THIS BOOK IS OF POOR LEGIBILITY DUE TO LIGHT PRINTING THROUGH OUT IT'S ENTIRETY.

THIS IS AS RECEIVED FROM THE CUSTOMER.

THE SYNTHESIS OF METHYL α AND β-D-XYLOPYRANOSIDES-5-^{18}O

by

WILLIAM DEAN RITZ

B.S., Kansas State University, 1971

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Graduate Group in Biochemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1974

Approved by:

Paul A Seib
Major Professor

Department of Grain Science
and Industry

THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH THE ORIGINAL
PRINTING BEING
SKEWED
DIFFERENTLY FROM
THE TOP OF THE
PAGE TO THE
BOTTOM.

THIS IS AS RECEIVED
FROM THE
CUSTOMER.

TABLE OF CONTENTS

List Of Figures

List Of Tables

INTRODUCTION

Many polysaccharides are important in commerce, and since the monosaccharide units of these polymers are linked by glycosidic bonds, it is important to understand the reaction mechanism by which these linkages are cleaved during acid hydrolysis. An accurate description of the reactive intermediate through which this reaction procedes is essential to the prediction of reactivity and to the achievement of optimal processing conditions for various carbohydrate raw materials.

A large amount of data has been gathered as to the molecularity of acid-catalyzed pyranoside hydrolysis and the reactive intermediate involved. The evidence is not conclusive, but indicates a rapid, pre-equilibrium protonation followed by a unimolecular substitution at the C-1 carbonium ion of the conjugate acid of the pyranoside. Since pyranosides are unsymmetric acetals, two different mechanisms, differing in the site of initial protonation are consistent with the proposed mechanism.

It is for the purpose of adding to the knowledge of this mechanism, in particular, knowledge concerning the structure of the intermediate in the rate determining step of the reaction, that this study was undertaken.

LITERATURE REVIEW

The subject of pyranoside hydrolysis has received much attention in the literature and has been reviewed by several authors (1-5). Much of this attention has been concentrated upon the determination of the order and type of reaction that is occurring during the acid catalyzed hydrolysis of pyranosides and the larger class of acetals, of which the pyranosides are a special category.

Bronsted and Grove (6) showed that acetals undergo rapid, hydrogen ion catalyzed hydrolysis in aqueous acid, but are stable in alkaline solution. These reactions are on the order of 10^{11} times more rapid than the hydrolysis of simpler dialkyl ethers under the same conditions. Ingold (7) presumed this increase in reactivity to be due to the formation of the conjugate acid of the acetal, with subsequent nucleophilic, unimolecular substitution (S_n1) to form the alcohol. Skrabal and Eger (8) investigated the hydrolysis of a series of alkyl-acetals and showed that reactivity could be explained by inductive effects in the protonated acid form of an acetal. O'Gorman and Lucas (9) reached a similar conclusion by noting that the acetal of an optically active alcohol, when hydrolyzed, gives the optically active alcohol, indicating that the reaction procedes by way of the carbonium ion of the aldehyde carbon.

Hammett and Paul (10) explained the linear relationship between the acidity function, H_o in strong acids, and the logarithm of the rate of hydrolysis for various compounds, including sucrose, by

the rapid formation of an ionized species. The hydrolytic breakdown of this ion is then much slower than its formation and allows an equilibrium to be reached between the ion and the non-ionized substrate. McIntyre and Long (11) determined the dependence of the logarithm of the hydrolysis rate for methal upon the Hammett acidity function, H_o, and explained the linear relation as further evidence for a rapid pre-equilibrium protonation and a unimolecular mechanism.

Orr and Butler (12) first measured a rate constant increase of a factor of three for the hydrolysis of methal in D_2O as compared to H_2O. Bunton and Shiner (13) calculated ratios for k_{D_2O}/k_{H_2O} from 1.7 to 2.5. Kilpatrick (14) observed a hydrolysis rate increase in D_2O of 2.7 times the rate in H_2O for methal and ethylene acetal. These authors argue that this effect indicates the existence of a protonation step and a protonated intermediate in the reaction mechanism. The observed rate increase is due to the greater proportion of the deuterated species in D_2O than the protonated species in H_2O.

The molecularity of the hydrolysis of acetals has also been indicated by the magnitude of the entropy of activation, $\Delta S^{\ddagger}$. Long and co-workers (15) noted that the entropy of activation for the hydrolysis of many acetals, which had previously been shown to undergo an A-1 reaction, exhibited positive or only slightly negative values of $\Delta S^{\ddagger}$. Compounds such as primary alkyl halides, which are known to undergo hydrolysis by an A-2 reaction, show large negative values of $\Delta S^{\ddagger}$. Schlager and Long (16) proposed

that the water molecule bound by the activated complex in the S_n2 transition state gives added structure to the overall system, and Overend and co-workers (17) argue that the positive entropy of activation for an A-1 type hydrolysis is due to a less ordered intermediate in the breakdown of the conjugate acid.

All the criteria for an A-1 reaction established for general acetals have also been applied to the pyranosides. That pyranosides of optically active alcohols retain optical activity of the alcohol upon acid hydrolysis is readily observable in the acid hydrolysis of oligosaccharides. The various other evidences for an A-1 mechanism have been applied by many other investigators. Several authors (17, 18, 19) have noted that the logarithm of the hydrolysis rate constant for various pyranosides is linear with respect to the Hammett acidity function, but not with respect to pH. This criteria for unimolecularity has, however, been criticized by Koskikallio et al. (20) as being unreliable in certain cases, and by Bunnett (21) who noted that the slope of the logarithm of k_{hyd} versus H_o departed from unity for different acids.

Activation entropies have also been determined for many pyranosides by Timell (18) and by Overend and co-workers (17). As previously stated, the $\Delta S^{\ddagger}$ of a reaction proceeding by an A-1 mechanism has been shown to be positive in many cases. Overend's work showed a mean value of +13.7 e.u. for the 22 different pyranosides studied.

Whalley (22) argued that the volume of activation, $V^{\ddagger}$, will be more positive in the case of an A-1 reaction than in the case of an S_n2 reaction. Many later experiments by the same investigator on reactions whose mechanisms had been studied by other means indicated that these initial arguments were correct. Withey and Walley (23) later measured $V^{\ddagger}$ for methyl α-D-glucopyranoside to be +5.1 cm^3mole^{-1}, indicating an S_n1 mechanism for the breakdown of the conjugate acid.

The ratio of hydrolysis rates in H_2O and D_2O has also been measured for pyranosides. A ratio of k_{D_2O}/k_{H_2O} of 1.8 was observed by Overend et al. (17) for methyl α-D-glucopyranoside, and a k_{D_2O}/k_{H_2O} of 2.5 was measured by Armour et al. (24) for methyl 2-deoxy-α-D-glucopyranoside.

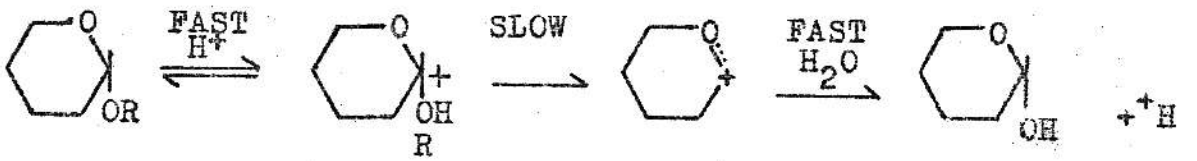
Bunton and co-workers (13) have also shown by ^{18}O tracer studies that in methyl α and β and in phenyl α and β-D-glucopyranosides, the breakdown of the conjugate acid intermediate proceeds by the cleavage of the C-1 to aglycone oxygen bond, rather than the oxygen to aglycone carbon bond.

The same authors in a later study noted, however, that the acid-catalyzed hydrolysis of t-butyl glucosides proceeds by the cleavage of the alkyl-oxygen bond (24).

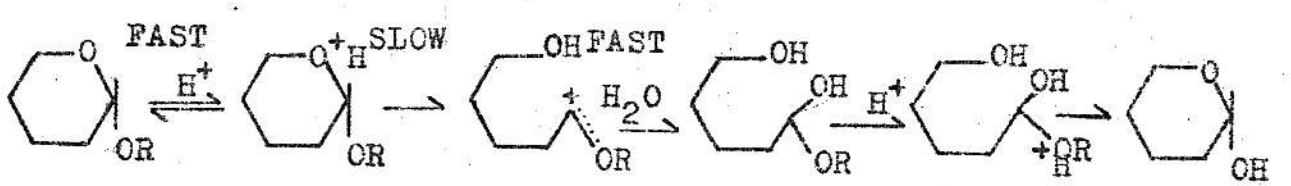
As stated before, the subject of pyranoside hydrolysis has been extensively reviewed, and this short discussion has not attempted to completely cover the literature, but only to present the more difinitive evidence for the A-1 mechanism. While these studies strongly suggest an A-1 mechanism, there are weaknesses

in the arguments based upon thermodynamic values of the activated state, because as measured, they include the properties of the protonation step as well as the rate limiting breakdown.

Since pyranosides are unsymmetrical acetals however, there are two possible mechanisms which fit the requirements of the A-1 mechanism, but which differ in the site of the position of the initial protonation and bond cleavage.



MECHANISM A



MECHANISM B

One of the two oxygen atoms bonded to the C-1 carbon of the pyranoside must be protonated to satisfy the requirements of the A-1 mechanism, but none of the evidence for that mechanism can distinguish between initial protonation of the aglycone oxygen (mechanism A) or the ring oxygen (mechanism B). Bunton has stated that some experimental difficulty is present in trying to distinguish between the two mechanisms, however, evidence exists for both mechanisms.

The sterochemical configuration at C-1 of the products of pyranoside hydrolysis might give an indication as to the structure of the reactive intermediate. This technique is not applicable to the hydrolysis of pyranosides, however, since the rate of anomerization of the aldose produced is much greater than the rate of hydrolysis. Banks and co-workers (25) however, interpreted the fact that the methanolysis of phenyl α and β -D-glucopyranosides gives the slowly anomerizing methyl glucopyranosides of predominantly the opposite configuration, as evidence for a cyclic carbonium ion intermediate.

The relative hydrolysis rates of various thio glycosides have also been taken as evidence of a cyclic carbonium ion in the hydrolysis of glycosides in general. Bumford et al. (26) explained the slow rate of hydrolysis of phenyl 1-thio-α-D-glucopyranoside relative to that of phenyl α-D-glucopyranoside, by the fact that the protonated thiophenol is a much poorer leaving group than the protonated phenol. This explanation indicates protonation at the aglycone position and, therefore, implies a cyclic carbonium ion intermediate in the hydrolysis.

Whistler and Van Es (27) also found that methyl 1-thio-α-D-xylopyranoside hydrolyzed at approximately one-half the rate of methyl α-D-xylopyranoside. Furthermore, the same authors found that the methyl α-and β-5-thio-D-xylopyranosides were hydrolyzed respectively 10 and 14 times faster than their 5-oxygen analogs. Both these observations were explained in terms of the cyclic

carbonium ion intermediate, in that sulfur in the ring position should allow greater charge delocalization than oxygen in the same position, due to a greater inductive effect. The more stable intermediate should exist in higher concentration, and the reaction rate should increase. In later work, Whistler and Rowell (28) found that the hydrolysis rate of methyl 1-thio-α-D-5-thio-xylopyranoside is only slightly less than that of methyl α-D-xylo-5-thio-pyranoside, implying that the inductive effect of the ring sulfur still predominates in the control of the hydrolysis rate.

Banks et al. (25) have supplied the most convincing, and the only direct evidence for the cyclic carbonium ion intermediate, by measuring an oxygen isotope effect for the breakage of the aglycone-oxygen bond during the partial hydrolysis of methyl α-D-glucopyranoside. A ratio of k_{16_O}/k_{18_O} of 1.03 was measured by noting a 3% decrease in the natural abundance of ^{18}O in the methanol produced during a 7% hydrolysis of methyl α-D-glucopyranoside as compared to that produced during a 100% hydrolysis. The observed kinetic isotope effect should be noted only if the reaction involves the breakage of the C-1 aglycone oxygen bond during the rate limiting step of the reaction. The observed effect is, however, only one-half the magnitude of the predicted effect of 1.064.

There is no positive evidence for the existence of the acyclic carbonium in the hydrolysis of glycosides; however, results by at least two authors make its existence a possibility. Lemieux (29)

noted that while the NMR spectra of N-(tetra-O-acetyl-β-D-glucopyranosyl)-4-methyl-pyridinium bromide indicated that the compound existed in the normal C 1 conformation, the anomer of the same compound was shown to exist in the energetically unfavorable 1 C conformation. If this compound is an accurate model of a pyranoside protonated in the aglycone position, it implies that protonation of the aglycone oxygen in the case of α-glycosides might produce a much less stable oxonium ion than protonation of the ring oxygen. In this manner, the acyclic hydrolysis mechanism might predominate.

In later studies, Clayton et al. (30) isolated small but significant amounts of methyl 1-thio- α and β-D-ribofuranosides and methyl 1-thio-β-D-ribopyranoside from the partial hydrolysis of pure methyl 1-thio-α-D-ribopyranoside. The existence of these anomeric forms indicates the presence of an open-ring structure at some point in the reaction mechanism.

While none of these studies positively identify the structure of the two possible reactive intermediates, the cyclic mechanism has gained wide acceptance, principally on the basis of the isotope effect measured by Banks. The fact that this effect was measured on only one anomer of one pyranoside and was of unexpectedly low magnitude leaves questions about the universality of the cyclic mechanism. Furthermore, the findings of other authors, such as Lemieux, are not explainable in terms of the cyclic mechanism. Greater confidence could be placed in the validity of the cyclic

intermediate if the isotope effect associated with the breakage of the C-1, aglycone oxygen bond were checked for other pyranosides. Also, the acyclic mechanism could be either proved or disproved by checking the possible oxygen isotope effect for the breakage of the C-1, ring oxygen bond during pyranoside hydrolysis. This study is then the beginning of an investigation of these two points, in particular the existence of the C-1 - ring oxygen, isotope effect.

RESULTS AND DISCUSSION

^{18}O Enrichment at C-5 of Xylose

It was initially felt that the most direct route to enrichment of the 5-oxygen of D-xylose would be nucleophilic replacement of a p-tolysulfonate group using enriched acetate or benzoate anion, enriched to at least 50 atom % ^{18}O at both carboxyl oxygens.

1,2-O-isopropylidene-5-O-p-tolysulfonyl-α-D-xylofuranose was prepared as planned, however initial studies showed that both sodium and potassium acetate were much too insoluble in dipolar aprotic solvents to be practical. Tetra-n-butylammonium benzoate has, however, been shown to give effective replacement at primary p-toulysulfonyl esters (40). Tetra-n-butyl ammonium acetate has also been reported; however, the method (41) involved the formation of the acetate salt of tetra-n-butylammonium hydroxide in water. Such an approach was not feasible for our needs since the exchange rates of the acetate oxygens with water is quite high. As an alternative approach, the formation of the acetate salt from tetra-n-butylammonium chloride in dry ethanol with potassium acetate was attempted. While the reaction proceeded in good yield (83%) as measured by the weight of the potassium chloride produced, the tetra-n-butylammonium acetate hydrated, either from water remaining in the ethanol or from atmospheric moisture during filtration and workup. Tetra-n-butylammonium acetate is known to form a hydrate with 15 moles of water per mole of salt (41).

