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 $(OD_\infty - OD_0)/(OD_\infty - OD_t)$ or log $(OD_0 - OD_\infty)/(OD_t - OD_\infty)$ *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}.

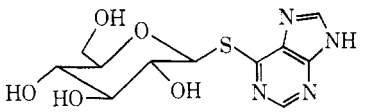
Acid Catalyzed and β-Glucosidase Catalyzed Hydrolysis of 6-Purinyl β-D-Glucothiopyranoside[1]

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 $[(OD_\infty - OD_i)/(OD_\infty - OD_t)]$ *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.

(2) I. Goodman, L. Salce, and G. H. Hitchings, *J. Med. Chem.*, **11**, 516 (1968).

(3) I. Goodman, J. R. Fouts, E. Bresnick, R. Menegas, and G. H. Hitchings, *Science*, **130**, 450 (1959).

(4) L. R. Fedor, N. C. De, and S. K. Gurwara, *J. Amer. Chem. Soc.*, **95**, 2905 (1973).

Table I. Rate Data for the Acid-Catalyzed Hydrolysis of Purine Thioglycosides[a-c]

Compd	a, min^{-1}	b, M^{-1} min^{-1}	c, M	[HCl], M	No. of k_{obsd}	
MPG[d]	0.00948	0.05626	0.0532	(0.006–1.0)	30	0.0000078[e]
MPG[d,f]	0.01197	0.10530	0.0268	(0.001–1.0)	16	0.00000118[e]
MPGU[g]	0.000228	0.001232		(0.4–1.0)	8	0.994[h]
MPGU[f,g]	0.000213	0.002489		(0.2–0.50)	8	0.984[h]

[a] $t = 30 \pm 0.1°$, $\mu = 1.0$ M(KCl). [b] Tabulated constants are those of eq 1. [c] Correlation coefficient for plots of k_{obsd} *vs.* acid concentration. [d] Data obtained by using NLIN-2 program from SUNY Computer Center Library. [e] Root mean square deviation for experimental *vs.* calculated first-order rate constants. [f] Reactions were run in D_2O. [g] Data obtained by plotting k_{obsd} *vs.* acid concentration. [h] See paragraph at end of paper regarding Supplementary Material.

values calculated on the basis of [H$^+$]. Acid-catalyzed hydrolysis of MPGU in water and in deuterium oxide is kinetically described by eq 1, [H$^+$] > c, so that in the acid concentration range employed, plots of k_{obsd} *vs.* [H$^+$] are linear with slope b and intercept a (Table I).

β-Glucosidase Catalyzed Reaction. Hydrolysis of MPG catalyzed by almond β-glucosidase obeys the Michaelis–Menten equation. Plots of the reciprocals of the initial velocities for MP formation *vs.* the reciprocals of [MPG] at constant enzyme concentration and constant pH gave intercepts $1/V_{max}$ and slopes K_m/V_{max} (Table II).

Table II. Rate Date for Hydrolysis of MPG Catalyzed by β-Glucosidase[a,d]

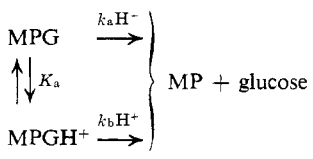
pH	K_m, M	$V_{max} \times 10^6$, M min^{-1}	r[b]	No. of runs
3.55	0.0135	6.74	0.997	5
4.10	0.0088	6.96	0.998	5
4.60	0.0043	6.04	0.993	5
5.15	0.0042	8.36	0.998	5
5.35	0.0057	9.73	0.998	5
5.35[c]	0.0096	8.90	0.975	5
5.62	0.0033	7.50	0.998	5
5.95	0.0029	6.19	0.960	5

[a] 0.1 M acetate buffer, $t = 25°$, enzyme concentration = 0.01 mg/ml, [MPG] = 1.206–6.03 × 10^{-3} M, five concentrations (five rates) for each value. [b] Correlation coefficient for plots of $1/v$ *vs.* 1/(S). [c] pD value of the D_2O solutions. [d] See paragraph at end of paper regarding Supplementary Material.

