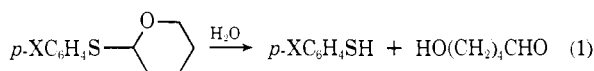# Acid Catalyzed and Mercuric Ion Catalyzed Hydrolysis of 2-(Para-substituted thiophenoxy)tetrahydropyrans[1]

L. R. Fedor* and B. S. R. Murty

*Contribution from the Department of Medicinal Chemistry,
School of Pharmacy, State University of New York at Buffalo,
Buffalo, New York 14214. Received August 9, 1973*

Abstract: Acid catalyzed hydrolysis of 2-(para-substituted thiophenoxy)tetrahydropyrans is characterized by a proportionality between rate and H_0, a deuterium solvent kinetic isotope effect, $k_D/k_H = 1.3$, an activation entropy of 7.4 eu, and a ρ value of -0.96. Mercuric ion catalyzed hydrolysis is characterized by a proportionality between rate and $[Hg^{2+}]$, an activation entropy of 45 eu, and a ρ value of 0.88. Rates of mercuric ion catalyzed hydrolysis are *ca.* 10^2–10^4-fold faster than their acid catalyzed counterparts. The A1 mechanism with protonation or mercuration on sulfur is favored.

Proton transfer occurs before the critical transition state for acid catalyzed hydrolysis of thioacetals such as oxathiolanes,[2-4] 6-purinyl β-D-glucothiopyranoside,[5] and benzaldehyde *o*-methyl phenyl thioacetals.[6] For the latter, C–S bond breaking of S-protonated thioacetals is rate determining. As an extension of our studies on electrophilic catalysis in thioacetal hydrolysis, we report herein results on the acid catalyzed and mercuric ion catalyzed hydrolysis of 2-(para-substituted thiophenoxy)tetrahydropyrans.

$$p\text{-}XC_6H_4S\!-\!\!\langle\!\!\!\!\!\overset{O}{}\!\!\!\!\!\rangle \xrightarrow{H_2O} p\text{-}XC_6H_4SH + HO(CH_2)_4CHO \quad (1)$$

Results

Hydrolysis of 2-(para-substituted thiophenoxy)tetrahydropyrans (**1–5**) to give para-substituted thiophenols and 5-hydroxypentanal is catalyzed by hydronium ions and mercuric ions. Under pseudo-first-order conditions the rate of reaction is given by eq 2. For acid

$$d(XPhSH)/dt = k_{obsd}[substrate] \quad (2)$$

catalyzed reactions, k_{obsd} is not linearly dependent on the molar concentration of the acid; however, log k_{obsd} is linearly dependent on H_0 (eq 3 and 4, Table I).

$$\log k_{obsd} = (-1.10 \pm 0.08)H_0^{HCl} - 0.088 \quad (3)$$

$$\log k_{obsd} = (-1.19 \pm 0.08)H_0^{HClO_4} - 1.01 \quad (4)$$

For mercuric ion catalyzed reactions in acid solutions, $k_{obsd} = k_{Hg}[Hg^{2+}] + b$ (b is the acid contribution to rate), and second-order rate constants, k_{Hg}, were evaluated from slopes of plots of k_{obsd} *vs.* $[Hg^{2+}]$ (Table II). The deuterium solvent kinetic isotope effect, k_D/k_H, = 1.3 (Table III) for hydrolysis of **2** and **4**. The sensitivity of the acid catalyzed reaction toward electronic effects of para substitutents is given by $\rho = -0.96$ (Table IV); the sensitivity of the mercuric ion catalyzed reaction toward electronic effects of para substituents is given by $\rho = +0.88$ (Table II). The Arrhenius activation entropies for the acid catalyzed

(1) This work was supported by grants from the U. S. Public Health Service, National Institutes of Health.
(2) N. C. De and L. R. Fedor, *J. Amer. Chem. Soc.*, **90**, 7266 (1968); **91**, 4017 (1969).
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(5) L. R. Fedor and B. S. R. Murty, *ibid.*, **95**, 8410 (1973).
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Table I. Rate Constants for Hydrolysis of 2-(*p*-Methylthiophenoxy)tetrahydropyran in Acidic 40% (v/v) Dioxane–Water (30 ± 0.1°)