It was feared that isotopic dilution would occur if the hydrated tetra-n-butylammonium acetate were used in the replacement reaction. In addition, no reaction could be detected in mixtures of 1,2-O-isopropylidene-5-O-p-tolysulfonyl-α-D-xylofuranose with slight excesses of the hydrated tetra-n-butylammonium acetate in N-methylpyrrolidone at either 60° or 150°, as evidenced by thin layer chromatography (TLC).

As sodium benzoate is known to replace the p-tolysulfonyl group at primary alcohols (40), its use as a carrier of ^{18}O was explored. Literature methods cite 200-400% molar excesses under forcing conditions to achieve even 60% yields. At current commercial prices for benzoic acid labeled at 70 atom% ^{18}O in both oxygen atoms ($765/gm, Miles Laboratories, Research Div., Kankakee, Ill.) and assuming a 60% yield in the replacement and 35% through the subsequent sequence of reactions, the cost to label 200 mg each of methyl α-D-xylopyranoside and methyl β-D-xylopyranoside can be calculated to be approximately $2,000.

To overcome this high cost of labeling, it was felt that the oxygen replacement might be carried out with the relatively cheaper ^{18}O hydroxide ion if a better leaving group were present at the C-5 position of 1,2-O-isopropylidene-α-D-xylofuranose. Accordingly, 1,2-O-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose was prepared. Upon refluxing the latter compound in basic conditions with a slight excess of water, TLC showed large amounts of a product with greater mobility than the starting material, whereas only traces of a compound corresponding in mobility to

1,2-O-isopropylidene- -D-xylofuranose (the desired product) were detected. It was assumed that the component of higher mobility was 1,2-O-isopropylidene-3,5-anhydro-α-D-xylofuranose (Figure 1.). The 3,5 anhydro compound is formed under similar, but anhydrous conditions (42).

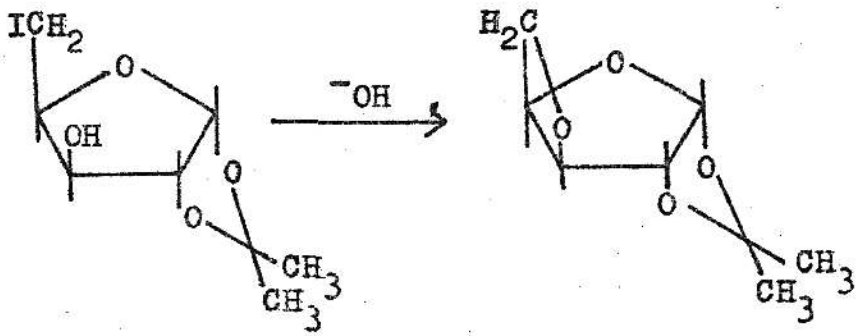


Figure 1. 1,2-O-isopropylidene-3,5-anhydro-α-D-xylofuranose

To block the formation of the 3,5-oxete ring; 1,2-O-isopropylidene-3-O-benzyl-5-deoxy-5-iodo-α-D-xylofuranose was prepared. Several attempts to effect replacement of the iodo group using water and various bases in polar solvents showed no appreciable reaction. Possibly the close proximity of the 3 and 5 positions on the furanose structure and the free rotation of the benzyl group causes steric interference during the attempted S_n2 displacement. The 5-iodo group in either the 3-hydroxyl or the 3-O-benzyl derivitive was however, easily removed by silver nitrate in the presence of water and a tertiary amine. TLC showed good conversion of the starting material to a component of intermediate mobility between the starting 5-iodo derivitive and the desired 1,2-O-isopropylidene-α-D-xylofuranose. The syrupy

product from the workup of the reaction mixture decolorized dilute bromine water. A literature search revealed that the suspected dehydrohalogenation product, 1,2-O-isopropylidene-4,5-dideoxy-α-D-threopent-4-enofuranose (Figure 2.) had been formed under similar but anhydrous conditions with mercuric salt catalysis (43).

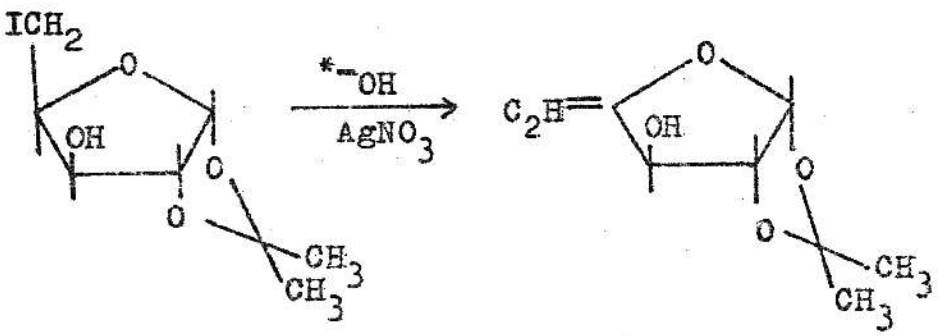


Figure 2. 1,2-isopropylidene-4,5-dideoxy-α-D-threopent-4-enofuranose

In view of the difficulty involved with side reactions in attempts at nucleophilic displacement reactions at the 5 position of xylose, an experimentally different, and in retrospect, a simpler technique involving $^{16}O/^{18}O$ exchange at a suitably positioned aldehyde group on 1,2-O-isopropylidene-α-D-xylofuranose was employed. The starting material exists as a dimer, bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)-3,5'-5,5' cyclic acetal (II), and has been studied rather extensively by Isbell (44) because of its use as an intermediate in the preparation of D-glucose-6-^{14}C. Data is available (45) showing that the exchange rates for $^{16}O/^{18}O$ at the 1-aldehydo position of aldoses is reasonably rapid, and that the exchange proceeds, with base

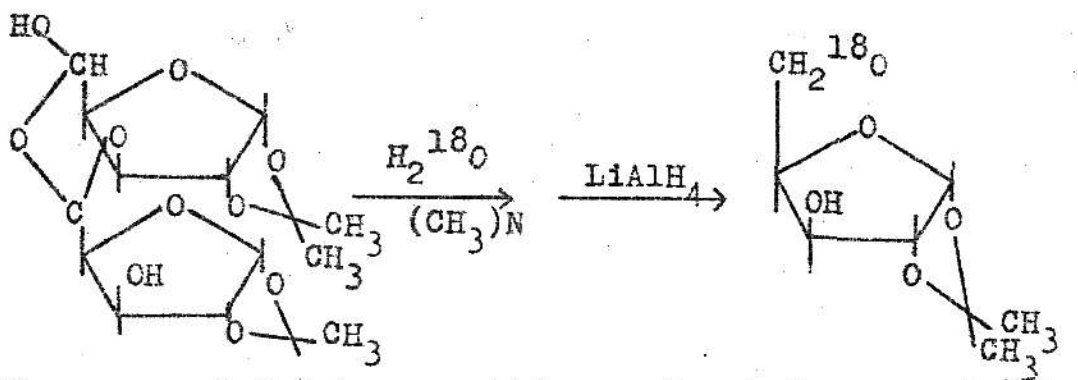
catalysis, to near 100% completion. Results by other investigators (46) also indicate that primary and secondary hydroxyl oxygen atoms show no exchange under basic or acid conditions over the same time span required for equilibrium to be established at the aldehyde oxygen.

The dimer (II) exists both as a syrupy product and as one of two crystalline solids. Our initial exchange studies were carried out on the syrupy product since crystallization could not be affected. Thin layer chromatography of the syrupy (II) showed four separate components, none of which corresponded by TLC mobility to the starting material, 1,2-O-isopropylidene-α-D-glucofuranose (I). Isbell (44) reported the compound to exist as a dimer in solution and in one of two crystalline forms, one hydrated and the other anhydrous. Also, Isbell stated the dialdose (II) tightly absorbs the formaldehyde produced during its formation by periodate oxidation of (I). The four components in syrupy (II) were assumed to be the dimer (II), the free aldehyde form, a hydrated form and a formaldehydrate. In our laboratory, formaldehyde could not be separated from the dialdose by absorbtion chromatography on silica gel, or by prolonged warming of the syrup under vacuum.

Isbell's method requires removal of the formaldehyde by freeze drying the reaction mixture from the periodate oxidation before extraction of the 5-aldehydo compound. In his method, the last traces of formaldehyde must be removed by crystallization

from water after prolonged standing in the cold. During our attempts at dehydration of the syrupy product, it was noted that formaldehyde could be removed by refluxing a solution of the syrupy product in benzene. The odor of formaldehyde was detected above the reflux condenser, and a white solid, presumable para-formaldehyde, formed at the lower end of the condenser. After refluxing for 10 hours, the benzene could be removed and the syrupy dimer (II) was easily purified by crystallization from water. The crystals obtained were dehydrated and recrystallized in anhydrous form from benzene.

Oxygen exchange at the potential 5-aldehyde group was first carried out using ammonia as the base catalyst and water containing 20.6 atom% ^{18}O. Reduction of the 5-aldehyde group was done by addition of the exchange reaction mixture to lithium aluminium hydride without preliminary removal of the excess water present in the exchange reaction mixture.



The syrupy 1,2-O-isopropylidene-α-D-xylofuranose-5-^{18}O (III) from the exchange reaction was trimethylsilylated, purified by preparative gas-liquid chromatography and its mass spectrum examined. A line graph of the spectrum is shown in Figure 3., page 21.

The method of calculation of the theoretical incorporation of ^{18}O at the 5-position of 1,2-O-isopropylidene-α-D-xylofuranose will be given here in detail for the first exchange experiment.

Oxygen-18 enriched water contained 20.6 atom% ^{18}O (Bio-Rad Laboratories analysis). The molecular weight of the enriched water is:

$0.206 \times 20.0 \text{ mol}^{-1} + 0.794 \times 18.0 \text{ g mol}^{-1} = 18.41 \text{ g mol}^{-1}$

Assuming the only exchangeable oxygen on (II) is the 5-aldehyde, and ignoring naturally occurring ^{18}O, (0.2%) the equilibrium content at the 5-position of (II) will be the moles of ^{18}O present in the exchange system divided by the total moles of exchangeable oxygen. The molecular weight of (II) is 377.40 g/mol, and there are two exchangeable oxygens per mole. If 0.3281 g of enriched water and 1.376 g of (II) are present in the exchange; the equilibrium percent of ^{18}O at O-5 of (II) is:

$$\frac{(0.206)(0.3281 \text{ g}/18.41 \text{ g mol}^{-1})}{(0.3281 \text{ g}/18.41 \text{ g mol}^{-1}) + 2(1.376 \text{ g})/377.40 \text{ g mol}^{-1}} \times 100 = 14.62\%$$

At equilibrium, the 5-oxygen of (II) should contain 14.62 atom% ^{18}O.

To determine the efficiency of exchange, the ratios of the peaks at m/e 319 to 321 and 103 to 105 in the mass spectrum of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsily)-α-D-xylofuranose were determined. The ratios were obtained by measuring the peak

heights with a steel rule, graduated in 1/64". The fragments at m/e 319 and 321 were assigned to the structure shown in Figure 3. (a) page 21. This corresponds to the molecular ion with loss of a $\cdot CH_3$ radical, a common fission in isopropylidene derivitives of aldoses (47). A measurement at the molecular ion region of the spectrum would be more satisfactory, but isopropylidene derivatives commonly do not exhibit molecular ions. The peaks at m/e 103 and 105 were assigned the structure shown in Figure 3. (b) page 21, and each contains only the oxygen originally at the 5 position in the intact molecule. Fragmentation at the exocyclic carbon has been shown to be a prominent disintegration in the mass spectra of other furanosides (48).

In using the mass spectrum to determine oxygen-18 enrichment, the relative intensity of the ion at two mass units higher than the base ion peak is the sum of the ^{18}O enrichment plus contributions from the natural abundance of other isotopes in the species corresponding to the base peak. Determination of the added ^{18}O in the D-xylose derivitives requires allowance for these natural abundance contributions.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

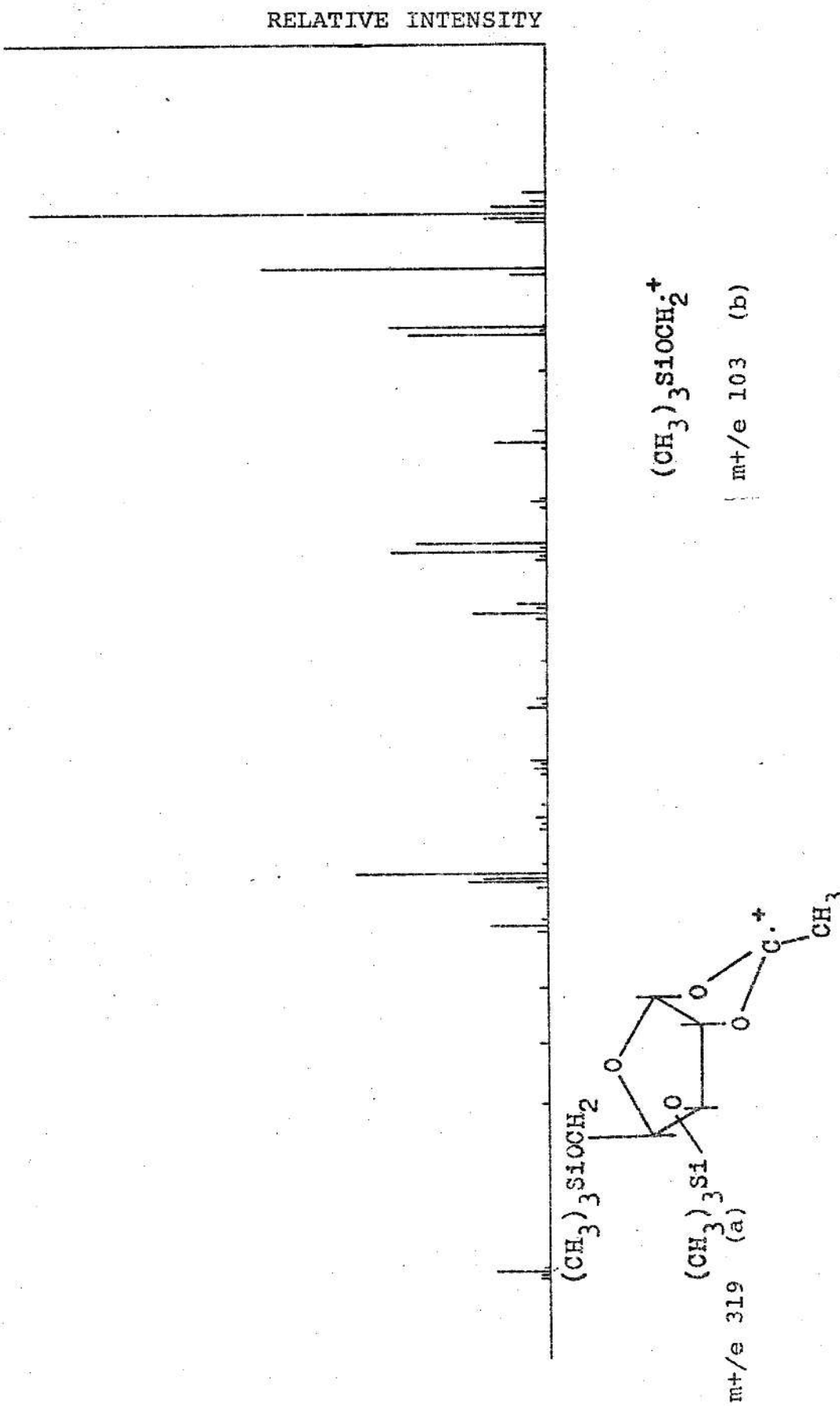


Figure 3. Mass Spectrum of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose.

