nonplanar benzylideneanilines, either in solution or in the solid. The frequency corresponding to this spacing is *ca.* 1600 cm^{-1} compared to 1342 and 1599 cm^{-1} for A and S, respectively.[17] The frequency has been assigned to a stretching mode of the central bridge atoms which is superimposed on the electronic transition polarized in nearly the same direction. The presence of this vibrational structure suggests a polarization consistent with that of the polarization of the major portion of the band in A and S. Structural and spectroscopic investigations of other benzylideneanilines are continuing in our laboratories.

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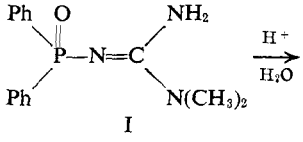
Acid-Dependent Associative and Dissociative Mechanisms of Displacement at Phosphorus. Hydrolysis of a Phosphinylguanidine Derivative

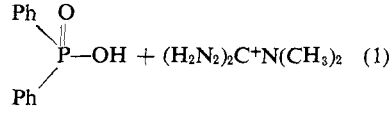
Sir:

The mechanism of hydrolysis of P–N bonds in phosphorus amides is strongly dependent on the nature of the substituents at phosphorus and at nitrogen. Creatine phosphate, a guanidine phosphate, is an important biological phosphorylating agent; hydrolysis of model guanidine phosphates occurs in very mild conditions with clear evidence for a metaphosphate intermediate,[1] but monoesters and diesters of guanidine phosphates are more stable.[1,2] Acid-catalyzed hydrolysis of diphenylphosphin*amides* proceeds by an associative (A2) mechanism,[3] but acid-catalyzed hydrolysis of diphenylphosphin*anilides* proceeds by a dissociative (A1 or A1-like) mechanism.[4]

We have studied the hydrolysis (eq 1)[5] of diphenyl-



I



(1)

phosphinyl-*N*,*N*-dimethylguanidine (I) over a wide range of concentrations of aqueous H_2SO_4 and find evidence for both associative and dissociative mechanisms of hydrolysis.

The first-order rate constants plotted *vs.* the acid concentration showed a bell-shaped profile at low acidity with a maximum at about 3 *M* H_2SO_4 and another rate increase above 10 *M* H_2SO_4 (Figure 1).[6] Such behavior cannot be explained in terms of a single mechanism.

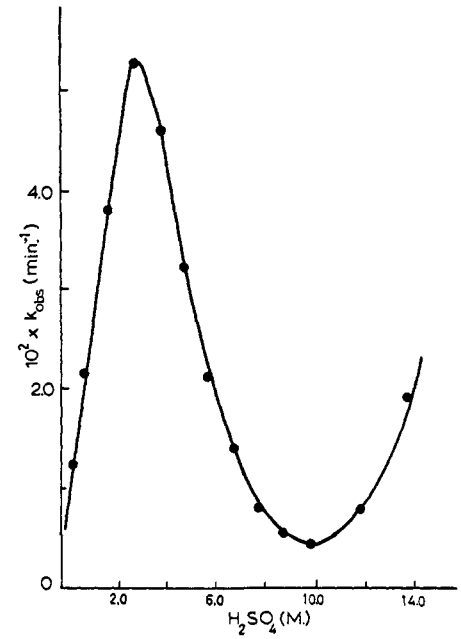
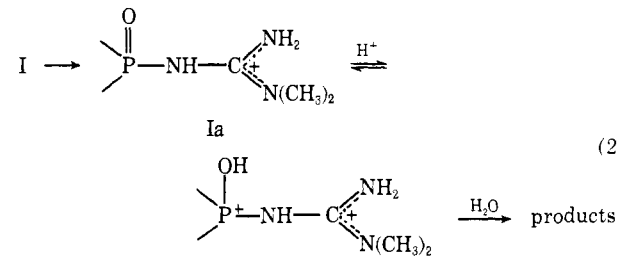


Figure 1. Dependence of rate of hydrolysis of I on acid concentration.

