogous homocyclic aromatic model, Ph$(CH_2)_n$Ph, that aromatic interaction was uniquely favored when n = 3.

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A Remarkably Stable Simple Thiepin

Sir:

Although thiepin and simple monoannulated derivatives of thiepin have not been described,[1] highly substituted thiepins which contain a cyclic eight-π-electron system have been prepared.[2] Generally these heterocycles undergo thermal rearrangement with loss of sulfur, their instability sharply increasing with decreasing ring substitution. Indeed, one attempt to synthesize an unsubstituted benzothiepin resulted only in the formation of sulfur-free products.[3] In marked contrast to these observations, we wish to report that thieno[3,4-*d*]-thiepin (I), an unsubstituted monoannulated thiepin system, exhibits remarkable thermal stability.

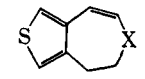
Periodate oxidation[4] of 4,5-dihydrothieno[3,4-*d*]-thiepin (II)[5] in aqueous methanol gave in 97% yield the corresponding vinyl sulfoxide III, mp 78°.[6] Brief treatment of III with freshly distilled acetic anhydride at 150° in the absence of oxygen followed by thick layer chromatography of the reaction mixture resulted in the isolation of bright yellow crystals of I, mp 149–151°, in 50% yield.[7]

The thiepin structure for I was indicated by its mass spectrum which showed a major fragment at *m/e* 134, the ion of benzo[*c*]thiophene (IV),[8] as well as the parent ion at 166.[9] Additionally, the nmr spectrum of I in $CDCl_3$ exhibits singlet resonance at δ 6.64 (two protons) and a quartet of bands centered at δ 6.06 and 5.22 (four protons), which is in accord with the assigned structure. Absorption maxima at λ_{max}^{MeOH} 210 mμ (log ϵ 4.13), 224 (4.12), 228 (4.15), 245 (4.29), 1251 (4.41), 260 (4.41), 308 (3.23), 318 (3.30), 331 (3.27), 346 (3.03), 369 (2.56), 378 (2.49), and 390 (2.43) indicate extended conjugation to be present in I.

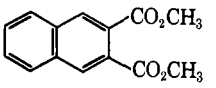
(1) R. Zahradnik, *Advan. Heterocyclic Chem.*, **1**, (1965).
(2) For examples, see (a) H. Hofmann and H. Westernacker, *Angew. Chem.*, **79**, 238 (1967); (b) M. J. Jorgenson, *J. Org. Chem.*, **27**, 3224 (1962); (c) J. D. Loudon and A. D. B. Sloan, *J. Chem. Soc.*, 3262 (1962); (d) V. J. Traynelis and R. F. Love, *J. Org. Chem.*, **26**, 2728 (1961).
(3) V. J. Traynelis and J. R. Livingston, *ibid.*, **29**, 1092 (1964).
(4) N. J. Leonard and C. R. Johnson, *ibid.*, **27**, 282 (1962).
(5) G. Eglinton, I. A. Lardy, R. A. Raphael, and G. A. Sims, *J. Chem. Soc.*, 1154 (1964).
(6) All melting points are uncorrected. Satisfactory analyses were obtained for all new compounds reported.
(7) For examples of the conversion of sulfoxides into vinyl sulfides, see (a) L. Horner and P. Kaiser, *Ann.*, **626**, 19 (1959); (b) W. E. Parham and R. Koncos, *J. Am. Chem. Soc.*, **83**, 4034 (1961); (c) W. E. Parham and M. D. Bharsar, *J. Org. Chem.*, **28**, 2686 (1963).
(8) R. Meyer, H. Kleinert, S. Richter, and K. Gewald, *J. Prakt. Chem.*, **20**, 244 (1963).
(9) Mass spectra were obtained at 70 ev. Peaks at *m/e* 166 and 134 showed correct ^{34}S isotopic abundance.

When heated above its melting point, thiepin I was found to decompose slowly to a mixture of products. However, on heating I at 150° with N-phenylmaleimide as the solvent, there was formed a single crystalline Diels–Alder adduct in nearly quantitative yield. This substance was identified as the previously reported *exo* adduct V of benzo[*c*]thiophene (IV) and N-phenylmaleimide.[10] Thiophene IV, on the other hand, gave in high yield a 1:1 mixture of the *exo* and *endo* adducts V and VI under identical conditions. Both I and IV when allowed to react with dimethyl acetylenedicarboxylate at elevated temperatures gave the naphthalene dimethyl ester VII in good yield.

