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Deuterium Isotope Effects in α - β Pyranose and in Pyranose-Furanose Interconversions[1]

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Rates of mutarotation, catalytic coefficients, and isotope effects are reported for the mutarotations of α-D-xylose, α-D-glucose, and β-D-fructose in H_2O and in D_2O at 20 °C. The isotope effects (k_H/k_D) for the mutarotation of β-D-fructose (a pyranose-furanose interconversion) parallel in striking manner the isotope effects for the mutarotation of α-D-glucose (an α-β pyranose anomerization). For each sugar, the isotope effect is lowest for the acid-catalyzed reaction, and highest for the water-catalyzed reaction. The parallelism of the values obtained for the isotope effects under various conditions shows that the rate-determining steps in the two reactions are similar. Presumably, in both instances, the overall mutarotation arises from concurrent reactions operating on different species of the sugar and showing substantially different isotope effects. The gradual change in the isotope effect indicates that, under the conditions studied, three reactions take place concurrently.

The following isotope effects were found for the mutarotations at 20 °C: For α-D-glucose, $k_{H_3O^+}/k_{D_3O^+} = 1.39$: $k_{H_2O}/k_{D_2O} = 3.87$; and $k_B/k_B^* = 1.83$. For β-D-fructose, $k_{H_3O^+}/k_{D_3O^+} = 1.39$; $k_{H_2O}/k_{D_2O} = 3.87$; and $k_B/k_B^* = 1.92$. Mechanisms are presented for the several concurrent acid- and base-catalyzed mutarotation reactions.

Key Words: Acid-base catalysis in D_2O, deuterium isotope effects, D-fructose, D-glucose, isotope effects, mechanism of mutarotation, mutarotation, pyranose-furanose interconversions, sugars in solution.

1. Introduction

The mutarotation process is known to involve the reversible interconversion of several modifications of the sugar [1, 2].[2] For most sugars, this results in an equilibrium that may include two pyranose forms, two furanose forms, the acyclic form, and solvated and ionic modifications of these. The mutarotation of D-glucose consists almost entirely of an α-β pyranose interconversion (anomerization). The mutarotations of D-galactose, D-talose, D-ribose, D-*glycero*-D-*ido*-heptose, and certain other sugars give clear evidence for two types of reaction, namely, α-β pyranose anomerization and pyranose-furanose interconversion [3]. The mutarotation of D-fructose, however, arises in large measure from a pyranose-furanose interconversion [4].

The α-β pyranose anomerizations have been investigated thoroughly, but relatively little work has been done on pyranose-furanose interconversions. Isbell and Pigman [4] showed that the two types of reaction differ in their heats of reaction, activation energies, rates of reaction, and sensitivity to acid and base catalysts. It has been tacitly assumed that both α-β pyranose anomerizations and pyranose-furanose interconversions take place through a common, acyclic intermediate. However, other mechanisms are possible, especially anomerization without ring opening, or a change in ring size by intramolecular ring contraction or expansion. In view of these possibilities, it seemed desirable to study and compare the two types of mutarotation, in H_2O and in D_2O. Prior to the present work, others [5, 6, 7, 8] had studied the mutarotation of D-glucose and certain other α-β pyranose anomerizations by means of deuterium isotope-effects, and had obtained important information on the reaction mechanisms, but no pyranose-furanose interconversion had been so investigated. For this reason, we undertook a comparative study of isotope effects in the mutarotation of D-glucose, a typical α-β pyranose anomerization, and the mutarotation of D-fructose, a typical pyranose-furanose interconversion.

The object of the work was to elucidate the mechanisms for pyranose-furanose interconversions and to show how these reactions differ from α-β pyranose anomerizations. In the work reported here, methods were successfully developed for preparing and purifying the *O*-deuterated analogs of α-D-glucopyranose,

[1] Abstracted, in part, from a thesis submitted by Clarence W. R. Wade to Georgetown University in May 1965, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Figures in brackets indicate the literature references at the end of this paper.

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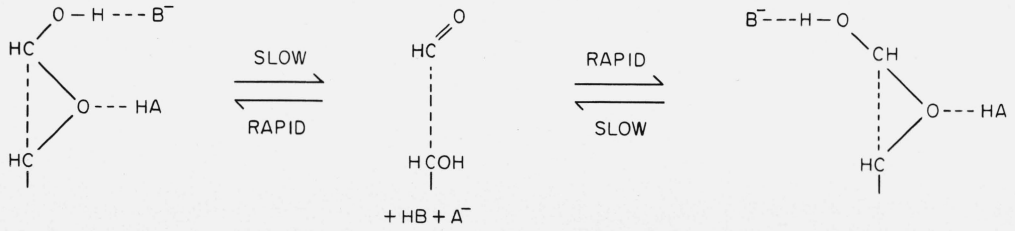

FIGURE 1. *Concerted mechanism of Lowry [10].*

α-D-xylopyranose, and β-D-fructopyranose. Accurate rates of mutarotation were determined for the *O*-hydrogenated sugars in H_2O and for the *O*-deuterated sugars in D_2O in the presence of certain acid and base catalysts. From these data, catalytic constants and deuterium isotope-effects were calculated. Mechanisms were formulated for the mutarotation reactions, and these are discussed in relation to isotope effects.

Numerous workers have sought to explain how mutarotation reactions take place. Lowry and coworkers [9] studied the mutarotation of tetra-*O*-methyl-D-glucopyranose in solvents of low dielectric constant and found that the reaction is slow in dry pyridine, or in dry cresol, but is fast in a mixture of the two solvents, or in either solvent when moist. From these observations, Lowry [10] concluded that, for mutarotation to occur, both an acid and a base must be present. He then proposed that the mutarotation of D-glucopyranose involves a ternary reaction of the sugar, an acid, and a base, as shown in figure 1. The rate-determining step was assumed to be the opening of the ring by the simultaneous attack of a base catalyst and an acid catalyst to form the acyclic aldehyde.

The mechanism involves simultaneous transfer of a proton from the acid to the sugar and of a different proton from the sugar to the base, with a rearrangement of the valence electrons. In aqueous solutions, water molecules provide both the acid and the base functions. The work of Swain and Brown [11] on the mutarotation of tetra-*O*-methyl-D-glucopyranose in the presence of the bifunctional catalyst, 2-pyridinol, and other evidence [12] support the concerted mechanism. However, there is considerable evidence for other reaction-paths, especially for a two-step mechanism [5, 13, 14, 15, 16, 17]. Regardless of whether proton transfer is by a concerted or a consecutive (two-step) mechanism, the overall process for the mutarotation requires that the sugar molecule accept a proton from an acid catalyst and release another proton to a base catalyst. The concerted and two-step mechanisms differ primarily in the mode of proton transfer. Deuterium isotope-effects, discussed in the next section. provide a means for testing various reaction mechanisms.

2. Discussion of Isotope Effects

In H_2O/D_2O systems, overall isotope-effects arise from a combination of *kinetic isotope-effects* and *solvent isotope-effects*. Ordinarily, kinetic isotope-effects are greater than unity, whereas solvent isotope-effects are less than unity. Hence, the overall isotope-effect, k_H/k_D, may have a value smaller than, equal to, or greater than unity. Kinetic isotope-effects depend on differences, in the H_2O and D_2O systems, in the energy involved in the alteration of bonds in the transition state; in general, the stronger the altered bond, the greater the kinetic isotope-effect [18, 19, 20]. Usually, the heavier isotope produces the lower reaction rate; hence, isotope effects greater than unity are termed "normal," and those less than unity are termed "inverse." A large, normal isotope-effect indicates that a proton is transferred in the rate-determining step, with rupture or formation of a bond.

