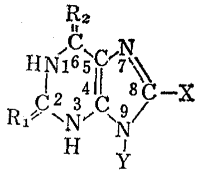
As a conculsion, it is revealed that among nucleic acid-bases, nucleosides and their related compounds, the compounds easily photodegradated with methylene-blue have a portial structure A and that they are not influenced by 8 and 9 substitutes of purine ring.



Portial structure A
R_1=O, NH
R_2=O, NH
Chart 5.

The authors are indebted to Dr. Waro Nakahara, Directer of this institute, for heartful encouragement.

Summary

As a study on mechanism of photodynamic action with methylene-blue, the authors examined structural correlation of 40 kinds of nucleic acid-bases, nucleosides and related compounds with their photodynamic degradation by visible light-irradiation in the presence of methylene-blue.

As a result, it became established that : 1) Above-mentioned compounds, in order to be easily photodynamically degradated, need to have the lactim structure with respect to N 1 and 3-position in pyrimidine moiety of purine ring.

2) Imidazol ring seems to be essential for photodegradation.

3) Existence of substituent in 8, 9-position in purine didn't affect on photochemical reaction.

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146. Edward R. Garrett, Pramod B. Chemburkar,*1 **and Tokuji Suzuki***2 :

Prediction of Stability in Pharmaceutical Preparations. XIV.*3 The Complete pH Dependent Solvolytic Degradations of an Iodinated Nucleoside, the Antiviral 5-Iodo-2′-deoxyuridine.*4

(*College of Pharmacy, University of Florida**5)

The biologically active nucleoside[1] 5-iodo-2′-deoxyuridine (IDU) is effective as an antitumor agent[2] and as an antiviral agent against herpes simplex in human keratitis.[3]

A complete knowledge of the stability of this compound in solution is vital for its pharmaceutical utility since a possible degradation product, iodouracil (IU), has greater toxicity and can inhibit the antiviral activity of IDU.[4] The observed reversal of the

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*5 Gainesvill, Florida, U.S.A.

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biological activity of a nucleoside by its solvolytic product appears to be a common phenomenon.[5]

Garrett and coworkers[6,7] have shown by spectrophotometric, polarographic and thin-layer chromatographic procedures that the solvolysis of IDU in acidic medium results in the final products of iodide ion, uracil and degraded deoxyribose. The intermediate products are deoxyribose and iodouracil.[7~9] Ravin and associates[8,9] by column chromatographic analysis[10] of reactions that proceeded only 10~30% were able to follow the kinetics of formation of these intermediates at several pH values and establish an operational pH dependency. The IDU molecule on its degradation in alkaline solution gives other products than these intermediates since the chromophore associated with the pyrimidine ring vanishes completely.[6~9] The Arrhenius parameters to describe temperature dependence have been reported only for hydrogen ion catalyzed solvolysis[7] and at pH 6.0.[9]

Since it has been shown that concomitant degradations of initial products occur when the IDU reactions are studied for the classical "more than two half-lives"[6,7] and since our preliminary studies had demonstrated a deviation from the apparent first order kinetics of appearance of products in the neutral pH region[7] as compared with the studies conducted for fractions of a half life at elevated temperatures,[8~10] it was necessary that the complete kinetics of IDU transformations be evaluated to predict its stability under pharmaceutical conditions.

The purposes of this paper are to report the complete rate-pH profile for the solvolytic degradation of IDU for 100% reaction and to determine the Arrhenius parameters for temperature dependence at all possible pH values. It was also desired to obtain insight into the complete solvolytic mechanisms of concomitant degradations of initial products by applications of analog computer techniques.

Experimental

Materials——The 5-iodo-2′-deoxyuridine (IDU), 5-iodouracil (IU), uracil (U), deoxyuridine (DU), deoxyribose (DR), 5-hydroxyuridine (5-OH-DU) and isobarbituric acid used in this study were obtained from the California Corporation for Biochemial Research, Los Angeles. All of these compounds were checked for purity by thin-layer chromatographic and spectrophotometric methods as previously described.[7]

Kinetic Procedure——A quantity of IDU (generally 35.0 mg.) was weighed into a 100 ml. volumetric flask and made up to volume with an appropriate buffer or NaOH solution so that the concentration in IDU was about $1\times10^{-3}M$. The buffer solutions were purged with nitrogen before use, and NaOH solutions were similarly purged and then standarized. All the solutions were equilibrated at the temperature of the kinetic studies in the constant temperature baths maintained within ±0.05°. The pH of the buffer solutions was measured at the temperature of the kinetic study with a Beckman Expanded Scale pH Meter, Model 76, which was standardized at the same temperature with Beckman standard buffers of pH 4, 7, and 10. The pH of the reaction mixture was measured over the entire period of a kinetic run and the average of these pH values was used for further kinetic calculations.

The ionic strengths of the reacting solutions were generally constant at 0.1. The effect of change in the ionic strength on the rate of degradation of IDU was studied at 80.0° by adjusting the ionic strength of the reaction mixtures with KCl to 0.1 and 0.5 in phosphate buffer at pH 7.10 and 7.87, and 0.05, 0.1, 0.15, and 0.2 in $0.0464N$ NaOH solution.

The degradation of IDU was studied in four-fold ranges of acetate, phosphate and borate buffer concentrations to evaluate the general acid-base catalysis. The conditions of the studies are given in detail in Table I and II.

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7) E.R. Garrett, T. Suzuki, D.J. Weber: J. Am. Chem. Soc., **86**, 4460 (1964)
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Assay Procedure——The assay procedure described previously[7)] was used to determine the concentration of IDU in buffer solutions up to pH 8.0. Two 2 ml. samples were drawn simultaneously at timed intervals from a reaction mixture which was initially $1\times10^{-3}M$ in IDU and diluted as follows: an appropriate quantity of $1N$ HCl was added to one of these samples so that the pH of the final 25 ml. solution was in the range of 2.0 to 2.5; an appropriate quantity of $1N$ NaOH was added to the other 2 ml. sample so that the pH of the final 25 ml. sample was in the range of 11.5 to 12.0. The absorbances of the final acidic and alkaline samples were measured on a Beckman DU spectrophotometer at wave lengths of 292 and 310 mμ respectively. The absorbance of the acidic sample was also measured at 259 mμ.

In the alkali-degrading solutions of IDU, neither the intermediates nor the final degradation products interfered with the decreasing absorbance of IDU at 286 mμ in the acidified samples. Hence the absorbance at 286 mμ in acid samples was taken to be proportional to the concentration of IDU. Complete absorption spectra of the acidic and alkaline samples of the IDU reactions were also obtained at timed intervals on a Cary Model 15 Recording Spectrophotometer.

Thin-layer Chromatographic Investigations——The degradation of IDU at pH 6.10, 7.10, and 7.87 in phosphate buffer, at pH 9.05 in borate buffer and in $0.093N$ NaOH at 80.0° was monitored by thin-layer chromatography. A small quantity of the reaction mixture was acidified with concentrated HCl and 20 mm^3 of this solution was spotted on a thin-layer chromatographic (TLC) plate. The TLC plates were prepared with 0.4 mm. layer of Silica Gel GF_{254} (containing phosphor indicator) Catalog No. 7730, Brinkmann Institute, Westbury, N. Y. The TLC plates were then developed in one of the two ways: in a mixture of chloroform-isopropyl alcohol (3:1); or first in 70% ethanol up to approximately 10 cm., dried and redeveloped in chloroform-isopropyl alcohol (3:1) up to about 15 cm. The two solvent system was specifically used for the identification and isolation of the decomposition products of IDU in alkali. The extent to which the developing solvent traveled was also carefully noted. The resulting separation of the degradation products was observed under UV light and the relative positions of the visible spots were noted. The plates were further developed by spray reagents such as 2% ferric chloride solution in butanol or concentrated sulfuric acid with subsequent drying at 120° for 20 min. The solutions of IDU, IU, DU, 5-OH-DU, and U were spotted on the TLC plates as standards for the purpose of identification of the degradation products.

