ORGANIC AND BIOLOGICAL CHEMISTRY

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Kinetics of the Hydrolytic Degradation of a Nucleoside, the Antibiotic Psicofuranine

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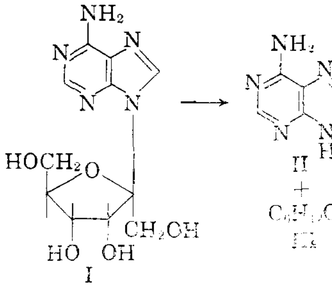
The nucleoside, the antibiotic psicofuranine, 6-amino-9-D-psicofuranosylpurine, is degraded by hydrogen and hydroxyl ions. The products are adenine and the sugar psicose with the former catalyst and possibly with the latter. The rates are linear functions of the concentrations of the neutral [P], protonated [PH$^+$] and anionic [P$^-$], nucleoside and can be expressed: $d[P]_{total}/dt = \{k_2[H^+] + k_3[OH^-]\}[P] + k_1[H^+][PH^+] + k_4[OH^-][P^-]$. The uncharged psicofuranine is hydrolyzed with bimolecular rate constants *ca.* 2.5 times faster than the charged species due to the repulsion of the catalytic species, H$^+$ or OH$^-$, by the positively or negatively charged psicofuranine ions, respectively. Estimates of the pK_a of the acid function in the sugar portion of the nucleoside have been made from the studies of the variations of the bimolecular rate constant for hydrolyses with pH *ca.* 12.

The new antibiotic, psicofuranine (I),[1] 6-amino-9-D-psicofuranosylpurine,[2a,b] is a unique nucleoside with antitumor and antibacterial properties.[3-5] Determination of the kinetics of degradation of I, possibly by hydrolysis to adenine (II) and the sugar psicose (III), is of pharmaceutical importance for the estimation of stability in aqueous formulations, to contribute to process design for maximum yield and to determine the least degrading conditions for bioassay.[6]



The determination of the pH profile of the rates of hydrolysis of a nucleoside at several temperatures is also of fundamental interest since, to the author's knowledge, a complete study of hydrolytic degradation in this class of compounds has not been reported.

Experimental

The characterization of the psicofuranine used in these studies has been reported recently.[1,2a]

Sufficient psicofuranine, generally 25 mg., previously dried at 60° under high vacuum for 48 hours, was weighed into a tared 25-ml. volumetric flask, and made up to volume with the aqueous solvent of the appropriate hydrochloric acid, acetic acid–sodium acetate, or sodium hydroxide concentration. Tables I and II list the final compositions. The solvent previously had been equilibrated in the appropriate constant temperature bath. The resultant solutions were placed in the constant temperature bath and assayed at recorded time intervals.

TABLE I

CONDITIONS AND OBSERVED FIRST-ORDER RATE CONSTANTS (k IN SEC.$^{-1}$) AT CONSTANT pH FOR THE ACID HYDROLYSIS OF PSICOFURANINE AT 1 MG./ML.

Run	°C.	Obsd., pH	Buffer composition, M [HCl]			10^5k^a
1	30.2	2.03	0.01			1.54
2	30.2	1.58	.025			4.65
3	30.2	1.26	.05			16.8
4	30.2	1.10	.075			16.8
5	50.5	2.08	.01			19.7^b
6	50.2	1.59	.025			61.3
7	50.2	1.36	.05			130
8	50.2	1.15	.075			205
9	50.2	0.97	.10			262
10	60.6	1.97	.01			59.6
11	60.6	1.60	.02			139
12	60.6	1.32	.04			293
13	60.6	1.24	.06			424
			[CH$_3$-COOH]		[CH$_3$-COO$^-$]	
14	50.2	3.33	0.075			1.64^c
15	50.2	3.61	.1756		0.0122	1.11^d
16	50.7	4.04	.1334		.0333	0.520^e
17	52.1	5.06	.0326		.0837	.0731^f
18	52.1	5.68	.0094		.0953	.0146^g
19	40.4	5.75	.0100		.0900	.00439
20	50.0	5.75	.0100		.0900	.0126
21	60.2	5.75	.0100		.0900	.0481
22	69.5	5.75	.0100		.0900	.151
				[KCl]		
23	50.5	4.02	.400	0	.100	.608^h
24	50.5	4.01	.2667	0.334	.0666	.608^h
25	50.5	4.02	.2000	0.0500	.050	.608^h

^{a} Rate constants calculated on the basis of equation 8 are in agreement with the experimental listed. Typical calculated $10^5 k$ values are ^{b} 23.6, ^{c} 1.83, ^{d} 1.11, ^{e} 0.519, ^{f} 0.0623, ^{g} 0.0153 and ^{h} 0.541.

