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Furanose ring anomerization: kinetic and thermodynamic studies of the D-2-pentuloses by ^{13}C-n.m.r. spectroscopy

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ABSTRACT

The tautomeric compositions of D-*erythro*-2-pentulose (D-ribulose) and D-*threo*-2-pentulose (D-xylulose) in aqueous solution have been studied by ^{13}C-n.m.r. spectroscopy at various temperatures using 2-^{13}C-substituted compounds. The α-furanose, β-furanose, and acyclic carbonyl (keto) forms were detected at all temperatures, whereas the acyclic hydrate (*gem*-diol) form was not observed. The percentage of keto form increased with increasing temperature, at the expense of the furanose forms. Thermodynamic (ΔG^0, ΔH^0, ΔS^0) and kinetic parameters for the interconversion of α- and β-furanoses with the acyclic carbonyl form were determined and compared with those determined under similar conditions for the structurally-related aldotetrofuranoses. The ring-opening rate constants (k_{open}) measured by ^{13}C saturation-transfer n.m.r. spectroscopy in 50mM sodium acetate (pH 4.0) at 55° were as follows: β-threofuranose (0.65 s^{-1}) > α-erythrofuranose (0.51 s^{-1}) > β-erythrofuranose (0.37 s^{-1}) ≈ β-*threo*-2-pentulofuranose (0.35 s^{-1}) > α-threofuranose (0.25 s^{-1}) > α-*threo*-2-pentulofuranose (0.20 s^{-1}) ≈ α-*erythro*-2-pentulofuranose (0.18 s^{-1}) ≈ β-*erythro*-2-pentulofuranose (0.18 s^{-1}). Within each structural type the pentulofuranose anomer having O-2 and O-3 cis (O-1 and O-2 cis in aldotetrofuranoses) opens faster than, or at a similar rate to, the alternative anomer having these oxygen atoms trans. Ring-closing rate constants (k_{close}), calculated from k_{open} and K_{eq}, decrease in the order β-erythrofuranose (15 s^{-1}) > β-threofuranose (12 s^{-1}) > α-erythrofuranose (9.9 s^{-1}) > α-threofuranose (6.2 s^{-1}) > β-*threo*-2-pentulofuranose (0.71 s^{-1}) > α-*erythro*-2-pentulofuranose (0.38 s^{-1}) > α-*threo*-2-pentulofuranose (0.13 s^{-1}) ≈ β-*erythro*-2-pentulofuranose (0.13 s^{-1}). Replacement of H-1 in aldotetrofuranoses by a hydroxymethyl group (*i.e.*, conversion to 2-pentuloses) significantly decreases the ring-opening and ring-closing rate constants of furanose anomerization.

INTRODUCTION

Keto sugars, and their phosphate mono- and di-esters, are important metabolites in biological systems. For example, phosphate esters of 2-ketoheptoses (*e.g.*, D-sedoheptulose), 2-ketohexoses (*e.g.*, D-fructose) and 2-ketopentoses (*e.g.*, D-*erythro*-2-pentulose, **1**) (Scheme 1) are important metabolic intermediates in glycolysis and the pentose monophosphate shunt in heterotrophs, and play a key role in carbon dioxide fixation in autotrophs (*e.g.*, D-ribulose 1,5-bisphosphate). The D-2-pentuloses, D-*erythro*-2-pentulose (D-ribulose, **1**) and D-*threo*-2-pentulose (D-xylulose, **2**), form the simplest 2-pentulofuranose rings [α-D-*erythro*-2-pentulofuranose (**1a**), β-D-*erythro*-2-pentulofuranose (**1b**), α-D-*threo*-2-pentulofuranose (**2a**), and β-D-*threo*-2-pentulofuranose (**2b**) (Scheme