[HCl]	H_0^a	$k_{obsd} \times 10^3$, min^{-1}	[HClO$_4$]	H_0^b	$k_{obsd} \times 10^3$, min^{-1}
0.382	2.06	3.8	0.6	0.88	9.2
0.597	1.88	5.8	0.9	0.54	22.2
0.778	1.76	9.2	1.2	0.28	49.4
0.985	1.62	15.6	1.5	0.03	70.1
1.19	1.46	24.5	1.8	−0.18	148
1.38	1.34	36.4	2.1	−0.4	311
1.56	1.19	37.8			
1.76	0.98	50.2			

^{a} B. Torck, M. Hellin, and F. Coussemant, *Bull. Soc. Chim. Fr.*, 1657 (1962). ^{b} M. A. Paul and F. A. Long, *Chem. Rev.*, **53**, 31 (1957).

Table II. Second-Order Rate Constants for the Mercuric Ion Catalyzed Hydrolysis of 2-(Para-substituted thiophenoxy)tetrahydropyrans in 1.96 M Hydrochloric Acid in 40% (v/v) Dioxane–Water (30 ± 0.1°)

Compd	k_{Hg}, M^{-1} min^{-1}	$[Hg^{2+}] \times 10^3$, M	No. of k_{obsd}
1 (*p*-CH$_3$O)	58	0.03–0.4	10
2 (*p*-CH$_3$)	50	0.05–1	16
3 (H)	91	0.05–0.8	12
4 (*p*-Cl)	142	0.05–1	16
5 (*p*-NO$_2$)	415	0.08–1	12

Table III. Deuterium Solvent Kinetic Isotope Effects for the Reactions of 2-(Para-substituted thiophenoxy)tetrahydropyrans in 40% v/v Dioxane–Water (Deuterium Oxide) (30 ± 0.1°)

Compd	k_H, M^{-1} min^{-1} ^{a,c}	k_D, M^{-1} min^{-1} ^{b,c}	k_D/k_H
2 (*p*-CH$_3$)	0.0159	0.0199	1.25
4 (*p*-Cl)	0.0053	0.0068	1.28

^{a} k_{obsd} divided by 0.985 M hydrochloric acid. ^{b} k_{obsd} divided by 0.9917 M deuterium chloride. ^{c} Average of three runs.

and mercuric ion catalyzed hydrolyses of **2** are (Table V) 7 and 45 eu, respectively.

Discussion

The most generally accepted mechanism for acid catalyzed hydrolysis of acetals and ketals is that of eq

Table IV. Rate Constants for the Acid Catalyzed Hydrolysis of 2-(Para-substituted thiophenoxy)tetrahydropyrans in 1.96 M Hydrochloric Acid in 40% v/v Dioxane–Water (30 ± 0.1°)

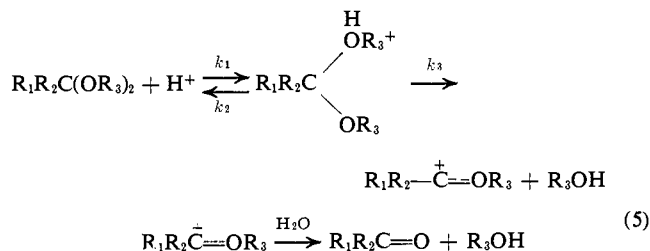| Compd	k_{obsd}^a $\times 10^2$, min^{-1}	Compd	k_{obsd}^a $\times 10^2$, min^{-1}
1 (p-CH$_3$O)	7.48 ± 0.1	4 (p-Cl)	3.14 ± 0.08
2 (p-CH$_3$)	7.68 ± 0.1		0.526 ± 0.004^b
	1.56 ± 0.1^b	5 (p-NO$_2$)	0.89 ± 0.004
3 (H)	9.21 ± 0.08		

^{a} Average of three runs. ^{b} For reaction in 0.985 M HCl in 40% (v/v) dioxane–water.

