[CONTRIBUTION FROM THE POLYMER RESEARCH INSTITUTE, POLYTECHNIC INSTITUTE OF BROOKLYN]

Reaction Rates of Polyelectrolyte Derivatives. II. Effect of Neighboring Carboxyl on the Reactivity of *p*-Nitroanilide Groups[1]

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The hydrolysis rate of an acrylic acid copolymer with 3 mole % of *p*-nitroacrylanilide between *p*H 2 and 8 indicates that the reaction rate is governed by the attack of un-ionized neighboring carboxyl groups on the nitroanilide. Kinetic data in strong base solution were obtained for the four copolymers of *p*-nitroacrylanilide or *p*-nitromethacrylanilide with acrylic acid or methacrylic acid. The rates were two to four orders of magnitude slower than those observed with nitroanilides of trimethylacetic, glutaric or succinic acids, with the copolymers containing one acrylic and one methacrylic component particularly resistant to hydrolysis. The results were interpreted in terms of nitroanilide stabilization with neighboring carboxylate groups and this assumption was confirmed by the pronounced catalytic effect of barium ion which forms complexes with the stabilizing carboxylates. The nitroanilides formed by condensing *p*-nitroaniline with a methacrylic acid–maleic anhydride copolymer are not hydrolyzed in acid or neutral solution, but split off water to form the imide. The rate of imidization is a maximum around *p*H 5 indicating that one ionized and one un-ionized neighboring carboxyl are required. The analogy of the observed effects of intramolecular catalysis and the activation of enzyme–substrate association complexes is discussed.

Introduction

Whenever a polymeric acid carries a reactive substituent whose reaction rate is governed by its interaction with hydroxyl ion, the large electrostatic potential in the neighborhood of the polyion will strongly repress the base-catalyzed reaction.[3,4] This effect is analogous to the relative stability of monoesters[5,6] or monoamides[6] of dicarboxylic acids in basic solution. However, a previous investigation of the solvolysis of a polycarboxylic acid carrying a small number of *p*-nitrophenyl ester groups has shown that the reaction rate does not depend on interactions with hydroxyl ions but is controlled by the attack of a neighboring ionized carboxyl on the ester group. As a consequence, the reactivity of the ester groups attached to the polymer was not inhibited at *p*H 5–6 as might have been expected on electrostatic grounds, but was on the contrary 6 orders of magnitude above the reactivity of a similar monocarboxylic acid ester.[7]

The present study was undertaken to find whether similar neighboring group effects are operative with other hydrolyzable groups. The *p*-nitroanilide group was chosen because of the ease with which the reaction can be followed by spectroscopy.

Experimental

Monomers.—Glacial acrylic acid (Goodrich) and glacial methacrylic acid (Rohm and Haas) were distilled at reduced pressure through copper-packed columns and stored frozen at 3°. Reagent grade maleic anhydride was twice recrystallized from chloroform. The *p*-nitroanilides of acrylic and methacrylic acid were prepared by adding dropwise acrylyl chloride or methacrylyl chloride (Monomer–Polymer Co.) in cold ether solution to a solution of *p*-nitroaniline (Fisher, highest purity, recrystallized from aqueous ethanol) in ether and pyridine at −10°. The solution was allowed to warm to room temperature and was washed with sodium carbonate solution and water. The ether was evaporated and the residue recrystallized from aqueous ethanol.

Other Reagents.—*p*-Nitro-α-trimethylacetanilide (NTA) was made by refluxing for 20 min. trimethylacetic acid with a slight excess of sulfuryl chloride, cooling and adding slowly with stirring to a chilled solution of *p*-nitroaniline in ether and pyridine. The product was isolated as described above for the *p*-nitroanilide of acrylic acid, recrystallizing until colorless crystals were obtained. Mono-*p*-nitroanilides of succinic and glutaric acid (SNA and GNA) were made by refluxing for 10 min. equimolar amounts of the acid anhydrides and *p*-nitroaniline in dioxane. The solution was poured into 0.01 *N* HCl, the anilide filtered off and recrystallized from butanol. Titration with base gave equivalent weights of 238 (calcd. 238) and 247 (calcd. 252). *N*-*p*-Nitrophenylsuccinimide was prepared by grinding together equimolar quantities of *p*-nitroaniline, succinic anhydride and potassium acid sulfate and fusing the mixture until the melt became gray and thick. The cooled mass was pulverized and extracted with water; the residue was recrystallized from glacial acetic acid; m.p. 211–212° (lit.[8] 210°). The poly-(acrylic acid) used was obtained from Dr. A. M. Kotliar and its preparation has been described previously.[9]

