TABLE II

OXYGEN EXCHANGE DURING THE DIAZOTIZATION OF BENZAMIDE-O^{18}

Reaction, %	Atom per cent. O^{18}
Unreacted	0.576[b]
Unreacted[a]	.550
50	.563
65	.558

[a] This sample was subjected to the entire procedure except that no sodium nitrite was introduced. The difference in the O^{18} content of this sample and the first sample indicates the small error due to isolation. [b] The O^{18} content of this labeled benzamide previously had been measured as 0.589 using the modified Doering and Dorfman pyrolysis.[31]

The data of Table II show no oxygen exchange during the diazotization of benzamide, within experimental error. The lack of oxygen exchange leads to no definite conclusion concerning the mechanistic pathway. In general, lack of exchange does not add much information to the mechanistic conclusions. For example, in the acid hydrolysis of benzamide, lack of exchange does not differentiate between an addition intermediate and the direct displacement by water. Again, in the present instance, the lack of oxygen exchange does not differentiate among a number of possibilities which include the paths mentioned above, although exchange would eliminate the proposed mechanism.

The use of benzamide-O^{18} thus does not differentiate between an addition intermediate and an acylium ion intermediate. The acyldiazonium ion is a species of such high instability that it must certainly react by an SN1 mechanism. In other cases involving acid chloride hydrolyses and hydrolysis of esters in strong acids, the oxygen exchange criterion should be of more importance.

Acknowledgment.—The authors 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.

CHICAGO 16, ILL.

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

The Hydrolysis of *p*-Nitrophenyl Acetate Catalyzed by *o*-Mercaptobenzoic Acid[1]

BY GREGORY R. SCHONBAUM AND MYRON L. BENDER

RECEIVED SEPTEMBER 30, 1959

The rate of the reaction of *p*-nitrophenyl acetate with *o*-mercaptobenzoic acid in approximately neutral solution at 25° was determined both by spectrophotometric measurement of the liberated *p*-nitrophenol and by iodine titration of the remaining *o*-mercaptobenzoic acid. The results indicate that the dianion of *o*-mercaptobenzoic acid is the effective species in reaction with *p*-nitrophenyl acetate. The second-order rate constants obtained by the two methods are considered consistent with one another. The initial product of the reaction is thioaspirin; thioaspirin hydrolyzes in neutral solution but at a rate slower than its formation. The hydrolysis of thioaspirin is postulated to proceed *via* intramolecular catalysis aided by the *o*-carboxylate ion, analogously to the aspirin hydrolysis. The two steps: (a) conversion of *p*-nitrophenyl acetate to thioaspirin and (b) hydrolysis of thioaspirin to acetate ion and *o*-mercaptobenzoic acid constitute an over-all catalytic process (brought about by the reagent *o*-mercaptobenzoic acid), the rate-determining step being the second step. The functional groups involved, the pK's of these groups and the over-all pH dependence of this catalytic process parallel those postulated for the action of the enzyme, papain, and reproduce in part those postulated for the action of the enzyme, ficin.

Introduction

In recent years increasing attention has been paid to the study of the mechanism of ester hydrolysis in the presence of nucleophiles other than hydroxide ion. For example it has been shown that imidazole and other tertiary amines catalyze the hydrolysis of certain esters.[2,3] The reasons for this interest in nucleophilic catalysis of ester hydrolysis are twofold: (1) the elucidation of the reaction mechanism in the system under investigation and (2) the possibility that such studies will aid our understanding of more complex catalytic processes, *e.g.*, enzymatic catalysis.