Table V. Temperature-Dependent Second-Order Rate Constants and Arrhenius Activation Parameters for the Hydrolysis of 2-(p-Methylthiophenoxy)tetrahydropyrans in 1.96 M Hydrochloric Acid^a

Temp, °C	k_H, M^{-1} min^{-1} ^{b}	$\Delta H^\ddagger$, kcal mol^{-1}	$\Delta S^\ddagger$, eu
25	0.0198		
30	0.0394	24.4 ± 0.4	7.4 ± 0.2
38.8	0.128		
	k_{Hg}, M^{-1} min^{-1} ^{b}		
25	74		
30.6	200	30.7 ± 0.4	45 ± 0.5
38.8	770		

^{a} Solvent, 40% (v/v) dioxane–water. ^{b} Average of two runs.

5.[7] Two limiting cases of this mechanism have been recognized: for $k_2 \gg k_3$, carbonium ion formation is rate determining (A1 mechanism); for $k_3 \gg k_2$, protonation of the acetal or ketal is rate determining (ASE2 mechanism). The most convincing evidence for the ASE2 mechanism is the result that general acid catalysis of hydrolysis is observed for those acetals having good leaving groups and/or which give rise to relatively stable oxocarbonium ions.[7] If such catalysis is not experimentally detectable for various reasons, then mechanism assignment, with respect to the timing of proton transfer, may be ambiguous.


$$R_1R_2\overset{+}{C}{=}OR_3 + R_3OH$$

$$R_1R_2\overset{\cdot}{C}{=}OR_3 \xrightarrow{H_2O} R_1R_2C{=}O + R_3OH \quad (5)$$

Further difficulty is encountered in assigning mechanisms of hydrolysis to thioacetals containing the O–C–S linkage; the additional complicating feature of the site of proton transfer, sulfur vs. oxygen, is introduced. The following discussion of results leads to a conclusion of mechanism of hydrolysis of 1–5, which must be tempered by the limitations of the kinetics methods used. Considered in turn they are electrophilic catalysis, the

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deuterium solvent isotope effects, the Hammett ρ values, and the Arrhenius activation parameters.

The Mercuric Ion Catalyzed Reaction. In the following discussion, "catalysis" is used in the sense of facilitated hydrolysis of 1–5. Mercuric ion is 10^2–10^4 times more efficient than hydronium ion as an electrophilic catalyst. In contrast to hydrolysis of 1–5 catalyzed by hydronium ion, the catalytic site for mercuration, the sulfur atom, is unambiguous. Thus, mercuric ion catalyzes hydrolysis of 2-phenyloxathiolane but not of 2-phenyldioxolane. Further, hydrolysis of 2-(p-tolyl)-1,3-dithiane is catalyzed by mercuric ion (k_{Hg} = 3 M^{-1} min^{-1}), under conditions wherein hydronium ion catalysis is undetectable.[8] In terms of the mechanism of eq 5, it remains then to attempt identification of the rate-determining step in the reaction k_1 vs. k_3.

