evidence indicates, however, that both the Drude equation constant (λ_c) and the Moffitt equation constant (b_0) are not altered in a manner which would suggest close similarity of secondary structural change for the two cleavage products with respect to the parent protein. The alterations of the λ_0 value producing the best linear fit of the data as noted in Table III for these transformations are also different for the two types of cleavage.

5. Similarly, expectation of structural changes ensuing upon cleavage of the disulfide bonds of insulin are not fulfilled experimentally. The conversions I(Zn) → OI and I(Zn-free) → SO_3-I produce changes in λ_c, b_0 and λ_0 for the corrected curves which are not similar with respect to the native molecule (it is necessary to use the Zn free insulin values in the one case since SO_3-I does not contain appreciable Zn). The cleavage of the disulfide bridges in insulin results in a dissociation of the A and B chains, of course; this is properly an alteration in tertiary structure, but could conceivably have an unaccounted effect on the optical rotatory dispersion of the system.

6. Basically, there does not appear to be any real over-all improvement in correlation of constants for the *corrected* curves over that for the *uncorrected* ones. One must conclude that if a common secondary structural characteristic does exist exclusive of the disulfide bond contribution, it is not manifest in a common set of optical rotatory constants derived from the Moffitt or one-term Drude equations.

7. It is further evident that one cannot *a priori* account for optical rotatory changes observed when the disulfide bonds of a protein are cleaved solely in terms of the rotatory changes occurring in the S-containing amino acid residues. The observed o.r.d. changes are a function both of the solvent and of the manner of cleavage. The changes in secondary structural order which must occur when these bonds are cleaved obviously *do* have a significant effect on the optical rotatory dispersion. A more satisfactory interpretation of this relationship awaits a more detailed evaluation of the contributions of the various segments of a protein molecule.

Acknowledgment.—The author gratefully acknowledges many helpful discussions of this work with Prof. C. M. Kay, Prof. F. Haurowitz and Dr. E. O. Davisson. Mr. John G. Williams of these laboratories was largely responsible for the design of the IBM 610 computer program for solving for the Moffitt equation constants. Personnel of the Analytical Development departments of Eli Lilly and Co. were most helpful in providing product characterization data. Thorough technical assistance was given throughout all experiments by Mr. John M. Carson.

[CONTRIBUTION FROM THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS, CALIF.]

The Reaction of *p*-Nitrophenyl Acetate with Thiols[1,2]

BY JOHN R. WHITAKER

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Cysteine, and other thiols studied, hydrolyze *p*-nitrophenyl acetate at a rate dependent upon the concentration of anion, RS^-, present. The initial products of the reaction are *p*-nitrophenol and thiol ester as determined spectrophotometrically. E_a, ΔH^*, ΔF^* and ΔS^* for the reaction of cysteine with *p*-nitrophenyl acetate are 8.0, 7.4, 16.7 kcal./mole and −30.7 e.u., respectively.

Introduction

During investigation of hydrolysis of *p*-nitrophenyl acetate (NPA) by the proteolytic enzyme, ficin, it was found that cysteine, added as an activator of ficin, could also hydrolyze this ester. Previously, reduced glutathione had been found to hydrolyze *p*-nitrophenyl benzoate in essentially neutral solution.[3] Dirks and Boyer[4] reported that cysteine splits NPA by a reductive process. While the present investigation was in progress Schonbaum and Bender[5] reported the results of a study of the catalyzed hydrolysis of NPA by *o*-mercaptobenzoic acid.

The present study was undertaken to determine the mechanism by which cysteine hydrolyzes NPA with the hope that it might aid in understanding the mechanism by which such sulfhydryl enzymes as ficin and papain catalyze the hydrolysis of susceptible substrates.

Experimental

Materials.—*p*-Nitrophenyl acetate was prepared by the method of Chattaway[6] and had a melting point of 82.4–83.1° (melting point block). Reagent grade *p*-nitrophenol (NP) (Fisher) was dried at 72° in a vacuum oven for 18 hours for standard curve preparation. *n*-Propyl mercaptan was Eastman Kodak Co. white label product. The other compounds tested were obtained from Nutritional Biochemicals Corporation. Ficin was a three-times acetone-precipitated (80% v./v.) vacuum-dried powder prepared from Kadota fig latex in this Laboratory. Deionized water (Barnstead mixed-bed resin) was used throughout this work.

