372. *Kinetic and Isotopic Studies of the Hydrolysis of* NN-*Diethylcarbamoyl Phosphate.*
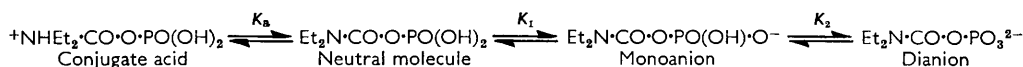
By A. Lapidot and David Samuel.

The kinetics of the hydrolysis of *NN*-diethylcarbamoyl phosphate have been studied in the range from pH 7 to 3·1N-perchloric acid. In basic solution it is hydrolysed very slowly at 100° with C–O bond fission. From pH 7 the rate of hydrolysis increases with increasing acidity to a maximum at pH 4, where hydrolysis occurs with C–O bond fission, and a deuterium solvent isotope effect $k_D/k_H = 0.75$. It is suggested that the monoanion, which is predominant at pH 4, is hydrolysed by a unimolecular mechanism to phosphate and an *NN*-diethylcarbamoyl cationic intermediate which then reacts rapidly with the solvent. At more acidic pH the hydrolysis of *NN*-diethylcarbamoyl phosphate is acid-catalysed, the rate being a function of the stoicheiometric concentration of acid, indicating an $A2$ type of reaction with P–O bond fission. In the strongly acid region there is a solvent isotope effect, $k_D/k_H = 1.4$, due to displacement of the protonation equilibrium.

Recent studies [1,2] of the decomposition of carbamoyl phosphate in aqueous solutions showed that above pH 6, in addition to hydrolysis, an elimination reaction occurs in which cyanate and phosphate are formed. Since carbamoyl phosphate can exist in aqueous solution in at least five different ionic forms, depending on the pH, a variety of reactions are possible. From the equilibrium constants between the different species, the rate of formation of various products, and the extent of P–O bond fission, the mechanism of decomposition of each ionic species of carbamoyl phosphate was determined.[2] It was suggested that the neutral molecule (or zwitterion), the monoanion, and the dianion undergo heterolytic C–O bond fission with the formation of phosphate and an unstable " carbamoyl " intermediate which then reacts rapidly with the solvent to give the products. In order to obtain additional evidence for this novel type of reaction mechanism, the decomposition of an *NN*-disubstituted carbamoyl phosphate in aqueous solution has been examined, where neither elimination nor dissociation to a trianion is possible. *NN*-Diethylcarbamoyl phosphate * was synthesised for this purpose, and its decomposition in aqueous solution was studied by the kinetic and isotope techniques used [2] for the parent compound.

Results

NN-Diethylcarbamoyl phosphate can exist in four ionic forms:

$$^+NHEt_2 \cdot CO \cdot O \cdot PO(OH)_2 \xrightleftharpoons{K_a} Et_2N \cdot CO \cdot O \cdot PO(OH)_2 \xrightleftharpoons{K_1} Et_2N \cdot CO \cdot O \cdot PO(OH) \cdot O^- \xrightleftharpoons{K_2} Et_2N \cdot CO \cdot O \cdot PO_3{}^{2-}$$

Conjugate acid Neutral molecule Monoanion Dianion

The position of the additional proton in the conjugate acid is depicted on nitrogen although its actual position is not yet known; similarly, the neutral molecule may, in fact, exist as a zwitterion, $^+NHEt_2 \cdot CO \cdot O \cdot PO(OH) \cdot O^-$.

The acid dissociation constants of the neutral molecule (K_1) and of the monoanion (K_2) were determined by potentiometric titration, giving $pK_1 = 3.46$ and $pK_2 = 5.4$, at 25·0°.

Since *NN*-diethylcarbamoyl phosphate is fairly stable at pH 1, the rate of hydrolysis can be determined by following the rate of formation of phosphate colorimetrically. The results at various pH's are given in Table 1. The rate of hydrolysis rises to a maximum at pH 4 and no reaction occurs in 0·09N-sodium hydroxide during 14 days at 50°. The

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* We found that *NN*-dimethylcarbamoyl phosphate is much less stable than the diethyl compound, in agreement with previous reports.[3]

[1] Jones and Lipmann, *Proc. Nat. Acad. Sci. U.S.A.*, 1960, **46**, 1194.
[2] Halmann, Lapidot, and Samuel, *J.*, 1962, 1944.
[3] Balsiger, Jones, and Montgomery, *J. Org. Chem.*, 1959, **24**, 434.

TABLE 1.

Hydrolysis of *NN*-diethylcarbamoyl phosphate at 50·0°.

