Evidence for a Hydride Shift in the Alkaline Rearrangements of D-Ribose[1]

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The synthesis of D-ribose-2-t from D-arabinose is described and its conversions in aqueous alkali in the presence and absence of oxygen reported. In both situations a significant amount of label is transferred from C-2 to -1 and a relatively small proportion is released to the solvent. It is concluded that hydride transfer is occurring and that enolization, which requires the loss of hydrogen from C-2, is not an obligatory first step in base-catalyzed rearrangements of D-ribose.

On décrit une synthèse du D-ribose-2-*t* à partir du D-arabinose et on rapporte ce qui lui survient en milieu alcalin en présence et en l'absence d'oxygène. Dans chaque cas une quantité significative de tritium est transférée du C-2 au -1 et une faible proportion se retrouve dans le solvant. On en conclut qu'un transfert d'hydrure se produit et qu'une enolisation, qui implique une pente d'hydrogène du C-2, n'est pas nécessairement la première étape lors du réarrangement du D-ribose au milieu alcalin.

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Introduction

In the preceding paper (1) circumstantial evidence was presented that the pentoses in aqueous potassium hydroxide, in the presence of oxygen, cannot react only by a simple enolization mechanism initiated by abstraction of the hydrogen at C-2. To further test this conclusion, we have studied the behavior of D-ribose-2-*t* in aqueous potassium hydroxide under both aerobic and anaerobic conditions.

Experimental

The n.m.r. spectra were obtained on a Varian A-60 spectrometer and i.r. spectra were obtained on a Perkin–Elmer Model 521. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee. Evaporations were carried out at water-aspirator pressure. Melting points are corrected.

A Beckman LS-100 scintillation counter equipped with automatic external standardization was used for radioactivity determination. Aqueous samples were added in a volume of 0.100 to 10.0 ml of scintillation fluid containing 5 g PPO and 100 g napthalene with dioxane added to make 1 l. Water insoluble samples were added directly to the scintillation fluid and then 0.100 ml H_2O added to this mixture.

Gas chromatographic analyses were performed using a Varian Aerograph Hy-Fi, Model 600 D, with a flame ionization detector (hydrogen flow rate of 30 ml/min). The column was 1/8 in. × 6 ft copper tubing packed with 10% Hi-eff-3-CP (Applied Sciences Laboratories, Inc.) with helium carrier gas at 30 ml/min. Silyl derivatives were prepared by dissolving 10 mg of material in 0.5 ml pyridine and adding 0.2 ml hexamethyl disilazane and 0.1 ml trimethylchlorosilane (Applied Sciences Laboratories). The derivative mixture (2 μl) was injected after 10 min. Acetylated derivatives were prepared by warming a 10 mg sample in 0.5 ml pyridine and acetic anhydride (1:1) for 30 min. Peak areas were determined by planimetry.

The t.l.c. was carried out on plates prepared with Silica gel G (Merck) using isopropyl ether as solvent. Spots were visualized by spraying with 10% sulfuric acid and heating at 110° for 5 min.

Acidic products were separated by paper chromatography on Whatman 3MM paper to which a wick of Whatman 1 (10 × 57 cm) had been sewn. The freshly prepared solvent, *n*-butanol : formic acid : water (75:10:15), gave an adequate separation provided that samples of 100 mg or less were used. Standards of glyceric (R_f = 0.46), glycollic (R_f = 0.61), and erythronic or threonic (R_f = 0.31) acids were applied 3 cm from each edge and were used to locate the main reaction components. Acidic compounds could be located as yellow spots on a blue background with the indicator bromocresol green (0.05%) in slightly alkaline ethanol if the chromatograms were allowed to dry for several days to free them of formic acid. Alternate cycles of steaming and drying at 100° in an oven was used also to remove formic acid more rapidly.

For quantitation of the reaction products, strips were cut from the chromatogram and eluted with water. The eluted acids were titrated to p*H* 7.0 with 0.050 *M* potassium hydroxide. In cases where lactonization had occurred (indicated by a rapidly fading end-point) an excess of alkali was added and the sample was back titrated with 0.050 *M* hydrochloric acid to determine the total amount of acid and lactone.

