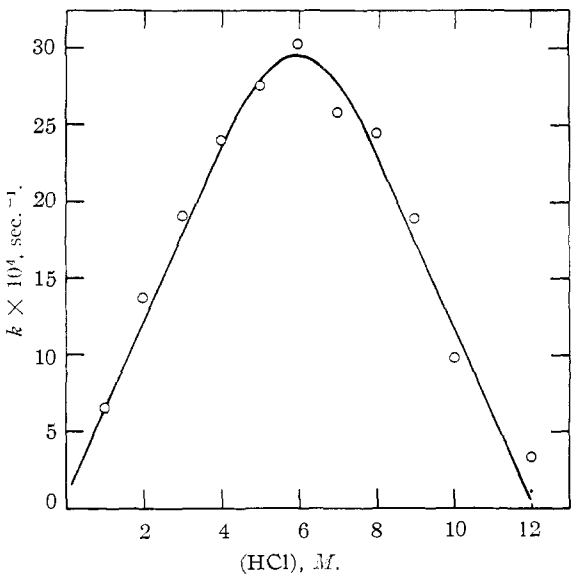

Fig. 3.—The hydrolysis of phthalamic acid in concentrated hydrochloric acid at 47.3°.

hydrolysis of normal amides including a rate maximum at about 6 M hydrochloric acid and the approximate dependence of the rate on the stoichiometric acid concentration up to the position of the maximum. This behavior is exhibited by benzamide, for example, as well as by a number of other simple amides. The position of the rate maximum is related to the ionization constant of the protonated amide and leads to an estimate of the pK of -2.5 for the protonation of phthalamic acid. It was not possible to determine this ionization constant directly because of the facile hydrolysis of phthalamic acid in concentrated acid solution.

The similarity in behavior of phthalamic acid in concentrated acid solution to the behavior of benzamide suggests that under these conditions phthalamic acid hydrolyzes *via* the direct attack of a water molecule on the protonated amide group. This change in mechanism of the hydrolysis of phthalamic acid from dilute to concentrated acid solution is apparent in the relative rate constants shown in Table III. The difference in rates of benzamide and phthalamic acid at 0.001 M HCl is 75,800 whereas it is only 240 at 4 M HCl, reflecting a change in mechanism.

TABLE III

A COMPARISON OF THE HYDROLYSIS OF BENZAMIDE AND PHTHALAMIC ACID

Amide	k_1, sec.$^{-1}$ 0.001 M HCl	k_1, sec.$^{-1}$ 4 M HCl
Phthalamic acid	2.35×10^{-4}	240×10^{-5}
Benzamide	3.1×10^{-9}	1×10^{-5}
Phthalamic acid/benzamide	75,800	240

Unfortunately it is not possible to test this hypothesis by conducting a (double-label) tracer experiment in concentrated hydrochloric acid since exchange of oxygen-18 between the reactant or product and the solvent is too fast in this medium. The change in mechanism in concentrated acid is consistent with the mechanism proposed earlier since the undissociated *o*-carboxylic acid group could not serve as a nucleophile as well as the *o*-carboxylate ion, thereby permitting an external water molecule to serve as nucleophile in the rate-determining attack on the protonated amide group.

Acknowledgment.—The authors acknowledge with pleasure valuable discussions with Drs. G. J. Buist, J. T. Edward, E. S. Kosower, F. A. Long and R. W. Taft, Jr., and wish to express their appreciation to Dr. H. Taube of the University of Chicago through whose courtesy the mass spectrometer under AEC Contract At(11-1)92 was made available. We thank the Simonize Co. for its contribution to the departmental fund for research.

CHICAGO 16, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY]

Intramolecular Catalysis of Hydrolytic Reactions. III. Intramolecular Catalysis by Carboxylate Ion in the Hydrolysis of Methyl Hydrogen Phthalate[1,2]