Methods.—Reactions were followed spectrophotometrically in a DU spectrophotometer equipped with a thermostated cell compartment. Temperatures were determined inside the reaction cuvette with thermocouples. In most cases, the rate of appearance of *p*-nitrophenolate at 402 mμ was followed; the rate of disappearance of NPA measured at 272 mμ gave identical results. The rate of formation of thiol ester was followed at 240.7 mμ, the isosbestic point of NPA and NP. The reactions were carried out in the presence of 0.064 *M* phosphate buffers and 5.0% (v./v.)

(1) This research was supported in part by a research grant, RG-5216, from the National Institutes of Health.

(2) Presented at the 140th A.C.S. Meeting, Chicago, Ill., Sept. 3–8, 1961.

(3) L. Perényi, *Acta Physiol. Acad. Sci. Hung.*, **5**, 87 (1954).

(4) B. M. Dirks and P. D. Boyer, *Cereal Chem.*, **28**, 483 (1951).

(5) G. R. Schonbaum and M. L. Bender, *J. Am. Chem. Soc.*, **82**, 1900 (1960).

(6) F. D. Chattaway, *J. Chem. Soc.*, **134**, 2495 (1931).

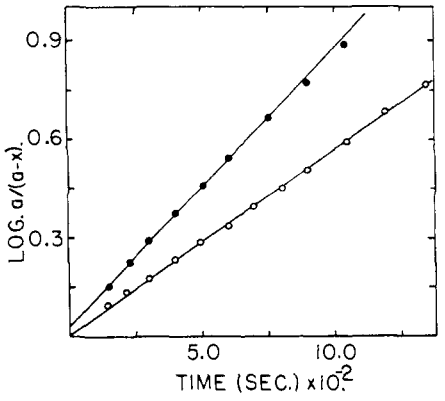


Fig. 1.—Hydrolysis of *p*-nitrophenyl acetate by cysteine and by ficin at *p*H 6.90 and 29.6°: ●, 1.00 × 10^{-5} *M* cysteine, 4.84 × 10^{-5} *M* NPA; O, 7.69 × 10^{-6} *M* ficin, 4.19 × 10^{-5} *M* NPA.

ethanol. The *p*H of the reaction mixtures was measured with a Beckman model *G* *p*H meter. Reactants were brought to temperature in a water-bath before mixing and then transferred to a cuvette with a 1.0-cm. light path (NPA in 100% ethanol added to initiate the reaction). Kinetic experiments were carried out with thiol in great excess so that pseudo-first-order kinetics were followed. Average rate constants were determined from calculated individual rate constants for at least 10 points corrected for hydrolysis in buffer alone. Each point was the result of at least three individual duplicate measurements. The pseudo-first-order rate constants were determined at a number of different concentrations of thiol. The second-order rate constants reported are the averaged values obtained by dividing by thiol concentration.

p-Nitrophenyl acetate concentration in each reaction mixture was determined spectrophotometrically by measuring *p*-nitrophenolate several hours after termination of a run. Extinction coefficients of *p*-nitrophenolate were determined under identical conditions used in the reaction mixtures. Concentrations of freshly prepared stock solutions of thiols were measured by use of N-ethylmaleimide.[7] Change in cysteine concentration during reaction with NPA was determined by the ferricyanide method[8] as NPA interfered with determination by the N-ethylmaleimide method.