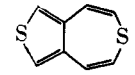
A number of explanations may be given to account for the great stability and Diels–Alder behavior of I. For example, the stability of I may be due to the contribution of charge-separated species such as VIII. The stereospecificity of I in adduct-forming reactions may be accounted for either by assuming appropriate Coulombic interaction of VIII with an electron-deficient dienophile or by assuming a nonplanar conformation for the thiepin portion of I. Additionally, the behavior of I may be rationalized in term of a common reaction intermediate such as the *o*-quinonoid episulfide IX.[11] Adducts of *exo* configuration would be expected to arise from IX, due to its unsymmetrical nature. The tetracovalent sulfur atom present in the quinonoid portion of IX should cause the extrusion of sulfur from I to be a high-energy process.[12]



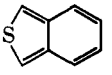
II, X = S
III, X = SO



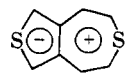
VII



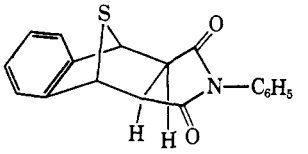
I



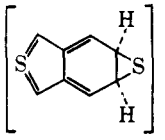
IV



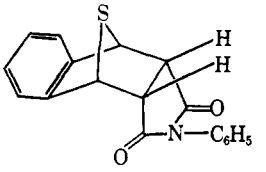
VIII



V



IX



VI

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(10) M. P. Cava and N. M. Pollack, *J. Am. Chem. Soc.*, **88**, 4112 (1966).

(11) Sulfur extrusion from thiepins long has been considered to proceed through episulfide intermediates; see J. D. Loudon in "Organic Sulfur Compounds," Vol. 1, N. Kharasch, Ed., Pergamon Press, Inc., New York, N. Y., 1961, p 299; also see ref 2a.

(12) For examples of high-energy tetracovalent sulfur species, see (a) M. P. Cava and N. M. Pollack, *J. Am. Chem. Soc.*, **89**, 3639 (1967); (b) M. P. Cava, N. M. Pollack, and D. A. Repella, *ibid.*, **89**, 3640 (1967); (c) R. H. Schlessinger and I. S. Ponticello, *ibid.*, **89**, 3641 (1967). This type of argument also may be used to account for the stability trends observed for other substituted thiepins; see B. P. Stark and A. J. Duke, "Extrusion Reactions," Pergamon Press, Inc., New York, N. Y., 1967, p 97.

The Kinetics of the *Escherichia coli* Alkaline Phosphatase Catalyzed Hydrolysis of 2,4-Dinitrophenyl Phosphate[1]

Sir:

Important progress in the elucidation of the mechanism of a multistep reaction can be achieved by observing individually the rate-limiting steps involved and by determining their kinetic parameters. In the *E. coli* alkaline phosphatase catalyzed hydrolysis of phosphate monoesters the transient formation of a phosphoryl–enzyme intermediate is well supported by experimental evidence[2] and, by using 4-nitrophenyl phosphate as a substrate, direct spectrophotometric observation of the reaction of phosphorylation of the enzyme has been accomplished.[3,4] However, in these experiments a satisfactory quantitative analysis of the results is impossible due to the unfavorable spectral properties of the substrate and the instability of the enzyme at low pH values where the reaction is slow enough to be measured by ordinary spectrophotometric techniques. These experimental difficulties can be eliminated by employing a rapid mixing technique and a substrate with negligible absorption in the spectral region of maximum absorbance of the reaction product. At pH 5.7—where the enzyme is sufficiently stable—2,4-dinitrophenyl phosphate satisfies these requirements; in the following we wish to report the results obtained by the use of this substrate.

Upon mixing an aqueous solution of an excess of the substrate at pH 5.5 (0.1 *M* acetate buffer) with a solution of alkaline phosphatase at pH 7.8 (0.01 *M* Tris-HCl, 0.1*M* NaCl) in a Durrum-Gibson stopped-flow spectrophotometer we observed at 400 mμ an initial rapid liberation of 2,4-dinitrophenolate ion for about 150 msec, followed by a slow, zero-order hydrolysis of the substrate. The kinetics of the presteady-state and the steady-state reactions were amenable to analysis by methods described earlier for this type of reactions,[5] and the appearance of 2,4-dinitrophenolate ion (P_1) has been found to obey an equation of the form: $P_1 = Vt + B(1 - e^{-bt})$, where V, B, and b are constants during the course of the reaction. By varying the initial concentration of the substrate and the enzyme and by adding an excess of phosphate ion (P_i) to the reaction mixture we found that B is proportional to the initial enzyme concentration and that the variation of b can be adequately described by $b = b_{lim}S/(S + K_S^{app})$, with S being the initial substrate concentration, b_{lim} a constant, and $K_S^{app} = K_S(1 + P_i/K_I)$.

(1) This research was supported by U. S. Public Health Service Medical Research Grant No. GM 13885. The authors wish to thank Professor J. H. Law and Professor E. A. Evans, Jr., for helpful suggestions.
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(3) A. Williams, *Chem. Commun.*, 676 (1966).
(4) W. K. Fife, *Biochem. Biophys. Res. Commun.*, **28**, 309 (1967).
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