Table IV. Rates of Reaction of Chloroaziridines with Thiophenolate

Aziridine	Wt., g.	NaS-Ph, g.	$Bu_4N^+Br^-$, g.	Temp., °C.	Rate × 10^2, sec.$^{-1}$
V	0.24	0.53	...	24.8	1.19 ± 0.05
V	0.23	0.23	...	24.8	0.92 ± 0.06
V	0.23	0.25	0.88	24.8	1.77 ± 0.08
III	0.26	0.54	...	71.9	0.94 ± 0.07

moved and quenched in *ca.* 25 ml. of glacial acetic acid. A standard iodine solution was used to titrate the unconsumed thiophenol. Typical results are shown in Table IV.

Acknowledgment. We wish to thank Professor John O. Edwards for his helpful discussions and information concerning his results prior to publication.

Kinetics and Mechanism of the Hydroxide Ion and Morpholine-Catalyzed Hydrolysis of Methyl *o*-Formylbenzoate. Participation by the Neighboring Aldehyde Group[1]

Myron L. Bender, Jerome A. Reinstein,[2] Marc S. Silver,[3] and Robert Mikulak[4]

Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois. *Received May 17, 1965*

The hydroxide ion, hydronium ion, and morpholine-catalyzed hydrolyses of methyl o-formylbenzoate have been investigated. The hydroxide ion and morpholine catalyses lead to exceedingly rapid hydrolysis of this methyl ester around neutrality. In the morpholine-catalyzed hydrolysis the formation and decomposition of an unstable intermediate could be observed spectrophotometrically. With 0.085 M morpholine, the maximal concentration of the intermediate occurred after 2 sec. and the decomposition was virtually complete in another 8 sec. The intermediate was isolated from the reaction mixture at low temperature after a short time and identified as 3-morpholinophthalide. The intermediacy of this compound was proven by demonstrating that it was converted to product at exactly the same rate that the intermediate was converted to product, and that morpholine affected the rate of these conversions in exactly the same manner. Morpholine affected the rate of hydrolysis of the intermediate since this hydrolysis is reversible under the conditions employed. The pH–rate profiles of the hydrolysis of 3-morpholinophthalide and of 3-methoxyphthalide were determined. Both hydrolyses show a reaction independent of pH near neutrality and a reaction dependent on hydroxide ion at higher pH. The hydrolysis of 3-morpholinophthalide is faster than that of 3-methoxyphthalide by 10^3–10^4 in both regions. Mechanisms of these processes are discussed as is the relationship of these catalyses to enzymatic catalysis.

Introduction

Intramolecular catalysis by neighboring carboxylate ion, carboxylic acid, imidazole, carboxamide, and aromatic and aliphatic hydroxyl groups constitutes an effective avenue of ester hydrolysis.[5-12] Interest in these intramolecular catalyses has been high because of their application as possible models for the intracomplex and intramolecular catalyses in enzymatic reactions, since each of these groups is present as a substituent in enzymes.

Other functional groups may also participate as intramolecular catalysts in ester hydrolysis. Recently Newman and Hishida[13] explained the exceptional hydrolytic reactivity of certain methyl-substituted *o*-benzoylbenzoates in terms of the initial attack of hydroxide ion on the keto group of the substrate. We report here the hydrolytic reactions of the similar compound, methyl *o*-formylbenzoate, using hydroxide ion and morpholine as catalysts.[14]

Experimental Section

Materials. Methyl *o*-formylbenzoate was prepared from phthalaldehydic acid (Eastman Kodak Co.) and diazomethane in ether. The ether was evaporated *in vacuo*, leaving methyl *o*-formylbenzoate, which was placed in the freezer. One such preparation gave material which was not contaminated by the corresponding pseudo-ester, as determined by the different characteristic spectra of these two compounds, while another preparation was contaminated with about 10% of pseudo-methyl ester (3-methoxyphthalide) as shown by the lactone carbonyl absorption at 5.63 μ.

(1) Supported by grants from the National Science Foundation.
(2) National Institutes of Health Postdoctoral Research Fellow.
(3) National Science Foundation Postdoctoral Research Fellow on leave from Amherst College.
(4) Participant in National Science Foundation Summer Research Program.

(5) M. L. Bender, *Chem. Rev.*, 60, 53 (1960).
(6) T. C. Bruice, *Brookhaven Symp. Biol.*, 15, 52 (1962).
(7) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Am. Chem. Soc.*, 84, 2421 (1962).
(8) T. C. Bruice and S. J. Benkovic, *ibid.*, 85, 1 (1963).
(9) E. J. Fuller, *ibid.*, 85, 1777 (1963).
(10) M. L. Bender, F. J. Kézdy, and B. Zerner, *ibid.*, 85, 3017 (1963).
(11) S. M. Kupchan, S. P. Eriksen, and Y.-T. Shen, *ibid.*, 85, 350 (1963).
(12) T. C. Bruice and T. H. Fife, *ibid.*, 84, 1973 (1962).
(13) M. S. Newman and S. Hishida, *ibid.*, 84, 3582 (1962).
(14) A portion of this work was communicated previously: M. L. Bender, and M. S. Silver, *ibid.*, 84, 4589 (1962).

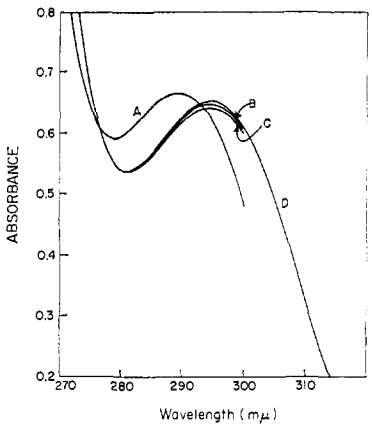


Figure 1. Ultraviolet spectra of methyl *o*-formylbenzoate (curve A), its alkaline hydrolysis product (curve B), its morpholine-catalyzed (0.017 *M*) hydrolysis product (curve C), the hydrolysis product of morpholinophthalide in 0.0125 *M* morpholine (curve D), and *o*-formylbenzoate ion in 0.0125 *M* morpholine (curve D). The latter two curves were exactly superimposed; pH 8.8, μ = 1.0, concentration = 3.69×10^{-4} *M*, Cary 14 PM recording spectrophotometer.

The latter sample was thrice purified by partial liquefaction of frozen material. The purified material utilized in these studies contained less than 1% of pseudo-ester by infrared estimate and melted at *ca.* 14°: λ^{CCl_4} 5.83 and 5.93 μ (lit.[15] λ^{CHCl_3} 5.82 and 5.92 μ); $n^{20}D$ 1.5426 (lit. $n^{20}D$ 1.5411,[16] $n^{20}D$ 1.5410 to 1.5423[17]).