Solvent isotope-effects have their origin in differences, in the behavior of the reactants, that are due to differences in the isotopic solvents; usually, they are inverse effects. The ion product of D_2O at 20 °C is $10^{-15.05}$, whereas that of H_2O is $10^{-14.17}$ [21]. Thus, a water molecule has a greater tendency to form an H_3^+O ion than a D_2O molecule has to form a D_3^+O ion. Because of the difference in the base strengths of the solvents, a sugar molecule is able to compete more effectively with the D_2O molecule for the deuteron than with the H_2O molecule for the proton. This situation leads to a higher concentration of the conjugate acid of the sugar in D_2O than in H_2O [22]. The same situation exists in certain other reaction systems. Examples are (a) the acid hydrolysis of sucrose [23]; (b) the base-catalyzed enolization of acetone [24]; (c) the acid-catalyzed decomposition of ethyl diazoacetate [25]; and (d) the acid-catalyzed anomerization of acetylated sugars [26]. These reactions are subject to specific hydrogen (deuterium) ion catalysis, and the isotope effect, k_H/k_D, ranges from 0.39 to 0.69. All of these reactions arise from solvent isotope-effects; they involve a rapid proton (deuteron) transfer from the catalyst to the substrate, followed by a rate-determining step with no proton transfer. Thus, these isotope effects arise from differences in the concentration of the conjugate acid, and not from a rate-determining cleavage reaction.

Reactions showing kinetic isotope-effects differ from those just cited in that they are more rapid in H_2O than in D_2O. Examples are (a) the decomposition of nitramide [27]; (b) the mutarotation of sugars [5, 6, 7, 8]; (c) the bromination of 3-methyl-2,4-pentanedione [28]; and (d) the hydrolysis of acetamide by strong acids [29]. The latter reactions are subject to general

acid-catalysis. In each instance, the kinetic isotope-effect is superimposed on a solvent isotope-effect.

For base catalysis, the deuterium isotope-effects are also greater than unity. Thus, (a) enolization of 3-methyl-2,4-pentanedione by base [30], (b) hydrolysis of acetamide by base [29], and (c) reaction of phenyl acetate with glycine and with ammonia [31] give overall isotope-effects which range from 1.1 to about 1.6.

For elucidation of the course of mutarotation reactions, knowledge of the timing of the addition and elimination of the proton is important. When the proton transfer occurs after the rate-controlling step, it has no primary kinetic consequence, but when it occurs *in* the rate-controlling step, it has a strong effect. Thus, the magnitude of the isotope effect provides an insight into the reaction mechanism.

Numerous comparative studies have been made of the mutarotation of α-D-glucopyranose in H_2O and D_2O. Pacsu [6] found that the rate of mutarotation of D-glucopyranose is higher in H_2O than in D_2O. Later, Hamill and La Mer [7] discovered that, regardless of the catalyst, the rate is higher in H_2O than in D_2O, and that the ratio of the rates in H_2O and D_2O is dependent upon the strength of the catalyst. Thus, they found the isotope effect to be 1.37 for catalysis by strong acids, and 3.80 for catalysis by the water molecule. Nicolle and Weisbuch [32] determined the rates of mutarotation of a number of sugars, and found that the overall isotope effect varied from 3.0 to 3.8; however, they did not relate the values to the *p*H or to the type of reaction.

Challis, Long, and Pocker [5] observed that the values of k_H/k_D for the mutarotation of tetra-*O*-methyl-α-D-glucopyranose do not differ greatly from those for α-D-glucopyranose. From a study of the reactions, they concluded that the acid-catalyzed mutarotation proceeds *via* a two-step mechanism consisting of a rapid exchange between hydrogen (or deuterium) of the solvent and that of the anomeric hydroxyl group, followed by slow rupture of the anomeric O–H bond in H_2O (or the O–D bond in D_2O) with simultaneous ring-opening.

Long and Bigeleisen [8] extended the theory of Challis and co-workers by relating k_H/k_D and its magnitude to (1) the strength of the catalyst, (2) specific hydrogen-ion and general acid-base catalysis, and (3) the exchange equilibrium between protons of the solvent and protons at the reaction center of the substrate. With these relationships mathematically represented, they accounted, on the basis of general acid-catalysis, for differences in the isotope effects for acids of various strengths. They pointed out that the isotope effects found for catalysis by strong acids (1.37) and by acetic acid (2.6) are of the expected magnitude, and that the isotope effect found for water catalysis is consistent with a reaction "in which the water molecule is acting simultaneously as a proton acceptor and donor" (i.e., the concerted mechanism).

The isotope effects and other experimental facts clearly indicate that the mutarotation of D-glucose, an α-β pyranose anomerization, takes place by acid-catalyzed and base-catalyzed mechanisms, involving acyclic intermediates.

In the present investigation a striking parallelism has been found in the values of k_{H_2O}/k_{D_2O}, $k_{H_3O^+}/k_{D_3O^+}$, and k_B/k_B^* for the mutarotations of β-D-fructose (a pyranose-furanose interconversion) and α-D-glucose (an α-β pyranose anomerization) under a variety of conditions. This result shows that the rate-determining steps in the two reactions are similar. Presumably, in both instances, the overall mutarotation arises from concurrent reactions involving the acid and base catalysts present. The mechanisms will be considered in detail after a brief discussion of the mutarotation measurements.

TABLE 1. *Mutarotation constants for sugars in* H_2O *and in* D_2O *solutions at 20° C*

Serial No.[a]	Solvent	Catalyst	Concn. sugar g/100 ml	*p*H meter reading[b]	(k_1+k_2)[c]
		α-D-GLUCOSE			
1	H_2O	0.015*N* sulfuric acid	4	1.72	0.0103
1*	D_2O	0.015*N* sulfuric acid	4	1.32	.00448
2	H_2O	0.005*N* sulfuric acid	4	2.10	.00789
2*	D_2O	0.005*N* sulfuric acid	4	1.71	.00277
3	H_2O	0.0025*N* sulfuric acid	4	2.38	.00719
3*	D_2O	0.0025*N* sulfuric acid	4	2.00	.00226
4	H_2O	0.001*N* K acid phthalate	4	4.40	.00634
4*	D_2O	0.001*N* K acid phthalate	4	4.70	.00163
5	H_2O	0.00002*N* sodium hydroxide	4	7.30	.00780
5*	D_2O	0.00002*N* sodium hydroxide	4	7.20	.00177
6	H_2O	0.00002*N* sodium hydroxide	18	6.70	.00660
6*	D_2O	0.00002*N* sodium hydroxide	18	7.40	.00198
7	H_2O	0.00006*N* sodium hydroxide	4	9.45	.1600
7*	D_2O	0.00006*N* sodium hydroxide	4	9.80	.0669
		β-D-FRUCTOSE			
8	H_2O	0.015*N* sulfuric acid	4	1.72	0.220
8*	D_2O	0.015*N* sulfuric acid	4	1.32	.138
9	H_2O	0.005*N* sulfuric acid	4	2.10	.124
9*	D_2O	0.005*N* sulfuric acid	4	1.71	.0623
10	H_2O	0.0025*N* sulfuric acid	4	2.38	.0952
10*	D_2O	0.0025*N* sulfuric acid	4	2.00	.0390
11	H_2O	0.001*N* K acid phthalate	4	4.40	.0580
11*	D_2O	0.001*N* K acid phthalate	4	4.70	.0153
12	H_2O	0.00002*N* sodium hydroxide	4	7.35	.247
12*	D_2O	0.00002*N* sodium hydroxide	4	8.22	.332
13	H_2O	0.00002*N* sodium hydroxide	18	6.70	.118
13*	D_2O	0.00002*N* sodium hydroxide	18	7.33	.0485
		α-D-XYLOSE			
14	H_2O	0.015*N* sulfuric acid	4	1.72	0.0353
14*	D_2O	0.015*N* sulfuric acid	4	1.32	.0151
15	H_2O	0.001*N* K acid phthalate	4	4.40	.0201
15*	D_2O	0.001*N* K acid phthalate	4	4.70	.00552

[a] Similar measurements in H_2O and D_2O are numbered alike, but are distinguished by use of asterisks.
[b] *p*D for the D_2O solutions equals the *p*H-meter reading + 0.4.
[c] $(k_1+k_2)=1/t\ [\log (r_0-r_\infty)-\log (r_t-r_\infty)]$.

