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Intramolecular Participation of the Amide Group in the Hydrolysis of p-Nitrophenyl N-(bromoacetyl) Anthranilate

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The rate of hydrolysis of *p*-nitrophenyl *N*-(bromoacetyl) anthranilate (Ib) has been measured in aqueous solution between pH 1 and 6.5 and has been found to increase linearly with pH at pH higher than 3. An abnormally large apparent alkaline rate constant of 3.8×10^6 M^{-1} sec^{-1} has been determined. Intramolecular nucleophilic displacement by the amide group at the carbonyl carbon of the ester occurred and a cyclic intermediate was formed. This intermediate has been detected by direct isolation and by measurements of the proton release accompanying the reaction. The rates of hydrolysis of analogous derivatives (IIb–IIIb–IV), for which this intramolecular assistance was not possible, were slower by a factor of about 5×10^5. Such an example of intramolecular catalysis may be useful for a better understanding of the enzymatic catalysis.

INTRODUCTION

Amides are very efficient nucleophiles in intramolecular displacement reactions, and examples of intramolecular catalysis by amide groups involving attack by nitrogen or oxygen are numerous (*1–3*). Interest in this type of reaction follows from the presence of this chemical function in the primary structure of proteins, and it has been suggested that some peptide bonds present in enzyme or substrate molecules might be involved in the mechanism of action of proteolytic enzymes (*4*, *5*).

We want to report an example of intramolecular participation of the amide group in the hydrolysis of *p*-nitrophenyl *N*-(bromoacetyl) anthranilate (compound Ib). The rate of hydrolysis of the ester Ib has been compared to those of analogous derivatives in which the amide function is either *N*-methylated (IIb), or in para position with regard to the carbonyl carbon of the ester bond (IIIb), or is lacking (IV). It has been found that the hydrolysis of ester Ib is about 5×10^5 times faster than the hydrolysis of the other species. The efficiency of such a model was suggested by Bender, Schonbaum and Hamilton (*6*) some years ago and verified in the case of the acidic hydrolysis of *o*-benzamido-*N*,*N*-dicyclohexylbenzamide (*7*). Recently, intramolecular displacement by the ureido group at the carbonyl carbon of esters and amides of *o*-ureidobenzoic acid (compounds VII) has been investigated (*8*) and the mode of ureido participation (oxygen or nitrogen attack) has been shown to be dependent upon the nature of the leaving group.

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EXPERIMENTAL PROCEDURE

Materials

Sodium chloride, formic acid, triethylamine, pyridine, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Calbiochem.), 2-(*N*-morpholino) ethanesulfonic acid (Calbiochem.) were reagent grade and were used without further purification.

Dioxane, tetrahydrofuran, absolute ethanol were purified according to Fieser (*9*) and acetonitrile was purified by the method of Coetzee (*10*). *N,N*-Dimethylformamide was reagent grade (Merck) for spectroscopic use.

Preparation of Compounds I–VI (Fig. 1)

Melting points were determined on a Büchi apparatus and were uncorrected. Yields were currently 30–50%, except in some cases as it is indicated, and their improvement