Results

Reaction of *p*-Nitrophenyl Acetate with Cysteine and with Ficin.—Both cysteine and ficin (in absence of activators) rapidly hydrolyze NPA at *p*H 6.90 and 29.6° according to pseudo-first-order reaction kinetics (Fig. 1). The second-order rate constants, k_2, for these reactions are 0.208 for cysteine and 173 for ficin. Ficin concentration is calculated from protein content (biuret method[9]) and a reported molecular weight of 26,000.[10] (This ficin preparation had 58.5% as much activity on casein at *p*H 7.0 in the absence of activators as in the presence of 1.25 × 10^{-2} *M* each cysteine and versene.) Even in absence of activators and as an impure preparation ficin is approximately 830 times a more efficient catalyst than is cysteine for NPA hydrolysis. This is the order of magnitude usually found between a simple model and an enzyme.[11]

(7) E. Roberts and G. Rouser, *Anal. Chem.*, **30**, 1291 (1958).
(8) H. L. Mason, *J. Biol. Chem.*, **86**, 623 (1930).
(9) E. Layne, in "Methods in Enzymology," S. P. Colowick and N. O. Kaplan, Ed., Academic Press, Inc., New York, N. Y., 1957, Vol. 3, p. 450.
(10) S. A. Bernhard and H. Gutfreund, *Biochem. J.*, **63**, 61 (1956).
(11) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960).

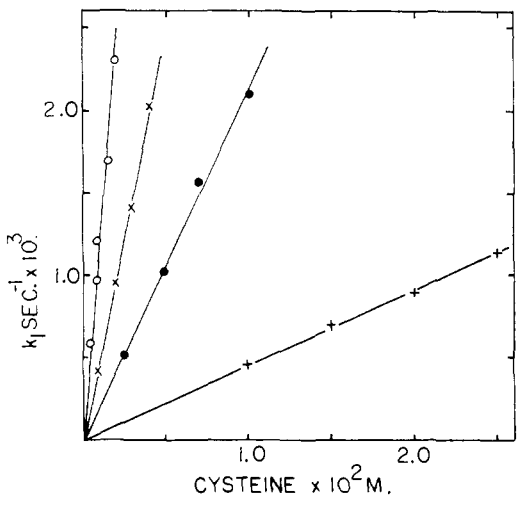


Fig. 2.—Effect of cysteine concentration and *p*H on rate of hydrolysis of *p*-nitrophenyl acetate at 29.6°: O, *p*H 7.80, 2.80 × 10^{-5} *M* NPA; X, *p*H 7.38, 2.76 × 10^{-5} *M* NPA; ●, *p*H 6.90, 4.78–11.7 × 10^{-5} *M* NPA; +, *p*H 6.20, 1.03 × 10^{-4} *M* NPA.

The rate of reaction of cysteine with NPA is a function of both NPA and cysteine concentrations over the *p*H range 6.20 to 7.80 (Fig. 2). Cysteine concentration (RSH + RS^-) was in large excess so that the reactions follow pseudo-first-order rate kinetics. At constant cysteine concentration (1.0 × 10^{-3} *M*) changing the NPA concentration from 1.25 × 10^{-5} *M* to 5.00 × 10^{-5} *M* gave a constant $t_{1/2}$ of 650 sec.

Effect of *p*H.—The second-order reaction rate constant, $k_2 = k_1/(\text{cysteine})$, increases as the *p*H increases as shown by Fig. 2 and Table I. A linear relationship is found between log k_2 and *p*H with a slope of 0.97 at 21.0° and 0.78 at 29.6° determined by the method of least squares. The data indicate that the reactive species is the RS^- and that RSH does not participate in the reaction of cysteine with NPA to produce the initial products (Table I). This will be discussed in more detail later.

TABLE I

EFFECT OF *p*H ON HYDROLYSIS OF *p*-NITROPHENYL ACETATE BY CYSTEINE

*p*H	Cysteine, *M* × 10^2	NPA, *M* × 10^5	k_2[a] liters/mole	k_2'[b] sec.
		21.0°		
6.20	12.0–14.2	10.0	0.0189	4.36
6.90	1.00–2.00	4.74	.102	4.77
7.38	0.500–1.00	2.94	.288	4.64
7.80	0.200–0.300	3.02	.658	4.42
		29.6°		
6.20	1.00–4.00	10.3	0.0446	7.45
6.90	0.250–1.00	4.78	.208	7.10
7.38	.100–0.400	2.76	.474	5.66
7.80	.0500–0.200	2.80	1.19	6.16

[a] $k_2 = k_1/(\text{cysteine})$. [b] $k_2' = k_2/(RS^-)$; pK_a cysteine at 21.0°, 8.56; at 29.6°, 8.42, calculated from data of ref. 13 using ΔH_{ion} = 6.5 kcal./mole.