Benzyl 3,4-O-Isopropylidene-β-D-ribopyranoside (4)

(A) By Dimethyl Sulfoxide – Acetic Anhydride Oxidation

The reaction sequence used to synthesize this compound is shown in Scheme 1. D-Arabinose was converted

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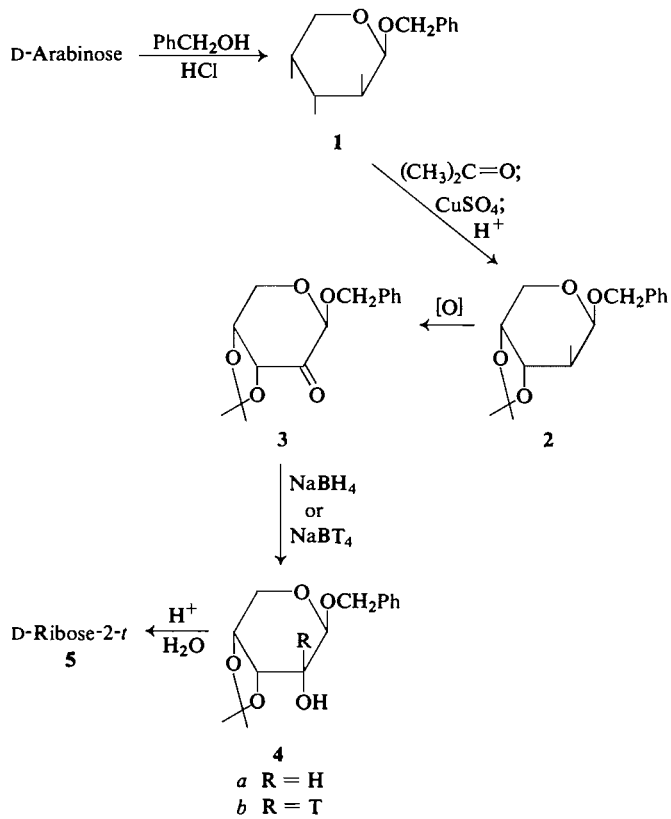


SCHEME 1

to benzyl β-D-arabinopyranoside (**1**) by the method of McCormick (2). Benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (**2**) was prepared from (**1**) by the method of Ballou (3).

Benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (**2**), 3.0 g, was oxidized in a mixture of 30 ml dimethylsulfoxide and 20 ml acetic anhydride (4). The progress of the reaction was monitored by t.l.c.; the starting material had $R_f = 0.15$. After 8 h reaction two new spots of about equal intensity appeared with $R_f = 0.37$ and 0.46. After 24 h only the two product spots were present. At this time the reaction mixture was quite yellow. Dimethylsulfoxide and acetic anhydride were removed by evaporation at 55°. The residue, a thin sirup, was reduced at 0° in 150 ml ethanol–water (3:1) using 3 g sodium borohydride. After 2 h acetic acid was added to destroy the excess borohydride and the reaction mixture was passed over 70 ml Rexyn 101 (H^+) and the eluate evaporated to dryness. Finally, boric acid was removed as its volatile methyl ester by three evaporations from 100 ml portions of methanol.

The t.l.c. of the mixture indicated that material of $R_f = 0.46$ was still present, but that the material of $R_f = 0.37$ had disappeared and a new spot with R_f approximately equal to that of the starting material had appeared. The mixture was dissolved in 15 ml ether and petroleum ether added to turbidity. After several hours at 4°, a cotton-like precipitate was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in a minimum amount of absolute ethanol and petroleum ether was added to produce turbidity. The product, **4***a*, 1.25 g (41% from **2**), could be recrystallized from ether – petroleum ether and melted at 94–96°; $[\alpha]_D^{25} -105 \pm 5°$ (*c* 3.1, EtOH). Follmann and Hogenkamp report m.p. 92–94° (5). The n.m.r. data: τ 2.65 (singlet, 5 protons, phenyl ring hydrogens); 5.06 (doublet, 1 proton H-1, $J_{1,2}$ 3.5 Hz); 5.20, 5.36, and 5.55 (multiplet, 3 protons, H-2, -3, and -4); 6.20 (multiplet, 4 protons, H_2-5 and benzyl methylene hydrogens); 2.47 and 2.63 (singlets, 6 protons, acetal methyl hydrogens).