It has been postulated that the efficiency of enzymatic processes may be due to the combination of a number of factors including (a) the fixation of the substrate at the enzymatic catalytic site by specific adsorption[4-6]; (b) the use of more than one functional group giving rise to a concerted process; and (c) the use of coupled reactions, that is, interaction of the catalytic groups of the enzyme with the substrate in a consecutive fashion.[7,8] It has been shown that certain kinds of nucleophilic catalysis in simple organic systems approximate the processes mentioned above. A number of examples of intramolecular catalysis approximate the conditions set forth in (a)[4-6] although the fixation in this instance is not due to adsorption. A classical example of (b) is the bifunctional action of 2-hydroxypyridine in the mutarotation of tetramethylglucose.[9] The consecutive processes involved in the imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate[1,3] and the spontaneous hydrolysis of aspirin[10] exemplify point (c) in simple systems.

(1) This research was supported by Grant H-2416 of the National Institutes of Health.

(2) M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1656 (1957).

(3) T. C. Bruice and G. L. Schmir, *ibid.*, **79**, 1663 (1957).

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

(5) M. L. Bender and M. C. Neveu, THIS JOURNAL, **80**, 5388 (1958).

(6) T. C. Bruice and J. M. Sturtevant, *Biochim. et Biophys. Acta*, **30**, 208 (1958).

(7) H. Gutfreund and J. M. Sturtevant, *Proc. Natl. Acad. Sci., U. S.*, **42**, 719 (1956).

(8) E. L. Smith, *J. Biol. Chem.*, **233**, 1392 (1958), and preceding papers.

(9) C. G. Swain and J. F. Brown, THIS JOURNAL, **74**, 2534 (1952).

(10) J. D. Chanley, E. M. Gindler and H. Sobotka, *ibid.*, **74**, 4347 (1952).

In the enzymatic sequence of events adsorption precedes any catalytic steps. In a model system adsorption is not to be expected: the first step of the reaction is envisaged to be the attack by the nucleophilic group of the catalyst on the carbonyl carbon atom of the ester; the following steps of the catalysis involve processes leading to over-all hydrolysis. The present study was undertaken to examine an organic catalyst which has the possibility of both consecutive and intramolecular catalysis.

In the present work a kinetic study has been made of the mechanism of hydrolysis of *p*-nitrophenyl acetate (NPA) in the presence of the *o*-mercaptobenzoic acid (MBA). Reduced glutathione has previously been shown to react with *p*-nitrophenyl benzoate in essentially neutral solution.[11] Furthermore, cysteine has been reported to react with *p*-nitrophenyl acetate.[12] However, neither of these studies provided information concerning the mechanism of action of the potentially powerful nucleophile, the mercapto group. *o*-Mercaptobenzoic acid was chosen as the mercapto compound in this investigation not only because it contains a mercapto group but also because it contains a carboxylate group in close, rigid configuration with respect to the mercapto group. These two groupings were selected for the present study because it has been postulated that the active center of the enzymes papain and ficin contains, at least, a mercapto group and a carboxylate ion.[13,14] Therefore it was conceivable that any catalytic properties exhibited in the hydrolysis of *p*-nitrophenyl acetate by *o*-mercaptobenzoic acid might be a reflection of the mechanism of the enzyme action.

Experimental

Materials.—*o*-Mercaptobenzoic acid (Matheson, Coleman and Bell practical grade) was purified by the method of Allen and McKay.[15] Recrystallization was carried out in both glacial acetic acid and alcohol–water solutions. The acid was further purified by sublimation to give colorless needles, m.p. 164°. *p*-Nitrophenyl acetate has been described previously.[2] Stock solutions of the ester were prepared either in acetone or acetonitrile. Phosphate buffers of *p*H 6.25, 6.98 and 8.02 were prepared using degassed water, immediately before carrying out kinetic measurements. The buffer concentration varied from 0.09 to 0.11 *M* with respect to total phosphate. Solutions of MBA were prepared by titrating the acid with sodium hydroxide solution to approximately *p*H 7 and diluting the solution to the required volume with a given phosphate buffer. Immediately before and after the kinetic runs, the molarity of *o*-mercaptobenzoic acid was checked by iodometric titration in glacial acetic acid. This precaution was necessary because of the ready oxidation of *o*-mercaptobenzoic acid. Thioaspirin was prepared by the method of Hinsberg, m.p. 126–127°.[16]