The kinetics results of hydrolysis of methyl para-substituted phenyl acetals of benzaldehyde 6[9,10] show
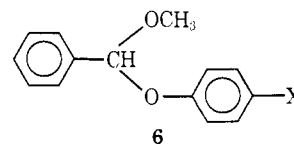
that this reaction is general acid catalyzed, k_1, eq 5, rate determining, and that the para-substituted phenoxy moiety is the leaving group. In contrast, hydrolysis of methyl para-substituted phenyl thioacetals of benzaldehyde 7 is catalyzed only by hydronium ion
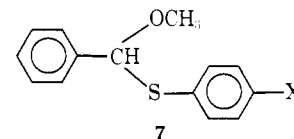
(not by acids generally), k_3, eq 5, rate determining, and for these compounds the para-substituted thiophenol moiety is the leaving group.[6] Additionally, for the 2,4-dinitro derivative noncatalyzed, spontaneous hydrolysis occurs.[6] On the basis of the above results it was reasonably concluded that C–S bond breaking in 7 is sufficiently difficult, compared to C–O bond breaking in 6, that k_3, eq 5, is rate determining for reactions of 7. For reactions of 1–5, carbonium ion stability should be less than for 7 because of less favorable geometry of pyrans 1–5 for oxygen p electron overlap with the incipient carbonium ion and because carbonium ion stabilization by phenyl is at least 30 times more effective than that by methyl using relative rates of benzaldehyde diethyl acetal and acetaldehyde diethyl acetal as a criterion.[7,11] Therefore, C–S bond cleavage in 1–5 should be even more difficult than for 7. Given the great propensity for mercuric ion to bind to sulfur,[2] mercuration of 1–5 at sulfur should be rapid with respect to C–S bond breaking and the A1 mechanism should be favorable.

Generally, the A1 mechanism of acetal hydrolysis is attended by positive activation entropies compared with negative activation entropies for A2 and ASE2 processes. Hydrolysis of 1–5 catalyzed by mercuric ion is characterized by $\Delta S^\ddagger$ = 45 eu, suggestive of an A1

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mechanism. The closest literature analogy is that for mercuric ion catalyzed hydrolysis of 2-phenyloxa-thiolane for which $\Delta S^{\ddagger} = -30$ eu and for which reaction the A2 mechanism was favored.[2] ρ is 0.88 for mercuric ion catalyzed hydrolysis of 1-5. If the A1 mechanism is assumed it can be shown that $\rho(\text{exptl}) = \rho(k_3) - \rho(k_2/k_1)$. Since $\rho(k_2/k_1)$ must have some positive value, it follows that $\rho(k_3)$ must also be positive, consistent with the A1 mechanism in which C–S bond breaking occurs in the rate-determining step. Such a process should be facilitated by electron withdrawal by para substituents. The result that $\rho = 0.88$ is also compatible with the ASE2 mechanism wherein mercuration of sulfur is concerted with C–S bond breaking but not solely with mercuration. Taken in total, the results are most consistent with the A1 mechanism of hydrolysis.

The Acid Catalyzed Reaction. Kinetically, acid catalyzed hydrolysis of 1-5 is characterized by (i) proportionality between log k_{obsd} and H_0, but not between log k_{obsd} and log C_{H^+} nor between k_{obsd} and C_{H^+}, (ii) $\Delta S^{\ddagger} = 7.4$ eu, (iii) $k_{\text{D}}/k_{\text{H}} = 1.3$, (iv) $\rho = -0.96$. These data favor the A1 mechanism, eq 5, k_3 rate determining, as the following discussion will show. They are also in accord with sulfur protonation, although this feature of the mechanism may be less certain than it is in mercuric ion catalyzed hydrolysis.

In acetal hydrolysis, following the Zucker–Hammett hypothesis,[12] the H_0 acidity function has been used as a criterion of mechanism to distinguish between A1 and A2 mechanisms.[13] However, this criterion of mechanism which has been discussed by Long and Schaleger[14] and Bunnett,[15] among others,[16] is unreliable. Be that as it may, hydrolyses of 1-5 catalyzed by HCl and HClO$_4$ obey H_0, with slopes -1.10 and -1.19, respectively, in accord with the A1 mechanism. Fife and Jao[3] reported a slope of -1.23 for hydrolysis of 2-(p-nitrophenyl)-1,3-oxathiolane and cited this result as support for an A1 mechanism. For hydrolysis of 2-methyl- and 2,2-dimethyl-1,3-oxathiolane, the slopes are -1.27 and -1.34, and the A1 mechanism was similarly invoked.

General acid catalysis of hydrolysis, indicative of rate-determining proton transfer, was not detected; 1-5 are too stable in buffer solutions of general acids to permit detection of such catalysis, even at 70°.

