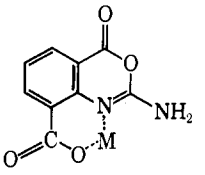
Given the intramolecular general acid catalysis mechanism for the hydrolysis of **11b** proposed above (**16**), it is perhaps surprising that metal ions do not have a more pronounced effect on the hydrolysis of **11**. The formation of a chelate complex such as **18** could be imagined, from which the reactivity of the a species associated with nitrogen protonation might be expected. The strangely varied results from an admittedly cursory examination of the hydrolysis of **11** in the presence of several metal ions, along with changes in the substrate spectrum in the presence of some of the metals (*e.g.*, Cu^{2+}), certainly point toward complex formation but these complexes clearly have no exceptional hydrolytic reactivity.



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Despite the uncertainties discussed in the preceding four paragraphs, the main point of this paper—that the reaction of nucleophiles with the carbonyl group of 2-amino-4-oxo-3,1,4-benzoxazine is strongly enhanced by addition of a neutral carboxyl group to the 8 position—seems indisputable. This enhancement is most likely an example of intramolecular general acid catalysis (**16**) involving proton donation by the carboxyl group to the ring nitrogen atom simultaneously with nucleophilic attack at the carbonyl group. The catalysis observed seems particularly efficient, the reactive species **11b** being only a few times less susceptible to nucleophilic attack than the species where the ring nitrogen atom is fully protonated. Of more importance perhaps is the fact that this catalysis ensures the reactivity of the benzoxazine system with nucleophiles at pH's just below neutrality where the basic system is inherently inert. Incorporation of a carboxyl group at the 5 position of the benzoxazine, ortho to the reactive carbonyl group (**13**), yields hydrolysis rates practically indistinguishable from those of a 6-carboxyl isomer (**12**) where participation is impossible. Thus the carboxyl at the 5 position apparently does not participate as a general acid, general base, or nucleophile to assist intermolecular nucleophilic attack.

The considerable advantage of a carboxyl group adjacent to an imidazoline nitrogen atom in biotin in terms of the proposed biotin mechanism (I) has thus been demonstrated.

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The Hydrolysis of *N*-Aryl Carbamyl Phosphate Mono- and Dianions[1]

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Abstract: Pseudo-first-order rate constants for the hydrolysis of *N*-phenyl, *N*-*p*-ethoxyphenyl, *N*-*p*-nitrophenyl, and *N*-*m*-chlorophenyl carbamyl phosphate have been obtained at 37° and ionic strength 0.60 *M* over a pH range in which mono- and dianionic species predominate. The rates of hydrolysis of the monoanionic species are markedly less sensitive to the electron-delocalizing ability of para substituents than those found for the dianionic species. The effects of varying temperature, ionic strength, organic solvent, and buffer concentrations on the k_{obsd} values for both mono- and dianions are consistent with unimolecular reaction mechanisms. Methanolysis studies indicate that the monoanionic and dianionic species of the substituted carbamyl phosphates undergo primarily P–O bond fission. However, at least in the case of *p*-nitrophenyl carbamyl phosphate monoanion, some C–O bond fission also occurs. Azide trapping studies support this conclusion since it can be shown that the *p*-nitrophenyl carbamyl group cannot be trapped during *p*-nitrophenyl carbamyl phosphate dianion hydrolysis in 1 *M* azide, whereas some carbamyl trapping is observed during hydrolysis of the monoanion. The data presented here indicate that mono-substituted carbamyl phosphate monoanions may hydrolyze *via* more than one mechanism, one of which is similar to that for the monoanions of carbamyl phosphate and other acyl phosphates whereas the mono-substituted carbamyl phosphate dianions hydrolyze *via* a mechanism similar to that for other acyl phosphates but different from the unsubstituted carbamyl phosphate dianion.

Acyl phosphates have been studied with regard to the rates and mechanism of hydrolysis with a variety of results. Studies of the uncatalyzed hydrolysis of the aliphatic acyl[2,3] and benzoyl phosphates[2] have led to the general conclusion that the mechanisms for the mono- and dianionic species involve unimolecular elimination reactions, with P–O bond fission, resulting in the release of the free carboxylic acid or carboxylate anion with the formation of the postulated monomeric metaphosphate ion as an intermediate to inorganic phosphate formation.

The mechanism of hydrolysis of carbamyl phosphate and its substituted derivatives appears, however, to be

(1) We are grateful to the National Institute of Arthritis and Metabolic Disease (Grant No. AM 12193) and to the College of Medicine, University of Florida, for financial support.

(2) G. Di Sabato and W. P. Jencks, *J. Amer. Chem. Soc.*, 83, 4400 (1961).

(3) D. R. Phillips and T. H. Fife, *J. Org. Chem.*, 34, 2710 (1969).

subject to the type and degree of substitution on the carbamyl nitrogen. The hydrolysis of the carbamyl phosphate monoanion[4] proceeds *via* a unimolecular elimination mechanism similar to that of other acyl phosphate monoanions. The decomposition of the dianionic species[4,5] is, however, different from the other acyl phosphates. In this case the mechanism involves a unimolecular elimination of isocyanate to yield inorganic orthophosphate *via* a fission of the C–O bond. The hydrolysis of *N,N*-diethyl carbamyl phosphate monoanion has been reported[6] to proceed *via* a monomolecular dissociation to give the carbamyl cation and orthophosphate by C–O bond fission. Other preliminary work[7] has shown that the substitution of a single aryl or aliphatic substituent on the carbamyl nitrogen of carbamyl phosphate results in an apparent shift in the mechanism of hydrolysis of the dianionic species such that P–O bond cleavage instead of C–O bond cleavage is observed. The data presented here indicate that monosubstituted carbamyl phosphate monoanions may hydrolyze *via* more than one mechanism whereas the monosubstituted carbamyl phosphate dianions hydrolyze *via* a mechanism similar to that for other acyl phosphates.

Experimental Section

Materials. The N-substituted carbamyl phosphates were prepared as the monotriethylammonium salts by the general procedures previously described.[8] Dilute solutions of each of the carbamyl phosphate derivatives were prepared and stored frozen between kinetic runs. In the case of *N-p*-nitrophenyl carbamyl phosphate fresh solutions were prepared at least every 2 weeks because of its lability. *p*-Nitrophenyl isocyanate used in the azide experiments was purchased from the Fluka Co. and was "purum grade." The triethylamine, acetonitrile, and dioxane were redistilled before use. Dioxane was also passed over an alumina column to remove any peroxides.[9] All other chemicals were of reagent grade and were used without further purification.

Spectra. The ultraviolet and visible spectra were all determined with a Carey 14 recording spectrophotometer in silica cells of 1 cm light path at room temperature.