Kinetic Measurements.—The rate of the reaction of *p*-nitrophenyl acetate with *o*-mercaptobenzoic acid was followed in two ways: (1) by spectrophotometric measurement of the formation of *p*-nitrophenoxide ion at 400 mμ and (2) by iodometric titration of the *o*-mercaptobenzoic acid remaining at any given time. The iodometric titrations were carried out in glacial acetic acid to the visual end-point, the accuracy of the determinations being at least 7%. The spectrophotometric measurements were carried out with excess *o*-mercaptobenzoic acid, the reaction being initiated by the direct addition of a small volume of ester solution (10 to 50 λ) to a known volume of *o*-mercaptobenzoic acid in a phosphate buffer solution.

Two methods also were used to determine the rate of hydrolysis of thioaspirin: (1) spectrophotometric measurement of the rate of formation of *o*-mercaptobenzoic acid at 350 mμ and (2) iodometric titration of the liberated *o*-mercaptobenzoic acid. Throughout this work degassed buffers were used to minimize autoxidation of *o*-mercaptobenzoic acid.

Determination of the pK_a of the Sulfhydryl Group of *o*-Mercaptobenzoic Acid.—The pK_a of the sulfhydryl group was necessary for calculation of the relative concentrations of the monoanion and dianion of the catalyst at a given *p*H. The pK_a was obtained by titration of the monoanion of *o*-mercaptobenzoic acid with sodium hydroxide solution and by titration of the dianion with hydrochloric acid solution using a Beckman model G *p*H meter. The value of the pK_a of the mercapto group of *o*-mercaptobenzoic acid was found to be 8.40 ± 0.04 in water and 9.30 ± 0.05 in 30% acetonitrile–water (by volume). The pK_a's were determined from the inflection point of the titration curves.

Determination of the Extinction Coefficients of the Mono- and Dianion of *o*-Mercaptobenzoic Acid.—The absorption curve of *o*-mercaptobenzoic acid exhibits interesting features in the region 300–400 mμ. A representative curve is shown in Fig. 1 and the results summarized in Table I.

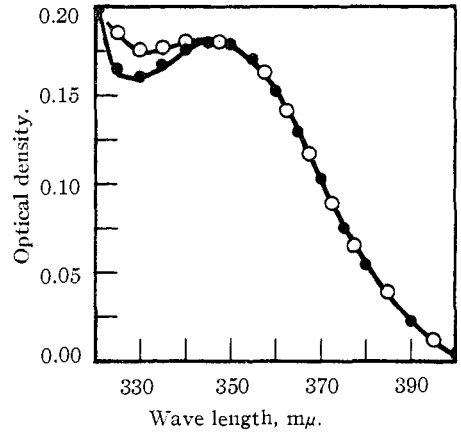


Fig. 1.—Absorption spectrum of *o*-mercaptobenzoic acid at 25° in 0.1 *M* phosphate buffers; stoichiometric concentration of *o*-mercaptobenzoic acid, 4.18×10^{-4} *M*: ●, *p*H 6.18; ○, *p*H 6.98.

The results in Table I indicate that the extinction coefficient of the dianion is very small. For practical purposes absorption at 350 mμ can be considered to be due to the monoanion, the average molecular extinction coefficient being ε 410.

TABLE I

ABSORPTION OF *o*-MERCAPTOBENZOIC ACID

Stoichiometric MBA, $M \times 10^4$	*p*H[a]	Monoanion of MBA, $M \times 10^4$	Dianion of MBA, $M \times 10^4$	Optical density, 350 mμ
4.18	6.18	4.16	0.02	0.180
4.18	7.00	4.03	.15	.175
3.97	6.60	3.91	.06	.180
5.30	6.98	5.10	.20	.215
10.60	7.00	10.18	.42	.418
11.30	8.02	8.08	3.22	.360
21.20	6.98	20.40	0.80	.820

[a] *p*H measurements at 25° with an error of ±0.02.