The TLC technique was also used to isolate and quantitatively determine the amount of some of the degradation products. A known volume (generally 1 ml.) of the degraded solution was streaked across a 1 mm. thick Silica Gel GF_{254} plate using a syringe microburet. The plate was then developed as previously described, first in 70% ethanol and then in chloroform-isopropyl alcohol (3:1). The separation of the degradation products into well defined bands was observed under UV light and the boundaries of these bands were marked on the plate itself. The silica gel from each marked area was scraped off and collected in separate beakers. The silica gel so collected was extracted with three 15 ml. portions of hot methanol. The methanol extracts were evaporated to dryness on a Rinco using a water aspirator. The residue was dissolved in water and the necessary dilutions made for the spectrophotometric analysis. Approximately the same weight of silica gel scraped from a control plate (no degraded IDU solution spotted) was treated in a similar manner and used as the spectrophotometric blank.

Polarography of Alkali-Degrading Solutions of IDU——The production of iodide ion in the alkali-degrading solutions of IDU was followed as a function of time using the Sargent Model XV Polarograph. An apparent $E_{1/2}=-0.06$ v. was established in $0.093N$ NaOH and pH 9.05 borate buffer after neutralization with sulfuric acid to a pH of 2.5. The diffusion current at −0.6 v., as used by the previous investigators[7)] was determined to be proportional to the iodide ion concentration as measured against a reference cell of saturated calomel in a 20 ml. capacity H-cell with an agar potassium nitrate bridge.

Five ml. aliquots of $2\times10^{-3}M$ IDU solution in pH 9.05 borate buffer and $0.093N$ NaOH were neutralized with sulfuric acid to pH 2.5, diluted with water to 15 ml., purged with nitrogen for 6 min. and a polarographic wave between +0.1 to −0.8 v. was obtained. The height of the polarographic wave at −0.6 v. was measured and the concentration of the iodide ion in the original sample was obtained from calibration curves of potassium iodide of known molarity in alkaline or borate buffer solutions were treated similarly with sulfuric acid.

The Analog Computer Programming——The proposed mechanism for the degradation of IDU in acetate buffer was programmed on the PACE TR-10 analog computer, Electronic Associated, Inc., Long Beach, New Jersey. The absorbance data from spectral readings of IDU solvolysis with time were converted to concentration data of IDU and IU from a knowledge of the molar absorptivities at 292 mμ in acid solution and at 310 mμ in alkaline solution. The concentrations were plotted on the graph paper used by the Sargent SRL recorder. The chart paper was run through the recorder at a constant rate of 12 inches a minute. The data was plotted on a scale designed to require from 2∼4 min. running time per graph. There was negligible drift of the amplifiers of the recorder over this time period. The values of the rate constants involved in the degradation mechanism were determined by setting the potentiometers, representing the rate constants on the analog computer, to a set of values giving the best computer fit to the experimental data.

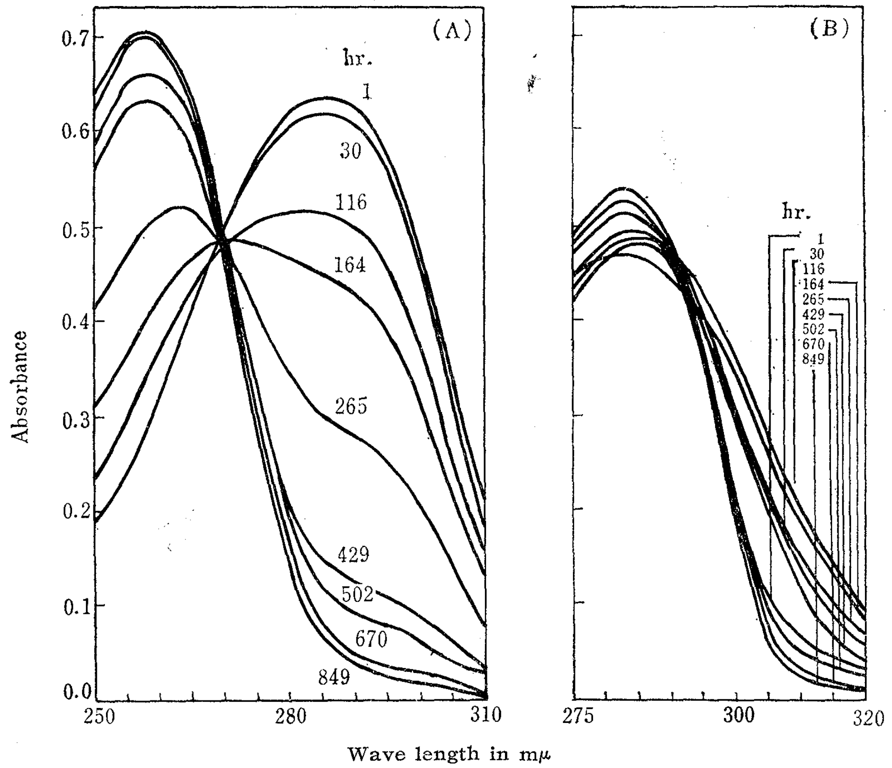

Fig. 1. Typical Curves of the Spectral Changes of Acetate Buffer Degraded 5-Iodo-2′-deoxyuridine

$1\times10^{-3}M$ IDU was degraded at pH 3.90 at 70.0°. The spectra were recorded after 2:25 dilution. Each curve is labelled as to the number of hours after the start of the degradation.
(A) Spectra at pH 2.0 (B) Spectra at pH 11.5

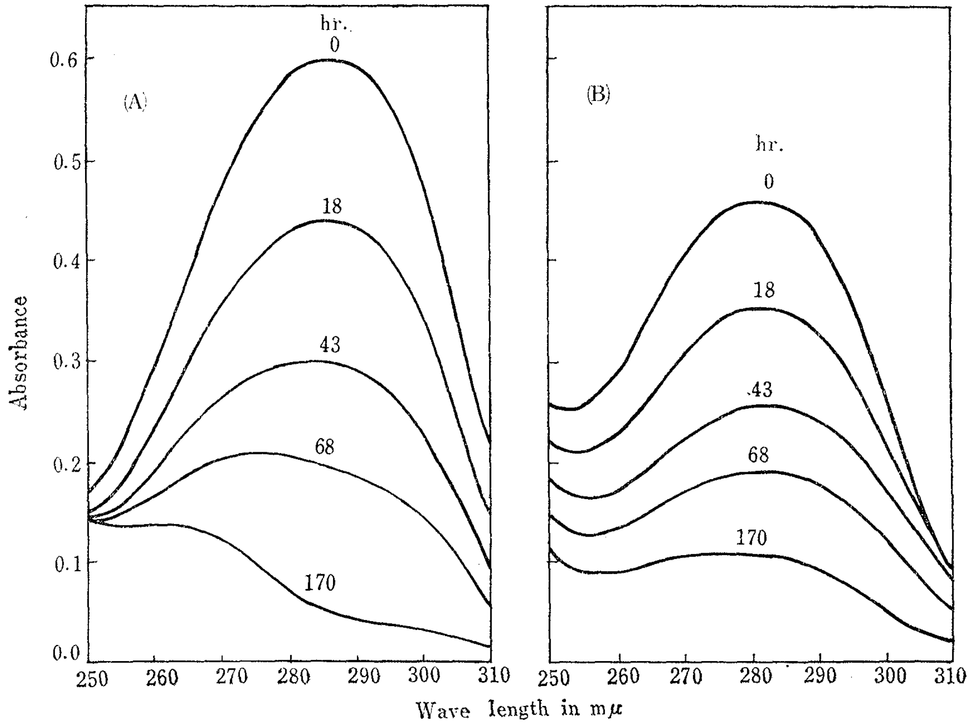

Fig. 2. Typical Curves of the Spectral Changes of Phosphate Buffer Degraded 5-Iodo-2′-deoxyuridine

$1\times10^{-3}M$ IDU was degraded at pH 7.90 at 70.0°. The spectra were recorded after 2:25 dilution. Each curve is labelled as to the number of hours after the start of the degradation. (A) Spectra at pH 2.0 (B) Spectra at pH 11.5

Results and Discussion

Spectral Changes at Various pH Values

The changes in the typical spectra of the acidic and alkaline samples of 5-iodo-2′-deoxyuridine (IDU) degraded in acetate buffer and sodium hydroxide solutions are given in Figs. 1, 2, and 3. The spectral changes in borate buffer were the same as those in alkali (Fig. 3).