Copolymer Preparation.—Copolymers of acrylic acid–*p*-nitroacrylanilide, (A/An) acrylic acid–*p*-nitromethacrylanilide, methacrylic acid–*p*-nitroacrylanilide and methacrylic acid–*p*-nitromethacrylanilide were all prepared under similar conditions. A solution containing about 0.1 mole of the mixed monomers with 3 mole % of the anilide and 4 mg. of azo-bis-isobutyronitrile (AIN) in 50 ml. of butanone was carefully degassed and heated for 1 hr. to 61°, resulting in 10–17% conversions to copolymer. The copolymers were precipitated into ether, dissolved in water, dialyzed and freeze dried. Spectroscopic examination showed the copolymers to have the same composition as the mixed monomers. Titration in 0.5 *N* sodium chloride gave 96–98% of the theoretical carboxyl content and since the copolymers were highly hygroscopic it could be assumed that there had been no decarboxylation during the polymerization. Copolymer A/An had $[\eta] = 1.30$ in 2 *N* sodium hydroxide, corresponding to a molecular weight of about 340,000.[10]

Another acidic copolymer carrying anilide groups was made by modification of a resin prepared by heating for 36 hr. at 58° 0.2 mole of maleic anhydride and 0.1 mole of methacrylic acid in 100 ml. of freshly distilled dioxane containing 10 mg. of AIN (yield 4.5 g.). A solution of 4 g. of this copolymer and 9 g. of *p*-nitroaniline in 150 ml. of dioxane was shaken for three days at room temperature, the modified copolymer M/Mal–An was precipitated three times into ether and after careful washing (to remove unreacted nitroaniline) it was vacuum dried for 10 hr. at 65°. Spectroscopic analysis gave a nitroanilide content of 0.22 millimole per gram.

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[1] Financial support of this research by grants of the Eli Lilly Co. and the Monsanto Chemical Co. are gratefully acknowledged.

[2] Abstracted from a Ph.D. thesis submitted by E. W. Westhead, Jr., to the graduate school of the Polytechnic Institute of Brooklyn, June, 1956.

[3] H. Deuel, K. Hutschneker and J. Solms, *Z. Elektrochem.*, **57**, 172 (1953).

[4] A. Katchalsky and J. Feitelson, *J. Polymer Sci.*, **13**, 385 (1954).

[5] C. K. Ingold, *J. Chem. Soc.*, 1375 (1930).

[6] F. H. Westheimer and M. W. Shookhoff, THIS JOURNAL, **62**, 269 (1940).

[7] H. Morawetz and P. E. Zimmering, *J. Phys. Chem.*, **58**, 753 (1954).

[8] R. Meyer and J. Maier, *Ann.*, **327**, 49 (1903).

[9] A. M. Kotliar and H. Morawetz, THIS JOURNAL, **77**, 3692 (1955).

[10] P. M. Doty and A. Oth, *J. Phys. Chem.*, **56**, 43 (1952).

Standardization of Barium Hydroxide.—Solutions of reagent grade barium hydroxide dissolved in distilled water which had been boiled and cooled under nitrogen were centrifuged to remove any precipitated barium carbonate and the barium was determined gravimetrically as barium sulfate.

Buffers.—All buffers used had a sodium ion concentration of 0.1 *N* rather than a constant ionic strength, since a constant counter-ion concentration was believed to be more significant in solutions of polymeric acids. They were prepared by titrating stock solutions of reagent grade sodium acetate or disodium hydrogen phosphate with hydrochloric acid to the desired *p*H. Acetate buffers were used for *p*H 3.9–5.7, phosphate buffers for *p*H 6.0–7.9. All *p*H values were determined at 24° on a Cambridge Research model *p*H meter. The *p*H values at the reaction temperature of 135.4° were estimated from the formula given by Harned and Owen[11] to be higher by 0.45 for acetate and by 0.41 for phosphate buffers. At 84.4° the applied correction was 0.14 for acetate and 0.05 for phosphate buffers.

Kinetic Runs.—All analyses were carried out spectroscopically using a Beckman model DU spectrophotometer with the cell compartment thermostated at 20°. Millimolar extinction coefficients (ϵ) used in the calculations were for *p*-nitroaniline (*p*H 5–14) ϵ_{375} 13.0, ϵ_{380} 13.3; *p*-nitrophenolate ϵ_{375} 13.2; *p*-nitroanilide ϵ_{315} 11.7, ϵ_{375} 1.60, ϵ_{380} 1.17; N-*p*-nitrophenylimide (in 5% aqueous ethanol acidified with HCl) ϵ_{270} 9.50. All of the investigated reactions followed first-order kinetics.

