SYNTHESIS AND KINETICS OF HYDROLYSIS OF SOME GLYCOSIDE-GLYCOSIDASE MODELS

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ABSTRACT

SYNTHESIS AND KINETICS OF HYDROLYSIS OF SOME GLYCOSIDE-GLYCOSIDASE MODELS

by Willard L. Madson

A series of acetal and ketal models for the glycoside-glycosidase system were prepared. These included 1,2-O-isopropylidene-α-D-glucose, 6-O-methyl-1,2-O-isopropylidene-α-D-glucose, 6-deoxy-6-methanethiol-1,2-O-isopropylidene-α-D-glucose, methyl-(6-O-methyl)-α-D-galactoside, methyl-(6-deoxy-6-methanethiol)-α-D-galactoside, and 4(5)-(γ-oxobutyl)-imidazole diethyl ketal. The rates of the acid-catalyzed hydrolysis of these compounds were determined by means of direct spectrophotometric, polarimetric, and colorimetric methods. The kinetics of these hydrolysis reactions indicate that anchimeric assistance by nucleophiles does occur in some of these cases. Participation by both the sulfur of the $-SCH_3$ group and by the imidazole nitrogen is indicated; increased hydrolysis rates of acetal and ketal derivatives containing these groups were observed in several instances. These results are compatible with the well-established mechanism of acetal and ketal hydrolysis involving an intermediate carbonium ion. A properly oriented nucleophile can stabilize this transition state and thus increase the rate of hydrolysis. It seems that enzymatic hydrolysis by glycosidases might well proceed in a similar fashion with the enzyme positioning a nucleophile in such a way that it can participate in stabilization of the glycosyl carbonium ion. This type of "onium" ion may also be involved in the transosylase activity of these enzymes; the transferring species may be an immonium or sulfonium ion.

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By

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TABLE OF CONTENTS

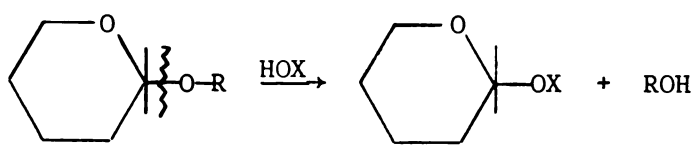
TABLE OF CONTENTS (Cont.)

LIST OF TABLES

SYNTHESIS AND KINETICS OF HYDROLYSIS OF SOME GLYCOSIDE-GLYCOSIDASE MODELS

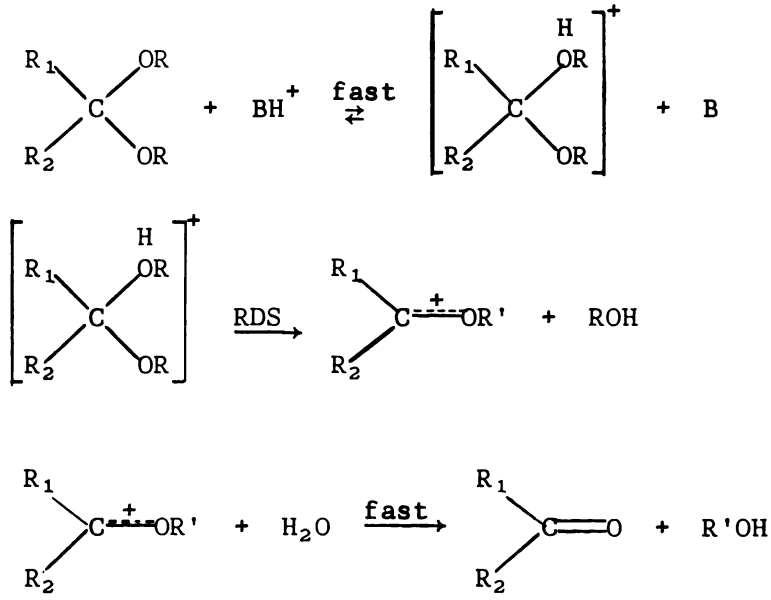
I. INTRODUCTION

Glycosidases and transosylases have in common the ability to catalyze cleavage of the carbon-oxygen bond of glycosides as shown in the following expression involving a pyranoside. Glycosidases without transosylase activity catalyze only hydrolysis, whereas glycosidases having transosylase activity catalyze transfer of the glycosyl moiety to substances other



than water. There is some evidence to indicate that this class of enzymes may include those which catalyze hydrolysis or formation of glycosides having nitrogen or other heteroatoms attached to the glycosyl carbon.

Since the substrates of these enzymes (glycosides) may be considered as cyclic acetals, it has appeared that a better understanding of the factors influencing acetal hydrolysis might give some insight into the mechanism of action of these enzymes. The mechanism of hydrolysis of acetals, as shown in the general expression below, has been well established (1). Polar, resonance, and steric effects on the reactivity of acetals have been determined and correlated (1, 2). It is interesting to note, however, that no evidence of neighboring group participation has been shown previously, although several of the substituents studied are known to be effective in this manner.



If the above mechanism is accurate, the transition state must have considerable carbonium ion character (as evidenced by hyperconjugative and steric effects). It is reasonable, then, to expect that neighboring groups on the acetal (i.e., nucleophiles) might stabilize this transition state and give rise to increased hydrolysis rates. If this is shown to be true, then enzymatic catalysis might proceed in a similar manner with an appropriate nucleophile positioned so as to participate in transition state stabilization.

Initial work in this laboratory indicated that there is an effect by neighboring nucleophiles. When the rates of hydrolysis of methoxy and methanethiol acetal were investigated, a thousand-fold difference was observed even though the inductive effects of methoxy and methanethiol are very similar (3).

The possible participation of imidazole nitrogen as a nucleophile is important to the investigation because of the obvious implication to

histidine as a participant in enzyme catalysis. No direct evidence has been obtained previously for the involvement of histidine in enzyme catalysis. Thus, it has seemed that investigation of the rates of hydrolysis of acetals containing nitrogen at positions suitable for intramolecular participation would be most valuable. In addition, it appeared that the acid-catalyzed hydrolysis of glycosides containing nucleophiles, especially at the 6 position of the glycosyl portion, might show any neighboring group effect more directly.

This thesis will present the work done to prepare some of these model compounds, the kinetic data that was collected, and some of the possible implications to the mechanism of action of glycosidases -- and perhaps to transosylases in general.