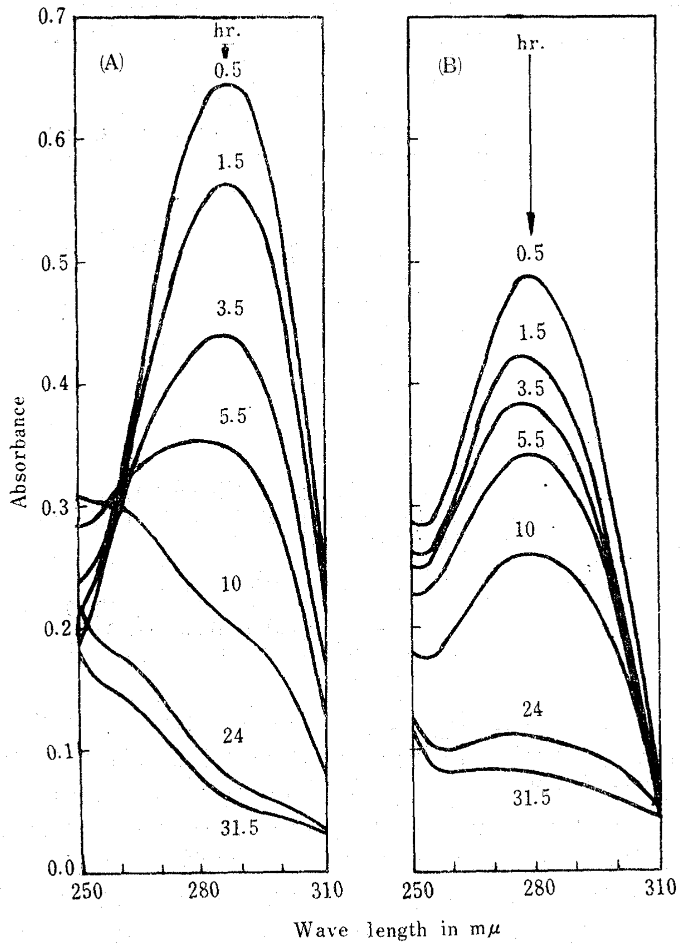

Fig. 3. Typical Curves of the Spectral Changes of Sodium Hydroxide Degraded 5-Iodo-2′-deoxyuridine

$1\times10^{-3}M$ IDU was degraded in $0.0462N$ NaOH at 80.0°. The spectra were recorded after 2:25 dilution. Each curve is labelled as the number of hours after the start of the degradation.

(A) Spectra at pH 2.0 (B) Spectra at pH 11.5

The absorbance at 286 mμ of the acidified samples of IDU degraded at all pH values decreased with time (Fig. 1A). In the case of IDU degraded in acetate buffer and the lower pH phosphate buffer the acidified samples showed the appearance of a new band at 259 mμ while the alkaline samples showed an initial rise in absorbance at 304 mμ decreasing to zero with time (Fig. 1B). The absorbance at 282 mμ in alkaline samples decreased only slightly and a shift of λ_{max} to 286 mμ was observed. This information coupled with the thin-layer chromatographic data demonstrated that 5-iodouracil (IU) (λ_{max} : 283 mμ in acid, 306 mμ in alkali) and uracil (U) (λ_{max} : 259 mμ in acid, 286 mμ in alkali) were the intermediate and the final product respectively of the IDU degradation in acetate and lower pH phosphate buffers.

The acidified samples of IDU degraded in alkali showed a new band appearing at 259 mμ after the first half-life of IDU (Fig. 3A) while the alkaline samples showed the appearance of a new band at about 265 mμ (Fig. 3B). Neither of the bands showed a sharp λ_{max}. The thin-layer chromatographic data obtained by monitoring $5\times10^{-2}M$ IDU degraded in $0.093N$ sodium hydroxide at 80.0° showed that 2′-deoxyuridine (DU) (λ_{max} : 261 mμ in acid, 263 mμ in alkali) and 5-hydroxy-2′-deoxyuridine (5-OH-DU) (λ_{max} : 280 mμ in acid, 303 mμ in alkali) could be major initial products of alkaline degradation. Thus the appearance of a band at 259 mμ in the acid samples and at about 265 mμ in the alkaline samples could be associated with the formation of DU.

Thin-layer Chromatographic Analysis

The thin-layer chromatographic (TLC) plates spotted with acidified reaction mixture from $1\times10^{-3}M$ IDU degraded in acetate and low pH phosphate buffers were developed with chloroform-isopropyl alcohol (3:1). The developed plates, when viewed under the ultraviolet light, showed a spot with an Rf value corresponding to IDU (0.54) in the zero hour sample. The intensity of the spots in the subsequent samples corresponding to this Rf value diminished with time. A faint spot with an Rf value corresponding to IU (0.67) appeared in the early samples, intensified with time, reached a maximum and then decreased in intensity with no corresponding spot in the final degraded solution. After the appearance of the IU spot, another spot with an Rf value (0.41) corresponding to that of uracil was observed on the plates. The uracil spot intensified with time and in the final degraded solution was the only spot to be observed under the ultraviolet light. The thin-layer plates were similar to those reported previously.[7]

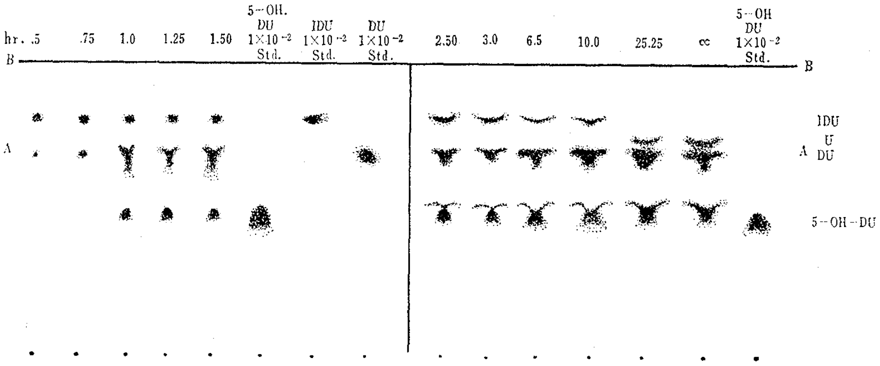

Fig. 4. Thin-Layer Chromatogram of $5\times10^{-2}M$ 5-Iodo-2′-deoxyuridine Degraded in $0.093N$ Sodium Hydroxide at 80.0° as observed under Ultraviolet Light

20 cm; of the reaction mixture was spotted on 0.4 mm. thick silca gel plates and developed first in 70% ethanol [(A) solvent front], dried and then developed in $CHCl_3$-isopropyl alcohol (3:1) [(B) solvent front]. Original chromatogram has been modified to account for uneven solvent fronts.

The alkaline degradation of $1\times10^{-3}M$ IDU monitored on the TLC plates showed only gradually fading spots corresponding to the Rf value of IDU. The degradation of $5\times10^{-2}M$ IDU in $0.093N$ sodium hydroxide at 80.0° was monitored by TLC to identify and isolate any intermediate and final degradation products which would be present in low percentages in the reaction mixture. The two solvent system previously described was used to differentiate between 5-OH-DU, IDU, IU, DU, and U. The 5-OH-DU does not move ($Rf=0.0$) in the chloroform-isopropyl alcohol (3:1) but has a distinctive Rf value in 70% ethanol ($Rf=0.58$). Thus IDU, IU, DU, and U, which move with the solvent front in 70% ethanol ($Rf=1.0$) are separable in chloroform-isopropyl alcohol (3:1). The separation of the degradation products as observed under ultraviolet light is shown in Fig. 4. It was observed that after about 1 hour, a faint spot corresponding to the relative position of the standard 5-OH-DU spot appeared on the plate, intensified until about 6.5 hours, decreased in intensity thereafter and disappeared completely after 24 hours. The said spots, when present, gave a violet color with ferric chloride spray, as did the standard 5-OH-DU spot. It may be noted here that the production of a color with ferric chloride is typical of phenols and enols.[11] A sopt corresponding to the relative position of standard DU spot on the plate appeared from the 15 minute sample and intensified until about 24 hours. The intensity of the DU spot in the 100 hour sample was much less than that in the 24 hour sample. After about 12 hours another spot occupying the same relative position as uracil was observed. This uracil spot intensified throughout the kinetic run and was quite intense in the 100 hour sample.

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[11] S. Soloway, S. H. Wilen: Anal. Chem., **24**, 979 (1952).

The wing shaped spot seen in Fig. 4 below the spot corresponding to the standard DU spot and a little bit above the 5-OH-DU spot, is due to some unidentified compound.