Absorption Characteristics of *p*-Nitrophenol in the Presence of *o*-Mercaptobenzoic Acid.—In phosphate buffers the

(11) L. Perényi, *Acta Physiol. Acad. Sci. Hung.*, **5**, 87 (1954); *C. A.*, **48**, 13382 (1954).

(12) B. M. Dirks and P. D. Boyer, *Cereal Chem.*, **28**, 483 (1951); *C. A.*, **46**, 2101 (1952).

(13) A. Stockell and E. L. Smith, *J. Biol. Chem.*, **227**, 1 (1957).

(14) S. A. Bernhard and H. Gutfreund, *Biochem. J.*, **63**, 61 (1956); B. R. Hammond and H. Gutfreund, *ibid.*, **72**, 349 (1959).

(15) C. F. H. Allen and McKay, "Organic Syntheses," Coll. Vol. II, J. Wiley and Sons, Inc., New York, N. Y., 1943, p. 580.

(16) O. Hinsberg, *Ber.*, **43**, 654 (1910).

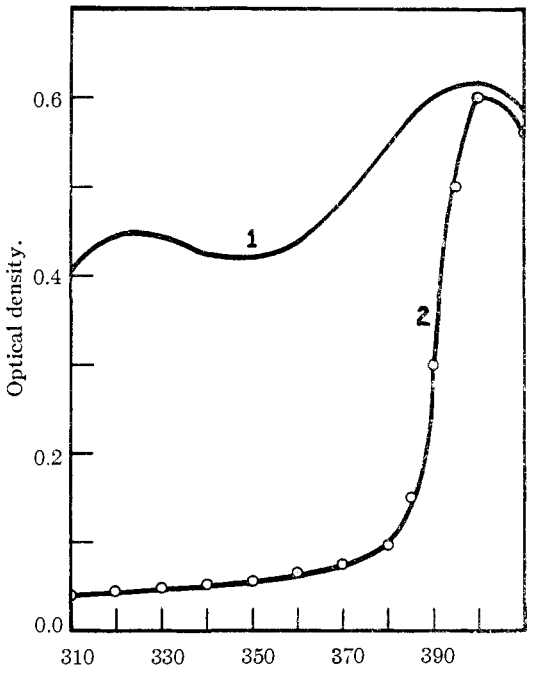


Fig. 2.—Absorption spectrum of *p*-nitrophenol (stoichiometric concentration 7.0×10^{-5} *M*) at 25° in 0.1 *M* phosphate buffers, *p*H 7.0: curve 1, no added substance; curve 2, *o*-mercaptobenzoic acid added (stoichiometric concentration 6.4×10^{-2} *M*).

values of the extinction coefficients of *p*-nitrophenol and *p*-nitrophenolate anion were found to correspond closely to those reported in the literature. However, in the presence of high concentrations of *o*-mercaptobenzoic acid (~0.01 *M*) a striking change in spectral characteristics takes place. This is illustrated in Fig. 2. Curve 1 represents the absorption spectrum of a mixture of *p*-nitrophenol and *p*-nitrophenolate anion. Curve 2 is a difference spectrum of *p*-nitrophenol and 6.36×10^{-2} *M* *o*-mercaptobenzoic acid *vs.* 6.36×10^{-2} *M* *o*-mercaptobenzoic acid. The spectral change in Curve 2 may be due to either an instrumental artifact or to an interaction between the *o*-mercaptobenzoic acid and *p*-nitrophenol species.

Results

The Formation of a Complex between the Substrate and the Catalyst.—On addition of *p*-nitrophenyl acetate to the buffer solution containing *o*-mercaptobenzoic acid an immediate increase in optical density was observed at 400 mμ. Since the molecular extinction coefficient of the ester at 400 mμ is negligible in the absence of *o*-mercaptobenzoic acid, it follows that the change in optical density must be due to some interaction between the ester and *o*-mercaptobenzoic acid.