3. Mutarotation Measurements

Mutarotation measurements, reported in table 1, were made for the normal forms and the *O*-deuterated forms of α-D-glucopyranose, α-D-xylopyranose, and β-D-fructopyranose, using H_2O and D_2O solutions containing acids and bases. The H_2O and D_2O solutions were designed to be molecularly equivalent, and the equivalence of the buffered solvents was compared by measuring the *p*H after dilution (to the same composition) of the H_2O solvent with D_2O, and of the D_2O solvent with H_2O. The mutarotation measurements were conducted in the conventional manner

[33], and the equilibrium rotations, specific catalytic effects, and isotope effects were calculated and compared.

Comparison of the equilibrium optical rotations (see table 2) of the sugars in H_2O and in D_2O shows that D-glucose is slightly less dextrorotatory in D_2O than in H_2O, whereas D-fructose is slightly less levorotatory in D_2O than in H_2O.

TABLE 2. *Equilibrium rotations of* D-*glucose and* D-*fructose in* H_2O *and in* D_2O

Sugar	Solvent	g/100 ml	°C	°S [a]	$[\alpha]_D$
D-Glucose	H_2O	4	20.0	+12.18	+52.7
	D_2O	4	20.0	+11.88	+51.4
	H_2O	4	3.9	+12.09	+52.3
	D_2O	4	3.9	+11.80	+51.1
D-Fructose	H_2O	4	20.0	−21.35	−92.4
	D_2O	4	20.0	−20.76	−89.8
	H_2O	4	3.9	−23.41	−101.3
	D_2O	4	3.9	−22.76	−98.5

[a] The solutions were read in a 2-dm tube with a Bates saccharimeter, using a dichromate filter and either a white light or a sodium light. No significant difference was found in the reading obtained with the two lights. The readings were converted into $[\alpha]_D$ on the assumption that 1 °S = 0.3462 angular degree.

4. Catalytic Effects in α-β Pyranose Anomerizations and Pyranose-Furanose Interconversions

Isbell and Pigman showed that pyranose-furanose interconversions are much more sensitive than α-β pyranose anomerizations to acid and base catalysts, and that the effect on the rate of mutarotation of small changes in acidity (or basicity) is greater for D-fructose than for D-glucose or D-xylose. Therefore, it is of interest to intercompare the rates of mutarotation of these sugars in H_2O and in D_2O at various pH (or pD) values (see table 3).

The mutarotation ratios of D-xylose to D-glucose at two widely different pH values (4.40 and 1.72) show only a small difference in either H_2O or D_2O. Thus, changes in the acid and base catalysts appear to affect the mutarotation constants of D-xylose and D-glucose to almost the same degree. On the other hand, the mutarotation ratios of D-fructose to D-glucose show wide variation in both solvents over the same range of pH. The high values of the ratios in acid solution arise from the greater sensitivity of the mutarotation of D-fructose to acid catalysts. In weakly acid solution (region of minimum rate), the mutarotation ratios of D-fructose to D-glucose are almost the same in H_2O and D_2O (9.2 and 9.4, respectively). This agreement shows that the catalytic effects of the H_2O molecule and the D_2O molecule on the rates of mutarotation are alike.

TABLE 3. *Relative rates of mutarotation of* α-D-*glucose (G), β-*D-*fructose (Fru) and* α-D-*xylose (Xyl) at 20 °C at approximately equal acidities*

Solvent	Catalyst	pH (or pD) of solution			Ratio of (k_1+k_2)	
		G	Fru	Xyl	Fru/G	Xyl/G
H_2O	0.015*N* sulfuric acid	1.72	1.72	1.72	21.4	3.43
D_2O	0.015*N* sulfuric acid	1.72	1.72	1.72	30.8	3.37
H_2O	0.005*N* sulfuric acid	2.10	2.10	.....	15.7	.....
D_2O	0.005*N* sulfuric acid	2.11	2.11	.....	22.5	.....
H_2O	0.0025*N* sulfuric acid	2.38	2.38	.....	13.2	.....
D_2O	0.0025*N* sulfuric acid	2.40	2.40	.....	17.3	.....
H_2O	0.001*N* K H phthalate	4.40	4.40	4.40	9.2	3.17
D_2O	0.001*N* K H phthalate	5.10	5.10	5.10	9.4	3.39
H_2O	0.00002*N* sodium hydroxide	7.30	7.35	.....	31.7	.....
D_2O	0.00002*N* sodium hydroxide	7.80	7.73	.....	24.5	.....

Because the mutarotations are affected to different degrees by acids and bases, evaluation of isotope effects in D_2O and H_2O requires consideration of the specific catalytic effects of the catalysts present. According to Brönsted and Guggenheim, and others [34, 35, 36], the mutarotation constant (k_1+k_2) for a sugar in the presence of acid and base catalysts can be represented by an equation of the following type:

$$(k_1+k_2)=k_{H_2O}+\Sigma_j k_{HA_j}[HA_j]+\Sigma_n k_{B_n}[B_n].$$

where the symbols in brackets represent the concentrations of the catalysts. The term k_{H_2O} represents the catalytic effect of the solvent, water, and the coefficients k_{HA_j}, k_{B_n}, etc., represent the specific catalytic constants of the substances indicated in the subscripts. In aqueous solutions at various pH values, the principal catalysts are water molecules, hydronium ions, hydroxyl ions, and sugar anions (S^-), and the mutarotation constant is represented by the following equation, from which the catalytic constants may be calculated:

$$(k_1+k_2)=k_{H_2O}+k_{H_3O^+}[H_3O^+] + k_{OH^-}[OH^-]+k_{S^-}[S^-]. \quad (1)$$

The catalytic constants of the deuterium analogs may be obtained from a similar equation by substitution of deuterium for hydrogen.

In evaluating catalytic effects, values of $[H_3O^+]$ and $[D_3O^+]$ were obtained by measurements with a glass electrode at 20 °C, assuming that $p\mathrm{D}=p\mathrm{H}+0.4$. The values of k_{H_2O} and k_{D_2O} given in tables 4 and 5 were estimated by plotting the mutarotation constants obtained in acid solutions against the hydrogen ion (or deuterium ion) concentrations. In each instance, a line was obtained whose intercept on the vertical axis

[3] Although k_{H_2O} represents the catalytic effect of the solvent, water, the specific (molar) catalytic constant for the water molecule, k'_{H_2O}, is $k_{H_2O}/55.5$.

TABLE 4. *Catalytic constants* [a] *for mutarotations in* H_2O *solutions at 20 °C*

Serial No.	*p*H	$[H_3O^+]$	$[OH^-]$ [b]	(k_1+k_2)	k_{H_2O}	$k_{H_3O^+}$	k_B [c]
			α-D-GLUCOSE				
1	1.72	1.9×10^{-2}	[d]	0.0103	(0.0063)	0.21	.....
2	2.10	7.9×10^{-3}	[d]	.0103	(0.0063)	.20	.....
3	2.38	4.2×10^{-3}	[d]	.0103	(0.0063)	.21	.....
4	4.40	[d]	[d]	.0103	(0.0063)	.....	.....
5	7.30	[d]	1.35×10^{-7}	.0103	(0.0063)	.....	1.1×10^{4}
6	6.70	[d]	3.39×10^{-8}	.0103	(0.0063)	.....	0.9×10^{4}
7	9.45	[d]	1.91×10^{-5}	.0103	(0.0063)	.....	0.9×10^{4}
				Average	(0.0063)	0.21	0.9×10^{4}
			β-D-FRUCTOSE				
8	1.72	1.9×10^{-2}	[d]	0.220	(0.058)	8.5	.....
9	2.10	7.9×10^{-3}	[d]	.124	(0.058)	8.4	.....
10	2.38	4.2×10^{-3}	[d]	.0952	(0.058)	8.9	.....
11	4.40	[d]	[d]	.0580	(0.058)	.....	.....
12	7.35	[d]	1.51×10^{-7}	.248	(0.058)	.....	1.3×10^{6}
13	6.70	[d]	3.39×10^{-8}	.118	(0.058)	.....	1.8×10^{6}
				Average	(0.058)	8.6	1.5×10^{6}
			α-D-XYLOSE				
14	1.72	1.92×10^{-2}	[d]	0.0353	(0.0201)	0.7	.....
15	4.40	[d]	[d]	.0201	(0.0201)	.....	.....
				Average	(0.0201)	0.7	.....

