Hydrolysis of Idoxuridine

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Data are presented for the hydrolytic degradation of idoxuridine (5-iodo-2′-deoxyuridine) in aqueous solution over a wide range of hydrogen ion concentration (pH 1.3 to 12.0). The over-all reaction rate was experimentally equal to $k_1 \frac{[H^+]}{[H^+] + K_a} + k_2 \frac{K_a}{K_a + [H^+]} + k_3 \frac{K_a}{K_a + [H^+]} [OH^-]$. The heat of activation for the hydrolysis of idoxuridine at pH 6.0 was found to be approximately 25 Kcal. per mole. A probable pathway for the hydrolysis of idoxuridine in acid and alkaline solution is discussed.

IDOXURIDINE (5-iodo-2′-deoxyuridine) has been reported to be an effective chemotherapeutic agent for the treatment of herpetic keratitis (1). Its chemical, biological, and pharmaceutical properties have been described previously (2). Results of clinical studies have also been reported (3, 4). There are several reports that discuss the enzymatic pathways for the metabolism of idoxuridine and related pyrimidines (5–7). However, the literature is lacking in information concerning its chemical degradation. The scope of the present work was concerned with the hydrolytic degradation of idoxuridine in acidic and alkaline solution.

EXPERIMENTAL

Materials.—The idoxuridine, 5-iodouracil, 2′-deoxyuridine, and uracil used in this study were obtained from California Corporation for Biochemical Research, Los Angeles, Calif. All reagents used were of analytical grade.

Apparatus.—A Cary model 11 recording spectrophotometer, Beckman zeromatic pH meter, constant temperature oven, and Pyrex glass chromatographic columns 19 mm. in diameter and 25 cm. long fitted with a stopcock were used.

Chromatographic Columns.—The chromatographic columns were packed according to the previously described procedure (8).

Assay.—A satisfactory partition chromatography system was developed and subsequently used to separate the ingredients of the hydrolyzed samples (8). A typical chromatogram of a known equimolar mixture of idoxuridine, 5-iodouracil, 2′-deoxyuridine, and uracil is shown in Fig. 1. The chromatogram of an artificially decomposed sample is also shown in Fig. 1. The results indicate that idoxuridine can be separated from its breakdown products by this procedure.

Hydrolysis Experiments.—In most cases the following procedure was used: 100 mg. (0.0028 mole) of idoxuridine was dissolved in 100 ml. of the appropriate buffer solution. The solution was placed in 10-ml. ampuls. The ampuls were sealed and heated at 60° in a constant temperature oven Ampuls were removed at the appropriate time intervals, cooled, and analyzed.

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RESULTS AND DISCUSSION

Order of Reaction.—The rate of disappearance of idoxuridine from the solution was an over-all first-order reaction with respect to idoxuridine at pH values from 1.3 to 12.0 at constant temperature. There was a linear relationship between time and logarithm of residual idoxuridine concentration as shown in Fig. 2. The observed rate constants at the respective pH values are listed in Table I. There was no significant change in the apparent first-order rate constant when the initial concentration of idoxuridine was varied.

Primary Salt Effect.—A primary salt effect should be expected where a reaction between two charged species is involved in a hydrolytic process. Since idoxuridine exists primarily in the unionized form in highly acidic and relatively neutral solutions, one would expect that the primary salt effect would be negligible. Experiments carried out at pH 1.3 and 6.3 showed that there was no primary salt effect. However, at pH 12.0, where idoxuridine exists in the ionized form, a primary salt effect was expected. This was borne out during the study so that all

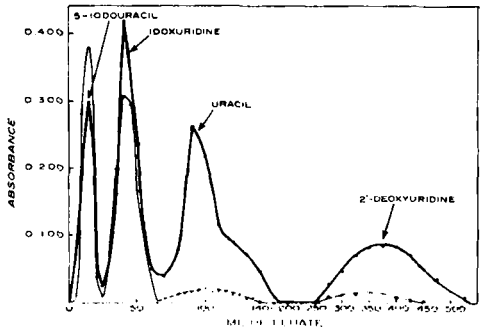

Fig. 1.—Comparison of partition chromatograms for a known equimolar mixture of idoxuridine and its possible degradation products and an artificially degraded sample stored for 18 months at 37°. 0.1 *N* HCl was used as the internal phase; a mixture consisting of five parts chloroform and one part *n*-butanol was used as the eluant. Data for the known mixture were previously reported (8). Key: ● known mixture; O, artificially degraded sample.