The degradation products were quantitatively isolated by the TLC technique described previously. The isolated products were identified as DU and U by the spectrophotometric and TLC characteristics. It was observed that after 100 hours the reaction mixture ($5\times10^{-2}M$ degraded IDU solution) contained DU and U in concentrations of $1.15\times10^{-2}M$ and $5.5\times10^{-3}M$ respectively.

Results of the Polarographic Investigation

A plot showing the loss of IDU and the appearance of iodide ion as a function of time in $2\times10^{-3}M$ IDU degraded in $0.093N$ sodium hydroxide at 80.0° is given in Fig. 5. From the figure it is seen that the rate of formation of the iodide ion monitored polarographically, closely parallels the spectrophotometrically measured disappearance of IDU. This was found also to be true in the case of degradation of IDU studied in pH 9.05 borate bufler.

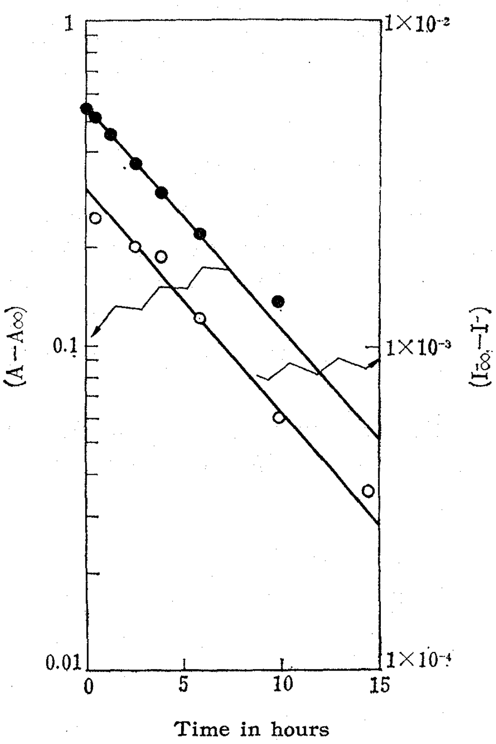

Fig. 5. Apparent First-Order Plots Showing the Degradation of 5-Iodo-2′-deoxyuridine Measured Spectrophotometrically and Formation of Iodide Ion Measured Polarographically in $2\times10^{-3}M$ IDU Degraded in $0.093N$ NaOH at 80.0°

IDU Transformations

The observations of the spectral changes at various pH values and the thin-layer chromatographic data imply that in the acetate buffer region and the low pH phosphate buffer region, IDU degrades to IU which in turn degrades to U in the same manner as was observed for the strong acid solutions.⁷⁾ The mechanism of degradation of IDU above a pH of 8.0 must differ from the mechanism for lower pH region since the products are different.

Calculation of IDU Concentration

The molar absorptivities (ε) of IDU at wave lengths of 259, 286, 292, and 310 mμ in acid solutions were 3397, 7650, 7523, and 717, respectively. The ε values for IU at these wave lengths were 1705, 6150, 5523, and 1231, respectively. The ε values for U in acid solutions at these wave lengths were 8027, 175, 73, and 15, respectively. In alkaline solutions the ε values for IDU at wave lengths of 259, 292, and 310 mμ were 3888, 5093, and 722, respectively; and for IU were 3198, 6231, and 6386, respectively. The ε values for U at the wave lengths 259, 286, 292, and 310 mμ were 3621, 5432, 4466, and 72, respectively.

The concentrations of IDU in acetate and phosphate buffer-degraded solutions of IDU were calculated by using the simultaneous equations derived by earlier workers⁷⁾

$$A_{310}^{\text{alk.}}=\varepsilon_{\text{IUD}}^{\text{alk.}}[\text{IDU}]+\varepsilon_{\text{IU}}^{\text{alk.}}[\text{IU}] \quad (1)$$

$$A_{292}^{\text{acid}}=\varepsilon_{\text{IUD}}^{\text{acid}}[\text{IDU}]+\varepsilon_{\text{IU}}^{\text{acid}}[\text{IU}] \quad (2)$$

Since the molar absorptivity of U is negligible at these wave lengths the concentration of IDU and IU at any time can be given by the following expressions⁷⁾

$$[\mathrm{IU}]=1.7077\times10^{-4}\mathrm{A}^{\mathrm{alk.}}_{310}-1.6395\times10^{-5}\mathrm{A}^{\mathrm{acid}}_{292} \quad (3)$$

$$[\mathrm{IDU}]=1.3292\times10^{-4}\mathrm{A}^{\mathrm{acid}}_{292}-0.7342[\mathrm{IU}] \quad (4)$$

The concentration of IDU in borate buffer and alkaline-degraded solutions of IDU is directly proportional to the absorbance at 282 mμ in the alkaline solutions and at 286 mμ in the acidified solutions. Fortunately the various products which may contribute to the absorbance at these wave lengths either have small absorptivities for the concentrations in which they occur (*e.g.* DU), or they appear much later as products of solvolysis of intermediates and thus do not interfere (*e.g.* U), or degrade so rapidly to non-absorbing species that they are not present in sufficient quantity for significant interference (*e.g.* 5-OH-DU). This fact is demonstrated by good first-order plots shown in Fig. 7.

Rate Constants for Loss of IDU

The first-order rate constants k in sec^{-1} for the degradation of IDU in acetate and phosphate buffers were calculated from the slopes of the plots of the logarithms of IDU against time in accordance with the equation

$$\log[\mathrm{IDU}]=-\frac{kt}{2.303}+\log[\mathrm{IDU}]_0 \quad (5)$$

Typical first-order plots for the degradation of IDU in acetate and phosphate buffers at three different pH values are given in Fig. 6.

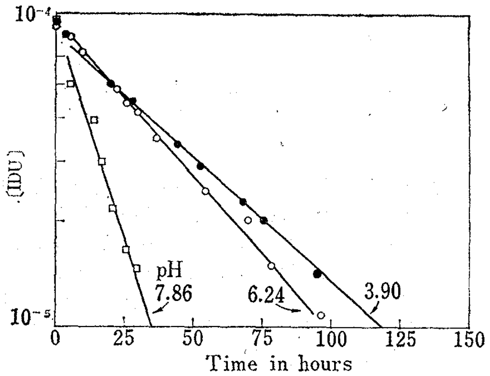

Fig. 6. Typical First-Order Plots for the Decrease of 5-Iodo-2′-deoxyuridine Degraded in Acetate Buffer (pH 3.90) and Phosphate Buffer (pH 6.24 and 7.86) at 80.0°

The concentrations of IDU in the degrading solutions were calculated from the spectral readings at 292 mμ in acidic solution and 310 mμ in alkaline solutions.

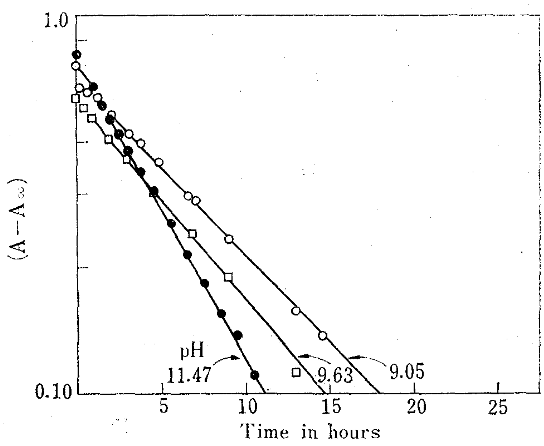

Fig. 7. Typical First-Order Plots for the Decrease of 5-Iodo-2′-deoxyuridine Degraded in Borate Buffer (pH 9.05 and 9.63) and 0.093 *N* NaOH (pH 11.47) at 80.0° as Measured by the Decrease in Absorbance at 286 mμ.

The first-order rate constants for degradation of IDU in borate buffer and sodium hydroxide solutions were calculated from the slopes of the plots of the logarithms of the difference in absorbance A at any time t and the final absorbance, A_∞, at 286 mμ for the acidified solution, against time in accordance with the equation

$$\log(\mathrm{A}-\mathrm{A}_\infty)=-\frac{kt}{2.303}+\log\mathrm{A}_0 \quad (6)$$

Typical first-order plots for the degradation of IDU in borate buffers and sodium hydroxide solution at various pH values are given in Fig. 7.