The rate of nitroanilide hydrolysis in buffer solutions was studied at 135.40 ± 0.02°. Buffered samples were heated in Pyrex tubes sealed after careful removal of oxygen by repeated freezing, evacuating and thawing under nitrogen. For the HCl solutions it was found to be sufficient to flush the tubes with nitrogen before sealing, avoiding the danger of a change in the acid concentration. For each kinetic point a tube was cooled, opened and after mixing an aliquot with an equal volume of 0.4 *N* NaOH the optical density D was read at 380 mμ. The initial nitroanilide concentration was found from the optical density at 315 mμ and this value was used to calculate D_∞, the optical density at 380 mμ corresponding to complete hydrolysis of the nitroanilide. The first-order rate constant (sec.$^{-1}$) was found from the slope of a plot of ln $(D_\infty - D)$ against time.

The nitroanilide hydrolysis in sodium hydroxide solutions was studied at 84.4°. Samples were contained in Teflon tubes threaded to match stainless steel caps. The caps were fitted with Teflon gaskets providing a very effective seal. No change in concentration was detected in 0.1 *N* acetic acid heated for 24 hr. in such a tube immersed in the bath at 84.4° and no absorption in the ultraviolet developed on heating 1 *N* sodium hydroxide for a similar period. The nitroaniline liberated from nitroanilides reacted with hydroxyl ion to yield *p*-nitrophenolate (second-order rate constant 3 × 10^{-5} liters-mole^{-1} sec.$^{-1}$) and the progress of the reaction was, therefore, determined by optical density readings at 375 mμ, where *p*-nitroaniline and *p*-nitrophenolate had the same extinction coefficients. In strongly basic solution the extinction coefficients were different for each nitroanilide and depended on hydroxyl ion concentration, so that the $\epsilon_{315}/\epsilon_{375}$ ratio had to be determined for each run. For each kinetic point the reaction tube was removed from the bath, opened and a 3-ml. aliquot withdrawn. This procedure was sufficiently rapid to be without appreciable effect on the reaction rate of the solution remaining in the tube.

The imidization of copolymer M/Mal-An was studied at 84.4° in buffer and HCl solutions. The progress of the reaction was followed by optical density readings at 270 mμ.

Results and Discussion

Hydrolysis Rates of *p*-Nitroanilides in Acid and Buffer Solutions.—The *p*H dependence of the hydrolysis rate at 135.4° of the acrylic acid–*p*-nitroacrylanilide copolymer (A/An) differs in a striking manner from the behavior of the low molecular weight analogs TNA and GNA (Fig. 1). The reaction rate of A/An first falls with rising *p*H, then levels off to a constant value between *p*H 2 and 6 and continues to decrease in more basic media. This behavior suggests that hydrogen ion is the effective catalyst only in the most acid solutions and that above *p*H 2 the reaction rate is controlled by a second catalytic species, whose concentration approaches a maximum below *p*H 6. It is believed that this catalysis is due to un-ionized carboxyl groups adjoining the nitroanilide on the macromolecular chain. This behavior would be analogous to the catalysis of ester hydrolysis by *ionized* carboxyl neighbors reported previously[7,12,13] for acrylic acid–*p*-nitrophenyl methacrylate copolymers. However, the effect of neighboring carboxylate on ester hydrolysis also had been observed with the monoester of glutaric acid[12,13] while both GNA and TNA behave in the "normal" manner accounted for by hydrogen ion and hydroxyl ion catalysis. This could indicate the involvement of both neighboring carboxyl groups in the nitroanilide hydrolysis of A/An and a series of runs was carried out in partially neutralized 0.01 *N* poly-(acrylic acid) to obtain information on the manner in which the reaction rate depends on the degree of ionization, α, of the carboxyl groups. The results are reproduced in Fig. 2 and they seem to exclude the possibility of a coöperative effect of two un-ionized or of one ionized and one un-ionized carboxyl. The rates are also not proportional to $(1 - \alpha)$ as they would be if neighboring un-ionized carboxyls acted independently of each other. The data are fitted more closely by assuming a rate proportional to $(1 - \alpha^2)$. It should be pointed out, however, that the probability of ionization of a group adjoining the uncharged nitroanilide residue should, in general, be higher than α, the *average* degree of ionization. Moreover, electrostatic interactions will make the ionization of one carboxyl neighbor depend, to some extent, on the state of ionization of the other neighboring group. Bearing in mind these uncertainties, the proportionality of the rate on $(1 - \alpha^2)$ may be interpreted in two ways:

(1) The result is consistent with a mechanism in which un-ionized carboxyl catalyzes the hydrolysis of nitroanilide groups, but two COOH neighbors are no more effective than one such neighboring group. This interpretation is supported by evidence that the amide hydrolysis rate in asparagine peptides[14] and in phthalamic acid[15] is proportional to the fraction of un-ionized carboxyl. The failure of GNA to give any indication of neighboring group catalysis may be due to the high flexibility of the molecule as compared to phthalamic acid or copolymer A/Am. (2) Hydrolysis rates proportional to $(1 - \alpha^2)$ also could be accounted for by assuming that two ionized carboxyl neighbors inhibit the uncatalyzed hydrolysis of anilide groups. This interpretation is supported by the observation that the hydrolysis rate of the glutaric acid derivative

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[11] H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," Reinhold Publ. Corp., New York, N. Y., 1950, p. 512.