Phthalaldehydic acid (Eastman Kodak Co.) was recrystallized twice from water, m.p. 99.8–100.5° (lit.[18] m.p. 99°). 3-Methoxyphthalide was prepared by refluxing phthalaldehydic acid with a 20-fold molar excess of methanol for 4 hr. The solution was poured into a tenfold molar excess of cold water and the precipitate was collected. The product was recrystallized twice from hexane, m.p. 42–44° (lit.[19] m.p. 44°).

Morpholine (Eastman Kodak Co.) was refluxed for 1 hr. over potassium hydroxide, and then distilled through a 35-cm., vacuum-jacketed column packed with glass helices, b.p. 128° (750 mm.). 3-Morpholinophthalide[20] was recrystallized from heptane, m.p. 127.8–128.2° (lit.[21] m.p. 127–128°). Reagent grade buffers, salts, and acids were used. Acetonitrile solvent was Eastman Kodak Spectro Grade material. Double-distilled water was used throughout.

Kinetics. The kinetics of the hydroxide ion and hydronium ion catalyzed hydrolyses of methyl *o*-formylbenzoate were carried out using a Cary 14 PM recording spectrophotometer equipped with a thermostated cell compartment. Reactions of long duration were thermostated in a water bath, and aliquots were withdrawn at suitable times for spectral analysis. The reaction was initiated by adding a 1-μl. quantity of a solution of the ester in acetonitrile to an appropriate thermostated buffer solution. The reaction was ordinarily followed at 220 mμ, although occasionally 265 or 310 mμ was used. The rate constant was shown to be independent of the wave length used.

The kinetics of hydrolysis of 3-methoxyphthalide was followed at 260 mμ, using a Beckman DU spectrophotometer equipped with a thermostated cell compartment.

The kinetics of the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate and the kinetics of the hydolysis of morpholinophthalide were carried out using a Cary Model 14 PM recording spectrophotometer equipped with a thermostated cell compartment. Both of these reactions are first order in substrate. Since these reactions are quite rapid, with half-lives down to a few seconds, the method of initiation will be described. The substrate solution (usually 50 μl. of *ca.* 10^{-2} *M* solution in acetonitrile) was added on the tip of a stirring rod which had a flat, disk-shaped head whose plane was perpendicular to the rod. The solution was stirred rapidly with an up-and-down motion, taking care not to withdraw the stirrer from the solution, and the instrument was turned on. The reactions were followed at 292.5 mμ. The initial time was taken as the moment of insertion of the stirring rod into the buffer. It was possible to perform these experiments with a lag of about 3 sec. between initial time and turning on the instrument. The pH was taken before and after the reaction; the change was never greater than 0.02 pH unit.

The morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate was also followed using a spectrophotometer equipped with a stopped-flow mixing device[22] since the initial phase of this reaction could not be observed on the time scale of the Cary spectrophotometer. A solution of ester in 1% acetonitrile–water and a solution of half-neutralized morpholine at pH 8.82 ± 0.02 (both at μ = 1) were mixed in the instrument, and the intensity of transmitted light at 290 mμ (the apparent isosbestic point for reactant and product on this instrument) was recorded *vs.* time. Mixing time was estimated to be about 10 msec. A chart speed of 25 divisions (12.5 cm.) per second was used. The rate constants and absorptivities obtained from the terminal reaction observed on the Cary spectrophotometer agreed with the corresponding data from the stopped-flow instrument.

Identification of the Reaction Products. The infinity spectra of the alkaline hydrolysis of methyl *o*-formylbenzoate and of the hydrolysis of 3-methoxyphthalide at pH 4, 5, and 9 were shown to be identical with the spectrum of *o*-formylbenzoate ion at the appropriate pH, indicating that the reactions being followed were indeed the hydrolytic reactions.

The identification of the product of the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate and of the hydrolysis of morpholinophthalide is a more crucial question. Therefore, both product analyses and spectral analyses were performed on these reactions. Figure 1 shows that the spectra of *o*-formylbenzoate ion (in 0.0125 *M* morpholine), the product of the alkaline hydrolysis of methyl *o*-formylbenzoate, the product of the morpholine-catalyzed hydrolysis of methyl

(15) R. E. Tarney, Ph.D. Thesis, University of Wisconsin, University Microfilms, L. C. Card No. Mic 60-6793, p. 171; *Dissertation Abstr.*, **21**, 2901 (1961).
(16) E. L. Eliel and A. W. Burgstahler, *J. Am. Chem. Soc.*, **71**, 2251 (1949).
(17) K. v. Auwers and A. Heinze, *Ber.*, **52B**, 595 (1919).
(18) E. Bernatek, *Acta Chim. Scand.*, **14**, 785 (1960).
(19) S. Gabriel, *Ber.*, **49**, 1612 (1916); H. Meyer, *Monatsh.* **25**, 497 (1904).
(20) The authors acknowledge with thanks the gift of this material from Dr. D. C. Young, Dow Chemical Co., Midland, Mich.
(21) D. D. Wheeler, D. C. Young, and D. S. Erley, *J. Org. Chem.*, **22**, 547 (1957).
(22) F. J. Kézdy and M. L. Bender, *Biochemistry*, **1**, 1097 (1962).

o-formylbenzoate, and the product of the hydrolysis of morpholinophthalide (in 0.0125 *M* morpholine) are identical.

o-Formylbenzoic acid was extracted by ether from acidified product solutions of the ester and morpholinophthalide hydrolyses, and was identified by melting point, mixture melting point, and ultraviolet spectrum. The results of four such experiments are summarized in Table I. Besides the identification of *o*-formylbenzoic acid as the product of these reactions, Table I indicates that when the hydrolysis of morpholinophthalide is carried out in 1 *M* (free) morpholine buffer, the reaction does not proceed to completion. Spectral and kinetic evidence of this equilibrium process will be presented later.

Table I. Product Analysis of the Hydrolysis of Morpholinophthalide and of the Alkaline Hydrolysis of Methyl *o*-Formylbenzoate[a]

Reactants	*o*-Formylbenzoic acid Yield, %	M.p., °C.
Morpholinophthalide + 1 *M* KCl	95	85–90
Morpholinophthalide + 1 *M* KCl, pH 6.81[b]	88	84–93
Morpholinophthalide + 1 *M* (free) morpholine buffer, pH 8.8[c]	19	97–98
Methyl *o*-formylbenzoate + 0.025 *M* (free) morpholine buffer, pH 8.8	60	96–97[d]

[a] The product solutions were acidified with 1 *N* hydrochloric acid and extracted with ether. The ether was removed almost to dryness, and the *o*-formylbenzoic acid was recovered. All of the yields are crude yields. Melting points of the first and second experiments are of crude products. In the third and fourth experiments, the melting points are of once recrystallized products. [b] In this experiment, the solution was extracted with ether before acidification to determine if any nonionic species were present. Nine milligrams of a semisolid material was isolated in this manner, corresponding to about 2% of the original morpholinophthalide. [c] Morpholinophthalide was isolated by extracting with ether before acidification; yield 82%, m.p. 127–127.5°. Thus the material balance of this reaction is 101% of the theoretical yield. [d] Mixture melting point with *o*-formylbenzoic acid, 96.5–97.5°.