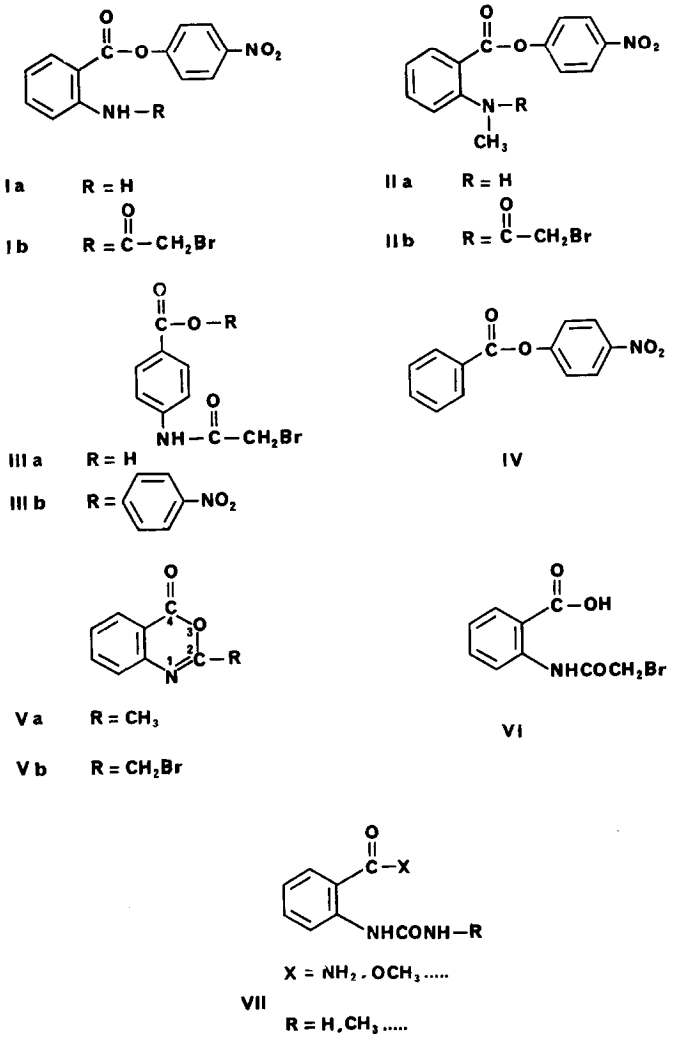


FIG. 1. Structure of compounds.

was not searched for. Elemental analyses were performed by the "Laboratoire de Microanalyse," Gif-sur-Yvette, 91, France.

p-Nitrophenyl anthranilate (Ia) was prepared following the procedure of Haugland and Stryer (*11*). It had mp 123–124°C [lit. (*11*) 127–128°C]. The infrared absorption spectrum (in $CHCl_3$) showed strong absorptions at 3510, 3390, 1710, 1620 cm^{-1}; the ultraviolet spectrum (in dioxane) showed λ_{max} at 256 and 350 nm (shoulder at 270 nm).

p-Nitrophenyl N-(bromoacetyl) anthranilate (Ib) was prepared by the dicyclohexylcarbodiimide method (*12*). The *p*-nitrophenyl ester of anthranilic acid (Ia) (6 mmoles) was dissolved in a minimal volume of tetrahydrofuran with 6 mmoles of bromoacetic acid. Dicyclohexylcarbodiimide (6.6 mmoles) dissolved in the same solvent was then added dropwise to the stirred solution at 0°C; after 30 min the mixture was allowed to come to room temperature and was held there for 5 hr. The insoluble *N,N'*-dicyclohexylurea was filtered off and washed with tetrahydrofuran. The filtrate was evaporated *in vacuo* and the crystalline residue was redissolved in tetrahydrofuran. Previously uneliminated dicyclohexylurea precipitated again and was filtered off. After evaporation of the solvent, the residue was dissolved in a mixture of tetrahydrofuran–ethanol and was recrystallized several times in the cold. The product (1.25 g of pale yellow crystals; 55% yield) had mp 156–160°C (decomp.). The infrared spectra (in $CHCl_3$) absorption maxima were at 3280, 1720–1695, 1590 cm^{-1}; the ultraviolet spectrum (in dioxane) showed λ_{max} at 262.5 and 315 nm.

Anal. Calcd for $C_{15}H_{11}N_2O_5Br$: C, 47.51; H, 2.92; N, 7.39; Br, 21.07. Found: C, 47.53; H, 2.91; N, 7.48; Br, 21.08.

p-Nitrophenyl N-(methyl) anthranilate (IIa) was synthesized according to the method of Staiger and Miller (*13*), with *N*-methyl isatoic anhydride (Schuchardt), and under conditions similar to those described for the preparation of *p*-nitrophenyl anthranilate (*11*).

The product (yellow needles) after recrystallization from absolute ethanol had mp 104–106.5°C. The infrared spectrum (in $CHCl_3$) showed absorption maxima at 3400, 1700, 1580 cm^{-1}; the ultraviolet spectrum (in dioxane) showed λ_{max} at 259 and 367.5 nm (shoulder at 275 nm).

Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.79; H, 4.47; N, 10.48.

p-Nitrophenyl N-(methyl), N-(bromoacetyl) anthranilate (IIb) was synthesized by the dicyclohexylcarbodiimide method (*12*) as described for the preparation of compound Ib. The coupling of *p*-nitrophenyl *N*-(methyl) anthranilate (IIa) with bromoacetic acid in presence of dicyclohexylcarbodiimide occurred with a low yield (<10%). After several recrystallizations from ethanol, pale yellow crystals (mp 129–131°C with decomposition) were obtained. The infrared spectrum (in $CHCl_3$) showed absorption maxima at 1745 and 1665 cm^{-1}; the ultraviolet spectrum (in dioxane) showed λ_{max} at 268 nm.

Anal. Calcd for $C_{16}H_{13}N_2O_5Br$: C, 48.87; H, 3.33; N, 7.12; Br, 20.32. Found: C, 49.00; H, 3.32; N, 7.22; Br, 20.30.

p-(N-Bromoacetyl-) aminobenzoic acid (IIIa) was prepared by action of bromoacetyl bromide upon *p*-aminobenzoic acid in aqueous solution in presence of sodium hydroxide and bromothymol blue, following the procedure of Steiger (*14*). After decoloration of the solution with Norit and addition of ethanol (to prevent the precipitation of the

sodium salt), hydrochloric acid was added to the mixture and the product precipitated after cooling. It had mp 218–220°C (decomp.). The ultraviolet spectrum (in dioxane) showed λ_{max} at 275 nm.

p-Nitrophenyl ester of p-(N-bromoacetyl-) aminobenzoic acid (IIIb) was prepared by the dicyclohexylcarbodiimide method with a very low yield. The acid IIIa (4 mmoles) was dissolved in a minimal volume of dimethylformamide with 4 mmoles of *p*-nitrophenol. Dicyclohexylcarbodiimide (4.4 mmoles) dissolved in the same solvent was then added dropwise to the stirred solution at 0°C; after 30 min the mixture was allowed to come to room temperature and was held there for 24 hr. The insoluble *N,N'*-dicyclohexylurea was filtered off and washed with dimethylformamide. The solvent was evaporated under reduced pressure and the solid residue was dissolved in ethyl acetate. It was then washed with 5% Na_2CO_3 and water, and dried over anhydrous $MgSO_4$. After evaporation of the solvent *in vacuo*, the compound was recrystallized from ethanol and then from acetone–water mixture. Some dozen milligrams of a partially pure product were obtained. It had mp 160.5–164.5°C (decomp.) and about 90% purity based on the amount of *p*-nitrophenol released on complete alkaline hydrolysis of a known weight of ester (spectrophotometric measurement at 400 nm). The ultraviolet spectrum (in dioxane) showed λ_{max} at 290 nm.

Anal. Calcd for $C_{15}H_{11}N_2O_5Br$: C, 47.51; H, 2.92; N, 7.39; Br, 21.08. Found: C, 48.52; H, 3.29; N, 7.35; Br, 19.92.

p-Nitrophenyl benzoate (IV) was obtained by coupling in tetrahydrofuran, benzoic acid and *p*-nitrophenol in presence of dicyclohexylcarbodiimide (*12*). After recrystallization from ethanol, it had mp 143–144°C [lit. (*15*) 142–143°C].

2-Methyl-3,1-benzoxazin-4-one (Va) was synthesized according to the procedure of Zentmyer and Wagner (*16*) and had mp 77–78.5°C [lit. (*16*) 80–81°C; (*17*) 77–78°C]. The infrared spectrum (in $CHCl_3$) showed strong absorptions at 1750, 1645, 1610 cm^{-1}; the ultraviolet absorption spectrum (in dioxane) showed λ_{max} at 304 and 248 nm.