The analyses involved the reading of spectrophotometric absorbance in a Beckman model B at 630 mμ of the acid-developed colored diphenylamine reaction product subsequent to alkaline sodium borohydride reduction of interfering sugars. The procedure was that of Forist[7] which has been applied to the chemical determination of psicofuranine in blood plasma and serum.[8]

Prior to the assay a 1-ml. aliquot was pipetted into a 5-ml. volumetric flask containing sufficient NaOH to neutralize the aliquot of the acid-catalyzed hydrolysate so as to halt hydrolysis prior to the alkaline borohydride reduction of the liberated psicose. This solution was then brought up to

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[1] T. E. Eble, H. Hoeksema, G. A. Boyack and G. M. Savage, *Antib. and Chemo.*, **9**, 419 (1959).

[2] (a) W. Schroeder and H. Hoeksema, THIS JOURNAL, **81**, 1767 (1959). (b) This structure also has been proposed for angustmycin C by Hsu Yuntsen, *J. Antibiotics (Japan)*, **11A**, 244 (1958).

[3] J. J. Vavra, A. Dietz, B. W. Churchill, P. Siminoff and H. J. Koepsell, *Antib. and Chemo.*, **9**, 427 (1959).

[4] C. Lewis, H. R. Reames and L. E. Rhuland, *ibid.*, **9**, 421 (1959).

[5] J. S. Evans and J. E. Gray, *ibid.*, **9**, 675 (1959).

[6] E. R. Garrett, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 169 (1959).

[7] A. A. Forist, *Anal. Chem.*, **31**, 1767 (1959).

[8] A. A. Forist, S. Theal and H. Hoeksema, *Antib. and Chemo.*, **9**, 685 (1959).

TABLE II

CONDITIONS AND OBSERVED FIRST-ORDER RATE CONSTANTS (k IN SEC.$^{-1}$) FOR THE ALKALINE DEGRADATION OF PSICOFURANINE AT 1 MG./ML.

Run	°C.	[NaOH]	Calculated pH[a]	10^5k
26	40.4	0.100	12.44	0.0649
27	40.4	.200	12.73	.0919
28	40.5	.300	12.89	.150
29	40.5	.400	13.00	.193
30	50.2	.100	12.17	.221
31	50.2	.200	12.45	.306
32	50.2	.300	12.62	.449
33	50.2	.400	12 73	.613
34	60.4	.025	11.35	.361
35	60.4	.050	11.63	.591
36	60.4	.075	11.80	.671
37	60.4	.100	11.92	.734
38	59.9	.200	12.21	1.14
39	59.9	.300	12.37	1.35
40	59.9	.400	12.48	1.77
41	69.5	.010	10 74	0.311
42	69.5	.020	11.03	0.556
43	69.4	.050	11.39	1.13
44	69.4	.100	11.68	1 59
45	69.5	.200	11.97	2.72
46	69.5	.300	12.13	3.49
47	69.5	.300	12.13	3.92
48	69.5	.400	12 25	3.51
49	69.5	.400	12.25	4.41

[a] Calculated from $pH = pK_w - pOH$; where pK_w's are 13.535 at 40°, 13.262 at 50°, 13.017 at 60° and 12.778 at 70° from ref. 11; and $pOH = -\log f[\text{NaOH}]$, [NaOH] is the experimental, and f the mean activity coefficient for NaOH at the appropriate temperature from ref. 12.

volume with water and the assay procedure applied. There was no need to neutralize the solutions subjected to alkaline hydrolysis.

Calculations and Results

The hydrolysis of psicofuranine is subject to specific hydrogen ion and hydroxyl ion catalysis and at constant pH, or constant acid and alkali concentration, is excellently first order. Figure 1 presents typical apparent first-order plots for the acid-catalyzed hydrolysis of psicofuranine as measured by less absorbance of the developed color at 630 mμ. Similarly, typical apparent first-order plots for the alkaline hydrolysis are given in Fig. 2. The upper part of Fig. 2 shows that in alkaline medium the color decreases to a reasonably consistent asymptote of *ca.* 0.100 for 1 mg./ml. of psicofuranine initially. This asymptote is accounted for in the first-order plots given in the lower part of Fig. 2.