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.27; H, 7.29.

The cotton-like precipitate which was removed from the product by filtration as described above proved to be a methylthiomethyl ether, presumably benzyl 3,4-*O*-isopropylidene-2-*O*-methylthiomethyl-β-D-arabinopyranoside. It could be isolated in yields of approximately 40% and crystallized readily from ether – petroleum ether. It melted at 43.8–45°; $[\alpha]_D^{25} -175 \pm 8°$ (*c* 1.2, EtOH). The n.m.r. data: τ 2.67 (singlet, 5 protons, phenyl ring hydrogens); 5.06 (doublet, 1 proton, H-1, $J_{1,2}$ 3.3 Hz); 5.23, 5.31, and 5.39 (multiplet, 3 protons, H-2, -3, and -4); 5.76 (singlet, 2 protons, methylthiomethyl methylene hydrogens); 6.06 (multiplet, 4 protons, H_2-5 and benzyl

methylene hydrogens); 2.47 and 2.67 (singlets, 6 protons, acetal methyl hydrogens).

Anal. Calcd. for $C_{17}H_{24}O_5S$: C, 59.97; H, 7.11; S, 9.42. Found: C, 59.90; H, 6.98; S, 9.28.

This compound proved to be the material of $R_f = 0.46$ observed in thin layer chromatograms of the reaction mixture.

(B) Alternate Oxidation Procedures

Because the dimethyl sulfoxide – acetic anhydride oxidation gave such a large amount of side-product, other oxidation methods were investigated.

(1) Pfitzner–Moffat Oxidation (6)

Benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (837 mg) was added to a mixture of 1.85 g dicyclohexylcarbodiimide (DCC), 5 ml dimethylsulfoxide, 10 ml benzene, 0.24 ml pyridine, and 0.12 ml trifluoroacetic acid. After 24 h, benzene was removed by evaporation and 20 ml water added to the reaction mixture. After 2 h the DCC had been converted to insoluble dicyclohexylurea, which was removed by filtration. The filtrate was evaporated to a sirup and extracted three times with 10 ml portions of chloroform. The chloroform extract was dried to a sirup. This sirup was applied to a 60 × 1.5 cm column of silica gel (60–200 mesh, W. R. Grace and Co.) and eluted with isopropyl ether, 3 ml fractions were collected at a flow rate of 0.2 ml per min. The first peak, found in fractions 1–14, contained at least three components as judged by t.l.c. Fractions 14–30 contained only the product, **3**: overall yield 38–42%.

(2) Catalytic Ruthenium Dioxide – Sodium Metaperiodate Oxidation (7)

Benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (1.0 g) was dissolved in 20 ml carbon tetrachloride containing 20 mg of ruthenium dioxide (Englehard Industry). Sodium metaperiodate (5%) was added by a burette to a rapidly stirred solution. The p*H* was maintained between 6.0 and 7.0 by the addition of dilute sodium bicarbonate. After 3 h and the addition of 23 ml of periodate, the end of the reaction was signalled by a permanent yellow ruthenium tetroxide color which was not darkened by conversion to the dioxide. The excess oxidant was destroyed by adding *n*-propanol. The organic layer was removed, filtered, and evaporated to a colorless sirup (0.78 g) which gave one spot on t.l c ($R_f = 0.37$ in isopropyl ether) and a carbonyl absorption (5.8 μ) in the i.r.

Benzyl 3,4-O-isopropylidene-β-D-ribopyranoside-2-t (4)

Benzyl 3,4-*O*-isopropylidene-β-D-*erythro*-pentopyranos-2-uloside (150 mg) was dissolved in a mixture of 7.5 ml ethanol (distilled from 2,4-dinitrophenol) and 5 ml distilled water. Sodium borohydride-*t* (3 mg, New England Nuclear, 200 mc/mmol), dissolved in 5 ml H_2O, was added drop by drop. The solution was allowed to stand overnight at 4° and then 25 mg of unlabeled borohydride was added. The t.l.c. indicated complete reduction. The reaction mixture was partitioned between ether and water; the ether extracts were evaporated at reduced pressure, and the residue was taken-up in a small volume of ether and 1.5 g of unlabeled benzyl 3,4-*O*-isopropylidene-β-D-ribopyranoside was added. The addition of petroleum ether gave 1.40 g of chromatographically pure product which had a specific activity of 0.232 mc/mmol, a radiochemical yield of 77%.