2-Bromomethyl-3,1-benzoxazin-4-one (Vb) was assumed to be transiently formed during the hydrolysis of *p*-nitrophenyl *N*-(bromoacetyl) anthranilate (*vide infra*) and was isolated following a procedure analogous to that one used by Bernhard et al. (*4*) in the preparation of the benzyloxycarbonyl-L-aminosuccinimide. Compound Ib (150 mg) was dissolved in a large enough volume of tetrahydrofuran and aqueous pH 7 buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid–NaOH) was added dropwise to the stirred solution at room temperature. The reaction medium became rapidly yellow. Cold water was then added and a precipitate formed. The precipitate was dissolved in ethanol and crystallized from it after cooling. It had mp 112–114°C. The ultraviolet spectrum (in dioxane) showed λ_{max} at 304 and 265 nm; the infrared spectrum (in $CHCl_3$) showed absorptions at 1760 cm^{-1} (O–C=O group), 1640 cm^{-1} (C=N group) and 1610 cm^{-1}. These spectra were comparable to those of the analogous derivative Va.

Anal. Calcd for $C_9H_6NO_2Br$: C, 45.03; H, 2.52: N, 5.83; Br, 33.29. Found: C, 45.35; H, 2.57; N, 6.13; Br, 32.96.

This compound was also prepared from *N*-bromoacetyl-anthranilic acid (VI) by the method of Zentmyer and Wagner (*16*) with a good yield (50%). The product, after recrystallization from ethyl acetate–hexane, had the same physical properties as those reported above.

Anal. Calcd for $C_9H_6NO_2Br$: C, 45.03; H, 2.52; N, 5.83; Br, 33.29. Found: C, 45.29; H, 2.77; N, 5.95; Br, 33.32.

N-Bromoacetyl-anthranilic acid (VI) was synthesized following the procedure of Uskokovic and Wenner (*18*). After recrystallization from ethanol, it had mp 168–171°C [lit. (*18*) 165.5–172°C). The ultraviolet spectrum (in dioxane) showed λ_{max} at 309 and 254 nm.

Methods

Ultraviolet absorption spectra were measured using a Cary Model 14 recording spectrophotometer, and infrared spectra, with a Perkin-Elmer Model 237 spectrophotometer.

Kinetic measurements were carried out at 25°C in aqueous solutions (0.5 *M* NaCl) containing 1 to 4.6% (v/v) dioxane (or indifferently acetonitrile, in some experiments). Employed buffers (at the concentration 0.025 *M*) were formic acid–NaOH between pH 3 and 4.5; 2-(*N*-morpholino) ethanesulfonic acid–NaOH between pH 4.5 and 6.5; triethylamine–HCl between pH 9.5 and 11. Pyridine–HCl buffer (pH 5.32) was also used at different concentrations.

The rates of hydrolysis of *p*-nitrophenyl esters were determined with a Cary Model 16 spectrophotometer equipped with a thermostatically controlled cell compartment, and coupled to a Sefram graphispot enregistror. For the hydrolysis of the ester Ib, the release of *p*-nitrophenol was followed at 347.5 nm (isosbestic wavelength) where the molar absorption change was found equal to 4700 under our experimental conditions. The cyclic intermediate Vb or the hydrolysis product VI did not absorb at this wavelength. For the hydrolysis of the other studied *p*-nitrophenyl esters, the release of *p*-nitrophenol (at pH >9) was followed at 400 nm, where the molar extinction coefficient of *p*-nitrophenolate ion was 18 300. Before and after spectrophotometric measurements, pH of the buffered solutions was controlled with a Tacussel pH meter type TS 60 N. The pseudo-first-order rate constants were calculated from the slopes of plots of log $[(OD_\infty - OD_0)/(OD_\infty - OD_t)]$ versus time *t*.

In potentiometric measurements of the hydrolysis of Ib or Vb, the proton release was followed as a function of time with a Tacussel model EPL 1 linear potentiometric recorder coupled with a Vibron pH meter Model 33 B (EIL). The pH change was followed on 0.05–0.1 unit and now and then small known volumes of 0.05 *N* NaOH were added with an Agla microsyringe to keep constant the pH of the unbuffered solution (under nitrogen) in this limit of 0.05–0.1 unit. The number of released protons could then be calculated from the quantity of 0.05 *N* NaOH necessary to carry the medium pH at its initial value.

A specific bromide ion electrode (Beckman Instruments Co.), in conjunction with a Beckman pH meter was also used to control if bromide ion was released during the alkaline hydrolysis of the compound Vb.