Initial Products of Reaction of Cysteine with *p*-Nitrophenyl Acetate.—*p*-Nitrophenol, measured as change in absorption at 402 mμ due to *p*-nitro-

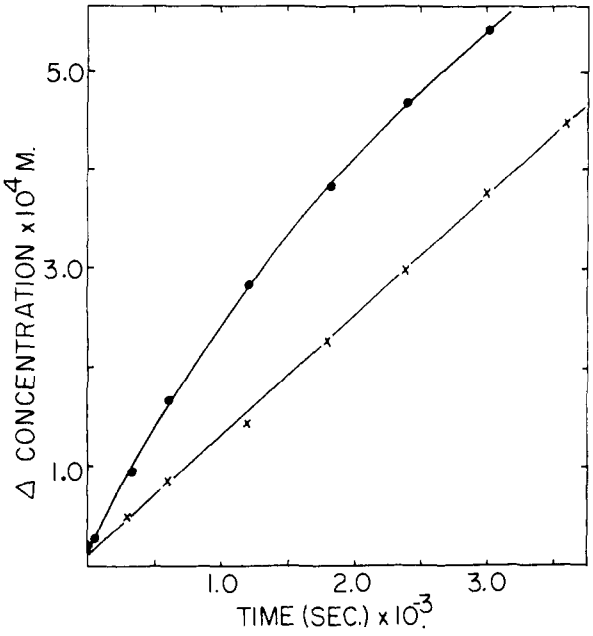


Fig. 3.—Rate of formation of *p*-nitrophenol and disappearance of cysteine during reaction of *p*-nitrophenyl acetate with cysteine at *p*H 6.20, 29.6°, 1.80 × 10^{-3} *M* cysteine and 2.50 × 10^{-3} *M* NPA: ●, rate of formation of *p*-nitrophenol; X, rate of disappearance of cysteine.

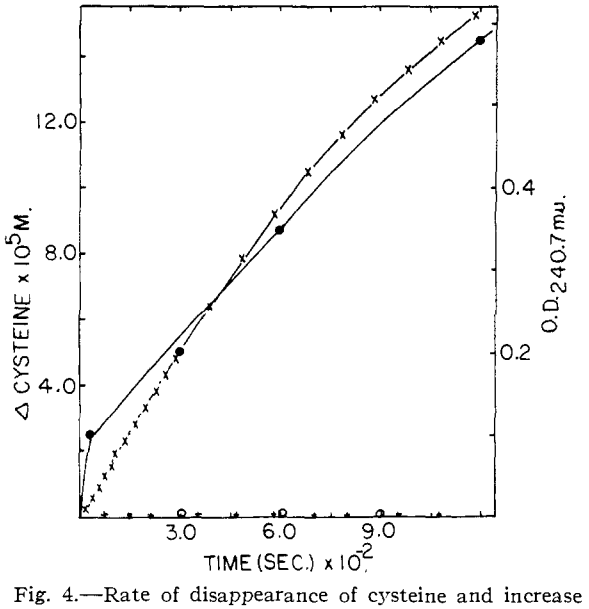


Fig. 4.—Rate of disappearance of cysteine and increase in absorbance at 240.7 mμ during reaction of *p*-nitrophenyl acetate with cysteine at *p*H 6.20, 5.50 × 10^{-3} *M* cysteine and 2.50 × 10^{-3} *M* NPA: ●, rate of disappearance of cysteine; X, increase in absorbance at 240.7 mμ. At *p*H 6.90, 1.80 × 10^{-3} *M* cysteine and 2.67 × 10^{-3} *M* NPA: O, rate of disappearance of cysteine; +, increase in absorbance at 240.7 mμ.

phenolate ion, is produced during the reaction. At *p*H 6.20, cysteine also disappears from the reaction mixture, but at a slower rate than NP is produced (Fig. 3). (Cysteine concentration was measured by the ferricyanide method which measures reactive sulfhydryl groups.) During