D-*Ribose-2-t*

Benzyl 3,4-*O*-isopropylidene-β-D-ribopyranoside-2-*t* (250 mg) was hydrolyzed with 10 ml of 1 *N* HCl at 100° for 3 h. The reaction mixture was passed through Rexyn 203 (OH^-) and 6 g unlabeled D-ribose was added to the eluate as carrier. After removal of the solvent the product was recrystallized three times from ethanol to a constant specific activity of 4.14 μCi/mmol; 4.0 g of material equivalent to a radiochemical yield of 54% was obtained.

To show that label was present only at C-2 the following experiments were performed. D-Ribose-2-*t* was reduced with borohydride to ribitol which then was oxidized with periodic acid – bicarbonate as described by Reeves (8) and the dimedon derivative of formaldehyde isolated. The dimedon derivative from a ribose sample containing 215 000 c.p.m. had 78 c.p.m.; indicating that less than 0.04% of the radioactivity was at C-1. The phenylosazone of ribose was then prepared and found to be free of radioactivity, indicating that there was no tritium bound to C-1, -3, -4, or -5 and that the counts observed in the dimedon derivative could have been due to contamination with formic acid or to migration of label under the alkaline conditions of the borohydride reduction.

Treatment of Ribose-2-t with Alkali

Into a 10 ml vial equipped with a rubber septum 1 ml of sugar solution was injected. An outlet needle was inserted in the septum and the vial was flushed with nitrogen (or oxygen) for 10 min and then degassed potassium hydroxide (5 ml) was injected. Vials were incubated in a constant temperature water bath and aliquots of approximately 0.5 ml were removed at various times and assayed for tritium as described below.

For larger scale experiments the reaction vessel consisted of a flask having a side-arm equipped with a rubber septum and an outlet to a gas reservoir. The reaction vessel was immersed in a constant temperature bath and attached to a shaker mechanism. The reaction vessel was filled with an alkaline solution and flushed repeatedly with oxygen or nitrogen with shaking. The reaction was initiated by injecting the sugar solution with a calibrated syringe. Aliquots were removed at intervals by syringe through the septum.

Assay of Tritium Release to Solvent

Samples were placed in a standard taper test-tube and shell-frozen in liquid nitrogen. The sample tube was attached to one side of a standard taper U-tube equipped with a stop-cock and another standard taper test tube was attached to the other arm. The system was evacuated to 0.1 mm Hg while the sample side was immersed in liquid nitrogen. After the system had been evacuated, the stop-cock was closed and the receiver tube was placed in liquid-nitrogen. In 15 min enough water had sublimed so that 0.100 ml could be obtained and assayed for tritium by scintillation counting.

Isolation of Products from the Alkaline Treatment of D-Ribose-2-t

The oxidation mixture was passed through a column containing 100 ml Dowex 50 (H^+) to remove potassium

ions. The acidic solution was then passed over 25 ml Rexyn 203 (free base form) to remove acids; and the acids were recovered by elution with potassium hydroxide. The salts so obtained were converted back to the acids by treatment with Dowex 50 (H^+). The acidic fraction was resolved by preparative paper chromatography. The neutral sugar fraction was concentrated and applied to a column of Rexyn 101 (Ba^+) for fractionation (21).

Reduction of D-*Arabinose-t*

Unlabeled D-arabinose (53.5 mg) was added to a sample of 1.96 mg arabinose-*t* isolated from a reaction mixture; the mixture was dissolved in 7 ml of water and the solution was stirred at 0°. The p*H* was monitored and kept below 9 with dilute acetic acid while 50 mg of sodium borohydride dissolved in 5 ml water was added dropwise. The reaction was kept for 24 h at 4°. Excess borohydride was destroyed with acetic acid and the reaction mixture was passed over a small column of Dowex 50 (H^+). The eluate was evaporated to dryness, with five additional evaporations from 10 ml of methanol. The yield was 50.5 mg. The product gave a quantitative yield of pentabenzoate m.p. 151.0–151.7, undepressed on admixture with the authentic material.