RESULTS AND DISCUSSION

Hydrolysis of p-Nitrophenyl N-(bromoacetyl) Anthranilate (Ib)

The rate of hydrolysis of *p*-nitrophenyl ester Ib was measured spectrophotometrically at 25°C in aqueous solution between pH 1 and 6.3 (Fig. 2) and was found to obey

the expression:

$$v = k_{obs} \cdot S = (k_0 + k_{OH^-} OH^-) S,$$

where S is the substrate concentration, k_{OH^-} is the second-order rate constant for specific-base catalyzed hydrolysis, and k_0 is the first-order rate constant for spontaneous hydrolysis. Based on the release of *p*-nitrophenol, the ester has an abnormally high apparent alkaline rate constant k_{OH^-} of 3.8 10^6 M^{-1} sec^{-1}. Spontaneous hydrolysis in water was detected with difficulty ($k_0 \neq 6.3 \times 10^{-6}$ sec^{-1}) and no buffer catalytic effect was observed for 2-(*N*-morpholino) ethanesulfonate or pyridine buffer at different concentrations between 2.5 m*M* and 0.25 *M* at pH 5.3.

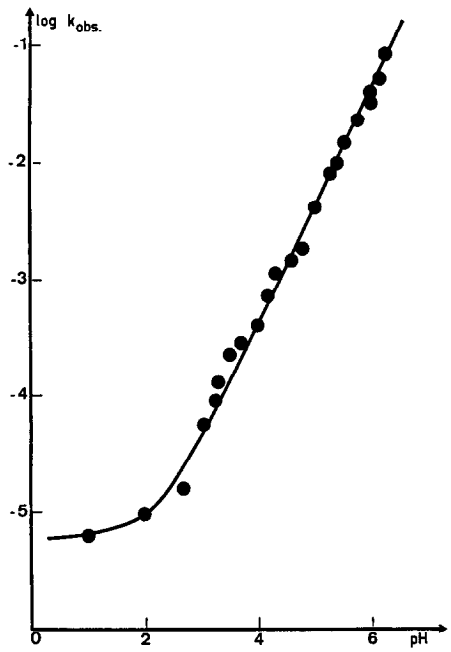


FIG. 2. pH-rate profile for the hydrolysis of *p*-nitrophenyl *N*-(bromoacetyl) anthranilate (Ib), as determined by release of *p*-nitrophenol. Experimental conditions: temp, 25°C; 0.5 *M* NaCl; 4.6% (v/v) dioxane in reaction mixtures. The initial concentration of substrate was 1.1×10^{-5} or 2.25×10^{-5} *M*. Units of k_{obs}, sec^{-1}.

The high value of the alkaline rate constant suggested that the hydrolysis of Ib might be facilitated by neighbouring amide group participation, and a cyclic intermediate might be formed during the hydrolysis reaction (Scheme I). When the hydrolysis of Ib was studied potentiometrically above pH 8.0, a sharp immediate pH change corresponding to the appearance of about one proton by mole of substrate was followed by a first-order release of protons (Fig. 3). At the end of the reaction, two protons per mole of substrate had been released, and it was verified with a specific bromide ion electrode that there was no appearance of bromide ions in the medium.[2] These two protons must

[2] In this experiment, the medium contained no interfering chloride ion.

then arise from the dissociation of the hydrolysis products, *p*-nitrophenol ($pK_a \neq 7$) and *N*-(bromoacetyl) anthranilic acid (VI) which do not appear simultaneously in the