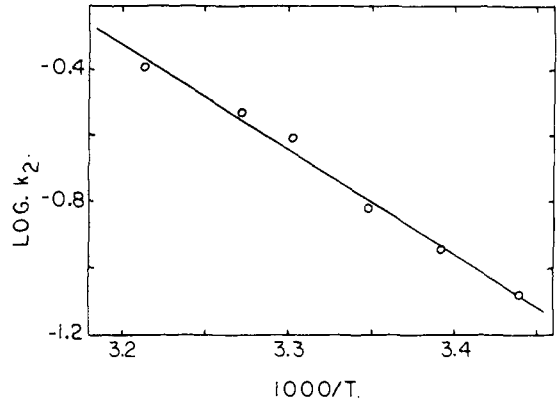


Fig. 5.—Effect of temperature on hydrolysis of *p*-nitrophenyl acetate by cysteine at *p*H 6.90, 0.400−3.00 × 10^{-2} *M* cysteine, 4.41 × 10^{-5} *M* NPA (average).

reaction at *p*H 6.20 with cysteine/NPA = 2.2 there is also an increase in absorption in the region of 220–250 mμ with maximum absorption at 233 mμ. Figure 4 shows the correlation between this rate of increase in absorption at 240.7 mμ (isosbestic point of NPA and NP) and decrease in cysteine.

Calculation of an extinction coefficient for this compound gives a value of 4100 at 240.7 mμ (5000 at 233 mμ). While this is only an approximate value, it agrees quite well with the values of 4510 and 5190 reported for β-(N-methylacetamino)-ethyl thioacetate and γ-acetaminopropyl thioacetate, respectively.[12]

Thus, both absorption maximum and extinction coefficient indicate that a thiol ester is one of the initial products. At *p*H 6.90 and cysteine/NPA = 0.72, however, there is no observed change in cysteine concentration or increase in absorption at 240.7 mμ (Fig. 4). This is true also at *p*H 7.80. If the reaction at *p*H 6.20 is carried out with cysteine/NPA of 400 there is no change in absorption at 240.7 mμ. Evidently, under these conditions, the thiol ester is being destroyed as rapidly as it is formed. At *p*H 6.90 and cysteine/NPA = 0.72, the rate of formation of *p*-nitrophenolate no longer follows pseudo-first-order rate kinetics but gives a very good second-order rate plot with k_2 = 0.204 liter/mole sec. This is in excellent agreement with k_2 = 0.208 liter/mole sec. (Table I) when the reaction is run in the presence of a great excess of cysteine.

Effect of Temperature.—Effect of temperature on reaction of cysteine with NPA at *p*H 6.90 is shown in Fig. 5. Treatment of the data by the method of least squares gives an energy of activation, E_a, of 14.5 kcal./mole. Changes in temperature affect not only rate of reaction of cysteine with NPA but also ionization of cysteine (we are concerned with its effect on the mercapto group here) and NP. Effect of temperature on ionization of NP has been compensated for by preparing the standard curve (NP concentration *vs.* absorption) under identical conditions used in the reaction. Therefore, the value of 14.5 kcal./mole includes

(12) D. S. Tarbell and D. P. Cameron, *J. Am. Chem. Soc.*, **78**, 2731 (1956).

the heat of ionization of RSH $\rightleftarrows$ $RS^- + H^+$ and energy of activation, E_a, for reaction of cysteine with NPA. Using a value of 6.5 kcal./mole as the heat of ionization of a mercapto group,[13] E_a for hydrolysis of NPA by cysteine would be 8.0 kcal./mole. From this value of E_a, ΔH^*[14] is 7.4 kcal./mole, ΔF^* is 16.7 kcal./mole and ΔS^* is -30.7 e.u. at 29.6°. ΔH^* is about the same as reported for imidazole-catalyzed hydrolysis of NPA (7.4 *vs.* 7.8), ΔF^* is a little smaller (16.7 *vs.* 18.5) and ΔS^* is a little more positive (-30.7 *vs.* -35.3).[15]