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The hydrolytic reaction of methyl hydrogen phthalate is characterized by a region near neutrality in which the observed first-order rate constant is independent of the pH. This phenomenon has been observed previously in the hydrolyses of mono-*p*-nitrophenyl glutarate and acetylsalicylic acid and has been interpreted as catalysis of the hydrolytic reaction by an internal nucleophile, carboxylate ion. It is suggested that in addition to the phenyl esters, aspirin and mono-*p*-nitrophenyl glutarate, the methyl ester, methyl hydrogen phthalate, is subject to intramolecular catalysis of hydrolysis by carboxylate ion, involving an anhydride intermediate. This reaction is the first example of the hydrolysis of an unactivated ester by a nucleophilic catalyst. The enthalpy and entropy of activation, 33.7 kcal./mole and 7.5 e.u., respectively, are consistent with an intramolecular process. The finding that oxygen-18 labeled salicylic acid is formed in the hydrolysis of aspirin in H_2O^{18} at pH 6 is consistent with intramolecular catalysis in this process. These observations lend credence to the hypothesis that intramolecular catalysis by carboxylate ion occurs during the catalytic process of the enzymes ficin and papain.

A general investigation has been initiated to ascertain what hydrolytic processes can be catalyzed by internal nucleophiles and/or electrophiles.[2,3] One nucleophile that occurs in many organic compounds is the carboxylate ion. Carboxylate ion is

(1) This research was supported by Grant H-2416 of the National Institutes of Health, and also by a grant from the Upjohn Co.

(2) Previous paper in this series, M. L. Bender, Y. L. Chow and F. Chloupek, THIS JOURNAL, **80**, 5380 (1958). A portion of this research was presented at a symposium on "Reaction Mechanisms and Solvent Effects" at Queen Mary College, London, July, 1957.

(3) M. L. Bender, *ibid.*, **79**, 1258 (1957).

a nucleophilic catalyst[2] for the hydrolysis of a number of ester hydrolyses including phenyl acetate,[4] *p*-nitrophenyl acetate,[4,5] 2,4-dinitrophenyl acetate[6] and methyl pyrrolidylacetylsalicylate.[7] The carboxylate ion is a relatively ineffective nucleophile, which is related in part to its low basicity; furthermore, it is less effective than nitrogen bases of the same pK by a factor of several powers of ten.[4-6]

Even though the carboxylate ion is an unfavorable case from the point of view of nucleophilicity, it apparently participates catalytically in a number of intramolecular catalyses of esters. The hydrolysis of aspirin may be regarded as a classical example. Edwards carried out a complete *p*H–rate profile of the hydrolysis of aspirin from *p*H 0 to 13;[8] Garrett has extended this work to include a number of acyl salicylates in a very complete investigation.[9] In the hydrolysis of acylsalicylates there exists catalysis by external hydrogen ion at low *p*H values (*p*H 1–3) and catalysis by external hydroxide ion at high *p*H values (*p*H 8–14). However from *p*H 4 to 8, the rate constant is independent of *p*H. This is a region of spontaneous hydrolysis which has been described by Bell[10] as usually due to catalysis by a water molecule. However, if such a reaction were a water reaction, it would be reasonable that external acetate ion would have an effect on the rate of hydrolysis, which is not the case.[8] Hydrolysis of aspirin at *p*H 4 to 8 is most reasonably interpreted as a spontaneous reaction of the aspirin anion which occurs by an intramolecular attack of carboxylate ion on the carbonyl carbon atom of the ester to produce an anhydride intermediate which subsequently hydrolyzes rapidly to furnish the products of the hydrolysis, salicylate and acetate. Proposals in essentially this form have been made by Chanley, *et al.*,[11] Davidson and Auerbach[12] and Garrett.[9] Stereochemically, such a pathway looks attractive. It gains added support from the fact that it has been demonstrated that acetate ion is indeed a catalyst for the hydrolysis of a number of phenyl acetates.[4-7]

Other reactions in which a carboxylate ion apparently participates in the hydrolysis of an ester group involve a copolymer of acrylic acid and *p*-nitrophenyl methacrylate and also its monomeric analog, mono-*p*-nitrophenyl glutarate.[13] The *p*H–rate profiles for these hydrolyses are similar in shape to the aspirin reaction in the region around neutrality. Furthermore, the rate of ester hydrolysis in the copolymer and in the monoester is about 10^5 faster than the hydronium or hydroxide ion-catalyzed reactions of similar systems which do not contain internal carboxylate ion. It was postulated by Morawetz that these rapid hydrolyses proceed through a substituted glutaric anhydride intermediate (containing a six-membered ring) formed by the attack of a neighboring carboxylate ion on the ester group.