The rate constants obtained at 0.99 *M* H_2SO_4 gave $\Delta H^*_{75.8}$ = 17.6 kcal/mol, $\Delta S^*_{75.8}$ = −24 eu, and $\Delta F^*_{75.8}$ = 26.0 kcal/mol. The value of ΔS^* is in agreement with an A2 mechanism[7] and the decreasing reaction rate from 3 to 10 *M* H_2SO_4 also supports this hypothesis: acid catalysis is due to protonation on oxygen which is catalytic for the attack of water and formation of the pentacoordinate species; acid inhibition (3–10 *M* H_2SO_4) is due to decrease of water activity.[8] Because of the high basicity of the guanidine residue, I has to be completely protonated at the P–N nitrogen in the range of acidity studied. Therefore, the hydrolysis of I at low acidity can be interpreted in terms of an addition–elimination process through a pentacoordinate phosphorus intermediate. This reaction shows an unusual solvent–deuterium isotope effect, k_H/k_D = 1.4, at 0.99 *M* H_2SO_4; k_H/k_D is normally about 0.7 in A2 reactions.[9] Possible explanations include the unusual importance of proton transfer in the rate-determining step or the unusual solvation requirements for II which is a guanidinium ion and a dication.



Ia

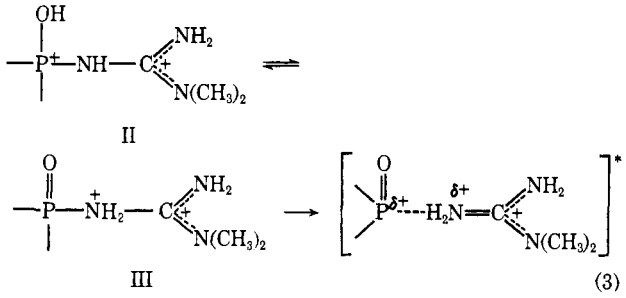
II

(2)

The rate increase at acidities greater than 10 *M* H_2SO_4 could be due to the increased ionizing power as acidity

(1) P. Haake and G. W. Allen, *Proc. Nat. Acad. Sci. U. S.*, **68**, 2691 (1971).
(2) V. M. Clark, A. R. Todd, and S. G. Warren, *Biochem. Z.*, **338**, 591 (1963); V. M. Clark and S. G. Warren, *Nature (London)*, **199**, 657 (1963).
(3) P. Haake and T. Koizumi, *Tetrahedron Lett.*, 4845 (1970).
(4) P. Haake and D. A. Tyssee, *ibid.*, 3513 (1970).
(5) The rates were measured spectrophotometrically on a Cary-16 spectrophotometer at 216 nm.
(6) Similar phenomena have been found in carboxylate derivatives: K. Yates, *Accounts Chem. Res.*, **4**, 136 (1971), and C. R. Smith and K. Yates, *J. Amer. Chem. Soc.*, **93**, 6578 (1971).
(7) L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).
(8) P. Haake and G. H. Hurst, *J. Amer. Chem. Soc.*, **88**, 2544 (1966).
(9) C. A. Bunton and V. J. Shiner, *ibid.*, **83**, 3214 (1961).

increases which would be expected to increase the concentration of the N-protonated tautomer III (eq 3) be-



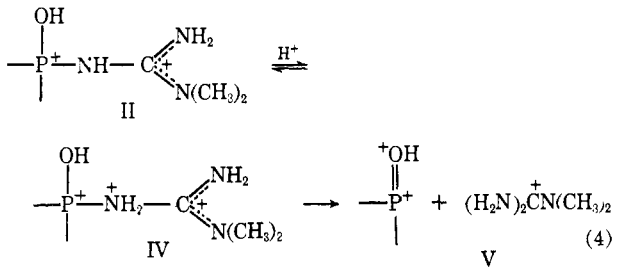
II

III (3)

cause III has a higher charge density than II and its concentration should therefore increase as the ionizing power of the solvent increases.

This is a similar tautomerism to that observed for phosphinanilides[4] in which case the analog of III is the reactive species. Because of the excellent leaving group, III would be expected to be highly reactive and would undergo rapid cleavage of the P–N bond with dissociative character (A1-like) in the transition state.[10] Therefore, although the concentration of III may be small relative to II, reaction could proceed through this. This hypothesis is supported by the solvent isotope effect determined in 12 M H_2SO_4, $k_H/k_D = 0.5$, which is in agreement with an acid-catalyzed reaction with dissociative character.[9,11]

Although this is not the only possible mechanism for the hydrolysis of I in acidities above 10 M H_2SO_4, the only other pathways result in unlikely species such as the pathway involving intervention of a trication IV. This seems unlikely since: (1) a pK_a value of -11 for the *diprotonated* tetramethylguanidine has been determined,[12] and (2) reaction through IV would produce highly unstable intermediates (*e.g.*, V) of prohibitively high energy.