Effect of Other Compounds on Hydrolysis of *p*-Nitrophenyl Acetate.—Elimination of the mercapto group (alanine) completely destroys the ability to hydrolyze NPA (Table II). Thiourea is also without effect. Tryptophan can hydrolyze NPA to some extent. The other thiols tested are also very good nucleophiles, being somewhat better than cysteine. Cysteine may be less effective than other thiols tested because of proximity of the ammonium group. It may also be due to the difficulty of assigning a single representative pK_a for ionization of the mercapto group because of the presence and similar acidity of the ammonium group. This problem has been discussed by Benesch and Benesch.[13] k_2'-Values for 2-mercaptoethanol, sodium mercaptoacetate and hydroxylamine agree well with values reported by Jencks and Carriuolo[16] (see Table II). These values are considerably higher than those reported for *o*-mercaptobenzoic acid.[5]

TABLE II

EFFECT OF SEVERAL COMPOUNDS ON *p*-NITROPHENYL ACETATE HYDROLYSIS AT 29.6°

Compound	pK_a	Concn., $M \times 10^2$	NPA, $M \times 10^5$	k_2, liters/mole sec.	k_2',[a] sec.
		*p*H 6.90			
Cysteine	8.42[b]	0.250–1.00	4.78	0.208	7.10
Glutathione	9.09[b]	0.712–1.43	4.10	.148	23.1
2-Mercaptoethanol	9.32[c]	2.00–3.50	4.50	.0493	13.0
Sodium mercaptoacetate	9.90[d]	4.00–7.00	4.60	.0472	49.5
Sodium β-mercaptopropionate	10.03[c]	9.00–13.5	4.12	.0149	20.1
n-Propyl mercaptan	10.5[e]	0.788	4.40	.0315	147
Hydroxylamine	6.2[f]	0.0600–0.100	3.99	1.92	2.3
	Reported k_2'	*p*H 7.80			
Cysteine		0.0500–0.200	2.30	1.19	6.16
Glutathione		.0950–0.190	2.78	1.02	20.9
2-Mercaptoethanol	11.0[g]	.500–1.00	2.74	0.343	11.8
Sodium mercaptoacetate	41.7[g]	.500–2.00	2.77	.265	33.5
Sodium β-mercaptopropionate		1.00–2.00	2.75	.0988	16.8
n-Propyl mercaptan		1.18	2.76	.146	73.5
Hydroxylamine	1.8[f]	0.0500–0.100	2.79	2.16	2.2
o-Mercaptobenzoic acid	1.0[h]				
Imidazole	0.48[f]				
Histidine		1.00–2.00	2.81	0.0832	0.085[i]
Trytophan		0.800	2.76	0.0199	
Thiourea		4.00	2.78	0	
Alanine		1.00	2.76	0	

[a] $k_2' = k_2$/(active species). [b] Calculated from ref. 13 using $\Delta H_{ion} = 6.5$ kcal./mole. [c] J. P. Danehy and C. F. Noel, *J. Am. Chem. Soc.*, **82**, 2511 (1960). [d] Titration pK_a at 20° from previous ref. *c* corrected to 29.6° using $\Delta H_{ion} = 6.5$ kcal./mole. [e] D. L. Yabroff, *Ind. Eng. Chem.*, **32**, 257 (1940), corrected to 29.6° using $\Delta H_{ion} = 6.5$ kcal./mole. [f] W. P. Jencks, *J. Am. Chem. Soc.*, **80**, 4581 (1958). [g] Ref. 16 at 25.0°. [h] Ref. 5. [i] $pK_a = 6.0$; E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

Discussion

Hydrolysis of NPA by thiols is believed to proceed according to the reaction

$$RCOR + R''S^- \underset{k_2}{\overset{k_1}{\rightleftarrows}} \left[\begin{matrix} O \\ | \\ R'COR \\ | \\ S \\ | \\ R'' \end{matrix} \right]^- \underset{k_4}{\overset{k_3}{\rightleftarrows}} RO^- + R'\overset{O}{\overset{\|}{C}}SR'' \quad (1)$$

Treatment of the tetrahedral intermediate, $\left[\left(\begin{matrix} O \\ \| \\ R'COR \end{matrix} \right) (R''S^-) \right]$, as a high energy intermediate by the method of stationary state gives the following differential equation for rate of appearance of *p*-nitrophenolate ion, RO^-

$$d(RO^-)/dt = [k_1k_3(NPA)(RS^-) - k_2k_4(RO^-)(\text{thiol ester})]/(k_2 + k_3) \quad (2)$$