A feature common to the intermolecular and intramolecular catalyses by carboxylate ion described above is that the group displaced from the ester molecule consisted of a phenoxide ion or substituted phenoxide ion. It was thought that an ordinary ester such as ethyl acetate could not be hydrolyzed by nucleophilic catalysis since imidazole, the best of the intermolecular catalysts, failed with ethyl acetate.[6] However, it is conceivable that in an intramolecular hydrolysis, nucleophilic catalysis of a methyl ester or ethyl ester might be feasible. That is, in such a case, the usual Brönsted type relationship found between catalytic rate constant of the nucleophile[6,14] for intermolecular catalysis would not hold so that intramolecular carboxylate ion could compete with catalysis by the solvent. For this reason we have investigated the hydrolysis of methyl hydrogen phthalate.

Experimental

Materials.—Methyl hydrogen phthalate was prepared from phthalic anhydride and absolute methanol according to the method of Eliel and Burgstahler.[15] Sodium acetyl salicylate was an Eastman Kodak Co. white label product.

Kinetics.—The kinetics of hydrolysis of methyl hydrogen phthalate were followed using sealed ampoules at 109 ± 0.5° and following the rate of disappearance of the ester grouping with a Beckman DK2 spectrophotometer at 275–279 mμ (ε 570) depending on the *p*H. The product, *o*-phthalic acid, was confirmed after each run by taking a complete spectrum at infinite time. The method of Guggenheim[16] was used for the determination of the first-order rate constants. The buffers used for *p*H's 2.6 to 7 were citrate–phosphate buffers; from *p*H 0.8 to 2.3 the *p*H was controlled by an excess of hydrochloric acid. It was found that a tenfold change in the ionic strength produced no change in the rate constant at *p*H 2.6 so that no special precautions were taken to maintain constant ionic strength.

The kinetics of hydrolysis of methyl benzoate using acetate ion as catalyst were followed using sealed ampoules at 109 ± 0.5°, following the rate of disappearance of the ester grouping with a Beckman DK2 spectrophotometer at 236, 238 and 240 mμ (ε 4350). The acetate buffer at *p*H 5.5 was maintained at constant ionic strength (the total salt concentration was 0.895 *M*) by the addition of the suitable concentration of sodium chloride.

Hydrolysis of Aspirin in H_2O^{18}.—Aspirin (50 mg.) was added to a solution containing 1.0 ml. of water (4.3 atom % oxygen-18, Atomic Energy Research Establishment, Harwell, Berks., England), 82 mg. of anhydrous sodium acetate and 8 lambda of concentrated hydrochloric acid (37 weight per cent.). The solution was refluxed for 22 hours. Two drops of concd. hydrochloric acid were added to the warm solution. The salicylic acid which crystallized practically immediately was filtered and washed with water, m.p. 159–160°. A duplicate run was made using ordinary water. The salicylic acid samples were pyrolyzed to carbon dioxide using the modified procedure of Doering and Dorfman[17] described previously[18] and the oxygen-18 content of the carbon dioxide samples was determined in a Consolidated–Nier model 21-201 isotope-ratio mass spectrometer by measurement of the 46/44 ratios.

(4) M. L. Bender and M. C. Neveu, THIS JOURNAL, **80**, 5388 (1958).
(5) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958).
(6) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1656 (1957).
(7) E. R. Garrett, *ibid.*, **79**, 5206 (1957).
(8) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).
(9) E. R. Garrett, THIS JOURNAL, **79**, 3401 (1957).
(10) R. P. Bell, "Acid–Base Catalysis," Oxford Univ. Press, London, 1941, Chap. V.
(11) J. D. Chanley, E. M. Gindler and H. Sobotka, THIS JOURNAL, **74**, 4347 (1952).
(12) D. Davidson and L. Auerbach, *ibid.*, **75**, 5984 (1953).
(13) H. Morawetz and P. E. Zimmering, *J. Phys. Chem.*, **58**, 753 (1954); H. Morawetz and E. W. Westhead, Jr., *J. Polymer Sci.*, **16**, 273 (1955); P. E. Zimmering, E. W. Westhead, Jr., and H. Morawetz, *Biochim. et Biophys. Acta*, **25**, 376 (1957).
(14) T. C. Bruice and G. L. Schmir, THIS JOURNAL, **80**, 148 1958).
(15) E. L. Eliel and A. W. Burgstahler, *ibid.*, **71**, 2251 (1949).
(16) E. A. Guggenheim, *Phil. Mag.*, [7] **2**, 538 (1926).
(17) W. E. Doering and E. Dorfman, THIS JOURNAL, **75**, 5595 (1953).
(18) M. L. Bender and K. C. Kemp, *ibid.*, **79**, 117 (1957).