Although no detailed investigations concerning the nature of this interaction have been undertaken, it has been shown that the increase in optical density is: (a) proportional to the ester concentration at a given concentration of the monoanion of *o*-mercaptobenzoic acid and (b) not proportional to the concentration of the dianion.

The Reaction of *p*-Nitrophenyl Acetate with *o*-Mercaptobenzoic Acid.—The rate of the reaction of *p*-nitrophenyl acetate with *o*-mercaptobenzoic acid was determined at 25.0 ± 0.2°, in phosphate buffers, over a *p*H range of 6.2 to 8.1. The results

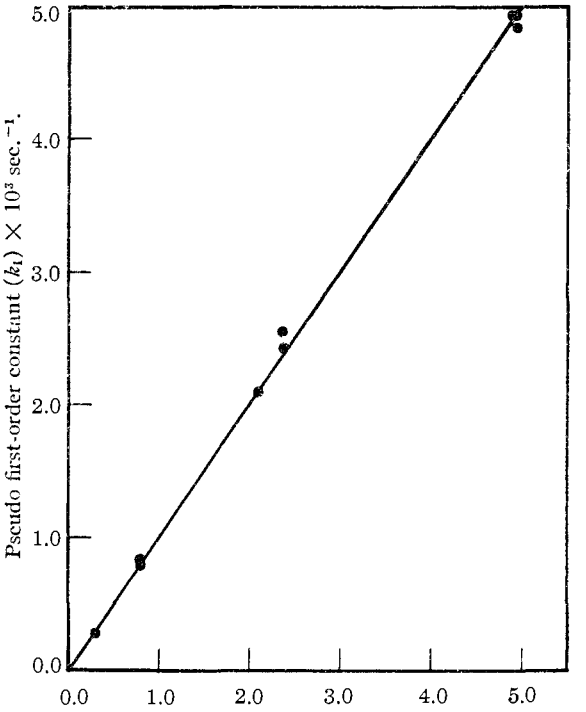


Fig. 3.—The reaction of *o*-mercaptobenzoic acid and *p*-nitrophenyl acetate in 0.1 *M* phosphate buffers at 25°.

given in Table II and presented graphically in Fig. 3 indicate that the reactive species is the dianion of *o*-mercaptobenzoic acid. The pK_a of the mercapto group of *o*-mercaptobenzoic acid was found to be 8.40 ± 0.04 and was used in the calculations of the species present at a given *p*H.

TABLE II

KINETICS OF THE REACTION OF *p*-NITROPHENYL ACETATE WITH *o*-MERCAPTOBENZOIC ACID

Stoichiometric *o*-mercaptobenzoic acid, $M \times 10^2$	*p*H	Dianion, $M \times 10^{3a}$	NPA, $M \times 10^5$	$10^3 k_1$, sec.$^{-1}$	k_2, l./mole sec.
12.9	6.23	0.81	9.2	0.84	1.04
12.9	6.23	0.81	18.4	0.79	0.98
12.9	6.98	4.90	4.6	4.85	0.98
12.9	6.98	4.90	4.6	4.95	1.00
12.9	6.98	4.90	9.2	4.95	1.00
6.2	7.00	2.38	9.2	2.43	1.02
6.2	7.00	2.38	4.6	2.57	1.08
5.4	7.00	2.10	9.6	2.10	1.00
0.113	8.02	0.311	9.0	0.310[b]	1.00

[a] The values reported for the dianion concentrations may be in error. The uncertainty arises due to existence of complex equilibria reported in the Experimental section. [b] The observed pseudo first-order constant was 0.34×10^{-3} sec.$^{-1}$. The k_1 reported above was obtained after correcting for the spontaneous hydrolysis at this high *p*H.