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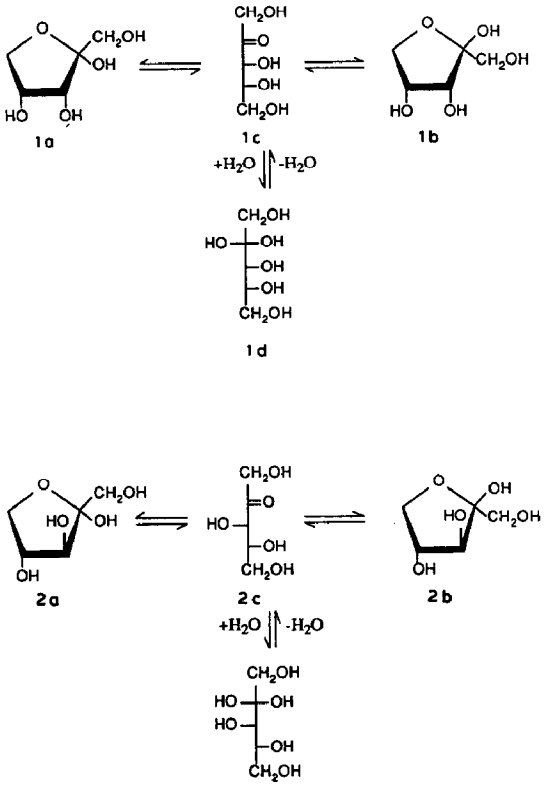
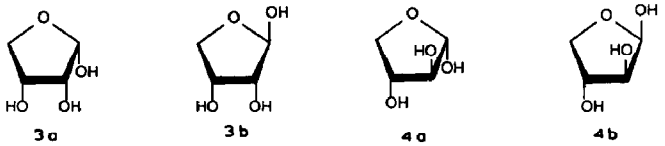


Scheme 1

1)], and may be considered the parent 2-ketofuranose rings. They may also be viewed as alkylated (hydroxymethylated) derivatives of the aldotetrofuranoses α- and β-D-erythrofuranose (**3a, 3b**) and α- and β-D-threofuranose (**4a, 4b**), having a hydroxymethyl group substituted for a hydrogen atom at the furanose anomeric carbon.

In a previous study[1] we described chemical and enzymic methods for the preparation of **1** and **2** substituted with ^{13}C at C-1 and C-2. The ^{1}H- and ^{13}C-n.m.r. spectra of the natural, and the 1-^{13}C- and 2-^{13}C-substituted compounds were fully assigned, and the conformational properties of the three n.m.r.-observable forms (α- and β-furanose and acyclic carbonyl forms) in deuterium oxide solution were inferred from their ^{1}H–^{1}H, ^{13}C–^{1}H and ^{13}C–^{13}C spin-coupling constants.

In the present study, we have examined the effect of temperature on the distribution of 2-ketopentose tautomers in aqueous solution by ^{13}C-n.m.r., and have evaluated the ring-opening (k_{open}) and ring-closing (k_{close}) rate constants of 2-pentulofuranose



anomerization by saturation-transfer n.m.r. methods[2–5]. The data are compared to those obtained under the same conditions on the aldotetrofuranoses (**3, 4**) in order to assess the effect of alkylation of the anomeric carbon on the thermodynamics and kinetics of furanose-ring anomerization.

EXPERIMENTAL

Materials. — Deuterium oxide (98 atom-% ^2H) was purchased from Cambridge Isotope Laboratories. D-(2-^{13}C)2-Pentuloses **1** and **2** and the D-(1-^{13}C)aldotetroses **3** and **4** were synthesized and purified by methods described previously[1,6].

Preparation of sample solutions. — Aqueous solutions of the D-pentuloses were concentrated to ~0.5 mL at 30° *in vacuo* after batchwise and separate treatment with excess Dowex HCR-W2 (H^+) and Dowex 1-X8 (OAc^-) ion-exchange resins. Sodium acetate buffer and ^2H_2O were added to achieve a final sugar concentration of 0.3M as determined by g.l.c.[1]. The pH of the solution was adjusted to 4.0 with M HCl and M NaOH. Final solutions (2 mL) were transferred to 10 mm n.m.r. tubes (Wilmad), which were fit with teflon vortex suppressors and sealed with plastic caps.