The conditions and the first-order rate constants obtained for the solvolytic degradation of IDU in acetate and phosphate buffers are given in Table I. The rate constants for the solvolytic degradation of IDU in borate buffer and sodium hydroxide solutions are given in Table II. The pH values were measured at the specified temperature except in the case of sodium hydroxide solutions where they were calculated from the equation (7)

Table I. Conditions and Observed First-Order Rate Constants k in sec^{-1} for the Solvolysis of 5-Iodo-2′-deoxyuridine ($10^{-3}M$) in Acetate and Phosphate Buffers[a)]

		pH[f)]	10^7k			
			60.0°	70.0°	75.0°	80.0°
[CH_3COONa]	[CH_3COOH]					
0.1	0.400	4.00	3.38	12.6[d)]	26.7	48.9
0.1	0.100	4.55	3.29	13.0		47.9
0.1	0.0316	5.10	3.35	13.4	26.9	46.0
[NaH_2PO_4]	[Na_2HPO_4]					
0.0770	0.0077	6.25	4.78	16.6	32.5	59.4
0.0250	0.0250	7.10	8.14	35.2[e)]	60.8	115.0[b)]
0.0050	0.0317	7.90	14.80	47.2	48.3	143.0[c)]

a) Ionic strength μ adjusted to 0.1 with KCl.
b) For μ=0.5, 10^7 k=116 sec^{-1}.
c) For μ=0.5, 10^7 k=153 sec^{-1}.
d) For 0.75, 0.50, and 0.25 times this buffer concentration (μ=0.1) 10^7 k in sec^{-1} were 12.6, 12.1, and 14.1 respectively.
e) For 0.75, 0.50, and 0.25 times this buffer concentration (μ=0.1) 10^7 k in sec^{-1} were 35.7, 34.6, and 31.1 respectively.
f) Measured at pertinent temperature averaged over measured values throughout a kinetic run. Given values are within ±0.1 pH unit.

Table II. Conditions and Observed First-Order Rate Constants, k in sec^{-1} for the Solvolysis of 5-Iodo-2′-deoxyuridine ($1 \times 10^{-3}M$) in Borate Buffer[a)] and Sodium Hydroxide Solutions[a)] at 80.0°

		pH[b)]	10^7k
[NaOH]	[H_3BO_3]		
0.05	0.55	8.25	200[c)]
0.05	0.208	8.60	255
0.05	0.100	9.05	266[e)]
0.05	0.066	9.40	272
0.05	0.055	9.63	300
0.0232		10.87	328
0.0464		11.17	393[d)]
0.0698		11.34	429
0.0928		11.47	469[g)]
0.200		12.00	809[f)]

a) Ionic strength μ adjusted to 0.1 with KCl, except where stated otherwise.
b) Measured at pertinent temperature and the given values are averaged over the entire period of kinetic run and are within ±0.10 pH unit. In NaOH solutions the pH was calculated from the hydroxyl ion activities where the activity coefficients are obtained from the literature.[13,14)]
c) For 0.75, 0.50, and 0.25 times this buffer concentration the 10^7 k in sec^{-1} were 203, 198, and 194 respectively. The ionic strength was adjusted 0.05 with KCl.
d) For ionic strength μ equal to 0.05, 0.15, and 0.2, adjusted with KCl, the 10^7 k in sec^{-1} were 363, 353, and 370 respectively.
e) The 10^7 k in sec^{-1} at 60.0°, 70.0°, and 75.0° were 26.5, 89.3, and 152 respectively.
f) The 10^7 k in sec^{-1} at 60.0°, 70.0°, and 75.0° were 117, 293, and 420 respectively. The ionic strength was μ=0.2.
g) The 10^7k in sec^{-1} for degradation of $2 \times 10^{-3}M$ IDU was 475.

$$pH = pKw - pOH = pKw + \log f_{NaOH}\,[NaOH] \tag{7}$$

where pKw is the negative logarithm of the equilibrium constant of water[12] and f_{NaOH} is the mean activity coefficient calculated by using the extended Debye-Hückel equation[13,14]

$$\log f_{\pm} = -\frac{A\sqrt{C}}{1+A'\sqrt{C}} + BC \tag{8}$$

where C is the concentration; A, A′, and B are constants which were obtained from the literature.[13,14]

Rate Dependency on Ionic Strength and Catalytic Species

The ionic strength effect on the rate of solvolysis of IDU in either its dissociated or undissociated forms is negligible in the ranges studied. Specific examples are cited in Table I for pH 7.10 and 7.87 phosphate buffers and in Table II for 0.046*N* NaOH at 80.0°. It has been shown previously[7] that no significant changes in the apparent first-order rate constant for the solvolysis of IDU occurs for variations of substrate concentrations in the acid pH region. Similarly, no significant changes in k were observed with substrate concentrations in the alkaline pH region (Table II).

General acid-base catalysis was not observed when the reactions were studied at four-fold ranges of buffer concentrations in acetate, phosphate and borate buffers (Tables I and II).

The log k-pH Profiles

The complete log k-pH profiles for the solvolysis of IDU at 60.0°, 70.0°, 75.0°, and 80.0° are given in Fig. 8. The data of Garrett, *et al.*[7] for the acid-degradation of IDU has been used to complete the log k-pH profile in the strong acid region.

The pH of the acid solutions was calculated from equation (9)

$$pH = -\log f_{HCl}\,[HCl] \tag{9}$$

where the mean activity coefficient f_{HCl} was obtained or extrapolated from the literature.[14]

The complete kinetic expression for the first-order loss of IDU may now be written as

$$\frac{d[IDU]}{dt} = -k[IDU]_T \tag{10}$$

$$\frac{d[IDU]}{dt} = -k\{[IDU]+[IDU^-]\} \tag{11}$$

where $[IDU]_T$ is the total IDU and includes both the ionized and unionized forms. The apparent first-order rate constant k for the solvolysis of IDU can be written in terms of microscopic rate constants[15] as

$$\begin{aligned} k = {} & \{k_{H^+}[H^+] + k_o + k_{OH^-}[OH^-]\}\frac{[H^+]}{[H^+]+K_a} \\ & + \{k'_{H^+}[H^+] + k'_o + k'_{OH^-}[OH^-]\}\frac{K_a}{[H^+]+K_a} \end{aligned} \tag{12}$$

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[12] H. S. Harned, B. B. Owen: "The Physical Chemistry of Electrolytic Solutions," 3rd Ed., p. 638 (1958), Reinhold Publishing Corporation, New York.

[13] *Idem*: *Ibid.*, pp. 498, 729, 735.

[14] R. Parsons: "Handbook of Electrochemical Constants," pp. 13, 31 (1959), Butterworths Scientific, Publications, London.

[15] E. R. Garrett: J. Pharm. Sci., **51**, 811 (1962).

where K_a is the dissociation constant of IDU, k_{H^+} and k_{OH^-} are the bimolecular rate constants for hydrogen and hydroxyl ion catalyzed solvolysis of the undissociated form of IDU, k'_{H^+} and k'_{OH^-} are the bimolecular rate constants for the hydrogen and hydroxyl ion catalyzed solvolysis of the dissociated form of IDU, and k_o and k'_o may be the rate constants for the solvent catalyzed degradations of the undissociated and dissociated IDU respectively. In equation (12), the $[H^+]$ and $[OH^-]$ are defined in terms of hydrogen and hydroxyl ion activities, respectively.

A plot of the observed first-order rate constants k[7] against the hydrogen ion activity estimates k_{H^+} from the slope and k_o from the intercept. The hydrogen ion activities were calculated from the hydrogen ion concentration previously reported[7] by the use of equation (9) and the literature values.[14] The functional dependency obtained by this procedure for 80.0° was

$$\begin{aligned} k &= k_o + k_{H^+}[H^+] \\ &= 4.60\times10^{-6} + 8.36\times10^{-5}[H^+] \end{aligned} \qquad (13)$$

Similarly a plot of k against hydroxyl ion activities allows us to estimate k'_{OH^-} from the slope of the plot and k'_o from the intercept.

The functional dependency obtained by this procedure for 80.0° was

$$\begin{aligned} k &= k'_o + k'_{OH^-}[OH^-] \\ &= 2.79\times10^{-5} + 2.90\times10^{-4}[OH^-] \end{aligned} \qquad (14)$$

where $[OH^-] = f_{NaOH}$ NaOH where the NaOH are given in Table II and the mean activity coefficient f_{NaOH} was obtained or extrapolated from the literature.[13,14]

A typical example of such plots is given in Fig. 9.