Results and Discussion

This study was undertaken to determine the role of enolization in the oxidative degradation of sugars, specifically the pentoses. Two hypotheses have been advanced for the analogous degradation in the hexose series. Bamford and Collins (9), supported by subsequent experiments of Dubourg and Naffa (10), proposed that in the oxidation of glucose an enediolate anion intermediate is attacked by oxygen to produce a peroxide which cyclizes and decomposes to give formic and arabinonic acids. This mechanism requires that the starting material lose the proton at C-2.

Samuelson and Thede (11) have recently reported that after 26 h in 4.5 *N* sodium hydroxide, at 1 atm oxygen pressure, glucose was converted to a mixture of at least 13 monoprotic carboxylic acids, as well as small amounts of mannose, fructose, and arabinose. These workers suggested the intermediate formation of osones, because D-glucosone gives approximately the same mixture of products as D-glucose does. This mechanism also requires loss of the proton from C-2 of the starting material.

Because the reaction of the pentoses with oxygen at neutral p*H* values is negligible compared to the base-catalyzed reaction, it is apparent that some reaction of the sugar with base must occur prior to the actual oxidation. Thus, it is also of interest to study the reaction of the labeled sugar with alkali in the absence of oxygen. Many other base-catalyzed sugar reactions such as the Lobry de Bruyn – Alberda van Ekenstein rearrangement and the formation of saccharinic acids occur by mechanisms that have been much debated but which usually are thought to involve enolization as the first step (12). Although D-ribose-2-*t* might appear to be an ideal substrate to test the proposal that alkaline rearrangements of sugars involve an initial enolization resulting in the loss of the C-2 hydrogen, it is not. Reactions involving breaking the carbon–tritium bond in the rate-determining step can be expected to differ in rate from those involving the carbon–hydrogen bond by a factor of 10, or more. In addition, ribose is an unusual sugar, it reacts many times faster than other sugars in reactions involving ring opening. Nevertheless, one would expect that the loss of tritium to solvent would occur if enolization is a principal event and that other reactions involving the hydrogen at C-2 would be reflected by qualitatively, if not quantitatively, similar reactions of tritium.

The release of tritium to water in the absence of oxygen in 0.88 *M* potassium hydroxide solution is shown in Fig. 1. The conditions were chosen to be pseudo-first order in D-ribose-2-*t*, that is with a large relative excess of alkali so that the formation of acidic products would not decrease the effective hydroxide ion concentration. The first 50% of the reaction follows first-order kinetics, but it is clear that a portion of the tritium (40%) is incorporated into a position from which base-catalyzed exchange with solvent is very slow. The initial rate of tritium release to solvent is strongly temperature dependent and increases five-fold between 30 and 40°.

Although no extensive study was made, we find that tritium release is not first-order with respect to base concentration.

To investigate the effect of oxygen on tritium release from D-ribose-2-*t*, experiments were carried out at 25° in the presence and absence of oxygen. The results obtained are given in Table 1. The presence of oxygen depresses the rate of tritium release to the solvent by a factor of two. The release of tritium in volatile acids was estimated by distillation of an acidified sample of the reaction mixture; the acid was shown to be formic acid by DuClaux distillation (13) and by n.m.r. spectroscopic examination of the neutralized distillate.

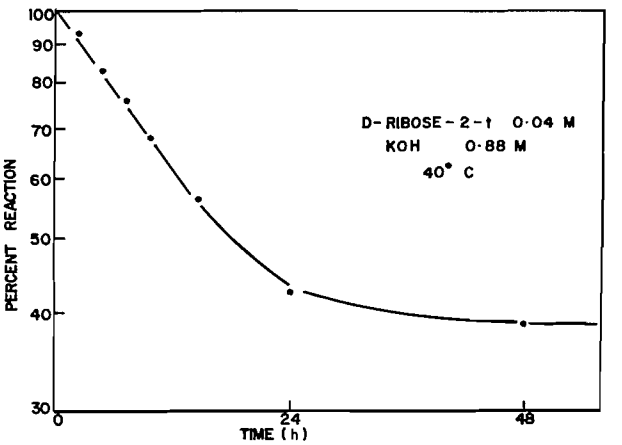


FIG. 1. The release of tritium to solvent from D-ribose-2-*t* in 0.88 *M* aqueous potassium hydroxide at 40°.