[12] H. Morawetz and E. W. Westhead, Jr., *J. Polymer Sci.*, **16**, 273 (1955).

[13] P. E. Zimmering, E. W. Westhead, Jr., and H. Morawetz, *Biochem. Biophys. Acta*, **25**, 376 (1957).

[14] S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 915 (1953).

[15] M. L. Bender, This Journal, **79**, 1258 (1957).

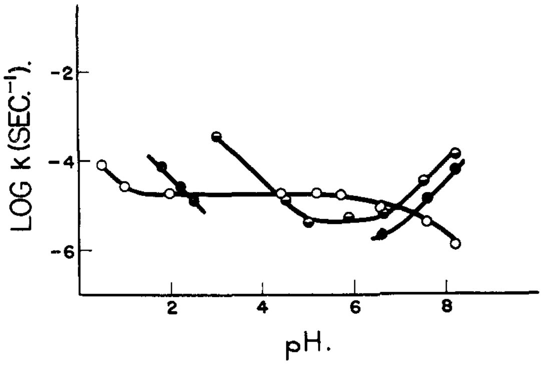


Fig. 1.—Hydrolysis of copolymer A/An and its analogs at 135.4°: O, copolymer A/An; ●, PNA; ◒, GNA.

remains nearly constant from *p*H 5 to 7, being comparable with the *p*H-independent rate observed for A/An (Fig. 1). Also, Pinner has reported that the alkaline hydrolysis of amide groups in methacrylic acid–methacrylamide copolymers is completely inhibited whenever two ionized carboxyls flank the amide residue.[16]

The effect of the addition of barium ion on the reaction rate, to be discussed below, indicates that both catalysis by neighboring un-ionized carboxyl and inhibition by hydrogen-bonding with carboxylate may be factors affecting nitroanilide hydrolysis under different conditions. The contrast between the catalysis of ester hydrolysis by ionized carboxyl and the catalysis of amide hydrolysis by un-ionized carboxyl is in line with the relative susceptibility of these groups to hydrogen and hydroxyl ion. The ratio of the catalytic coefficients k_{OH^-}/k_{H^+} in hydrolyses at 25° is 2.8×10^6 for *p*-nitrophenyltrimethylacetate,[7] 1.95×10^4 for phenyl acetate,[17] 2500 for methyl acetate[18] and 6.7 for acetamide.[19] The contrast would tend to become less important at higher temperatures because of the generally higher activation energies for acid hydrolysis.

The Imidization of Copolymer M/Mal–An.—When copolymer M/Mal–An was heated at 84.4° in acid or buffer solution, the maximum in the absorption spectrum shifted from 315 mμ, characteristic of *p*-nitroanilides, to a new peak at 270 mμ. This was in striking contrast with the appearance of the *p*-nitroaniline peak at 380 mμ accompanying the hydrolysis of *p*-nitroanilides. The product of the reaction of M/Mal–An had a spectrum identical with that of *p*-N-nitrophenylsuccinimide implying that the reaction observed with this copolymer in acid and neutral solution involves the condensation of a *p*-nitroanilide group with a neighboring carboxyl. This interpretation was confirmed by the regeneration of the original spectrum when the imidized copolymer was treated with sodium hydroxide at room temperature.

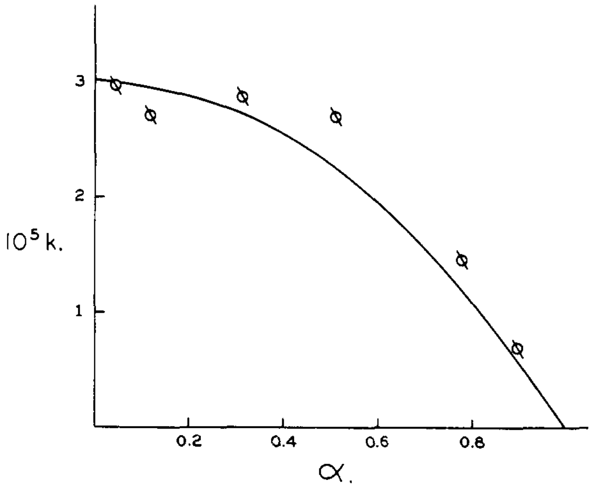
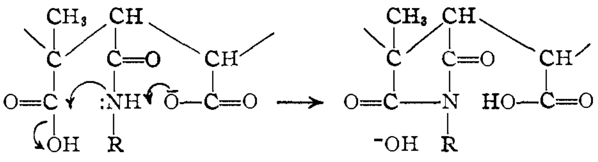


Fig. 2.—Dependence of the hydrolysis rate on the degree of neutralization of A/An: ⦰, experimental results; solid line, $k = 3 \times 10^{-5}\,(1 - \alpha^2)$.