[a] $(k_1+k_2)=k_{H_2O}+k_{H_3O^+}[H_3O^+]+k_B[OH^-]$.

[b] Calculated from *p*H, assuming that the ion product at 20 °C is $10^{-14.17}$.

[c] $k_B[OH^-]=k_{S^-}[S^-]+k_{OH^-}[OH^-]$. k_B is concentration-dependent, as shown in eqs (2) and (8).

[d] Concentration of catalyst considered negligible.

TABLE 5. *Catalytic constants* [a] *for mutarotations in* D_2O *solutions at 20 °C*

Serial No.	*p*D	$[D_3O^+]$	$[OD^-]$ [b]	$(k_1^*+k_2^*)$	k_{D_2O}	$k_{D_3O^+}$	k_B^* [c]
			α-D-GLUCOSE				
1*	1.72	1.9×10^{-2}	[d]	0.00448	(0.00163)	0.150	.....
2*	2.11	7.8×10^{-3}	[d]	.00277	(0.00163)	.146	.....
3*	2.40	4.0×10^{-3}	[d]	.00226	(0.00163)	.158	.....
4*	5.10	[d]	1.12×10^{-10}	.00169	(0.00163)	.....	.....
5*	7.60	[d]	3.55×10^{-8}	.00177	(0.00163)	.....	3940
6*	7.80	[d]	5.62×10^{-8}	.00198	(0.00163)	.....	6230
7*	10.20	[d]	1.41×10^{-5}	.0669	(0.00163)	.....	4630
				Average	(0.00163)	0.157	4930
			β-D-FRUCTOSE				
8*	1.72	1.9×10^{-2}	[d]	0.1380	(0.015)	6.47	.....
9*	2.11	7.8×10^{-3}	[d]	.0623	(0.015)	6.06	.....
10*	2.40	4.0×10^{-3}	[d]	.0390	(0.015)	6.00	.....
11*	5.10	[d]	1.12×10^{-10}	.0153	(0.015)	.....	.....
12*	8.62	[d]	3.72×10^{-7}	.3320	(0.015)	.....	8.5×10^{5}
13*	7.73	[d]	4.79×10^{-8}	.0485	(0.015)	.....	7.0×10^{5}
				Average	(0.015)	6.19	7.8×10^{5}
			α-D-XYLOSE				
14*	1.72	1.9×10^{-2}	[d]	0.0151	(0.0055)	0.5	.....
15*	5.10	[d]	[d]	.0055	(0.0055)	.....	.....
				Average	(0.0055)	0.5	.....

[a] $(k_1^*+k_2^*)=k_{D_2O}+k_{D_3O^+}[D_3O^+]+k_B^*[OD^-]$.

[b] Calculated from *p*D, assuming that the ion product at 20 °C is $10^{-15.05}$.

[c] $k_B^*[OD^-]=k_{S^-}^*[S^-]+k_{OD^-}[OD^-]$.

[d] Concentration of catalyst considered negligible.

was k_{H_2O} or k_{D_2O}. The values of $k_{H_3O^+}$ and $k_{D_3O^+}$ were calculated from the measurements in acid solution, assuming that

$$(k_1+k_2)=k_{H_2O}+k_{H_3O^+}[H_3O^+] \text{ in } H_2O,$$

and

$$(k_1^*+k_2^*)=k_{D_2O}+k_{D_3O^+}[D_3O^+] \text{ in } D_2O.$$

The values of k_{H_2O}, k_{D_2O}, $k_{H_3O^+}$, and $k_{D_3O^+}$ found for D-glucose are in fair agreement with values reported by Hamill and La Mer [7] and other workers [34, 35, 36]. No similar data have heretofore been reported for the mutarotation of D-fructose.

Evaluation of the catalytic constants k_{OH^-} and k_{S^-} is complicated by the fact that the mutarotation reaction becomes too rapid for accurate measurement in hydroxyl ion concentrations greater than 10^{-6}. Separate determination of $k_{OH^-}[OH^-]$ and $k_{S^-}[S^-]$ is especially difficult because the latter term is overshadowed by the former. Hence, we represented the overall effect of base catalysis in H_2O with hydroxyl ion concentration [OH] as:

$$k_B[OH^-]=k_{OH^-}[OH^-]+k_{S^-}[S^-], \qquad (2)$$

and in D_2O as

$$k_B^*[OD^-]=k_{OD^-}[OD^-]+k_{S^-}[S^-]. \qquad (3)$$

In neutral and alkaline solutions, catalysis by hydrogen (or deuterium) ions is negligible, and values for k_B and k_B^* were calculated from the equations

$$(k_1+k_2)=k_{H_2O}+k_B[OH^-], \qquad (4)$$

and

$$(k_1^*+k_2^*)=k_{D_2O}+k_B^*[OD^-]. \qquad (5)$$

In the calculations, the values for k_{H_2O} and k_{D_2O} were derived as previously described, and those for $[OH^-]$ and $[OD^-]$ were obtained from *p*H or *p*D measurements, on the assumption that $[H_3O^+][OH^-]=10^{-14.17}$, and that $[D_3O^+][OD^-]=10^{-15.05}$ [21].

The values of k_B and k_B^* found by solving eqs (4) and (5) are given in tables 4 and 5. The catalytic constants were then used to obtain the isotope effects given in table 6. The results show a striking parallelism in the isotope effects for the water-catalyzed, the acid-catalyzed, and the base-catalyzed mutarotations of α-D-glucose and β-D-fructose. In each instance, the isotope effect is lowest for the acid-catalyzed reaction and highest for the water-catalyzed reaction. The parallelism supports the hypothesis that the two reactions, one an α-β pyranose anomerization and the other a pyranose-furanose interconversion, take place by similar mechanisms. Isotope effects had not been reported previously for the mutarotation of D-fructose.

As already mentioned, the catalytic constant k_B for base catalysts includes the effect of the hydroxyl ion

and that of the sugar anion. Necessary data are available for the separate evaluation of the two effects in H_2O but not in D_2O.

TABLE 6. *Isotope effects in the mutarotations of* D-*glucose*, D-*fructose*, *and* D-*xylose at 20 °C* [a]

Sugar	k_{H_2O}/k_{D_2O}	$k_{H_3O^+}/k_{D_3O^+}$	k_B/k_B^*
α-D-Glucose	3.87	1.39	1.83
β-D-Fructose	3.87	1.39	1.92
α-D-Xylose	3.65	1.40	.....

[a] The values are based on the averages for the catalytic constants given in tables 4 and 5.

According to Brönsted [37] the catalytic constant for a base is given by the following expression:

$$k_B = GK_B^y, \tag{6}$$

where G and y are parameters evaluated from measurements with a series of comparable base-catalysts, and K_B is the reciprocal of the dissociation constant of the conjugate acid. From the measurements of Brönsted and Guggenheim [36], y has a value of about 0.4 for the base-catalyzed mutarotation of D-glucose. The values of G for the mutarotation of D-glucose and D-fructose may be estimated from the respective catalytic constants for the water molecule, given in table 4. Thus,

$$k'_{H_2O} = GK_B^y, \tag{7}$$

where k'_{H_2O} is the specific (molar) catalytic constant for the water molecule (i.e., $k_{H_2O}/55.5$), y is 0.4, and K_B, by definition, is the reciprocal of the dissociation constant of the conjugate acid, H_3O^+; for this acid, K_A is 55.5. For D-glucose at 20 °C, k'_{H_2O} is 0.0063/55.5, and G, calculated from eq 7, is $10^{-3.25}$. Similarly, for D-fructose at 20 °C, k'_{H_2O} is 0.058/55.5, and G is calculated to be $10^{-2.28}$.