Instrumentation. — ^{13}C-N.m.r. spectra were obtained on a Nicolet NT-300 300 MHz superconducting FT-n.m.r. spectrometer operating at 75 MHz and equipped with a variable temperature accessory, quadrature phase-detection, and a 293B pulse programmer. ^1H-Decoupled ^{13}C-n.m.r. spectra used to evaluate the solution composition of 2-^{13}C-substituted 2-pentuloses were obtained with long relaxation delays (>90 s), and with broadband ^1H-decoupling applied only during signal acquisition to eliminate potential differential n.O.e. effects between tautomers. Spin-lattice relaxation times (T_1) were determined by the inversion-recovery method[7].

Saturation-transfer n.m.r. (ST-n.m.r.) spectra (^{13}C) were obtained on the NT-300 spectrometer, which was equipped with a broadband F3-decoupler to supply the saturating r.f. signal at the carbonyl resonance. Saturation-transfer spectra were recorded with saturation times ranging from 5 μs to 25 s and relaxation delays of >90 s. At least ten (10) saturation times were employed in each experiment (4 transients per spectrum), and signal intensities were plotted semilogarithmically as described previously[2] in order to evaluate τ_1 from the slope. Using T_1 and τ_1 values, ring-opening rate constants (k_{open}) in s^{-1} were determined from the relationship[2] $1/\tau_1 = k_{open} + 1/T_1$. Ring-closing rate constants (k_{close}) were calculated from k_{open} and the relevant equilibrium constant, K_{eq}.

Solution pH measurements were made at 23° with a microelectrode purchased from Microelectrodes, Inc. and a Corning Model 125 pH meter. Since the proportion of ^2H_2O in the sample solutions was small (15% v/v), no correction was made to account for deuterium isotope effects[8].

Sample temperature in the spectrometer probe was measured with a Fluke Model 2160A digital thermometer equipped with a copper–constantan thermocouple. The thermocouple was inserted securely with the aid of a plastic cap into a 10 mm n.m.r. tube containing an unenriched sample, the assembly was lowered into the probe, and the

solution temperature was recorded after it had stabilized (~0.5 h). The tube was then replaced by one containing an enriched sample, otherwise identical to the previous one, and this was allowed to equilibrate for 0.5 h before measurements were initiated. This procedure was adopted in order to avoid potential contamination of the enriched experimental sample by the metal thermocouple.

RESULTS AND DISCUSSION

Tautomeric composition of 2-pentuloses **1** *and* **2** *in aqueous solution.* — ^{13}C-Substitution at the anomeric carbon (C-2) of the 2-pentuloses facilitates the detection and quantitation of their tautomeric forms in solution by ^{13}C-n.m.r. The ^{13}C spectra of D-*erythro*-(2-^{13}C)-2-pentulose (D-ribulose, **1**) and D-*threo*-(2-^{13}C)-2-pentulose (D-xylulose, **2**) in aqueous solution each contain three major signals. For **1**, the C-2 signals have been assigned previously[1] to the α-furanose (**1a**; 104.0 p.p.m.), β-furanose (**1b**; 107.0 p.p.m.), and keto (**1c**; 213.9 p.p.m.) forms, which constitute 54.8, 18.9, and 26.4% of the mixture, respectively, at 55° (Table I). For **2**, the C-2 signals (Fig. 1) are assigned to the α-furanose (**2a**; 107.2 p.p.m.), β-furanose (**2b**; 104.4 p.p.m.), and keto (**2c**; 214.4 p.p.m.) forms[1], whose proportions are 16.9, 55.8, and 27.5%, respectively, at 55° (Table I). Spin-lattice relaxation times (T_1) for the unprotonated C-2 carbons of **1** and **2** (Table II) were significantly longer than those for the protonated C-1 carbons of aldoses[9]; but the long relaxation delays employed in determining the spectra should ensure reliable quantitation of the data.