Hydrogen Ion-catalyzed Hydrolysis.—Variation of acetic acid–sodium acetate concentration at constant pH and ionic strength (see runs 23, 24, and 25 in Table I and curve C in Fig. 1) did not significantly affect the apparent first-order rate constant and thus did not implicate general acid–base catalysis. Variation of initial psicofuranine concentrations at 0.42, 0.61 and 1.00 mg./ml. in hydrolysis studies at 40° in 0.100 M HCl also did not significantly affect the apparent first-order rate constant. Variation in pH, however, had a significant effect on first-order rate constants as is shown by the varying slopes of the plots in Fig. 1 so that the rate dependence may be postulated as

$$d[\text{P}]_\text{T}/dt = k_{\text{H}^+}[\text{H}^+][\text{P}]_\text{T} = k[\text{P}]_\text{T} \qquad (1)$$

where k_{H^+} is the catalytic rate constant for specific hydrogen ion catalysis, $[\text{P}]_\text{T}$ is the total psicofuranine concentration and k is the apparent first-order rate constant at constant pH. The conditions and observed first-order rate constants for the hydrolysis are given in Table I except for the 40° hydrolysis data in HCl solutions which have been included in another publication.[9]

The rate constants were determined from the slopes of plots such as are shown in Fig. 1

$$\log [\text{P}]_\text{T} = -[k/2.303]t + \log[\text{P}]_0 \qquad (2)$$

where $[\text{P}]_0$ is the initial concentration of psicofuranine. In actuality, the absorbance of the color developed at 630 mμ by the analytical method was used in the determination of the rate constants.

The plots of the observed first-order rate constants, k in sec.$^{-1}$, against $[\text{H}^+]$ for the various temperatures are given in Fig. 3 and the resultant slopes are listed in Table III. These slopes as per equation 1 are the values in l./mole/sec. for the specific hydrogen ion catalytic rate constant, k', for the hydrolysis of protonated psicofuranine. These plots do not go through the origin as the psicofuranine of pK_a 3.9 actually consumes some of the hydrogen ion concentration in its salt formation so that the molarity of psicofuranine (0.037 M at 1 mg./ml.) must be subtracted from the apparent HCl molarity to obtain the true HCl concentration. This correction should have no effect on the slope of such plots.

TABLE III

CATALYTIC RATE CONSTANTS, k_1 AND k_2, IN L./MOLE/SEC. FOR THE ACID HYDROLYSIS OF PROTONATED AND NON-PROTONATED PSICOFURANINE

T, °C.	$10^3k_1'$[a]	$\log k_1$[b]	$10k_2'$[c]	$\log k_2$[d]
30	2.43	−2.72	...	....
40	8.58	−2.04	0.247	−1.62
50	27.7	−1.56	0.708	−1.16
60	73.9	−1.16	2.70	−0.58
70	...	....	8.5	+0 01

[a] Determined from the slope of the observed pseudo first-order rate constant, k in sec.$^{-1}$, against the molarity of HCl. [b] Determined from the intercept of the line formed by log k *vs.* pH with a slope of unity and based on pH values less than 2.5. [c] Calculated from k in sec.$^{-1}$ at pH 5.75 divided by $[\text{H}^+] = 10^{-5.75}$. [d] Estimated from the intercept of the line formed by log k *vs.* pH with a slope of unity and based on pH values greater than 4.5.

An alternative estimate of k_{H^+} involving protonated psicofuranines, *i.e.*, k_1, may be determined by the plot of log k against pH assuming a slope of unity

$$\log k = \log k_1 - pH \qquad (3)$$

The intercepts for such plots from the studies at the several temperatures are derived from the plots of the acid branch in Fig. 4 and are given in Table III.

Arrhenius plots according to the classical relation of the specific rate constant, k or k_1', are given in

$$\log k_1 = -(\Delta H_a/2.303R)(1/T) + \log C \qquad (4)$$

Fig. 5 as plots of log k_1 *vs.* $1/T$, where R is 1.987 cal. deg.$^{-1}$ mole^{-1} and T is the absolute temperature.

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[9] E. R. Garrett and L. J. Hanka, submitted for publication to *J. Am. Pharm. Assoc., Sci. Ed.*

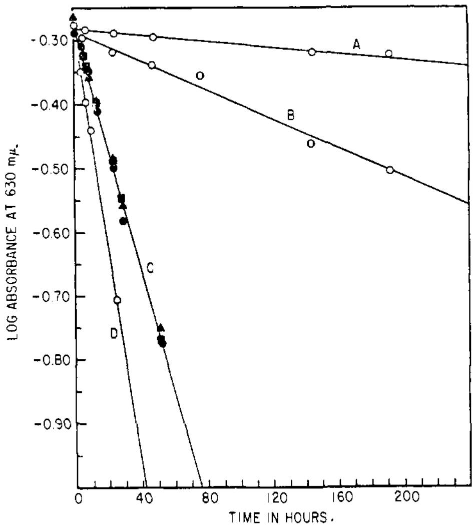

Fig. 1.—Typical pseudo first-order plots for the acidic degradation of psicofuranine as followed by absorbance of developed color (50°). The curves, *p*H and run number are: A, 5.68, 18; B, 5.06, 17; D, 3.61, 15. For curve C at *p*H 4.02, the acetate ion concentration and run numbers are ●, 0.100 *M*, 23; ▲, 0.067 *M*, 24; ■, 0.050 *M*, 25.