These values can now be used to estimate k_S^-, the catalytic constant for the sugar anion. From the data of Urban and Schaffer [38], we calculated the acid dissociation constants for D-glucose and for D-fructose in water at 20 °C to be $10^{-12.25}$ and $10^{-12.00}$, respectively. Therefore, from eq (6), for D-glucose,

$$k_{S^-} = 10^{-3.25}(10^{12.25})^{0.4} = 45,$$

and for D-fructose,

$$k_{S^-} = 10^{-2.28}(10^{12.00})^{0.4} = 330.$$

The latter value is based on the assumption that the parameters of eq (6) are the same for the mutarotation of D-fructose as those determined for the mutarotation of D-glucose. The above values were used to obtain the catalytic effects of the sugar anions given in table 7. There are no data on the ionization of sugars in D_2O, and, therefore, calculations similar to those in table 7 could not be made for the D_2O system.

TABLE 7. *Calculated catalytic effects for the sugar anions in the mutarotations of* α-D-*glucose and* β-D-*fructose in water at 20 °C*

Serial No.	Sugar	Concn. mole/l	*p*H	$[S^-]$ [a] mole/l	Catalytic constant k_{S^-}	Catalytic effect for sugar anion $k_{S^-}[S^-]$	Percent of (k_1+k_2)
5	D-Glucose	0.22	7.30	$10^{-5.61}$	45.	0.00011	1.7
6		1.00	6.70	$10^{-5.55}$	45.	.00013	2.1
		1.00	12.25	0.50	.....	.....	[b]
12	D-Fructose	0.22	7.35	$10^{-5.31}$	330.	0.00162	2.83
13		1.00	6.70	$10^{-5.30}$	330.	.00166	2.86
		1.00	12.00	0.50	.....	.....	[b]

[a] Calculated from the *p*H, the concentration of the sugar, and the acid dissociation constant of the sugar.
[b] Mutarotation too rapid to be determined.

A simple calculation shows that the relative importance of catalysis by the sugar anion depends on the concentration of the sugar. Thus, if K is the acid dissociation constant of the sugar, and K_w is the ion product of water, then

$$[S^-] = \frac{K[HS]}{[H_3O^+]} = \frac{K[HS][OH^-]}{K_w}$$

and

$$k_{S^-}[S^-] = \frac{k_{S^-}K[HS][OH^-]}{K_w}. \tag{8}$$

Substitution of the appropriate values in eq (8) gives the following expressions:

$$\text{for D-glucose, } k_{S^-}[S^-] = \frac{44.7 \times 10^{-12.25}[HS][OH^-]}{10^{-14.17}}$$

$$= 3720[HS][OH^-];$$

$$\text{and for D-fructose, } k_{S^-}[S^-] = \frac{331 \times 10^{-12.00}[HS][OH^-]}{10^{-14.17}}$$

$$= 49{,}000[HS][OH^-].$$

These values may now be used in eq (2) to calcu- k_{OH^-} from $k_B[OH^-]$. Thus, for D-glucose,

$$k_B[OH^-] = (k_{OH^-} + 3720[HS])[OH],$$

or

$$k_{OH^-} = k_B - 3720[HS].$$

For D-fructose,

$$k_{OH^-} = k_B - 49{,}000[HS].$$

At *p*H values less than 8, [HS] is substantially equal to the molar concentration of the sugar. It follows from the above equations that the values of k_B and k_B^* should be dependent on the concentration of the sugar. However, because of the high catalytic effect of base catalysts, the errors in measuring k_B and k_B^* in the present study were too large to reveal the concentration factor. The sugar concentrations for the measurements in D_2O were the same as those for the cor-

responding measurements in H_2O; hence, the concentration factor is partially eliminated in the ratios of k_B/k_B^* given in table 6.

Table 8 presents values for the catalytic constants for the mutarotations of D-glucose and D-fructose in H_2O. The high sensitivity of the mutarotation of D-fructose to hydroxyl ion catalysis is shown by the large value of k_{OH^-}, nearly 200 times that for the mutarotation of D-glucose. The values of k_{H_2O}, $k_{H_3O^+}$ and k_{S^-} for D-fructose, however, are only approximately ten times those found for D-glucose. The exceptionally high sensitivity of the mutarotation of D-fructose (and presumably of other pyranose-furanose interconversions) to catalysis by hydroxyl ion is anomalous and should be investigated further.

TABLE 8. *Comparison of catalytic constants for the mutarotations of* D-*glucose and* D-*fructose in* H_2O *at 20 °C*

Serial No.	Sugar	Molar concn. of sugar	pH	$[OH^-]$	k_{H_2O}	$k_{H_3O^+}$	k_{S^-} [a]	k_{OH^-} [b]	k_B
5	D-Glucose	0.22	7.30	1.35×10^{-7}	6.3×10^{-4}	0.21	45.	10,200.	1.1×10^4
6		1.00	6.70	3.39×10^{-8}				5,300	0.9×10^4
7		0.22	9.45	1.91×10^{-5}				7,200	0.8×10^4
	Average	0.5			6.3×10^{-4}	0.21	45.	7,600	0.9×10^4
12	D-Fructose	0.22	7.35	1.51×10^{-7}	5.8×10^{-3}	2.2	330.	1.29×10^6	1.3×10^6
13		1.00	6.70	3.39×10^{-8}				1.74×10^6	.....
	Average	0.6			5.8×10^{-3}	2.2	330.	1.5×10^6	1.3×10^6
Ratio of constants fructose/glucose					9.2	10.5	7.3	197	

[a] Calculated by means of eq (6).
[b] Calculated by means of eqs (2) and (8).

5. Reaction Mechanisms

In the preceding section, it was shown that the isotope effects for the mutarotations of D-glucose and D-fructose in H_2O and in D_2O are similar under a variety of conditions. This result is rather surprising, because the mutarotation of D-fructose involves a change in the size of the ring, whereas the mutarotation of D-glucose involves a change in the configuration of the anomeric carbon atom. A change in anomeric configuration could take place either through a cyclic or an acyclic intermediate.

5.1. Cyclic Mechanisms

Excellent discussions of epimerization and ring isomerization of sugar derivatives have been written [42, 43, 44]. It was pointed out that anomerization of acetylated sugars in aprotic solvents, and certain rearrangements of methyl glycosides, presumably take place by cyclic mechanisms. Thus, Bonner [26] found that the rates for the anomerization of penta-*O*-acetyl-D-glucopyranose and tetra-*O*-acetyl-D-xylopyranose in 1:1 $AcOD-Ac_2O$ with D_2SO_4 catalyst are approximately 1.7 times those in 1:1 $AcOH-Ac_2O$ with H_2SO_4 catalyst (i.e., $k_H/k_D=0.6$, an inverse isotope effect). He explained the result by a solvent isotope-effect in the reaction depicted in figure 2. Undoubtedly, the reaction takes place by way of a cyclic intermediate, without rupture of the pyranose ring.

In striking contrast to this reaction, the mutarotation of the unsubstituted sugar is slower in D_2O than in H_2O under all conditions studied. There is no evidence for a mutarotation reaction of a sugar that proceeds with an inverse isotope-effect. Elimination and addition of the anomeric hydroxyl group in a cyclic mechanism would result in hydroxyl exchange. Experimentally, oxygen exchange in water labeled with ^{18}O is much slower than the mutarotation reaction [39]. Hence, we conclude that the cyclic mechanism is of little importance in the mutarotation of the free sugar in water.

Because a pyranose-furanose interconversion requires ring contraction, the cyclic mechanism can be excluded in the mutarotation of D-fructose. An intramolecular cleavage reaction analogous to the rearrangement of methyl 3,6-anhydro-α-D-glucopyranoside to methyl 3,6-anhydro-α-D-glucofuranoside [40] can be envisaged [41] for the mutarotation of D-fructopyranose, but this seems unlikely in light of the similarity in the isotope effects for the mutarotations of D-fructose and D-glucose.

5.2. Acyclic Mechanisms

In the discussion that follows, it is postulated that the mutarotation of free sugars takes place by way of an acyclic intermediate, and that cyclization of the intermediate gives either a pyranose or a furanose form of the sugar, depending on the position of ring closure.