Solvent	HCl aq.	A.B.	KHP	KHP *	DB	NaOH aq.
Concn. (M)	0·0786	0·1	0·05	0·05	0·05	0·0882
pH	—	3·4	4·0	—	7·03	—
$10^4 k_{obs}$ (sec.$^{-1}$)	1·18	1·85	2·25	1·66	0·084	No reaction after 14 days

* Rate in D_2O.   A.B. = Acetate buffer.   KHP = Potassium hydrogen phthalate.   DB = Diethyl barbiturate.

usual switchback type of pH profile is observed.   At pH 4 there is a reverse deuterium isotope effect, $k_D/k_H = 0.74$, as was found [2] in the hydrolysis of carbamoyl phosphate.

The energy and entropy of hydrolysis at pH 4 were calculated from the rate constants at three temperatures (Table 2).   The energy of activation is similar in magnitude to that found for amides [4] and for mixed anhydrides [2,5] of phosphoric acid.   After passing through

TABLE 2.

First-order rate constants for hydrolysis at pH 4.

Temp. (°c)	37·0	50·0	61·0	$\Delta E = 24.4$ kcal. mole^{-1}
$10^4 k$ (sec.$^{-1}$)............	0·53	2·25	9·8	$\Delta S = -0.4$ e.u.

TABLE 3.

Hydrolysis of *NN*-diethylcarbamoyl phosphate in acid solution at 50·0° and constant ionic strength ($\mu = 3$).

[HClO$_4$] (M)	0·0925	0·24	0·435	0·925	1·94	2·74	3·1
Solvent	D_2O	H_2O	H_2O	H_2O	H_2O	D_2O	H_2O
$10^4 k_{obs}$ (sec.$^{-1}$).........	1·1	1·8	2·6	4·3	8·6	16·5	13·1

a minimum at about pH 0 the rate of hydrolysis increases with increasing acid concentration.   First-order rate constants at constant ionic strength are shown in Table 3 and Fig. 1.   It is seen from Table 3 that the rate is acid catalysed.   The rate constant for the neutral species, k_0, was determined by plotting k_{obs} against acidity at constant ionic strength and extrapolating to zero acidity (Fig. 1).   The value obtained is $k_0 = 0.7 \times 10^{-4}$ sec.$^{-1}$ at 50°.   The rate constant for the hydrolysis of the conjugate acid, k_A, is obtained from the slope of this plot; $k_A = 4.0 \times 10^{-4}$ l. mole^{-1} sec.$^{-1}$ at 50·0°.   The dependence of log ($k_{obs} - k_0$) on the stoicheiometric concentration of acid and on the Hammett acidity function H_0 is shown in Fig. 2.   A linear relationship with log [H$_3$O]$^+$ is obtained, with a slope of 1·0.

The rates of hydrolysis of *NN*-diethylcarbamoyl phosphate, measured in deuterium oxide as solvent under various conditions of acidity, are also included in Table 3.   On plotting the observed rate constant in both H_2O and D_2O solvents against acidity (Fig. 2) it is seen that the non-acid-catalysed reation (in 0·09M-perchloric acid) appears to be unaffected by substituting deuterium for hydrogen in the solvent.   However, in strongly

TABLE 4.

Hydrolysis of *NN*-diethylcarbamoyl phosphate in ^{18}O-enriched solvents at 100°.

Solution	A, Atom % excess ^{18}O in water	B, Atom % excess ^{18}O in phosphate	B/A	% P–O fission
N-HClO$_4$	8·29	1·84	0·222	89
pH 4	8·29	0·11	0·0132	5
0·1N-NaOH.................	9·29	0·083	0·01	4

acid solution (2·74M-HClO$_4$ in D_2O) a kinetic isotope effect is evident for which $k_D/k_H = 1.42$, as was found [4] in many other acid catalysed reactions.

[4] Halmann, Lapidot, and Samuel, *J.*, 1960, 4672; 1961, 3158.
[5] Bentley, *J. Amer. Chem. Soc.*, 1949, **71**, 2765; Park and Koshland, *J. Biol. Chem.*, 1958, **233**, 986.

The position of bond fission in the hydrolysis of NN-diethylcarbamoyl phosphate was determined in the usual way,[2] using ^{18}O-enriched solvents. From Table 4 it can be seen that in basic solution (under forcing conditions) and at pH 4 virtually no P–O bond fission
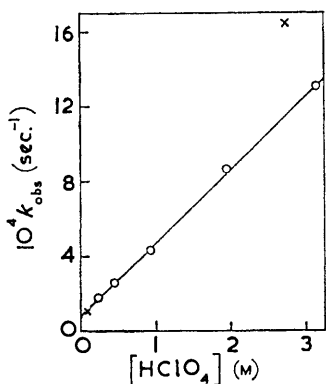

FIG. 1.   Acid-catalysed hydrolysis of NN-diethylcarbamoyl phosphate at constant ionic strength $\mu = 3$, at $50 \cdot 0°$.