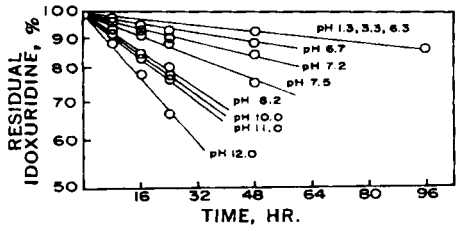

Fig. 2.—Plot showing the over-all first-order character of the hydrolysis of idoxuridine at different pH values at 60°. For the actual runs, the pH was adjusted as follows: 0.1 *N* hydrochloric acid (pH 1.3), 0.02 *M* citrate (pH 3.3), 0.036 *M* phosphate (pH 6.3, 6.7, 7.2, 7.5), 0.06 *M* borate (pH 8.2), and 0.1 *N* NaOH (pH 10.0, 11.0, and 12.0).

experiments at pH values above 10.0 were carried out in solutions adjusted to constant ionic strength with sodium chloride.

pH Dependence.—The pH-rate profile indicated in Fig. 3 shows the relationship between pH and the observed reaction rate over a pH range from 1.3 to 12.0. The nature of the curve suggests that the over-all degradative rate for the hydrolysis of idoxuridine represents a summation of the following reactions:

$$\text{Idoxuridine} \xrightarrow{k_1} \text{products}$$

$$\text{Idoxuridine}^- \xrightarrow{k_2} \text{products}$$

$$\text{Idoxuridine}^- + \text{OH}^- \xrightarrow{k_3} \text{products}$$

The over-all reaction velocity is equal to the sum of the rates of these reactions and can be represented by

$$k_{obs.} = k_1 \frac{[H^+]}{[H^+] + K_a} + k_2 \frac{K_a}{K_a + [H^+]} + k_3 \frac{K_a}{K_a + [H^+]} [OH^-] \quad \text{(Eq. 1)}$$

The theoretical rate of disappearance of idoxuridine was calculated for the pH range of 1.3 to 12.0 using the following coefficients in the above rate equation: $k_1 = 0.0015$ hour^{-1}; $k_2 = 0.0105$ hour^{-1}; $k_3 = 0.5600$ hour^{-1}; K_a (acid dissociation constant) $= 10^{-8.0}$.[1] The calculated rate appears as the solid line in Fig. 3. It is in good agreement with the experimentally determined rate.

The data indicate that the rate of breakdown is independent of pH over the range 1.3 to 6.3. In this region $[H^+]$ is high compared to K_a; therefore, the values of $k_2 \frac{K_a}{K_a + [H^+]}$ and $k_3 \frac{K_a}{K_a + [H^+]} [OH^-]$ are negligible compared to $k_1 \frac{[H^+]}{[H^+] + K}$ and may be omitted, thus

$$k_{obs.} = k_1 \frac{[H^+]}{[H^+] + K_a} \quad \text{(Eq. 2)}$$

Between pH 6.3 and approximately 9.0 there is a sharp increase in the observed reaction rate. This coincides with an increase in the ionized form in solution. $k_3 \frac{K_a}{K_a + [H^+]} [OH^-]$ is still negligible in this region. Thus in pH range 6.3 to 9.0, Eq. 1 may be expressed as

$$k_{obs.} = k_1 \frac{[H^+]}{[H^+] + K_a} + k_2 \frac{K_a}{K_a + [H^+]} \quad \text{(Eq. 3)}$$

Between pH 9.0 and 11.0 the observed reaction rate tends to level off. In this region idoxuridine exists mainly in the ionized form. k_1 is small compared to k_2 and k_3, and the $[OH^-]$ is low, which makes the values of $k_1 \frac{[H^+]}{[H^+] + K_a}$ and $k_3 \frac{K_a}{K_a + [H^+]} [OH^-]$ negligible compared to $k_2 \frac{K_a}{K_a + [H^+]}$, and they may be disregarded giving

$$k_{obs.} = k_2 \frac{K_a}{K_a + [H^+]} \quad \text{(Eq. 4)}$$

Above pH 11.0 the reaction rate increases rapidly. In this region $k_1 \frac{[H^+]}{[H^+] + K_a}$ is negligible and may be eliminated from Eq. 1 which may be written

$$k_{obs.} = k_2 \frac{K_a}{K_a + [H^+]} + k_3 \frac{K_a}{K_a + [H^+]} [OH^-] \quad \text{(Eq. 5)}$$

In this pH range the reaction is base catalyzed and changes from first order to pseudo first order.

TABLE I.—EXPERIMENTAL RATE CONSTANTS FOR THE HYDROLYSIS OF IDOXURIDINE AT 60° C.

pH	Observed First-Order Rate Constant hr.$^{-1}$ × 10^3	
1.3	1.51	
3.3	1.51	
6.3	1.51	1.54[a]
6.7	2.44	
7.2	3.31	
7.5	5.73	
8.2	8.80	
10.0	10.35	
11.0	10.90	
12.0	16.70	

[a] Initial concentration, 0.075% (0.0021 *M*).