When a sugar is dissolved in water, various reactions occur. Hydrogen bonds and ion pairs are formed between the solvent and polar groups; protons of the hydroxyl groups are exchanged; ionization takes place with the formation of both cations and anions; and activated complexes are formed with any catalysts present. The various molecular species exist in dynamic equilibrium and take part in the mutarotation reactions. The observed mutarotations are, however, dominated by kinetic conditions (not by true equilibrium conditions). It was mentioned that

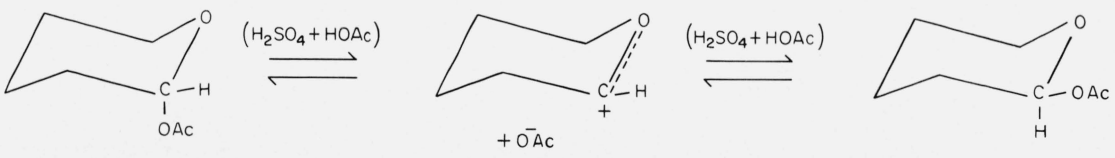

FIGURE 2. *Anomerization of an* O-*acetylpyranose in a mixture of acetic acid, acetic anhydride, and sulfuric acid* [26].

oxygen exchange in water labeled with ^{18}O is slow [39] in comparison with the mutarotation, and that this fact precludes a (cyclic) mutarotation mechanism involving hydroxyl exchange. Reversible formation of a *gem*-diol by the carbonyl form of the sugar would also result in oxygen exchange. The slow exchange with ^{18}O is in accord with the premise that hydroxyl groups attached to the carbon chain of the sugar react more readily than water molecules with the nascent carbonyl group. In the reaction mixture, equilibrium conditions are established slowly, and this fact accounts for ^{18}O exchange at elevated temperatures and during long reaction periods.

In the acid-catalyzed mutarotation the reaction sequence starts with the rapid, reversible addition of the acid, HA, to the ring oxygen atom. This addition is followed by a series of reactions, resulting in an acyclic intermediate which establishes equilibrium with other acyclic forms. By recyclization, the acyclic intermediate yields the ring isomers (or anomers). Presumably, the activated complex formed by the addition of HA to the ring oxygen atom can take part in the cleavage reaction directly, or the complex can ionize prior to cleavage. The first path gives rise to general acid-catalysis (fig. 3); in this process, rupture of the ring, with release of anion A^-, is rate-determining. In accordance with the interpretation of Long and Bigeleisen [8], in the transition state, the HA bond is stronger for weak acids than for strong acids. Rupture of a strong, isotopic bond in a rate-determining step gives a large isotope-effect; this accounts for the fact that the isotope effect is larger weak than for strong acids.

Figure 4 illustrates the general acid-catalyzed mutarotation reaction with hydronium ion as the catalyst. Cleavage of the hydronium ion and rupture of the ring in the transition state yields the acyclic cation and a molecule of water. The bond joining the proton to the anomeric oxygen atom must remain intact in the transition state; otherwise, the reaction would be the concerted mechanism of figure 1. The isotope effect is small because the bond joining the proton and the water molecule is weak and easily ruptured.

Protonation of the ring oxygen atom by strong acids prior to ring cleavage results in specific acid catalysis (see fig. 5). The rate of the subsequent reaction depends on the concentration of the cyclic sugar cation formed, which, in turn, depends on the concentration of the hydronium ion and not on that of any other acid. Thus, the reaction should be subject to *specific* hydronium ion catalysis. This reaction differs from general acid-catalysis (see figure 4) in that the H_3O^+

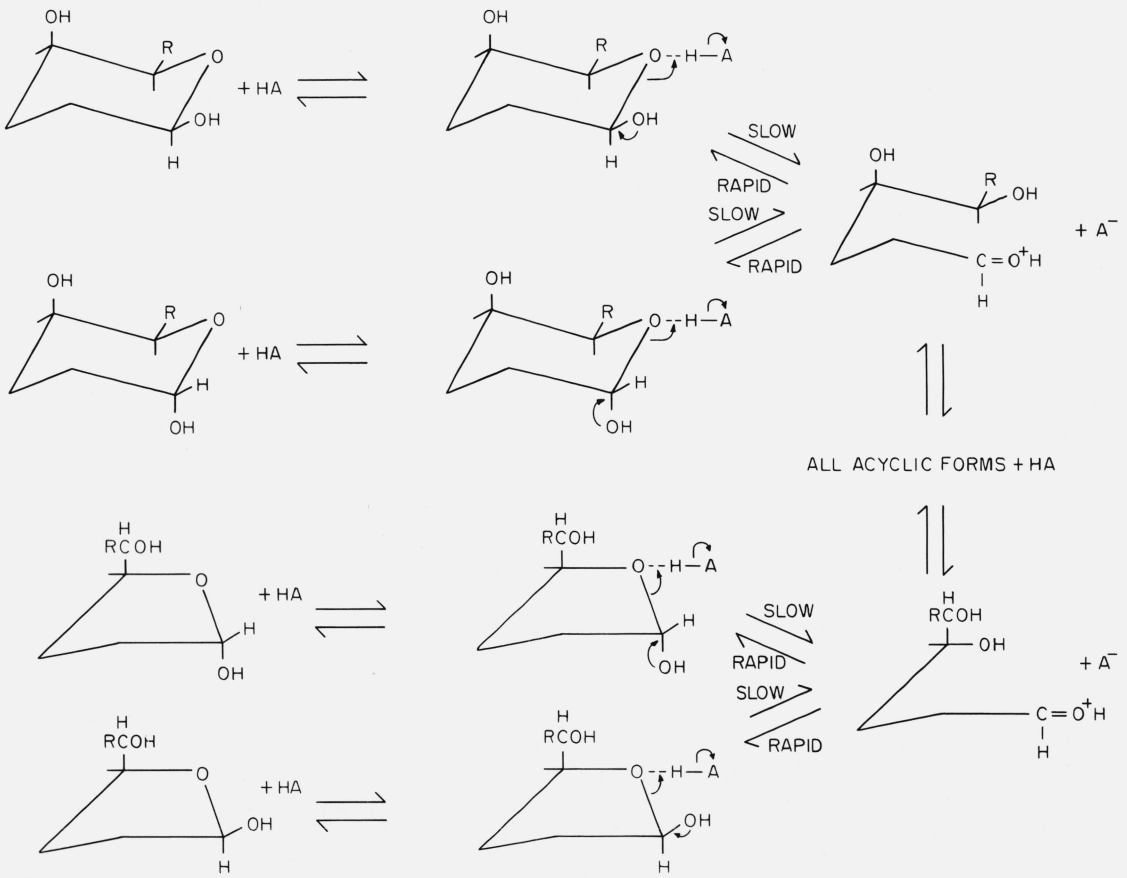

FIGURE 3. *General acid-catalysis.*

bond is broken prior to, not in, the rate-determining step. For this reason, there is no primary isotope-effect. However, the concentration of the sugar cation is higher in D_2O than in H_2O, and this higher concentration of the sugar cation in D_2O should give rise to an inverse, solvent isotope-effect, as in the acid hydrolysis of sucrose [23]. Experimentally, it has not been possible, under any circumstances, to obtain an inverse isotope-effect for the mutarotation reaction of sugars in aqueous solutions. Hence, we conclude that specific hydronium-ion catalysis plays, at most, only a minor part in the overall mutarotation.

To account for the very rapid mutarotation of the sugars in alkaline solution, a base-catalyzed mechanism is necessary. The base-catalyzed reaction must involve the anomeric hydroxyl group, because, when this hydroxyl group is replaced by an amino group or by an alkoxyl group, base-catalyzed anomerization does not occur [17].

A base-catalyzed step, in combination with general acid-catalysis, accounts for the behavior of the sugars in slightly alkaline solution. Strong bases cause rapid, reversible ionization of the sugar; and the ionization step is followed by transfer of a proton from the solvent (or other general acid-catalyst) to the ring oxygen atom, and slow rupture of the ring, as illustrated in figure 6. A moderately large, kinetic isotope-effect arises in H_2O and D_2O from alteration of the relatively strong H—OH bond in the transition state.