Attempts also were made to determine k_2 directly by carrying out the reaction at nearly equimolar stoichiometric concentrations of *o*-mercaptobenzoic acid and ester. However, since NPA is only sparingly soluble in water, a considerable amount of an organic solvent, acetonitrile,

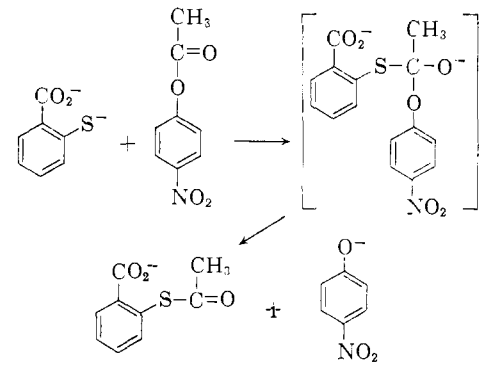
had to be added to ensure homogeneity of the reaction mixture. The rate of reaction was followed by iodometric titration of the remaining *o*-mercaptobenzoic acid. The second-order rate constant obtained for the reaction in 30% acetonitrile–water at 25.0° is 0.45 ± 0.1 l./mole sec. which is in a reasonable agreement with the value found in aqueous solution, 1.0 l./mole sec., by the spectrophotometric method. The difference in the rate constants may be due to medium effects, for example, differences in dielectric constants and effects on the complex equilibria mentioned previously.

Although the titration procedure was subject to rather large errors it would appear justifiable to claim that the second-order rate constant obtained by titration is consistent with the previous spectrophotometric results and that a compound, not subject to an attack by iodine, is formed in the initial step of the reaction. Since it was suspected that this compound is thioaspirin, it was decided to study its rate of hydrolysis.

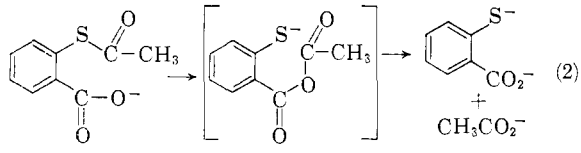
Hydrolysis of Thioaspirin.—The rate of hydrolysis of thioaspirin was determined at 25° in 0.1 *M* phosphate buffers over the *p*H range 6.20–7.90. It was mentioned in the Experimental section that two methods, spectrophotometric and iodometric, were used to estimate the liberated *o*-mercaptobenzoic acid. In our hands neither of these methods proved to be entirely satisfactory. Only an approximate value of the pseudo first-order rate constant can be reported: $k_1 = 7.0 \pm 3.0 \times 10^{-6}$ sec.$^{-1}$ compared to 3.6×10^{-6} for the hydrolysis of aspirin.[17,18] Significantly this rate constant is independent of the *p*H of the solution within the range of *p*H from 6.2 to 7.9 paralleling the behavior of aspirin. The postulate that thioaspirin constitutes an intermediate in the *o*-mercaptobenzoic acid-catalyzed hydrolysis of *p*-nitrophenyl acetate is thus seen to be consistent with the experimental data given above.

Discussion

The present work was undertaken to determine the possible catalytic properties of *o*-mercaptobenzoic acid. Because there are two functional groups in the molecule, the first question to be decided is whether the thiophenoxide ion or the carboxylate ion is the attacking nucleophile in the primary step of the reaction of *o*-mercaptobenzoic acid with *p*-nitrophenyl acetate. Since an ionized carboxylate group is only a very weak nucleophile and since it has been clearly demonstrated that the sole effective species is the dianion of *o*-mercaptobenzoic acid, it is justified to assume that the initial step of the reaction is the attack by the sulfur anion on the ester. Since the rate of disappearance of *o*-mercaptobenzoic acid is in reasonably good agreement with the rate of appearance of *p*-nitrophenoxide ion, it can be postulated that the products of the initial step are thioaspirin and *p*-nitrophenoxide ion. Thioaspirin has been found to hydrolyze in neutral aqueous solution, at a rate which is slow and reasonably similar to that of aspirin.[17,18] The spontaneous hydrolysis of aspirin has been postulated to proceed through an intramolecular catalysis of the *o*-carboxylate ion.[18,19] On the basis of the close structural analogy of aspirin and thioaspirin it is postulated that the thioaspirin formed in the initial reaction hydrolyzes according to the mechanism