Isolation of the Intermediate in the Morpholine-Catalyzed Hydrolysis of Methyl o-Formylbenzoate. When methyl *o*-formylbenzoate and morpholine were mixed in the pure state at room temperature, crystallization began in a few minutes. The melting point of the crude crystalline material was 118–123°, which was raised to 127–128° after one recrystallization from carbon tetrachloride; the melting point of a mixture with authentic 3-morpholinophthalide was 127–128°. Ultraviolet and infrared spectra of the compound were identical with those of 3-morpholinophthalide and the recovery from the mixture of reactants was 85% of theoretical.

Since the 3-morpholinophthalide was isolated from the reaction in the absence of solvent, the possibility existed that it was an artifact of the conditions and not the intermediate formed in aqueous alkaline solution. At the temperature of the kinetic studies, 25°, the maximum concentration of intermediate was reached in about 2 sec. with the half-life for disappearance of the intermediate also about 2 sec. (*vide infra*). To obviate this difficulty, the reaction was carried out at about −8°

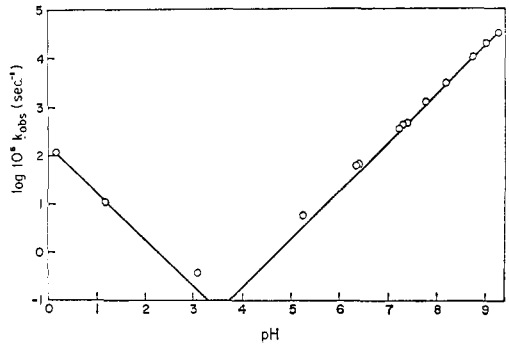


Figure 2. The hydrolysis of methyl *o*-formylbenzoate in aqueous solution at 25.0°, μ = 0.1.

(in an ice–salt bath). The reactants were mixed rapidly and the solution was immediately frozen in an acetone–Dry Ice bath to stop the reaction. The frozen reaction mixture was lyophilized, the remaining solid was washed with ether, and the ether was evaporated to dryness. The recovered solid had a melting point of 126–127°. It showed an undepressed melting point on admixture with authentic 3-morpholinophthalide and thus could be identified as 3-morpholinophthalide. It was obtained in 65% yield.

Results

The hydrolysis of methyl *o*-formylbenzoate in aqueous solution was followed from pH 0 to 9.23 using hydrochloric acid, acetate, phosphate, and carbonate buffers at constant ionic strength of 0.1. The results of these experiments are displayed in Table II and Figure 2. Variation of acetate, phosphate, or carbonate buffer concentration at constant pH and constant ionic strength had no effect on the rate, indicating that the buffer components did not participate in the hydrolytic reaction. In fact, Figure 2 may be simply described as the pH–rate profile of the hydronium and hydroxide ion catalyzed hydrolysis of methyl *o*-formylbenzoate. The rate data from pH 0 to 1 show direct proportionality to the hydronium ion concentration and the rate data from pH 7.2 to 9.2 show strict proportionality to the hydroxide ion concentration. The rate data at pH 3.08 and from pH 5.2 to 6.4 show a slight incursion of a water reaction, but such a reaction appears to make only a minor contribution to the over-all rate.

The kinetics of hydrolysis of 3-methoxyphthalide (the corresponding pseudo-ester of methyl *o*-formylbenzoate) were determined in aqueous solution at 25° from pH 4 to 11. The results of these determinations are shown in Table III and in Figure 3. Like the hydrolysis of methyl *o*-formylbenzoate, the hydrolysis of 3-methoxyphthalide shows no effect of buffer concentration, or indeed of ionic strength, as is seen in Table III. However, in contrast to the former hydrolysis, the latter hydrolysis shows a quite marked "water" reaction, as is evident in Figure 3. Above pH 10, the rate of the hydrolysis is approximately proportional to the hydroxide ion concentration, but from pH 4 to pH 9, the hydrolytic reaction is essentially independent of pH.

Also shown in Figure 3 are kinetic data on the hydrolysis of 3-morpholinophthalide in aqueous solution at 25°. These data, which cover the range from

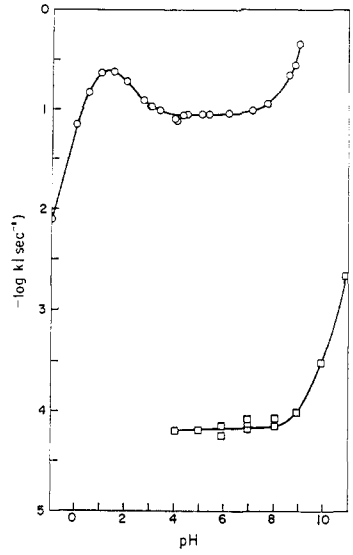


Figure 3. The hydrolysis of 3-methoxyphthalide (lower curve, □) and 3-morpholinophthalide (upper curve, O) in aqueous solution at 25°. The latter is at $\mu = 1.0$. −1 on the abscissa is 3.16 M HCl ($H_0 = -1.0$).

pH −1 to 9, are more extensive than the 3-methoxyphthalide data and, furthermore, show that the hydrolysis of morpholinophthalide is much more facile than the hydrolysis of the corresponding 3-methoxyphthalide. (See Table IV.)

Since nucleophiles other than hydroxide ion or water can potentially add to carbonyl groups, such reagents were tested as catalysts for the hydrolysis of methyl *o*-formylbenzoate. Of the bases and/or nucleophiles tested, imidazole, diethanolamine, carbonate, bicarbonate, monohydrogen phosphate, dihydrogen phosphate, and azide ions were without significant effect. That is, 0.01 to 0.1 M solutions of these various species at a pH $\simeq$ pK_a did not increase the rate of hydrolysis of methyl *o*-formylbenzoate by more than 50%. N-methylhydroxylamine, hydroxylamine, bisulfite ion, and Tris gave reactions considerably faster than normal hydrolytic reaction. However, these reactions were not simple hydrolytic reactions in character. For example, the reaction with hydroxylamine gave a product which spectrophotometrically was neither *o*-formylbenzoate ion nor the oxime of this ion. Likewise, reaction with Tris gave a product whose spectrum was different from that of the *o*-formylbenzoate ion in the same buffer. N-methylhydroxylamine gave a product which absorbed much more strongly in the ultraviolet than *o*-formylbenzoate which product was not stable with time but underwent further reaction to a product of even higher absorbance. Finally, bisulfite gave a series of extremely complex reactions, which were a function of many variables such as pH, concentration, etc. However, in no instance did spectrophotometric analysis indicate a clean-cut, over-all hydrolytic reaction.