For 2, $\Delta S^{\ddagger} = 7.4$ eu comparable with the $\Delta S^{\ddagger}$ values for phenyl 1-thio-β-D-glucopyranoside[17] (10.8 eu) and for ethyl 1-thio-β-D-glucopyranoside (12.4 eu)[17] for which the A1 mechanisms are postulated. In contrast, De and Fedor[2] reported $\Delta S^{\ddagger}$ values of -18 and -25 eu for the acid catalyzed hydrolysis of 2-phenyl-1,3-oxothiolane and 2-methyl-2-phenyl-1,3-oxothiolane for which the A2 mechanism was suggested.

The deuterium solvent isotope effects $(k_{\text{D}}/k_{\text{H}} = 1.3)$ for 2 and 4 are considerably less than generally obtained in A1 acetal hydrolysis reactions where ratios

from 2.7 to 3.0 are normally seen.[18-20] The small solvent isotope effect probably reflects the differences arising from protonation of sulfur rather than of oxygen which have been observed in the case of 7 where rate-determining C–S bond cleavage occurs.[6]

For acid catalyzed hydrolysis of 1-5 $\rho = -0.96$, which is comparable with $\rho = -0.9$ for hydrolysis of para-substituted thiophenyl-β-glucopyranosides[21] which are suggested to hydrolyze via the A1 mechanism. The following, nonrigorous estimate of ρ (k_3) may be made. If $\rho = 1.8$ for dissociation of 1H$^+$-5H$^+$, the value for ionization of para-substituted thiophenols is 1.80;[22] it can be shown (vide supra) that $\rho(k_3) = 0.9$, a reasonable value for rate-determining C–S bond breaking via the A1 mechanism.

Various kinetic parameters determined in the present study for the acid catalyzed and mercuric ion catalyzed hydrolysis of 1-5 best support the A1 mechanism of hydrolysis. If sulfur protonation as well as sulfur mercuration are on the reaction pathway, then Hg^{2+} is indeed a potent electrophilic catalyst for hydrolysis of 1-5.

Experimental Section

Reagents and Compounds. Certified ACS grade inorganic salts were purchased from Fisher Scientific Co. Benzenethiols were purchased from Aldrich Chemical Co., Inc. Dioxane (Fisher) was purified by refluxing with lithium aluminum hydride for 10–12 hr followed by distillation. Deuterium chloride and deuterium oxide were purchased from Diaprep Inc. All the solvents were Fisher supplied and were dried and distilled before use. Cleland's reagent (dithiothreitol) was purchased from Calbiochem. The elemental analyses were performed by Galbraith Microanalytical Lab., Knoxville, Tenn. 2-(Para-substituted thiophenoxy)tetra-hydropyrans were synthesized by the following procedure. Equimolar quantities of para-substituted thiophenol and dihydropyran in benzene containing a few crystals of p-toluenesulfonic acid were heated at reflux for 6–8 hr. Upon cooling, the solution was washed with saturated NaHCO$_3$ solution and water, dried (MgSO$_4$), concentrated, and distilled (or crystallized from benzene in the case of 5) to give the product. Reported λ_{max} values are for 40% (v/v) dioxane–water. 2-(p-Methoxythiophenoxy)tetrahydropyran (1), 82% yield, had bp 126–130° (0.3 mm), λ_{max} 241 nm (ϵ 10,030). *Anal.* Calcd for C$_{12}$H$_{16}$O$_2$S: C, 64.28; H, 7.14. Found: C, 64.50; H, 7.05. 2-(p-Methylthiophenoxy)tetrahydropyran (2), 70% yield, had bp 112–114° (0.4 mm), λ_{max} 242 nm (ϵ 9800). *Anal.* Calcd for C$_{12}$H$_{16}$OS: C, 69.23; H, 7.69. Found: C, 69.18; H, 7.61. 2-Thiophenoxytetrahydropyran (3), 62% yield, had bp 100–102° (0.9 mm), λ_{max} 250 nm (ϵ 7100). *Anal.* Calcd for C$_{11}$H$_{14}$OS: C, 68.04; H, 7.22. Found: C, 68.27; H, 7.39. 2-(p-Chlorothiophenoxy)tetrahydropyran (4), 57% yield, had bp 128–130° (0.4 mm), λ_{max} 254 (ϵ 10,630). *Anal.* Calcd for C$_{11}$H$_{13}$-ClOS: C, 57.78; H, 5.69. Found: C, 58.00; H, 5.60. 2-(p-Nitrothiophenoxy)tetrahydropyran (5), 50% yield, had mp 59–60°, λ_{max} 330 (ϵ 9940). *Anal.* Calcd for C$_{11}$H$_{13}$NO$_3$S: C, 55.23; H, 5.44; N, 5.86. Found: C, 55.06; H, 5.43; N, 5.65. The nmr spectra and integrations were in agreement with assigned structures.