Historical Background

A. Acetal Hydrolysis

The rates of hydrolysis of acetals and the factors influencing these rates have been under investigation for over fifty years. As early as 1908, Fitzgerald and Lapworth (4) looked at the hydrolysis of acetals and were able to make estimates of their speeds. The first systematic study of acetal hydrolysis was made in the early 1920's by Skrabal and workers (5). They investigated the hydrolysis of alkyl and acyl (substituted at the alcohol) acetals under both alkaline and acidic conditions. A step-wise mechanism was postulated for these hydrolyses:

$$RCH(OX)_2 + H_2O \xrightarrow{k_1} RCH(OX)(OH) + XOH \qquad (1)$$

$$\downarrow k_2$$

$$R\text{—}CHO + XOH \qquad (2)$$

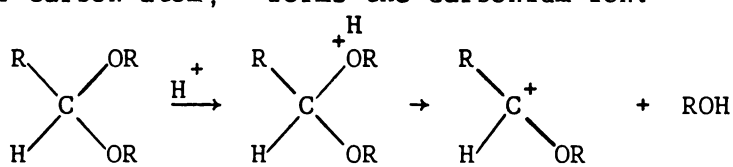
Based on the observation that the rate constants for acid hydrolysis of the alkyl acetals were constant, Skrabal suggested that the measured constants represented the velocity at which the half acetals were formed and that the decomposition to the aldehydes occurred at a much higher velocity. Later work by Skrabal (6) in which he varied the aldehyde rather than the alcohol, showed a rapid change in the hydrolysis rates going from the pentaerythritol acetals of formaldehyde, acetaldehyde, propionaldehyde, to acetone. Still later, in 1939, Skrabal (7) showed that the effects of alkyl substituents (on the carbonyl portion) on the rates of hydrolysis of acetals were additive.

In 1924, Hermans (8), looking at the reaction of acetone with glycols, said that only the C–O bonds of the carbon atom of the carbonyl, and never those of the alcohol, were broken since no change in configuration was observed. This view was supported by Palomaa and Aalto (9) in 1933 in their work to determine the influence of oxygen on the aldehyde component. More recent work by Alexander, et al. (10), O'Gorman and Lucas (11), and Garner and Lucas (12) on the hydrolysis of acetals derived from optically active alcohols [(a) D(+)–2–butanol, (b) D(+)–2–octanol, and (c) D(-)–2,3butanediol] substantiated these conclusions; in each instance, the alcohol recovered from the hydrolysis of the acetal had not undergone racemization.

Looking at some cyclic acetals, Leutner (13) in the early 1930's was able to show that the speed of hydrolysis is increased by replacing hydrogen in the alcohol portion by methyl. He also showed a six-fold

increase in stability as the ring size went from five to six and a similar increase between the six-membered and seven-membered rings.

The presently accepted mechanism was first suggested by O'Gorman and Lucas (11) after examining rate studies by earlier workers [Skrabal (7) and Hammett (14)] and their own observations on the hydrolysis of acetals of optically active alcohols. They again noted that the effect of change in structure operates on the aldehyde carbon rather than on the alkyl carbon atom since changes in the nature of the aldehyde part of the molecule exerted more profound effects on the hydrolysis rate than changes in the alcohol portion. They stated that the hydrolysis may be considered to be a typical S_N1 reaction in which the aldehyde carbon atom, not the alkyl carbon atom, forms the carbonium ion:



McIntyre and Long (15), observing the acid-catalyzed hydrolysis of methylal, showed a direct relationship between the logs of the first-order rate constants and the Hammett acidity functions. This supports the idea that the activated complex behaves like the conjugate acid of methylal (i.e., there are no water molecules firmly bound in the transition state). Hammett and Zucker (16) showed this relationship to be valid in earlier work on acid-catalyzed hydrolysis reactions.

The most recent work on the mechanism of acetal hydrolysis and the effects of substituents on the hydrolysis was carried out by Taft and Kreevoy (1, 2, 17). They correlated the inductive, resonance, and steric

effects on the reactivity of the acetals and further supported the concept that the transition state has considerable carbonium ion character at the carbonyl carbon atom. Taft and Kreevoy found that the second-order rate constant for the acid-catalyzed hydrolysis of acetal in fifty percent aqueous dioxane showed a three-fold increase when run in deuterium oxide. Orr and Butler (18) observed the same phenomenon, earlier, in water. Kilpatrick (19) obtained similar results with dimethyl and ethylene acetals in deuterium chloride-deuterium oxide solutions. The greater hydrolysis rates in deuterium oxide indicate that a prototropic equilibrium precedes the rate determining step.

B. Glycosidases

Koshland, in 1954, proposed three main schemes to account for the facts that were known about enzymatic transfer (20). Single displacement, in which the substrate and reactant are fixed on the enzyme and the attacking group enters on the side opposite the leaving group, was the first. This implies inversion at the glycosidic bond. This has been proposed for the maltose phosphorylase reaction (21). The second was a double displacement in which the preliminary attack is on the substrate by a nucleophile on the enzyme surface, followed by splitting of the glycosidic bond and the formation of a covalent bond between the enzyme and the residue to be transferred; the enzyme-substrate intermediate then is attacked by the entering group resulting in a double inversion and retention of configuration. This mechanism is supported by the exchange of phosphorus by sucrose phosphorylase (22). Third, a frontal displacement mechanism was suggested in which the substrate is fixed on the enzyme's surface in such a way that the

attacking group can come in on the side on which the leaving group is attached, again with retention of configuration.

This third possibility of Koshland's has been supported by Jermyn (23) who has considered fixation of the acceptor molecule on the binding site as necessary before reaction occurs, and by Stringer and Tsuchiya (24) in their work on the activation of dextran sucrase by methyl-α-D-glucoside.

Mayer and Larner (25), in examining the α-amylases from hog pancrease and B. subtillus and the β-amylase from sweet potatoes, suggested formation of a carbonium ion at carbon 1 of the glucose moiety (after orientation of the enzyme surface) followed by a stereospecific hydration. Thoma and Koshland (26) found a quantitative inversion in sweet potato β-amylase and stated that no carbonium ion can be formed and that the mechanism probably involves a single displacement (a displacement of the -OR group) in which the approach of water is guided by the enzyme.

Thoma and Koshland (27), looking at the inhibition of β-amylase by cycloamyloses, have suggested an induced fit theory in which (a) a change is induced in the protein's geometry by the substrate when it becomes attached to the binding site, (b) a precise orientation of the catalytic groups is required, and (c) the substrate induces this orientation by the modification it produces in the protein geometry. This is in opposition to the theories which suggest some kind of preformed fit between enzyme and substrate which contributes to the specificity and proper orientation of the catalytic centers.

Cohn (28) was the first to show that the transferred moiety in transosylase catalyzed reactions was glycosyl. Using O^{18} labeled inorganic phosphate, glycogen and muscle phosphorylase, she showed that all of the O^{18} was in the inorganic phosphate and glucose phosphate at the end of the reaction. No loss to water occurred as would have been the case if the phosphorus-oxygen bond had broken. Koshland and Stein (29), in examining yeast invertase in the presence of H_2O^{18}, found the glucose formed did not contain any O^{18}; the fructose, therefore, is liberated as fructosyl. Similar results with H_2O^{18} were obtained with hog pancrease and B. subtillus α-amylase and sweet potato β-amylase by Mayer and Larner (25, 30). Using glycogen as a substrate, they found that 90 percent of the O^{18} used was contained in the 1 position of the saccharides that were formed. Maltose, used as a control, contained only a trace of O^{18}. They concluded that both of the amylases hydrolytically rupture the C1-O bond of the 1,4 linkages. Bunton and workers (31, 32), examining the acid-catalyzed hydrolysis of a number of glycosides in H_2O^{18}, stated that the results are best interpreted in terms of a mechanism involving pyranosyl carbonium ions. They concluded that the hydrolysis proceeds by a unimolecular decomposition of the conjugate acid and involves breaking of the hexose-oxygen bonds only. Their results, however, do not distinguish between cyclic and acylic pyranosyl carbonium ions. The one exception noted by Bunton was that of the t-butyl-β-D-glycopyranoside which involves a bond breaking between the t-butyl group and the glycoside oxygen atom. The formation of such an alkyl carbonium ion will presumably occur in any glycoside for which the stability of such an ion is greater than that of the glycopyranosyl carbonium ion.