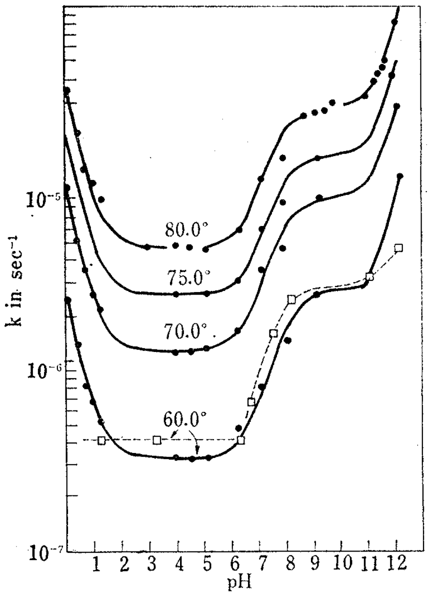

Fig. 8. Log k-pH Profiles for the Degradation of 5-Iodo-2′-deoxyuridine at 60.0°, 70.0°, 75.0°, and 80.0°

● Experimental value
—— Theoretical log k-pH profiles
···□··· Data of Ravin, *et al.*[9]

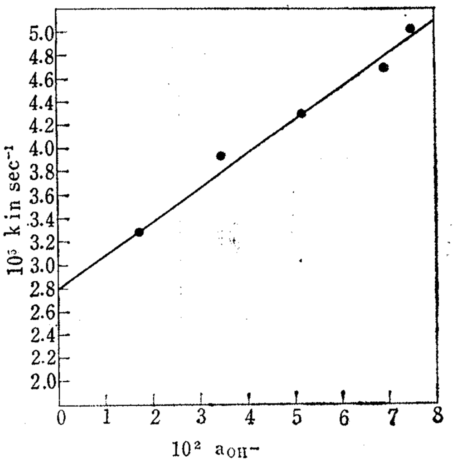

Fig. 9. A Typical Plot of Apparent First-Order Rate Constants, k in sec^{-1} for the Alkaline Degradation of 5-Iodo-2′-deoxyuridine at 80.0° as a Function of Hydroxyl Ion Activity, a_{OH^-}

In the equation (12), $k'_{H^+}[H^+]\cdot\frac{K_a}{[H^+]+K_a}$ and $k_{OH^-}[OH^-]\cdot\frac{[H^+]}{[H^+]+K_a}$ are kinetically indistinguishable from $k_o\cdot\frac{[H^+]}{[H^+]+K_a}$ and $k'_o\cdot\frac{K_a}{[H^+]+K_a}$ respectively.[15] Thus, equation (12) simplifies to

$$k=\{k_{H^+}[H^+]+k_o\}\frac{[H^+]}{[H^+]+K_a}+\{k'_{OH^-}[OH^-]+k'_o\}\frac{K_a}{[H^+]+K_a} \tag{15}$$

Thus in the acid and the neutral pH regions where IDU is predominantly present in its undissociated form, the solvolysis of IDU may be considered as catalyzed by hydrogen ions and solvent. In the alkaline region where IDU is mostly present in its dissociated form IDU^-, the solvolysis may be considered as catalyzed by hydroxyl ions and solvent. The kinetically equivalent mechanisms to solvent attack on undissociated and dissociated IDU are, hydrogen ion attack on dissociated IDU and hydroxyl ion attack on undissociated IDU, respectively.

The Microscopic Rate Constants

The value of the microscopic rate constants k_{H^+}, k'_{OH^-}, k_o, and k'_o at 80.0° were estimated directly from the plots of the observed first-order rate constants k against the activity of the hydrogen and hydroxyl ions as has been previously described. The values of the microscopic rate constants k_o and k_{H^+} at other temperatures were calculated similarly from the data given in Table I and II and the data previously reported.[7] The values of the observed first-order rate constants k at pH 9.05 at temperatures of 60.0°, 70.0°, and 75.0° (Table II) were taken as representing the k'_o values since at this pH the contributions from $k_{H^+}[H^+]$, k_o, and $k'_{OH^-}[OH^-]$ are negligible (Fig. 8). The value of the microscopic rate constant k'_{OH} was obtained from the equation

$$k'_{OH^-}=\frac{(k-k'_o)}{[OH^-]} \tag{16}$$

applied to the k values obtained in the strongly alkaline solutions.

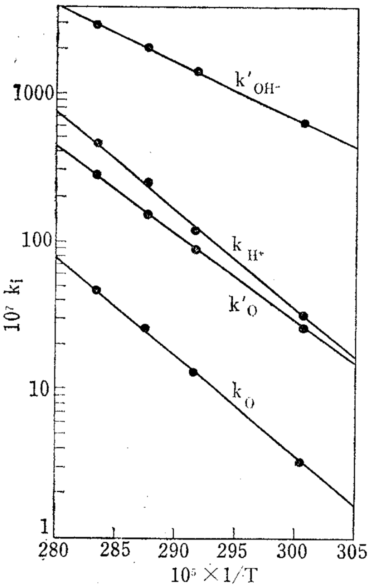

Fig. 10. Arrhenius Plots for the Specific Rate Constants, k_i in sec^{-1} for the solvolytic Degradation of 5-Iodo-2′-deoxyuridine

The microscopic rate constants at different temperatures and the calculated pKa values obtained from the best fit of the data of Table I and II to the logk-pH profiles of Fig. 8 as based on the equation (15) are given in Table III. The drawn curves of Fig. 8 were calculated from the k_i of Table III and equation (15).

Arrhenius Parameters

The logarithmic version of the Arrhenius expression is

$$\log k_i=-\frac{\Delta H_a}{2.303R}\cdot\frac{1}{T}+\log P \tag{17}$$

where ΔH_a is the heat of activation in cal. $mole^{-1}$, R is 1.987 cal. $deg.^{-1}$ $mole^{-1}$, T is the absolute temperature and log P is the intercept of the plot of log k_i against the reciprocal of the absolute temperature. The k_i are the microscopic rate constants of equation (15) listed in Table III. The Arrhenius plots for the specific rate constants are shown in Fig. 10. The ΔH_a and log P values obtained from the slope and the intercept, respectively, of the Arrhenius plots for the specific rate constants, are listed in Table III.

TABLE III. Microscopic Rate Constants[b)] (k_{H^+} and k'_{OH^-} in L./mole sec., k_o and k'_o in sec^{-1}) and Arrhenius Parameters[a)] for the Solvolytic Degradation of 5-Iodo-2'-deoxyuridine and pKa Values Calculated from log k-pH Profiles

	$10^6k_{H^+}$	10^7k_o	$10^5k'_{OH^-}$	$10^6k'_o$	pKa
60.0°	3.25	3.31	6.28	2.65	7.75
70.0°	12.10	13.07	14.30	8.93	7.60
75.0°	25.20	26.08	19.90	15.20	7.57
80.0°	45.00	47.60	29.00	27.90	7.46
ΔH_a kcal. mole^{-1}	30.4	30.9	17.6	27.1	
log P	14.5	13.8	7.4	12.2	

a) where $\log k_i = -\frac{\Delta H_a}{2.303\,RT} + \log P$

b) where $k_{apparent} = \{k_{H^+}[H^+] + k_o\}\frac{[H^+]}{[H^+]+K_a} + \{k'_o + k'_{OH^-}[OH^-]\}\frac{K_a}{[H^+]+K_a}$

TABLE IV. Arrhenius Parameters for the Degradation of 5-Iodo-2'-deoxyuridine as Function of pH

pH	1.31	4.00	7.10	7.90	9.05	11.77
ΔH_a kcal. mole^{-1}	30.9	30.9	29.5	26.1	27.0	20.2
log P	14.0	13.8	13.3	11.3	12.1	8.4

The Arrhenius parameters for the observed first-order rate constants k at various are recorded in Table IV and are actually functions of ΔH_a and log P values (Table III) obtained from the microscopic rate constants. It is apparent that the assumption of only one ΔH_a for all pH values[9)] is invalid for the prediction of stability at any specific pH value and temperature.