Discussion

Acid-Catalyzed Reaction. The dependence of rate on hydronium ion concentration is accommodated by the mechanism of Scheme I which involves acid-catalyzed hydrolysis of MPG and its conjugate acid, MPGH$^+$. For Scheme I, the rate of MP formation, which is equal to the rate of MPG loss, is given by eq 2

Scheme I



$$v/[MPG] = k_{obsd} = (k_aK_a[H^+] + k_b[H^+]^2)/(K_a + [H^+]) \quad (2)$$

which has the form of eq 1. The k_a term for reaction of MPG with a proton is kinetically indistinguishable from one for spontaneous hydrolysis of MPGH. The former interpretation, as written in Scheme I, is favored on the basis that ρ is negative for the preferred interpretation while it is positive for the one involving a spontaneous decomposition of MPGH.[5] In the benzene series, ρ is negative for acid-catalyzed hydrolysis of para-substituted phenyl β-D-glucothiopyranosides,[6] 2-aryloxytetrahydropyrans,[7] benzaldehyde methyl *S*-(substituted phenyl) thioacetals,[8] 2-(para-substituted phenylthio)tetrahydropyrans,[9] etc.[10] Also, deuterium solvent kinetic isotope effects are more consistent with the mechanism of Scheme I than with its kinetically indistinguishable variant (*vide infra*).

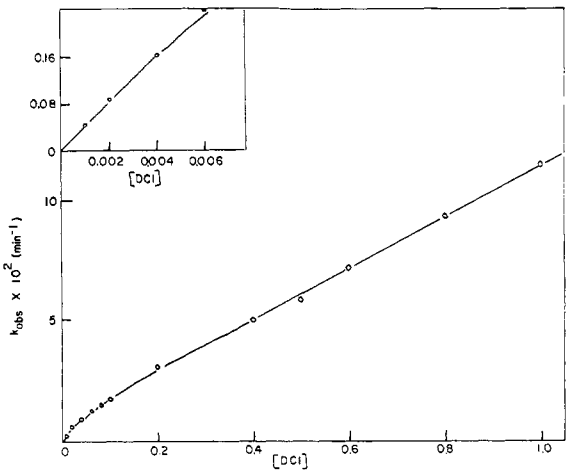


Figure 1. Plot of the pseudo-first-order rate constants, k_{obsd}, *vs.* the molar concentration of DCl in D_2O for hydrolysis of MPG, $t = 30°$, $\mu = 1$ M (KCl). The solid lines are calculated from the constants of Table I and the symbols represent experimental values of rate constants for given DCl concentrations.

The mechanism of Scheme I requires further elaboration with respect to the nature of the proton transfer reactions represented by k_a and k_b. The result that hydrolysis of MPG and MPGH is not general acid catalyzed effectively rules out hydrolysis mechanisms involving rate-determining proton transfer. No examples of general acid catalysis of hydrolysis of thioacetals have been reported: most likely this class of compounds hydrolyzes by the A-1 or A-2 mechanism.[6,8,9,11-13] Accordingly, the most conservative mechanism for hydrolysis of MPG and MPGH is the A-1 mechanism shown in Scheme II. From eq 2, k_a =

(5) ρ is here used in a very qualitative sense (positive *vs.* negative value).

(6) G. Wagner and M. Wagler, *Arch. Pharm. (Weinheim)*, **297**, 348 (1964).
(7) T. H. Fife and L. K. Jao, *J. Amer. Chem. Soc.*, **90**, 4081 (1968).
(8) T. H. Fife and E. Anderson, *ibid.*, **92**, 5464 (1970).
(9) L. R. Fedor and B. S. R. Murty, *ibid.*, **95**, 8407 (1973).
(10) E. H. Cordes, *Progr. Phys. Org. Chem.*, **4**, 1 (1967).
(11) N. C. De and L. R. Fedor, *J. Amer. Chem. Soc.*, **90**, 7266 (1968); N. C. De and L. R. Fedor, *ibid.*, **91**, 4017 (1969).
(12) T. H. Fife and L. K. Jao, *ibid.*, **91**, 4217 (1969).
(13) K. Pihlaja, *ibid.*, **94**, 3330 (1972).