Allowance for the natural abundance peak may be accomplished either by actually determining the ratio of the mass +2 peak to the base peak of the ion cluster in the spectrum of the unlabeled compound or by calculation of the relative intensity of the molecular ion +2 (m +2) peak using the natural abundances of higher mass isotopes of atoms contained in the ion. In this work, calculation of the m +2 intensity due to naturally occurring isotopes was done to eliminate the added error caused by subtraction of the measured value in determination of the $^{18}O/^{16}O$ ratio.

Because of the importance of this calculation in interpreting changes in the $^{18}O/^{16}O$ ratio and changes in the ^{18}O content at the 5-position, it is given in detail below for the m/e 319 peak.

When more than one atom is present in an ion, the probability of any one of these atoms being a higher mass isotope is found by using a binomial expansion of (49):

$$(a + b)^m$$

Where:

a = the percentage natural abundance of the light isotope

b = the percentage natural abundance of the heavy isotope

m = the number of atoms of an element present in the ion.

The relative magnitude of the terms in the expansion corresponded to the relative abundance of ion species containing zero through m atoms of an isotope of higher mass. Contributions to the intensity of the peak two mass units higher than the base peak for the ion cluster will be made by ions containing one atom of

an isotope of two mass units higher than the most abundant isotope. This contribution will be represented by the second term of the expansion. Contributions to the mass +2 peak intensity will also be made by ions containing two atoms of an isotope one mass unit higher than the most abundant isotope for an element. This contribution may be calculated by the sum of the third terms of the binomial expansions for the isotopes one mass higher than the most abundant isotope for each atom in an ion. In addition, the m +2 peak will contain species with two mass +1 isotopes from different elements. This contribution is calculated by cross multiplying the second terms of two binomial expansions where each expansion represents the probability of zero through m mass +1 isotopes of an atom occurring at the same time in an ion.

The exponents and coefficients for the second and third terms of a binomial expansion are given by the general formulas:

$$\text{second term} = (m)a^{(m-1)}b$$

$$\text{third term} = \frac{m(m-1)}{2!}a(m-2)b^2$$

The elemental formula for the (M-15) peak in the spectrum of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose is $C_{14}H_{27}O_5Si_2$, and the natural abundance percentages of the elements with the most abundant isotope of each taken as 100 are as follows:

^{29}Si = 5.07%

^{30}Si = 3.31%

^{17}O = 0.04%

^{18}O = 0.20%

^{13}C = 1.08%

^{2}H = 0.016%

The individual contributions to the intensity of the peak at 2 mass units higher i.e. (M-13) by the occurrence of two isotopes of the same element, each one mass unit higher than the most abundant isotope of that element is calculated by i) determining the third term of the binomial expansion for that particular element, and ii) converting the value calculated to a percentage of the peak containing only the most abundant isotope by dividing the value found for the third term in part i) by the first term of the expansion and then multiplying the quotient by 100.

For two ^{29}Si

$$\text{Part i)} \quad \frac{2(2-1)}{2!} (100)^{(2-2)} (5.07)^2 = 25.7$$

$$\text{Part ii)} \quad \frac{25.7}{(100)} 2 \times 100 = 0.257\%$$

The value found in part ii) of the calculation gives the magnitude of the contribution of an ion species containing two isotopes of one mass unit higher than the most abundant isotope for the atom to the (M-13) peak as a percentage of the magnitude of the (M-15) peak.

For two ^{17}O

Part i) $$\frac{5(5-1)}{2!}(100)^{(5-2)}(0.04)^2 = 1.6 \times 10^4$$

Part ii) $$\frac{1.6 \times 10^4}{(100)^5} \times 100 = 1.6 \times 10^{-4}\%$$

For two ^{2}H

Part i) $$\frac{27(27-1)}{2!}(100)^{(27-2)}(0.016)^2 = 8.99 \times 10^{48}$$

Part ii) $$\frac{8.99 \times 10^{48}}{(100)^{27}} \times 100 = 8.99 \times 10^{-4}\%$$

For two ^{13}C

Part i) $$\frac{14(14-1)}{2!}(100)^{(14-2)}(1.08)^2 = 9.79 \times 10^{25}$$

Part ii) $$\frac{9.79 \times 10^{25}}{(100)^{14}} \times 100 = 0.978\%$$

The value found in part ii) of the calculation gives the magnitude of the contribution of an ion species containing two isotopes of one mass unit higher than the most abundant isotopes to the (M-13) peak as a percentage of the magnitude of the (M-15) peak.

The contributions to the (M-13) peak by two ^{2}H's or two ^{17}O's were regarded as insignificant in view of the contribution by two ^{29}Si's and two ^{13}C's, and were disregarded in further calculations and in the summation of the total natural abundance of the (M-13) peak.

Contributions to the (M-13) peak by a combination of one ^{29}Si and one ^{13}C occurring simultaneously in the ion may be calculated by the product of the second terms of their respective binomial expansions as follows:

For one ^{29}Si

Part i) $2(100)^{(2-1)}\ (5.07) = 1014$

Part ii) $\frac{1014}{(100)^2}\ 2 \times 100 = 10.14\%$

For one ^{13}C

Part i) $14(100)^{(14-1)}\ (1.08) = 1.512 \times 10^{27}$

Part ii) $\frac{1.512 \times 10^{27}}{(100)^{14}} \times 100 = 15.12\%$

The probability then of one ^{13}C occurring given the occurrence of one ^{29}Si is:

$15.12\% \times 10.14\% = 1.53\%$

The similar contributions to the (M-13) peak from the other possible contributions of mass +1 isotopes were calculated and the values are as follows:

$^{2}H + {}^{29}Si = 0.044\%$

$^{2}H + {}^{13}C = 0.065\%$

$^{17}O + {}^{29}Si = 0.020\%$

$^{17}O + {}^{13}C = 0.030\%$

The contribution to (M-13) due to the occurrence of one isotope of two mass units higher than the most abundant isotope for an element is calculated by the second term of its binomial expansion. These contributions, with the base peak of the ion cluster normalized to 100 are;

For one ^{18}O

Part i) $5(100)^{(5-1)}\ (0.20) = 1 \times 10^8$

Part ii) $\frac{1 \times 10^8}{(100)^5} \times 100 = 1.0\%$

For one ^{30}Si

Part i) $2(100)^{(2-1)}\ (3.31) = 6.62 \times 10^2$

Part ii) $\frac{6.62 \times 10^2}{(100)^2} \times 100 = 6.62\%$

Summing these contributions gives the total height of the m/e 321 peak as a percent of the height of the m/e peak as 10.54%.

Since we are labeling only one oxygen position in the molecule, it is of interest to note here that to determine the ratio of $^{18}O/^{16}O$ in a fragment containing the ^{18}O label, only the intensities of the base peak in the ion cluster and the m/e +2 peak in the cluster need be considered.

Similar calculations show the contribution of the m/e 103 peak to the m/e 105 peak as 3.53% of the m/e 103 intensity.

To within experimental error, the measured intensities of the peaks at m/e 321 and 105 in the spectrum of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose agree with the calculated natural abundances as shown in Table 1. page 28. The

Table I. Ratios of Peak Intensities at m+/e 321/319 and Peak Intensities at m+/e 105/103 in the Mass Spectrum of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose (unlabeled)

Spectrum	Peak Height in 1/64"				Peak Height Ratio	
	m^+/e 321	m^+/e 319	m^+/e 105	m^+/e 103	321/319	105/103
A	7.0	73	3.0	78	0.0959	0.0385
B	3.5	32	2.5	72	0.1094	0.0342
				mean	0.1026	0.0358
				standard deviation	0.0096	0.0023

experimental error is, however, nearly 10%. Much of this error is due to the difficulty in measuring the heights of the small peaks at m/e 321 and 105. The noise, both at the top of the peaks and at the baseline must be neglected, which leads to difficulty in reproducing the measurements. The error involved in reading the intensity of peaks from the spectrum, however can be considerably reduced by analyzing 30 to 50 scans of the ion region of interest. These results do not rule out the possibility of introducing a systematic error by the use of the calculated contribution to the (M-13) /e peak to correct for the natural abundance of other isotopes in the ^{18}O labeled compounds. It was felt, however, that a calculated correction would be preferable to an experimentally measured correction determined from the spectrum on an unlabeled compound, since the latter approach would again introduce the error involved in measuring intensities from a spectral chart.

In estimating ^{18}O content of labeled compounds then, the m +2 peaks were corrected for the contribution by heavier isotopes using the theoretically calculated corrections.

The results in Table II show that within the experimental error, all the ^{18}O enrichment is at the O-5 position of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose. The ratio of $^{18}O/^{16}O$ in our first sample of ^{18}O labeled (II) was determined to be 0.1274 using the peaks at m/e 321 and 319 whereas it was 0.1437 using the peaks at 103 and 105. Calculating

the percent ^{18}O at O-5 from the ratio of $^{18}O/^{16}O$ equal to 0.1274 gives 11.30% ^{18}O at O-5 as compared to the maximum value of 14.62%. The exchange efficiency was 77.4%.

In further studies of the exchange reaction, difficulties were encountered due to the formation of apparent base degradation products. TLC showed three distinct components of slower mobility then 1,2-O-isopropylidene- -D-xylofuranose following exchange and reduction. It was first felt that these by-products might be formed during the reduction by the action of alkali on the carbohydrate; the alkali base would arise from the excess water remaining from the exchange reaction with lithium aluminum hydride. Removal of the water prior to reduction resulted in no great improvement, however a slightly weaker base, trimethylamine, was substituted as catalyst. Due to some insolubility problems during the reduction step, the solvent used in both the exchange and the reduction was changed from diethyl ether to the more polar tetrahydrofuran. Refluxing crystalline (II) in THF with trimethylamine for up to 8 hours have no side-reactions as evidenced by TLC. At 10 hours reaction time, small amounts of the slower moving components became noticeable and by 16 hours of reaction time, the third component appeared and considerable degradation had taken place.

The efficiency of the newly devised exchange system was tested in a reaction designed to produce (II) with 50 atom% ^{18}O at O-5. The average molecular weight of the 95 atom% water used

Table II. Ratios of Peak Intensities at m+/e 321/319 and Peak Intensities at m+/e 105/103 in the Mass Spectrum of 1,2,-O-Isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose-5-^{18}O. (20 atom% ^{18}O water used in exchange)

Spectrum	Peak Height in 1/64"				Peak Height Ratios			
	m^+/e 321	m^+/e 319	m^+/e 105	m^+/e 103	m^+/e 321 corrected[a]	$^{18}O/^{16}O$[b]	m^+/e 105 corrected[c]	$^{18}O/^{16}O$[d]
A	20	84	17	96	11.205	0.1334	13.61	0.1418
B	26	115	17	94	13.66	0.1214	13.68	0.1456
					mean	0.1274	mean	0.1437
					standard deviation	0.0085	standard deviation	0.0027

a. Corrected value = (m^+/e 321) - (0.1054)(m^+/e 319)
b. Ratio = Corrected value/ (m^+/e 319)
c. Corrected value = (m^+/e 105) - (0.0353)(m^+/e 103)
d. Ratio = Corrected value/ (m^+/e 103)

in the experiment was 19.9 g mol^{-1}, and the amount of (II) required to give an increase in ^{18}O at O-5 of 50% when exchanged with 0.05 g of enriched water was calculated as follows:

$$\text{Moles (II)} = \frac{0.95(0.05\text{ g}/19.9\text{ g mol}^{-1}) - 0.5(0.05\text{ g}/19.9\text{ g mol}^{-1})}{0.5}$$

$$= 0.00225 \text{ mol}$$

The exchange, reduction and mass spectral analysis on 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose were repeated at the higher level of enrichment. The contribution of the natural abundance of higher mass isotopes was corrected using theoretical values of 0.1054 for m+/e 321 and 0.0353 for m+/e 105. Results are given in Table III.

The $^{18}O/^{16}O$ ratio calculated from the mass spectral data in Table III indicates an increase in ^{18}O at the O-5 position of 1,2-O-isopropylidene-α-D-xylofuranose of 31.2% (62.4% efficiency). Calculations using the m/e 321/319 and m/e 105/103 ratios agree within experimental error.

In an attempt to increase incorporation of ^{18}O in (II), solvents were repurified to preclude isotopic dilution with normal water. The tetrahydrofuran used in the exchange was passed through an Alumina column to remove possible peroxide contamination. Also, the reaction vessel was flushed with dry, gaseous trimethylamine before addition of the other reactants. These precautions gave no increase in exchange efficiency.

Table III. Relative Abundance of $^{18}O/^{16}O$ in 1,2-O-Isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose (Trimethylamine catalyzed exchange)

Spectrum	Peak Height in 1/64"				Peak Height Ratios			
	m^+/e 321	m^+/e 319	m^+/e 105	m^+/e 103	m^+/e 321 corrected[a]	$^{18}O/^{16}O$[b]	m^+/e 105 corrected[c]	$^{18}O/^{16}O$[d]
A	12	22	12	25	9.70	0.4409	11.12	0.4448
B	12	21	11	23	9.80	0.4667	10.19	0.4430
C	10	18	10	20	8.12	0.4511	9.29	0.4645
					mean	0.4529	mean	0.4507
					standard deviation	0.0130	standard deviation	0.0119

a. Corrected value = $(m^+/e\ 321) - (0.1054)(m^+/e\ 319)$
b. Ratio = Corrected value/ $(m^+/e\ 319)$
c. Corrected value = $(m^+/e\ 105) - (0.0353)(m^+/e\ 103)$
d. Ratio = Corrected value/ $(m^+/e\ 103)$

Since the starting material (II) is known to exist in a hydrated state, the possibility of water contamination due to a hydrated reactant was checked. Isbell (44) observed a melting point of 182° for (II) which was dehydrated and crystallized from benzene. The same compound, crystallized from water undergoes a transition at 145°, and then melts at 182°. These properties were also noted in this investigation. As further evidence that our starting material (II) was in the anhydrous form, a sample of (II) was dissolved in dimethylsulfoxide-d_6 and the NMR spectrum was checked for the appearance of a water spike. The spectra showed no water spike at δ3.3 ppm from tetramethylsilane (50).

From these studies it was concluded that an oxygen exchangeable contaminant in the quantity required to give the observed low exchange efficiency was not present. A likely explanation for the low exchange efficiency is the stability of the 3,5'-5,5' acetal ring present in the dimerized compound. Bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)-3,5'-5,5' cyclic acetal has been shown to give no phenyl-hydrazone upon refluxing in ethanol (44). This stabilizing might result in a slow exchange rate, requiring longer reaction times to achieve equilibrium. This hypothesis was not tested in view of the difficulty encountered with side reactions during the base catalyzed exchange. Another possible explanation which might merit investigation is dilution by water absorbed on the glass of the exchange vessel.