Prediction of Stability

The quantitative relation between the specific reaction rates and the temperature is the Arrhenius expression given in the equation (17). The microscopic rate constants for the solvolytic degradation of IDU at any temperature can be calculated from the Arrhenius parameters listed in Table III. Also a plot of pKa against the reciprocal of the absolute temperature based on the expectation of the integrated form of the Van't Hoff equation[16)]

$$\mathrm{pKa} = -\log K_a = \frac{H^\circ}{2.303R}\cdot\frac{1}{T} + \mathrm{constant} = S\cdot\frac{1}{T} + (\mathrm{pKa})_{T=\infty} \qquad (18)$$

where the specific heat at constant pressure C_p is considered constant for a first approximation, yields a straight line with a slope S of 1650 and the intercept $(\mathrm{pKa})_{T=\infty}$ of 2.79. The equilibrium constant K_a for the dissociation of IDU at any temperature can be calculated from equation (18). The substitution of these calculated values of the microscopic rate constants and the equilibrium constant into equation (15) allows the calculation of the apparent first-order rate constant for the degradation of IDU at any pH value. It is interesting to note that equation (18) predicts a pKa of 8.25 at 31.0°, where 8.25 was experimentally determined value at 31.0° previously.[7)]

[16)] G.N. Lewis, M. Randall: "Thermodynamics", 2nd Edition, p. 173 (1961), McGraw-Hill Book Company, Inc., New York.

It can be seen from Fig. 8 that the log k-pH profile exhibits two plateaus, one in the pH range of 2.5 to 5.5 and the other in the pH range of 9.0 to 10.5. The predicted values of the microscopic rate constants k_{H^+}, k_o, k'_o, and k'_{OH^-} and the equilibrium constant K_a at 25.0° are 1.67×10^{-8}, 1.43×10^{-9}, 2.19×10^{-8}, 3.18×10^{-6}, and 4.753×10^{-9} respectively from equations (17) and (18). At 25.0°, the apparent first-order rate constant k in $\sec^{-1}$ as calculated from equation (15) is

$$k=\{1.67\times10^{-8}[H^+]+1.43\times10^{-9}\}\frac{[H^+]}{[H^+]+4.753\times10^{-9}}$$
$$+\{2.19\times10^{-8}+3.18\times10^{-6}[OH^-]\}\frac{4.753\times10^{-9}}{[H^+]+4.753\times10^{-9}} \qquad (19)$$

where $[H^+]=10^{-pH}$ and $[OH^-]=10^{pH-pK_w}$. The time required in days for 1% degradation of IDU at 25.0°, as predicted from equation (5), at pH 4.0, 5.0, 6.0, 7.0, and 8.0 is 82.0, 81.5, 76.0, 47.5, and 10.0, respectively.

Programming and Application of the Analog Computer to the Complex Sequential Degradations in the Acetate Buffer Region

The experimental data for the acid degradation of IDU had been fitted by an analog computer⁷⁾ to the sequential first-order reactions.

$$\text{IDU} \xrightarrow{k_1} \text{IU} \xrightarrow{k_2} \text{U} \qquad (20)$$

However, the actual mechanism for the acid solvolysis of IDU was more complex and could be explained⁷⁾ by

$$\text{IDU} \xrightarrow{k_1} \text{DR}+\text{IU} \underset{k_3}{\overset{k_2}{\rightleftharpoons}} \text{U}+\text{I}^+ \qquad (21)$$

where the deoxyribose (DR), product of nucleoside solvolysis, is transformed under those acidic conditions⁷⁾ in a non-rate determining step

$$\text{DR} \xrightarrow[H^+]{k_4} \text{DDR} \qquad (22)$$

to a degraded deoxyribose (DDR)¹⁷⁾ which has the property of reducing the iodonium ion equilibrated with IU

$$m\text{I}^+ + \text{DDR} \xrightarrow{k_5} m\text{I}^- + \text{products} \qquad (23)$$

$$\text{I}^+ + \text{I}^- \longrightarrow \text{I}_2 \qquad (24)$$

$$m\text{I}_2 + \text{DDR} \longrightarrow 2m\text{I}^- + \text{products} \qquad (25)$$

where m is the stoichiometric number of iodonium and iodine species reduced by DDR. Since the reaction of equations (22~24) are extremely fast and non-rate-determining, the effective rate constant for the solvolysis of IU under the acidic conditions was k_2 and equation (21) was effectively equation (20). However, the analog computer program of equation (20) did not describe the kinetic data of IDU and IU obtained as functions of time in the acetate buffer region. This is understandable if equation (22) for the production of DDR becomes rate determining. The relations of equations (21) to (23) can be expressed in the following differential equations

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17) J. K. Seydel, E. R. Garrett: Anal. Chem., **37**, 271 (1965).

$$\frac{d[IDU]}{dt}=-k_1[IDU] \tag{26}$$

$$\frac{d[IU]}{dt}=-k_2[IU]+k_3[U][I^+]+k_1[IDU] \tag{27}$$

$$\frac{d[I^+]}{dt}=k_2[IU]-k_3[U][I^+]-k_5[I^+]^m\cdot[DDR] \tag{28}$$

$$\frac{d[DR]}{dt}=-k_4[DR]+k_1[IDU] \tag{29}$$

$$\frac{d[U]}{dt}=k_2[IU]-k_3[U][I^+] \tag{30}$$

$$\frac{d[DDR]}{dt}=k_4[DR]-k_5[I^+]^m\cdot[DDR] \tag{31}$$

where equations (28) and (31) summarize the stoichiometry of the equations (23)~(25).

The total number of iodonium ions I^+ produced by equation (21) is proportional to the [U] less those that have reacted with DDR by equation (23). If the reaction of equation (23) is not rate determining, it follows that from the stoichiometry of equation (23) the unreacted iodonium ion concentration at any time is

$$[I^+]=[U]-m[DDR]_T \tag{32}$$

where $[DDR]_T$ is the total DDR formed at that time whether or not it has been further converted to products. The use of equation (32) simplifies the computer program which is given in Fig. 11. The value of $m=7$, based on the previous work,[7] was chosen. For the purpose of simplification of this program the second term in the equation (31) ($k_5[I^+]^m[DDR]$) was neglected on the premise that the concentration of DDR at any time will be very small making the term negligibly small.

This program, for which the loss of IU was determined by the rate of degraded deoxyribose production as well as the rate of IU dissociation, was thoroughly consistant with the [IDU] and [IU] obtained as a function of time in the acetate buffer region. A typical fitting of such data by the program of Fig. 11 is given in Fig. 12. The k_1 values used are given in Table I. For IDU degradation in pH 4.55 acetate buffer at 70.0° a good fit was given for $k_4=2.5\times10^{-7}\ sec^{-1}$.

Mechanism of Degradation

The spectrophotometric and thin-layer chromatographic investigations have proved that in the acetate buffer region and in the near neutral pH region, where IDU is predominantly present in the undissociated form, IDU degrades to uracil through an IU intermediate. A mechanism for the degradation of IDU in the near neutral pH region as given in equations (21) to (25) has been proposed previously.[7] This mechanism is consistent with the experimental data in the acetate buffer region as seen from the excellent analog computer fit with the experimental data of the program (Fig. 11) based on the above mentioned mechanism (Fig. 12).

The alkaline degradation of IDU yields DU and U as two experimentally confirmed products. The presence of 5-OH-DU as an intermediate has been indicated by thin-layer chromatography (Fig. 4). It has been observed that 5-OH-DU degrades about two times faster than IDU in 0.1*N* sodium hydroxide.[18] If 5-OH-DU is an intermediate in the sequential degradation of IDU, its highest concentration in the reaction

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[18] E. R. Garrett, J. K. Seydel: Unpublished data (1965). To be published.