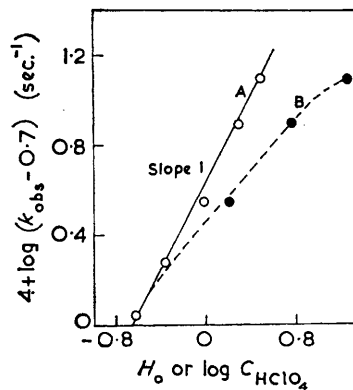○ Rate in H_2O; × rate in D_2O.

FIG. 2.   Dependence of $\log k_{obs}$ in the acid-catalysed hydrolysis of NN-diethylcarbamoyl phosphate on (A) acid concentration (C_{HClO_4}) or (B) acidity function (H_0), at $50 \cdot 0°$.

occurs, whilst in acid solution, in contrast to the reaction of carbamoyl phosphate,[2] nearly 90% P–O bond fission takes place.

DISCUSSION

The kinetic and isotopic results presented above for the hydrolysis of NN-diethyl-carbamoyl phosphate have some unusual features. Although this compound may be considered a mixed anhydride of NN-diethylcarbamic and phosphoric acid there appears to be virtually no reaction in basic solution. Owing to disubstitution on nitrogen, the elimination reaction found [1,2] in carbamoyl phosphate obviously cannot occur; also, apparently, the ready nucleophilic attack of hydroxide on the carbonyl group cannot take place, as has been found in the hydrolysis of acetyl phosphate.[5] The reason for this stability may be the proximity of a tertiary nitrogen atom which prevents the polarisation of the carbonyl group,[4] or possibly steric hindrance to attack on the carbonyl carbon due to the ethyl groups. Since attack at phosphorus is prevented by electrostatic repulsion, the stability of NN-diethylcarbamoyl phosphate in basic solution is similar to that of monoalkyl phosphates,[6] and distinctly in contrast to the lability of other phosphoric acid anhydrides.[1,2,5] The maximum at pH 4 in the rate of hydrolysis (Table 1) also superficially resembles the pH profile for the hydrolysis of esters [6] and amides [4] of phosphoric acid, where a cyclic mechanism involving proton transfer and P–O bond fission from the monoanion has been suggested. In contrast to ester hydrolysis, however, the breakdown of NN-diethylcarbamoyl phosphate at pH 4 occurs with almost exclusive C–O bond fission (Table 4), and with a solvent deuterium isotope effect of $k_D/k_H = 0 \cdot 74$. A similar isotope effect was found in the hydrolysis of carbamoyl phosphate at pH 4, where it was suggested [2] that the first step of hydrolysis is a slow ionisation to a carbamoyl cation and phosphate.

Robertson and his co-workers [7] showed, in an extensive study of the hydrolysis of

[6] Westheimer, *Chem. Soc. Special Publ.*, No. 8, 1957, p. 1; Vernon, *ibid.*, p. 17.
[7] Laughton and Robertson, *Canad. J. Chem.*, 1959, **57**, 1494; 1961, **39**, 2155; Leffek, Llewellyn, and Robertson, *ibid.*, 1960, **38**, 2171.
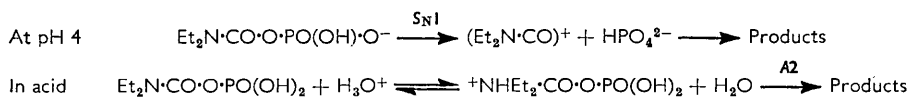
alkyl sulphonates and halides, that the deuterium solvent isotope effect for unimolecular reactions in water is (for t-alkyl halides, for instance) of the order of $k_D/k_H = 0.70$—0.75. We have confirmed that similar solvent isotope effects are found for phosphate esters, where the mechanism of hydrolysis is S_N1 with C–O bond fission. Thus, in the hydrolysis of t-butyl phosphate,[8] $k_D/k_H = 0.60$—0.73, depending on pH.

Further support for a unimolecular hydrolysis of the monoanion of NN-diethylcarbamoyl phosphate is obtained from the work of Hall[9] on the hydrolysis of NN-dimethylcarbamoyl chloride. He showed, by means of both kinetics and " trapping " experiments, that a transient "dimethylcarbamoyl cation" is formed by a rate-determining S_N1 heterolysis of the N–Cl bond.