Weak bases and sugars form complexes which may react by way of general base-catalysis depicted in figure 7. In this process, cleavage of the proton from the anomeric hydroxyl group and rupture of the ring occur in the rate-determining step. Coordination of the solvent (H_2O) with the ring oxygen atom presumably facilitates the reaction, but transfer of the proton to the ring oxygen atom must be subsequent to ring cleavage; otherwise, the reaction would be concerted. Presumably, slow cleavage of the proton from the anomeric hydroxyl group, with rupture of the ring, is followed by rapid addition of a proton to the nascent anion. The isotope effect is moderately large because the anomeric O—H bond is ruptured in the rate-determining step.

Considerable uncertainty exists as to whether the water-catalyzed reaction follows the concerted mechanism of Lowry (fig. 1) or a two-step process. In both, one molecule of water acts as a general acid-catalyst and another acts as a general base-catalyst. In both mechanisms, rupture of the complex at the ring oxygen atom yields a hydroxyl ion, and transfer of the anomeric proton to a water molecule yields a hydronium ion. If, as depicted in figure 8, addition and elimination of the protons take place simultaneously, the mechanism is concerted. The isotope effect is high (3.87) because proton transfers occur in the transition state at the ring oxygen atom and at the anomeric hydroxyl group, and both bonds are strong.

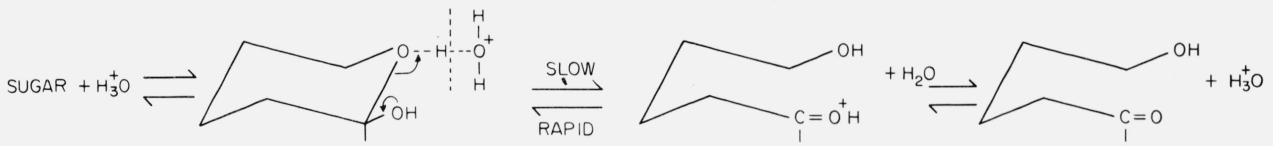

FIGURE 4. *General acid-catalyzed reaction with hydronium ion as catalyst.*

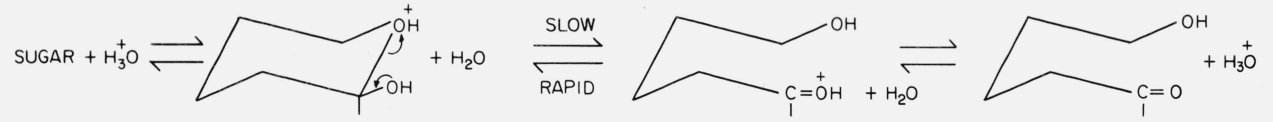

FIGURE 5. *Specific acid-catalyzed reaction.*

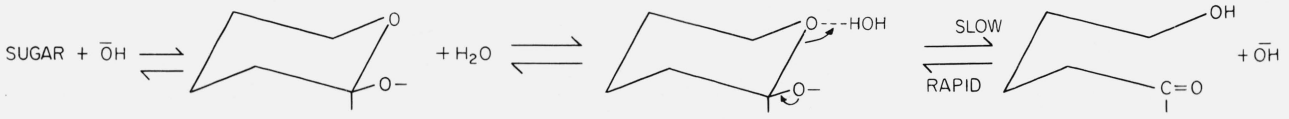

FIGURE 6. *Strong-base catalysis.*

FIGURE 7. *Weak-base catalysis.*

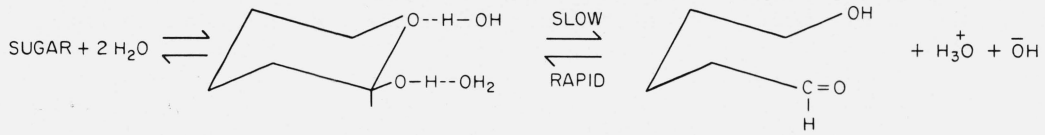

FIGURE 8. *Concerted reaction with* H_2O *as catalyst.*

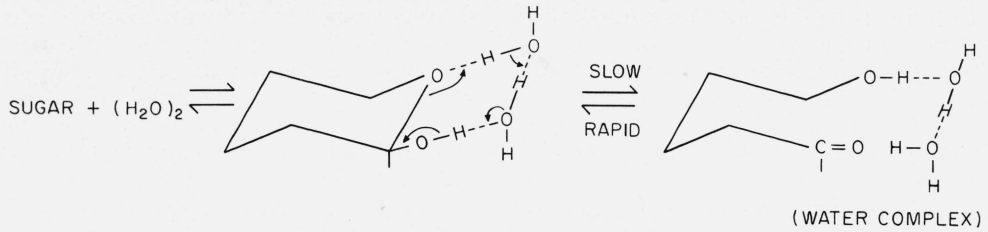

FIGURE 9. *Dimeric* H_2O *as a bifunctional catalyst.*

If transfer of the proton from the anomeric hydroxyl group to a water molecule occurs either before or after ring cleavage, the reaction consists of a two-step process in which water acts as a general acid-catalyst. The isotope effect for this process would be expected to be slightly less than that for the concerted mechanism, because, in this mechanism, proton transfer occurs in the transition state at only one point in the complex, instead of at two points. Figure 9 depicts a possible mechanism in which dimeric water acts as a bifunctional catalyst. The mechanism is speculative, but seems plausible in view of the high activity of the bifunctional catalysts of Swain and Brown [11].

Comparison of the results obtained under various conditions reveals that, in passing from highly acid to slightly acid and to alkaline solutions, there is no *abrupt* change in the magnitude of the isotope effects. The isotope effects increase from about 1.4 in highly acid solution to about 4 in slightly acid solution, and then decrease in alkaline solutions. The gradual change in the isotope effects is in accordance with the view that, under the conditions studied, three reactions take place concurrently. In highly acid solution, the acid-catalyzed mechanism predominates. The isotope effect is small for strong acids and large for weak acids, because the rate-determining step involves transfer of a proton from the catalyst, HA, to the ring oxygen atom. In the transition state, the proton is bonded more strongly to A^- in weak acids than in strong acids; hence, the isotope effect with the former is greater. In the region of minimum rate, the solvent molecules (H_2O or D_2O) are the principal catalysts, and presumably, the reactions take place by the two-step mechanism as well as by the concerted mechanism of Lowry. In neutral and alkaline solution, the base-catalyzed mechanism predominates. In the concerted mechanism, with H_2O or D_2O as a catalyst, both proton transfer at the ring oxygen atom and proton elimination at the anomeric hydroxyl group take place in the rate-controlling step. Both of the bonds are relatively strong; consequently, the isotope effect is large. In the base-catalyzed reaction, the sugar molecule is principally in the form of the anion, and the rate-controlling step is shifted to the opening of the ring, with transfer of a proton to the ring oxygen atom. The size of the isotope effect depends, as in general acid-catalysis, on the strength of the bond joining the conjugate base to the ring oxygen.

The mechanisms outlined accommodate the experimental results. All of the reactions proceed concurrently, and complex equilibrium states are established.