The question of a cyclic versus an acylic carbonium ion in the transition state is still an open one. There does appear to be more evidence for the acylic form in acid-catalyzed hydrolysis. Some of this evidence comes from the interconversion of furanosides and pyranosides. In the formation of glycosides, the aldoses, normally present as pyranoses, give a mixture of the pyranosides and furanosides (33). The proportion of the less stable furanosides initially present gradually decreases as the reaction reaches equilibrium, suggesting cleavage of the oxygen ring. The hydrolysis of the glycosides may proceed by a similar mechanism. Additional evidence in support of the acyclic mechanism is found in the acetolysis of methyl tri-O-acetyl-β-D-arabinopyranoside which, when catalyzed with zinc chloride or sulfuric acid gives a mixture of the two isomeric penta-O-acetyl-D-arabinose methyl hemicaetals in good yield (34).

Many suggestions have been made concerning the groups present at the active sites of transosylases. Involvement of the sulfhydryl group has been proposed for a number of these enzymes (35). Competitive protection by the substrate against inhibition by sulfhydryl reagents has been shown (36). A quantitative relationship between the binding of sulfhydryl groups by p-mercuribenzoate and the decrease of activity for a β-galactosidase has been observed (37). Reversible inactivation of β-amylase using p-chloro-mercuribenzoate is known to involve at least six sulfhydryl groups (38). None of these results, however, give any direct evidence for the involvement of sulfhydryl at the active site; these groups may be necessary for the configurational requirements of the enzymes.

Wallenfels found (39), by using the pH activity curve for a β-galactosidase from E. coli, indication of two groups at pK 5.8 to 6.7 and 7.8 to 8.6. The first is supposed to be an imidazole group, and the latter a sulfhydryl group. Thoma and Koshland (40) explained the V_m versus pH plot of sweet potato β-amylase on the basis of two active groups, one having pK 4.3 and the other pK 7.1, and suggested that they are a carboxyl and an imidazole group, respectively. They considered that a sulfhydryl group is involved also.

Neely (41) found two pK values for dextran sucrase at 4.9 to 5.1 and 5.5 to 5.7. Neely proposed that a carboxyl group (pK 4.9-5.1) acts as a nucleophile and an imidazole group (pK 5.5-5.7) as an electrophile. Neely also exposed solutions of the enzyme and methylene blue to intense light and observed inactivation of the enzyme and concommitant destruction of histidine; substrate protected the activity and suppressed the destruction of histidine. These results suggest that histidine may participate in the enzymatic catalysis.

II. EXPERIMENTAL

A. Reagents

1. General

All inorganic salts, concentrated acids, general organic reagents, etc. were reagent grade.

2. Solvents

a. Acetone, anhydrous

Reagent grade acetone was dried over anhydrous calcium sulfate (Drierite) and filtered through paper before use.

b. Benzene, anhydrous

Reagent grade benzene was dried by azeotropic distillation.

c. Chloroform, anhydrous

Reagent grade chloroform was washed with concentrated sulfuric acid, then water, and dried over anhydrous sodium sulfate before being distilled.

d. Dioxane-1,4, anhydrous

Reagent grade dioxane (2 l) was refluxed with 27 ml of concentrated hydrochloric acid and 200 ml of water under nitrogen for 12 hours. Solid potassium hydroxide was added until it no longer dissolved and a layer separated. The dioxane was decanted, fresh potassium hydroxide added, decanted again into a clean flask, and refluxed with sodium for 12 hours. The dioxane was then distilled under nitrogen. This purified dioxane was redistilled from sodium just before use.

e. Ethyl alcohol, anhydrous

Gold Shield (Commercial Solvents Corporation) 200-proof ethyl alcohol was refluxed with and distilled from barium oxide.

f. Methyl alcohol, anhydrous

Reagent grade methyl alcohol was refluxed with magnesium shavings and distilled.

g. Pyridine, anhydrous

Reagent grade pyridine was distilled from calcium hydride.

3. Organics

a. Fructose (Levulose)

Nutritional Biochemicals Corporation

b. Galactose, anhydrous

Pfanstiehl

c. Glucose (Dextrose), anhydrous

Baker reagent grade or Pfanstiehl

d. p-Toluenesulfonyl chloride

Eastman Kodak p-toluenesulfonyl chloride was sublimed before using.

4. Others

2,4-Dinitrophenylhydrazine solution

0.4 g of 2,4-dinitrophenylhydrazine was dissolved in 10 ml of 72 percent perchloric acid and 20 ml of water.

B. Preparation of 1,2-O-Isopropylidene-α-D-Glucose

The method used is that of Mehltretter, et al. (42), as described by Schmidt (43).

C. Preparation of 1,2-O-Isopropylidene-6-O-(p-Toluenesulfonyl)-α-D-Glucose

The procedure is a modification of the method described by Tipson (44).

A solution of 33 g (0.15 mole) of 1,2—O—Isopropylidene—α—D—glucose and 150 ml of dry pyridine was cooled to 5° in a 500-ml two-neck round-bottom flask equipped with a drying tube and an addition funnel. A solution of 30 g (0.154 mole) of p—toluenesulfonyl chloride in 150 ml of dry chloroform was added slowly, with swirling, over a period of 1 hour while cooling was continued. The temperature of the mixture was kept at 0° for 7 hours and then the temperature was allowed to rise gradually to room temperature. Ten hours after the flask was removed from the cold bath, it was cooled again to 0° in ice-salt. A few pieces of ice were added to the mixture and it was transferred to a Teflon-stoppered separatory funnel and washed with the following:

(1) five 100-ml portions of ice-cold 20 percent potassium bisulfate,

(2) two 100-ml portions of ice-cold water,

(3) five 100-ml portions of ice-cold 20 percent potassium bisulfate,

(4) two 100-ml portions of ice-cold water,

(5) two 100-ml portions of ice-cold saturated sodium bicarbonate, and

(6) one 100-ml portion of ice-cold water.

The last three washings (4, 5, and 6) were done by decantation in a beaker since the mixture had become too thick to handle in the funnel. The resulting solid was filtered and dried over silica gel in a vacuum desiccator in the cold room (1°). The filtrate was placed in a separatory funnel and the chloroform layer separated, dried over anhydrous sodium sulfate, and then evaporated to dryness under reduced pressure (water pump). The solid that formed was added to that obtained above.

The crude product was recrystallized by dissolving it in ether (about 100 ml for each 35 g of crude product), filtering, clouding the filtrate with ligroin, and placing it in the refrigerator. The crystals that formed were filtered and dried over silica gel in the cold room (1°). The yield was about 30 g. The melting point was 98 - 100°; the literature value (45) is 101 - 108°.