In the acid range up to 3·1M-perchloric acid, the rate of hydrolysis is proportional to the stoicheiometric acid concentration (see Fig. 2). The Zucker–Hammett hypothesis therefore suggests that a water molecule is involved in the rate-determining step. Acid catalysis of this type, known as $A2$, has been found in the hydrolysis of alkyl phosphates[6] and of some phosphoramidates.[10]

The hydrolysis of acetyl[5] and carbamoyl[2] phosphate is also acid-catalysed with mainly C–O bond fission, but in NN-diethylcarbamoyl phosphate at least 89% P–O bond fission occurs, which means that the water molecule must attack the phosphorus atom rather than the carbonyl group. Here again, some resistance to nucleophilic attack on the carbonyl carbon is found, which may be either electronic or steric in origin. Owing to the greater general stability of the substituted compound, the rate of hydrolysis could be measured up to quite high acidities, and a deuterium solvent isotope effect of $k_D/k_H = 1.42$ was found in this region. This is of a similar magnitude to the usual deuterium solvent isotope effect found in acid-catalysed reactions[11] and is probably due to the shift in the equilibrium when deuterium oxide is substituted for water.

The mechanisms of hydrolysis of NN-diethylcarbamoyl phosphate may therefore be summarised:

At pH 4 $Et_2N\cdot CO\cdot O\cdot PO(OH)\cdot O^- \xrightarrow{S_N1} (Et_2N\cdot CO)^+ + HPO_4^{2-} \longrightarrow$ Products

In acid $Et_2N\cdot CO\cdot O\cdot PO(OH)_2 + H_3O^+ \rightleftharpoons\ ^+NHEt_2\cdot CO\cdot O\cdot PO(OH)_2 + H_2O \xrightarrow{A2}$ Products

The formation of a diethylcarbamoyl cation at intermediate pH lends further support to our original suggestion[4] that such an intermediate is formed in the hydrolysis of carbamoyl phosphate. The relative stability of the " carbamoyl " intensity may account for the many transcarbamoylation reactions[12] that occur in biological systems.

EXPERIMENTAL

The magnesium salt of diethylcarbamoyl phosphate was prepared[3] from diethylcarbamoyl chloride. Potentiometric titration of the product with 0·1N-hydrochloric acid using a TTl Radiometer pH meter indicates that the monomagnesium salt is formed, $[Et_2N\cdot CO\cdot O\cdot PO_3H]_2Mg$, and not as reported in the literature. The acid dissociation constants were determined from the titration curve as described previously.[4]

Deuterium oxide (99·8%) was used to prepare the solvents for the kinetic experiments. Acidic solvents were prepared from 72% perchloric acid and D_2O. Water enriched in ^{18}O was obtained from the Isotope Separation Plant of the Weizmann Institute.

Kinetic Measurements.—The rate of formation of phosphate was measured by keeping buffer (or acid) solutions of NN-diethylcarbamoyl phosphate (10^{-3}—10^{-2}M) in a thermostat-bath and withdrawing samples for spectrophotometric analysis of phosphate.[13]

[8] Lapidot, Samuel, and Weiss-Broday, *J.*, 1964, 637.
[9] Hall, jun., *J. Amer. Chem. Soc.*, 1955, **77**, 5993.
[10] Chanley and Feageson, *J. Amer. Chem. Soc.*, 1958, **80**, 2686.
[11] Wiberg, *Chem. Rev.*, 1955, **55**, 713.
[12] Jones, *Science*, 1963, **140**, 1373.
[13] Fiske and Subbarow, *J. Biol. Chem.*, 1925, **66**, 375.

Determination of the Position of Bond Fission.—Magnesium NN-diethylcarbamoyl phosphate (100 mg.) was dissolved in the appropriate solution (20 ml.). After 30 min. at 100° the solution was brought to room temperature and pH 1, and nitrogen was bubbled through it to remove carbon dioxide. After hydrolysis with 0·1N-sodium hydroxide the solution was left for 2 days at 100°. The solutions were brought to pH 9 (phenolphthalein) with N-sodium hydroxide, and sufficient M-barium chloride was added to precipitate barium phosphate, which was centrifuged off, washed with water, dissolved in N-hydrochloric acid (1 ml.), and re-precipitated by addition of sodium hydroxide to pH 9. After several washings with water and ethanol, the barium phosphate was dried in a vacuum and analysed for [18]O.

[18]O *Analyses.*—The [18]O content of the water was determined by equilibrium with normal carbon dioxide. The [18]O analyses of barium phosphate were made [14] by conversion into carbon dioxide by reaction with mercuric chloride and mercuric cyanide at 400°. The isotopic content of the carbon dioxide was determined using a Consolidated Engineering Corp. model 21-401 mass spectrometer.

THE ISOTOPE DEPARTMENT, THE WEIZMANN INSTITUTE OF SCIENCE,
REHOVOTH, ISRAEL.   [*Received, August 26th*, 1963.]

[14] Anbar and Guttmann, *Internat. J. Appl. Radiation Isotopes*, 1959, **3**, 233.