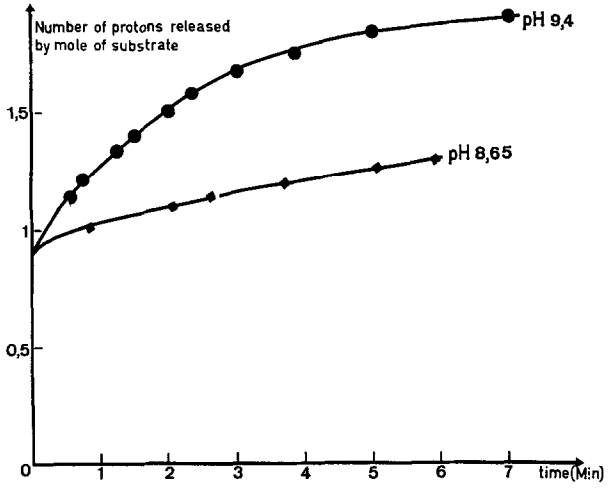


FIG. 3. Proton release accompanying the hydrolysis of *p*-nitrophenyl *N*-(bromoacetyl) anthranilate (Ib) as a function of time. Experimental conditions: temp, 25°C; 0.5 *M* NaCl; 1 % (v/v) dioxane; initial substrate concentration, 10^{-4} *M*. The medium was unbuffered; (■) pH 8.65; (●) pH 9.4.

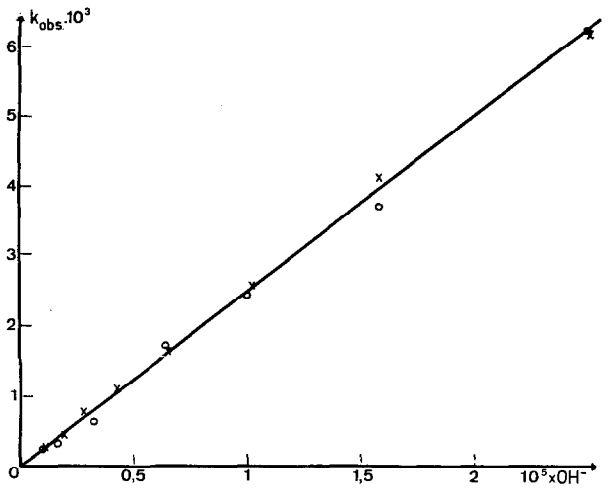
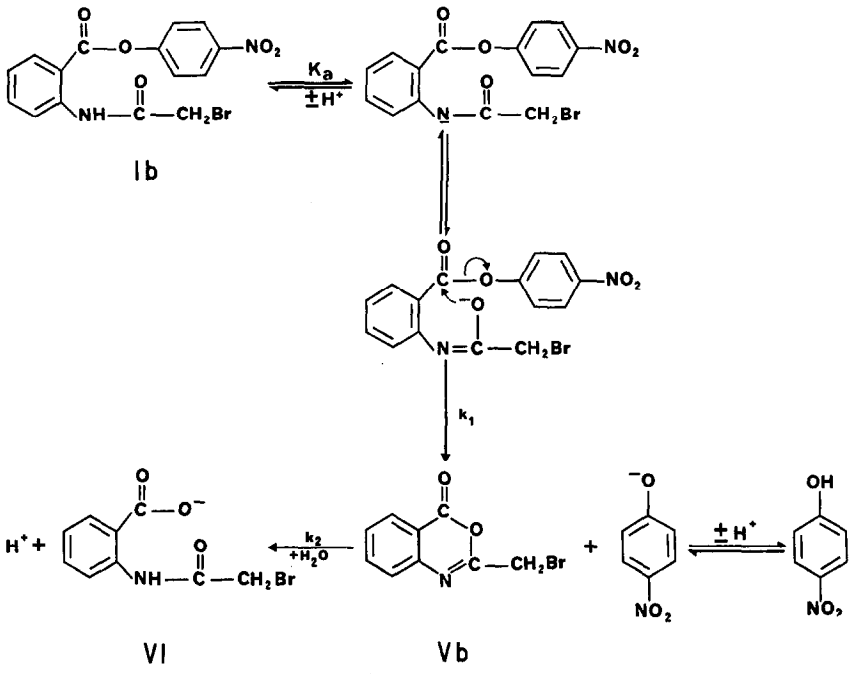


FIG. 4. First-order rate constant for the hydrolysis of 2-bromomethyl-3,1-benzoxazin-4-one (Vb) plotted against hydroxide ion concentration. Experimental conditions: temp, 25°C; 0.5 *M* NaCl; 1 % (v/v) dioxane. Units of k_{obs}, sec^{-1}. (○) non-isolated 2-bromomethyl-3,1-benzoxazin-4-one; (×) isolated 2-bromomethyl-3,1-benzoxazin-4-one.

solution. The rate of the time-dependent release of protons increased linearly with hydroxide ion concentration between pH 8.0 and 9.6 (Fig. 4) and a second-order rate constant of 250 M^{-1} sec^{-1} at 25°C was determined.