Separation of Methyl α-D-xylopyranoside-5-^{18}O (IV) and Methyl β-D-xylopyranoside-5-^{18}O (V)

Methanolysis of the 1,2-O-isopropylidene-α-D-xylofuranose produced after reduction of bis-(1,2-O-isopropylidene-α-D-xylo-pentodialdofuranose)-3,5'-5,5' cyclic acetal gives an equilibrium mixture containing 65% (IV) and 29.8% (V). Separation of the two main components in this mixture (the anomeric pyranosides) has been previously accomplished by fractional crystallization of the free glycosides (37) and by fractional crystallization of their triacetate derivitives (37). Both of these techniques gave the anomer in low yield and were difficult to use on the small amounts of ^{18}O-labeled glycosides prepared in our laboratory.

Attempts to eliminate the β anomer from the mixture by enzymolysis with β-glucosidase from almond emulsin failed despite the fact that β-xylosidase activity has been reported in the crude almond emulsin (51).

The anomeric forms of several pyranosides have also been resolved by chromatography on stongly basic, anion exhange resins in the hydroxide form (52). However, separation of the methyl α and β-D-xylopyranosides proved to be incomplete on the scale required for preparative work.

Another approach to obtain pure methyl α-D-xylopyranoside is through anomerization. The equilibrium mixture of methyl 2,3,4-tri-O-acetyl-α and β-D-xylopyranosides produced upon heating the peracetates with Lewis acid catalysis, has been

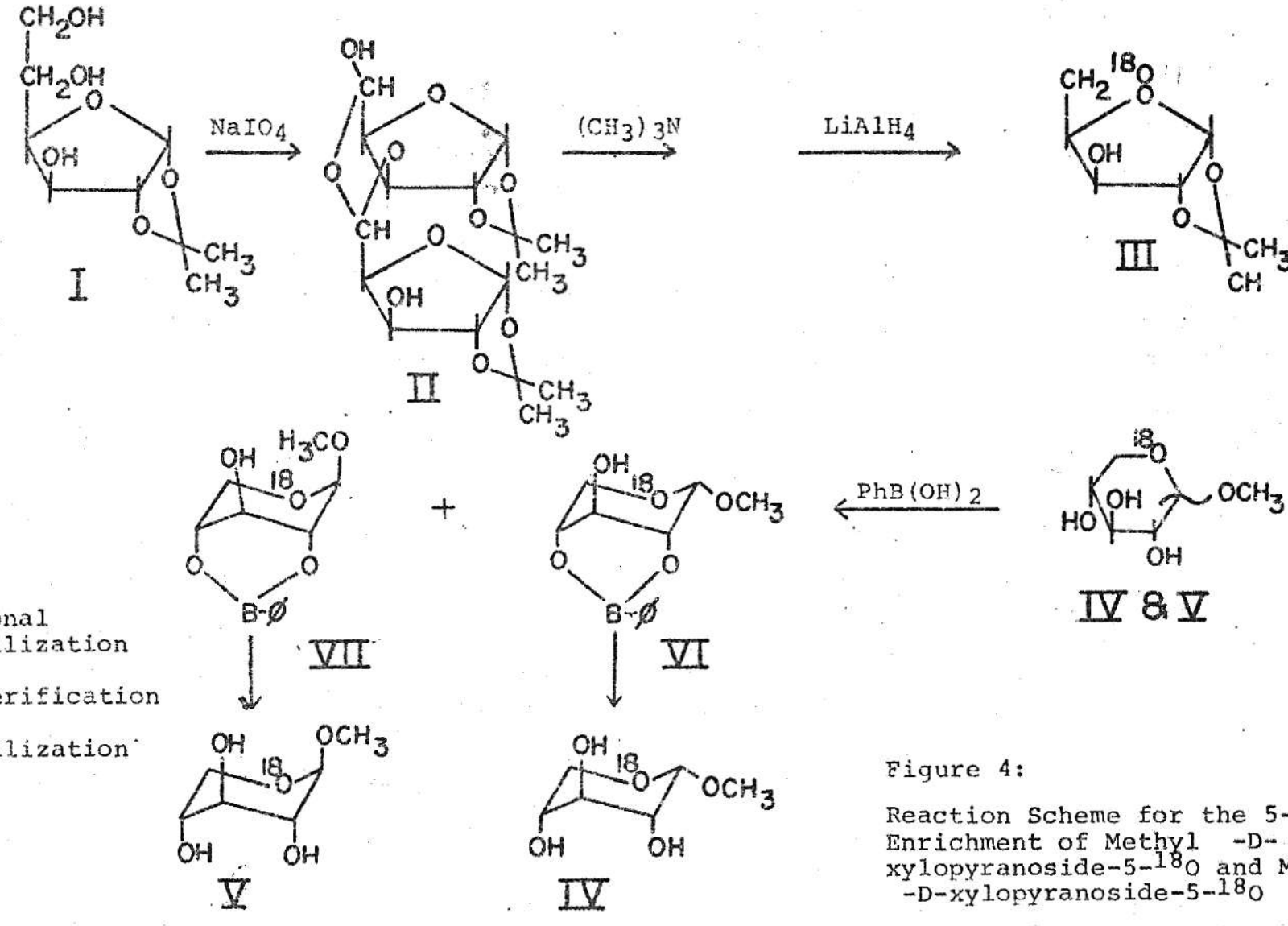
shown to contain predominantly the α-anomer. In this work catalysis by titanium tetrachloride or boron trifluoride was evaluated by gas-liquid chromatography of reaction mixtures at various reaction times. Reaction by both catalysts reached equilibrium after approximately 2½ hours. The titanium tetrachloride catalyzed reaction formed an equilibrium mixture having an α/β pyranoside ratio of 85/15, whereas the boron trifluoride catalysis gave a ratio of 95/5. Fractional crystallization of the 2,3,4-tri-O-acetyl-α-D-xylopyranoside followed by removal of the acetyl groups and crystallization gave pure methyl α-D-xylopyranoside (IV).

Overall yields of (IV) produced through anomerization were quite low. Also, a separate sequence of reactions would have to be devised to produce the ^{18}O enriched β-anomer. For these reasons, the preparation of the anomeric D-xylopyranosides by the method of Ferrier (38) was employed in the final ^{18}O enrichment scheme adopted in this work. The method (as described) involves formation of the cyclic 2,4 phenyl boronate esters of methyl α and β-D-xylopyranoside, followed by fractional crystallization of the easily crystallized methyl α-D-xylopyranoside 2,4-O-phenyl boronate. The 2,4 phenyl boronate ester of the β anomer, which remains in the mother liquors following crystallization of the ester of the α anomer, shows much higher solubility in non-polar

solvents, possibly due to partial masking of the 3-hydroxyl group by its 1-methoxyl group. De-esterification followed by removal of the phenyl boric acid relased, and subsequent crystallization gave pure α and β anomers, (IV) and (V), in good yield.

The final scheme for the preparation of methyl α-D-xylopyranoside-5-^{18}O and methyl β-D-xylopyranoside-5-^{18}O is shown in Figure 4., page 38. Because exchange of the 5-aldehydo oxygen of (II) with $H_2{}^{18}O$ is not stoicheometric, to achieve labeling of (II) at O-5 in the 50% range, higher molar excesses of $H_2{}^{18}O$ (calculated to give 65 atom% labeling) were used. Beginning with 0.5 g of 95 atom% ^{18}O water, the sequence of reactions shown in Figure 4., page 38, gave (IV) and (V) in overall yield from (II) of 19.7% and 48.8% respectively.

The percent label in each of the xylopyranosides was determined by complete hydrolysis followed by trimethylsilylation of the hydrolysis products and preparative glc to give 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranoside-5-^{18}O. The mass spectrum of the pertrimethylsilylated β-D-xylopyranose was taken and the peaks at m+/e 438 and m+/e 440 (molecular ions) in the spectrum were used to calculate the ^{18}O content. Theoretical calculation of the contribution to the m+/e 440 peak due to naturally occurring isotopes by the same method used for the case of the peak m+/e 319 in the spectrum of 1,2-O-isopropylidene-3,5-bis-O-(trimethylsilyl)-α-D-xylofuranose,



1. Fractional crystallization
2. De-esterification
3. Crystallization

Figure 4:

Reaction Scheme for the 5-^{18}O Enrichment of Methyl -D-xylopyranoside-5-^{18}O and Methyl -D-xylopyranoside-5-^{18}O .

showed the contribution of heavier isotopes to the intensity of the line at m+/e 440 was 21.43% of the intensity of the line at m+/e 438. The $^{18}O/^{16}O$ ratios in the ^{18}O-labeled xylosides as determined by mass spectrometry are shown in Tables IV and VI pages 47 and 49. The percent ^{18}O at the O-5 position was calculated from the average of the $^{18}O/^{16}O$ ratios found for the complete hydrolysis product of both methyl α-D-xylopyranoside-5-^{18}O ($^{18}O/^{16}O$ = 1.19) and methyl β-D-xylopyranoside-5-^{18}O ($^{18}O/^{16}O$ = 1.19) the relative ^{18}O content at O-5 is 54.5%. The observed abundance of 54.5% ^{18}O represents an exchange efficiency of 83.8%. The oxygen-18 recovered in the labeled xylosides was 7.3% of the total ^{18}O added to the exchange reaction. The cost to label 244 mg of (IV) and 277 mg of (V) was $210.

Separation of 1,2,3,4-Tetrakis-O-(trimethylsilyl)-β-D-xylopyranose from a 95/5 Mixture of Methyl 2,3,4-Tris-O-(trimethylsilyl) α or β-D-xylopyranoside

Separation of the xylose produced in the partial hydrolysis of xylosides was accomplished by preparative gas-liquid chromatography of the pertrimethylsilylated mixture from the partial hydrolysis. From data compiled by Sweely et al. (39), one would predict that non-polar columns such as SE-30 or SE-52 will give the best separation

of the tris-(trimethylsilyl)-D-xylosides from the α and β pertrimethylsilylated xyloses derivitives. In this study, complete resolution of the three main components of a trimethylsilylated hydrolysate was achieved on an analytical scale; the first component eluted, depending on the starting glycoside was either methyl 2,3,4-tris-O-(trimethylsilyl)-α or β-D-xylopyranoside, while the other two peaks were pure 1,2,3,4-tetrakis-O-(trimethylsilyl)-α-D-xylopyranose and 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose. On a scale practical for preparative work on an 8' x 1/4" column, however, the first appearing peak containing methyl 2,3,4-tris-O-(trimethylsilyl)-α or β-D-xylopyranoside overlapped with the second peak, 1,2,3,4-tetrakis-O-(trimethylsilyl)-α-D-xylopyranose, but the slowest moving component, 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose, was cleanly separated. Injecting 7-10 μl of the nearly solvent free mixture of trimethylsilyl derivitives allowed collection of a sufficient amount of the pertrimethylsilylated- -D-xylose for mass spectral analysis after 5-6 collections.

Isotope Effects in the Hydrolysis of Methyl α and β-D-xylopyranosides

A calculation of the maximum kinetic isotope effect expected in the cleavage of the C-1 to O-5 bonds during the hydrolysis of a methyl D-xylopyranoside is necessary

in the evaluation of any observed effect. A small secondary effect might be expected in the cleavage of the C-1 to O-1 bond. Secondary effects are, however, much smaller in magnitude than primary effects (54), and should be distinguishable from a primary effect.

Exact calculation of the theoretical magnitude of the isotope effect expected during glycoside hydrolysis is very difficult, especially when little is known about the exact nature of the transition state of the reaction. Fortunately, such detailed calculations are not required in this investigation. A rough calculation can be made using Bigeleisen's approximate treatment for heavy isotopes (55).

$$\frac{k_1}{k_2} = \frac{\nu_1^{\ddagger}}{\nu_2^{\ddagger}} \cdot \left[1 + \left(\frac{1}{u_1} - \frac{1}{2} - \frac{1}{e^{(u_1-1)}}\right) (u_1 - u_2)\right] \quad 1$$

Where:

k_1 = the hydrolysis rate constant for the lighter isotope

k_2 = the hydrolysis rate constant for the heavier isotope

$\nu_1^{\ddagger}$ = the frequency of breakdown of the activated species containing the lighter isotope

$\nu_2^{\ddagger}$ = the frequency of breakdown of the activated species containing the heavier isotope

$u_1 = h\nu_1/kT$

$u_2 = h\nu_2/kT$

Where ν_1 and ν_2 are the assymetric stretching frequencies (lighter and heavier isotopes respectively) of an activated molecule's bond which undergoes cleavage during the breakdown of the activated complex;

h = Plank's constant

k = Boltzman's constant

T = the reaction temperature (absolute)

The function $\left(\frac{1}{u_1} - \frac{1}{2} - \frac{1}{e^{(u_1-1)}}\right)$ is defined as -G(u) and has been tabulated by Bigeleisen and Goeppert-Mayer (56). The stretching frequency in the ^{18}O substituted molecule ($_2$) was calculated from the classical formula describing the frequency of a harmonic occilator:

$$= \frac{1}{2\pi}\left(\frac{K}{\mu}\right)^{\frac{1}{2}} \qquad 2$$

Where:

K = the force constant for the bond

μ = the reduced mass of the bonded atoms

In calculating the vibrational frequency of the C-^{18}O bond, the force constant, K, is assumed to remain the same as for the C-^{16}O bond.

The ratio of the frequency of breakdown of the activated complex is estimated by Bigeleisen's modification of the Slater approach (55).

$$\frac{\nu_1^*}{\nu_2^*} = \left[\frac{1/M_1' + 1/M_1''}{1/M_2' + 1/M_2''}\right]^{\frac{1}{2}} \qquad 3$$

Where:

M' and M" care the masses of the separating fragments, and the subscripts 1 and 2 denote the light and heavy isotopes as before.

The calculation of the isotope effect for the breaking of the C-1 to O-5 bond in the hydrolysis of glycosides involves one further complication in using equation 3 above. If the C-1 to O-5 bond is broken, two separate fragments are not released, but remain a part of the same molecule during the transition state. Bigeleisen (57) has shown that, at least for vibrational contributions, atoms more than one bond removed from the breaking bond have little effect on the magnitude of the isotope effect. Cutting off atoms more than one bond removed from C-1 and O-5 and assuming breakage of the C-1 and O-5 bond gives the two fragments (labeled A and B) in Figure 5. with masses of 29 and 41 respectively.

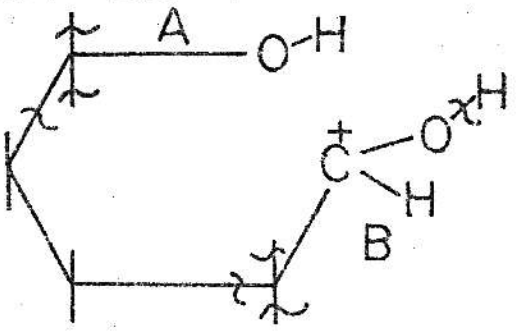


Figure 5. Fragments Defined for the Calculation of the Possible Oxygen Isotope Effect in the Breakage of the C-1 to O-5 Bond During the Hydrolysis of Glycosides.