Kinetics. Stock solutions of compounds 1-5 were prepared in dry methanol. All the reactions were carried out in redistilled water at 30 ± 0.1° unless otherwise specified. The courses of the acid catalyzed reactions were monitored at wavelengths 236 (1, 3), 242 (2, 4), and 325 (5). For mercuric ion catalyzed reactions, the wavelengths used were 235 (1), 260 (2), 254 (3), 270 (4), and 310 (5). Calculated ionic strength was adjusted with potassium chloride. pH values were determined before and after all runs to ensure constancy of pH ±0.02 unit for lower acid concentrations. Re-

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actions were started by adding a microdrop of the appropriate substrate in methanol to 3-ml cuvettes filled to the stopper level with the proper solutions and equilibrated at appropriate temperatures (final concentration of the substrate is $3–5 \times 10^{-4}\,M$). Reactions were monitored to completion. Pseudo-first-order rate constants, determined by multiplying the slopes of plots of log (OD_∞ − OD_0)/(OD_∞ − OD_t) or log (OD_0 − OD_∞)/(OD_t − OD_∞) vs. time by 2.303, were linear to ca. 2–3 half-lives. Dithiothreitol (0.02–0.03 M) was added to acidic solutions to prevent the formation of disulfide from the product thiols. For 1×10^{-4} solutions of 1 in 1.87 M HCl, k_{obsd} values were identical in 0.013, 0.026, and 0.039 M dithiothreitol.

Product Analysis. Hydrolysis of 2-(p-methylthiophenoxy)tetrahydropyran in 1.87 M HCl in 40% aqueous dioxane gave p-methylbenzenethiol. The uv spectrum of this hydrolysis product (ϵ 9450 at 242 nm) was identical with the uv spectrum of an authentic sample (p-methylthiophenol + hydroxypentanal) under identical conditions (ϵ 9800 at 242 nm). This corresponds to a product yield of 96.4%.

Authentic p-methoxybenzenethiol in 1.87 M HCl in 40% aqueous dioxane was autoxidized to disulfide with a pseudo-first-order rate constant of 0.0142 min⁻¹. Under identical conditions, p-methoxybenzenethiol obtained from the hydrolysis of 1 underwent autoxidation with k_{obsd} = 0.0141 min⁻¹.

Acid Catalyzed and β-Glucosidase Catalyzed Hydrolysis of 6-Purinyl β-D-Glucothiopyranoside[1]

L. R. Fedor* and B. S. R. Murty

Contribution from the Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214. Received August 18, 1973

Abstract: Hydrolysis of 6-purinyl β-D-glucothiopyranoside (MPG) to 6-mercaptopurine and glucose is catalyzed by hydrogen ion and by almond β-glucosidase. This thioacetal is ca. 10^6-fold more reactive toward hydronium ion than is phenyl β-D-glucothiopyranoside. The dependency of rate on acidity in H_2O and in D_2O suggests that both acidic and neutral forms of MPG undergo acid-catalyzed hydrolysis via the A-1 mechanism. β-Glucosidase catalyzed hydrolysis of MPG in the pH range 3.55–5.95 is characterized by a very shallow bell-shaped V_{max}–pH profile with a maximum at ca. pH 5.3; K_m decreases as pH increases. The deuterium solvent kinetic isotope effect $V_{max}(H_2O)/V_{max}(D_2O) = 1$ (pH = pD = 5.35).