(1)



(2)

The over-all process illustrated in equations 1 and 2 constitutes a catalysis of the hydrolysis of *p*-nitrophenyl acetate by *o*-mercaptobenzoic acid. It is of interest to compare this catalytic process with the catalytic processes exhibited by the enzymes papain and ficin. These two enzymes require an SH group for catalytic activity since it has been shown that these enzymes are inactivated by a single equivalent of a mercuric compound[8,13,14,20] (as is the present catalyst). Furthermore, both these enzymes exhibit a *p*H–rate profile of the catalytic (k_3) step[21] which indicates the involvement of a group with a *pK* of about 4 (4.3 for ficin and 3.7 for papain[20]) in the anionic form, presumably a carboxylate ion.[14,20] In ficin-catalyzed hydrolysis a third group with a *pK* of 8.4 appears to be involved, possibly an ammonium ion. In the system outlined above two functional groups are necessary, a mercapto group and a carboxylate ion. If one were to plot a *p*H–rate profile of the over-all catalytic action of this system, the profile would be dependent on the second slow step and would thus exhibit the same *p*H behavior as that of the papain system. It would also parallel the *p*H behavior of the ficin system in the acid region. Therefore it may be said that the catalyst *o*-mercaptobenzoic acid is a reasonable model for the enzyme papain. This catalyst also reproduces a part of the postulated mechanism for the enzyme ficin, the difference being that in the second step of the enzyme reaction,

(17) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).

(18) E. R. Garrett, THIS JOURNAL, **79**, 3401 (1957).

(19) J. D. Chanley, E. M. Gindler and H. Sobotka, *ibid.*, **74**, 4347 (1952).

(20) E. L. Smith and M. J. Parker, *J. Biol. Chem.*, **233**, 1387 (1958).

(21) The usual Michaelis–Menten formulation of an enzyme-catalyzed reaction is written:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where E, S and P are enzyme, substrate and product, respectively. The step pertinent to chemical catalysis is denoted by k_3.

TABLE III

KINETIC COMPARISON OF NUCLEOPHILES IN THEIR REACTION WITH *p*-NITROPHENYL ACETATE

Catalyst	Reaction conditions	$k_c \times 10^2$, l./mole sec.	log k_c	pK_a of catalyst	Ref.
Imidazole	26.2°, 5% dioxane	46.9	−0.323	7.04	2
Mercaptobenzoate	25°, phosphate buffer	100	0	8.4	[a]
$XC_6H_4O^-$	30°, 28.5% EtOH–H_2O	3	−1.523	8.4	[b]
Salicylate	25°, phosphate buffer	...	.....	13	[a]

[a] This investigation. [b] Calculated from the data of T. C. Bruice and R. Lapinski, THIS JOURNAL, 80, 2622 (1958).

corresponding to equation 2, an ammonium ion facilitates the attack of the carboxylate ion.

The efficiency of the catalyst *o*-mercaptobenzoic acid does not approach that of the enzymes papain and ficin. However, a comparison, shown in Table III, of the nucleophilicities of *o*-mercaptobenzoic acid and other compounds in reaction with *p*-nitrophenyl acetate reveals that *o*-mercaptobenzoic acid is indeed a powerful nucleophile. For example, *o*-mercaptobenzoic acid is a better nucleophile than a phenoxide ion of comparable pK_a. Furthermore *o*-mercaptobenzoic acid is a more effective catalyst around neutrality than salicylate because the latter does not form an appreciable amount of dianion at neutral *p*H's.

