taken for mass spectral analysis. For fast-reacting pyridine arylboranes 3 hr was sufficient, while 8 hr were taken for the slow-reacting pyridine arylboranes. Enough measurements were taken for each sample to determine a standard deviation of the measurement.

The same procedure was followed for total hydrolysis reactions, except the solution in the first side arm was 15 ml of 1 *M* perchloric acid, and the reaction was run to completion. The solution was frozen and a gas sample taken for mass spectral analysis.

The ultraviolet spectra of the compounds were examined in base solutions containing 25% tetrahydrofuran. The spectra were determined on a Perkin-Elmer Spectronic 505 recording ultraviolet spectrophotometer and are given in Table V. The rates of the hydrolysis of the pyridine arylboranes were studied using a Beckman DU ultraviolet spectrophotometer. This spectrphotometer was equipped with a brass cooling block for the cells through which water from a constant temperature flowed to maintain the proper temperature (±0.02°). The change of the pyridine arylborane absorption at 275 to 280 mμ was used to follow the reaction. Plots of log $(A_\infty - A)$ gave (*vs.* time) straight lines for runs in buffer solutions, thus showing first-order dependence in substrate.

Samples were dissolved in Eastman tetrahydrofuran that was distilled from calcium hydride and run through alumina to remove peroxides. The solution of 1–4 ml was diluted to 25 ml with a buffer of pH 6.86 prepared from 0.025 *M* each of potassium dihydrogen phosphate and disodium hydrogen phosphate.

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Neighboring N-Carboxyalkyl Group Participation in the Hydrolysis of Phthalimide[1]

Peter D. Hoagland[2] and Sidney W. Fox[3]

Contribution from the Chemistry Department of the Florida State University, Tallahassee, Florida. *Received March 18, 1966*

Abstract: A number of new 3- or 4-nitrophthaloyl-ω-amino acids have been synthesized. The effect of N-carboxyalkyl substitution on intramolecular hydrolysis of imides near neutrality has been investigated. A pH–rate profile for phthaloylglycine revealed that the rate of hydrolysis between pH 2 and 7 depends upon the degree of ionization of the carboxyl group. The magnitude of the enhancement of the rate of hydrolysis of an imide linkage by a neighboring carboxylate indicates that the neighboring group can be an intramolecular catalyst for hydrolysis of imide.

Intramolecular catalysis of the hydrolysis of phthalimide by a carboxyl group has been established for *o*-carboxyphthalimide by Zerner and Bender.[4] Neighboring carboxyl groups may influence the hydrolysis of polyimides generated by thermal copolymerization of aspartic acid with amino acids.[5,6] Because the kinetics of hydrolysis of such polyimides is complex, we selected phthaloyl derivatives of some amino acids as models for investigating the influence of an N-alkylcarboxy group on the hydrolysis of a cyclic imide. Intramolecular catalysis of ester hydrolysis by a neighboring carboxyl group has received considerable attention for some time.[7–12] Carboxyl groups have also been assigned roles in the mechanisms of action of several enzymes.[11,13,14]

This report will show that a carboxylate group linked through an alkyl group to the phthalimide nitrogen atom may participate as an anionic intramolecular catalyst in the hydrolysis of the imide ring.

Experimental Section

Characterization. Uncorrected melting points were determined with the Mel-Temp apparatus. Elemental analyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill. Acids were titrated potentiometrically with potassium hydroxide solution. Imide linkages were quantitatively hydrolyzed at constant pH with the same base, using a pH-Stat. When hydrolysis of an imide interfered with titration of an acid, total base consumed for neutralization of the carboxyl group and for hydrolysis of the imide was used for characterization.

Materials. A general procedure was employed for synthesis of imides.[15–17] An equimolar mixture of dicarboxylic acid anhydride and amino acid was fused under reduced pressure above 150° for about 1 hr. In Table I are listed the nitrophthaloylamino acids synthesized. The following derivatives were prepared: phthaloylglycine, mp 194–195° (lit.[18] 192–194°); phthaloyl-β-

(1) Contribution No. 082 of the Institute for Space Biosciences, The Florida State University, Tallahassee, Fla.

(2) From the Ph.D. dissertation of P. D. Hoagland, 1964.

(3) Institute of Molecular Evolution and Biochemistry Department, University of Miami, Coral Gables, Fla. 33134.