D. Preparation of 1,2-O-Isopropylidene-6-Deoxy-6-Methanethiol-α-D-Glucose

The method used is a modification of the procedure described by Raymond (46).

A 100-ml round-bottom flask was equipped with a gas inlet tube, reflux condenser (for which a gas exit tube was arranged leading to an ethyl alcohol bubbler), and an electric heating mantle. Fifty ml of absolute methyl alcohol and 2.07 g (0.09 mole) of freshly cut sodium were placed in the flask. After all of the sodium had reacted, methyl mercaptan was bubbled into the solution until it was saturated (8 g added). The system was flushed with nitrogen and 2.18 g (0.06 mole) of 1,2-O-isopropylidene-6-(p-toluenesulfonyl)-α-D-glucose was added. The nitrogen flushing was continued. After about 5 minutes, the mixture became thick due to the precipitation of sodium tosylate. The mixture was allowed to stand at room temperature for 4 hours. It then was poured into a flask and 240 ml of ether added to precipitate the salts. The mixture was filtered and an additional 30 ml of ether used to rinse the flask. The filtrate was refiltered into a 500-ml round-bottom flask and an additional 30 ml of ether was used to rinse the original flask (total volume of ether used was 300 ml). An alternate and preferred method used to remove the salts was to filter the

original mixture containing the sodium tosylate and then add about 120 ml of absolute methyl alcohol. One ml of water was added to the solution and carbon dioxide was bubbled through the mixture to precipitate the sodium ion as sodium carbonate. The precipitate was removed by centrifugation. The precipitate was washed with a little dry methyl alcohol and the combined washing and supernatant solutions evaporated under reduced pressure (water pump). The resulting syrup-precipitate was extracted with six 50-ml portions of dry ether and the filtered extracts evaporated (water pump). The product then was distilled as indicated below.

The ether-methanol solution was evaporated under reduced pressure (water pump). The dark syrup was dissolved in 25 ml of dry acetone and treated with acetone-washed Amberlite MB-1 until the pH was about 7. The mixture was much lighter in color after this treatment. The mixture was filtered, the flask and resin rinsed well with dry acetone, and the solution stored overnight at room temperature.

The solution was transferred portion-wise to a small distillation apparatus, the solvent removed under reduced pressure (water pump), and the syrup distilled under a nitrogen atmosphere at reduced pressure. The bath temperature was 150°, the distillation temperature was 110 - 120°, and the pressure about 0.1 mm. The yield was 7 g. $[\alpha]_{578}^{24}$ -7.6° in ethyl alcohol (c. 11.9); the literature values (46) are $[\alpha]_{D}^{23}$ -3.5° and -7.6° in ethyl alcohol (c. 2.3).

E. Preparation of 1,2-O-Isopropylidene-6-O-Methyl-α-D-Glucose

This was prepared from 1,2-O-isopropylidene-6-O-(p-toluenesulfonyl)-α-D-glucose according to the method of Levene and Raymond (47). $[\alpha]_{578}^{25}$ -6.9 in chloroform (c. 17.3); literature value (47) is $[\alpha]_{D}^{20}$ -6.0 in chloroform.

F. Preparation of 1,2:3,4-Di-O-Isopropylidene-α-D-Galactose

This product was prepared using a modification of the procedure described by Schmidt (48).

A three-neck 5-l round-bottom flask was equipped with a thermometer, stirring motor (or Teflon-coated magnetic stirring bar), and an addition funnel. Two-hundred g of powdered anhydrous galactose (powdered in a Waring Blender) and 4 l of acetone were placed in the flask and mixture cooled while stirring to 10°. Concentrated sulfuric acid (160 ml) then was added with stirring in 20-ml portions at 15 minute intervals. During addition of the acid, the temperature of the mixture was kept at 4 to 10°. Stirring, then, was continued after all of the acid had been added and the mixture allowed to warm gradually to room temperature over a period of about 5 hours. At the end of this reaction time, the mixture was cooled again to approximately 10° and a solution of sodium hydroxide (245 g in 300 ml of water) was added slowly so that the temperature did not rise above 15° (an alternate and preferred method of neutralization is to use dry ammonia gas). After the mixture reached pH 9, solid sodium bicarbonate (20 g) and sodium carbonate (5 g) were added to keep the mixture near neutrality.

After standing overnight, the pH was near 5 so an additional 10 g of sodium bicarbonate and 10 g of sodium carbonate were added to bring the pH to 7. The mixture was filtered and the filtrate evaporated under reduced pressure (water pump). After all of the acetone had been removed, about 1 l of water was added and the evaporation continued to remove acetone condensation products. Anhydrous ether (150 ml) was added to the

resulting salt-syrup mixture and the mixture filtered. The filtrate was evaporated under reduced pressure (water pump). One-hundred and fifteen g of product was distilled (this was about one-half of the product obtained) under reduced pressure at a bath temperature of 130 - 140°C and a pressure of 0.5 - 0.3 mm.

G. Preparation of 1,2:3,4-Di-O-Isopropylidene-6-O-(p-Toluenesulfonyl)-α-D-Galactose

This compound was prepared using a modification of the method described by Tipson (44).

Twenty-six g (0.1 mole) of 1,2:3,4-di-O-isopropylidene-α-D-galactose was poured into a 250-ml flask equipped with a Teflon-coated magnetic stirring bar. Anhydrous acetone (27.5 ml) and anhydrous pyridine (17.5 ml) were added and the flask stoppered. The mixture was stirred until the isopropylidene derivative dissolved. The solution was cooled in cold water and the flask closed with a stopper fitted with a thermometer. While the solution was stirred, 22.8 g (0.12 mole) of p-toluenesulfonyl chloride was added in small portions over a 1 hour period so that the temperature was kept below 30°. A precipitate formed after most of the tosyl chloride had been added. The mixture was kept at room temperature overnight (12 hours). After the mixture had been cooled to 0° in an ice-salt bath, 10 ml of water was added slowly so that the temperature did not rise above 5°. The mixture was poured into 250 ml of cold water. The syrup that separated became thick and white but did not crystallize. The mixture then was extracted with three 100-ml portions of chloroform. The chloroform solution was washed in a separatory funnel with the following:

(1) five 100-ml portions of ice-cold 20 percent potassium bisulfate,

(2) two 100-ml portions of ice-cold water,

(3) two 100-ml portions of saturated sodium bicarbonate, and

(4) one 100-ml portion of water.

The chloroform portion then was dried over anhydrous sodium sulfate. The chloroform solution was filtered and the sodium sulfate rinsed several times with dry chloroform. The combined filtrate and rinses were evaporated under reduced pressure (water pump). The resulting thick syrup was stored under vacuum over silica gel in the cold room (1°). After about 10 days, the syrup crystallized.