The *p*H dependence of the imidization rate is plotted in Fig. 3 and the data show that the reaction proceeds at a maximum velocity around *p*H 5, with both acid and base acting as inhibitors. The result indicates clearly that the reaction mechanism requires the nitroanilide group to be flanked by one ionized and one un-ionized carboxyl. The mechanism of the reaction may be represented by[20]



It is significant that the mono-*p*-nitroanilide of succinic acid, in which the nitroanilide group has only one carboxyl neighbor, does not imidize in aqueous solution.

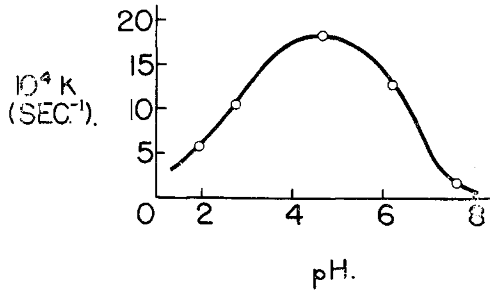


Fig. 3.—Imidization rate of copolymer M/Mal–An at 84.4°.

It should be emphasized that no deviations from first-order kinetics were found with the imidization of M/Mal–An, even when the reaction was run to 90% conversion. This is highly significant because of the manner in which M/Mal–An was prepared. Copolymers of maleic anhydride are known to have the maleic anhydride residues flanked by units of the co-monomer[21] and the condensation of a methacrylic acid–maleic anhydride copolymer with *p*-nitroaniline could, in principle, take two courses

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(16) S. H. Pinner, *J. Polymer Sci.*, **10**, 379 (1951).

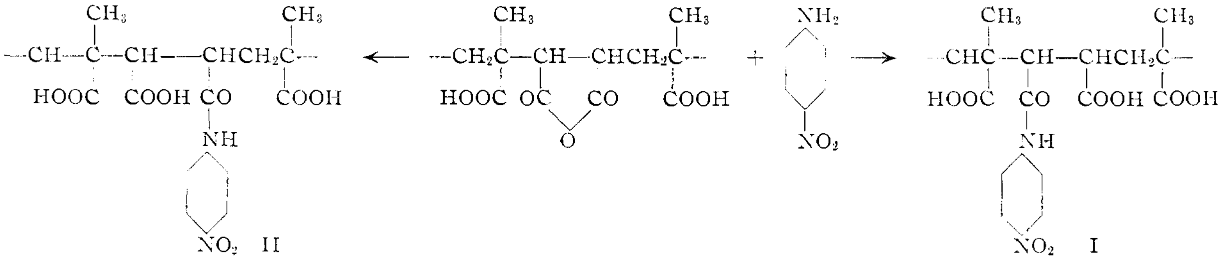
(17) E. Tommila and C. N. Hinshelwood, *J. Chem. Soc.*, 1801 (1938).

(18) W. B. S. Newling and C. N. Hinshelwood, *ibid.*, 1357 (1936).

(19) N. v. Peskoff and J. Meyer, *Z. physik. Chem.*, **82**, 129 (1913).

(20) We are indebted to Dr. L. A. Cohen of the National Institutes of Health, Bethesda, Md., for suggesting this mechanism.

(21) T. Alfrey, Jr., J. J. Bohrer and H. Mark, "Copolymerization," Interscience Publishers, Inc., New York, N. Y., 1952, p. 45.



It seems certain that I and II would imidize at different rates and the observation that the reaction was strictly first order suggests strongly that only one of the two possible structures was, in fact, obtained. This means that the neighboring carboxyl group was also operative in directing the condensation of *p*-nitroanilide with the anhydride residue.

Hydrolysis Rates of *p*-Nitroanilides in Sodium Hydroxide Solution.—Second-order rate constants for the hydroxyl ion catalyzed hydrolysis of *p*-nitroanilide substituents carried by various copolymers and their low molecular weight analogs are listed in Table I. The results were found to be independent of the chain length of the copolymer or of its composition up to 9 mole % of the nitroanilide component. For copolymer A/An $k_2 = 4 \times 10^{-5}$ was obtained in a solution of 0.41 N sodium hydroxide, but the second-order rate constant increased to 10.2×10^{-5} on addition of 0.61 N sodium chloride. This indicates that even at high electrolyte concentration the reaction rate is depressed to some extent by the repulsion of the catalytic hydroxyl ion by the polyanion, but only a small fraction of the very large difference between the reactivity of the analogs and the copolymers can be assigned to this cause.