II

IV V (4)

Therefore, the reasons for the characteristic rate–acidity profile for the hydrolysis of I can be summarized at low acidity by protonation of an increasing fraction of substrate until decrease in water activity reduces the rate of the A2 process and at higher acidity by the fraction of the N-protonated dication which is the reactive species and which undergoes cleavage of the P–N bond by a mechanism with considerable dissociative character.

(10) P. Haake and P. S. Ossip, *J. Amer. Chem. Soc.*, **93**, 6924 (1971).

(11) From the rate of hydrolysis of I in 13.9 M H_2SO_4, we determined $\Delta S^*_{75.8} = -30$ eu. Although ΔS^* values are normally near zero in a reaction proceeding by an A1 mechanism, the high-solvation requirement of III would be expected to lead to a high negative entropy of formation of III so that the entropy change associated with the rate-determining step could be very small. These entropy considerations have been discussed briefly[4] and will be treated in more detail in a paper now in preparation (P. Haake and D. A. Tyssee).

(12) S. Limatibul and J. W. Watson, *J. Org. Chem.*, **36**, 3805 (1971).

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Laser Raman Spectra of Native and Denatured Insulin in the Solid State

Sir:

In order to demonstrate the sensitivity of Raman spectroscopy to protein conformation, we have selected a small protein, insulin, as a model and undertaken a systematic Raman spectroscopic study of it under various conditions. Recently we have reported a preliminary spectrum of native zinc-insulin crystal[1] in which a total of 45 lines were observed and tentative assignments were made. At present, we wish to report a higher resolution spectrum of the same sample together with a spectrum of denatured insulin for the purpose of comparison (Figure 1). These two spectra were obtained under comparable experimental conditions. Comparison of these two spectra (see Figure 2) reveals some striking and interesting spectral changes, indicating that the structure and conformation of insulin have undergone considerable changes during denaturation. It is obvious that a detailed analysis of these spectral changes will certainly provide detailed information about the nature of this structural transformation.

Biologically, insulin is a hormone concerned in the regulation of carbohydrate metabolism. It is well known that it undergoes a transformation from globular to fibrous form in dilute HCl solution by heating, with a concomitant loss in its activity and solubility.[2] However, both the activity and solubility can be recovered by treating the inactivated insulin with a 10% aqueous phenol solution.[3,4] The nature of this transformation has been extensively studied by various techniques.[2]

In 1951, Ambrose and Elliott[5] reported an infrared study of insulin in the amide I and amide II regions. It was found that the denaturation by heating at pH 2 causes a weakening of the C=O stretching band (amide I) at 1657 cm^{-1} and produces a new band at 1637 cm^{-1}. They also have shown that if the denatured insulin is dissolved in aqueous phenol and precipitated with acetone, the 1657-cm^{-1} band reappears. This observation is quite different from our present Raman results. We have observed that the amide I line at 1664 cm^{-1} (with a shoulder near 1680 cm^{-1}) is shifted to 1673 cm^{-1} and sharpened quite considerably upon denaturation. Obviously the infrared and Raman techniques are not detecting the same amide I vibrations in proteins. The coupling of adjacent peptide groups may be

(1) Nai-Teng Yu, C. S. Liu, J. Culver, and D. C. O'Shea, *Biochim. Biophys. Acta*, **263**, 1 (1972).

(2) D. F. Waugh, *Advan. Protein Chem.*, **9**, 326 (1954); see references cited therein.

(3) T. D. Gerlough and R. W. Bates, *J. Pharmacol.*, **45**, 19 (1932).

(4) V. du Vigneaud, R. H. Sifferd, and R. R. Sealock, *J. Biol. Chem.*, **102**, 521 (1933).

(5) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc., Ser. A.*, **208**, 75 (1951).