The crude product (42 g) was recrystallized in three batches in the following manner. The crude product was dissolved in a volume of hot isopropyl alcohol equal to the weight of product. The solution was cooled to room temperature and then nucleated with a small seed of the crude product. As soon as crystallization began, a volume of n-hexane equal to the volume of isopropyl alcohol was added, the mixture stirred for a few minutes, and then filtered. The crystals were washed with a little dry n-hexane. After a few minutes of standing, another crop of crystals appeared in the filtrate and these were recovered also. The combined crystalline material was dried under vacuum over phosphorous pentoxide. The yield was 31.2 g. The melting point of the product was 88 - 89°; the literature value (49) is 89 - 91°.

H. Preparation of 1,2:3,4-Di-O-Isopropylidene-6-Deoxy-6-Methanethiol-α-D-Galactose

A 1-l three-neck round-bottom flask was equipped with reflux condenser,

gas inlet tube, and drying tube. Two-hundred ml of dry n-propyl alcohol was placed in the flask and the system flushed with dry nitrogen. Freshly cut sodium (3.45 g, 0.15 g-atom) was added. After all of the sodium had reacted, the solution was saturated with methyl mercaptan (8.2 g added). The system was again flushed with dry nitrogen and 37.9 g (0.094 mole) of 1,2:3,4-di-O-isopropylidene-6-O-(p-toluenesulfonyl)-α-D-galactose was added. The mixture was heated under reflux for 3.5 hours. A precipitate formed. The mixture was cooled, filtered, and the precipitate washed well with dry n-propyl alcohol. Two ml of water were added to the solution and then carbon dioxide bubbled through the solution to precipitate the excess base as sodium carbonate. The mixture was centrifuged, the precipitate washed well with dry n-propyl alcohol, and the combined washing and supernatant solutions evaporated under reduced pressure (water pump). The residue (containing a small amount of precipitate) was taken up in 100 ml of anhydrous acetone, filtered, and the filtrate evaporated to a light yellow syrup. Yield was 20.6 g. The syrup can be distilled under reduced pressure at a bath temperature of 128°, a distillation temperature of 102 - 104°, and a pressure of 0.3 - 0.5 mm to yield a water-white product. $[\alpha]_{578}^{21}$ -72.4° in methyl alcohol (c. 6.8).

Analysis:

Calculated:	% C	53.77	% H	7.64	% S	11.04
Found:		53.87		7.66		11.11

I. Preparation of Methyl-(6-Deoxy-6-Methanethiol)-α-D-Galactoside

A solution of 11.6 g (0.04 mole) of 1,2:3,4-di-O-isopropylidene-6--deoxy-6-methanethiol-α-D-galactose in 12 ml of absolute methyl alcohol

was placed in a glass tube (1-inch gauge glass sealed at one end). Twelve ml of a solution of dry hydrogen chloride gas in absolute methyl alcohol (3.5 percent in hydrogen chloride) was added to the tube. An additional 12 ml of absolute methyl alcohol was used to rinse the original flask and this was added to the tube. The tube was cooled in an ice-salt bath and sealed. It then was heated in a water bath for 50 hours at 100° (after about 5 to 19 minutes, the contents became very dark but became lighter about 2 hours later). The sealed tube was cooled, opened, and the contents evaporated under reduced pressure (water pump). The product precipitated as soon as the alcohol was removed. The solid was taken up in a minimum of absolute methyl alcohol, filtered, washed with dry ethyl ether, and dried over phosphorous pentoxide. Yield was about 5 g. It was recrystallized from hot ethyl alcohol. The melting point of twice-recrystallized product was 167 - 167.5°; $[\alpha]_{578}^{20}$ +156° in methyl alcohol (c. 1.1). Analysis:

Calculated:	% C	42.84	% H	7.19	% S	14.30
Found:		42.95		7.07		14.16

J. Preparation of 1,2:3,4-Di-O-Isopropylidene-6-O-Methyl-α-D-Galactose

This derivative was prepared by the method of Goldstein, Hamilton, and Smith (50).

A 2-l three-neck round-bottom flask was equipped with a Teflon-coated magnetic stirrer, reflux condenser, two addition funnels, a thermometer, and a pH electrode. A solution of 50 g (0.19 mole) of 1,2:3,4-di-O-isopropylidene-α-D-galactose in 95 ml of acetone was placed in the flask. A 30 percent aqueous solution (405 ml) of sodium hydroxide was placed in one of

the addition funnels. Dimethyl sulfate (162 ml) was placed in the other funnel (CAUTION - dimethyl sulfate is very toxic. Ammonia is a specific antidote). Some of the sodium hydroxide solution was added to the flask to make the solution in the flask basic (pH 11). Dimethyl sulfate then was added, with stirring, in small portions. The pH was kept between 10 and 11 by additions of the sodium hydroxide solution. During the addition of the first 20 to 30 ml of dimethyl sulfate, the temperature was kept at about 15° by using an ice bath. The bath then was removed and the addition of the sodium hydroxide solution and dimethyl sulfate controlled so that the temperature remained below 50°. The addition of the dimethyl sulfate required about 5 hours. At the end of this time, the remainder of the sodium hydroxide solution was added dropwise so that the temperature did not rise above 50°C. The mixture then was stirred overnight (12 hours).

The resulting mixture was extracted with chloroform. During the first extraction (100 ml) the chloroform layer was on top, while in the following two extractions (each with 50 ml), the chloroform layer was on the bottom. The combined chloroform extracts were dried over anhydrous sodium sulfate overnight.

The filtered chloroform solution was evaporated under reduced pressure (water pump). The resulting light yellow syrup was distilled under reduced pressure. The bath temperature was 110° and the pressure 0.05 - 0.1 mm.

The almost water-white syrup was redistilled on a "Bantamware" set-up using a small fractionating column. The product distilled at a bath temperature of 110 - 120°C, a distillation temperature of 86 - 92°, and a pressure of 0.10 - 0.20 mm. The product was water-white. Yield was about 35 g.

K. Preparation of Methyl-(6-O-Methyl)-α-D-Galactoside

A solution of 7.0 g (0.025 mole) of 1,2:3,4-di-O-isopropylidene-6-O-methyl-α-D-galactose in 10 ml of absolute methyl alcohol was placed in a 1-inch gauge-glass tube sealed at one end. The original flask was rinsed with an additional 10 ml of dry methyl alcohol and this added to the tube. Three ml of a solution of dry hydrogen chloride in absolute methyl alcohol (12 percent in hydrogen chloride) then was added to the tube followed by another 7 ml of absolute methyl alcohol. The tube was cooled in an ice-salt bath and sealed. The contents of the tube were mixed and the tube heated in a water bath for 26.5 hours at 100°. The contents of the tube became almost black after 30 minutes of heating but became lighter in color as heating was continued. The tube was cooled to room temperature, opened, and the contents transferred to a small round-bottom flask (the tube was rinsed with a little dry methyl alcohol). The solution was evaporated under reduced pressure (water pump). The syrupy residue did not crystallize and was dissolved in dry methyl alcohol, Norite added to the solution, and this mixture filtered and evaporated again under reduced pressure (water pump). After standing at room temperature for a week, the syrup crystallized.