(4) B. Zerner and M. L. Bender, *J. Am. Chem. Soc.*, **83**, 2267 (1961).

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(10) A. Agren, U. Hedsten, and B. Jonsson, *Acta Chem. Scand.*, **15**, 1532 (1961).

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(12) L. Eberson, *Acta Chem. Scand.*, **18**, 2015 (1964).

(13) (a) S. A. Bernhard and H. Gutfreund, *Biochem., J.*, **63**, 61 (1956); (b) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. A. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).

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Table I. Properties of Imides Synthesized

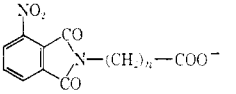
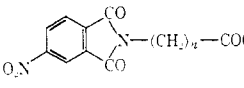
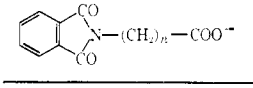
Compound[a]	Yield, %	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Equiv wt Calcd	Equiv wt Found[d]
3-Nitrophthaloylglycine	33	211–213[b]	$C_{10}H_6O_6N_2$	48.01	2.42	11.20	48.08	2.66	11.26	250.2	251.2
3-Nitrophthaloyl-β-alanine	53	145–148	$C_{11}H_8O_6N_2$	50.00	3.05	10.61	50.07	3.29	10.78	264.2	260.0, 259.7
3-Nitrophthaloyl-γ-aminobutyric acid	68	134.5–136	$C_{12}H_{10}O_6N_2$	51.80	3.62	10.07	52.20	3.68	10.20	278.2	275.4, 281.8
3-Nitrophthaloyl-ε-aminocaproic acid	83	152–154	$C_{14}H_{14}O_6N_2$	54.90	4.61	9.15	55.20	4.54	9.20	306.3	304.8
3-Nitrophthaloyl-α-aminoisobutyric acid	50	205–207	$C_{12}H_{10}O_6N_2$	51.80	3.62	10.07	52.04	3.77	10.30	278.2	274.9
4-Nitrophthaloylglycine	51	195–197[c]	$C_{10}H_6O_6N_2$	48.01	2.42	11.20	48.45	2.62	11.18	250.2	245.7, 254.2
4-Nitrophthaloyl-β-alanine	52	207–209	$C_{11}H_8O_6N_2$	50.00	3.05	10.61	50.65	3.38	10.46	264.2	259.4, 267.9
4-Nitrophthaloyl-γ-aminobutyric acid	59	165–167	$C_{12}H_{10}O_6N_2$	51.80	3.62	10.07	52.02	3.59	10.20	278.2	281.0

[a] Imides were recrystallized from hot absolute ethanol. [b] L. R. Caswell and P. C. Atkinson [*J. Org. Chem.*, **29**, 3151 (1964)] reported mp 208–209°. [c] Lit.[b] mp 189–192°. [d] Calculated from base consumed to neutralize carboxyl group and to hydrolyze the imide linkage.

alanine, mp 150–152° (lit.[19] 150–151°); phthaloyl-γ-aminobutyric acid, mp 115–117° (lit.[20] 113–115°); and phthaloyl-ε-aminocaproic acid, mp 107–108° (lit.[21] 107°). N-Methylphthalimide was obtained from Eastman and phthaloylglycylglycine was obtained from Sigma Chemical Co.

Kinetics. Hydrolysis of imide linkages was followed by the pH-Stat method with a Radiometer Titrigraph Type SBR 2C.[12] About 100 μmoles of imide in 12.0 ml of 0.60 *M* KCl was hydrolyzed at constant pH at 40.0°. Observed pseudo-first-order rate constants were calculated from plots of log base consumed *vs.* time. The electrodes were standardized with 0.10 *M* sodium borate (pH 9.07 at 40°). Carbon dioxide was excluded with a layer of Nujol mineral oil, which also served to prevent evaporation of water during long periods of hydrolysis. Uniformity of solutions was maintained by vigorous magnetic stirring. Linear first-order plots beyond half-life times were obtained. Rates for the hydrolysis of phthaloylglycine and of N-methylphthalimide at 100° were determined by the method of Zerner and Bender.[4]