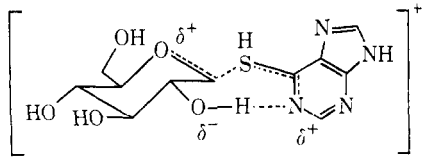
Scheme II

$$\underset{(\text{MPGH})}{\text{MPG} + \text{H}^+} \underset{K_1(K_2)}{\overset{}{\rightleftharpoons}} \underset{(\text{MPGH}_2^+)}{\text{MPGH}^+} \xrightarrow{k_1(k_2)} \text{products}$$

k_1/K_1 and $k_b = k_2/K_2$. The deuterium solvent kinetic isotope effects, $k_a(D_2O)/k_a(H_2O) = 2.5$ and $k_b(D_2O)/k_b(H_2O) = 1.9$, provide support for the A-1 mechanism shown in Scheme II.[10,14] Although the timing of the proton transfer reaction is established, the site of protonation, O *vs.* S, is uncertain. Guinot[15] has shown that protonation of 2,2-dimethyl-1,3-oxathiolane in FSO_3H–SbF_5 leads exclusively to the carbonium–sulfonium cation (**1**) suggesting that for oxathiolanes, O-protonation followed by C–O bond cleavage may be the path to hydrolysis. This suggestion is supported by results of the effect of α-deuterium substitution on hydrolysis rates of oxathiolanes derived from 4-*tert*-butylcyclohexanone.[16] Further, partial hydrolysis of methyl 1-thio-α-D-ribopyranoside in HCl followed by product isolation gives β-pyranoside and β-furanoside derived from O-protonated, ring-opened pyranoside.[17] However, some of these results may not be related to mechanisms of thioacetal hydrolysis. Fife and Anderson[8] established that hydrolysis of benzaldehyde methyl *S*-tolylthioacetal and benzaldehyde methyl *S*-(*p*-chlorophenyl) thioacetal occurs *via* S-protonation followed by C–S bond cleavage to give the products. Further, for these reactions $k(D_2O)/k(H_2O) = 1.5$, a rather small solvent isotope effect for an A-1 mechanism,[10] but nevertheless, one associated with S-protonation. For MPG and MPGH, similar small isotope effects as well as analogous good leaving groups favor S-protonation.

$$(CH_3)_2C{=}\overset{+}{S}{-}CH_2CH_2O\overset{+}{H}_2$$
1

A particularly interesting aspect of the hydrolysis of MPG is its great reactivity compared with that of phenyl β-D-glucothiopyranoside. From the dependence of rate on temperature the calculated rate constant for hydrolysis of this compound is 10^{-7} min^{-1} in 1 *M* HCl at 30°.[18] Under these conditions MPG and MPGH hydrolyze 2×10^6 and 5×10^5 more rapidly. Such rate enhancements find analogy in the large hydrolytic rates of glucothiopyranosides having nitrogen-containing heterocyclic aglycones.[19] For none of these compounds was a rate–acidity dependence determined so that the rate contributions of possible reactants are unknown. It seems likely that this enhanced reactivity is due, at least in part, to the excellence of heterocyclic thiones such as 2- and 4-thiopyridone as leaving groups. However, the magnitude of the reactivity difference between phenyl β-D-glucothiopyranoside and MPG appears to be too great to be accommodated strictly in terms of relative leaving tendencies of the aglycones. In the case of 2-pyridyl β-D-glucothiopyranoside, N-protonation (pK of 2-methylmercaptopyridine = 3.23)[20] provides for generation of S-protonated 2-thiopyridone as the leaving group. The site of ring protonation in MPG is ambiguous. We suggest MPG accepts a proton (K_a) in the imidazole ring to give MPGH. A number of substituted 6-thiopurines, for which imidazole protonation is likely, have pK_a values in the range 0.9–2.2[21] (*cf.* the kinetic pK_a (MPGH) = 1.3) while the kinetic pK_a for various thiolimidate esters is 3.8–7.[22] If this suggestion is correct, then MPG may in fact be deprived of a possible kinetic advantage available to the pyridyl compounds, unless another proton source is available to the N^1 atom of MPG. Such a source could be the 2′-OH of the glycone. This hydrogen bonded structure should enjoy great reactivity because the aglycone