Results and Discussion

Methyl Hydrogen Phthalate.—The kinetics of hydrolysis of methyl hydrogen phthalate in aqueous solution at 109 ± 0.5° shown in Table I indicate a minimum in the observed first order rate constant at about *p*H 3. In addition the region from approximately *p*H 4 to 7 is one in which the first-order rate constant is independent of *p*H. This behavior is reminiscent of the hydrolysis of aspirin.[8,9] In fact, Fig. 1 indicates *p*H–rate profiles

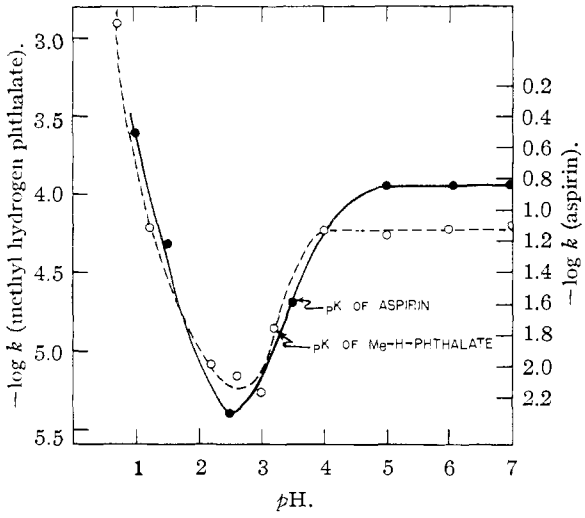


Fig. 1.—The hydrolysis of methyl hydrogen phthalate at 109°, O; and of aspirin at 17°, ●. The aspirin data were taken from ref. 8.

for the hydrolyses of methyl hydrogen phthalate and aspirin can be essentially superimposed upon one another although the absolute rates of the two hydrolyses are somewhat different.

TABLE I

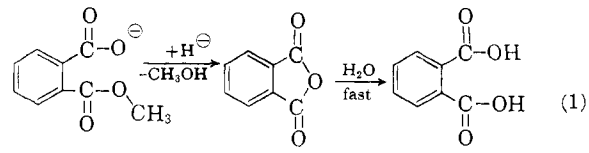
THE KINETICS OF THE HYDROLYSIS OF METHYL HYDROGEN PHTHALATE

*p*H	$k_{obs} \times 10^5$, sec.$^{-1}$ 109°	*p*H	$k_{obs} \times 10^5$, sec.$^{-1}$ 109°
0.8	124	3.2	1.38
1.3	5.8	4	5.9
2.3	0.78	5	5.6
2.6	.67	6	6.4[a]
3.0	.54	7	6.7

[a] 0.26 at 84°.

If the hydrolysis in the aspirin plateau can be explained by an intramolecular catalysis by *o*-carboxylate ion, then certainly the hydrolysis in the plateau of the methyl hydrogen phthalate curve must be explained by the mechanism in eq. 1 involving attack of *o*-carboxylate ion on the methyl ester to form phthalic anhydride as an intermediate which is subsequently hydrolyzed by water. It is seen that the *pK* of methyl hydrogen phthalate (3.22)[19] occurs at approximately the point of inflection of its *p*H–rate profile, as demanded by the mechanism in eq. 1. This observation also has been made in the case of aspirin.[8,9]

(19) F. H. Westheimer and O. T. Benfey, THIS JOURNAL, **78**, 5309 (1956). Apparently the change in ionization constant with temperature is of small magnitude in this case as is usual with carboxylic acids.



(1)

The subsequent hydrolysis of phthalic anhydride has been shown to have a rate constant of 4.61×10^{-3} sec.$^{-1}$ at 25°,[20] much larger than the rate constant of the over-all reaction.