The crystalline mass was recrystallized once from methyl alcohol and twice from absolute ethyl alcohol. The yield was about 2 g. The rotation of this thrice crystallized product was $[\alpha]_{578}^{22}$ = +173° in water (c. 2.9); the literature value (50) is $[\alpha]_{D}^{20}$ = +165° in water (c. 1).

L. Preparation of 4(5)-Hydroxymethyl Imidazole Picrate

This product was prepared according to the procedure of Totter and Darby (51).

M. Preparation of 4(5)-Chloromethyl Imidazole Hydrochloride

This product was prepared from 4(5)-hydroxymethyl imidazole picrate using the procedure of Mehler, Tabor, and Bauer (52).

N. Preparation of 4(5)-(γ-Oxobutyl)-Imidazole

This procedure combines the method described by Marvel, Johnson, and Hager(53,54) for the preparation of ketones via acetoacetic ester and a modification of the procedure described by Pyman (55) for the preparation of 4(5)-(γ-oxobutyl)-imidazole.

A 500-ml round-bottom flask was equipped with a Teflon-coated magnetic stirring bar, addition funnel, and reflux condenser. Two-hundred ml of absolute ethyl alcohol (distilled from barium oxide) and 9.2 g (0.4 mole) of freshly cut sodium were placed in the flask. After all of the sodium had reacted, 26 g (0.2 mole) of freshly distilled acetoacetic ester was slowly dripped into the stirred solution. After all of the ester had been added, the solution was cooled in an ice bath and 30 g (0.2 mole) of 4(5)-chloromethyl imidazole hydrochloride was added. A precipitate formed immediately. The mixture was stirred at room temperature for 1 hour and then refluxed (bath temperature about 85°) for 3 hours. The mixture was cooled to room temperature and the salt removed by centrifugation. The salt was washed three times with absolute alcohol. After each washing, the salt was separated by centrifugation. The combined supernatant solutions were evaporated under reduced pressure (water pump).

The residual syrup from this operation was poured, with stirring, into 200 ml of 5 percent aqueous sodium hydroxide in a 500-ml round-bottom flask. The mixture was stirred for 4 hours. At the end of this time,

the unsaponified material was removed by an ether extraction (about 50 ml of ether used). The aqueous layer was placed in a 500-ml flask and 20 ml of 50 percent sulfuric acid added dropwise with stirring. Stirring was continued until the evolution of carbon dioxide stopped (about 3 hours). The mixture then was treated with solid potassium carbonate until the pH was about 7. The mixture was stored overnight at room temperature. The dark solution then was placed in a 2-l round-bottom flask and evaporated under reduced pressure (water pump). When all but about 50 to 75 ml of water had been removed, the mixture was extracted with chloroform. The aqueous layer must be slightly basic during the extractions. Three extractions were made, each with about 75 ml of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure (water pump). The dark syrup was then put in a vacuum desiccator over barium oxide at 0.1 to 0.5 mm pressure until the syrup crystallized. The crude product was then purified by two sublimations at a bath temperature of 80 - 90° and a pressure of 0.1 - 0.2 mm. The yield was 8 g. The melting point of the twice sublimed product was 79 - 80°; the literature value (55) is 80 - 81°.

O. The Preparation of 4(5)-(γ-Oxobutyl)-Imidazole Diethyl Ketal

A mixture of 5.7 g(0.04mole) of 4(5)-(γ-oxobutyl)-imidazole, 14 ml of absolute ethyl alcohol (distilled from barium oxide), and 26 ml of freshly distilled triethylorthoformate were placed in a 100-ml round-bottom flask equipped with a drying tube. The ketone was dissolved by swirling the contents of the flask. Concentrated sulfuric acid was added; enough to redissolve the white precipitate that formed during the initial addition

of the acid plus about a three-drop excess past this point (total volume of acid added was about 1.6 ml). The solution was allowed to stand overnight (12 hours) at room temperature. The solution was transferred to a 300-ml flask and a solution of sodium ethoxide was added until the pH was $\geq$ 11. The flask was equipped with a reflux condenser, drying tube, and eletric heating mantle. The mixture was refluxed one hour. After standing at room temperature for 2 days, the mixture was evaporated under reduced pressure (water pump). The salt-syrup residue was extracted with about 50 ml of dry benzene. The salts were removed by centrifugation. The salts were washed twice with benzene; in each case the salts were removed again by centrifugation. The combined supernatant solutions were evaporated under reduced pressure (water pump). After the benzene was removed, the thin syrup was transferred to a small distillation setup and distilled under reduced pressure. The product distilled at a bath temperature of 115 - 123°, a distillation temperature of 75 - 77°, and a pressure of 0.05 - 0.01 mm. The yield was 2.2 ml. Assuming an ε_{270} of 30 for the ketone (obtained from the absorbance at 270-mμ using weighed samples of the pure ketone), absorbance measurements of the product indicated that it contained from 15 - 25 percent ketone. All attempts to purify this product have failed. Gas chromatography with a number of columns operated under a wide range of conditions did not give a pure sample (as indicated by the absorbance at 270-mμ). Treatment of a solution of the product with basic alumina (Brockman Activity I) did not remove an appreciable amount of the ketone impurity. An attempt to prepare a larger amount of product and then to fractionally distill this product failed.

If the impurity is the parent ketone, this should not affect the rate of hydrolysis and, thus, some preliminary rate studies were made of this impure product (see Results and Discussion).

P. Methods Used to Determine Rates of Hydrolysis

1. Direct Spectrophotometric Method

A Beckman Model DU Spectrophotometer equipped with a water-jacketed temperature-controlled cell compartment was used. A circulating water bath (Precision Scientific Company) was connected to the cell compartment and the water temperature adjusted so that the temperature within the cells was maintained at 25 ± 0.05°.

In a typical run, a solution of the acetal or ketal and a solution of the appropriate buffer or acid were equilibrated in a water bath at 25° for about 3 minutes. The solutions then were mixed thoroughly, a portion of the resulting solution placed in a 1.0 cm silica cell and the absorbance at 270-mμ of this solution determined at 2-minute intervals. The reaction was followed well past half-life, usually to 70 - 80 percent of completion.

The logarithms of increments in 270-mμ absorbance of these reaction mixtures (usually over 6 - 8 minutes) were plotted versus time, and the k_1 values then were determined from the slopes of these plots.

2. Polarimetric Method

The Carl Zeiss Photoelectric Precision Polarimeter was used for polarimetric measurements. The hydrolysis reactions were carried out in a water-jacketed 1-decimeter polarimeter cell. Using a circulating water bath (Precision Scientific Company), the temperature in the cell was maintained at 60 ± 0.1° (corrected).

Before each run, the cell was equilibrated at 60° for about 15 minutes. A sample of the glycoside was weighed in a beaker and 8 ml of 1.003N hydrochloric acid was added. After thorough mixing, the solution was placed in the polarimeter cell. The cell was kept outside of the polarimeter during most of the run because of the obvious danger of damage to the instrument in case of a leak. At regular intervals, usually 4 hours, the electronics of the instrument were allowed to warm up, the zero point was checked, and the optical rotation of the reaction solution determined. The reaction was followed to 80 - 90 percent completion.