(RO^-) was followed spectrophotometrically and was very small ($\sim 10^{-5}$) as was (thiol ester). Therefore, if it may be assumed that $k_1k_3(NPA)(RS^-) >> k_2k_4(RO^-)(\text{thiol ester})$, eq. 2 would reduce to

$$d(RO^-)/dt = \frac{k_1k_3}{k_2 + k_3}(NPA)(RS^-) = k_2'(NPA)(RS^-) \quad (3)$$

For kinetic studies, the concentration of thiol was much greater than NPA concentration; therefore, eq. 4 was found to describe the rate of reaction.

$$d(RO^-)/dt = k_2'(NPA)(RS^-) = k_1(NPA) \quad (4)$$

where k_1 is the observed pseudo-first-order reaction rate constant. Equation 4 would be expected to be valid at the lower *p*H values studied as well, even though (RS^-) is smaller than (NPA). This is so because (RS^-) is maintained constant through replenishment according to eq. 5. Combining

$$(RS^-) = K_{eq}(RSH)/(H^+) \quad (5)$$

eqs. 3 and 5 gives eq. 6.

$$d(RO^-)/dt = k_2'K_{eq}(RSH)(NPA)/(H^+) = k_2(RSH)(NPA) \quad (6)$$

It should be clear that k_2 is *p*H dependent so that a plot of log k_2 *vs.* *p*H would be expected to give a straight line with unit slope. This was found experimentally (Table I) where the slope, determined by the method of least squares, was

(13) R. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).

(14) From the value of E_a, ΔH^* was obtained from the relationship $\Delta H^* = E_a - RT$. The value of ΔF^* was obtained by means of the equation $-\Delta F^* = 2.3\,RT \log k_{rate}\,h/KT$ (see A. Frost and R. Pearsons, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 95); ave. $k_2' = 6.59$ liters/mole sec. at 29.6° (see Table I).

(15) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **79**, 1663 (1957); calculated from data at 30°.

(16) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).

0.97 at 21.0° and 0.78 at 29.6°. Equation 6 also explains why a plot of log k_2 *vs.* $1/T$ should give a combined value including ΔH_{ion} of $RSH \rightleftarrows RS^- + H^+$ and E_a for reaction of NPA with RS^-.

It appears that eq. 1 adequately describes the experimental data and that the attack on NPA is by RS^- which is shown to be a strong nucleophile. Formation of thiol ester as one of the products is supported by spectrophotometric data. Evidence for formation of thiol ester was found only at the lower *p*H values and at low, nearly equal, concentrations of reactants indicating that the thiol ester is hydrolyzed quite rapidly. Thiol esters which contain a hydroxyl or amine group in a suitable position would be expected to also be split by intramolecular attack by these groups[17,18] as well as by hydroxide ion catalysis. Rate of reaction was also found to be independent of ionic strength (up to 0.50; above this it decreased slowly) as would be expected from eq. 1. The reaction cannot be carried out in the presence of acetone because of its inhibition of the reaction.

(17) W. P. Jencks, S. Cordes and J. Carriuolo, *J. Biol. Chem.*, **235**, 3608 (1960).

(18) T. Wieland, W. Schafer and E. Bokelmann, *Ann. Chem.*, **573**, 99 (1951).

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF G. D. SEARLE AND CO., CHICAGO 80, ILL.]