The enthalpy and entropy of activation have been determined from the data at *p*H 6 in Table I and are 33.7 ± 2 kcal./mole and 7.5 ± 5 e.u., respectively. Although these values are somewhat crude they are certainly significantly different from those for the hydrolysis of methyl benzoate by hydroxide ion, which has an enthalpy of activation of 13.6 kcal./mole and an entropy of activation of −20.3 e.u.[21] The activation parameters of the methyl hydrogen phthalate reaction are seen to be markedly different from an ordinary ester hydrolysis and differ in the direction that would be expected from an intramolecular process.[4] The positive entropy of activation in the intramolecular hydrolysis of methyl hydrogen phthalate is the most striking feature exhibited by the kinetic data and certainly indicates a process in which the contiguous positions of catalyst and substrate are of primary importance for reaction.

An attempt has been made to determine the kinetics of the intermolecular reaction corresponding to methyl hydrogen phthalate, namely, the acetate ion-catalyzed hydrolysis of methyl benzoate. The kinetics of hydrolysis of methyl benzoate by acetate ion in aqueous solution at 109° at constant *p*H and constant ionic strength is shown in Table II.

TABLE II

ATTEMPTED ACETATE ION-CATALYZED HYDROLYSIS OF METHYL BENZOATE[a]

Acetate ion,[b] *M*	$k_{obs} \times 10^5$, sec.$^{-1}$
0.3511	4.45
.5267	4.75
.7900	5.0

[a] 109°. [b] Constant *p*H (5.5); constant ionic strength maintained by the addition of sodium chloride.

The data are not of the highest accuracy due to the extreme conditions employed, and lead to the low catalytic constant of 1.2×10^{-6} l./mole sec. for the acetate ion-catalyzed hydrolysis of methyl benzoate. Bell[22] has voiced objections to the utilization of salt solutions up to one molar, which may lead to primary and secondary salt effects depending largely on the nature of the ions present. It is therefore tentatively concluded that these experimental data do not constitute a valid proof of the acetate ion catalysis.

The demonstration of the catalysis of hydrolysis of a methyl ester by intramolecular carboxylate ion appears significant because of the failure to

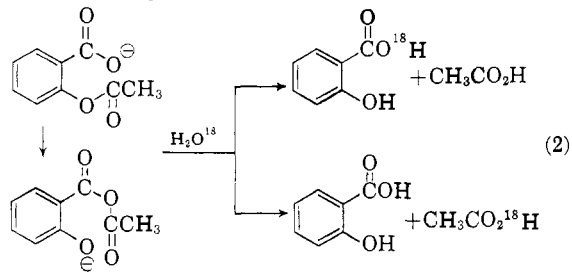
(20) A. C. D. Rivett and N. V. Sidgwick, *J. Chem. Soc.*, **97**, 1683 (1910).

(21) E. Tommila and C. N. Hinshelwood, *ibid.*, 1801 (1938); the alkaline hydrolysis of methyl benzoate in 56% by weight acetone.

(22) *Cf.* R. P. Bell, "Acid–Base Catalysis," Oxford Univ. Press, London, 1941, p. 80.

effect hydrolysis of simple alkyl esters by an intermolecular nucleophilic catalyst, including imidazole, a catalyst more powerful than carboxylate ion by about six powers of ten.[20] This result demonstrates the powerful nature of intramolecular catalysis and indicates that the use of imidazole as an intramolecular catalyst by an enzyme or otherwise would indeed lead to a powerful catalytic system.[23]

Aspirin.—Hydrolysis of the aspirin anion at *p*H 4 to 8 previously has been postulated to proceed by an intramolecular attack of carboxylate ion on the carbonyl carbon atom of the ester to produce acetyl salicyl anhydride which subsequently hydrolyzes rapidly to produce acetate ion and salicylate ion.[9,11,12] Alternatively it has been proposed that the addition of the carboxylate ion to the carbonyl group of the ester is followed by some reaction with water leading to the products.[9,11] The existence of a phthalic anhydride intermediate in the hydrolysis of phthalamic acid is strongly suggested by the double label isotopic tracer experiment demonstrated in the previous paper.[2] The hydrolysis of aspirin anion in H_2O^{18} should yield the isotopic species shown in eq. 2 if acetyl salicyl anhydride is the intermediate. It can be calculated from the relative rates of hydrolysis of ethyl acetate and ethyl salicylate that the reaction producing salicylic acid-O^{18} should occur to the



(2)

extent of 2.5% and that the other reaction should occur to the extent of 97.5%.[24] The hydrolysis of aspirin at *p*H 6 in water containing 4.3 atom % of oxygen-18 produced salicylic acid containing 6% of the excess oxygen-18 in the water. Since the calculation on the relative breakdown of the unsymmetrical anhydride was based on the obviously limited assumption that the departing groups were identical to one another,[24] the agreement between the theoretical prediction and the experimental result is considered to be reasonable and consistent with the reaction postulated in eq. 2.