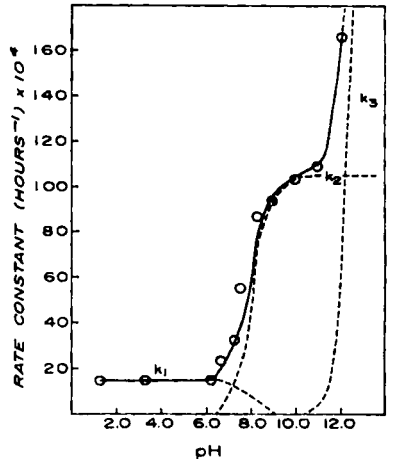

Fig. 3.—Plot showing the pH-rate profile for the hydrolysis of idoxuridine at 60°. Key: O, experimental results; ———, theoretical pH-rate profile; -------, contribution of k_1, k_2, and k_3 at any pH value.

[1] Determined spectrophotometrically at 60°.

TABLE II.—COMPARISON OF APPROXIMATE R_f VALUES OBTAINED FOR IDOXURIDINE, SOME POSSIBLE DECOMPOSITION PRODUCTS, AND AN ARTIFICIALLY DECOMPOSED ALKALINE SOLUTION OF IDOXURIDINE

Material	Known Soln.	Artificially Decomposed Alkaline Soln., (pH 10.0)
Unknown	...	0.00 (significant)
2′-Deoxyuridine	0.28	0.28 (small)
Uracil	0.33	...
Idoxuridine	0.34	0.36 (large)
2-Deoxyribose	0.42	...
5-Iodouracil	0.55	...

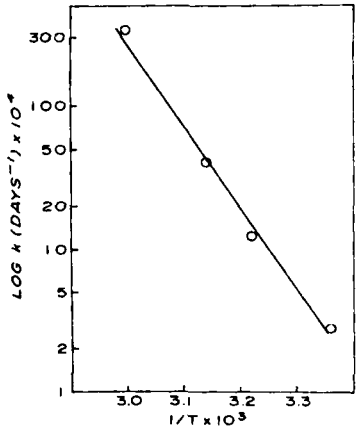

Fig. 4.—Typical Arrhenius-type plot showing the temperature dependency for the hydrolysis of idoxuridine at pH 6.0.

Probable Pathway for the Hydrolysis of Idoxuridine.—Data were presented in an earlier report (9) indicating that 5-iodouracil and 2-deoxyribose were the principal products formed during the hydrolysis of idoxuridine in acid solution. Preliminary work has indicated that alkaline hydrolysis at pH 10.0 results in a complex mixture. A paper chromatogram of this mixture (system: pH 8 buffer-2-methyl-3-butyn-2-ol) showed that the principal breakdown product is one which has lost its ultraviolet absorption characteristics (U.V. absorbance) but retained its deoxyribose moiety (gives a red coloration with cysteine in sulfuric acid). This suggests that the pyrimidine ring has been destroyed. 2′-Deoxyuridine was also found to be a product of alkaline hydrolysis. This is indicated in Table II, which contains a comparison of the R_f values of idoxuridine, some possible decomposition products, and an artificially decomposed alkaline solution (pH 10.0) of idoxuridine. The R_f values were obtained by paper chromatographic analysis using a *n*-butanol-3 *N* ammonia system.

On the basis of the previous data (9) we can assume that idoxuridine is hydrolyzed in acid solution at the riboside linkage to form 5-iodouracil. Garrett and Suzuki (10) presented data suggesting that uracil was the principal product of hydrolysis in strong acid solution. Uracil was not detected in an appreciable amount under the conditions of our study. In alkaline solution the ultraviolet absorption spectrum disappears, suggesting that the pyrimidine ring is destroyed. Since some 2′-deoxyuridine is found, the pathway for degradation may proceed through 2′-deoxyuridine.

Temperature Dependency.—Rate constants for the over-all disappearance of idoxuridine were obtained over the temperature range from 25 to 60° at pH 6.0. A typical Arrhenius-type plot of the data is presented in Fig. 4. From these data the apparent heat of activation for the process was determined as approximately 25 Kcal. per mole.

SUMMARY

(*a*) The hydrolysis of idoxuridine was first order with respect to idoxuridine over a wide range of hydrogen ion concentration (pH 1.3 to 12.0).

(*b*) The over-all rate of disappearance of idoxuridine in aqueous solution as a function of pH can be described using

$$k_{obs.} = k_1 \frac{[H^+]}{[H^+] + K_a} + k_2 \frac{K_a}{K_a + [H^+]} + k_3 \frac{K_a}{K_a + [H^+]} [OH^-]$$

A theoretical pH-rate profile calculated using the above equation is in good agreement with the experimentally determined values.

(*c*) The probable pathway for the hydrolysis is presented. The data suggest that 5-iodouracil is the principal product of acid hydrolysis. Alkaline hydrolysis results in a complex mixture containing 2′-deoxyuridine and a compound with no ultraviolet absorption spectrum. The data suggest that the pyrimidine ring is destroyed during alkaline hydrolysis.

(*d*) The heat of activation (ΔH_a) for the reaction was approximately 25 Kcal. per mole at pH 6.0.

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