Table II. Observed First-Order Rate Constants for Hydrolysis of Some Phthaloyl- and Nitrophthaloyl-ω-Amino Acids

	n	pH	$10^2 k_{obsd}$, min^{-1} [a]
	1	7.0	3.48 ± 0.34 (2)[b]
	2		3.32 ± 0.38 (2)
	3		1.82 ± 0.03 (2)
	5		1.07
	1	7.0	1.89 ± 0.10 (2)
	2		1.46 ± 0.03 (2)
	3		0.96
	1	8.5	1.62 ± 0.09 (3)
	2		1.96 ± 0.05 (4)
	3		1.68 ± 0.08 (2)
	5		1.58 ± 0.03 (2)

[a] 0.60 *M* KCl, 40.0°. [b] A mean value and average deviation of *n* determinations.

Results and Discussion

At pH 8.5 (Table II) the length of alkyl chain between the phthalimide ring and carboxylate group has little effect on the rate of imide hydrolysis. At this pH the hydrolysis of the imide linkage should be under control of the rate of attack of hydroxide ions.[4,22,23] As the length of alkyl chain increases, the electrostatic repulsion of attacking hydroxide ions should decrease. This tendency of the rate of hydrolysis to increase with increasing separation of phthalimide ring from carboxylate is presumably offset by a steric factor associated with the bulky alkyl chain. On the other hand, in both the 3- and 4-nitrophthaloyl series in Table II the rate data show that N-methylcarboxylate and N-ethylcarboxylate derivatives at pH 7.0 are hydrolyzed at an appreciably greater rate than derivatives having a longer alkyl chain between carboxylate and imide nitrogen atom. These data provided the first indication that a neighboring carboxylate group might participate in imide hydrolysis. At pH 8.5 such participation may be swamped out by hydroxide ion catalyzed hydrolysis.

The effect on hydrolysis on the phthalimide ring by a neighboring carboxylate was next examined by partial pH–rate profiles extending from an alkaline region down into the neutral region.[24] As the curves in Figure 1 show, below pH 8 all phthaloyl derivatives of glycine or β-alanine are hydrolyzed at rates greater than can be accounted for by extrapolation of the linear region above pH 8 to below pH 8. The apparent acid dissociation constant of phthaloylglycine was found to be 3.5 from the half-neutralization point of a titration curve. We have calculated that at pH 7 deionized phthaloylglycine would have to be at least 500 times more reactive than ionized phthaloylglycine to cause the observed curvature below pH 8. This difference in reactivity is too great to be explained only as an electrostatic effect.

The electrostatic effect of an ionized carboxyl of phthaloylglycine may be evaluated from data in Figure 1. N-Methylphthalimide is hydrolyzed at pH 8.5 nearly three times faster than its carboxylate derivative. This relationship can be attributed to the charge of the ionized phthaloylglycine. Hydroxide ions are repelled from an imide by a proximate negative charge, as was first established by Edward and Terry for ionized succinimide.[22] Phthaloylglycylglycine, however, is hydrolyzed at a rate nearly seven times greater than the phthaloyl-ω-amino acids in Table II. This reactivity can be attributed to withdrawal by the carboxamide group of electrons from the imide ring. A similar inductive effect should obtain for an un-ionized carboxyl group of phthaloylglycine. Therefore, un-ionized phthaloylglycine should hydrolyze less than ten times as fast as the ionized species in alkali. This evaluation, coupled with the low concentration of deionized phthaloylglycine (apparent pK_a = 3.5) above pH 7, prompted us to discard the idea that only an electrostatic effect could account for the curvature of the pH–rate profiles in Figure 1.

The hydrolysis of phthaloylglycine is compared directly to the hydrolysis of *o*-carboxyphthalimide in

(19) A. Schöberl and H. Braun, *Ann.*, **542**, 274 (1939).
(20) E. Späth, F. Kuffner, and N. Platzer, *Ber.*, **68**, 699 (1935).
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(24) Below pH 6 under the conditions employed the nitrophthaloyl derivatives are very insoluble and also hydrolysis does not necessarily go to completion.