In the 2-ketopentofuranoses, anomers having O-2 and O-3 *cis* predominate over those having these atoms *trans*[1], whereas in the aldotetrofuranoses, the O-1, O-2-*cis* anomers are less abundant[2,6,10a]. Presumably, the *cis*-2,3 interactions (OH–CH_2OH, OH–H) present in the O-2,O-3-*trans* anomers **1b** and **2a** are more destabilizing than those present (OH–OH, H–CH_2OH) in the O-2,O-3-*cis* anomers **1a** and **2b**. The effect of C-3 configuration on anomeric distribution in the 2-ketopentofuranoses is small, in contrast to the analogous effect of C-2 configuration on the anomeric distributions of aldotetrofuranoses[6]; the ratio of more to less abundant anomer in **1** is the same as that in **2** (Table I), whereas the ratios differ significantly for **3** and **4** (ref. 6). It is interesting to note that aqueous solutions of 6-*O*-methyl-D-fructose (**5**), which is structurally related to **2**, show a smaller α:β ratio (0.26) at 40° (ref. 3), than solutions of **2** (0.30, Table I). Presumably the 1,3-interaction between the exocyclic CH_2OCH_3 substituent at C-4 and the exocyclic CH_2OH group at C-2 reduces the stability of the α-furanose anomer of **5**.

Acyclic carbonyl forms are more abundant in aqueous solutions of **1** and **2** (~ 27% at 55°) than in aqueous solutions of aldotetroses **3** and **4** (~ 2.4% at 51°)[2]. However, the acyclic hydrate (*gem*-diol) forms are significantly more abundant in the aldotetroses (~ 10% at 25°)[2]; the acyclic hydrate forms **1d** and **2d** could not be detected in solutions of 2-^{13}C-substituted 2-pentuloses. It is interesting to note that aqueous solutions of D-(2-^{13}C)ribulose 1,5-bisphosphate at pH 7.6 contain 88% carbonyl form (**6a**) and 12% hydrate (**6b**) (ref. 10b). If the carbonyl:hydrate ratio of 7:3 were maintained in the 2-pentuloses, aqueous solutions of **1** would contain ~3% hydrate form at

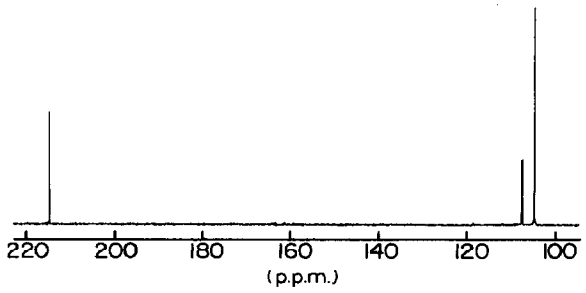


Fig. 1. The ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-(2-^{13}C)xylulose [0.3M, 50mM acetate buffer, 15% (v/v) $^{2}H_2O$, pH 4.0, 50°], showing signals for three tautomeric forms: α-furanose (**2a**), 107.2; β-furanose (**2b**), 104.4; and acyclic keto (**2c**), 214.4 p.p.m.

TABLE I

Composition of D-pentuloses in solution at various temperatures[a]

Compound	*Percent of total*[b]			
	Temp.(°)	*α-f*	*β-f*	*keto*
D-*erythro*-Pentulose (1)	20	62.8	20.4	16.8
	32	60.9	20.4	18.7
	42	60.5	18.7	20.8
	48	56.3	18.8	24.9
	53	56.1	18.8	25.1
	55	54.8	18.9	26.4
	58	54.3	18.6	27.1
D-*threo*-Pentulose (2)	26	18.1	62.3	19.6
	35	17.5	62.3	20.2
	40	17.9	59.8	22.4
	45	16.8	59.8	23.4
	50	17.0	58.9	24.2
	55	16.9	55.8	27.5

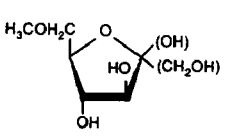
[a]Conditions: 0.3M pentulose, 15% (v/v) $^{2}H_2O$, 50mM acetate buffer, pH 4.0. [b]*α-f* = α-furanose, *β-f* = β-furanose.

TABLE II

^{13}C Spin-lattice relaxation times[a] (T_1) for D-pentuloses

Compound[b]	*Carbon* T_1 (*S*)		
	C-2α	*C-2β*	*C-2, keto*
D-*erythro*-Pentulose **1**	17.0	16.6	18.8
D-*threo*-Pentulose **2**	21.8	20.1	22.1

[a]Values were obtained by the inversion-recovery method[7]. [b]Conditions: 0.3M D-(2-^{13}C)pentulose, 50mM acetate buffer, 15% (v/v) $^{2}H_2O$, pH 4.0, 55°.