Plant and animal thioglycosidase, almond β-glucosidase, and aqueous acid hydrolyze 6-purinyl-β-D-glucothiopyranoside (MPG) to give the antileukemia

MPG
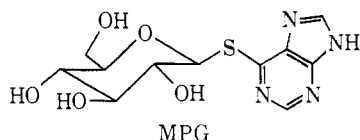
drug 6-mercaptopurine (MP) and glucose.[2,3] The high hydrolytic lability of MPG in acid solution may signal an unusual mechanism of thioglucoside hydrolysis which in turn may be related to the β-glucosidase mechanism of hydrolysis. The present investigation is concerned with the details of the acid catalyzed and β-glucosidase catalyzed hydrolysis of MPG. Less extensively studied was the acid-catalyzed hydrolysis of 6-purinyl β-D-glucothiopyranosiduronic acid (MPGU).

Experimental Section

Materials. 6-Mercaptopurine (MP) was obtained from Nutritional Biochemicals Co. 6-Purinyl β-D-glucothiopyranoside (MPG) and 6-purinyl β-D-glucothiopyranosiduronic acid (MPGU) were obtained from Dr. G. H. Hitchings. β-Glucosidase was obtained from Worthington Biochemical Corp., and deuterium oxide was obtained from Diaprep, Inc.

Apparatus. The apparatus used was previously described.[4]

Kinetics. Formation of MP was monitored at 320–325 nm following addition of MPG or MPGU contained in a microdrop of H_2O/DMF to temperature equilibrated solutions of aqueous acid (3 ml) or aqueous enzyme (0.5 ml) contained in cuvettes. For acid-catalyzed reactions, pseudo-first-order rate constants were obtained by multiplying slopes of plots of log [(OD_∞ − OD_t)/(OD_∞ − OD_t)] vs. time by 2.303. Plots were linear to at least 3 half-lives and OD_∞ values were stable. For β-glucosidase catalyzed reactions, rates (v) were obtained from initial slopes of plots of OD vs. time. V_{max} and K_m were then evaluated from the intercepts and slopes of plots of $1/v$ vs. 1/[MPG] at constant enzyme concentration.

Product Analysis. Uv spectra taken after kinetic runs were identical with those of authentic MP: at pH 4.10, ϵ_{325} 17,876 for MP; for reaction products, ϵ_{325} 17,518 which corresponds to a 98% conversion of MPG to mercaptopurine.

Results

Acid-Catalyzed Reaction. Acid-catalyzed hydrolysis of MPG to MP and glucose in water solution and in deuterium oxide solution is kinetically described by eq 1. At high acidity $[H^+] > c$ and plots of k_{obsd} vs.

$$k_{obsd} = (a[H^+] + b[H^+]^2)/(c + [H^+]) \qquad (1)$$

$[H^+]$ are linear with slope b and intercept a: at low $[H^+]$, $c > [H^+]$, $[H^+]^2 \rightarrow 0$, and plots of k_{obsd} vs. $[H^+]$ are linear with slope a/c and intercept zero (Figure 1). No hydrolysis was detectable at pH 6 during 60 days. The values of a, b, and c, determined using a computerized nonlinear regression analysis, are provided in Table I. The rate of hydrolysis of MPG is unaffected by formate buffer, 0.2–1.0 M, pH 3.15, $t = 49.4°$, $k_{obsd} = (1.66 \pm 0.01) \times 10^{-3}$ min⁻¹. Similarly, no catalysis by 1 M phosphate buffer, pH 1.1, nor by 1 M dichloroacetate buffer, pH 0.77, was detected: hydrolysis rates were depressed to ca. 85–90% of the

(1) This work was supported by a grant from the U. S. Public Health Service.

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