leaves as the thermodynamically more stable thioamide, rather than as the thioimidate,[23] and importantly, to the extent that there is an excess electron density at 2′-OH, the incipient oxocarbonium ion is further stabilized. Both of these features should be rate accelerating. A variant of this mechanism involves general base catalysis by N^1 of 1,2-epoxide formation concerted with loss of the protonated aglycone. The experimentally observed isotope effects, although indicative of specific acid catalysis, could nevertheless reflect a component of general base catalysis depending on the extent (~0, ~100%) of proton transfer in the transition state.[24] No buffer catalysis by the weak base formate ion was detected, a result which may be used to argue against general base catalysis of epoxide formation. However, such catalysis could be difficult to detect if β approached zero or one. Also, an intermolecular proton transfer reaction could go undetected because its rate would be much less than that of an intramolecular reaction. Bearing on this general question of mechanism, the configuration of the glucose product was not examined because the rate of mutarotation of glucose exceeds the rate of MPG hydrolysis.[25]

6-Purinyl β-D-glucothiopyranosiduronic acid (MPGU) undergoes acid-catalyzed hydrolysis to MP and glucuronic acid *ca.* 50-fold more slowly than does MPG. Capon and Ghosh[26] reported 2-naphthyl β-D-glucopyranosiduronic acid undergoes acid-catalyzed hydrolysis *ca.* 65-fold more slowly than does 2-naphthyl β-D-glucopyranoside and attributed the rate difference to transition state destabilization of the incipient

(14) F. A. Long, *Ann. N. Y. Acad. Sci.*, **84**, 596 (1960).
(15) F. Guinot, G. Lamaty, and H. Munsch, *Bull. Soc. Chim. Fr.*, 541 (1971).
(16) F. Guinot and G. Lamaty, *Tetrahedron Lett.*, 2569 (1972).
(17) C. J. Clayton, N. A. Hughes, and S. A. Saeed, *J. Chem. Soc.*, 644 (1967).
(18) C. Bamford, B. Capon, and W. G. Overend, *J. Chem. Soc.*, 5138 (1962).
(19) G. Wagner and R. Metzner, *Naturwissenschaften*, **52**, 83 (1965).
(20) A. Albert, "Physical Methods in Heterocyclic Chemistry," Vol. 1, A. R. Katritzky, Ed., Academic Press, New York, N. Y., 1963, p 90 ff.
(21) D. Lichtenberg, F. Bergmann, and Z. Neiman, *Isr. J. Chem.*, **10**, 805 (1972).
(22) R. K. Chaturvedi, A. E. MacMahon, and G. L. Schmir, *J. Amer. Chem. Soc.*, **89**, 6984 (1967).
(23) P. Beak, J. Bonham, and J. T. Lee, Jr., *ibid.*, **90**, 1569 (1968).
(24) In order to reconcile the experimental deuterium solvent isotope effects with preequilibrium sulfur protonation, for which $k(D_2O)/k(H_2O)$ appears to be smaller than for acetals,[8] and with proton transfer from 2′-OH to N^1, Referee II suggested that the latter interaction might better be viewed as a hydrogen bond. Thus instead of being in transit, the proton would be in a potential well and the contribution of this interaction to the isotope effect should be small.
(25) C. S. Hudson, *J. Amer. Chem. Soc.*, **32**, 889 (1910).
(26) B. Capon and B. C. Ghosh, *J. Chem. Soc. B*, 739 (1971).

glycosyl oxocarbonium ion by electron withdrawal by CO_2H ($\rho = -6.5$ for CO_2H, CO_2^-, CH_2OH). A similar explanation suffices to account for the 50-fold rate difference between MPG and MPGU.

β-Glucosidase Catalyzed Reaction. As an enzyme class, glycosidases catalyze hydrolysis of various glycosides.[27] The most extensively studied glycosidase is hen's egg white lysozyme whose detailed mechanism of action, as with all enzymes, remains an open question.[28,29] It is generally accepted by workers in the field that Glu-35 functions as a general acid in the hydrolytic reaction, facilitating departure of the aglycone; the role of Asp-52 is less certain. It has been suggested that this carboxylate residue electrostatically stabilizes the incipient oxocarbonium ion, although there is little evidence for this in model systems,[30] or acts as a nucleophile to form a covalently bound glycosyl–enzyme intermediate. For lysozyme, oxocarbonium ion formation is supported by model studies[31] but the role of Asp-52 remains unknown. For almond β-glucosidase, similar studies[31] support a nucleophilic displacement mechanism although here the nucleophilic role of carboxylate is qualified to include nucleophilicity toward the proton of 2′-OH or H_2O (general base catalysis). The fact that lactones derived from substrates are potent inhibitors of glycosidases in general, a point which speaks to transition-state structure, suggests possible similarities in mechanisms.[32–34] Qualitatively, almond β-glucosidase mimics lysozyme action in that hydrolysis is characterized by a bell-shaped pH–initial velocity profile suggesting involvement in catalysis of carboxylate and carboxylic acid residues.[32]