TABLE I

HYDROLYSIS OF *p*-NITROANILIDE FROM COPOLYMERS AND THEIR ANALOGS IN SODIUM HYDROXIDE SOLUTION AT 84.4°

Copolymers			
p-Nitroanilide	Acid	NaOH, M	$10^5 k_2$, l.-mole^{-1} sec.$^{-1}$
Acrylic	Acrylic	1.02	9.2
Acrylic	Methacrylic	1.02	0.12
Methacrylic	Acrylic	1.02	0.07
Methacrylic	Methacrylic	1.02	2.2
Maleic	Methacrylic	0.102	35.7
Analogs			
TNA		0.041	1080
GNA		.041	1540
SNA		.041	2280

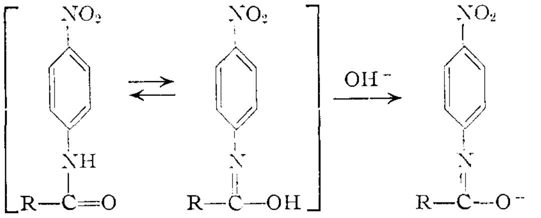
It is revealing that *p*-nitroacrylanilide residues were 77 times more reactive with acrylic acid than with methacrylic acid co-monomers, while *p*-nitromethacrylanilide was conversely 31 times more reactive in a methacrylic acid chain. These are obviously not inductive effects. Fisher–Hirshfelder models show the profound difference in the conformations of the acrylic and methacrylic acid copolymers and it is suggested that this might control the ease of hydrogen-bonding of the nitroanilide residues with neighboring carboxylate. It has been shown previously that even in an aqueous medium such intramolecular hydrogen bonding may lead to sharply reduced reactivities.[16,22]

The change of nitroanilide spectra in strong base solutions pointed to the shift from an acid form A to a basic form B, defining an equilibrium constant $K = (B)/(A)(OH^-)$. It can be shown that

$$1/(OH^-) = (C/\Delta)K(\epsilon_B - \epsilon_A) = K \qquad (1)$$

where C is the stoichiometric nitroanilide concentration, Δ the change in optical density per cm. path length due to the addition of base, while ϵ_A and ϵ_B are the molar extinction coefficients of species A and B at the wave length employed. Plots of $1/(OH^-)$ against C/Δ at an ionic strength of 2.0 were linear for all polymers and analogs yielding the same value of $K = 0.5$ l.-mole^{-1}. The slopes of the plots varied considerably, however, yielding for 380 mμ $\epsilon_B - \epsilon_A = 13400$ for TNA and values ranging from 5100 to 7000 for the nitroanilide residues of the copolymers.

The relative susceptibility of the acid and basic form of TNA to alkaline hydrolysis was studied in a series of runs at 25° in which the sodium hydroxide concentration was varied at a constant ionic strength of 2. The second-order rate constant changed only from 1.02×10^{-4} to 1.00×10^{-4} l.-mole^{-1} sec.$^{-1}$ for an increase of base concentration from 0.67 to 1.33 N, corresponding to an increase in the fraction present in the basic form from 0.25 to 0.40. This seems to indicate that A and B are equally susceptible to hydroxyl-catalyzed hydrolysis.[22a] In the copolymers, both forms of the nitroanilide must be equally stabilized by hydrogen-bonding to neighboring carboxylate groups, since the second-order rate constant at constant ionic strength changes little with base concentration. The ionization of the nitroanilide enol would not be expected to meet these criteria so that no consistent interpretation of the spectral shift in basic solutions can be offered at present.



A rather surprising effect was observed when poly-(acrylic acid) was added to copolymer A/An in 0.41 N sodium hydroxide. The addition of 0.04 N PAA to 0.0015 N A/An increased the hydrolysis rate by 35% in spite of the lowered basicity of the solution. Acetate or glutarate addition had no effect on the second-order rate constant. Rate

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[22] C. A. Grob, *Helv. Chim. Acta*, **33**, 1787 (1953).

[22a] By contrast, J. T. Edward and K. A. Terry (*J. Chem. Soc.*, 3527 (1957)) found that only the un-ionized forms of succinimide and diacetylamine participated in base catalyzed hydrolysis.

constants in neutral and acid solution had a similar dependence on polymeric acid concentration. It should be emphasized that the addition of the polymeric acid in these experiments could not have produced any major effect on ionic activity coëfficients, since simple electrolytes were in large excess over the polyion.