Titration Experiments. Titrations were carried out on solutions of *N-p*-nitrophenyl, *N*-phenyl- and *N-p*-ethoxyphenyl carbamyl phosphate whose initial concentrations were 1.9×10^{-3}, 5×10^{-3}, and 5×10^{-3} *M*, respectively. A solution of the monoanion, held at either 37 or 25° at a constant ionic strength of 0.60 *M*, was titrated with dilute KOH, using a Radiometer automatic titrator, Model TTTl, with a syringe buret attachment and recorder. Titrations were completed in 5–10 min to minimize hydrolysis of substrates. Values of pK_a were determined from the inflection points of the titration curves. For *N-p*-nitrophenyl carbamyl phosphate, which is most labile in the dianion region, the pK_a values were the same within experimental error at 37 and 25°.

Kinetic Measurements. The pseudo-first-order rate constants for the hydrolysis of the substituted carbamyl phosphates were obtained from measurements of inorganic orthophosphate release, substituted aniline formation, or substituted carbamyl phosphate disappearance.

The assay method and reaction conditions used to obtain the rate constants by determining inorganic orthophosphate release were the same as those previously described.[4]

Hydrolytic rate constants were measured in most cases, however, by a spectrophotometric technique making use of the difference in light absorption properties between the substituted carbamyl phosphates and their substituted aniline products at various pH values. Direct and indirect methods were used for the spectrophotometric assay. In the direct method, after temperature equilibration of a 3-ml buffer–KCl solution, the reaction was initiated by the introduction of 0.1 ml of a solution of the substituted carbamyl phosphate. The rates of hydrolysis of *N-p*-ethoxyphenyl, *N*-phenyl, and *N-m*-chlorophenyl carbamyl phosphate monoanions were measured in most cases by this method following directly the loss in absorbance of the reactants at 250, 235, and 240 mμ, respectively. These wavelengths are near the absorption maximum of *N-p*-ethoxyphenyl carbamyl phosphate (λ = 239 mμ, ϵ = 15,200), *N*-phenyl carbamyl phosphate (λ = 233 mμ, ϵ = 13,650), and *N-m*-chlorophenyl carbamyl phosphate (λ = 238 mμ, ϵ = 13,950). In the range where *N-p*-nitrophenyl carbamyl phosphate monoanion predominates and below about pH 7 for the corresponding dianion, the appearance of *p*-nitroaniline was measured directly by the increase in absorbance at 430 mμ. The unhydrolyzed sample has an absorption maximum at 324 mμ (ϵ = 11,400), whereas the hydrolysis product, *p*-nitroaniline, has an absorption maximum at 382 mμ. The rates of hydrolysis of *N-p*-nitrophenyl carbamyl phosphate below pH 2 were measured directly by the loss in absorbance at 315 mμ.

The employment of an indirect method was usually necessary for measurements of the hydrolytic rate constants of the N-substituted carbamyl phosphate diainons in order to obtain good first-order kinetics. The stability of reaction intermediates such as the substituted carbamates under mildly basic conditions probably delays formation of the substituted anilines. The indirect method takes advantage of the rapid decomposition of aryl carbamates[10,11] and isocyanates[12] (also possible reaction intermediates) to the anilines at acid pH. In the indirect method, aliquots of 1 ml were removed from 20-ml reaction mixtures at various times and added to a dilute HCl solution to bring the final pH to about 2–3. The absorbance of this acid solution was then immediately read in a spectrophotometer at 235, 240, or 430 mμ for the *N*-phenyl, *N-m*-chlorophenyl, or *N-p*-nitrophenyl derivatives, respectively.

The hydrolysis of *N-p*-nitrophenyl carbamyl phosphate or *p*-nitrophenyl isocyanate in azide was carried out at 37° in solutions containing 0.06 *M* buffer, 0.59 *M* KCl, and 1.0 *M* NaN_3 adjusted with HCl to the pH of the buffer being used. The hydrolytic rate constants were determined from measurements of *p*-nitroaniline formation using the direct spectrophotometric method.

Experiments to measure the rate of *p*-nitroaniline release from aqueous solutions of *N-p*-nitrophenyl carbamyl phosphate containing methanol, acetonitrile, or dioxane were carried out in reaction mixtures similar to those used in the azide experiments except that the organic solvent was substituted for azide.

The reaction kinetics were followed for at least two half-lives in the case of *N*-phenyl and *N-p*-ethoxyphenyl carbamyl phosphate and at least three half-lives in the case of *N-p*-nitrophenyl and *N-m*-chlorophenyl carbamyl phosphate. The pseudo-first-order rate constants were determined from semilogarithmic plots of $(A_\infty - A_t)$ *vs.* time for those reactions in which product appearance was measured and $(A_t - A_\infty)$ *vs.* time for those reactions in which the loss of starting material was measured. Typical first-order plots are shown in Figure 1. Values of $t_{1/2}$ were determined graphically and the rate constants calculated using the relationship $k_{obsd} = 0.693/t_{1/2}$. The experimental values are accurate to within ±5%. The desired levels of ionic strength were attained in all cases by the appropriate addition of KCl unless otherwise stated.

Methanolysis Studies. For determining the extent of P–O bond cleavage, buffered methanol water solutions containing 1×10^{-3} *M* substituted carbamyl phosphate were incubated at 37° for about 18 hr. Aliquots of the incubation mixtures were taken and assayed for inorganic orthophosphate.[13] Since methyl phosphate does not react in the assay for orthophosphate, the amount of inorganic orthophosphate not recovered on the complete solvolysis of the substituted carbamyl phosphate can be assumed to be methyl phosphate.[14] The fraction of the total inorganic orthophosphate released in the presence of methanol compared with total inorganic orthophosphate released from an equivalent amount of substituted carbamyl phosphate hydrolyzed in the absence of methanol gives an

(4) C. M. Allen, Jr., and M. E. Jones, *Biochemistry*, 3, 1238 (1964).
(5) M. E. Jones and F. Lipmann, *Proc. Nat. Acad. Sci. U. S.*, 46, 1194 (1960).
(6) A. Lapidot and D. Samuel, *J. Chem. Soc*, 1931 (1964).
(7) C. M. Allen, Jr., E. Richelson, and M. E. Jones, "Current Aspects of Biochemical Energetics," N. O. Kaplan and E. P. Kennedy, Ed., Academic Press, New York, N. Y., 1966, p 401.
(8) F. Cramer and M. Winter, *Chem. Ber.*, 92, 2761 (1959).
(9) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, N. Y., 1967, p 333.
(10) I. Christenson, *Acta Chem. Scand.*, 18, 904 (1964).
(11) C. Faurholt, *J. Chim. Phys.*, 22, 1 (1925).
(12) P. M. Mader, *J. Org. Chem.*, 33, 2253 (1968).
(13) L. Spector, M. E. Jones, and F. Lipmann, *Methods Enzymol.*, 3, 653 (1957).
(14) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574 (1958).