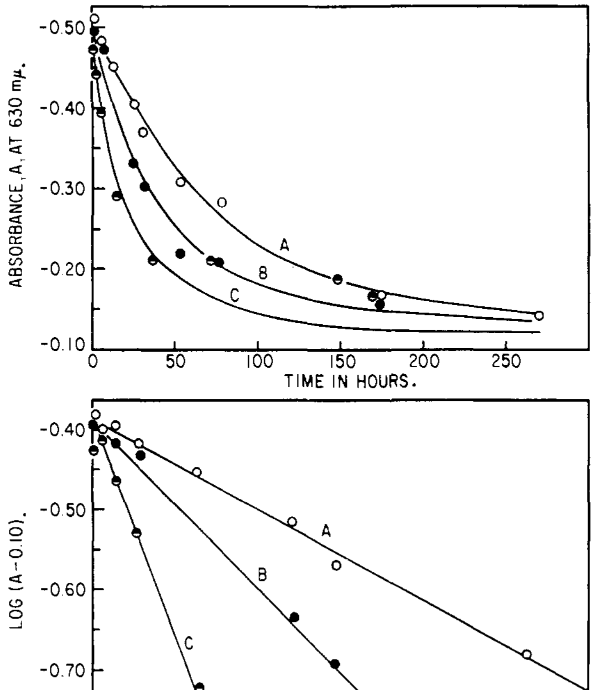

Fig. 2.—Typical plots of the pseudo first-order alkaline degradation of psicofuranine as followed by absorbance of developed color (60°). The curves, NaOH concentration and run number are: A, 0.025 *M*, 34; B, 0.075 *M*, 36; C, 0.400 *M*, 40.

The slope of this plot is -5.056 so that the heat of activation, ΔH_a, is 23.1 kcal./mole and log C is 14.060.

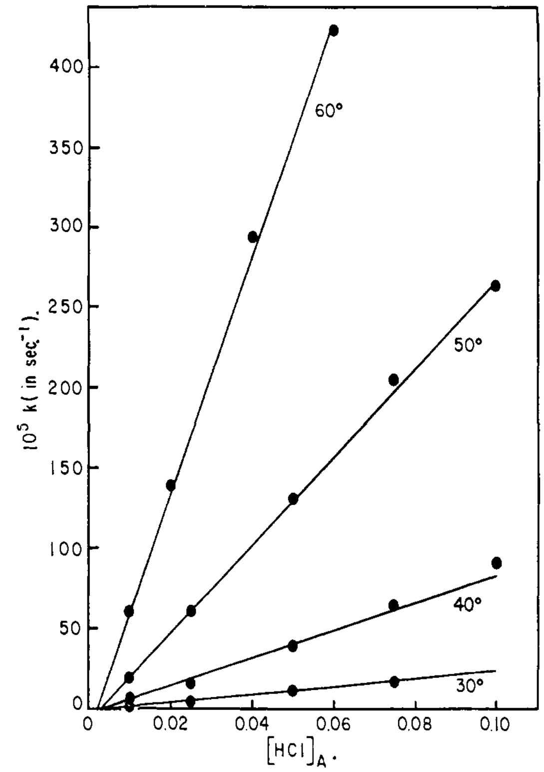

Fig. 3.—Plots of pseudo first-order rate constants, *k* in sec.$^{-1}$, for the acidic hydrolysis of psicofuranine as a function of the apparent molarity of HCl, $[HCl]_A$.

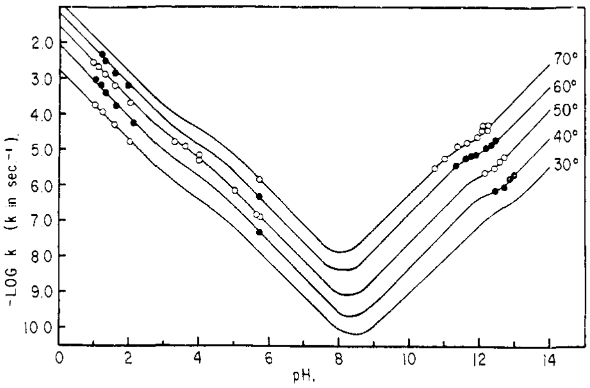

Fig. 4.—*p*H profiles of the logarithm of the apparent first-order rate constant, *k* in sec.$^{-1}$, for the hydrolytic degradation of psicofuranine.