The change in optical rotation was plotted versus time and the k_1 values determined from the slopes of these plots.

3. Colorimetric Determination of Reducing Sugars

This method is based on the amount of color produced by the reaction of a reducing sugar (one of the hydrolysis products) with 3,5-dinitrosalicylic acid (56).

The reagent was prepared by moistening 10 g of 3,5-dinitrosalicylic acid with a few drops of water, adding this to 200 ml of 2N aqueous sodium hydroxide and bringing the solution to 500 ml with water. Three-hundred g of potassium sodium tartrate were added and the mixture brought to 1 l with water.

The hydrolysis reactions were run in glass-stoppered flasks in a temperature-controlled circulating oil bath maintained at 75 (or 85) ± 0.1°. The samples were weighed into a flask and the appropriate amount of 0.46N perchloric acid (preequilibrated in the oil bath for 15 minutes) added. At regular intervals, aliquots were withdrawn from the flask and placed in

50-ml volumetric flasks. An amount of 0.5N aqueous sodium hydroxide equal to the aliquot was added to each volumetric flask to stop the reaction. After a number of samples had been obtained in this manner, the color was developed by adding 4 ml of the 3,5-dinitrosalicylic acid reagent to each flask, heating the flasks (unstoppered) in a boiling water bath for 6 minutes, removing the flasks, and then diluting the contents to the 50-ml mark with distilled water. After thorough mixing, the absorbances at 540 mμ were determined on a Beckman Model DU Spectrophotometer in 1.0 cm Corex cells.

Plots were made of the change in absorbance versus time and the k_1 values calculated from the slopes of these plots.

Q. Preparation of Buffers

Buffer No. 1

A mixture of 1.7 g acetic acid and 9.51 ml of 2.584N aqueous sodium hydroxide was diluted to 100 ml with distilled water. The pH of the resulting solution was found to be 5.37. The concentration of acetic acid is 0.2833M; the concentration of acetate ion is 0.3457M.

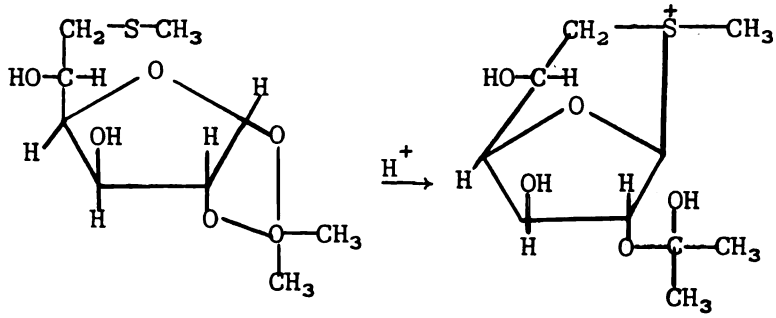
Buffer No. 2

A mixture of 45 g of acetic acid and 150 ml of 1.638N aqueous sodium hydroxide was diluted to 1 l with distilled water. The pH of the resulting solution was found to be 4.10. The concentration of acetic acid was 0.750M; the concentration of acetate ion was 0.2457M.

III. RESULTS AND DISCUSSION

A. Derivatives of 1,2-O-Isopropylidene-α-D-Glucose

The rates of hydrolysis of three isopropylidene derivatives of α-D-glucose are shown in Table I. The substitution at position 6 of the glucose moiety was varied from -OH to $-OCH_3$ to $-SCH_3$ to determine whether or not a nucleophile could operate from this position by anchimeric assistance to give increased hydrolysis rates. The observed rates indicate that there is no effect in changing -OH to $-OCH_3$, even though the latter is a somewhat better nucleophile. There is, however, a significant change in rate with the $-SCH_3$ substituted derivative suggesting that this group may participate in a transition state stabilization (as shown below) and thus result in an increased hydrolysis rate. One must remember in examining this effect that



there are three possible bond cleavages in these hydrolysis reactions and the nucleophile can influence the rate of hydrolysis of only one of these (i.e., at bond 1). Thus, the overall effect on the hydrolysis rate will be decreased.

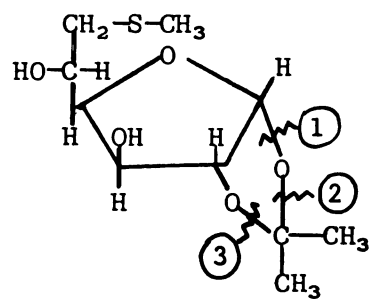


TABLE I. Rates of Acid-Catalyzed Hydrolysis of Some Derivatives of 1,2-O-Isopropylidene-α-D-Glucose

Compound	k_1 Values†	Average k_1 Values	k_2 Values‡
	(10^{-4} sec.$^{-1}$)	(10^{-4} sec.$^{-1}$)	(10^{-4} x 1.mole^{-1} sec.$^{-1}$)
1,2-O-Isopropylidene-α-D-Glucose	1.15		
	1.20	1.16 ± 0.04	1.03 ± 0.04
	1.11		
	1.17		
1,2-O-Isopropylidene-6-O-Methyl-α-D-Glucose	1.13		
	1.17		
	1.07	1.12 ± 0.05	1.00 ± 0.05
	1.11		
1,2-O-Isopropylidene-6-Deoxy-6-Methanethiol-α-D-Glucose	1.27		
	1.31		
	1.34	1.31 ± 0.04	1.16 ± 0.04
	1.31		

† These values were determined from the slopes of the plots of the change in absorbance versus time; see the experimental section for the details of the method. The values are expressed in terms of Briggs' logarithms.

‡ The acid concentration in all of these runs was 1.121N (perchloric acid).

B. Rates of Hydrolysis of Methyl-(6-Deoxy-6-Methanethiol) and Methyl-(6-O-Methyl)-α-D-Galactosides

The rates of hydrolysis of these two glycosides were determined by both polarimetric and colorimetric methods (see experimental section for details of methods). Table II lists the various k_1 and k_2 values that were calculated from these runs. Some difficulty was experienced in obtaining good linear plots with the colorimetric method; this was probably due to slight variations in the color development procedure since it was impossible to develop the color in all of the samples at the same time. The polarimetric method was much better since it involved the direct measurement of a physical property of the reaction solution.

Comparison of the k_2 values for the acid-catalyzed hydrolysis of these two galactosides shows that the $-SCH_3$ substituted (at the 6 position of the galactosyl) hydrolyzes at a rate greater than the $-OCH_3$ substituted derivative. This would support the results observed with the 1,2-O-isopropylidene glucose derivatives and again suggest the anchimeric participation of the $-SCH_3$ group as shown below.