In contrast to the complications noted above, morpholine catalyzes the hydrolysis of methyl *o*-formylbenzoate significantly, leading to a product which has been identified both spectrophotometrically and by isolation as *o*-formylbenzoate ion (see Experimental Section). Figure 4 illustrates a typical spectrophotometric curve of the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate, using the stopped-flow mixing device described earlier. Since the reaction is followed at the isosbestic point of the ester and acid product, the decrease followed by an increase in absorbance during the course of the reaction demonstrates the

Table II. The Hydrolysis of Methyl *o*-Formylbenzoate in Aqueous Solution at 25.0°, $\mu = 0.1$

pH	Buffer[a]	k_0, sec.$^{-1}$	$k_2 \times 10^{-3}$, M^{-1} sec.$^{-1}$
9.22	a	3.32×10^{-2}	1.98
9.23	a	3.31×10^{-2}	1.95
9.23	a	3.30×10^{-2}	1.94
9.23	a	3.27×10^{-2}	1.92
9.21	b	3.2×10^{-2}	1.95
9.15	c	3.09×10^{-2}	2.18
9.17	c	2.99×10^{-2}	2.00
9.12	d	2.90×10^{-2}	2.18
9.13	d	2.48×10^{-2}	1.82
9.12	d	2.53×10^{-2}	1.92
9.13	d	2.71×10^{-2}	2.01
9.06	e	2.3×10^{-2}	2.04
9.06	e	2.4×10^{-2}	2.12
9.03	f	2.18×10^{-2}	2.04
9.03	f	2.08×10^{-2}	1.94
8.72	g	1.08×10^{-2}	2.05
8.76	g	1.04×10^{-2}	1.82
8.19	h	3.05×10^{-3}	1.97
8.18	h	3.06×10^{-3}	2.02
8.17	h	2.96×10^{-3}	1.98
7.78	i	1.26×10^{-3}	2.10
7.38	j	4.83×10^{-4}	2.01
7.31	k	4.43×10^{-4}	2.18
7.24	l	3.87×10^{-4}	2.25
7.24	l	3.51×10^{-4}	2.02
6.36	m	6.2×10^{-5}	2.7
6.36	m	6.0×10^{-5}	2.6
6.41	n	6.8×10^{-5}	2.7
5.27	o	5.5×10^{-6}	2.9
5.25	p	5.7×10^{-6}	3.2
3.08	q	3.8×10^{-7}	
1.10	HCl	1.16×10^{-5}	1.17×10^{-7}
0.10	HCl	1.14×10^{-4}	1.15×10^{-7}

[a] a–c and e–g were carbonate buffers, made up to ionic strength solely with the buffer components; d was a carbonate buffer, 0.01 μ in components and 0.09 M in KCl; h–j, m, and n were phosphate buffers, made up as a–c and e–g; k was a phosphate buffer, 0.03 μ in components and 0.07 M in KCl; l was a phosphate buffer, 0.01 μ in components, 0.09 M in KCl; o and p were acetate buffers and q was dilute HCl plus KCl; p was buffer o diluted with an equal volume 0.1 M KCl.

Table III. The Hydrolysis of 3-Methoxyphthalide in Aqueous Solution at 25°

pH	Buffer	$k_{obsd} \times 10^5$, sec.$^{-1}$
4.05	*a*	6.3
4.99	*a*	6.4
5.92	*b*	5.6
5.92	*b*	7.0
6.94	*b, c*	6.7
6.94	*b, c*	7.6
6.94	*b, c*	8.2
8.04	*b*	7.0
8.04	*b*	8.2
8.92	*d*	9.5
9.92	*e*	30.4
10.90	*e*	221

[a] Acetate buffer. [b] Phosphate buffer. [c] Total buffer concentration was changed from 0.05 to 0.067 to 0.30 M in these experiments at constant pH. [d] Borate buffer. [e] Borate–carbonate buffer.

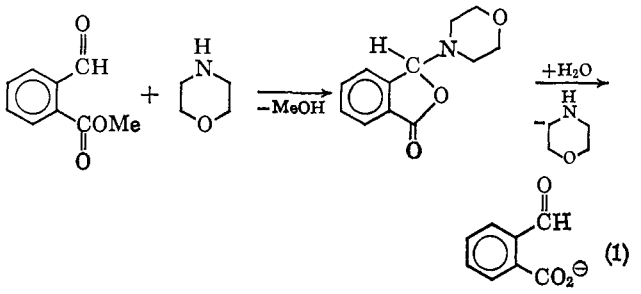
Table IV. The Hydrolysis of 3-Morpholinophthalide in Aqueous Solution at 25°, μ = 1.0

pH	Buffer[h]	$k_{obsd} \times 10^2$, sec^{-1}
−1.0[a]	*b*	0.8
0.02	*b*	7.2
0.51	*b*	14.5
1.03	*b*	21.4
1.52	*b*	23.7
2.05	*b*	19.0
2.70	*c*	12.3
2.96	*c*	10.8
3.03	*c*	10.8
3.17	*c*	11.6
3.35	*c*	9.7
3.97	*c*	8.1
4.07	*d*	7.7
4.26	*c*	8.8
4.44	*c*	9.0
5.03	*d*	9.2
5.31	*e*	9.1
6.12	*e*	9.3
7.08	*e*	9.9
7.70	*e*	11.3
8.55	*f*	22
8.79	*g*	28
8.98	*g*	45

[a] 3.16 *M* HCl (H_0 = −1.0). [b] Hydrochloric acid. [c] 0.01 *M* citrate buffer. [d] 0.01 *M* acetate buffer. [e] 0.011 *M* phosphate buffer. [f] 0.01 *M* borate buffer. [g] 0.01 *M* Tris–HCl buffer. [h] Ionic strength maintained at 1.0 by the addition of KCl.

formation and subsequent decomposition of an unstable intermediate.

The intermediate in the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate was identified as 3-morpholinophthalide by isolation from the reaction mixture after a few seconds of contact time (see Experimental Section). Furthermore, it was found that morpholinophthalide was hydrolyzed to *o*-formylbenzoate ion at exactly the rate at which the intermediate in the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate was hydrolyzed. This combination of isolation and kinetic evidence leaves little doubt that the morpholine-catalyzed hydrolysis may be described by the pathway shown in eq. 1.