The most complete log *k* *vs.* *p*H profile for the acid branch was obtained at 50° for 0–6 *p*H and is plotted in Fig. 4. The slope of this 50° plot is not constant; it shows an enhancement in log *k* in the 3.5–4.5 *p*H region. However, a ready explanation can be found for this phenomenon. Actually, the rate of total psicofuranine $[P]_T$ concentration change of equation 1 is the sum of the rates of change of the protonated, $d[PH^+]/dt$, and non-protonated, $d[P]/dt$, forms so that

$$\begin{aligned} d[P]_T/dt &= d([P] + [PH^+])/dt \\ &= -k_1[H^+][PH^+] - k_2[H^+][P] \\ &= -k_{H^+}[H^+]\{[PH^+] + [P]\} \qquad (5) \end{aligned}$$

The ionization equilibrium of

$$PH^+ \rightleftharpoons P + H^+ \qquad (6)$$

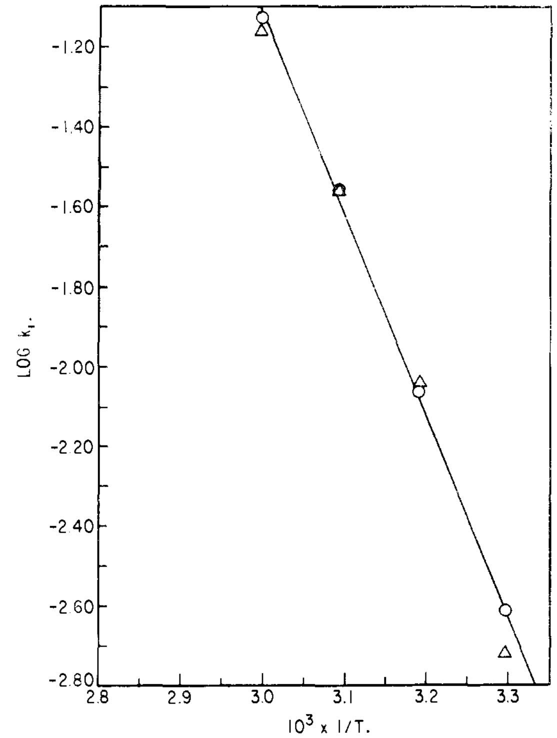

Fig. 5.—Arrhenius plots for the specific hydrogen ion-catalyzed hydrolysis of protonated psicofuranine: ⊙, k'_1 based on the slopes of k (sec.$^{-1}$) *vs.* [HCl]A plots; △, log k_1 based on intercept of log k *vs.* pH plots.

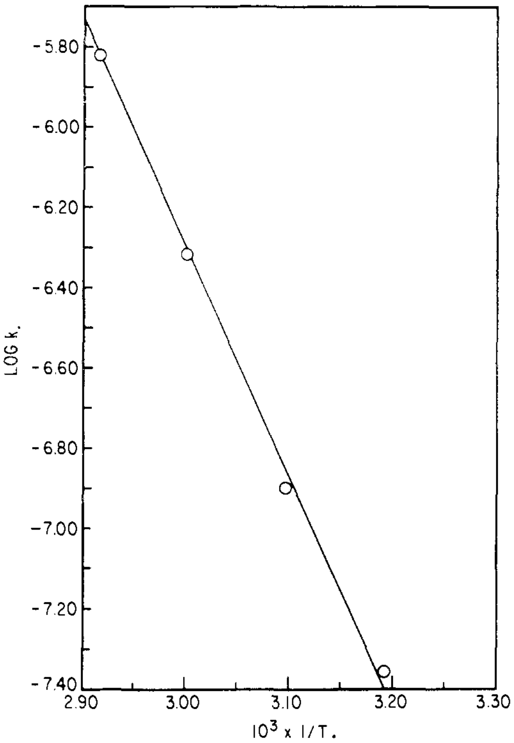

Fig. 6.—Arrhenius plot for the specific hydrogen ion-catalyzed hydrolysis of non-protonated psicofuranine at pH 5.75 in acetic acid–acetate buffer; k is the apparent first-order rate constant in sec.$^{-1}$.

may be formulated as

$$[P]/[PH^+] = K_a/[H^+] \qquad (7)$$

where K_a is the ionization constant of the equilibrium given in equation 6. By a method analogous to that of Edwards[10] it can be shown that

$$k_{H^+} = k_1/(1 + K_a/[H^+]) + k_2/(1 + [H^+]/K_a) \qquad (8)$$

The non-linear segment of the acid branch plots for 50° plotted in Fig. 4 may be calculated from the product of equation 8 and the $[H^+] = 10^{-pH}$. The equations are confirmed by the demonstrated agreement of calculation with experiment where the values are taken as $k_1 = 2.75 \times 10^{-2}$ l./mole/sec., $k_2 = 7.4 \times 10^{-2}$ l./mole/sec., and $K_a = 1.59 \times 10^{-4}$ at 50° for psicofuranine.[9] The rate constants calculated on this basis for the pH range 2 to 6 at 50° are given in a footnote to Table I for comparison with the actual values.