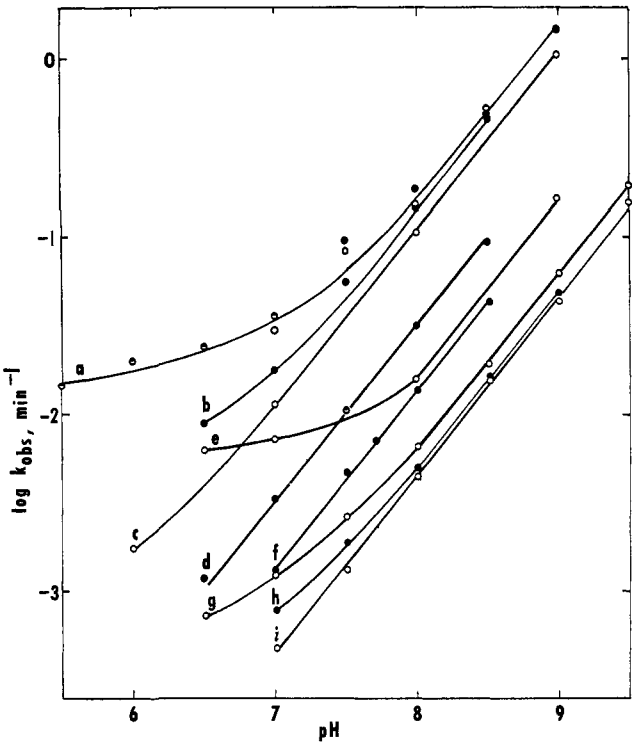


Figure 1. Observed first-order rate constants for a, 3-nitrophthaloylglycine, ●; and 3-nitrophthaloyl-β-alanine, ○; b, 3-nitrophthaloyl-γ-aminobutyric acid; c, 3-nitrophthaloyl-ε-aminocaproic acid; d, phthaloylglycylglycine; e, 3-nitrophthaloyl-α-aminoisobutyric acid; f, N-methylphthalimide; g, phthaloyl-β-alanine; h, phthaloylglycine; and i, phthaloyl-ε-aminocaproic acid; 40.0°, 0.60 *M* KCl.

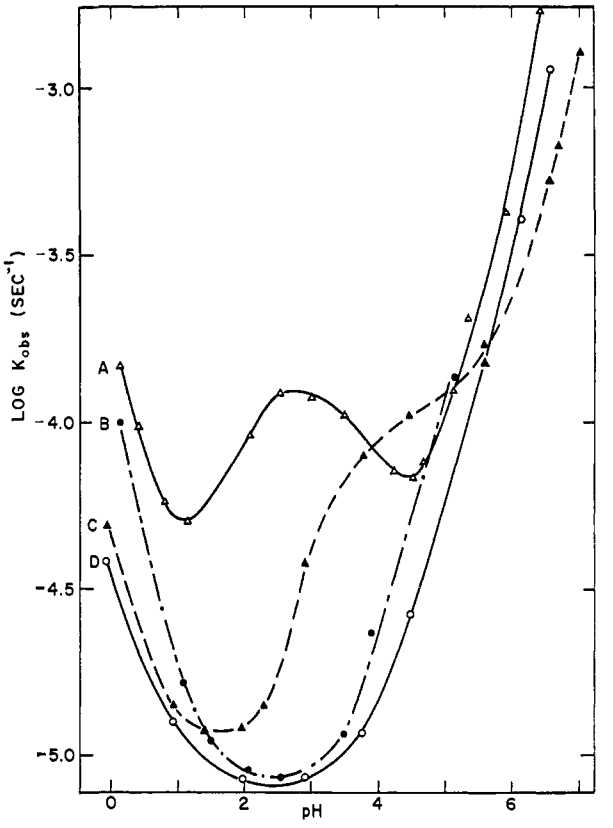


Figure 2. pH–rate profiles of A, *o*-carboxyphthalimide (ref 4); B, phthalimide (ref 4); C, phthaloylglycine; and D, N-methylphthalimide. Temperature 100°; rates for C and D determined by procedure in ref 4.

Figure 2. The pH–rate profile for N-methylphthalimide is similar to that for phthalimide, both having a region of acid catalysis and a region of base catalysis. The rate minimum occurs near pH 2.5. The pH–rate profile for phthaloylglycine bulges between pH 2 and 5. The bulge is distinctly different from the bell-shaped maximum observed by Zerner and Bender for carboxyl catalysis of *o*-carboxyphthalimide hydrolysis that involves two ionization steps among the reactive intermediates. The bulge in the profile of phthaloylglycine can be related to the ionization of the carboxyl group. The carboxylate group may increase the rate of hydrolysis of the phthalimide ring through intramolecular catalysis. Whether nucleophilic catalysis or general base catalysis prevails cannot be determined from the kinetic data. The curvature of some of the partial pH–rate profiles below pH 8 in Figure 1 is presumably produced by a bulge similar to that for phthaloylglycine.