(1)

Since the last reaction of eq. 1 could be observed separately using 3-morpholinophthalide, this reaction was investigated in detail. It was found that the rate of formation of *o*-formylbenzoate ion from 3-morpholinophthalide increased with increasing free morpholine concentration. In addition, a solution of 3-morpholinophthalide in 1 *M* KCl, pH 6.8, yielded nothing on extraction with ether, whereas a solution of 3-morpholinophthalide in 1 *M* (free) morpholine buffer at pH 8.7 yielded 82% unreacted 3-morpholinophthalide after three extractions with ether. Both of these observations may be explained if one assumes a reversible hydrolysis

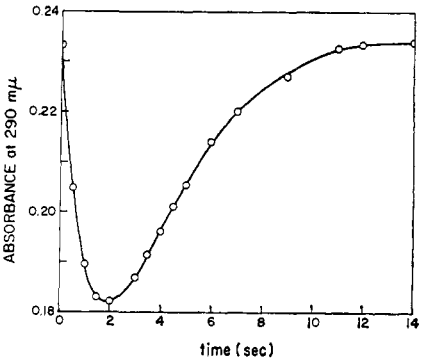


Figure 4. The morpholine-catalyzed (0.085 *M*) hydrolysis of methyl *o*-formylbenzoate. The reaction was followed using a spectrophotometer equipped with a stopped-flow mixing device.

of 3-morpholinophthalide to morpholine and *o*-formylbenzoate ion.

The reversible reaction described above was investigated from a kinetic standpoint by comparing the kinetics of reactions, starting either with 3-morpholinophthalide or with *o*-formylbenzoate ion, both in the presence of the same concentrations of morpholine and at the same pH. The reaction of *o*-formylbenzoate ion with morpholine, although more difficult to observe experimentally because of an unfavorable equilibrium constant, yielded kinetic results, at three concentrations of morpholine, which were within experimental error of those obtained for the reaction of 3-morpholinophthalide in the presence of morpholine, as shown in Table V.

Table V. Kinetics of the Reversible Hydrolysis of 3-Morpholinophthalide in the Presence of Morpholine at 25° and pH 8.7

	k_{obsd}, $sec.^{-1}$	
Free morpholine, *M*	3-Morpholinophthalide in morpholine	*o*-Formylbenzoate and morpholine
0.25	0.46	0.39
0.50	0.55	0.56
1.00	0.61	0.8

These observations are consistent with a reversible reaction which may be represented as eq. 2. When

$$\text{3-morpholinophthalide} + H_2O \underset{k_3(M)}{\overset{k_2}{\rightleftharpoons}} \text{o-formylbenzoate} + \text{morpholine} \quad (2)$$

morpholine is in great excess, k_2 is the pseudo-first-order constant including the concentration of water and k_3(M) is a pseudo-first-order constant including the concentration of morpholine. Thus, under conditions of excess morpholine, the kinetics are those of a simple, first-order, reversible reaction where the observed rate constant may be described as[23]

$$k_{obsd} = k_2 + k_3(M) \quad (3)$$

Equation 3 is the equation of a straight line whose slope is k_3 and whose intercept is k_2. Data testing eq.

(23) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1961, p. 186.

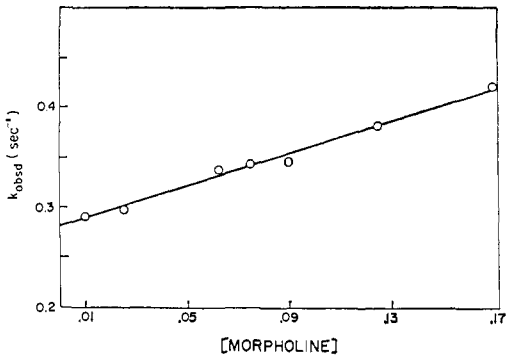


Figure 5. The hydrolysis of 3-morpholinophthalide in the presence of morpholine at pH 8.87 and 25°.

3 are shown in Table VI and Figure 5. It is seen that the data describe an excellent straight line.

Table VI. The Hydrolysis of 3-Morpholinophthalide in the Presence of Morpholine[a]

Free morpholine, M	k_{obsd}, sec.$^{-1}$
0.01	0.291 ± 0.005[b]
0.025	0.298 ± 0.004
0.0625	0.338 ± 0.009
0.075	0.343
0.090	0.346 ± 0.001
0.125	0.382 ± 0.002
0.17[c]	0.421 ± 0.002

[a] pH 8.87 and 25.0°. [b] Average deviation of two or more determinations. [c] At free morpholine concentrations higher than 0.17 M, k_{obsd} was no longer linear with morpholine, probably owing to the change in medium.

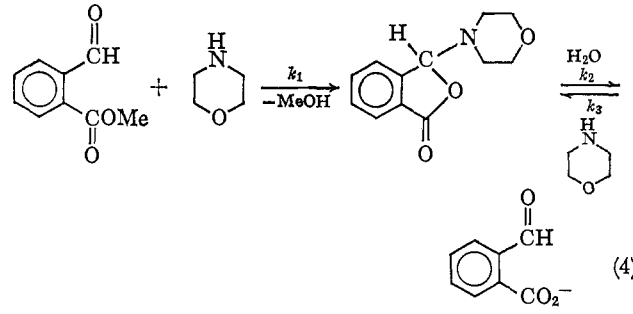
The equivalence of the second step in the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate and the hydrolysis of 3-morpholinophthalide implies that the rate of the second step of the former reaction should be dependent on morpholine concentration in a like manner to that described below. A comparison of the rate constants, k_2 and k_3, determined from the two sets of experiments, as shown in Table VII, agree within experimental error, but the values for the hydrolysis of 3-morpholinophthalide are more precise and probably more accurate.

Table VII. Comparison of Rate Constants[a] k_2 and k_3 for Both the Hydrolysis of 3-Morpholinophthalide and the Hydrolysis of the Intermediate in the Morpholine-Catalyzed Hydrolysis of Methyl *o*-Formylbenzoate

Reaction	k_2, sec.$^{-1}$	k_3, M^{-1} sec.$^{-1}$
Hydrolysis of 3-morpholinophthalide	0.28	0.78
Hydrolysis of the intermediate	0.25	0.83

[a] At 25°.