Further confirmation is obtainable from inspection of the rate constants evaluated at pH 5.75 at the several temperatures listed as runs 19 through 22 in Table I and plotted in Fig. 4 as per equation 3. These values representing the hydrolysis of non-protonated psicofuranine at this pH are not on the same linear plot of unit slope of log k *vs.* pH for the acid hydrolysis of the protonated psicofuranine, *i.e.*, below pH 2.5.

An Arrhenius plot as per equation 4 is given in Fig. 6 for log k at pH 5.75. The slope of this plot is $-5{,}700$ so that the heat of activation, ΔH_a, is 26.1 kcal./mole and the log C is 10.80. However, since $k = k_2'[H^+]$ where $[H^+] = 10^{-5.75}$, then the Arrhenius equation for k_2' may be estimated as

$$\log k_2' = -5{,}700(1/T) + 16.55 \qquad (9)$$

The log k_2 values are also listed in Table III as determined from the intercept of the unit slope line of best fit through the log k *vs.* pH at pH values greater than 4.5.

Hydroxyl Ion-catalyzed Hydrolysis.—The overall hydrolysis rate of psicofuranine decreased with increasing pH to a minimum and then increased with increasing hydroxyl ion concentration (Fig. 7) or pH (Fig. 4). This is indicative of hydroxyl ion-catalyzed hydrolysis. The conditions and observed first-order rate constants for the alkaline hydrolyses are given in Table II.

The pH of the solutions listed in this table are calculated from

$$pH = pK_w - pOH \qquad (10)$$

where the pK_w values determined from the literature[11] are listed in Table IV. Also

$$pOH = -\log f\,[NaOH] \qquad (11)$$

where [NaOH] is experimental and f the mean activity coefficient for NaOH at the appropriate temperatures as estimated from the literature.[12]

The plots of Fig. 7 indicate a variation in the mechanism of the alkaline hydrolysis of psicofuranine. Acid-catalyzed hydrolysis should be negligible at neutrality or $k \sim 0$ at the origin of Fig. 7. However, there is a large initial increase in the ap-

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[10] L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).

[11] H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold Publishing Corp., New York, N. Y., 1958, p. 638.

[12] H. S. Harned and B. B. Owen, *ibid.*, pp. 498, 729, 735.

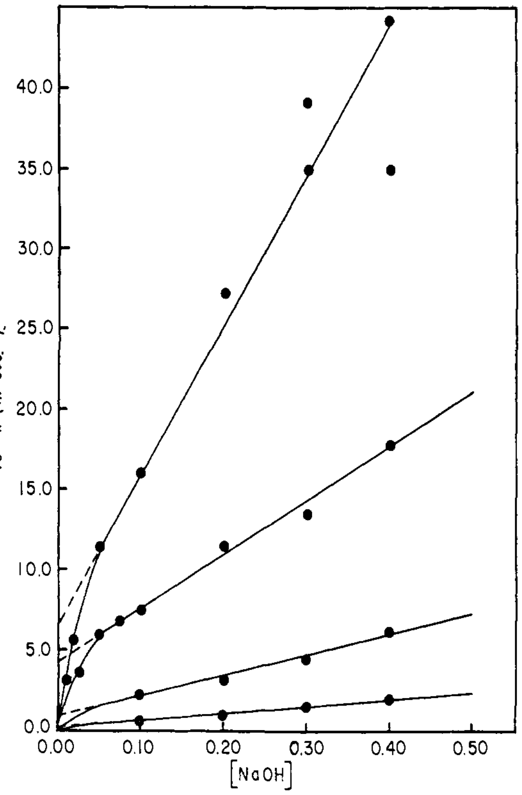

Fig. 7.—Apparent first-order rate constants (k in sec.$^{-1}$) for the alkaline degradation of psicofuranine as a function of sodium hydroxide concentration.

parent first-order rate constant, k, up to *ca.* 0.05 M NaOH before k becomes linear with respect to the hydroxide concentration.