6. Experimental Procedures

6.1. Preparation of Sugars

a. *α-D-Glucopyranose.* D-Glucose (10 g) was dissolved in 10 ml of water, and the solution was treated with 0.5 g of decolorizing carbon and filtered. The filtrate and washings were concentrated in a rotary evaporator at 35 to 40°, to about 15 g of clear solution; this was mixed with 20 ml of glacial acetic acid and nucleated with a few crystals of α-D-glucopyranose. The flask was stoppered and slowly rotated. After three days, the crystalline product was separated by filtration and washed successively with 5 ml each of 90 percent aqueous acetic acid, glacial acetic acid, and absolute ethyl alcohol. Finally, the crystalline α-D-glucopyranose was dried for 1 hr over phosphorus pentaoxide in an oven at 55°/1 mm. The product weighed 5.2 g. Measurements of optical rotation and of the infrared spectrum showed that the product was pure α-D-glucopyranose.

b. *β-D-Fructopyranose.* The following procedure proved satisfactory for the purification of β-D-fructopyranose. D-Fructopyranose (10 g) was dissolved in 10 ml of water, and the solution was treated with 0.15 g of decolorizing carbon and filtered. The filtrate was concentrated to a thick sirup, which was diluted with 5 ml of ethanol and again concentrated. The residue was dissolved in 10 ml of methanol, and the solution was re-evaporated to a sirup; this procedure was repeated 3 times. Finally, the sirup (about 12 g) was dissolved in *p*-dioxane (10 ml), and the clear solution was nucleated with β-D-fructopyranose crys-

tals and rotated overnight. The resulting crystals were separated by filtration, and washed successively with 10 ml each of 85 percent aqueous *p*-dioxane and *p*-dioxane. The product was dried for 1 hr over phosphorous pentaoxide in an oven at 55°/1 mm; weight 5.6 g. Measurements of optical rotation and of the infrared spectrum showed that the sugar was pure β-D-fructopyranose.

c. α-D-*Xylopyranose.* Commercial D-xylopyranose (10 g) was dissolved in 6 ml of boiling water, and the hot solution was mixed with 0.5 g of decolorizing carbon and filtered. The filtrate was cooled to room temperature, diluted with about 3.5 ml of *p*-dioxane (to incipient turbidity), nucleated with crystals of α-D-xylopyranose, and placed on a rotator. Crystallization began almost immediately, and, after 24 hr, the crystals were separated by filtration and washed successively with 5 ml each of 60 and 85 percent aqueous *p*-dioxane and pure *p*-dioxane. The crystals, 7.5 g, were filtered and twice recrystallized from water by the same procedure. After the crystals had been air-dried (with the exclusion of dust), they were dried for 1 hr over phosphorus pentaoxide in an oven at 50°/1 mm. The optical rotation and the infrared spectrum showed that the substance was pure α-D-xylopyranose.

d. O-*Deuterated Sugars.* The deuterium analogs of the hydrogen forms of the sugars were prepared by replacing the labile hydrogen atoms of each sugar with deuterium; this was accomplished by repeated dissolution of the sugar in D_2O followed by freeze-drying. The crude, deuterated sugars were recrystallized by dissolution in D_2O, followed by the addition of *p*-dioxane. This procedure avoids the use of large quantities of deuterated solvents, and gives high yields of desired products. The isotopic purity of the deuterated sugars was established by infrared absorption measurements which showed negligibly weak bands corresponding to OH groups and strong bands corresponding to OD groups.

In the preparations, commercial deuterium oxide (99.5%) was used without purification. The infrared absorption spectra showed a lack of appreciable H_2O absorption. Scrupulous precautions were taken to avoid deuterium exchange in the recrystallized, deuterated sugars, and to prevent dilution of the deuterium oxide by atmospheric moisture.

6.2. Solvents for Mutarotation Measurements

a. *Sulfuric Acid in* D_2O. To prepare 0.015 *M* sulfuric acid in deuterium oxide, 200 ml of 0.015 *M* aqueous sulfuric acid was transferred to a 1-liter, round-bottomed flask and lyophilized; then, the flask was placed in a dry box. After removal of moisture from the dry box by successive evacuation and filling of the dry box with dry nitrogen, 6 ml of D_2O was added to the flask, and the resulting solution was lyophilized. Successive dissolution in 6 ml of deuterium oxide and lyophilization of the solution was carried out twice more. Finally, the sirupy residue was transferred to a 200-ml volumetric flask and diluted with sufficient D_2O to make 200 ml of solution.

The same procedure was used for preparing 0.005 *M* and 0.0025 *M* sulfuric acid in deuterium oxide from the corresponding aqueous acids.

After preparation, all of the solutions were transferred to glass-stoppered bottles and tightly closed. The D_2O solutions were transferred, in the dry box, to 25-ml flasks, and were opened only under anhydrous conditions (so as to prevent dilution of the D_2O with H_2O from the air).

Proof that the quantity of sulfuric acid was the same in each of the water solutions and in the corresponding deuterium oxide solutions was obtained as follows: Two ml of the aqueous acid was mixed with 2 ml of D_2O; similarly, 2 ml of the deuterated acid was mixed with 2 ml of H_2O, giving D_2O-H_2O mixtures that should contain equal amounts of D_2O and H_2O. The pairs gave the following *p*H meter readings: 2.23 and 2.22; 2.76 and 2.76; and 2.91 and 2.91.

b. *Potassium Acid Phthalate in* D_2O. Two hundred ml of 0.001 *M* aqueous potassium acid phthalate was lyophilized; the residue was thrice successively dissolved in 2 ml of D_2O and lyophilized. The residue was then dissolved in enough D_2O to make 200 ml of solution. (The solution gave a reading of 4.70 on a *p*H meter; the corresponding *p*D is 5.10.)

c. *Sodium Hydroxide Stock Solution.* Approximately 8 g of sodium hydroxide was mixed with 8 ml of H_2O, and the mixture was allowed to stand until the sodium carbonate had precipitated, leaving a clear solution. A portion (2 ml) of this carbonate-free solution was transferred to a 250-ml volumetric flask and diluted with enough freshly boiled H_2O to make 250 ml of solution. The solution was standardized with potassium acid phthalate.

d. *Sodium Hydroxide-d in* D_2O. An aliquot of the standardized sodium hydroxide stock solution was transferred to a 500-ml, round-bottomed flask and lyophilized. After introduction of dry nitrogen, 2 ml of D_2O was added, and the solution was again lyophilized. The residue was diluted with D_2O under dry nitrogen to the desired concentration of alkali, and the solution was stored in a dry-box protected from air.

6.3. Measurement of Optical Rotation

For each measurement of optical rotation, approximately 0.4 g of the crystalline sugar was weighed into a dry, 25-ml Erlenmeyer flask, and about 10 ml of the solvent to be used was placed in another 25-ml flask. Both flasks were stoppered and placed in a thermostated bath at the temperature planned for the experiment. A 2-dm, glass-jacketed, polarimeter tube was placed in a Bates, adjustable-sensitivity saccharimeter. The temperature was controlled by circulating water from the thermostated bath through the jacket of the polarimeter tube, and measured by a thermometer placed in the line of flow. The zero reading of the instrument was determined before the measurement was started. After temperature equilibrium had been attained for the system (1 hr or longer), the solvent was quickly poured into the flask containing the sugar. Time was measured from the moment the solvent was added. The sugar was dissolved by swirling the partially submerged, stoppered

flask, and, upon dissolution of the crystals, the solution was immediately transferred to the polarimeter tube. Optical rotations were read continuously during the first 30 min for fast reactions and at regular intervals for slower reactions. The equilibrium rotation was read at the end of a 24-hr period for the fast reactions, and after 48 or more hours for the slow reactions. All optical rotations recorded in the tables are averages of 10 readings.

The rate constant for each buffered sugar solution was calculated by use of the conventional formula:

$$(k_1+k_2)=\frac{1}{(t_2-t_1)}\log\frac{(r_1-r_\infty)}{(r_2-r_\infty)}$$

where (k_1+k_2) is the mutarotation constant for the balanced reaction, r_1 is the rotation at time t_1, r_2 is the rotation at time t_2, and r_∞ is the equilibrium rotation, all in "degrees sugar."

After the equilibrium rotation had been taken, the *p*H of the solution was measured with a glass electrode and commercial *p*H meter. Only the experiments employing sodium hydroxide solution showed any substantial difference in the *p*H of the original solvent and of that of the sugar solution. Values for $[H_3O^+]$ and $[OH^-]$ were calculated from the *p*H and the ion product corresponding to *pK* 14.17 at 20 °C. Values for *p*D were obtained from the relationship *p*D = reading on *p*H meter + 0.4. Values for $[D_3O^+]$ and $[OD^-]$ were calculated from the values of *p*D and the ion product corresponding to *pK* 15.05 at 20 °C.

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