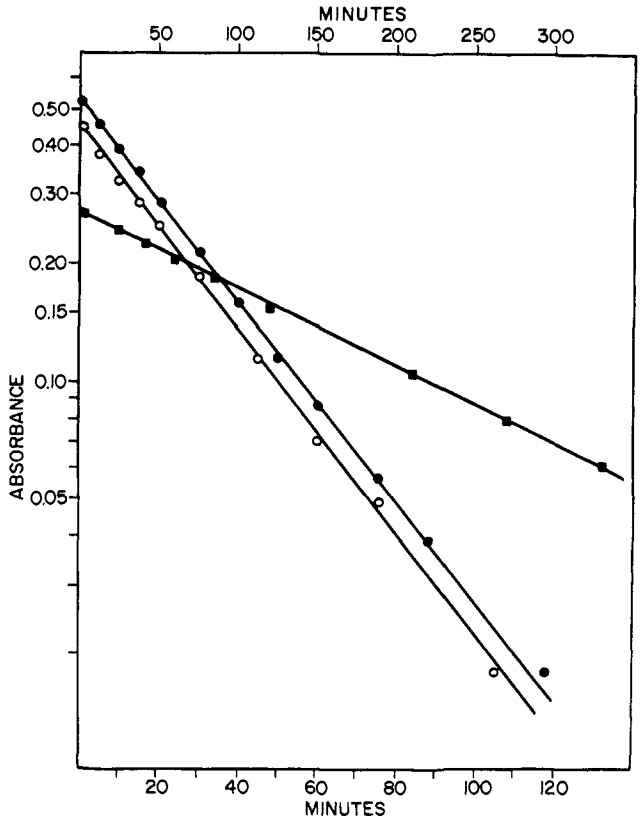


Figure 1. Plot of absorbance changes with time for the hydrolysis of *N*-aryl-substituted carbamyl phosphates in 0.06 *M* buffer, μ = 0.6, at 37°: ●, $(A_\infty - A_t)$ values at 430 mμ for the direct measurement of *p*-nitroaniline release from *N*-*p*-nitrophenyl carbamyl phosphate in maleate buffer, pH 5.5; ○, $(A_\infty - A_t)$ values at 430 mμ for the measurement of *p*-nitroaniline release, by the indirect method, from *N*-*p*-nitrophenyl carbamyl phosphate in borate buffer at pH 8.72; ■, $(A_\infty - A_t)$ values at 660 mμ for the measurement of phosphate release from *N*-phenyl carbamyl phosphate in succinate buffer, pH 6.1. The upper time scale is applicable only to the closed squares.

indication of the degree of P–O bond fission (*i.e.*, the percentage of total phosphate found as methyl phosphate).

Results

Kinetics. Pseudo-first-order rate constants for the hydrolysis of various N-substituted carbamyl phosphates at 37° and at various pH values are given in Table I. The results using assays for phosphate appearance generally give rate constants 5–10% lower than those determined by the spectrophotometric assays. The pH dependence of some of these rate constants is illustrated graphically in Figure 2. There is a marked difference in the hydrolytic rate constants for the mono- and dianionic species of the substituted carbamyl phosphates, as demonstrated by the difference in the rate constants in the pH regions 1–3 and 6–9, where the monoanion and dianions predominate, respectively.

Table II summarizes the pseudo-first-order rate constants for the hydrolysis of the mono- and dianionic species of the N-substituted carbamyl phosphates as well as similar data reported for substituted benzoyl phosphates.[2] The solid lines in Figure 2 represent theoretical curves of the pH dependence of the pseudo-first-order constants for the hydrolysis of the N-substituted carbamyl phosphates calculated from the

Table I. Observed First-Order Rate Constants for Hydrolysis of N-Substituted Carbamyl Phosphate in 0.06 *M* Buffer Solutions at 37°, Ionic Strength 0.6

A. *N*-Phenyl Carbamyl Phosphate

Buffer[a]	pH	$k_{obsd} \times 10^{+3}$, min^{-1}	Buffer[b]	pH	$k_{obsd} \times 10^{+3}$, min^{-1}
$HClO_4$ (0.513 *M*)[c,d]		10.68			
$HClO_4$ (0.308 *M*)[c,d]		8.82			
$HClO_4$ (0.103 *M*)[c,d]		7.41	HCl	1.38	6.48
Sulfate[c]	2.10	6.66	Formate	3.10	6.24
Sulfate[c]	2.23	6.67	Formate	3.45	5.92
Formate[c]	3.38	6.56	Succinate	3.90	5.74
Formate	3.60	6.18	Acetate	4.50	5.33
Succinate	3.82	6.41	Succinate	5.25	4.67
Succinate	4.01	6.30	Succinate	6.10	4.50
Acetate	4.40	5.86	Maleate	6.20	4.94
Succinate	5.45	5.17	Imidazole	7.25	4.88
Succinate	6.00	4.69	Imidazole	7.55	4.34
Borate	8.92	4.81	Borate	8.80	4.47
Carbonate	10.11	5.37	Carbonate	10.10	5.34

B. *N*-*p*-Nitrophenyl Carbamyl Phosphate

Buffer[c]	pH	$k_{obsd} \times 10^{+2}$, min^{-1}	Buffer[c]	pH	$k_{obsd} \times 10^{+2}$, min^{-1}
$HClO_4$ (0.60 *M*)[d,e]		2.02			
$HClO_4$ (0.50 *M*)[d,e]		1.89	Phthalate	5.01	2.63
$HClO_4$ (0.30 *M*)[d,e]		1.63	Maleate	5.55	2.95
$HClO_4$ (0.10 *M*)[d,e]		1.40	Maleate[f]	6.20	2.76
Sulfate	1.98	1.40	Imidazole[f]	7.02	2.74
Maleate[e]	2.00	1.47	Imidazole[g]	7.02	2.81
Formate	3.21	1.48	Imidazole[b,g]	7.02	2.79
Formate[e]	3.58	1.65	Tris[f]	7.52	2.86
Succinate	3.85	1.80	Tris[f]	8.18	2.86
Succinate	4.04	1.70	Tris[f]	8.35	3.04
Acetate	4.35	2.05	Borate[f]	8.72	3.04
Acetate	4.52	2.44	Borate[b,g]	8.78	2.86

C. *N*-*p*-Ethoxyphenyl Carbamyl Phosphate

Buffer[b]	pH	$k_{obsd} \times 10^{+3}$, min^{-1}	Buffer[b]	pH	$k_{obsd} \times 10^{+3}$, min^{-1}
HCl	1.20	6.93	Succinate	5.35	3.74
Sulfate[c,e]	2.05	6.75	Maleate	6.10	3.67
Formate[c,e]	3.35	7.25	Borate	8.68	3.60

