oxime ($\lambda_{max.}$ = 245 mμ, log ϵ = 4.02) which indicates an in-plane conformaton (9).

The reason for the different conformations for *1* and *4* is not apparent. The stability of *1* appears to be a unique property associated with the *o*-trifluoromethyl group and the aldoxime function.

EXPERIMENTAL

Nitrone Formation—The procedure of Buchler for the synthesis and isolation of nitrones and *o*-methyl benzaldoxime ethers was used (7).

o-Trifluoromethylacetophenone oxime (4)—Compound *4* was prepared by Bachmann's procedure using hydroxylamine hydrochloride in pyridine and absolute ethanol (10). Yield 75%, m.p. 118–120°.

Anal.—Calcd. for $C_9H_8F_3NO$: C, 53.20; H, 3.94; N 6.90. Found: C, 53.44; H, 4.08; N, 6.85.

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Kinetics of the Reaction of 1,3-Dihyro-1-hydroxy-3-oxo-1,2-benziodoxole with Cysteine

WALTER WOLF and THERESA WU*

Abstract □ The kinetics of the oxidation of cysteine by 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole were studied in buffered solutions at pH 6.8, 7.2, 7.6, and 8.0 by means of a rapid stop flow system. The second-order rate constants obtained were 50 moles/l. sec., 150 moles/l. sec., 835 moles/l. sec., and 1175 moles/l. sec., respectively. One or more reactive intermediates are postulated and their possible structure and the mechanisms involved are discussed, involving the intermediate formation of an (unstable) iodine-sulfur bond.

Keyphrases □ Cysteine oxidation—kinetics □ 1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole—cysteine oxidation □ UV spectrophotometry—analysis

"*o*-Iodosobenzoic acid" has been used for some time as a reagent for mercapto groups, and the reaction that occurs has a stoichiometry of 1 mole of oxidizing agent to 2 moles of the thiol (1). Since it has been proven that "*o*-iodosobenzoic acid" has a cyclic structure, namely, 1,3-dihydro-1-hydroxy-3-oxo-2-benziodoxole (I) (2), it was of interest to investigate the kinetics of this reaction, to determine if this reaction occurred as a three-center process involving 2 moles of cysteine and one of benziodoxole; or as a two-center reaction, involving 1 mole of benziodoxole and 1 mole of cysteine, to give in a first, rate-determining step, a reactive species. It had been noted before that the mechanism of oxidation of thiols depends upon the nature of the oxidant, and that the rate-determining step is the oxidation of the thiol to a reactive intermediate (3). The kinetics of this reaction have been studied by using potassium ferricyanide (3), potassium persulfate (4), hydrogen peroxide (5), and sodium 2,6-dichlorobenzophenone indophenol (6). Therefore a study to determine the kinetics of the oxidation of cysteine with 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole was undertaken, in the pH range 6.8–8.0, in order to compare the action of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole, a radiomimetic agent, with that of the above-mentioned, more conventional oxidizing agents.

EXPERIMENTAL

A mixing chamber, built by Nieman[1] was utilized. First, however, it had to be modified because of the easy formation of air bubbles in this type of manual-injection system. The authors, therefore, designed a simplified version (Fig. 1) consisting of a

[1] Made available through the Department of Chemistry, California Institute of Technology.

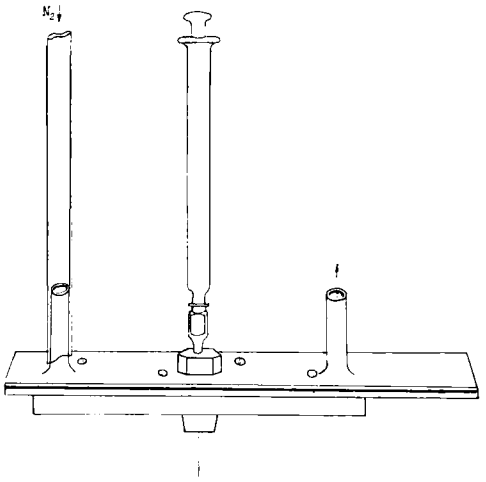


Figure 1—*Diagram of the manual injection system.*

single-injection inlet mounted directly on the cover of a spectrophotometer[2] and having gas inlet and outlet vents, so that the mixing system could be flushed with nitrogen and other gases. According to Sturtevant (7), a similar system would give a complete mixing time of 10 msec. and the authors' results appear to bear out his calculations.