A further analogy between the model system and the enzyme action is the formation of an acyl–catalyst compound which upon intramolecular reaction leads to the products of reaction and the regeneration of the catalyst. The two-step process envisaged for the enzyme was first suggested by Smith,[13] but has been criticized by him recently on the grounds that it is thermodynamically unsound.[8] He has instead suggested that the active site in papain is not a sulfhydryl group and a carboxylate group but a combination of these of higher free energy, namely, a thiol ester. It is difficult to assess the thermodynamic argument because of the paucity of data available for the cases under question. It should be pointed out that any mechanism for catalysis of ester hydrolysis by a thiol ester is extremely complicated and probably must involve another nucleophilic group.

CHICAGO 16, ILL.

[CONTRIBUTION FROM THE SCHOOL OF PHARMACY, UNIVERSITY OF WISCONSIN]

Kinetics and Mechanism of Formation of Sulfonate from Epinephrine and Bisulfite

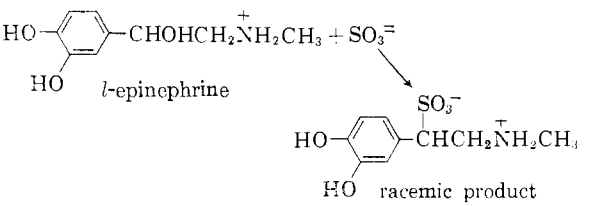
BY TAKERU HIGUCHI AND LOUIS C. SCHROETER

RECEIVED AUGUST 5, 1959

The over-all mechanism of the reaction between epinephrine and bisulfite leading to formation of a sulfonate has been studied. At *p*H values above 5 the reaction appears to be a simple SN2 reaction involving direct attack of sulfite ion on the catecholamine with an apparent heat of activation of 24 kcal. mole^{-1}. Below *p*H 5 there appears to be a parallel SN1 reaction which appears to pass through the same activated intermediate responsible for racemization of epinephrine exhibiting zero-order dependency on bisulfite or sulfite concentration. The rate expression developed on these assumptions has been found to approximate closely the observed *p*H profile.

Although some kinetic data already have been reported on the reaction observed between *l*-epinephrine and sodium bisulfite in aqueous solution, the over-all mechanism of this unexpected reaction has not been discussed in earlier publications. In the present communication it is shown that the rate of the observed reaction can best be explained on the basis of a combination of SN1 and SN2 reactions. The first-order reaction, according to our findings, seems to be directly involved with the racemization mechanism of the catecholamine. The bimolecular reaction appears to be parallel but independent of the other.

Previous studies have shown that *l*-epinephrine is gradually lost from aqueous solutions under nitrogen containing the drug and sodium sulfite between *p*H 4–7. The sulfur compound is normally present in aqueous preparations of the drug as an antioxidant. The isolated end-product and the stoichiometry of the reaction indicate the over-all reaction as illustrated in the formula diagram. The isolated product, m.p. 259° dec., has been found to be both optically and physiologically inactive.[1]



Experimental

Epinephrine solutions were prepared using synthetic *l*-epinephrine hydrochloride in acetate, phthalate or phosphate buffers of varying ionic strength. The *p*H was determined at 25°. Solutions were flushed with nitrogen and stored under a positive nitrogen atmosphere until ready for use. These solutions served as blanks. Identical solutions were prepared containing, in addition, varying concentrations of sodium bisulfite or sodium sulfite. The *p*H of these solutions was adjusted at 25° to that of the appropriate blank and then flushed with nitrogen.

Solutions employed in polarimetric studies were filled into a jacketed polarimeter tube designed so that a nitrogen atmosphere was maintained above the solution at all times. Polarimetric measurements were made on the reacting system with a Zeiss–Winkel polarimeter using 589 mμ filtered sodium light at the temperature of the thermostat. Tem-

(1) L. C. Schroeter, T. Higuchi and E. Schuler, *J. Am. Pharm. Assoc.*, **47**, 723 (1958).