D. *N*-*m*-Chlorophenyl Carbamyl Phosphate

Buffer[c]	pH	$k_{obsd} \times 10^{+3}$, min^{-1}	Buffer[a]	pH	$k_{obsd} \times 10^{+3}$, min^{-1}
Sulfate	1.90	9.24	Imidazole	7.28	8.40
Formate	3.30	9.47	Borate	8.73	8.45

[a] An indirect assay method was used in solutions 1.0–1.6 $\times 10^{-4}$ *M* in reactant unless otherwise stated. [b] Phosphate appearance was measured in solutions 1 $\times 10^{-3}$ *M* in reactant unless otherwise stated. [c] A direct assay method was used in solutions 1.3–1.6 $\times 10^{-4}$ *M* in reactant unless otherwise stated. [d] Ionic strength was adjusted with NaCl. [e] Reactant concentration was 0.69 $\times 10^{-4}$ *M*. [f] Indirect assay method was used in solutions 4.3 $\times 10^{-4}$ *M* in reactant. [g] Reactant concentration was 4.3 $\times 10^{-4}$ *M*.

pK_a's and pseudo-first-order rate constants for the individual mono- and dianionic species using the relationship $k_{obsd} = k_{mono}$ (mole fraction of monoanion) + $k_{dianion}$ (mole fraction of dianion).

The effects of varying buffer concentration and ionic strength are small. Varying formate buffer (pH 3.3) concentration from 0.03 to 0.30 *M* has a negligible effect on the pseudo-first-order constants for the hydrolysis of *N*-*p*-nitrophenyl and *N*-phenyl carbamyl

Table II. Pseudo-First-Order Rate Constants for the Solvolysis of Mono- and Dianionic Species of N-Substituted Carbamyl Phosphates at 37° and Ionic Strength 0.6

N substituent of carbamyl phosphate	$pK_a(37°)$[a]	$k \times 10^{+3}$, min^{-1} [b] Monoanion		Dianion	
H	5.1[c]	14.2[c]	(13)[d]	16.4[c]	(4.3)[d]
p-Nitrophenyl	4.6	14.4	(8.1)	29.5	(28)
m-Chlorophenyl		9.5		8.4	
Phenyl	5.0	6.6	(6.1)	4.4	(3.1)
Tolyl		5.6[e]	(5.7)	3.7[e]	(2.6)
p-Ethoxyphenyl	5.0	6.9	(4.8)[f]	3.6	(1.8)[f]

[a] For the dissociation of a proton from the monoanionic species. [b] Values in parentheses are from data of Di Sabato and Jencks[2] on solvolysis of substituted benzoyl phosphates at 39°. [c] Taken from work of Allen and Jones.[4] [d] Rate constants for solvolysis of acetyl phosphate at 39°.[2] [e] Taken from work of Allen, *et al.*[7] [f] Rate constant for *p*-methoxy substituent.

phosphate monoanions. Ionic strength changes from 0.20 to 2.0 *M* has no effect on the hydrolysis of the *N*-*p*-nitrophenyl carbamyl phosphate monoanion. Increasing imidazole buffer concentration from 0.03 to 0.30 *M* has little effect on the pseudo-first-order rate constant for the hydrolysis of either *N*-*p*-nitrophenyl (pH 6.9) or *N*-phenyl carbamyl phosphate (pH 7.5) dianions. Increasing ionic strength from 0.20 to 2.0 *M* does have, however, a small positive effect on the hydrolytic rate constants for the *N*-phenyl carbamyl phosphate monoanion and dianion as well as the *N*-*p*-nitrophenyl carbamyl phosphate dianion.

The kinetics of *N*-*p*-nitrophenyl carbamyl phosphate dianion hydrolysis were followed by the direct measurement of the appearance of both orthophosphate and *p*-nitroaniline at pH 8.78 in borate buffer and at pH 7.02 in imidazole buffer at ionic strength 0.60 *M*. At pH 7.02 the pseudo-first-order rate constants for *p*-nitroaniline and phosphate appearance at 37° were 2.81×10^{-2} and 2.79×10^{-2} min^{-1}, respectively. At pH 8.78 the pseudo-first-order rate constant for phosphate formation was 2.86×10^{-2} min^{-1} which compares favorably with the value of $k_{obsd} = 3.04 \times 10^{-2}$ min^{-1} obtained using the indirect method for measuring *p*-nitroaniline formation (see Table I). The measurement of the *p*-nitroaniline release at pH 8.78 by the direct spectrophotometric method showed a distinct concave shape to the rate curve early in the time course of the reaction. This phenomenon was even more apparent when the rates were observed in bicarbonate buffer at pH 10.1. A probable explanation for this failure to observe first-order kinetics using the direct assay in the mildly basic pH range may be ascribed to the increased stability of a reaction intermediate with increasing pH.

The spectrum of a solution of *N*-*p*-nitrophenyl carbamyl phosphate incubated in 0.01 *M* NaOH at 37° for 1 hr shows maximum absorption at 349 mμ. This is distinctly different from the absorption maximum of unreacted *N*-*p*-nitrophenyl carbamyl phosphate dianion (324 mμ) and from the ultimate product, *p*-nitroaniline (382 mμ) which accumulates after 48 hr at 37°. The same absorption maximum is found for the product of the reaction of *p*-nitrophenyl isocyanate with 0.01 *M* NaOH. These data indicate the accumulation of a reaction intermediate, probably *p*-nitrophenyl carbamate, which is relatively stable in dilute base.

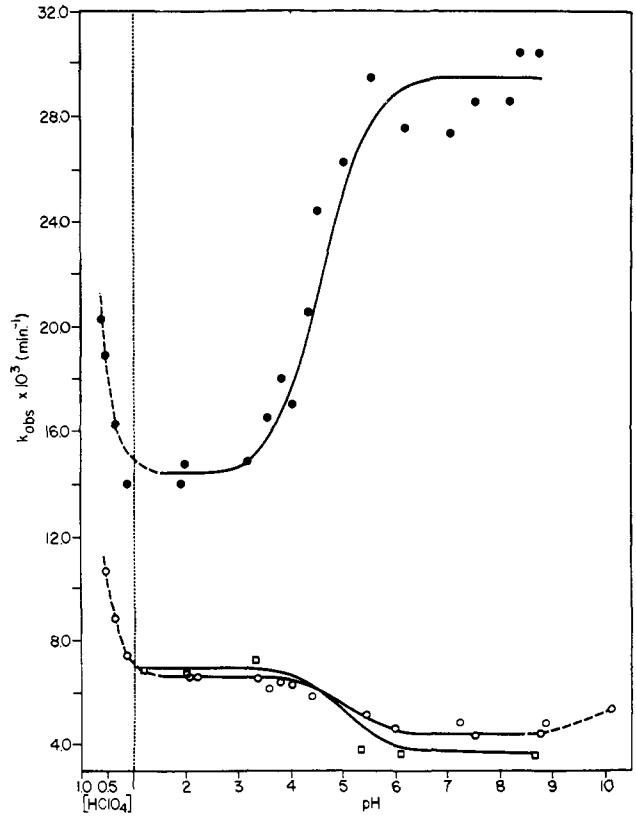


Figure 2. The pH dependence of k_{obsd} for the hydrolysis of N-substituted carbamyl phosphates in 0.06 *M* buffer of $\mu = 0.6$ at 37°. The specific buffers used are given in Table I: ○, *N*-phenyl carbamyl phosphate; □, *N*-*p*-ethoxyphenyl carbamyl phosphate; ●, *N*-*p*-nitrophenyl carbamyl phosphate. Below pH 1.0, the abscissa represents the molar concentration of perchloric acid.