Using these masses for the computation of $\frac{\nu_1^{\ddagger}}{\nu_2^{\ddagger}}$ and a value of 2.751×10^{13} sec^{-1}, assigned to the asymmetric stretch of the C-O-C bonds in the pyranose ring (58), the calculation of the isotope effect for the breakage of the C-1 to O-5 bond in glycosides is as follows:

Since

$$\nu_1 = \frac{1}{2} (k)^{\frac{1}{2}} (\mu_1)^{-\frac{1}{2}} = 2.751 \times 10^{13} \text{ sec}^{-1}$$

and

$$\mu_1 = \frac{16 \times 12}{16 + 12} \quad \text{and} \quad \mu_2 = \frac{18 \times 12}{18 + 12}$$

then

$$\frac{1}{2\pi} (k)^{\frac{1}{2}} = \nu_1 (\mu_1)^{\frac{1}{2}} = 7.204 \times 10^{13} \text{ sec}^{-1}$$

and

$$\nu_2 = \frac{1}{2\pi} (k)^{\frac{1}{2}} (\mu_2)^{-\frac{1}{2}} = 2.685 \times 10^{13} \text{ sec}^{-1}$$

From equation 3, the ratio of the breakdown frequencies is:

$$\frac{\nu_1^{\ddagger}}{\nu_2^{\ddagger}} = \frac{1/41 + 1/29}{1/41 + 1/31}^{\frac{1}{2}} = 1.0393$$

$$u_1 = \frac{h\nu_1}{kT} = \frac{6.626 \times 10^{-27} \text{ erg sec } (2.751 \times 10^{13} \text{ sec}^{-1})}{1.38 \times 10^{-16} \text{ erg molecule deg}^{-1} (373°)} = 3.541$$

$$u_2 = \frac{h\nu_2}{kT} = \frac{6.626 \times 10^{-27} \text{ erg sec } (2.685 \times 10^{13} \text{ sec}^{-1})}{1.38 \times 10^{-16} \text{ erg molecule deg}^{-1} (373°)} = 3.456$$

$$(u_1 - u_2) = 0.0850$$

G(u) from the values tabulated by Bigeleisen is 0.24746.

Substituting these values into equation 1:

$$\frac{k_1}{k_2} = 1.0393 \left[1 + 0.24746(0.0850)\right] = 1.061$$

To measure a C-1 to O-5 oxygen isotope effect, samples of methyl α-D-xylopyranoside-5-^{18}O and methyl β-D-xylopyranoside-5-^{18}O were hydrolyzed to 100% completion and to approximately 5% completion as stated. The mass spectrum of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose isolated from each hydrolysate was scanned in the molecular ion region repeatedly (12-29 scans). The peak intensities of m+/e 440 and m+/e 438 were measured from the base to the estimated top of the peak. The measured intensities at m+/e 440 were corrected for contributions by species other than the 5-^{18}O in the ion. The results are tabulated in Tables IV through VII page 47 and 50. The ratios of $^{18}O/^{16}O$ found in the xylose samples isolated from the four different hydrolysis experiments are summarized in Table VIII., page 51. The coefficient of error for each individually determined ratio of $^{18}O/^{16}O$ is also given in Table VIII.

The data does show a slight positive isotope effect of the α-xyloside ($\frac{k_1}{k_2} = 1.01$), but no firm conclusion regarding the possible kinetic isotope effect can be reached due to the large error of the mass spectral measurements of ^{18}O content. The large error is due, in the most part, to the

low relative intensity of the molecular ion exhibited by the trimethylsilyl derivitives of xylose. To achieve measurable values in the m+/e display, large samples must be used and electronic magnification must be quite high. This situation leads not only to variability in the peak ratios, but to "noise" which makes accurate measurement difficult.

More accurate measurement of the $^{18}O/^{16}O$ ratio in the trimethylsilyl derivitives may be possible through the use of chemical ionization mass spectrometry to obtain a more intense molecular ion.

The use of neutron or proton activation analysis as a possible alternative to mass spectrometry was explored in the literature. Whereas techniques have been described for the measurement of ^{18}O in solids by activation to ^{19}O (59), these require relatively large samples sizes and are limited in accuracy to the ± 2% range. A technique has been described (60) for the determination of the ^{18}O content by activation with an accuracy of ± 0.2%, however conversion of the solid to water was required.

The most promising method to achieve the required accuracy is conversion of the carbohydrate to carbon dioxide followed by mass spectral measurement of the $^{18}O/^{16}O$ ratio with an isotope ratio mass spectrometer. Existing

Table IV. Oxygen-18 to Oxygen-16 Ratio in the Mass Spectrum of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose from the 100% Hydrolysis of Methyl β-D-xylopyranoside-5-^{18}O.

	Peak Heights in 1/64"			
Spectrum	m+/e 438	m+/e 440	m+/e 440 (corrected)[a]	$^{18}O/^{16}O$[b]
1	43.0	58.5	49.3	1.15
2	38.5	53.5	45.2	1.17
3	39.0	55.0	46.6	1.19
4	41.0	57.5	48.7	1.19
5	39.5	57.0	48.5	1.23
6	41.0	56.0	47.2	1.15
7	36.5	51.0	43.2	1.18
8	43.0	59.5	50.3	1.17
9	42.0	57.5	48.5	1.15
10	42.0	58.0	49.0	1.17
11	40.0	57.0	48.4	1.21
12	45.5	66.0	56.2	1.24
13	40.0	56.5	47.9	1.20
14	43.0	63.0	53.8	1.25
15	40.5	58.0	49.3	1.22
16	45.5	64.5	54.7	1.20
17	44.0	62.5	53.1	1.21
18	43.0	60.0	50.8	1.18
19	42.5	60.5	51.4	1.21
20	41.0	57.5	48.7	1.19
21	30.5	43.5	37.0	1.21
22	41.0	57.0	48.2	1.18
23	48.0	68.0	57.7	1.20
24	46.0	64.0	54.1	1.18
25	44.5	63.5	54.0	1.21
26	44.5	62.5	53.0	1.19
27	33.5	48.5	41.3	1.23
28	35.0	48.0	40.5	1.16
29	36.0	50.0	42.3	1.18

a. Corrected Value = (m+/e 440) - (0.2143)(m+/e 438)
b. Ratio = corrected value/ (m+/e 438)

Table V. Oxygen-16 to Oxygen-18 Ratio in the Mass Spectrum of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose from the 5% Hydrolysis of Methyl β-D-xylopyranoside-5-^{18}O.

	Peak Heights in 1/64"			
Spectrum	m+/e 438	m+/e 440	m+/e 440 (corrected)[a]	$^{18}O/^{16}O$[b]
1	38.5	54.5	46.2	1.20
2	37.0	53.5	45.6	1.23
3	37.0	53.5	45.6	1.23
4	39.0	56.0	47.6	1.22
5	38.5	56.0	47.8	1.24
6	39.5	54.5	46.0	1.16
7	41.0	58.5	49.4	1.20
8	42.0	57.5	48.5	1.15
9	40.0	50.5	50.9	1.27
10	38.5	55.5	47.2	1.23
11	41.5	57.0	48.1	1.16
12	42.5	60.5	51.4	1.21
13	40.5	61.0	52.3	1.29
14	38.5	53.5	45.2	1.17
15	38.0	54.0	45.9	1.21
16	42.5	59.0	49.9	1.17
17	41.0	57.0	48.2	1.18
18	42.0	60.5	51.5	1.23
19	36.0	48.0	40.3	1.12
20	35.0	52.5	45.3	1.29
21	37.5	49.5	41.6	1.11
22	37.0	52.0	44.1	1.19
23	36.0	51.5	43.8	1.22
24	32.5	44.0	37.0	1.14
25	36.0	51.5	43.8	1.22
26	35.5	52.5	44.9	1.29
27	37.5	51.0	42.1	1.12
28	39.0	54.5	46.1	1.18
29	39.0	55.0	46.6	1.19

a. Corrected Value = (m+/e 440) - (0.2143)(m+/438)
b. Ratio = corrected value/ (m+/e 438)

Table VI. Oxygen-18 to Oxygen-16 Ratio in the Mass Spectrum of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose from the 100% Hydrolysis of Methyl α-D-xylopyranoside-5-^{18}O.

	Peak Heights in 1/64"			
Spectrum	m+/e 438	m+/e 440	m+/e 440 (corrected)[a]	$^{18}O/^{16}O$[b]
1	34	48	40.7	1.20
2	38	54	45.9	1.21
3	37	51	43.1	1.16
4	36	51	43.3	1.20
5	37	51	43.1	1.16
6	37	52	44.1	1.19
7	38	55	46.9	1.23
8	38	54	45.9	1.21
9	39	56	47.6	1.22
10	40	57	48.4	1.21
11	42	59	50.0	1.19
12	42	60	51.0	1.21
13	42	59	50.0	1.19
14	44	61	51.6	1.17
15	44	61	51.6	1.17
16	40	57	48.4	1.21
17	40	57	48.4	1.21
18	41	57	48.2	1.18
19	36	52	44.3	1.23
20	24	34	28.6	1.19
21	25	34	28.6	1.14
22	46	64	54.1	1.18
23	44	63	53.6	1.22

a. Corrected Value = (m+/e 440) - (0.2143)(m+/e 438)
b. Ratio = corrected value/ (m+/e 438)

Table VII. Oxygen-18 to Oxygen-16 Ratio in the Mass Spectrum of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose from the 5% Hydrolysis of Methyl α-D-xylopyranoside-5-^{18}O.

	Peak Heights in 1/64"			
Spectrum	m+/e 440	m+/e 438	m+/e 440 (corrected)[a]	$^{18}O/^{16}O$[b]
1	40.0	56.0	47.4	1.19
2	37.5	50.5	42.5	1.13
3	44.5	62.0	52.5	1.18
4	45.0	64.0	54.4	1.21
5	43.5	62.5	53.2	1.22
6	40.5	54.5	45.8	1.13
7	36.5	49.0	41.2	1.13
8	40.5	57.5	48.8	1.20
9	39.5	52.0	43.5	1.10
10	38.5	54.2	45.9	1.19
11	39.0	56.0	47.6	1.22
12	39.5	57.5	49.0	1.24

a. Corrected Value = (m+/e 440) - (0.2143)(m+/e 438)
b. Ratio = corrected value/ (m+/e 438)

Table VIII. Mean $^{18}O/^{16}O$ Ratios, Standard Deviations, and Coefficients of Error in Xylose Produced by the 100% and 5% Hydrolysis of Methyl α-D-xylopyranoside-5-^{18}O and Methyl β-D-xylopyranoside-5-^{18}O.

Glycoside	$^{18}O/^{16}O$	Standard Deviation	Coefficient Of Error
Methyl β-D-xylopyranoside-5-^{18}O. 100% Hydrolysis	1.19	0.026	2.18%
Methyl β-D-xylopyranoside-5-^{18}O. 5% Hydrolysis	1.20	0.038	3.15%
Methyl α-D-xylopyranoside-5-^{18}O. 100% Hydrolysis	1.19	0.031	2.60%
Methyl α-D-xylopyranoside-5-^{18}O. 5% Hydrolysis	1.18	0.045	3.81%

instruments are capable of error of less than 0.1% even at the natural abundance of ^{18}O, as demonstrated by Bank's measurement of a 3% change in the natural abundance of ^{18}O.

Several methods are available for the conversion of oxygen containing organic compounds to carbon dioxide. These catalytic conversions are of two types, either direct conversion to carbon dioxide, or conversion to carbon monoxide, followed by conversion to carbon dioxide with unenriched oxygen. The indirect conversion of organic compounds to carbon dioxide is carried out by pyrolysis to carbon monoxide over carbon or carbon-platinum, followed by conversion to carbon dioxide by passage through hot iodine pentoxide (61). This method has the advantage that no peak due to the presence of $^{12}C^{18}O^{18}O$ must be accounted for in the calculation of the $^{18}O/^{16}O$ ratio. The method has a serious disadvantage in that the carbon catalyst traps oxygen and may therefore contaminate another sample by either dilution or enrichment of the actual ^{18}O content. This is known as the memory effect and is difficult to overcome.

The direct conversion to carbon dioxide is done by heating the ^{18}O compound with a heavy metal catalyst in a vacuum tube at high temperature (62, 63). These methods

seem to be simpler and suffer from no memory effect. The disadvantage is that the $^{12}C^{18}O^{18}O$ peak must be accounted for and the magnitude of this peak in the mass spectrum will be dependant upon the ^{18}O level of the sample.

Completion of the study of the possible oxygen isotope effects during xyloside hydrolysis will require the establishment of a precise method for the evaluation of the $^{18}O/^{16}O$ ratio in small amounts of xylose. A study of other possible techniques for the separation of small amounts of xylose from the unreacted xylopyranoside after partial hydrolysis may be necessary, although removal of the trimethylsilyl groups from carbohydrates has been demonstrated (64) and may allow use of the existing separation technique. Also the dilution of the existing labeled xylosides with isotopically normal xylosides to increase the sample size available for hydrolysis may be possible. Conversion of the xylose to carbon dioxide would result in a 1 to 5 dilution of the O-5 ^{18}O, however even 10% abundances of ^{18}O in the carbon dioxide may be more than sufficient for the determination of an isotope effect.

It is hoped that continued investigation along these lines can bring a conclusion to the study begun here by the preparation of the O-5 labeled xylopyranosides.

MATERIALS AND METHODS

General:

Solutions were evaporated at 40° under water aspirator vacuum in a rotary evaporator. Thin-layer plates were coated with silica gel G (E. Merck Ag., Darmstadt, Germany); developing solvents are quoted in parenthesis. Components were visualized by spraying with fifty percent sulfuric acid, followed by oven heating at 130°. Gas-liquid chromatography of trimethylsilyl derivatives was done on a Hewlett-Packard Model 5750 gas chromatograph equipped with a 1/8" x 9' column packed with 3% SE-52 on Anachrom SD (90-100 mesh). Column temperature was 155° and the carrier gas (nitrogen) flow rate was 25 ml/min. Gas liquid chromatography of the triacetate derivatives was also done on the Hewlett-Packard Model 5750, equipped with a 1/8" x 9' stainless steel column packed with 3% ENCSS-M on Gaschrom Q (100-120 mesh). Preparative scale gas-liquid chromatography of trimethylsilyl derivatives was done on an Aerograph Model A-90-P (Wilkens Instrument) equipped with a 1/4" x 8' stainless steel column, packed with 3% SE-52 on Anachrom SD (90-100 mesh). The column was operated at 185° and the carrier gas (helium) flow rate was 25 ml/min. Melting points were determined with a Fisher-Johns melting point apparatus. Spectrotometric readings were taken on a

Beckman Model DU spectrophotometer. Mass spectra were run on a M.S. 9 double focusing, high resolution, mass spectrometer (Model M.S. 902, Associated Electrical Industries, Ltd., Urmstrom, Manchester, England).

Solvents:

Solvents, unless otherwise noted, were prepared as follows:

Tetrahydrofuran was refluxed over calcium hydride for 36 hours, distilled off and stored over calcium hydride. Tetrahydrofuran was passed through a column (2cm x 26 cm) of Alumina (Brockman Activity One) immediately before use.