TABLE IV

CATALYTIC RATE CONSTANTS, k_3 AND k_4, IN L./MOLE/SEC. FOR THE ALKALINE DEGRADATION OF UNCHARGED AND ANIONIC PSICOFURANINE

T, °C.	$10^6k_3'^a$	C^a	$10^5k_3^b$	$10^5k_4^b$	$pK_a'^c$	pK_w^d
69.5	93.2	6.70	38.9	15.5	11.5	12.78
60.4	33.8	4.25	15.9	5.89	11.9	13 02
50.2	12.6	1.00	5.38	2.09	12.1	13.26
40.5	4.24	0.25	1.74	0.676	12.3	13.54

[a] Determined from the plot of the apparent first-order rate constant, k in sec.$^{-1}$, against molarities of NaOH in excess of 0.05 M where $k = k_3'[\text{NaOH}] + C$. [b] As estimated from the log k *vs.* pH plots of unit slope above and below the pH of inflection where $pH = pK_w - pOH$ where $pOH = -\log f\ [\text{NaOH}]$ where f is the mean activity coefficient.[12] [c] Estimated from pH at maximum inflection. [d] Ref. 11.

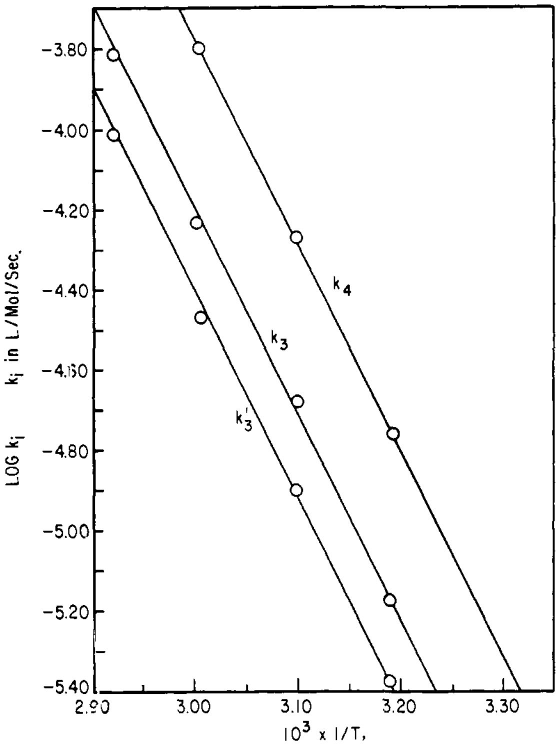

Fig. 8.—Arrhenius plots for the hydroxyl ion-catalyzed degradations of psicofuranine; k_3 and k_4 are specific hydroxyl ion catalytic rate constants in sec.$^{-1}$ for degradation of uncharged and anionic psicofuranine, respectively, where $[H^+] = 10^{-pH}$ and $[OH^-] = 10^{-pK_w} + pH$; k_3' is the rate constant for anionic psicofuranine where $k = k_3'[\text{NaOH}] + C$ for $[\text{NaOH}] > 0.05$.

An explanation analogous to that developed for the aberration in the apparent second-order rate constant, k_{H^+}, in the acid-catalyzed hydrolysis of psicofuranine can be used for the apparent second-order rate constant, k_{OH^-}, for alkaline hydrolysis. The psicofuranine, P, may be assumed to ionize as an acid at high alkalinity so that

$$P \rightleftarrows P^- + H^+ \qquad (12)$$

and

$$[P^-]/[P] = K_a'/[H^+] \qquad (13)$$

where K_a' is the ionization constant of the equilibrium given in equation 12. Thus

$$\begin{aligned} d[P]/dt &= -k_{OH^-}[OH^-][P]_T = \\ -k_{OH^-}[OH^-]\{[P] + [P^-]\} &= d([P] + [P^-])/dt = \\ &-k_3[OH^-][P] - k_4[OH^-][P^-] \qquad (14) \end{aligned}$$

Thus it can be shown, analogous to equation 8

$$k_{OH^-} = k_3/(1 + K_a'/[H^+]) + k_4/(1 + [H^+]/K_a') \qquad (15)$$

Inspection of Fig. 7 indicates that the apparent first-order rate constant at alkalinities in excess of 0.05M NaOH is completely determined by the hydroxyl ion catalysis of the anionic psicofuranine. The equations of the lines at these alkalinities adequately describe the first-order rate constant, k, as per the expression

$$k = k_{OH^-}[\text{NaOH}] + C; \ [\text{NaOH}] > 0.05 \qquad (16)$$

where [NaOH] is expressed in stoichiometric molarity and where k_{OH^-} as based on the molarity of sodium hydroxide reduces to k_3 and is termed k_3'. The values of k_3' and C are listed in Table IV. The k_3 and k_4 values may also be derived from Fig. 4 on application of the expressions

$$\log k = \log k'_{OH^-} - pOH = \log k'_{OH^-} - pK_w + pH \qquad (17)$$

The hydroxyl ion concentration used in equation 15 is defined as

$$[OH^-] = 10^{-pOH} = 10^{-pK_w + pH} \qquad (18)$$

The k_3 values at the various temperatures may be estimated by assuming that the straight line at the lower pH values, *i.e.*, below the pH of the plotted inflection, alkaline branch of the log k *vs.* pH profile of Fig. 4, is characteristic of the hydrolysis of the uncharged psicofuranine. The line may be extrapolated with a slope of unity to the pH equal to the pK_w at that temperature. This appropriate pK_w as derived from the literature[11] (Table IV) may be inserted into equation 17 so that the log k at this pH serves as an estimate of log k_3.