The hydrolysis of *N*-*p*-nitrophenyl carbamyl phosphate in dilute NaOH solutions at 0.60 *M* ionic strength can, however, be followed kinetically using the indirect method. This derivative shows a weakly base-catalyzed reaction with a second-order rate constant of 0.97 M^{-1} min^{-1} (Table III). A plot of log k_{obsd} *vs.*

Table III. Base-Catalyzed Hydrolysis of *p*-Nitrophenyl Carbamyl Phosphate at 37° and Ionic Strength 0.6[a]

Concentration of KOH (*M*)	$k_{obsd} \times 10^{+2}$, min^{-1}
0.01	4.32
0.03	6.43
0.05	7.67
0.06	10.16
0.08	10.73
0.10	13.15

[a] k_{obsd} was determined by the indirect assay method in solutions 4.3×10^{-4} *M* in reactant.

hydroxide ion concentration gives a line with a slope of one indicating the involvement of 1 mol of hydroxide per mole of carbamyl phosphate derivative.

The temperature dependence of the hydrolysis of *N*-phenyl and *N*-*p*-nitrophenyl carbamyl phosphate was studied in the range of both the mono- and dianionic species. A tabulation of the observed pseudo-first-order rate constants is presented in Table IV with a summary of the thermodynamic parameters $\Delta H^{\pm}$ and $\Delta S^{\pm}$ calculated from these data. These results are typical of acyl phosphates studied thus far.

Table IV. Temperature Effects and Activation Parameters for the Solvolysis of N-Substituted Carbamyl Phosphates in 0.06 *M* Buffered Solutions at Ionic Strength 0.6

Reactant	Temp, °C	$k_{obsd} \times 10^{+2}$, min^{-1}	$\Delta H^{\ddagger}_{37°}$, kcal/mol	$\Delta S^{\ddagger}_{37°}$, eu
N-*p*-Nitrophenyl carbamyl phosphate				
Monoanion[a]	55.5	14.1	24.2	+3.0
	47.0	4.91		
	37.0	1.48		
	33.0	0.82		
Dianion[b]	57.0	31.6	24.4	+4.7
	47.0	9.95		
	37.0	2.74		
	34.5	1.96		
N-Phenyl carbamyl phosphate				
Monoanion[c]	61.0	12.5	24.8	+3.2
	53.0	5.02		
	51.0	3.30		
	46.0	2.18		
	37.0	0.67		
	30.0	0.27		
Dianion[d]	60.0	6.36	23.0	−3.3
	51.0	2.16		
	45.0	1.26		
	37.0	0.42		
	30.0	0.19		

[a] In formate buffer at pH 3.30. [b] In imidazole buffer at pH 6.90. [c] In sulfate buffer at pH 2.10. [d] In borate buffer at pH 8.9.

Azide Experiments. The pseudo-first-order rate constants for the release of *p*-nitroaniline during the hydrolysis of *N*-*p*-nitrophenyl carbamyl phosphate in 1 *M* azide at pH 3.4, 4.55, 5.1, and 5.75 were 1.31, 2.27, 2.64, and 2.89 $\times$ 10^{-2} min^{-1}, respectively. Under similar conditions of buffer concentration and pH without added azide the first-order rate constants were 1.48, 2.44, 2.63, and 2.95 $\times$ 10^{-2} min^{-1}, respectively. The azide ion does not appear, therefore, to have any marked effect on the rate of *p*-nitroaniline release from either the mono- or dianionic species. The observed rate constant for phosphate release at pH 3.3 was 1.12 $\times$ 10^{-2} min^{-1}. At pH 5.75 and 5.1 in the presence of azide the amount of *p*-nitroaniline released at infinity was stoichiometric with respect to the amount of *N*-*p*-nitrophenyl carbamyl phosphate being used. Furthermore, the only nitrogenous product of the hydrolysis of *N*-*p*-nitrophenyl carbamyl phosphate was shown to be *p*-nitroaniline. On the other hand, the hydrolysis carried out at pH 3.4 resulted in the appearance of only about 70% of the total *p*-nitroaniline expected from stoichiometric conversion of *N*-*p*-nitrophenyl carbamyl phosphate to *p*-nitroaniline. The spectrum of the products of this hydrolysis in azide shows that *p*-nitroaniline is not the only aniline derivative formed. Analysis of the spectrum indicated that the other component has an absorption maximum at 323 mμ.

The hydrolysis of *p*-nitrophenyl isocyanate, a possible reaction intermediate, was studied over the pH range of 3.4–5.75 in the presence and absence of azide. In the absence of azide the isocyanate is very labile, since after 20 min incubation at 37° 91–100% of the total *p*-nitroaniline formed at infinity was already released. However, during the hydrolysis of *p*-nitrophenyl isocyanate in 1 *M* azide the amount of *p*-nitroaniline appearing after 20 min is less than 2% of the total available from the complete hydrolysis of an equivalent amount of *p*-nitrophenyl isocyanate in the absence of azide. The spectrum of the product of the reaction of *p*-nitrophenyl isocyanate with azide also showed the presence of a compound with an absorption maximum of 323 mμ which is the same as that found for the product formed from the reaction of *N*-*p*-nitrophenyl carbamyl phosphate with 1 *M* azide at pH 3.4. It is apparent then that *p*-nitrophenyl isocyanate can be trapped by azide as *N*-*p*-nitrophenyl carbamyl azide which is relatively stable under these pH conditions.

The fact that *p*-nitrophenyl isocyanate can be trapped as the azide under conditions where the rate and extent of *p*-nitroaniline release from the *p*-nitrophenyl carbamyl phosphate dianion is unchanged clearly rules out the possibility of C–O bond fission with the formation of *p*-nitrophenyl isocyanate during dianion hydrolysis. However, the trapping of the carbamyl azide during the hydrolysis of *N*-*p*-nitrophenyl carbamyl phosphate monoanion at pH 3.4, in the presence of azide, suggests the existence of an isocyanate or carbamyl cation as an intermediate in the hydrolytic pathway.