Benzene was refluxed over sodium for 36 hours, fractionally distilled, and stored over 4Å molecular sieves.

Trimethylamine was stored over calcium hydride in the cold. The liquid was allowed to come to room temperature in a sealed flask and used as a gas.

Methanol was refluxed over magnesium turnings, fractionally distilled and stored over 4Å molecular sieves.

Absolute ethanol (99%) was stored over 4Å molecular sieves and used directly.

Petroleum ether was fractionally distilled, the 38° - 45° fraction was collected and used.

Dichloromethane was washed twice with sulfuric acid, followed by three water washes. The slight yellow color

was removed by two washes each with 5% sodium thiosulfate and 5% sodium hydroxide. The salts were removed with three more water washes and the solvent was pre-dried over calcium chloride, distilled from phosphorous pentoxide and stored over 4Å molecular sieves.

Acetone was refluxed over potassium permanganate for 6 hours, distilled off and dried over anhydrous sodium sulfate.

Pyridine was fractionally distilled from potassium hydroxide pellets and stored over potassium hydroxide pellets.

N-methyl pyrrolidone and trimethylamine were used as received from Eastman Organic Chemicals, Rochester, N. Y.

Acetonitrile, diethyl ether and chloroform were used as received from Mallinckrodt Chemical Works, St. Louis, Mo.

N-N-dimethylformamide was used as received from J. T. Baker Chemical Company, Phillipsburg, Pa.

Replacement Reactions at C-5 of Various Derivatives of 1,2-O-Isopropylidene-α-D-xylofuranose

Initial label attempts were made by replacement reactions at C-5 of i) 1,2-O-isopropylidene-5-O-p-tolylsufonyl-α-D-xylofuranose ii) 1,2-O-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose and iii) 1,2-O-isopropylidene-3-O-benzyl-5-deoxy-5-iodo-α-D-xylofuranose.

1,2-O-Isopropylidene-5-O-p-tolylsulfonyl- -D-xylofuranose was prepared by the method of Lavene and Raymond (31). Tetra-n-butylammonium acetate for use in the replacement reaction was prepared by mixing tetra-n-butylammonium bromide, (5.0 g, 0.0155 mol, Eastman Organic Chemicals, Rochester, N. Y.) in 25 ml of ethanol with potassium acetate, (1.53 g, 0.0155 mol) in 50 ml of ethanol. The resultant precipitate was filtered off and the solvent was evaporated. The remaining colorless solid was dried under vacuum, over phosphorous pentoxide. The replacement reaction was attempted by holding 1,2-O-isopropylidene-5-O-p-tolylsulfonyl-α-D-xylofuranose, (0.36 g, 0.001 mol) and tetra-n-butylammonium acetate, (0.298 g, 0.0012 mol) in 5 ml of N-methyl pyrrolidone at 60° for 4 hours. Thin-layer chromatography (TLC) (90:100, ether: pet. ether) of the reaction mixture showed only the starting material and a small amount of tailing from the origin.

The reaction was attempted under identical conditions at 150°. TLC (90:100 ether:pet. ether) showed a fast running component corresponding to the starting material, a very light intensity lower running component, and extensive tailing from the origin.

In order to provide a better leaving group at the C-5 position, 1,2-O-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose was made by refluxing 1,2-O-isopropylidene-5-O-p-tolysulfonyl-α-D-xylofuranose (2.0 g, 0.0056 mol) with a sodium iodide

(2.5 g, 0.0167 mol) in 50 ml of acetone for 36 hours. The sodium tosylate produced was filtered off, and the acetone was evaporated. The residue was dissolved in chloroform and washed twice with water (15 ml). The chloroform layer was dried over anhydrous sodium sulfate, filtered and evaporated. The residue was dissolved in diethyl ether with warming, a small amount of petroleum ether was added and crystallization began upon standing in the cold. Yield, 1.02 g, 0.0032 mol, Melting point 136°-138°, literature value 138° (32).

Replacement of the 5-iodo group by hydroxide ion was attempted by refluxing 1,2-O-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose, (20 mg, 0.063 mol) with sodium hydroxide, (10 mg, 0.003 mol) and water, 10 ul, in 3 ml of acetonitrile for 2 hours. TLC (70:30 ether:pet. ether) showed two components, one corresponding to the starting material and one of greater mobility. Similar results were observed when the reaction was attempted using triethylamine (1 ml) in place of sodium hydroxide.

In further attempts at replacement at the C-5 position, 3-O-benzyl-1,2-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose was prepared. 1,2-O-Isopropylidene-5-O-p-tolylsulfonyl-α-D-xylofuranose (5.0 g, 0.0139 mol), 1-bromotoluene (6.47 g, 0.0378 mol, Eastman Organic Chemicals, Rochester, N.Y.) and silver oxide, prepared

by the method of Yale (33) (5.0 g, 0.040 mol) were stirred in 20 ml of N,N-dimethylformamide (DMF) for 5 hours at 60°. TLC (70:30 ether:pet. ether) showed the production of a faster running component and a very small amount of a component of corresponding mobility to the starting material. Silver oxide was filtered off and the excess 1-bromotoluene and DMF were removed by vacuum distillation at 70°. The heavy reddish brown oil which resulted gave a white precipitate upon addition of chloroform. The precipitate was filtered and the chloroform solution (50 ml) was washed twice with water (50 ml), twice with 0.1N sodium cyanide (50 ml), and the four times with water (total volume 250 ml). The solution was dried over anhydrous sodium sulfate, filtered and the chloroform was evaporated.

The oily 1,2-O-isopropylidene-3-O-benzyl-5-O-p-tolylsulfonyl-α-D-xylofuranose, described above, was dissolved in 25 ml of acetone and refluxed with sodium iodide, (5.0 g, 0.0335 mol) for 48 hours. The sodium tosylate produced was filtered off, the acetone was evaporated and the residue was redissolved in chloroform. The remaining insoluble sodium iodide was filtered and the chloroform solution was washed with water (50 ml), twice with 0.2N sodium thiosulfate, and finally with five portions of water, (total volume 250 ml). The chloroform solution was dried over anhydrous sodium

sulfate, evaporated to a thin syrup and chromatographed on a 400 gm of silica gel in a 7.5 cm x 69 cm column 60-200 mesh, Davison Chemical Company, Baltimore, Md.) Eluant fractions containing the 1,2-O-isopropylidene-3-O-benzyl-5-deoxy-5-iodo-α-D-xylofuranose were pooled, evaporated to a thick syrup and redissolved in petroleum ether with warming. Large crystals formed upon standing. Melting point 67°, literature 67° (32), yield 1.50 g (0.0061 mol).

Replacement of the 5-iodo group in the 3-O-benzyl derivative was attempted by refluxing 1,2-O-isopropylidene-3-O-benzyl-5-deoxy-5-iodo-α-D-xylofuranose (10 mg, 0.002 mmol) in acetonitrile (1 ml) containing water (5 1, 0.0277 mmol) and triethylamine (0.5 ml). TLC (50:50 ether:pet. ether) showed only one component of identical mobility to the starting material after two hours of refluxing.

Removal of the 5-iodo group in either the 3-hydroxyl or the 3-O-benzyl-1,2-O-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose was however, possible by silver nitrate catalysis. Refluxing 1,2-O-isopropylidene-5-deoxy-5-iodo-α-D-xylofuranose (1.0 g, 0.0059 mol) with silver nitrate (1.0 g, 0.0059 mol) in a mixture of water (0.05 g, 0.00283 mol), acetonitrile (2 ml) and pyridine (2 ml) for 45 minutes gave a product of much slower mobility in TLC (70:30 ether:pet. ether) than the starting material.

The yellow precipitate produced was filtered off, and the solvents were evaporated. The residue was redissolved in chloroform (10 ml) and refiltered. The chloroform solution was washed with 20 ml portions of water until the aqueous layer was no longer cloudy. The solution was washed with three portions of 0.2N sodium thiosulfate (10 ml), followed by three washes with 0.1N sodium cyanide (10 ml). The chloroform layer was washed four additional times with water (total volume 100 ml), then dried over anhydrous sodium sulfate. TLC (ethylacetate) showed the mobility of the product (assumed to be 1,2-O-isopropylidene-4,5-dideoxy-α-D-threopent-4-enofuranose) to be intermediate to that of the starting material and that of 1,2-O-isopropylidene-α-D-xylofuranose.

Oxygen Exchange at the 5-Aldehyde of Bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose-3.5'-5,5' cyclic acetal (II)

Bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)-3,5'-5,5' cyclic acetal (II) was prepared by the periodate oxidation of 1,2-O-isopropylidene-α-D-glucofuranose (I) which was prepared by the method of Mehltretter et al. (34). Compound (I) (22.5g, 0.101 mol) was reacted with sodium periodate (22.0 g, 0.103 mol) in 150 ml of 5% sodium hydrogen carbonate. The solution was stirred for 2 hours,

then extracted five times with chloroform (total volume 1500 ml). The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered and concentrated to a thick syrup under vacuum. The slight yellow color was removed by decolorization with Neutral Norit (5 g J. T. Baker Chemical Co.) in methanol. The methanol was removed and the product was dehydrated by three successive dissolutions and evaporations from benzene.

Alternately the cyclic acetal (II) was made by the method of Isbell and co-workers (35) with the following modifications. A part of the formaldehyde, which remains tightly bound in the syrupy (II), was removed by refluxing a 10% solution of syrupy (II) in benzene for 10 hours. The last traces of formaldhyde were removed by crystallization from water. The (II) was further dried by allowing a 10% solution of the hydrated crystals (Melting point 175°-178°) in ethanol to stand over 4Å molecular sieves for 36 hours. The sieves were then filtered off and the ethanol removed under pump vacuum. The residue was again dissolved in benzene and the benzene was evaporated under pump vacuum. Upon dissolution again in benzene and concentration to approximately 50% of the original volume, crystallization began. Concentration was stopped, and the solution was allowed to crystallize for 4 hours at room temperature. The crystals were filtered off and dried over

phosphorous pentoxide under vacuum, Melting Point 180°, literature 182°-184° (35).

Oxygen-16/oxygen-18 exchange at the potential C-5 aldehyde was done in an initial experiment by refluxing a syrupy sample of bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)3,5'-5,5' cyclic acetal (II), (2.569 g, 0.0136 mol) with water enriched to 20 atom % ^{18}O (Bio-Rad Laboratories, Richmond, California) (0.308 g, 0.0155 mol) in diethyl ether (65 ml). Dry ammonia was bubbled through the solution for 30 minutes and the solution was refluxed for 10 hours under protection from atmospheric moisture by use of a drying tube. The ether was removed under vacuum and the syrup dehydrated by three successive dissolutions and evaporations from dry benzene. TLC (90:100 Dichloromethane:methanol) showed two components corresponding in mobility to a reference standard of the starting material, and three light intensity, slower running components.

In later exchange reactions using 95 atom % ^{18}O enriched water (Bio-Rad Laboratories), the crystalline dimer (II), (2.19 g, 0.0116 mol), was refluxed with ^{18}O enriched water (0.500 g, 0.0251 mol) in tetrahydrofuran (60 ml), which had previously been saturated with trimethylamine. The solution was refluxed under protection from atmospheric moisture for 9 hours. The tetrahydrofuran was evaporated under vacuum, the residue was dissolved in dry benzene (20 ml), and the

benzene was evaporated under pump vacuum. The residue was further dehydrated by another dissolution and evaporation from benzene (20 ml). TLC in the same solvent system, showed only the two components corresponding in mobility to the starting material (II).

Reduction of the Potential 5-Aldehydo Group of (II) and Methanolysis of 1,2-O-Isopropylidene-α-D-xylofuranose 5-O^{18} (III)

Bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)-3,5'-5,5'-O^{18} (II) (2.19 g, 0.0116 mol) from the exchange reaction was dissolved in tetrahydrofuran (20 ml) and added dropwise to lithium aluminum hydride (0.41 g, 0.0116 mol) in tetrahydrofuran (15 ml). The solution was stirred and refluxed for an additional 20 minutes after all the reactants had been added. Water, (2 ml), was added dropwise and the mixture was stirred under reflux until the precipitate was completely white. The precipitate was filtered and washed with additional tetrahydrofuran. The solvent was evaporated and the resultant syrup was dried by four successive dissolutions and evaporations from ethanol. TLC (90:100 dichloromethane:methanol) showed one component of identical mobility with known 1,2-O-isopropylidene-α-D-xylofuranose (III). Yield 1.942 g (0.0102 mol), 88.2%.

The labeled 1,2-O-isopropylidene-α-D-xylofuranose (III) from the reduction was repeatedly dissolved in and evaporated from methanol, to remove the last traces of ethanol, before solvolysis in methanol to produce the equilibrium mixture of methyl-α-D-xylopyranoside 5-O^{18} (IV) and methyl β-D-xylopyranoside 5-O^{18} (V). The product (III) from the final evaporation was dissolved in methanol (20 ml), and the solution was added to methanol (20 ml) which contained acetylchloride (0.2 ml). The solution was refluxed for 8 hours under protection from atmospheric moisture, then concentrated to a thin syrup, redissolved in methanol, and again concentrated. Dowex AG-1x8 ($^-$OH form) was added with water (15 ml) and the slurry was stirred for 30 minutes. The resin was filtered off and washed with water. The aqueous filtrate containing (IV) and (V) was evaporated to dryness and further dehydrated by four successive dissolutions and evaporations from ethanol. TLC (85:15 ethylacetate: methanol) showed one component of identical mobility with a known equilibrium mixture of (IV) and (V). Yield, 1.546 g, (0.0094 mol) 92.1%.

Separation of Methyl α-D-xylopyranoside-5-^{18}O and Methyl β-D-xylopyranoside-5-^{18}O

Separation of the anomeric methyl D-xylosides was attempted by four methods; i) Enzymolysis of the anomer, ii) resolution on strongly basic ion exchange resin, iii)

anomerization of the mixture to predominantly the α anomer and iv) fractional crystallization of the anomeric 2,4-O-phenylboronate esters.

Removal of the methyl β-D-xylopyranoside from the equilibrium mixture of the α and β anomers by enzymolysis to xylose was attempted as a means of obtaining the pure α anomer. The equilibrium mixture of methyl α-D-xylopyranoside and methyl β-D-xylopyranoside obtained from the methanolysis of xylose (2.0 gm, 0.0128 mol) was dissolved in 0.1M acetate buffer pH 5.1. Ten mg of almond emulsin (Nutritional Biochemicals, Cleveland, Ohio) was added and the solution was kept at 37° for 24 hours. No reducing sugar could be detected using dinitro salicic acid reagent (DNS) (36).

Resolution of the anomers was attempted by column chromatography on strongly basic anion exchange resin. Three hundred ml of AG 1x8 anion exchange resin (Bio-Rad Laboratories, Richmond, California) in the chloride form was treated by slurring in 1N sodium hydroxide (250 ml), then filtered. The resin was washed with deionized water (6 liters), packed in a column (2.5 cm x 70 cm) and washed with additional deionized water until the pH of the effluent reached 8.2 (6 liters). Methyl α-D-xylopyranoside and methyl β-D-xylopyranoside were applied to the column in water (5 ml). The column was eluted with water at a flow rate of 0.5 ml/min. The α anomer eluted first but was extensively overlapped with the β anomer.