As *p*-nitrophenol and *N*-(bromoacetyl) anthranilic acid are liberated with very different rates at neutral or alkaline pH,[3] it was possible to isolate a reaction intermediate after incubation of the ester Ib, at neutral pH as described in the Experimental section. The intermediate was identified as 2-bromomethyl-3,1-benzoxazin-4-one (Vb): its infrared and ultraviolet absorption spectra were comparable with the spectra of the analogous derivative Va and it had the same physical properties as the benzoxazone prepared from *N*-bromoacetylanthranilic acid (VI) by the method of Zentmyer and



SCHEME 1

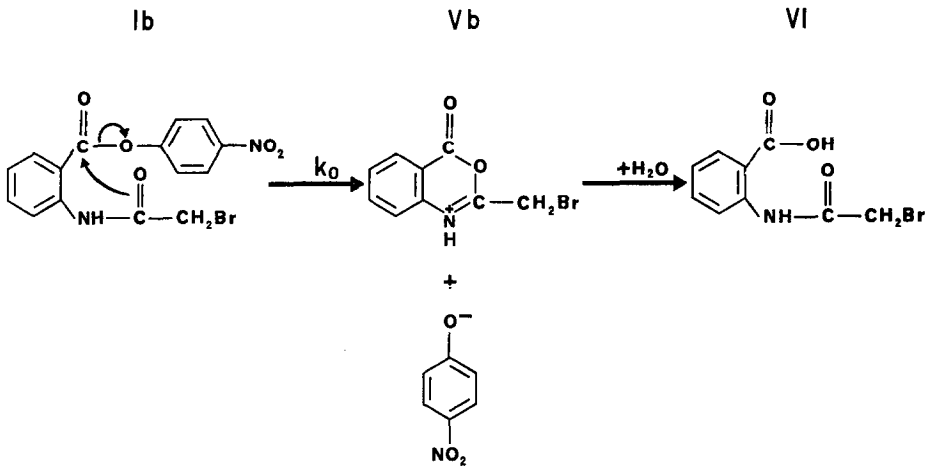
Wagner (*16*). This isolated compound was hydrolyzed at 25°C between pH 8.0 and 9.6 (Fig. 4) with a second-order rate constant of 250 M^{-1} sec^{-1} identical to the constant measured in the alkaline hydrolysis of the non-isolated intermediate. The spectrum of the hydrolysis product at alkaline pH was identical to that one of *N*-bromoacetylanthranilic acid (VI).

All these results are consistent with the following mechanism (Scheme 1) of amide group participation in the hydrolysis of *p*-nitrophenyl *N*-(bromoacetyl) anthranilate (Ib). The neutral amide group is in equilibrium with its ionized form (this last one being in very low concentration in the studied pH range) and the ionized oxygen attacks then the carbonyl carbon of the ester bond. A six-member ring intermediate, the 2-

[3] This is not the case in the acidic pH range where it has been shown benzoxazones are very susceptible to acid catalyzed hydrolysis (17). Thus 2-bromomethyl-3,1-benzoxazin-4-one (Vb) is hydrolyzed between pH 3 and 5 with a second-order rate constant $k_{H_3O^+}$ equal to 3.7 M^{-1} sec^{-1}; that rate constant was measured by following the time-dependent decrease in absorbance of the benzoxazone at 315 nm.

bromomethyl-3,1-benzoxazin-4-one (Vb) is formed. Subsequently at alkaline pH, this intermediate is hydrolyzed with a second-order rate constant k_2 equal to $250 M^{-1}$ sec^{-1}. Following this mechanism, the apparent alkaline rate constant k_{OH^-} for the release of *p*-nitrophenol is equal to $k_1 K_a / K_w$ where k_1 is the first-order rate constant for the intramolecular attack of the ionized amide function, K_a is the dissociation constant of this amide group, K_w is the dissociation constant of water. The respective values of k_1 and K_a cannot be separated due to the limited investigated pH range and the high hydrolysis rate of Ib.