TABLE 1. Products from ribose-2-*t* (0.05 *M*) in 100 ml of 0.833 *M* KOH for 12 h at 25.00 ± 0.05 °C

Gas	Acid produced (mmol)	Percent of total counts released to: Solvent	Formic acid	Other acids
O_2	6.03	2.1	17.5	—
	5.92	2.0	17.2	—
N_2	1.73	4.15	0.0	1.87
	1.67	4.14	0.0	1.91

TABLE 2. Specific activity of acids from the aerobic degradation of D-ribose-2-*t* in c.p.m. per mmol

	Experiment 1	Experiment 2
Erythronic acid	5.1×10^4	1.2×10^5
Glyceric acid	3.1×10^4	3.8×10^4
Glycollic acid	2.0×10^5	2.6×10^5

The acids formed in the aerobic degradation of D-ribose-2-*t* were separated by paper chromatography, titrated and counted; their specific activities are listed in Table 2. The data for erythronic acid do not agree well and the counts in that fraction may be due partly to contamination with small amounts of starting material. There is no doubt, however, that tritium is present in significant amounts in glyceric and glycolic acids.

Acids produced under anaerobic conditions when 22% of the reducing sugar had been transformed were isolated, after removal of cations, by adsorption on a weakly basic anion exchange resin and elution with alkali. The whole fraction contained 1.5×10^4 c.p.m., 1.9% of the original activity; equivalent to 50% of the tritium released to solvent. The acids were regenerated by removal of solvent, converted to silyl derivatives, and examined by g.l.c. Two main peaks and at least eight minor peaks were present. The major peaks are probably the saccharinic acids, 3-deoxy-D-*erythro*-, and 3-deoxy-D-*threo*-pentonic acids. Attempts to isolate and separate these acids by fractional crystallization of their quinine salts (14) were unsuccessful.

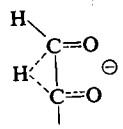
The reaction of D-ribose-2-*t* was further examined by isolating the pentoses (neutral sugar fraction) after 12 h reaction and determining the proportion of label present at C-1 (or C-5) by reduction to the penitol with sodium borohydride, oxidation with periodate, and isolation of the dimedon derivative. The results of these experiments are shown in Table 3. Ribose was not purified after reisolation and the label present in the dimedon fraction, which is significantly greater than in the dimedon fraction from the starting material, could be due to the presence of small amounts of other pentoses or pentuloses. The increase in specific activity of the neutral sugars allows an approximate calculation to be made of the isotope effect. Under the conditions used in the aerobic degradation experiments 50% of the protium sugar had reacted in the 12 h reaction period. During the same time 19% of the tritium sugar had reacted; an apparent isotope effect (k_H/k_T) of 3.3. A similar calculation gives an isotope effect of 4.1 in the anaerobic reaction in which approximately 22% of the reducing sugar had been converted. By themselves, isotope effects cannot be used to demonstrate the operation of a specific mechanism, however, the isotope effects observed in

TABLE 3. The distribution of tritium in the neutral sugar fraction after incubating D-ribose-2-*t* in 0.833 *N* potassium hydroxide

	Percent reaction	Total neutral sugars (c.p.m./mmol)	Dimedon derivative (C-1 and -5) (c.p.m./mmol)
Original ribose	0	7.83×10^5	$<1.5\times10^2$
"Ribose" from aerobic treatment	50	1.23×10^6 1.26×10^6	2.09×10^3 2.12×10^3
"Ribose" from anaerobic treatment	22	1.11×10^6 1.15×10^6	1.3×10^3 1.2×10^3

this study are of the order expected for a hydride shift (15) but are smaller than expected for a simple enolization reaction (16, 17). The principal reaction occurring in the absence of oxygen is in a large part attributable to enolization since tritium is lost to the solvent. The small isotope effect is not incompatible with an enolization reaction even though a much larger effect is observed with acetone (16). The magnitude of the isotope effect depends on the symmetry of the transition state (15), changes in bending frequencies (18), the linearity of the transition state (19), and the occurrence of prerate-determining equilibria (20). All of these factors could be important in producing the small isotope effect observed here; however, the data do not permit a more detailed analysis of the mechanism.