The Catalytic Effect of Barium Ion on the Hydrolysis of *p*-Nitroanilide Groups Attached to Polyanions.—Data plotted in Fig. 4 show that in 0.41 *N* sodium hydroxide solution as little as 0.0021 *M* barium hydroxide is sufficient to double the rate of hydrolysis of *p*-nitroanilide residues attached to poly-(acrylic acid). Moreover, the acceleration of the reaction is almost proportional to the square of the barium ion concentration. These results are consistent with the assumption that the low reactivity of nitroanilide residues in the copolymers investigated is due to hydrogen-bonding with neighboring carboxylate groups. Barium previously has been shown to form complexes with polycarboxylic acids[23] and such complex formation of two barium ions with the carboxylate groups flanking the nitroanilide residue would eliminate the stabilizing hydrogen bonds and increase the nitroanilide reactivity to a value comparable to that of, *e.g.*, TNA. We may then express the observed hydrolytic rate constant k as

$$k = k_0 + 2\beta(1 - \beta)\Delta k_{\mathrm{Ba}} + \beta^2\Delta k_{2\mathrm{Ba}} \qquad (2)$$

where β is the fraction of carboxylate groups complexed with barium, k_0 is the hydrolytic rate constant in the absence of barium, while Δk_{Ba} and $\Delta k_{2\mathrm{Ba}}$ are the increments of these rate constants due to the presence of one or two neighboring barium complexes, respectively. The elimination of one of the two neighboring carboxylates should double the probability that a nitroanilide group escapes hydrogen-bonding so that $\Delta k_{\mathrm{Ba}} \approx k_0$. The complexation equilibrium with the polymer is given by

$$\beta/(1 - \beta) = K_{\mathrm{Pol}}(\mathrm{Ba}^{++})$$

and the second ionization constant of barium hydroxide has been estimated as 0.23.[24]

Using the TNA rate constant for $k_0 + \Delta k_{2\mathrm{Ba}}$, the catalytic effect of barium ion is consistent with K_{Pol} of the order of 100. This value is of the expected magnitude for barium chelates with two neighboring carboxylates of a polycarboxylic acid.[22] It is revealing that the addition of 0.61 *N* sodium chloride did not alter the extent of the acceleration brought about by barium ion, indicating that the effect is due to specific interaction rather than general electrostatic interactions of polyion and counter-ion. On the other hand, the catalytic effect of barium ion decreased with increasing base concentration as would be expected because of the increased fraction of barium present as the hydroxo complex.

Preliminary experiments indicated that the effect of barium on the hydrolysis rate of the copolymers depends on their degree of ionization. The addition of 0.0025 *M* barium chloride to a solution of copolymer A/An at 135.4° reduced the hydrolytic rate by 25% at $\alpha = 0.12$ and by 35% at $\alpha = 0.51$, while the rate was accelerated by 15% at $\alpha = 0.78$ and by 53% for $\alpha = 0.90$. These results seem to show that in the region where the catalytic effect of neighboring un-ionized carboxyl is of primary importance, barium ion interferes with this catalysis, while in more basic media, where hydroxyl ion attack is rate determining, barium ion accelerates the reaction by the disruption of protecting hydrogen bonds.

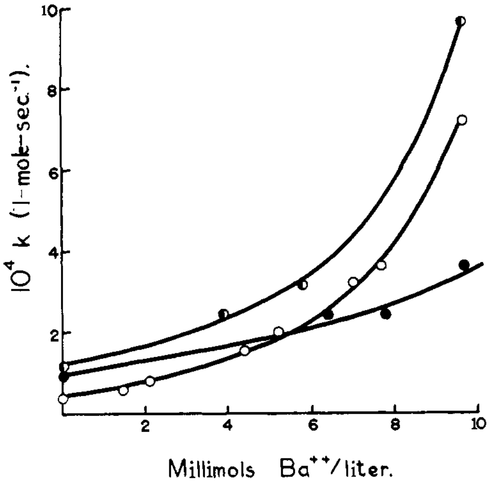
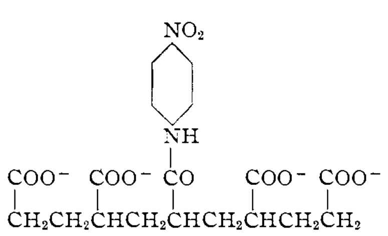


Fig. 4.—Catalytic effect of Ba^{++} on the hydrolysis of A/An at 84.4°: ○, 0.41 *M* NaOH; ◐, 0.41 *M* NaOH + 0.61 *M* NaCl; ●, 1.02 *M* NaOH.