The Rearrangement of Sulfoxides of Pyrimido[5,4-b][1,4]thiazines

BY ELMER F. SCHROEDER AND R. M. DODSON[1]

RECEIVED DECEMBER 7, 1961

The crystallization of the pyrimidothiazine-5-oxide (IV) from methyl alcohol, ethyl alcohol or acetic acid produced the corresponding 6-methoxy-, 6-ethoxy- or 6-acetoxypyrimidothiazine (VIIIb, a, or d). When IV was stirred with water or allowed to stand at room temperature in the crystalline state, it was rearranged to the corresponding 6-hydroxy compound VIIIc. The course of this rearrangement was established by the conversion of VIIIa, *via* the amide XIIIa, and desulfurization with Raney nickel, to 1-propyl-3-ethyl-6-aminouracil (XIV) and ethoxyacetamide (XV). The rearranged products VIIIa, b, c, d and XIa were also independently synthesized from the corresponding 6-halo- or 6,6-dihalopyrimidothiazines IXa, b, c. The 6-ethoxypyrimidothiazine-5-oxide (X) in aqueous ammonium hydroxide solution was rapildy converted to the corresponding thiazolopyrimidine-2-carboxamide (XVIa). The structure of the thiazolopyrimidine was established by its independent synthesis and by its conversion to the simpler thiazolo[4,5-d]pyrimidine (XVII). A mechanism, involving the intermediate formation of a 4,1-azathionium ion (XXIV) has been proposed to explain these extremely facile rearrangements.

Recently a series of 1,3-disubstituted-5-halo-6-aminouracils has been described.[2] Since several members of this series exhibited significant biological activity as bronchodilators and antihypertensive agents, it became desirable to prepare a number of derivatives for further testing. Accordingly, five of the chlorouracils I were converted into the corresponding 6-amino-5-(carboxymethylthio)-uracils (II) by reaction with mercaptoacetic acid in alkaline solution. On treatment with acetic anhydride for several hours at 100°, the mercaptoacetic acids cyclized with loss of water to yield derivatives III of the hitherto unknown pyrimido-[5,4-b][1,4]thiazine ring system.[3]

Tables I and II describe the compounds of types II and III, respectively, which have been prepared. The compounds of both series are stable, readily crystallizable solids. They are quite insoluble in water but dissolve in dilute sodium hydroxide solution from which they are reprecipitated upon acidification. The mercaptoacetic acids II could not be obtained when 5-bromo-6-aminouracils were used in place of the 5-chloro derivatives I. Instead, reductive debromination occurred, with formation of 6-aminouracils unsubstituted in position five.[4]

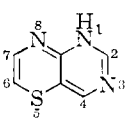
One representative pyrimido[5,4-b][1,4]thiazine, the 1-propyl-3-ethyl derivative IIIb, was selected for further chemical study because of the favorable effect of these particular alkyl groups on activity and toxicity of pyrimidines and purines tested as bronchodilators. It was found that the 5-oxide of this pyrimido[5,4-b]thiazine (IV) and the 5-oxide of one of its derivatives (X) were extremely labile compounds. The rearrangements of these sulfoxides and the proofs of structure of the compounds obtained from them are discussed in the following sections.

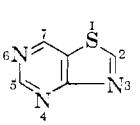
The Sulfoxide Rearrangement.—Oxidation of 1-propyl-3-ethyl-1*H*-pyrimido-[5,4-b][1,4]thiazine-2,4,7(3*H*,6*H*,8*H*)-trione (IIIb) in dry, alcohol-free chloroform with one equivalent of perbenzoic acid in benzene gave an excellent yield of a highly insoluble material which, after crystallization from butanone, gave good analytical values for the sulfoxide IV. The new compound was acidic as expected, but was unstable in aqueous suspension, giving rise to a highly colored purple product in a few hours even at room temperature. It was also quite insoluble in chloroform, benzene and ethyl acetate. It appeared very probable that the

(1) Present address: University of Minnesota, Minneapolis 14, Minn.

(2) E. F. Schroeder, U. S. Patents 2,731,465, Jan. 17, 1956, and 2,958,692, Nov. 1, 1960.

(3) The compounds in this paper are named as derivatives of 1H-pyrimido-[5,4-b][1,4]thiazine and thiazolo[4,5-d]pyrimidine. However, to aid in following the formulas, in all cases the pyrimidine ring





has been drawn on the left with the sulfur in the upper right. We are indebted to Dr. Leonard T. Capell of "Chemical Abstracts" for advice on nomenclature.

(4) V. Papesch and E. F. Schroeder, *J. Org. Chem.*, **16**, 1879 (1951). Similar reductive debrominations with mercaptans and thiamides have been detected previously; *e.g.*, see ref. 10.