The principal reaction in the presence of oxygen involves the transfer of hydrogen from C-2 to -1; it cannot be accomplished by an enolization reaction since tritium lost to solvent cannot return. In this case, the small isotope effect is probably attributable to the occurrence of a cyclic transition state, **6** (14, 18).



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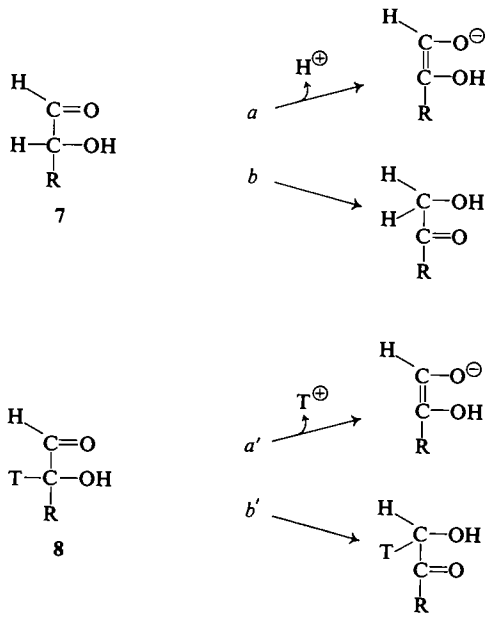
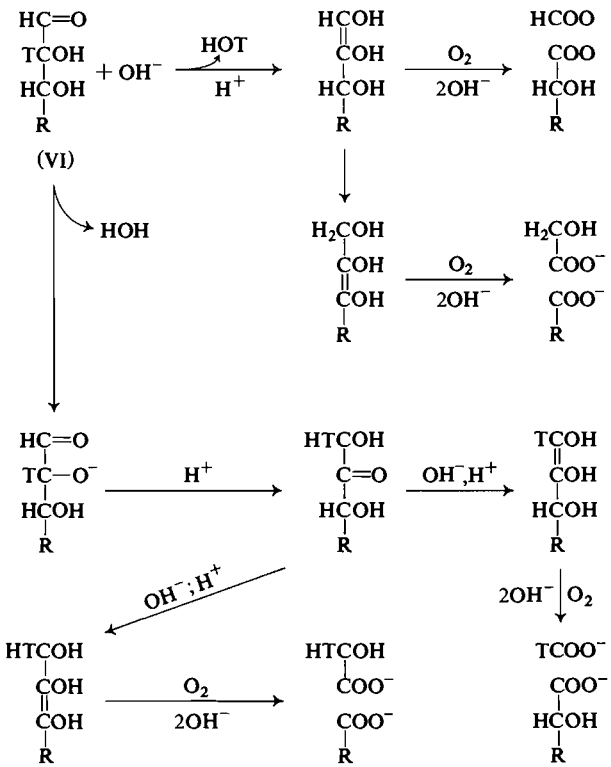
The possibility that tritium ribose and protium ribose react by different pathways must be considered. In Scheme 2 a hypothetical reaction sequence is shown in which two pathways are available to ribose; pathway *a* for enolization and pathway *b* for hydride shift. If we assume that protium ribose **7** reacts 100 times faster through pathway *a* than through pathway *b* and that the enolization isotope effect is 20 (pathway *a'*) while that for the hydride shift is 2 (pathway *b'*), then, of the tritium ribose **8** that



SCHEME 2

had reacted at any given time, 10 parts would have traversed pathway *a'* and one part pathway *b'*. Thus, the observation of an overall isotope effect less than 5 allows us to conclude that a substantial portion of the protium ribose is reacting by the same pathways as the tritium ribose, and that under both aerobic and anaerobic conditions a hydride shift to C-1 is important.