Only a very low spontaneous hydrolysis of the ester Ib in water is detected at pH below 3 ($k_0 \# 6.3 \times 10^{-6}$ sec^{-1}). It is likely due to an intramolecular nucleophilic displacement by the neutral amide group (Scheme 2) rather than to a direct nucleo-



SCHEME 2

philic attack by a water molecule on the carbonyl carbon of the ester bond. No spontaneous hydrolysis of the analogous derivative IIb is indeed detectable in similar experimental conditions ($k_0 < 10^{-6}$ sec^{-1}).

Hegarty and Bruice (*8*) have recently shown that, in the hydrolysis of *p*-nitrophenol 2-ureidobenzoate (compound VII where R = H, X = $OC_6H_4p \cdot NO_2$), oxygen attack by the ionized and un-ionized ureido group occurs. The second-order rate constant for the hydroxide ion-catalyzed hydrolysis is equal to 6.92×10^4 M^{-1} sec^{-1} and is therefore smaller than the one determined for the hydrolysis of Ib ($k_{OH^-} = 3.8 \times 10^6$ M^{-1} sec^{-1}), due to a difference in the acidities of the attacking amide group –NHCOR′ (with R′ = CH_2Br or NH_2). Indeed the bromomethyl substituent in Ib is strongly electron-withdrawing and lowers the ionization pK_a of the amide bond; the increase in the concentration of the ionized nucleophile then compensates largely the reduction of its nucleophilic reactivity. On the other hand, when R is a strong electron-releasing substituent as in *p*-nitrophenyl 2-ureidobenzoate, the attack by the neutral oxygen upon the carbonyl carbon of the ester bond occurs with a high efficiency. The first-order rate constant for spontaneous cyclization of *p*-nitrophenyl 2-ureidobenzoate is 0.024

sec^{-1} and is many times larger than the constant k_0 found for the spontaneous hydrolysis of Ib ($k_0 \# 6.3 \times 10^{-6}$ sec^{-1}). In the absence of added base, electron donating substituents increase the rate of cyclization probably by increasing the negative charge density on the attacking oxygen atom (*3*).

Evaluation of the Efficiency of the Amide Group as Intramolecular Nucleophile

The rate constants for the alkaline hydrolysis of analogous derivatives of Ib in which the amide group is either *N*-methylated (compound IIb), or located in para position with regard to the ester bond (compound IIIb), or lacking (compound IV), are considerably smaller by a factor of $2.5–7.5 \times 10^5$ (Table 1). The electronic effects of the

TABLE 1

KINETIC PARAMETERS FOR THE HYDROXIDE-ION-CATALYZED HYDROLYSIS OF *p*-NITROPHENYL ESTERS[a]

Compounds	pH range	k_{OH^-} (M^{-1} sec^{-1})	Relative rates
Ib	1–6.3	3.8×10^6	7.45×10^5
IIb	9.8–10.8	5.9	1.15
IIIb	9.5–10.5	14	2.8
IV	9.8–10.8	5.1	1

[a] Experimental conditions: temp, 25°C; 0.5 *M* NaCl; 4.6% (v/v) dioxane; the initial substrate concentration was equal to 10^{-5} or 2×10^{-5} *M*.

amide substituent to the reaction center are similar in the esters Ib, IIb, and IIIb. Furthermore, the steric hindrance of the bromoacetamide substituent in Ib is comparable, although a little smaller, to the one of *N*-methyl-bromoacetamide substituent in IIb. Then, the rate acceleration caused by the approximation of the amide group to the ester in Ib may be estimated to a mean value of 4.5×10^5. Similar rate accelerations have often been observed in the hydrolysis of carboxylic derivatives having a neighbouring amide group (4, 7, 8, 19, 20) and many authors have emphasized that amide groups are among the most effective intramolecular nucleophiles. The example reported here corroborates this view.

ACKNOWLEDGMENTS

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