Effect of Organic Solvents. The effects of organic solvents on the rates of solvolysis of *N*-*p*-nitrophenyl carbamyl phosphate are shown in Table V. It is apparent that the rate of *p*-nitroaniline release from the monoanionic species is only slightly inhibited by the presence of increasing concentrations of methanol and acetonitrile. However, the pseudo-first-order rate constants for *p*-nitroaniline release from the dianionic species are moderately accelerated by increasing concentrations of either methanol or dioxane.

Table V. Solvent Effects on the Hydrolysis of *p*-Nitrophenyl Carbamyl Phosphate at 37°

Organic solvent	% (v/v)	$k_{obsd} \times 10^{+3}$, min^{-1} [a] Monoanion[b]	Dianion[c]
Methanol	20	13.0	32.7
	50	11.7[d]	44.7
	60		49.5
Acetonitrile	33	12.3	27.2
	50	13.1	26.7
Dioxane	20		40.5
	50		65.0
None		14.7	29.5

[a] Rate constants were determined by measurement of *p*-nitroaniline released from reactant by the direct assay method. Each reaction mixture was identical with those described for kinetic experiments in Table I with the exception of the added organic solvent. [b] Formate buffer (0.06 *M*) at pH 3.30 and maleate buffer (0.06 *M*) pH 1.9 were used in the methanol and acetonitrile experiments, respectively. [c] Maleate buffer (0.06 *M*) at pH 6.06 was used for all experiments in the dianion range. [d] The rate constant for inorganic phosphate release from reactant in formate buffer at pH 3.30 was 8.4 $\times$ 10^{-3} min^{-1}.

The solvolysis of three N-substituted carbamyl phosphates in 50% methanol at several pH values shows varying degrees of orthophosphate formation (Table VI). *N*-Phenyl and *N*-*p*-ethoxyphenyl carbamyl phosphate dianions show essentially the same amount of inorganic orthophosphate release indicating about 53% of the phosphate product formed during methanolysis of this species was inorganic phosphate. During monoanion solvolysis about 62% of the total phosphate

Table VI. Phosphate Product Analysis Following Solvolysis of N-Substituted Carbamyl Phosphates in a Methanol-Water Mixture (50:50, v:v) at 37°

Buffer[a]	pH[b]	% orthophosphate formed from N-substituted carbamyl phosphate: *p*-Nitrophenyl	Phenyl	*p*-Ethoxyphenyl
Maleate	1.9	81	62	61
Formate	3.3	69	64	64
Maleate	6.1	42	52	51
Borate	9.1	43	53	52
0.01 *M* NaOH		45		

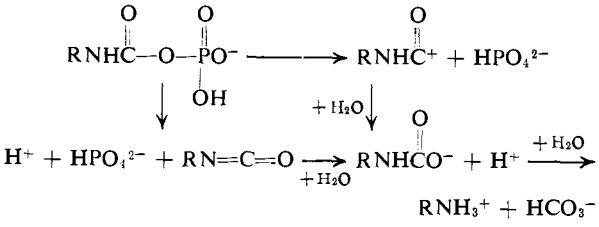
[a] Reaction mixtures are the same as those used in the kinetic experiments where inorganic orthophosphate was measured with the exception of the addition of methanol. [b] The pH given is that of the buffer being used, not the measured pH.

product was inorganic phosphate. Solvolysis of the *N*-*p*-nitrophenyl carbamyl phosphate monoanion, however, results in 69–81% of the phosphate product appearing as inorganic phosphate, whereas about 43% was inorganic phosphate in the dianion region and in moderately basic solutions.

The spectrum of the products of the methanolysis of the dianionic species of *N*-*p*-nitrophenyl carbamyl phosphate at pH 6.1 shows that *p*-nitroaniline is the only aniline derivative formed. This is not the case, however, for the methanolysis of *N*-*p*-nitrophenyl carbamyl phosphate monoanion in formate buffer, pH 3.3. The other product has an absorption maximum at 324 mμ which is the same as that found for the product formed from the reaction of small quantities of *p*-nitrophenyl isocyanate with a large excess of methanol. Since urethans are well known to be the products of the reaction of isocyanates with alcohols, it is most probable that the other aniline containing product from this solvolysis is methyl *p*-nitrophenyl carbamate. These results indicate, that for the *N*-*p*-nitrophenyl carbamyl phosphate monoanion, C–O bond cleavage is occurring to a measurable extent.

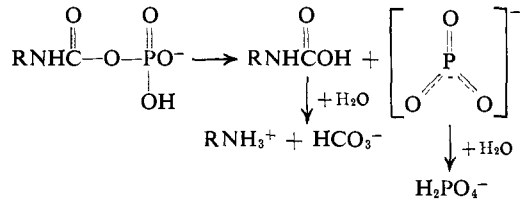
Discussion

The N-substituted carbamyl phosphates may hydrolyze by pathways involving either C–O or P–O bond fission. Possible pathways for the monoanions involving C–O bond fission with the formation of inorganic orthophosphate and the substituted carbamate are shown in eq 1. At acid and neutral pH the carbamate will rapidly decompose to the substituted aniline and bicarbonate.[10,11]



(1)

A reaction pathway involving P–O bond fission may proceed by the elimination of carbamic acid to form the reactive monomeric metaphosphate with subsequent reaction of both these intermediates with water to give the final products, eq 2.



(2)

Since schemes similar to (1) and (2) are also possible for the dianionic species, it is conceivable that either species may hydrolyze by one or both of these pathways.

Monoanion Hydrolysis. The reaction pathway for the hydrolysis of the monoanionic species of *N*,*N*-diethyl carbamyl phosphate[6] apparently proceeds *via* C–O bond cleavage with the formation of the carbamyl cation *via* a pathway similar to that shown in eq 1. However, the unsubstituted carbamyl phosphate monoanion[4] and other acyl phosphate monoanions[15] hydrolyze by P–O bond fission. In the present study the extent of P–O or C–O bond fission in the substituted carbamyl phosphates was measured by analysis of product composition and rates of solvolysis in methanol and azide solutions.

The appearance of methyl *N*-*p*-nitrophenyl carbamate and *N*-*p*-nitrophenyl carbamyl azide as products of the solvolysis of *N*-*p*-nitrophenyl carbamyl phosphate monoanion in solutions containing methanol and azide indicates that there was some C–O bond fission for the monoanionic species. The kinetic data demonstrate, furthermore, that it is unlikely that these derivatives are the result of a nucleophilic attack.