The evidence for a hydride shift in the aerobic degradation of D-ribose-2-*t* is very convincing, 17% of the label is present in formic acid and a small amount in the dimedon derivative from the recovered neutral sugars. In the anaerobic system, however, the evidence is weaker, although there is a significant increase in the tritium content of the dimedon derivative (*i.e.*, at C-1), and this is hard to explain by other than



SCHEME 3

a hydride shift mechanism. To further demonstrate that a hydride shift does occur in this system, D-arabinose was isolated from the neutral sugar fraction of the anaerobic degradation when the reaction had proceeded to 22% completion (as judged by reducing sugar content). D-Arabinose was isolated by chromatography on the barium form of Rexyn 101 as described by Jones and Wall (21). Two fractions were obtained, the first fraction containing arabinose had 4% of the radioactivity of the neutral sugar fraction; the second fraction had 96% of the radioactivity. Unlabeled D-arabinose was added to the material of the first peak and the whole reduced to D-arabinitol with sodium borohydride. After recrystallization a sample was subjected to periodate oxidation and the dimedon derivative of the formaldehyde so released was prepared. A second sample of the D-arabinitol was converted to the pentabenzoate and recrystallized to constant specific activity. The results of this experiment are presented in Table 4. It is clear that D-arabinose is formed from D-ribose in the anaerobic reaction and that it contains a significant amount of radioactivity.

TABLE 4. Radioactivity present in D-arabinose derived from ribose-2-*t* treated with 0.833 *M* KOH, 12 h at 25.00 ± 0.05 °C; oxygen excluded

	Specific activity (c.p.m./mmol)
Starting material (ribose-2-*t*)	6.36×10^6
Arabinitol pentabenzoate	$2.13 \pm 0.02 \times 10^4$
Formaldehyde dimedon of periodate oxidized arabitol	$7.53 \pm 0.07 \times 10^3$
Arabinose-*t* from reaction mixture (= 28.3 × specific activity penta-benzoate)	6.03×10^5

At least 70% of the activity in the D-arabinose is present in hydrogen attached to C-1. The overall isotope effect for the conversion of D-arabinose can be estimated to be approximately 10 from the specific activities of the starting material and product. The conversion by hydride shift involves two equivalent steps, that is, conversion of D-ribose to an intermediate and conversion of the intermediate back to the epimer D-arabinose. Both steps should show approximately the same isotope effect and the overall isotope effect is the multiple of those for the

individual steps. Thus, in the conversion of D-ribose to D-arabinose each step has an isotope effect of approximately 3.0 consistent with the observed effect for the transfer of tritium from C-2 to -1.

The results of this investigation indicate that an intermediate enediol is not a necessary intermediate in the alkaline conversion of one aldose to another. This may explain the many contradictory findings in the literature concerning the incorporation of solvent deuterium during the treatment of carbohydrates with alkali. Thus, one reviewer (22) concluded that a pathway for the Lobry de Bruyn – Alberda von Ekenstein which does not permit the exchange of carbon bound hydrogen with the solvent was invalid, while another author (23) concluded that the reaction can proceed by two mechanisms (enolization and hydride transfer) the operation of which depends on the nature of the catalyst, temperature, and structure of reactant. Scheme 3 is based on these mechanisms; in it the reactions of a tritiated molecule are shown. The protium isomer would follow the same pathways in different proportion. This scheme accounts only for the major products of the aerobic conversion of D-ribose in alkali. The overproduction of formic acid (1, 9) and the formation of saccharinic acids are not shown since our data do not permit us to comment on the validity of proposals that have been made to explain their formation (9, 11).

Isbell *et al.* (17) have shown that sugars take up tritium from solvent in alkaline medium and have interpreted their data in terms of the rate of primary enolization of the sugars with incorporation at C-2 of the aldoses. Our data would suggest that this incorporation may be a secondary event, depending upon the prior formation of a ketose anion.

It is possible that our findings are peculiar to the D-ribose-2-*t*, potassium hydroxide system, but it appears that this is the first direct demonstration of the operation of a mechanism other than enolization for carbohydrate interconversions. Experiments are in progress in this laboratory to investigate the interconversion mechanism for glucose, fructose, and mannose in order to test the generality of the findings for ribose-2-*t*.

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