Conclusions.—It is of interest to compare the hydrogen ion dependencies of the rate constants for the hydrolyses of phthalamic acid[2] and methyl hydrogen phthalate. These two reactions represent the hydrolysis of an amide and an ester, each involving possible catalysis of hydrolysis by an internal *o*-carboxylic acid grouping. Each of these hydrolyses involves a region of the *p*H range in which the rate constant of hydrolysis is independent of the hydrogen ion concentration. However the two reactions differ in the region in which they are independent of the hydrogen ion concentration, the phthalamic acid hydrolysis being *p*H independent from *p*H 1 to 3 while the methyl hydrogen phthalate is *p*H independent from *p*H 4 to 7. These different regions correspond of course to the fact that the un-ionized carboxylic acid group is the catalytic species in the former reaction while the carboxylate ion is the catalytic species in the latter case. It is interesting to note that in a comparable steric and electronic situation, the hydrolysis of the amide linkage is catalyzed by the carboxylic acid group while the hydrolysis of the ester linkage is catalyzed by the carboxylate group. Furthermore, the intramolecular hydrolysis of the amide phthalamic acid is of the order of 10^3 faster than the intramolecular hydrolysis of the ester, methyl hydrogen phthalate, although the hydrogen ion-catalyzed hydrolysis of a simple amide such as benzamide is much slower than the alkaline hydrolysis of ethyl benzoate. This reversal in relative reactivities in the intramolecular reactions is presumably due to the fact that the phthalamic acid hydrolysis is an electrophilic–nucleophilic catalyzed reaction while the methyl hydrogen phthalate hydrolysis involves only nucleophilic catalysis. The difference in the type of intramolecular catalysis is presumably due to the difference in basicity of the amide and carbomethoxy groups. It was estimated[2] that the pK_B of phthalamic acid is -2.5 while the pK_B of methyl hydrogen phthalate is probably several orders of magnitude more negative.[25]

It has been proposed by Bernhard and Gutfreund[26] that in the ficin-catalyzed hydrolysis of benzoyl-L-arginine ethyl ester, a group with a pK of 4.35, presumably an ionized carboxyl group, plays a dominant role in the rate-determining hydrolysis of the postulated acyl–thiol enzyme–substrate compound. This rate-determining process easily can be rationalized in terms of the intramolecular catalyses of ester hydrolyses discussed in this paper. In the ficin-catalyzed hydrolysis, the catalytic process can be described as involving an initial fast attack by a thiol group on the carboxylic acid derivative to form the acyl–thiol compound followed by the rate-determining attack of carboxylate ion to form a mixed anhydride of the acyl group and the enzyme which is spontaneously hydrolyzed by the aqueous solution in a final fast step.

Acknowledgment.—The authors acknowledge with pleasure discussions with Drs. T. C. Bruice, E. R. Garrett and H. Gutfreund.

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(23) See G. L. Schmir and T. C. Bruice, THIS JOURNAL, **80**, 1173 (1958).

(24) *Cf.* D. B. Denney and M. A. Greenbaum, *ibid.*, **79**, 979 (1957), for the nucleophilic reactions of unsymmetrical anhydrides.

(25) Morawetz, *et al.*,[13] have observed a comparable difference in the intramolecular catalysis of copolymers containing amide or ester groups. A copolymer containing *p*-nitroanilide groups was hydrolyzed by internal carboxylic acid while a copolymer containing *p*-nitrophenylate groups was hydrolyzed by internal carboxylate ion. See also E. W. Westhead, Jr., and H. Morawetz, THIS JOURNAL, **80**, 237 (1958).

(26) S. A. Bernhard and H. Gutfreund, *Biochem. J.*, **63**, 61 (1956).