For MPG, V_{max} at constant enzyme concentration shows a bell-shaped pH dependence with a maximum rate increase in the pH range 3.55–5.95 of *ca.* 50%. At low acidity V_{max} appears to be constant, suggesting possible incursion of a spontaneous rate of hydrolysis catalyzed by the enzyme. Concurrently, K_m decreases with increasing pH. The parameter V_{max}/K_m increases with increasing pH to a maximum value and appears to decrease as pH is further increased: data were not collected at sufficiently high pH to establish this convincingly. Although V_{max} and K_m values may be complex constants,[28] they qualitatively reflect catalysis and binding and suggest that catalysis is influenced by an acidic and a basic group of the enzyme and binding is enhanced by a basic group. The same base may influence V_{max} and K_m, *i.e.*, that base may generate the productive catalytic conformation of the ES complex to permit hydrolysis.

The deuterium solvent kinetic isotope effect, $V_{max}(H_2O)/V_{max}(D_2O) = 1.1$ for pH = pD = 5.35, is similar to that for lysozyme catalyzed reactions[28] and supports a mechanism involving rate-determining proton transfer. The twofold reverse isotope effect on K_m supports the role of a base in "binding:" acids are weaker in D_2O than in H_2O.[35]

Although these results for the β-glucosidase catalyzed hydrolysis of MPG do not permit assignment of mechanism, they do permit conservative speculation on possible mechanisms. Work concerned with mechanisms of acetal, thioacetal hydrolysis, and with the lysozyme mechanism supports the role of proton transfer to the leaving group as a feature of catalysis. Further, for lysozyme, and likely other glycosidases, a carboxylate residue is assigned a catalytic role (*vide supra*), and the present study suggests a carboxylate residue of almond β-glucosidase assists hydrolysis of MPG. For lysozyme, Glu-35 most likely does not function as a general base to abstract a proton from 2′-OH to generate 1,2-epoxide; for β-glucosidase, however, effects of isotopic substitution in phenyl β-D-glucoside do support such a role.[31] Also, 2′-*O*-methyl β-D-glucosides are not appreciably hydrolyzed by β-glucosidase,[32] suggesting a possible catalytic involvement of the 2′-OH group. Results of the model study, wherein the MP aglycone is assigned an enzyme-like catalytic role, support a β-glucosidase mechanism involving general base catalysis of epoxide formation concerted with loss of protonated aglycone.

Acknowledgment. We thank Dr. George Hitchings for the MPG and MPGU used in this study.

Supplementary Material Available. The primary kinetic data in Tables Ia and IIa will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-8410.

(27) M. Dixon and E. C. Webb, "Enzymes," Academic Press, New York, N. Y., 1964, p 224.
(28) T. Imoto, L. N. Johnson, A. C. T. North, D. C. Phillips, and J. A. Rupley, *Enzymes, 3rd Ed.*, 7, 665 (1972).
(29) B. M. Dunn and T. C. Bruice, *Advan. Enzymol. Relat. Areas Mol. Biol.*, 37, 1 (1973).
(30) T. H. Fife, *Accounts Chem. Res.*, 5, 264 (1972).
(31) F. W. Dahlquist, T. Rand-Meir, and M. A. Raftery, *Biochemistry*, 8, 4212 (1969).
(32) H. Baumann and W. Pigman, "The Carbohydrates," W. Pigman, Ed., Academic Press, New York, N. Y., 1957, p 562.
(33) J. Conchie, A. L. Gelman, and G. A. Levvy, *Biochem. J.*, 103, 609 (1967).
(34) I. I. Secemski, S. S. Lehrer, and G. E. Lienhard, *J. Biol. Chem.*, 247, 4740 (1972).
(35) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed, Butterworths, London, 1968, pp 517, 561.