A typical run was conducted in the following manner: a cell filled with 2.8 ml. of one of the reactants, usually I (10^{-3} to $10^{-4}M$ concentrations were used routinely), was placed in the light beam; 0.2–0.3 ml. of the other reagent (also at 10^{-3} to $10^{-4}M$ concentrations) was placed in a syringe fitted in its place on the modified cover; both solutions were in the appropriate 0.067 *M* phosphate buffer. The spectrophotometer was then turned on at a suitable wavelength. After displacing most of the air in the spectrophotometer compartments by dry nitrogen (in order to minimize air oxidation of cysteine), the optical monitoring was begun. The reducing solution was injected, rapidly and by hand, and the change in absorbance recorded. Figures 2 and 3 show, respectively, a recording and oscilloscope display of typical runs. All studies were conducted at room temperature (20 ± 3°). The results were analyzed by assuming that the only absorbing species are cysteine, cystine, I, and *o*-iodobenzoic acid. All chemicals used were analytical reagents and were purified by recrystallization before use. Knowing the initial concentration of cysteine and I, and the molar absorption coefficients of the four substances at the particular pH, the following formula was used to calculate the remaining concentration of I at the time *t*:

$$Y = \frac{X - CD - F(G - 2D + H)}{B - G + 2D - H}$$

where X = absorbance, observed; B = molar absorption coefficient, at 288 mμ, of benziodoxole; C = initial concentration of cysteine, at $t = 0$; D = molar absorption coefficient of cysteine at 288 mμ; F = initial concentration of benziodoxole at $t = 0$; G = molar absorption coefficient of cystine at 288 mμ; H = molar absorption coefficient of *o*-iodobenzoic acid at 288 mμ.

From the above, first-, second-, and third-order rate constants were computed. For these calculations, one of two computers was used.[3]

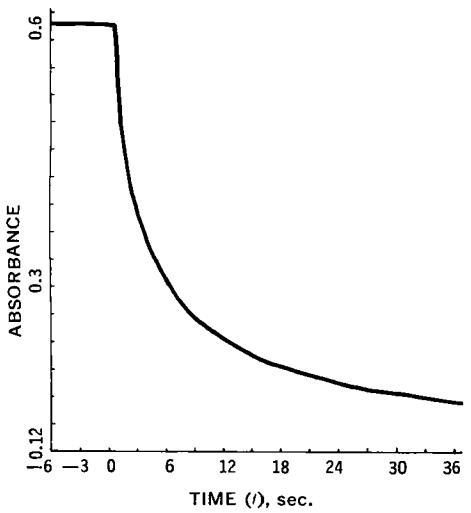


Figure 2—*Paper chart display of absorbance changes upon reaction of* I *with cysteine, pH 7.2.*

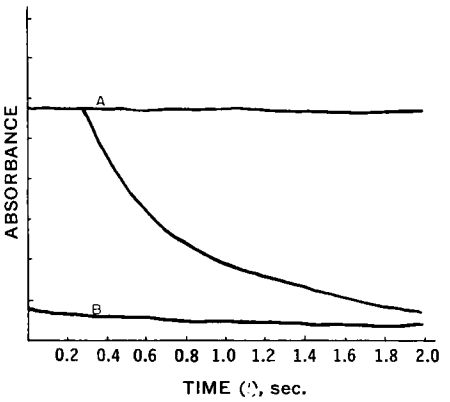


Figure 3—*Oscilloscope display of absorbance changes upon reaction of* I *with cysteine, pH 8.0. Key:* A, *initial absorbance;* B, *final absorbance.*

RESULTS AND DISCUSSION

The results obtained give a good fit, assuming a second-order rate, for the reaction of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole (I) with cysteine. The rate constants vary little after the initial first few percentages of the reaction and remain constant over approximately 90% of the reaction. The discrepancy in the very early stages may be due to errors in measurement, to poor mixing and/or to recorder inertia.

As the reaction, which stoichiometrically involves three molecules, one of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole and two of cysteine, fits a second-order rate, it follows that the rate-determining step involves only two molecules, one of I and one of cysteine, where $k_1 < k_2$.

$$\text{I} + \text{cysteine} \xrightarrow{k_1} \text{reactive intermediate(s)}$$

$$\text{reactive intermediate(s)} + \text{cysteine} \xrightarrow{k_2} \text{cystine} + \text{o-iodobenzoic acid}$$