Anomerization of the 2,3,4-tri-O-acetate derivatives made from the equilibrium mixture of methyl α-D-xylopyranoside and methyl β-D-xylopyranoside was used as a method of obtaining methyl α-D-xylopyranoside. The equilibrium mixture of methyl α and β-D-xylopyranosides (1.18 g, 0.0084 mol) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml), and the mixture was allowed to stand at room temperature for 10 hours. Water (15 ml) was added and the solution was stirred for 30 minutes, then extracted five times with chloroform (total volume 30 ml). The combined chloroform extracts were washed twice with water (10 ml), three times with 1N sulfuric acid (15 ml), three times with aqueous 7% sodium hydrogen carbonate (15 ml), and twice again with water (10 ml). The chloroform layer was evaporated to a thick syrup and further dried by four successive dissolution and evaporations from benzene. TLC (di-isopropyl ether, developed twice) shows two components, the component of higher mobility corresponded to a standard of methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside and the slower running component corresponded to the β anomer. Yield 1.97 g (0.0068 mol) 80.8%.

Titanium tetrachloride catalyzed anomerization was done by dissolving the mixed triacetate derivatives of the methyl α and β-D-xylopyranosides (2.0 g, 0.007 mol) in

dichloromethane (15 ml). Titanium tetrachloride (1.71 g, 0.009 mol) was added, and the solution was refluxed for 1 hour. The solution was added to cold water (20 ml), the dichloromethane layer was separated, washed with water (20 ml), 7% sodium hydrogen carbonate (20 ml), washed three additional times with water (total volume 30 ml) and dried over anhydrous sodium sulfate. Gas-liquid chromatography of a chloroform solution of the resulting syrup showed two peaks, corresponding to the α and β anomers in an approximate ratio of 85/15.

Anomerization catalyzed by boron triflouride was carried out by the following procedure. The anomeric mixture of the xyloside triacetates (1.23 g, 0.0043 mol) was dissolved in dichloromethane (15 ml) and the solution was added to a flask which had been flushed with nitrogen. Boron triflouride:etherate (2 ml, previously distilled from calcium hydride under vacuum) was added and the solution was refluxed for 2 hours. The darkened solution was cooled, washed twice with cold water (10 ml), three times with aqueous 7% sodium hydrogen carbonate (15 ml) and twice with water (10 ml). The dichloromethane was removed under vacuum and the resultant syrup was decolorized in methanol (20 ml) with Neutral Norit (1 gm). Several drops of water were added to the clear solution and

large crystals formed upon standing in the cold. Methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside was isolated and had a melting point of 82°, literature 86° (37). Yield 260 mg (0.897 mmol) 20.9%.

The methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside produced by anomerization and isolated by fractional crystallization of the equilibrium mixture (0.26 g, 0.009 mmol) was deacetylated in methanol (7 ml) with 1N sodium methoxide in methanol (0.5 ml). After standing for 1 hour the solution was passed through a column (1 cm x 10 cm) of Amberlite MB-3 (H^+, OH^- form) ion exchange resin. The methanol was removed under vacuum and the syrup was redissolved in 1N sodium hydroxide, stirred at 100° for 1 hour, cooled and again passed through an Amberlite MB-3 column (H^+, OH^- 2 cm x 10 cm). The water was removed under vacuum and the syrup dried by repeated dissolution and evaporation from ethanol. The product (methyl α-D-xylopyranoside) crystallized from ethanol upon standing in the cold. Melting point, 89° Literature 92° (37). Yield 140 mg (0.086 mmol) 94%.

The best method of isolating pure methyl α-D-xylopyranoside-5-^{18}O (IV) from the anomeric mixture of (IV) and (V), and at the same time obtaining ^{18}O labeled (V) in pure form involved the use of Ferrier's method for the complete resolution of the four methyl D-xylosides (38).

The steps in this method include i) preparation of the 2,4-phenyl boronate esters of the mixed xylosides (IV and V), ii) fractional crystallization of methyl α-D-xylopyranoside-5-^{18}O, 2,4 phenyl boronate, iii) de-esterification of the separated boronate derivatives, and iv) crystallization of the free xylopyranosides (IV) and (V).

Prior to formation of the 2,4 phenyl boronate esters of (IV) and (V), part of the methyl β-D-xylopyranoside-5-^{18}O, (V) was removed by fractional crystallization. The equilibrium mixture of (IV) and (V) (1.546 g 9.40 mmol), was dissolved in ethanol (10 ml) with warming and ethyl acetate (5 ml) was added. Crystalline (V) deposited upon standing in the cold. The crystals of methyl β-D-xylopyranoside-5-^{18}O were isolated, washed with cold ethanol, and recrystallized from ethanol. Yield, 164 mg (1.00 mmol); Melting Point 153°-156°, literature 156° (37).

The mother liquors from both crystallizations were combined and the solvent was evaporated. The residue, (1.381 g 8.42 mmol) was redissolved in ethanol, glass beads were added to the solution, and the ethanol was evaporated. Phenyl-boric acid (Aldrich Chemical Company Inc., Milwaukee, Wisconsin, 1.345 g, 0.011 mol) was added and the mixture was stirred under reflux in benzene with continual removal of the water produced. After 4 hours, no additional water production could be detected, and no insoluble material remained.

Refluxing was stopped, the glass beads and stirrer removed, and the solution concentrated to approximately 15 ml. Crystalline methyl 2,4-O-phenylboronly-α-D-xylopyranoside-5-^{18}O formed upon cooling. The crystals (688 mg) were filtered off, and further concentration of the mother liquor, with addition of a small amount of petroleum ether, gave additional crystalline (VI) (302 mg) upon standing in the cold. Recrystallization of the combined (VI) from benzene gave 962 mg, (3.40 mmol), yield 59.0% Melting Point 172°-175°, literature 175°-176° (38).

The crystalline (VI) was de-esterified by dissolution in acetone (5 ml) and slurrying with water (10 ml) and Dowex AG-1 x 8 anion exchange resin ($^-$OH form) for 1 hour. The solution was then passed through a column (1 cm x 10 cm) of Amberlite MB-3 mixed bed ion exchange resin (H^+, $^-$OH form), and washed with water (20 ml). The water was evaporated and the residue redissolved in ethanol. The solution did not contain phenylboric acid as shown by no UV absorbance at 260 nm (ethanol blank). The ethanol was evaporated and the syrupy (IV) was further dried by three dissolutions and evaporations from ethanol. Crystalline (IV) deposited upon dissolution in ethanol (5 ml) and standing in the cold. Recrystallization from ethanol (7 ml) gave 244 mg of methyl α-D-xylopyranoside-5-^{18}O (1.49 mmol) yield, 43.8%, Melting Point 88°-91°, literature 92° (36).

The mother liquor obtained from the fractional crystallization of α-D-xylopyranoside-5-^{18}O (VI) was evaporated to a thick syrup, redissolved in acetone (10 ml), water (10 ml) added, and the solution slurried with AG-1 x 8 anion exchange resin (^{-}OH form) at 55° for 1 hour. The solution was passed through an MB-3 column (2 cm x 10 cm, ^{+}H, ^{-}OH form), the column was washed with water (30 ml) and the column effluent was treated as described for (IV). Methyl β-D-xylopyranoside-5-^{18}O (V) crystallized upon dissolution of the syrup in ethanol (5 ml) and seeding with (V). 112 mg (0.68 mmol) yield 37.8%, Melting Point 151°-152°. The combined yield of (V) was then 61.97%.

Hydrolysis of Methyl α-D-xylopyranoside and Methyl β-D-xylopyranoside

The rate of hydrolysis of methyl α and β-D-xylopyranoside in 1.01N sulfuric acid at 100° was measured by determining the rate of appearance of xylose using dinitrosalicic acid reagent (36). A standard curve was prepared as follows. Aliquots (1 ml) of 1.01N sulfuric acid solutions containing 0.3, 0.4, 0.6 and 0.8 mg/ml of xylose were added to 0.3N sodium hydroxide (4 ml). Aliquots (2 ml) of the resulting solutions were added to DNS reagent (2 ml) and the color was developed by heating in a boiling water bath for 10 minutes. Absorbance at 540 nm was read. Solutions were blanked against 1.01N sulfuric acid (1 ml) which had been treated in the same way.

To measure the rate of hydrolysis, a 0.1M solution (10 ml) of methyl α or β-D-xylopyranoside was brought to temperature in a boiling water bath and added to a preheated solution (100°) of 2.02N sulfuric acid (10 ml). One ml aliquots were taken at 30 second intervals in the case of methyl α-D-xylopyranoside and at 15 second intervals in the case of the βanomer. The aliquots were immediately added to 0.3N sodium hydroxide (4 ml). The color was developed using 2 ml of the resulting solution and DNS reagent (2 ml). The reciprocal of the moles of xylopyranoside remaining was plotted against time, and the time required for the production of 0.5 mmole (5%) of xylose was noted for each anomeric xylopyranoside.

Five percent hydrolysis of the xylosides was accomplished by adding a 0.1M aqueous solution of either xyloside (3.05 ml), at boiling water bath temperature, to a solution of 2.02N sulfuric acid (3.05 ml) also at boiling water temperature. The resulting solution was held at the water bath temperature for 45 seconds in the case of methyl β-D-xylopyranoside, and 85 seconds in the case of methyl α-D-xylopyranoside. The hydrolysate was then poured into ice water (10 ml) and neutralized with barium carbonate. The barium sulfate and excess barium carbonate were filtered off and the filtrate was evaporated to dryness.

To achieve 100% hydrolysis, a sample of xylopyranoside (10 mg, 0.061 mmol) was dissolved in 1.01N sulfuric acid (1.22 ml), and the solution was held under reflux at boiling water bath temperature for 1 hour.

The solution was neutralized with barium carbonate, filtered, and the filtrate evaporated to dryness.

Formation of Trimethylsilyl Derivatives

Formation of trimethylsilyl ether derivatives of compounds was done by the method of Sweely et al. (39) with one modification. After the silylation reaction was complete, to the pyridine solution of the pertrimethylsilylated compounds was added enough water to completely dissolve the white precipitate. The resulting mixture in which the pertrimethylsilylated compounds form a separate layer was extracted twice with petroleum ether. The petroleum ether solution was concentrated under a stream of nitrogen before injection into a gas-chromatograph.

Trimethylsilyl ether derivatives of compounds, prior to injection into the mass spectrometer, were first purified by preparative gas-liquid chromatography.

Separation of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranoside from Methyl 2,3,4-tris-O-(trimethylsilyl)-α or β-D-xylopyranoside

The 95/5 mixture of xyloside/xylose produced during partial acid hydrolysis was trimethylsilylated and worked up

as stated. Seven to 10 ul of the resultant oil were injected into a gas-chromatograph and the slowest moving peak, corresponding to 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose, was captured in a U-tube immersed in a dry ice-acetone slurry at the outlet of the thermal conductivity detector.

Mass Spectrometry

Pertrimethylsilylated derivatives were introduced into the mass spectrometer by direct injection. Sample inlet temperature for 3,5-bis-O-(trimethylsilyl)-1,2-O-isopropylidene-α-D-xylofuranose was 120° and the electron beam energy was 70 e.v. Sample inlet temperature for 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose was 200° and the electron beam energy was 17 e.v.

SUMMARY

To add to our understanding of the mechanism by which pyranosides undergo acid-catalyzed hydrolysis, attempts were made to measure an isotope effect for the breakage of the C-1 to O-5 bond in pyranosides during acid catalyzed hydrolysis. The demonstration of an isotope effect associated with this bond during acid catalyzed hydrolysis would give proof of the existence of an open-ring intermediate in the hydrolysis mechanism. Conversely, the absence of an isotope effect associated with the bond, would make more convincing the evidence for the existence of a cyclic intermediate.

Measurement of a possible oxygen isotope effect associated with the C-1, O-5 bond requires enrichment of the oxygen at the 5 position of a pyranoside to near 50% ^{18}O for maximum accuracy. First attempts at formulation of an enrichment scheme involved nucleophilic replacement at C-5 of derivatives of 1,2-O-isopropylidene-α-D-xylofuranose. Numerous complications were encountered with this approach. The close proximity of the 3-hydroxyl group to the C-5 position greatly complicates attempts at S_n2 replacement at the C-5 position.

The label incorporation scheme which finally proved successful was $^{18}O/^{16}O$ exchange at the potential 5-aldehyde group of bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)-3,5'-5,5' cyclic acetal. Oxygen exchange at the 5-aldehyde with 95 atom% ^{18}O enriched water and trimethylammine catalysis

gave an exchange efficiency of 83.8% and after reduction of the 5-aldehyde, 1,2-O-isopropylidene-α-D-xylofuranose 5-^{18}O labeled to 54.5% ^{18}O at the 0-5 position was isolated in good yield. Mass spectral analysis of 1,2-O-isopropylidene-3,5, bis-O-(trimethylsilyl)-α-D-xylofuranose was consistent with label at the 5 position.

Separation of the pyranosides from the anomeric mixture of methyl D-xylosides produced by methanolysis of 1,2-O-isopropylidene-α-D-xylofuranose-5-^{18}O was attempted by selective enzymolysis, column chromatography and anomerization to the α-anomer. Only the later technique gave positive results. While anomerization was possible as a route to the pure α-xylopyranoside, the most successful separation method which gave the α and β xylopyranosides in 43.8% and 62.0% yields respectively, involved fractional crystallization of the cyclic 2,4-phenylboronate ester of methyl α-D-xylopyranoside-5-^{18}O. The anomerically homogeneous 2,4-phenylboronate esters were de-esterified, and methyl α and β-D-xylopyranosides-5-^{18}O, (IV) and (V) were crystallized in pure form.

In the final enrichment reaction scheme, the methyl α and β-D-xylopyranosides were obtained in overall yields of 19.8% and 48.8%, enriched to 54.5 atom% ^{18}O at 0-5.

Measurement of a possible oxygen isotope effect associated with the cleavage of the C-1 and 0-5 bond during the hydrolysis of pyranosides requires the following experiments; i) partial hydrolysis, ii) separation of xylose from a 95/5 mixture of

xyloside/xylose, and iii) determination of the $^{18}O/^{16}O$ ratio in the xylose. Separation of part of the xylose from the hydrolysis was accomplished by trimethylsilylation of the partial hydrolysate followed by isolation of pure 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose by preparative gas-liquid chromatography.

Measurement of the $^{18}O/^{16}O$ ratio in D-xylose released during hydrolysis was accomplished by examining the mass spectrum of the pertrimethylsilylated D-xylose-5-^{18}O (50 atom%). Specifically the ratio of the two molecular ions containing either ^{16}O or ^{18}O at the 0-5 position was measured. This technique the ^{18}O content with a precision of, at best only 2.18%, which was too large an error to permit the use of this approach to measure a kinetic isotope effect in the cleavage of the methyl D-xylopyranosides.

BIBLIOGRAPHY

1. Capon, B., Mechanism in Carbohydrate Chemistry Chem. Reviews 69 407 (1969).

2. BeMiller, J. N., Glycoside Hydrolysis Adv. Carbchydrate Chem. 15 11 (1960).

3. Capon, B., and Overend, W. G., Constitution and Physicochemical Properties of Carbohydrates Adv. Carbohydrate Chem. 15 11 (1958).