The question may be raised as to whether the first step, the conversion of methyl *o*-formylbenzoate to 3-morpholinophthalide, is also reversible. To this end, 3-morpholinophthalide was dissolved in excess absolute methanol and refluxed for several hours. No ester was recovered, and there was only a slight change in the spectrum indicating that at most a few per cent of the ester was present after refluxing. A large quantity of methanol was then distilled from the reaction mixture (about 20 times the original volume). Any free morpholine present should have distilled with the methanol. To test this possibility, the distillate was titrated with 0.1 N hydrochloric acid. Less than 10% of the theoretical amount of base was titrated. Furthermore, a 97% yield of 3-morpholinophthalide, m.p. 125–127°, was recovered. From these experiments we conclude that although the second step of the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate is reversible, the first step is sensibly not reversible. Thus, the equation that describes the system is



(4)

Two of the rate constants of eq. 4 have already been determined, but k_1 has not. Using the differential equation

$$dB/dt = k_1(M)A + k_3(M)C - k_2B \quad (5)$$

where A, B, and C are the concentrations of the three components of eq. 4, (M) is the concentration of morpholine, and k_1, k_2, and k_3 are the three rate constants of eq. 4, it may be shown that[23,24]

$$B = \frac{A_0(M)}{k_2 + k_3(M)}\left\{k_3 + \frac{1}{k_2 + (k_3 - k_1)(M)} \times [(k_1 - k_3)(k_2 + k_3(M))e^{-k_1(M)t} - k_1k_2e^{-(k_2 + k_3(M))t}]\right\} \quad (6)$$

Differentiating eq. 6 with respect to time and setting $dB/dt = 0$ gives an equation (7) where t_{max} is the time

$$\frac{k_2}{(k_1 - k_3)(M)} = e^{[k_2 - (k_1 - k_3)(M)]t_{max}} \quad (7)$$

at which the concentration of B is at a maximum, and where the concentration of free morpholine and its corresponding t_{max} are the only variables. Taking the logarithm of eq. 7 and rearranging terms gives

$$\log\frac{k_2}{(M)} - \frac{k_2t_{max}}{2.303} = -\frac{(k_1 - k_3)(M)t_{max}}{2.303} + \log(k_1 - k_3) \quad (8)$$

This equation should yield a straight line if the left-hand side is plotted against $(M)t_{max}$. Such a plot using previously determined values of k_2 and experimental values of t_{max} and morpholine concentration is shown in Figure 6. A satisfactory linear plot is obtained. Using previously determined values of k_3, both the intercept of the plot (log $k_1 - k_3$) and the slope ($-(k_1 - k_3)/2.303$) yield independent values of $k_1 = 11 \pm 1$ M^{-1} sec.$^{-1}$.

(24) A. Rakowski, *Z. physik. Chem.* (Leipzig), 57, 321 (1907).

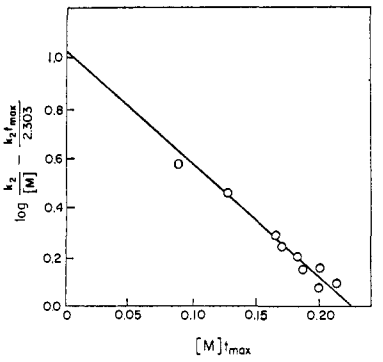


Figure 6. The morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate. t_{max} is time of maximum concentration of intermediate.

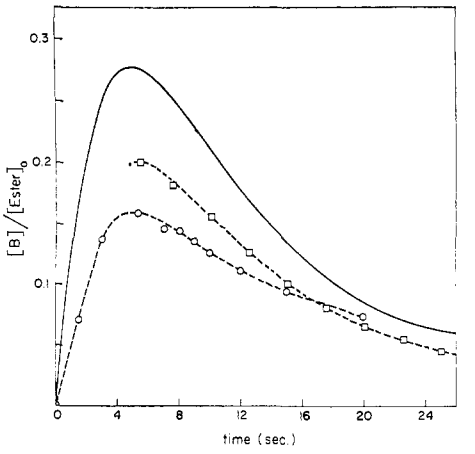


Figure 7. Comparison of experimental data (dashed lines) and calculated curve (solid line) for the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate. The calculated curve was obtained from a Model 31 R Electronic Associates, Inc. analog computer: O, stopped-flow experiments; □, Cary spectrophotometer experiments.

The differential equation for B may be programmed on an analog computer to plot the ratio of the concentration of B to the initial ester concentration as a function of time. The values of k_2 and k_3 calculated from the 3-morpholinophthalide hydrolysis data were used, and k_1 was varied to get the best fit to an experimental curve, obtained from the absorbance *vs.* time curves for methyl *o*-formylbenzoate such as Figure 4. The best value for k_1 was the same as that obtained from the plot of Figure 6. With this value of k_1, it was possible to fit the time of maximum concentration of intermediate as a function of morpholine concentration very well, but the calculated ratio of the concentration of B to the initial ester concentration was consistently low (see Figure 7). This failure is probably due mainly to a systematic error in the determination of the absorptivity of B. Since 3-morpholinophthalide (B) is essentially completely hydrolyzed within a few seconds after being placed in aqueous solution, it was not possible to measure its absorptivity directly. The log absorbance *vs.* time curve for the hydrolysis was extrapolated to zero time to estimate the absorptivity. Because the rate constant was so large, this extrapolation had to be made from 66% reaction to 0% reaction; thus a systematic error of 1 sec. in estimating the zero time could account for the difference between the computer curve and the calculated ratio of B to the initial ester concentration in Figure 7.

At higher concentrations of morpholine another systematic error could have been introduced by a medium effect of morpholine on the *o*-formylbenzoate spectrum, leading to erroneous calculations of the extinction coefficients of the latter. Medium effects were observed on the spectra of *o*-nitrobenzoic acid and *o*-chlorobenzoic acid in morpholine buffer, and with *o*-formylbenzoic acid in dioxane–water solutions, changes of 3–9% in 1 *M* solutions being observed. The discrepancy between the calculated and experimental curves may also have been caused partly by the presence of a second product, perhaps a Schiff base, which is convertible by hydrochloric acid to *o*-formylbenzoic acid and thus would not be observed in our product isolation experiments.

Discussion

The alkaline hydrolysis of methyl *o*-formylbenzoate is one of the fastest nonenzymatic hydrolyses of a methyl ester known in aqueous solution at 25°.[25] A calculated rate constant for this hydrolysis may be obtained using the ratio of rate constants of the *o*-nitrobenzoate and *p*-nitrobenzoate esters, and the rate

Table VIII. Rate Constants of the Hydrolysis of Some Ethyl Benzoates at 25°

Substituent	Catalyst	Solvent	k^2, M^{-1} sec.$^{-1}$	Ref.
p-NO_2	OH^-	56 wt. % acetone–water	2.44×10^{-1}	26
o-NO_2	OH^-	56 wt. % acetone–water	1.69×10^{-2}	27
p-CHO	OH^-	56 wt. % acetone–water	8.72×10^{-3}	28
H	OH^-	56 wt. % acetone–water	2.87×10^{-3}	26
H	OH^-	Water	3.0×10^{-2}	29
o-CHO	OH^-	Water	6.3×10^{-3}	Calcd.
o-CHO[a]	OH^-	Water	2000	Exptl.
H	H^+	Water	5.95×10^{-7}	30
o-CHO[a]	H^+	Water	1.16×10^{-4}	Exptl.

[a] These correspond to methyl esters which are ordinarily twice as reactive as ethyl esters.