Phosphorus–oxygen cleavage also occurs since solvolysis of three N-substituted carbamyl phosphate monoanions at pH 3.3 with 0.30 mol fraction of methanol present results in 31–39% of the total phosphate formed appearing as methyl phosphate. This percentage indicates that solvent incorporation into phosphate products during P–O bond cleavage is nearly proportional to the mole fraction of the solvent components. Such results might be expected from the reaction of a highly reactive intermediate such as metaphosphate. Similar results have been found for the methanolysis of *p*-nitrophenyl[16] and 2,4-dinitrophenyl phosphate[17] monoanions, where monomeric metaphosphate has been postulated as a solvolytic intermediate. Frequently, however, methanolysis of the dianions of nitrosubstituted phenyl phosphates[17,18] results in formation of methyl phosphate in concentration several times higher than the mole fraction of methanol used in the solvent indicating a selectivity toward methanol. This selectivity might also occur during methanolysis of the substituted carbamyl phosphate monoanions. If there were considerable C–O bond fission with a high selectivity for methanol incorporation into the phosphate product formed from P–O bond fission, then the percentage of methyl phosphate observed would be only fortuitously similar to the mole fraction of methanol being used. Evidence that this might be occurring here, at least in one case, is that measurable C–O bond fission is observed during methanolysis of

(15) W. P. Jencks, *Brookhaven Symp. Biol.*, 15 (BNL 738 (C-34)), 134 (1962).
(16) J. D. Chanley and E. Feageson, *J. Amer. Chem. Soc.*, 85, 1181 (1963).
(17) A. J. Kirby and A. G. Vargoglis, *ibid.*, 89, 415 (1967).
(18) C. A. Bunton, E. J. Fendler, and J. H. Fendler, *ibid.*, 89, 1221 (1967).

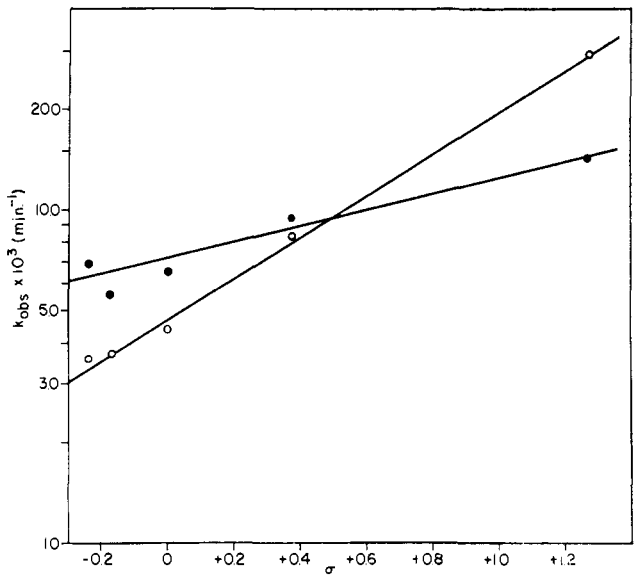
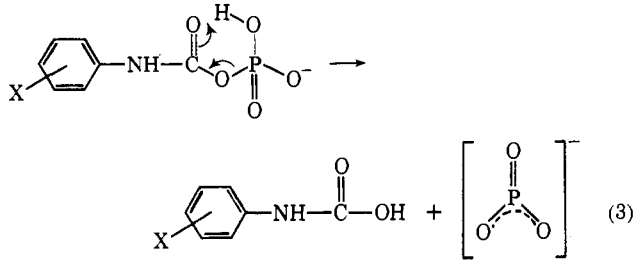


Figure 3. The hydrolytic rate constants for N-substituted carbamyl phosphate monoanions (●) and dianions (○) as a function of Hammett's substituent constants. The σ^- value of 1.27 is used for the *p*-nitro substituent.

the *p*-nitrophenyl derivative. The solvolysis of *N*-*p*-nitrophenyl carbamyl phosphate at pH 1.9, in the presence of 0.30 mol fraction methanol results in only 19% methyl phosphate formed. This clearly indicates a significant amount of C–O bond cleavage.

The rates of hydrolysis of the monoanions of the *N*-aryl-substituted carbamyl phosphates show a marked similarity to the rates of hydrolysis of the substituted benzoyl phosphate monoanions. A particularly striking similarity is the insensitivity of the hydrolytic rate constants to the electron delocalizing ability of the aromatic substituents. A Hammett $\rho\sigma$ plot for the various substituted carbamyl phosphate monoanions yields a ρ value of approximately 0.5 if the σ value of 0.78, assigned for *p*-nitro substitution on benzoic acid, is used. However, if the σ^- value of 1.27, suggested for *p*-nitro-substituted anilines,[19] is used instead, a ρ value of 0.24 is obtained (Figure 3). The substituted benzoyl phosphate monoanions give a ρ value of 0.2.

A reaction mechanism for the pathway of monoanion hydrolysis involving P–O bond cleavage similar to that proposed for the aliphatic acyl, benzoyl, and carbamyl phosphate monoanions is suggested in eq 3. The rather small effects of various aromatic substituents on the rate constants is probably because electronic con-



tributions which aid proton transfer retard P–O bond fission and *vice versa*.[2] Since the hydrolytic rate constants increase slightly with increasing ability of the substituents to withdraw electrons, it is presumed that P–O bond fission is more important than hydrogen transfer to the carbonyl oxygen.

The solvolysis of the *N*-*p*-nitrophenyl carbamyl phosphate monoanion, however, is obviously complex. The ability to form methyl *N*-*p*-nitrophenyl carbamate and *N*-*p*-nitrophenyl carbamyl azide under appropriate conditions demands an alternate mechanism to the one just proposed.[20] The formation of the carbamyl cation and inorganic phosphate seems unlikely because of the absence of any ionic strength effect on the hydrolytic rate constants; hence formation of the substituted isocyanate is more likely (eq 1). Substituted isocyanate has been suggested as a product in the pathway of the solvolysis of *N*-aryl-substituted carbamate esters in amine solvents although there the reaction involves solvent participation.[21] The reason for some C–O bond cleavage for the *p*-nitro derivative is not clear at this time.

Dianion Hydrolysis. The reaction pathway for the decomposition of the dianionic species of unsubstituted carbamyl phosphate has been shown to proceed *via* C–O bond fission with the formation of isocyanate and inorganic orthophosphate.[4] The present study clearly indicates that the hydrolysis of *N*-*p*-nitrophenyl carbamyl phosphate dianion does not proceed *via* C–O bond cleavage with the formation of the isocyanate or carbamyl cation as an intermediate. This conclusion is supported by the following evidence. The kinetics and products of hydrolysis of *N*-*p*-nitrophenyl carbamyl phosphate dianion are not affected by the presence of 1 *M* azide, whereas *p*-nitrophenyl isocyanate is quantitatively trapped as the acyl azide in a solution of 1 *M* azide. Hall and Lueck[22] have previously shown partial trapping of the *N*,*N*-dimethyl carbamyl cation at lower azide concentrations (0.5 *M*), so it is unlikely that the carbamyl cation is formed here. Finally, methyl *N*-*p*-nitrophenyl carbamate is not a product of the methanolysis of the *N*-*p*-nitrophenyl carbamyl phosphate dianion.