Table I—Second-Order Rate Constants

pH	k, moles/l. sec.
6.8	50 ± 16
7.2	150 ± 20
7.6	835 ± 75
8.0	1175 ± 120

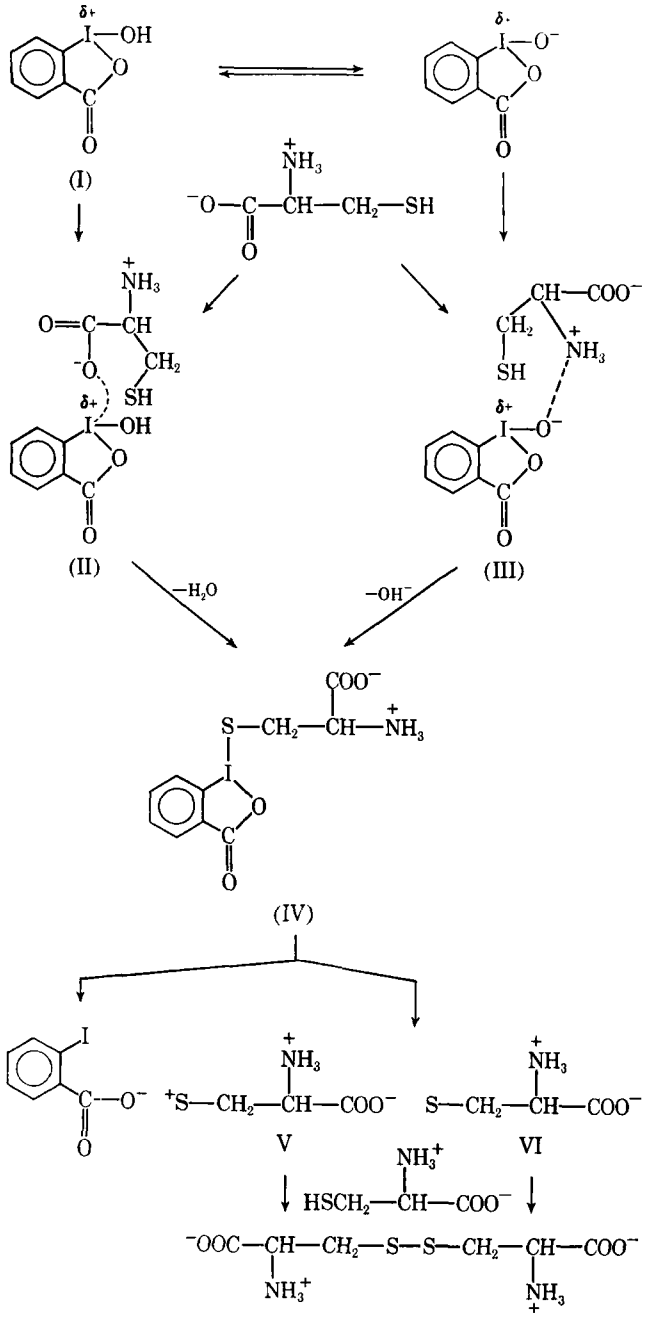
The problem now is: what is the nature of this reactive intermediate; and is it a single compound, or does it involve, in turn, a series of fast-reacting intermediate species?

Table I summarizes the change of the rate constant as a function of pH.

These values have been plotted in Fig. 4, and it can be seen that they do not fit a straight line. At pH 7.6 and 8.0, where the reaction

[2] Gilford-Beckman.

[3] Either a Honeywell 400 or a Mathatronics 480 desk model was used.



Scheme I—Suggested reaction mechanism for the interaction of I with cysteine, as a function of pH.

is much faster than in media of higher acidity, a large proportion of I is ionized (64% and 82%, respectively).

It had been noted that, in the crystalline state, the iodine in I acts as a weak Lewis acid (8). As cysteine, at *e.g.* pH 6.8, exists as a zwitterion, with only 4.4% of the thiol ionized, it can be assumed that the carboxylate ion can attach itself to the iodine, to form a complex such as II (Scheme I), where the steric relationship of sulfur and iodine would be such as to favor a reaction between these two atoms. When the ionized form of I predominates, a formal ionic interaction may occur between the ammonium group and the ionized oxygen (III), and the attachment of sulfur to iodine is favored by the increasing ionization of the thiol function. In both cases, an intermediate, perhaps best represented by IV, would be formed. Such an intermediate species is likely in view of the growing quantity of experimental evidence for structures having a reactive iodine-sulfur bond in transition

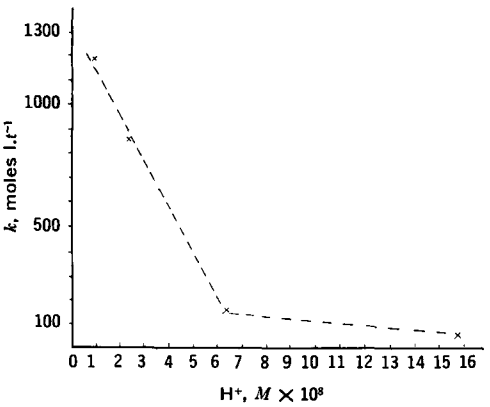


Figure 4—*Plot of the second-order rate constants* (k) versus *the hydrogen-ion concentrations in the pH range 6.8–8.0.*

states. Thus, both Cunningham (9) and Kaufman (10) have postulated sulfenyl iodides during the iodination of certain proteins, and the formation of stable iodonium-sulfur bonds has been proven by Sandin (11). In related work, Sandin's observations have been extended (12) and it seems reasonable to believe that trivalent iodine-sulfur bonds can be quite stable, specifically if the iodine atom is part of the heterocyclic ring. It has also been noted that when aromatic thiols are allowed to react with I, similar transient yellow products are formed, as in the studies mentioned above.

The problem unanswered so far is that of the subsequent fate of product IV. The iodine-sulfur bond can be broken either homolytically or heterolytically, to give sulfenium ion V or a free radical such as VI. Work is in progress to determine which of these two alternatives is operative.

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