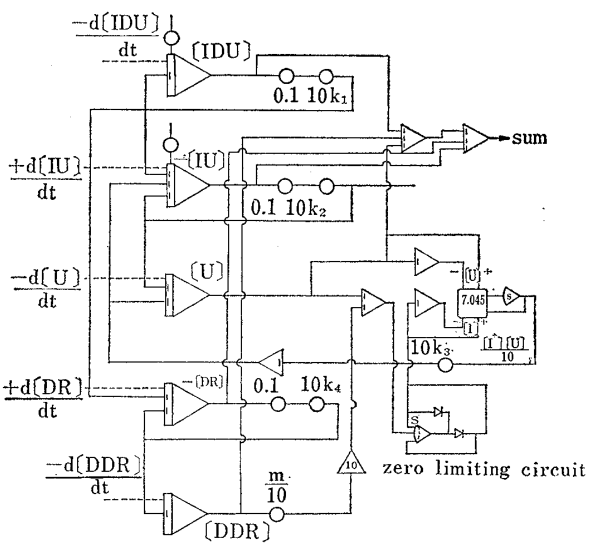

Fig. 11. The Analog Computer Program for the Sequential Degradation of 5-Iodo-2′-deoxyuridine in Acetate Buffer, based on Equations (26) to (32)

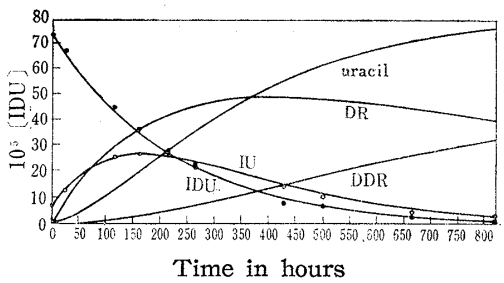

Fig. 12. Typical Analog Computer Fit of the Data obtained from the Solvolytic Degradation of 5-Iodo-2′-deoxyuridine in pH 4.55 Acetate Buffer at 70.0°

$k_1=1.30\times10^{-6}\,\text{sec}^{-1}$ $k_2=1.77\times10^{-6}\,\text{sec}^{-1}$ $m=7$
$k_3=6.61\times10^{-7}\,\text{sec}^{-1}$ $k_4=2.50\times10^{-7}\,\text{sec}^{-1}$

mixture of degrading IDU solution can be calculated[19] to be 4.5 hours after the start of the degradation. The experimentally observed maximum intensity of the spots corresponding to the Rf value of 5-OH-DU is between 3.5 and 6.5 hours as seen on the thin-layer chromatographic plate (Fig. 4). The spectra of the material eluted from the spots were the same as 5-OH-DU in both acid and alkali. This strongly suggests that 5-OH-DU is an intermediate in the alkaline degradation of IDU. No 5-OH-DU could be detected from thin-layer chromatography of IDU degraded in borate buffer, the region where solvolysis has been shown to be pH independent (Fig. 8). However this method is not very sensitive for detection of 5-OH-DU. Conversely a 5-OH-DU spot was obtained on degrading IDU in pH 8.4 bicarbonate buffer.

In one alkaline degraded solution of IDU, 30% of the original amount could be accounted for by the presence of DU and U. The remaining 70% may be postulated to degrade through the formation of 5-OH-DU which rapidly loses its spectral charcter[18] and/or by a destruction of the pyrimidine moiety.

The 5-OH-DU can be readily formed by the postulated S_N2 displacement of the iodine of the IDU by a hydroxyl group with the resultant formation of I^-. An alternative to this S_N2 mechanism is the addition of water across the 4—5 double bond of the pyrimidine ring of the IDU molecule with subsequent removal of HI. The rate of formation of I^- has been shown to be the same as the rate of degradation of IDU (Fig. 5). The 5-OH-DU so formed may degrade either through isobarbituric acid which decomposes very rapidly in alkali[18] with the destruction of its chromophore, or without the solvolysis of the nucleoside linkage. Consistent with these postulates is the fact that all 5-halogen and hydroxyl substituted deoxyuridines readily degrade in alkali whereas the 5-alkyl substituted deoxyuridines are extremely stable.[18]

The formation of DU may be postulated by the reaction of a water molecule with the anionic form of IDU with a subsequent elimination of hypoiodous acid. This hypoiodous acid may quickly dissociate and rearrange itself to form iodate ion. The iodate ion then may react with other decomposition products to form iodide ion. No significant amount of DU had been observed previously in the attack of water on the neutral IDU molecule.[7] The addition of a water molecule across the 4—5 double bond of the pyrimidine ring of the IDU molecule has been postulated by various

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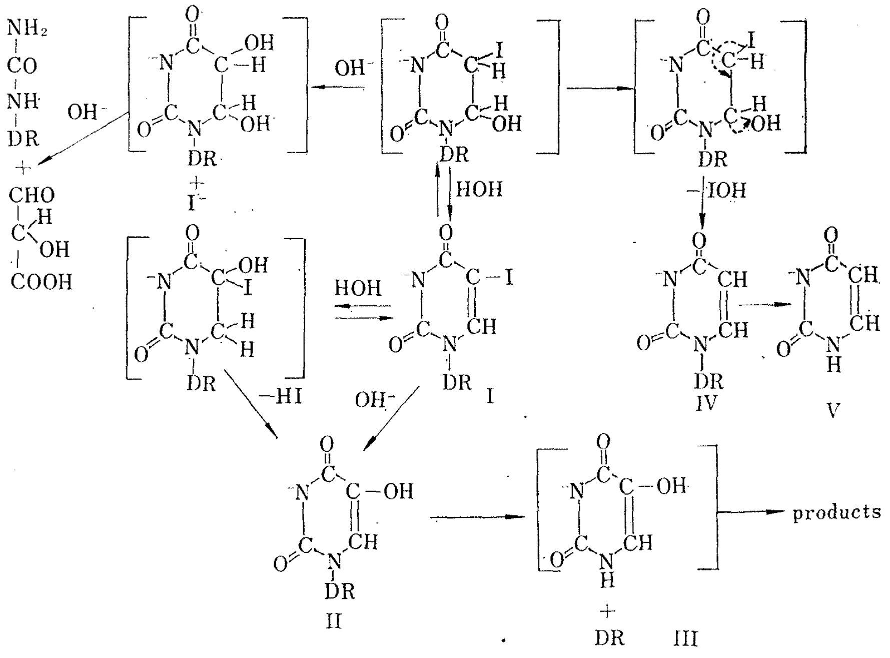
19) S. W. Benson: "The Foundation of Chemical Kinetics," p. 35 (1960), McGraw-Hill Book Company, New York.

workers[20~22] through the mediation of ultraviolet irradiation. Wang[21] advocated the saturation of the 4—5 double bond through the polarization of the α, β unsaturated ketone to the ionic intermediate. Although photolytic acitvation is absent in our studies, water attack followed by hydroxyl ion attack as postulated by Wang[20,21] may be used here to explain the formation of DU. Michelson[23] has given a pathway for the degradation of a 5-alkyl substituted nucleoside which, when coupled with observations on the addition of a water molecule,[20~22] explains the destruction of the pyrimidine ring with the postulated formation of a substituted urea compound. The production of ammonia in alkali subsequent to the destruction of the chromophore has also been observed in our studies. It was observed that DU degraded in alkali gives U as one of its products.[17] Hence it is postulated that uracil found in the alkali-degraded IDU solutions is formed by the degradation of DU.

The mechanistic schemes for the alkaline degradation of IDU are given in Chart 1.



$$IOH \rightleftharpoons IO^- + H^+$$

$$3IO^- \rightleftharpoons IO_3^- + 2I^-$$

$$IO_3 + \text{sugars} \rightarrow I^- + \text{products}$$

Chart 1. Proposed Mechanistic Schemes for the Alkaline Degradation of 5-Iodo-2′-deoxyuridine

I : 5-Iodo-2′-deoxyuridine II : 5-Hydroxy-2′-deoxyuridine
III : Isobarbituric acid IV : Deoxyuridine V : Uracil

The authors are thankful to Mrs. Susan D. Allen, Mr. Rafael Cazali and Mr. Y. Raghunathan for their excellent technical assistance.

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20) S.Y. Wang, M. Apicella, B.R. Stone : J. Am. Chem. Soc., **78**, 4180 (1956).
21) S.Y. Wang, *Ibid.*, **80**, 6196 (1958).
22) A.M. Moore, C.H. Thomson : Science, **122**, 594 (1955).
23) A.M. Michelson : "The Chemistry of Nucleosides and Nucleotides", 29 (1962), Academic Press, New York.

Summary

a) The solvolysis of the antiviral, 5-iodo-2′-deoxyuridine (IDU) is catalyzed by hydrogen ions and solvent, in the acetate and low pH phosphate buffer regions, and by hydroxyl ions and solvent, in the borate buffer and high pH alkaline region.

b) The solvolysis of IDU is first-order with respect to IDU over the pH range of 3.9 to 12.0.

c) The Arrhenius parameters at different pH values varied. These parameters have been evaluated at specific pH values.

d) The general procedure for the prediction of stability of IDU at any pH value has been discussed.

e) In the alkaline region the proposed mechanism involves two routes: (1) the production of uracil through the formation of the intermediate deoxyuridine as the minor route and (2) the formation of 5-hydroxy-2′-deoxyuridine which degrades further as the major route.

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