Analogy to Enzymatic Catalysis.—We have suggested previously[12,13] that intramolecular neighboring group catalysis is analogous to the substrate activation in an enzyme–substrate association complex. The observation that a neighboring carboxyl must be un-ionized to catalyze nitroanilide hydrolysis, but must be ionized to catalyze the solvolysis of nitrophenyl esters is of particular interest in that it points to one possible mechanism of enzyme specificity. The nature of the neighboring carboxylate catalysis in the condensation reaction of copolymer M/Mal–An may also have a bearing on the mechanism of group transfer to



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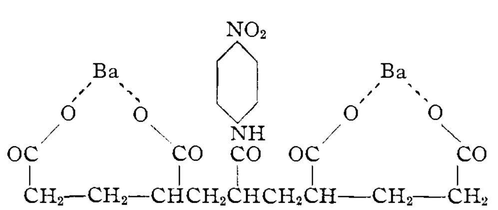

IV

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enzymes.[25-27] Finally, the striking catalytic effect of small concentrations of barium ion on the hydrolysis of copolymer A/An and its interpretation in terms of the elimination of an inhibitory neighboring group effect adds yet another possible mechanism to those enumerated previously[28] for the activation of enzymes by specifically required ions.

In many cases low-molecular weight model compounds should serve as well or better than polymers in such model studies. However, copolymerization is a very simple technique compared to the rather complex synthetic problems that might otherwise have to be faced. In addition, the use of polymers with a large number of hydrophilic groups makes it possible to keep in aqueous solution groupings which would otherwise be too insoluble for study. Both points may be strikingly illustrated on the barium catalysis of A/An hydrolysis. To demonstrate a similar effect on a low molecular weight compound, it would be necessary to study the hydrolytic rates of the structures III and IV.

It is clear that these materials would be very hard to prepare and that the chelate IV would be too insoluble for a kinetic study.

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The Reaction of D-Glycerose-3-C^{14} with Alkali[1]

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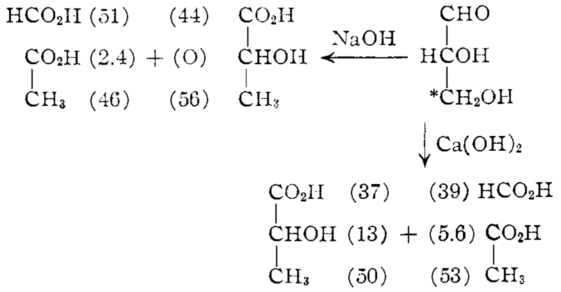
The isomerization of D-glycerose-3-C^{14} by 1.68 N sodium hydroxide at 25° leads to lactic acid labeled almost equally, and only, in C-1 and C-3. In contrast, saturated calcium hydroxide at 25° converts D-glycerose-3-C^{14} to lactic acid with significant amounts of label in all three carbon atoms. An interpretation of these results is offered in terms of the Nef–Isbell mechanism for the formation of saccharinic acids.

In their classical studies[2] of the interconversion of reducing sugars by alkali, Lobry de Bruyn and Alberda van Ekenstein recognized certain variations in the details of the reaction in the presence of different bases. Such differences have been attributed[3] more recently to specific, cationic effects of the reacting bases.

Different bases also have been observed to produce different products, although under very different reaction conditions, in the isomerization of reducing sugars to saccharinic acids. Thus, D-glucose with calcium hydroxide at room temperature gives "α"-D-glucosaccharinic acid as the principal six-carbon saccharinic acid,[4] whereas the same sugar with hot 8 N sodium hydroxide is reported to give no trace of this acid, but rather the D-glucometasaccharinic acids plus a small amount of "α"-D-isosaccharinic acid.[5] These results have been attributed[6] recently to variations in the ionization behavior of the intermediate sugar enediols at different pH values, and a resultant channeling of the reaction into one or other of the alternative pathways of the Nef–Isbell mechanism.[5,7]

In the present continuation of our studies[8] on saccharinic acid formation from C^{14}-labeled sugars, the action of saturated calcium hydroxide and of 1.68 N sodium hydroxide at 25° on D-glycerose-3-C^{14} have been examined in an attempt to elucidate further the course of the reaction in the presence of different bases. The lactic, acetic and formic acids[9] obtained in each case were separated by chromatography and radioassayed in the form of appropriate derivatives. The acetic acid was degraded[8,10] by way of 2-methylbenzimidazole, 2-styrylbenzimidazole and benzimidazole-2-carboxylic acid to benzimidazole, and the lactic acid[10] by way of 2-(α-hydroxyethyl)-benzimidazole and benzimidazole-2-carboxylic acid to benzimidazole, to obtain values for the relative distribution of radioactivity among the various carbon atoms. The percentages of the specific radioactivity of the lactic acid found in the individual carbon atoms of the products is shown in parentheses in the accompanying formulas.



With 1.68 N sodium hydroxide, D-glycerose-3-C^{14} yielded lactic acid labeled nearly equally, and en-

[1] Abstracted from a thesis submitted by Eva K. Pohlen in partial fulfillment of the requirements for the degree Master of Arts, Washington University, June, 1954.

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