This leads to the consideration of the direct evidence for P–O bond cleavage (the pathway found to predominate in the hydrolysis of other acyl phosphate dianions). Earlier hydrolysis studies of Allen, *et al.*,[7] have shown that the dianions of *N*-phenyl, *N*-tolyl, and *N*-*p*-nitrophenyl carbamyl phosphate all hydrolyze with 78% or greater P–O bond cleavage. Furthermore, the results of the methanolysis experiment presented here show nearly 50–60% methyl phosphate formation in a solvent of 0.30 mol fraction methanol, which is indicative of exclusive P–O bond fission. Similar results were obtained during solvolysis of the monoanionic species of unsubstituted carbamyl phosphate where P–O bond fission overwhelmingly predominates.[23] The methanolysis of the dianion could lead to a shift in mechanism such that a nucleophilic attack by

(19) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1940, p 188.

(20) Another explanation for these findings is that P–O bond fission is occurring with the formation of the carbamate which subsequently decomposes with the loss of OH^- to give isocyanate. The isocyanate in turn would react with methanol or azide to give the corresponding derivatives. Such a mode for carbamate decomposition has not been observed previously[10,12] and is quite unlikely.

(21) T. Mukaiyama and M. Iwanami, *J. Amer. Chem. Soc.*, 79, 73 (1957).

(22) H. K. Hall, Jr., and C. H. Lueck, *J. Org. Chem.*, 28, 2818 (1963).

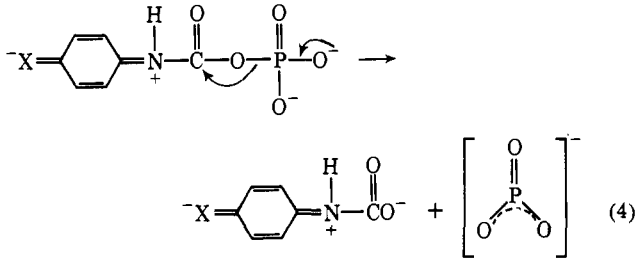
(23) C. M. Allen, Jr., Ph.D. Thesis, Brandeis University, Waltham, Mass., 1964.

methanol results in increased P–O bond cleavage and an increase in rate of *p*-nitroaniline release. This possibility tends to be supported by the increased rate of *p*-nitroaniline release with increasing methanol concentrations but is unlikely since the much weaker nucleophile, dioxane, results in an even greater rate increase. Such a rate increase in less polar solvent might be indicative of the stabilization of a transition state in which charge dispersal is occurring. The absence of any rate increase by acetonitrile does not, however, support this hypothesis. This type of data is similar to that observed earlier for the solvolysis of the dianionic species of 2,4-dinitrophenyl phosphate.[17]

In contrast to the N-substituted carbamyl phosphate monoanions the hydrolytic rate constants for the dianions are markedly influenced by the ability of the phenyl ring substituent to delocalize electrons. However, a particularly striking similarity is seen between the hydrolytic rate constants for the substituted carbamyl and benzoyl phosphate dianions as was shown for the monoanionic species. A Hammett $\rho\sigma$ plot for the substituted carbamyl phosphate dianionic species gives ρ values of about 0.94 and 0.62 (Figure 3) using values of $\sigma = 0.78$ and $\sigma^- = 1.27$, respectively, for the *p*-nitro substituent. A comparison of the ρ value of 1.2 for the benzoyl phosphate dianions with the value of 0.94 for the substituted carbamyl phosphates, using $\sigma = 0.78$, shows a close similarity in dependence of the hydrolytic reaction to the substituent's electron delocalizing ability.[24]

Studies of Thorne[25] on the thermal decomposition of related compounds, the *tert*-butyl *N*-aryl carbamates, show that these reactions have similar ρ values.

A reaction mechanism for the dianionic species similar to eq 2 and 4 is probable where hydrolysis proceeds by the elimination of carbamate with the formation of metaphosphate. Such a mechanism would be enhanced markedly by electron-withdrawing



(4)

aromatic substituents. The smaller ρ value for the carbamyl phosphate derivatives may reflect a shielding effect by the nitrogen atom interposed between the carbamyl function and the phenyl ring.

The hydrolytic rate constant for unsubstituted carbamyl phosphate dianion is about four times that of the acetyl phosphate dianion. However, the N-substituted carbamyl phosphates have hydrolytic rate constants comparable to the benzoyl phosphates. The difference in the hydrolytic rate constants for unsubstituted and substituted carbamyl phosphates is generally larger than the difference in the rate constants for acetyl phosphate and the benzoyl phosphates. This rate difference may be explained by the shift in the mechanism of decomposition of the carbamyl phosphates from C–O bond fission for the unsubstituted derivative to P–O bond fission for the substituted derivatives.

Carbon–oxygen fission observed for the unimolecular decomposition of unsubstituted carbamyl phosphate dianion and hydroxide catalyzed solvolysis of the singly N-substituted esters of carbamate[10,26,27] is presumably made possible by the feasibility of losing a proton from the amide group leading to the elimination of the isocyanates. The substitution of a single aryl group on the carbamyl nitrogen of the dianion results in a hydrolytic pathway involving only P–O bond fission.

Groups other than hydrogen substituted on the carbamyl nitrogen might sterically hinder the mechanism suggested by Allen and Jones[4] for the unsubstituted carbamyl phosphate dianion, where it is proposed that the negatively charged phosphate moiety may facilitate phosphate elimination by abstraction of a proton from the carbamyl nitrogen. Furthermore, if the resonance hybrids shown in eq 4 contributed significantly to the dianionic structure, its decomposition *via* P–O bond fission is more likely than C–O bond fission because of the electrophilic character of the positively charged nitrogen group.

This shift in the mechanism of dianion hydrolysis may explain in part why *N*-*p*-nitrophenyl carbamyl phosphate fails to carbamylate aspartic acid in the presence of aspartate transcarbamylase[28] even though it binds relatively well to the enzyme.

Acknowledgments. We are grateful to Dr. William P. Jencks and Dr. Eugene G. Sander for their constructive suggestions and criticism during the preparation of the manuscript and to Mrs. Bonnie Sitko for technical assistance.

(24) A striking example of the sensitivity of the rates of hydrolysis of the dianionic species to the electron delocalizing ability of the para substituent is seen with the *p*-dimethylamino derivative,[12] where the half-life at 25° is 0.97 day.

(25) M. P. Thorne, *Can. J. Chem.*, **45**, 2537 (1967).

(26) L. W. Dittert and T. Higuchi, *J. Pharm. Sci.*, **52**, 852 (1963).

(27) M. L. Bender and R. B. Homer, *J. Org. Chem.*, 30, 3975 (1965).

(28) R. W. Porter, M. O. Modebe, and G. R. Stark, *J. Biol. Chem.*, **244**, 1846 (1969).