The pH–rate profile for phthaloylglycine permits comparison of rates of hydrolysis of ionized species and deionized species. The difference in rates at pH 2.5 and at pH 7 is greater than 500-fold. The difference is much greater than a tenfold difference expected for only an electrostatic effect, as discussed earlier.

Near neutrality, the observed first-order rate constant for hydrolysis of phthaloylamino acids subject to intramolecular catalysis by a neighboring carboxylate group can be expressed as

$$k_{obsd} = [1 + (H^+)/K_A]k_{COO^-} + k_{OH^-}(OH^-)$$

When the pH is two units above the pK value of the carboxylate group, the rate equation simplifies to

$$k_{obsd} = k_{COO^-} + k_{OH^-}(OH^-)$$

Between pH 6 and 8, k_{obsd} is plotted against (OH^-) for 3-nitrophthaloyl-α-aminoisobutyric acid in Figure 3. Straight lines were obtained with slopes of k_{OH^-} and intercepts of k_{COO^-}. Approximate values for k_{COO^-} and for k_{OH^-} were also calculated from rate data in

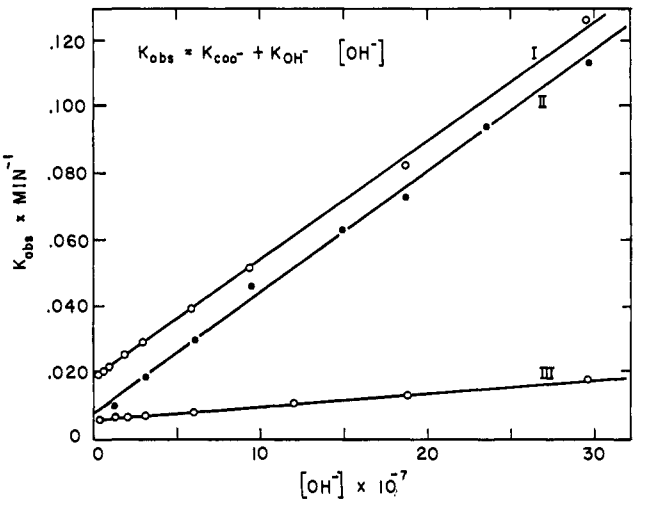
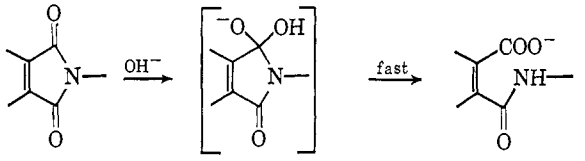


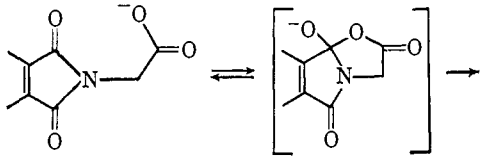
Figure 3. Graphical evaluation of k_{COO^-} and of k_{OH^-} for 3-nitrophthaloyl-β-alanine, I; 3-nitrophthaloyl-γ-aminobutyric acid, II; and 3-nitrophthaloyl-α-aminoisobutyric acid, III. Hydrolysis at constant pH at 40.0° in 0.60 *M* KCl.

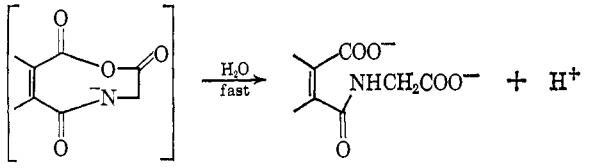
Figure 1. In the latter case, values for $k_{OH^-}(OH^-)$, as determined from the linear portion of the pH–rate profile, were subtracted from values for k_{obsd} at pH 7 to give values for k_{COO^-}. The values for k_{COO^-} in Table III suggest that both the position of the carboxylate group and the electrophilic character of the carbonyl carbon atoms of the imide ring influence the degree of intramolecular catalysis. When the carboxylate group is joined to the imide ring by a five-carbon alkyl chain, k_{COO^-} has a value much smaller than the value for lower homologs. The more electrophilic carbonyl carbon atoms of the 3-nitrophthaloyl derivatives are presumably responsible for the large values of k_{COO^-} as compared to the values for corresponding phthaloyl derivatives. Greater electrophilic character of a carbonyl carbon atom would favor nucleophilic attack by either OH^-, water, or a carboxylate group.