Similarly, the k_4 value may be estimated by extrapolation of the straight line above the pH of the plotted inflection on the alkaline branch of the log k *vs.* pH profile of Fig. 4. Such log k_3 and log k_4 values are listed in Table IV and their Arrhenius plots are given in Fig. 8. Also, Table IV lists the pK_a values as estimated from the points of maximum inflection in the alkaline branch of the profiles of Fig. 4.

The slopes of the Arrhenius plots in Fig. 8 are 5,110 so that by equation 4 the heat of activation is 23.4 kcal./mole. The respective intercepts, log C, of these plots are: for k_3', 10.92; k_3, 11.08; k_4, 11.55.

Discussion

The hydrolysis of a nucleoside such as psicofuranine is subject to both specific acid and base catalysis. The rates of hydrolysis are about 2.5 times faster when the catalytic species attacks the neutral nucleoside than when it reacts with the similarly charged species. The variation in the apparent bimolecular rate constant, k_{H^+}, for the acid hydrolysis is to be expected with respect to the degree of protonation of the adenine moiety of psicofuranine. The variation in the apparent bimolecular rate constant, k_{OH^-}, for the basic hydrolysis confirms the previously published evidence[13] presented for the acidity of the sugar portion of nucleosides of pK_a *ca.* 12.

The kinetic procedures presented herein permit a valid estimation of the apparent hydrogen ion dissociation constant of a nucleoside. Such derived values are presented in Table IV.

The possible products of alkaline degradation could be adenine and psicose as in the acid hydrolysis with the sugar subjected to further reaction under the influence of alkali. However, it must be realized that the method of assay used could not differentiate between non-hydrolyzed nucleoside or some possible degradation intermediate(s). Such an intermediate must survive borohydride reduction so that on the subsequent acid hydrolysis the necessary functionality to react with diphenylamine must be retained or generated.

The asymptotic value of the color assay approached with time on alkaline degradation is significant and indicative of alkali undegraded material and/or the presence of protected carbonyl. One of several possible explanations is that di- or polysaccharides are produced by the action of alkali.

It is of interest to note that the apparent first-order rate constants in HCl solution for this ketonucleoside do not agree, even in magnitude, with the findings of Levene and Sobotka[14] for aldonucleoside hydrolysis. Their value is $k = 7 \times 10^{-4}$ in 0.1 N HCl at 100°. It is assumed that the units are min.$^{-1}$. At 60° and in 0.1 N HCl, the value of k determined by extrapolation of the 60° data in Fig. 3 is much greater; $k = 7.25 \times 10^{-3}$ sec.$^{-1}$ or 0.435 min.$^{-1}$. This indicates that the ketonucleoside is more susceptible to acid hydrolysis than the aldonucleoside. Confirmation of this point may be obtained from the comparison of the hydrolysis rates of simple glucosides. The half-life, $t_{1/2}$, of methyl β-glucopyranoside in 0.05 N HCl at 60° is 1.04×10^5 min., but that of methyl β-fructopyranoside is 12.8 min.[15]

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KALAMAZOO, MICH.

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Hexane Isomer Equilibrium Studies

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The equilibrium hexane isomer distribution has been obtained over the temperature range 26.6–40.6°, using infrared spectrometry. There is substantial disagreement between the experimentally determined values and those calculated from entropies and enthalpies of combustion. The experimentally observed concentration of 2,2-dimethylbutane is lower than the calculated value, while that of 2-methylpentane is higher than the calculated value. The reason for these discrepancies is not known, but it does indicate that thermodynamic hydrocarbon properties should be used with caution.

Introduction

Several relatively recent papers have appeared on a direct experimental determination of the equilibrium concentration of hexane isomers in the liquid phase.[1–4]

van Eijk measured this equilibrium at one temperature, 80°.[2] He did not differentiate between the two dimethylbutane isomers and their sum was

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[3] H. Koch and H. Richter, *Angew. Chem.*, **A59**, 31 (1947).

[4] J. A. Ridgway, Jr., and W. Schoen, Abstracts of Papers, Division of Petroleum Chemistry, A.C.S. Meeting, Boston, Mass., April, 1959. This work appeared after the present paper had been written. In Dr. Ridgway's paper, the equilibrium determinations were carried out at higher temperatures than those reported here. The conclusions reached are similar to those of the present authors.