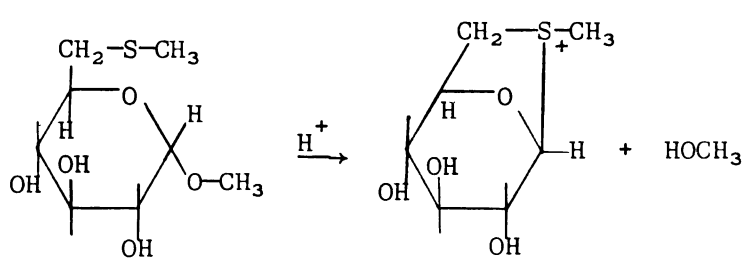


TABLE II. Rates of Acid-Catalyzed Hydrolysis of Methyl-(6-O-Methyl) and Methyl-(6-Deoxy-6-Methanethiol)-α-D-Galactosides

Compound	Method	Temp.	k_1†	k_2†
Methyl-(6-Deoxy-6-Methanethiol)-α-D-Galactoside	Colorimetric	85°	$6.61 \times 10^{-5} \times \text{sec.}^{-1}$	$1.44 \times 10^{-4} \times \text{l.mole}^{-1}\ \text{sec.}^{-1}$
Methyl-(6-O-Methyl)-α-D-Galactoside	Colorimetric	85°	$5.70 \times 10^{-5} \times \text{sec.}^{-1}$	$1.23 \times 10^{-4} \times \text{l.mole}^{-1}\ \text{sec.}^{-1}$
Methyl-(6-Deoxy-6-Methanethiol)-α-D-Galactoside	Polarimetric	60°	$6.87 \times 10^{-6} \times \text{sec.}^{-1}$	$6.68 \times 10^{-6} \times \text{l.mole}^{-1}\ \text{sec.}^{-1}$
Methyl-(6-O-Methyl)-α-D-Galactoside	Polarimetric	60°	$5.60 \times 10^{-6} \times \text{sec.}^{-1}$	$5.44 \times 10^{-6} \times \text{l.mole}^{-1}\ \text{sec.}^{-1}$

† These values are expressed in terms of natural logarithms.

C. Rate of Hydrolysis of 4(5)-(γ-Oxobutyl)-Imidazole Diethyl Ketal

The direct spectrophotometric method (see experimental section) was used to determine the rate of hydrolysis of this ketal. The ketal was dissolved in peroxide-free dioxane and an equal amount of the appropriate buffer (preparation of these buffers was described in the experimental section) used for the acid catalyst. The hydrogen ion concentration was calculated using the ionization constant for acetic acid in 50 percent aqueous dioxane at 25° interpolated from the data of Harned and Owen (59). Harned and Owen reported ionization constants for acetic acid as 4.93×10^{-7} in 45 percent dioxane at 25° and 4.78×10^{-9} in 70 percent dioxane at 25°. Interpolation gives a value of 2.63×10^{-7} in 50 percent dioxane at 25°.

The observed second order constants for the hydrolysis of 4(5)-(γ-oxobutyl)-imidazole diethyl ketal lie within the allowable experimental error and are approximately 100 times as great as the second order constants for the diethyl ketal of methyl ethyl ketone (1). This data strongly indicates anchimeric assistance in this hydrolysis reaction by nitrogen of the imidazole ring, and it demonstrates the possibility of nucleophilic assistance by imidazole (of histidine residues) in glycosidase-catalyzed hydrolysis reactions.

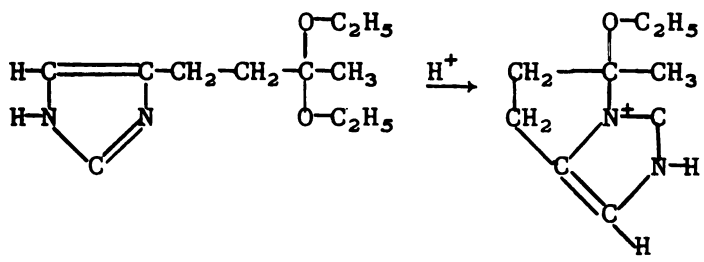


TABLE III. Rate of Acid-Catalyzed Hydrolysis of 4(5)-(γ-Oxobutyl)-imidazole Diethyl Ketal

Compound	Buffer[a]	(Acetic Acid)[b]	(Acetate Ion)[b]	k_1[c]	k_2[d]
4(5)-(γ-Oxobutyl)-Imidazole Diethyl Ketal	No. 1	0.1416 M	0.1729 M	1.97×10^{-4} sec.$^{-1}$	9.16×10^{-4} l.mole^{-1} sec.$^{-1}$
	No. 2	0.3750 M	0.1229 M	3.14×10^{-4} sec.$^{-1}$	3.92×10^{-4} l.mole^{-1} sec.$^{-1}$

a. The preparation of these buffers is described in the experimental section.

b. These are the concentrations in the final reaction solutions.

c. These values are expressed in terms of natural logarithms.

d. The H^+ ion concentrations were determined using the ionization constant of acetic acid in 50 percent dioxane at 25° interpolated from the data of Harned and Owen (59).

D. General Comments

Although the evidence for anchimeric assistance by potential nucleophiles in the hydrolysis of acetals, ketals, and glycosides is not conclusive, it strongly suggests such a possibility. Both the sulfur of $-SCH_3$ and the imidazole nitrogen appear to participate in this manner. Of these nucleophiles, the nitrogen of the imidazole ring appears to have the greatest potential as a participant in enzymatic hydrolysis by glycosidases. The imidazole immonium ion may also be involved in the transfer reactions of these enzymes; the transferred moiety being an imidazole immonium ion or, possibly, a sulfonium ion.

One aspect of acetal hydrolysis that has not been examined is the problem of electron-withdrawing groups located near the carbonyl carbon atom. In the glycosides, the -OH at carbon 2 of the glycosyl moiety results in a greatly increased stability. For example, the hydrolysis of 2-methoxytetrahydropyran proceeds rapidly with dilute acids at room temperature (60) while the pentopyranosides require elevated temperatures. During enzymatic catalysis, the enzyme must be able to overcome the effect of such electron-withdrawing groups on carbon 2 of the glycosyl moiety; possibly by means of hydrogen bonding.

IV. SUMMARY

1. A number of acetal and ketal models for the glycoside-glycosidase system were prepared. These included 1,2-O-isopropylidene-α-D-glucose, 6-O-methyl-1,2-O-isopropylidene-α-D-glucose, 6-deoxy-6-methanethiol-1,2-O-isopropylidene-α-D-glucose, methyl-(6-O-methyl)-α-D-galactoside, methyl-(6-deoxy-6-methanethiol)-α-D-galactoside, and 4(5)-(γ-oxobutyl)-imidazole diethyl ketal.

2. The acid-catalyzed rates of hydrolysis of the above compounds were determined using a number of methods including direct spectrophotometric, polarimetric, and colorimetric methods.

3. The kinetic data obtained suggest that some form of anchimeric assistance by nucleophiles does take place in these acid-catalyzed hydrolysis reactions. Both the sulfur of the $-SCH_3$ group and the imidazole nitrogen appear to participate in this manner; acetal and ketal derivatives containing these groups have increased hydrolysis rates.

4. It seems that enzymatic hydrolysis of glycosides may involve a similar participation of a nucleophile; positioned by the enzyme so as to stabilize the intermediate carbonium ion and thus increase the rate of hydrolysis. This "onium" ion may be the species involved in the enzymatic transfer reactions of these enzymes; the transferred moiety being an immonium or sulfonium ion.

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