Two mechanisms for intramolecular catalysis by a carboxylate can be proposed. The mechanism for hydroxide ion catalysis involves addition of OH^- to a carbonyl group.[4,22,23]

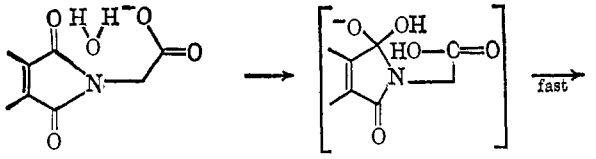


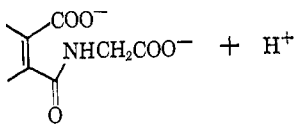
The carboxylate groups might also add to the carbonyl group in the case of nucleophilic catalysis.





Finally, the carboxylate group might promote the addition of water by accepting a proton in the case of general base catalysis.

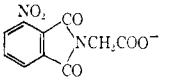
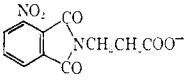
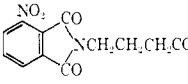
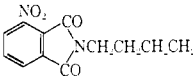
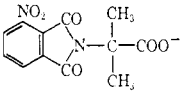
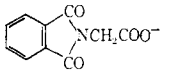
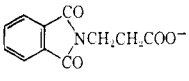




The mechanisms for carboxylate catalysis are indistinguishable by the kinetic data.

Values of k_{COO^-} for 3-nitrophthaloyl derivatives of glycine and β-alanine (Table III) are similar. This similarity supports the conclusion, reached earlier, that the enhancement of hydrolysis is not just an inductive effect of an undissociated carboxyl group. Such an inductive effect is significantly reduced by interposition of a methylene group, as is the case for 3-nitrophthaloyl-β-alanine. Apparently, the carboxylate group in this imide has a conformation as favorable as that for 3-nitrophthaloylglycine for intramolecular catalysis.

Table III. Catalytic Constants[a]

	Carboxylate catalytic constant, $10^3 k_{COO^-}$, min^{-1}	Hydroxide catalytic constant, k_{OH^-}, $mole^{-1}$ min^{-1}
	19	46,000
	18.5	35,700
	7.2	36,700
	0.9	36,600
	5.7	4,300
	0.33	1,730
	0.59	2,140

[a] Hydrolysis at 40.0°, 0.60 *M* KCl. Values for constants were determined from data in Figures 1 and 3 using the rate equation $k_{obsd} = k_{COO^-} + k_{OH^-}(OH^-)$.

The k_{OH^-} value for 3-nitrophthaloylglycine is reduced by a factor of ten when this imide is substituted with two methyl groups (see 3-nitrophthaloyl-α-aminoisobutyric acid, Table III). However, the value for k_{COO^-} is reduced by a factor of only three. If carboxyl group enhancement of imide hydrolysis were merely an inductive steric effect, k_{COO^-} should also be decreased by a factor of ten. The value for k_{COO^-} for 3-nitrophthaloyl-α-aminoisobutyric acid, then, also supports the conclusion that carboxylate catalysis contributes significantly to hydrolysis of the imide linkage near neutrality.

The methyl groups of 3-nitrophthaloyl-α-aminoisobutyric acid may impede the approach of the carboxylate to the imide ring. This would contrast to the usual "*gem* effect" in which dimethyl substitution promotes a favorable orientation of interacting groups.[8,25] However, Stuart models show that the methyl groups of 3-nitrophthaloyl-α-aminoisobutyric acid restrict the orientation of the carboxylate group toward the imide ring. Also, free rotation is not possible about all bonds since the imide ring is planar. Unfavorable orientation of the carboxylate group could, of course, reduce intramolecular catalysis.

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(25) For a general discussion of the "*gem* effect" with pertinent references see T. C. Bruice and W. C. Bradbury, *J. Am. Chem. Soc.*, 87, 4838, 4846, 4851 (1965).