4. Shafizadeh, F., Formation and Cleavage of the Oxygen Ring in Sugars Adv. Carbohydrate Chem. 13 9 (1958).

5. Overend, W. G., In: The Carbohydrates, Chemistry and Biochemistry, Eds., Pigman, W. W., and Horton, D., Academic Press, New York, 1970 p. 316.

6. Bronsted, J. N., and Grove, C., The Kinetic Determination of Hydrogen-Ion Concentrations in Aqueous Solution J. Am. Chem. Soc. 52 1394 (1930).

7. Ingold, C. K., In: Structure and Mechanism in Organic Chemistry Cornell University Press, Ithica, New York, (1953) p. 153.

8. Skrabal, A., and Eger, H. H., Die Hydrolysegeschwindigkeit der Einfacsten Formale Z. Physik. Chem. 349 (1926).

9. O'Gorman, J. M., and Lucas, H. J., Hydrolysis of the Acetal of D(+)-2-Octanol J. Am. Chem. Soc. 72 5489 (1950).

10. Hammett, L. P., and Paul, M. A., The Relation Between the Rates of Some Acid Catalyzed Reactions and the Acidity Function, Ho J. Am. Chem. Soc. 56 830 (1934).

11. McIntyre, D., and Long, F. A., Acid-Catalyzed Hydrolysis of Methal. I. Influence of Strong Acids and Correlation With the Hammett Acidity Function. J. Am. Chem. Soc. 76 3240 (1954).

12. Orr, W. J. C., and Butler, J. A. V., The Kinetic and Thermodynamic Activity of Protons and Deuterons in Water-Deuterium Oxide Solutions J. Chem. Soc. 330 (1937).

13. Bunton, C. A., and Shiner, V. J., Jr., Isotope Effects in Deuterium Oxide Solution. Part II. Reaction Rates in Acid, Alkaline and Neutral Solution, Involving only Secondary Solvent Effects J. Am. Chem. Soc. 83 3208 (1961).

14. Kilpatrick, M., Kinetics of Hydrolysis of Acetals in Protium and Deuterium Oxides. J. Am. Chem. Soc. 85 1036 (1963).

15. Long, F. A., Pritchard, J. G., and Stafford, F. E., Entropies of Activation and Mechanism for the Acid-Catalyzed Hydrolysis of Ethylene Oxide and Its Derivatives J. Am. Chem. Soc. 79 2362 (1957).

16. Schalger, L. L., and Long, F. A., Entropies of Activation and Mechanisms of Reactions in Solution Adv. Phys. Organic Chem. Vol. I 47 (1963).

17. Overend, W. G., Rees, C. W., and Sequeira, J. S., Reactions at Position 1 of Carbohydrates. Part III The Acid-Catalyzed Hydrolysis of Glycosides J. Chem. Soc. 1456 (1964).

18. Timell, T. E., Acid Catalyzed Hydrolysis of Pyranosides: General Conditions and the Effect of the Nature of the Aglycone Can. J. Chem. 42 1456 (1964).

19. Semke, L. K., Thompson, N. S., and Williams, D. G., The Rates of Acid Hydrolysis of the Phenyl β-D-Glucopyranosiduronic Acids and Phenyl β-D-Glucopyranosides of Phenol, p-Cresol and p-Chlorophenol J. Org. Chem. 29 1360 (1959).

20. Koskikallio, J., Pouli, D., and Walley, E., Pressure Effect and Mechanism in Acid Hydrolysis Can. J. Chem. 37 1360 (1964).

21. Bunnett, J. F., Kinetics of Reactions in Moderately Concentrated Aqueous Acids. I. Classifications of Reactions J. Am. Chem. Soc. 82 499 (1960).

22. Walley, E., Pressure Effect and Mechanism in Acid Catalysis Trans. Faraday Soc. 55 798 (1959).

23. Withey, R. J., and Walley, E., Pressure Effect and Mechanism In Acid Catalysis XIII. Hydrolysis of Methyl -D-Glucopyranoside Can. J. Chem. 41 1849 (1963).

24. Armour, C., Bunton, C. D., Patai, S., Selman, L. H., and Vernon, C. A., Mechanisms of Reactions in the Sugar Series. Part III. The Acid-Catalyzed Hydrolysis of t-Butyl β-D-Glucopyranoside and other Glycosides J. Chem. Soc. 412 (1961).

25. Banks, B. E., C., Meinwald, Y., Rhind-Tutt, A. J., Sheft, I., and Vernon C. A., Mechanisms of Reaction in the Sugar Series. Part III. The Structure of the Carbonium Ions Formed in the Acid Catalyzed Solvolysis of Glucopyranosides J. Chem. Soc. 3240 (1961).

26. Bumford, C., Capon, B., and Overend, W. G., Reactions at Position 1 of Carbohydrates. Part IV. The Kinetics and Mechanism of the Acid-Catalyzed Hydrolysis of Ethyl and Phenyl 1-Thio-β-D-Glucopyranosides J. Chem. Soc. 5138 (1962).

27. Whistler, R. L., and Van Es T., Solvolysis of Methyl D-Xylothia-Pyranosides and 2,3,4-tri-O-acetyl-α-D-xylothia Pyranosyl Bromide J. Org. Chem. 28 2303 (1963).

28. Whistler, R. L., and Rowell, R. M., Preparation and Hydrolysis of Methyl 1-thio-β-D-xylopyranoside J. Org. Chem. 29 3290.

29. Lemieux, R. U., and Morgan, A. R., The Abnormal Configurations of Pryidinium α-Glycopyranosides Can. J. Chem. 43 2205 (1965).

30. Clayton, C. J., Hughes, N. A., and Saeed, S. A., Synthesis of the Methyl 1-deoxy-1-thio-D-Riboses and the Hydrolysis of Methyl 1-deoxy-1-thio-α-D-ribose. J. Chem. Soc. (C) 644 (1967).

31. Lavene, P. A., and Raymond, A. L., Derivatives of Monoacetone Xylose J. Biol. Chem. 102 317 (1933).

32. Young, R. C., Kent, P. W., and Dwerk, R. A., The 220 MHz Proton Magnetic Resonance Spectra of Some 5-deoxy-5-Iodo a-D-xylo Pentofuranose Derivatives Tetrahedron 26 3983 (1970).

33. Yale, H. L., Some Derivatives of α-Amylcinnamic Acid J. Org. Chem. 16 713 (1951).

34. Mehltretter, C. L., Alexander, B. H., Mellies, R. L., and Rist, C. E., A Practical Synthesis of D-Gluconic Acid Through the Catalytic Oxidation of 1,2-O-Isopropylidene Glucose J. Am. Chem. Soc. 73 2424 (1951).

35. Schaffer, R., and Isbell, H. S., Improved Synthesis of D-Glucuronate-6-C^{14} and of D-Glucose-6-C^{14} J. Res. Natn'l Bureau of Stds. 56 191 (1956).

36. Dinitrosalicic Acid Reagent: In Methods in Enzymology Vol. I. Eds. Colowick, S. P., and Kaplan, N. O., Academic Press, New York 1954, p. 149.

37. Hudson, C. S., and Dale, J. K., Triacetyl-d-Xylose and Alpha Triacetylmethyl-d-xyloside J. Am. Chem. Soc. 40 997 (1918).

38. Ferrier, R. J., Prasad, D., and Rudowski, A., Boric Acid Derivatives As Reagents in Carbohydrate Chemistry. Part III. The Interaction of Phenylboric Acid with Methyl xylofuranosides and the Isolation of the Four Methyl Xylosides J. Chem. Soc. 858 (1965).

39. Sweeley, C. C., Bently, R., MaKita, M., and Wells, W. W., Gas-Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances J. Am. Chem. Soc. 85 2497 (1963).

40. Hughes, N. A., and Speakman, R. R. H., Benzoate Displacements of Sulfonate Esters of some 1,2-O-Isopropylidene-pentofuranoses Carbohydrate Res. 1 341 (1965).

41. Thompson, W. E., and Kraus, C. A., Properties of Electrolytic Solutions XXVII The Conductance of Several Quaternary Ammonium Salts in Ethylene Chloride at 25° J. Am. Chem. Soc. 69 1016 (1947).

42. Buchanan, J. G., and Oakes, E. M., Oxetans: Part I 3,5-anhydro-1,2-O-Isorpopylidene- -D-glucofuranose and -L-Idofuranose Carbohydrate Res. 1 242 (1965).

43. Hough, L., and Otter, B., Furanoid Vinyl Ethers Chem. Commun. 6 173 (1966).

44. Schaffer, R. S., and Isbell, H. S., Structure of 5-Aldo-1,2-O-isopropylidene-D-xylo-pentofuranose J. Am. Chem. Soc. 79 3864 (1957).

45. Sammuel, D., and Silver, B. L., Oxygen Isotope Exchange Reactions of Organic of Organic Compounds Adv. Physical Organic Chem. 3 123 (1965).

46. Murray, A., and Williams, D. L., In: Organic Synthesis with Isotopes Part II. Interscience Publishers, Inc. New York, (1958) p. 1894.

47. Dejongh, D. C., and Bieman, K., Mass Spectra of O-Isopropylidene Derivatives of Pentoses and Hexoses J. Am. Chem. Soc. 86 67 (1964).

48. Dejongh, D. C., Radford, T., Hribar, J. D., Hanession, S., Bieber, M., Dawson, G., and Sweely, C. C., Analysis of Trimethylsilyl Derivatives of Carbohydrates by Gas Chromatography and Mass Spectrometry J. Am. Chem. Soc. 91 1728 (1969).

49. Hill, C. H., In: Introduction to Mass Spectrometry Heydon & Son Ltd. London (1966) p. 20.

50. Rowe, J. J. M., Hinton, J., and Rowe, K. L., Nuclear Magnetic Resonance Studies on the Biochemistry of Biopolymers Chem. Rev. 70 42 (1970).

51. Fisher, D., Highman, M., Kent, P. W., and Pritchard, P., β-xylosidases of Animal and other Sources in Relation to the Degradation of Chondroitin Sulphate-Peptide Complexes Biochem. J. 98 46P (1969).

52. Austin, P. W., Hardy, F. E., Buchanan, J. G., and Addiley, J. B., The Separation of Isomeric Glycosides on Basic Ion-exchange Resins J. Chem. Soc. 5350 (1963).

53. Lindberg, B., Action of Strong Acids on Acetlyated Glucosides Acta Chim. Scand 6 949 (1952).

54. Thornton, E. K., and Thornton, E. R., In: Isotope Effects in Chemical Reactions Ed. Collins, C. J. and Bowman, N. S., ACS Monograph No. 169 Van Norstrand-Reinhold, New York (1970) p. 172.

55. Bigeleisen, J., and Wolfsberg, M., In: Advances in Chemical Physics Vol. I Ed. Prigogine, J., Interscience Publishers, New York (1959) p. 29.

56. Bigeleisen, J., and Goeppert-Mayer, M., Calculation of Equilibrium Constants for Isotopic Exchange Reactions J. Chem. Phys. 15 261 (1949).

57. Bigeleisen, J., and Friedman, L., C^{13} Isotope effect in the Decarboxylation of Malonic Acid J. Chem. Phys. 16 323 (1948).

58. Neely, N. B., Infrared Spectra of Carbohydrates Adv. Carbohydrate Chem. 12 13 (1957).

59. Aumann, D. C., and Born, H. J., In: Radiochemical Methods of Analysis Vol. I. Proceedings of the Symposium on Radiochemical Methods of Analysis. International Atomic Energy Agency, The Agency, Salzburg, Austria (1964).

60. Amiel, S., and Peisach, M., Determination of Deuterium Content in Heavy water by the reaction O-16 (d,n) Fluorine-17 Induced by Reactor Neutrons Analyt. Chem. 34 1305 (1962).

61. Taylor, J. W., and Chen, I., Variables in Oxygen-18 Isotopic Analysis by Mass Spectrometry Analyt. Chem. 42 224 (1970).

62. Lee, J. S., Determination of O^{18} Concentration of Sugars and Glycogen Analyt. Chem. 34 835 (1962).

63. Fong, B. J., Smith, S. R., and Tanaka, J., Re-evaluation of the Preparation of Organic Compounds for the Mass Spectrometric Analysis for Oxygen-18 Analyt. Chem. 44 224 (1970).

64. Hurst, D. T., and McInnes, A. G., The Alcoholysis of Trialkylalkoxysilanes Part II. The Preparation and Chemistry of Methyl 2,3,4-tri-O-trimethylsilyl-α-D-Glucopyranoside Can. J. Chem. 43 2004 (1964).

Acknowledgment

The author wishes to express his appreciation to the National Science Foundation for the grant number 6P-33948 by which this study was funded.

Thanks go also to Dr. Paul Seib for his patience and direction during the course of the study and to Michael Rennekamp for his assistance in the operation of the mass spectrometer.

A large part of any educational experience is the interaction and exchange of ideas between peers. To my fellow students in the Department of Grain Science, especially Robert Vander Zanden and Suntay Ediz, I wish to express my thanks for a rewarding experience.

THE SYNTHESIS OF METHYL α AND β-D-XYLOPYRANOSIDES-5-^{18}O

by

WILLIAM DEAN HITZ

B. S., Kansas State University, 1971

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Graduate Group in Biochemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1974

ABSTRACT

To further study the mechanism of the acid-catalyzed hydrolysis of pyranosides, methyl α-D-xylopyranoside-5-^{18}O and methyl β-D-xylopyranoside-5-^{18}O were prepared with 54.5% ^{18}O at the O-5 position. These compounds were needed to investigate possible kinetic isotope effects at the O-5 position during acid hydrolysis. The ^{18}O enrichment was accomplished as follows: $^{18}O/^{16}O$ exchange was carried out at the potential 5-aldehydo group of bis-(1,2-O-isopropylidene-α-D-xylo-pentadialdofuranose)-3,5'-5,5' cyclic acetal,(II), with 95 atom% water and base catalysis. The 5-aldehydo group was reduced to give 1,2-O-isopropylidene-α-D-xylofuranose-5-^{18}O, which was then solvolyzed in methanol to a mixture of methyl α-D-xylopyranoside-5-^{18}O and methyl β-D-xylopyranoside-5-^{18}O. The pyranosides were separated and purified by fractional crystallization of the corresponding cyclic 2,4-O-phenylboronate esters. Using this scheme, 244 mg and 277 mg of the 5-^{18}O labeled methyl α-and β-D-xylopyranosides were prepared in 19.8% and 48.8% yield respectively from the cyclic acetal (II).

In order to measure the kinetic isotope effect at the O-5 position, separation of D-xylose from a 95:5 mixture of xyloside: xylose was necessary. The separation was accomplished by preparative gas-liquid chromatography of the pertrimethylsilylated mixture from the partial hydrolysate.

Mass spectral measurement of the $^{18}O/^{16}O$ ratio at the five position of 1,2,3,4-tetrakis-O-(trimethylsilyl)-β-D-xylopyranose using the molecular ion region of the spectrum was not precise enough (2.18% - 3.81% error) to observe the maximum kinetic oxygen-isotope effect which might occur during hydrolysis.