(25) Both J. A. Shafer and H. Morawetz, *J. Org. Chem.*, **28**, 1899 (1963), and M. T. Behme and E. H. Cordes, *ibid.*, **29**, 1255 (1964), have found that the rate constant of the hydroxide ion catalyzed loss of methanol from methyl phthalamate is 3000 M^{-1} sec.$^{-1}$ However, the imide intermediate which is formed first in this reaction is 200–300 times less reactive toward hydroxide ion than is the starting material, so that the over-all hydrolytic reaction is slower than that reported here.

(26) E. Tommila and C. N. Hinshelwood, *J. Chem. Soc.*, 1801 (1938).

(27) E. Tommila, *Ann. Acad. Sci. Fennicae, Ser. A 57*, **No. 13**, 3 (1941).

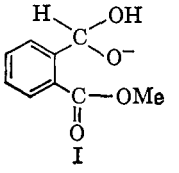
(28) E. Tommila, L. Brehmer, and H. Elo, *Ann. Acad. Sci. Fennicae, Ser. A 59*, **No. 9**, 3 (1942).

(29) M. L. Bender, *J. Am. Chem. Soc.*, **73**, 1626 (1951).

(30) E. W. Timm and C. N. Hinshelwood, *J. Chem. Soc.*, 862 (1938). This value has been corrected to aqueous solution by comparing the relative rate at 100° with that in water at 100°.[29]

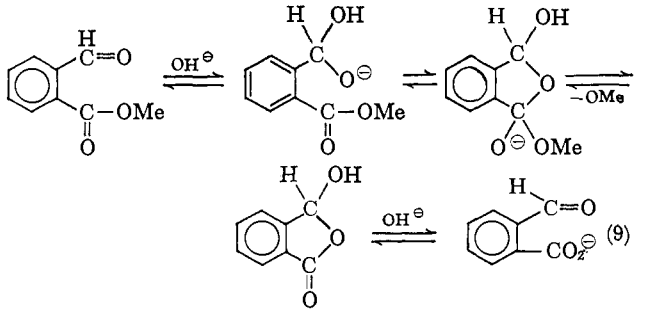
constant of the *p*-formylbenzoate ester (corrected to aqueous solution), as shown in Table VIII. Since the experimental rate constant is over 10^5 faster than that calculated for the compound solely on the basis of its substituent (electronic and steric) effect, the formyl group must participate directly in the mechanism. Similar large rate enhancements have been found in the alkaline hydrolyses of other *o*-formyl compounds, *e.g.*, cinnamoylsalicylaldehyde ($k_2 = 1.2 \times 10^4\ M^{-1}$ sec.$^{-1}$)[31] and *o*-acetoxybenzaldehyde ($k_2 = 11 \times 10^4\ M^{-1}$ sec.$^{-1}$ while the rate constant for the corresponding *para* compound was $k_2 = 25\ M^{-1}$ sec.$^{-1}$).[32,33] Rate enhancements have also been found in the alkaline hydrolyses of *o*-benzoylbenzoate esters, especially evident in the reactions of some very hindered esters of this kind.[13]

The simplest chemical explanation of the participation by the *o*-formyl group is that hydroxide ion adds to the formyl group leading to the adduct I. The adduct I may lead to catalysis in several ways: (1) intramolec-



I

ular nucleophilic catalysis involving the intramolecular displacement of methoxide by the alkoxide ion of I (presumably through the intermediacy of a tetrahedral addition compound); (2) intramolecular general basic catalysis in which the alkoxide ion of I removes a proton from an attacking water molecule; and (3) intramolecular general acidic–hydroxide ion catalysis in which the conjugate acid of I plus a hydroxide ion act to facilitate hydrolysis. We have previously suggested mechanism 1 as the most likely mechanism.[14] The most important argument for mechanism 1 is the analogous process for the morpholine-catalyzed hydrolysis described in this report. It is of course conceivable that the hydroxide ion reaction may take place by a mechanism entirely different from the morpholine reaction, but in the absence of any evidence to the contrary, the principle of scientific simplicity dictates that both processes be considered to be similar. Therefore it is suggested that eq. 9 is the mechanism of the alkaline hydrolysis of methyl *o*-formylbenzoate.



(9)

If mechanism 9 is correct, the rate constant for hydration of the aldehyde group cannot be slower than that of the ester hydrolysis. Unfortunately, the rate constant for the hydration of benzaldehyde is not known, but the hydroxide ion catalyzed hydration of acetaldehyde is $8 \times 10^4\ M^{-1}$ sec.$^{-1}$.[34] The hydration of benzaldehyde would be expected to be somewhat slower, leading to the prediction that the rate constant of hydration would be of the same order of magnitude as the rate constant observed for the ester hydrolysis. Thus the hydration may be the slow step of the reaction, or possibly the reaction may be concerted.

Mechanisms similar to this have been suggested before, applying both to compounds having *ortho*-aldehyde[30] and *ortho*-keto[13] groups. Furthermore, a similar process may be operative in the hydrolysis of a keto-substituted phosphate ester.[35]

An intermolecular analog of eq. 9 is the reaction of chlorate ion with *p*-nitrophenyl acetate.[36] The chlorate ion exhibits a nucleophilicity in this reaction in line with its basicity,[37] and thus the oxyanion I would be expected to do the same, with the added provision that the reaction is intramolecular rather than intermolecular.

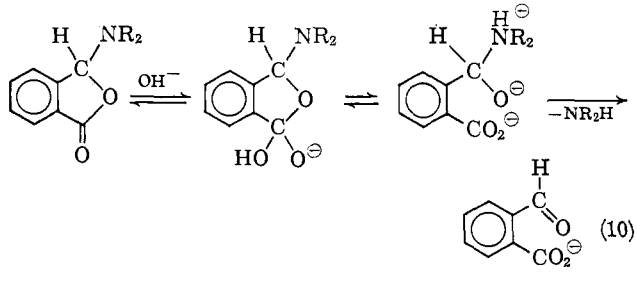
In contrast to the alkaline hydrolysis of methyl *o*-formylbenzoate, the acidic hydrolysis shows only a modest enhancement of rate. The comparison shown in Table VIII indicates that the observed rate constant for the *o*-formyl ester is about 200-fold faster than that of the unsubstituted ester. Since electronic effects in acid-catalyzed reactions are usually small and since the steric effect of one *ortho* substituent should decrease the rate to some extent, the acidic hydrolysis of the *o*-formyl ester shows an exceptional reactivity of somewhat greater than 200-fold. This kinetic result may reflect a mechanism analogous to eq. 9. However, hydronium ion catalysis of methyl *o*-formylbenzoate hydrolysis is 10^7 times slower than hydroxide ion catalysis, whereas hydronium ion catalysis of aldehyde hydration is only 10^2 times slower than hydroxide ion catalysis.[34] Therefore, the hydronium ion catalyzed hydrolysis of methyl *o*-formylbenzoate may be different from eq. 9.

The morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate is also an extremely facile reaction. Figure 4 indicates that with 0.085 *M* morpholine, the hydrolysis is essentially over in 10 sec. Comparison of this fast reaction with other systems is very difficult. Other hydrolytic reactions involving general bases or nucleophiles as catalysts are of course known. For example, imidazole catalyzes the hydrolysis of phenyl esters *via* nucleophilic catalysis, but methyl esters, such as methyl *o*-formylbenzoate, are not affected by nucleophilic catalysts.[5] Some alkyl esters, however, are hydrolyzed by imidazole and other bases *via* general basic catalysis. But known examples of this phenomenon are restricted to esters having activated acyl groups such as ethyl dichloroacetate and ethyl fluoroacetate.[38] Thus, it is not feasible to compare the rate of hydrolysis of the morpholine catalysis with any of the classical forms of catalysis of ester

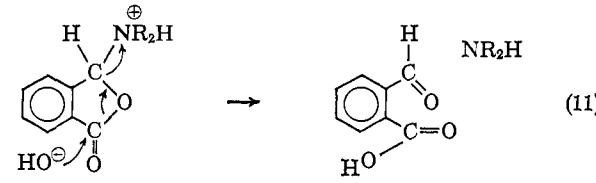
(31) Y. Shalitin and S. A. Bernhard, *J. Am. Chem. Soc.*, **86**, 2291 (1964).
(32) L. Holleck, G. A. Melkonian, and S. B. Rao, *Naturwiss.*, **45**, 438 (1958).
(33) See also G. Vavon and J. Scandel, *Compt. rend.*, **223**, 1144 (1946).
(34) R. P. Bell, M. H. Rand, and K. M. A. Wynne-Jones, *Trans. Faraday Soc.*, **52**, 1100 (1956).
(35) F. Ramirez, B. Hansen, and N. B. Desai, *J. Am. Chem. Soc.*, **84**, 4588 (1962).
(36) O. Gawron and F. Draus, *ibid.*, **80**, 5392 (1958).
(37) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).
(38) W. P. Jencks, and J. Carriuolo, *ibid.*, **83**, 1743 (1961).

hydrolysis, since the former catalysis is different in kind. Certainly there are nucleophiles other than morpholine which can catalyze the hydrolysis of *o*-formyl esters. One such example has been reported: 0.002 *M* potassium cyanide has been reported to accelerate the hydrolysis of cinnamoylsalicylaldehyde by tenfold.[31]

The intermediacy of 3-morpholinophthalide in the morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate and the pathway of the reaction has been amply demonstrated above. Questions still to be answered include the detailed mechanisms of the formation and decomposition of 3-morpholinophthalide. To that end, it is of interest to consider the kinetics and pH dependencies of the hydrolyses of 3-morpholinophthalide and 3-methoxyphthalide. Table IX presents a comparison of these reactions. The pH–rate constant profiles for the hydrolysis of 3-morpholinophthalide and of 3-methoxyphthalide above pH 4 are quite similar to one another in shape (Figure 3), although not in absolute magnitude. Above pH 4, each profile may be described in terms of a water reaction around neutrality and a hydroxide ion catalyzed reaction at high pH.[39] In both the water and hydroxide ion catalyzed reactions, 3-morpholinophthalide is much faster than is 3-methoxyphthalide. It is interesting that reactivities of nitrogen and oxygen members of this family are exactly the reverse of those found in the carboxylic acid derivative family. The differences in rate must be related to the fact that morpholine is about 10^9 better base than is methanol. The difference in rates between these compounds in both the water and hydroxide ion catalyses may be explained in several ways. The assumption will be made that the water and hydroxide ion reactions are mechanistically similar since the substituent effects on these reactions are similar. Two families of mechanisms are possible, involving either acyl or alkyl fission. Alkyl fission would lead to a protonated Schiff base–carboxylate ion ion pair. This reaction would not, however, be accelerated by hydroxide ion, and thus may be ruled out if the water and hydroxide ion reactions are parallel. Acyl fission would involve hydroxide ion or water attack at the carbonyl carbon atom. The hydroxide ion reaction is shown in eq. 10. The water reaction could be described as: (1) an intramolecular general basic catal-



ysis on water by the morpholino group; (2) the reaction of hydroxide ion with the protonated substrate; or (3) the reaction of a water molecule *per se*. Although it is not possible to distinguish between these alternatives at present, most facets of the facile water reaction of 3-morpholinophthalide can be explained in terms of eq. 11, which indicates a possible concerted process which leads to a transition state stabilized by the



resonance of the formyl group. This process could occur more readily with 3-morpholinophthalide than with 3-methoxyphthalide because of the greater basicity of the former compound. Unfortunately no means of measuring the protonated substrate described above exists at present. Alternatively, a rate-determining decomposition of the tetrahedral intermediate formed from the addition of water to the phthalide could account for the observed effect of structure on reactivity. This process would likewise occur more readily with 3-morpholinophthalide than with 3-methoxyphthalide because of the greater basicity of the tetrahedral intermediate of the former compound.

Table IX. The Hydrolysis of Some Substituted Phthalides[a]

Compd.	k_{OH^-}, M^{-1} sec.$^{-1}$	k_{H_2O}, sec.$^{-1}$
Phthalide[b]	0.255	
3-Methoxyphthalide	2.8	7×10^{-5}
3-Morpholinophthalide	4.5×10^4	9×10^{-2}
3-Hydroxyphthalide	$>10^8$[c]	

[a] At 25° in aqueous solution. [b] M. L. Bender, H. Matsui, R. J. Thomas, and S. W. Tobey, *J. Am. Chem. Soc.*, **83**, 4193 (1961). [c] Predicated on an assumed diffusion-controlled reaction.

The facile hydroxide ion and morpholine-catalyzed hydrolyses of methyl *o*-formylbenzoate around neutrality exhibit interesting formal analogies to the facile catalysis of ester hydrolysis by enzymes around neutrality. In both systems, the catalyst complexes with the substrate, in the former reactions by covalent bonding and in the latter by noncovalent bonding. In chymotrypsin the acyl group of the substrate becomes covalently attached to the enzyme during the catalytic process; in the catalyses described here, the acyl group of the substrate also is transferred to a new group during the catalytic process, although one cannot call that new group strictly the catalyst. In chymotrypsin, a hydroxyl group of the enzyme is the nucleophile to which the acyl group becomes attached; likewise in the present catalyses, a new acyl–oxygen bond is made. Thus these catalyses provide some small measure of insight into enzyme reactions.

Acknowledgment. The authors acknowledge valuable assistance with the morpholine kinetics by Dr. Ferenc J. Kézdy and with the analog computations by Dr. Robert E. Blakeley, and valuable discussions with Dr. D. C. Young.

(39) No explanation can be offered for pH behavior of 3-morpholinophthalide hydrolysis below pH 4.