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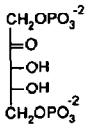
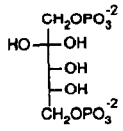


6a



6b

40°, which would be readily detected in the ^{13}C-n.m.r. spectrum of the 2-^{13}C-substituted compound, but is not observed. The enhanced hydrate:carbonyl ratio in the bisphosphate may, therefore, be due to the electron-withdrawing nature of the phosphate group at C-1, making the carbonyl carbon more prone to attack by water.

The amount of acyclic carbonyl form in aqueous solutions of 6-*O*-methyl-D-fructose (**5**) is notably smaller (3.6% at 40°) (ref. 3) than that found in solutions of the structurally-related D-*threo*-2-pentulose **2** (~22% at 40°, Table I). Apparently alkylation of furanoses at sites other than the anomeric carbon reduces the proportion of acyclic carbonyl form in solution[11], presumably because Thorpe-Ingold effects[12–14] favor the ring-closed forms both thermodynamically and kinetically.

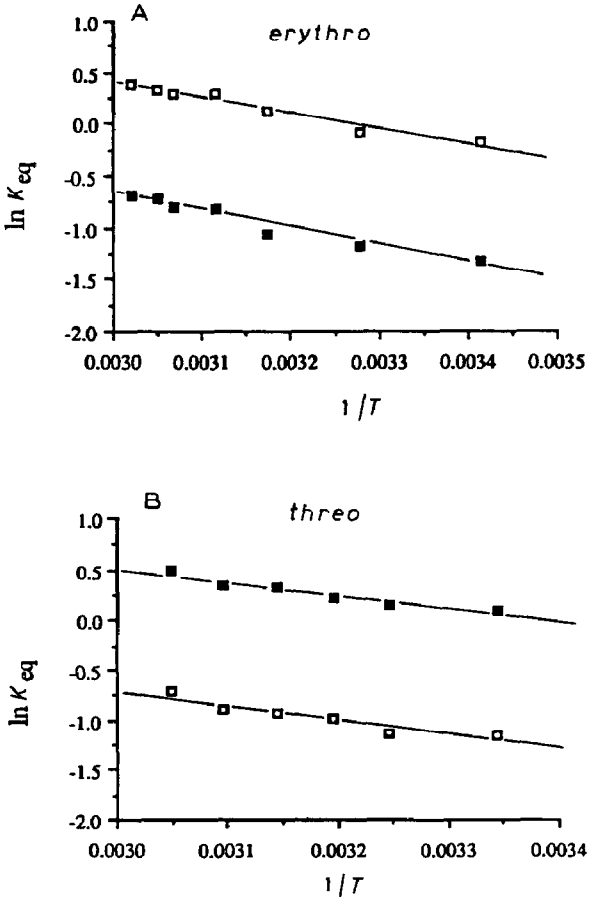


Fig. 2. Temperature dependence of K_{eq} ([carbonyl]/[cyclic]) for A, D-*erythro*-2-pentulose; B, D-*threo*-2-pentulose. Symbols: ■, *α*-furanose; □, *β*-furanose. Experimental conditions are described in Table I, footnote *a*.

Effect of temperature on the tautomeric composition of 2-pentuloses **1** *and* **2**. — The thermodynamic constants, ΔG^0, ΔH^0 and ΔS^0, for the tautomeric equilibria of the 2-pentuloses **1** and **2** were evaluated by measuring the proportions of furanose and carbonyl forms as a function of temperature. As shown in Table I, increasing temperature increases the proportion of carbonyl form and decreases the proportions of furanoses. Plots of ln K_{eq}, defined as [carbonyl]/[cyclic], against $1/T$ were approximately linear (Fig. 2) and yielded the following enthalpies of conversion (ΔH^0) for ring-closing: α-D-*erythro*-2-pentulofuranose (**1a**), -3.3 ± 0.8 kcal.mol^{-1}; β-D-*erythro*-2-pentulofuranose (**1b**), -3.1 ± 0.6 kcal.mol^{-1}; α-D-*threo*-2-pentulofuranose (**2a**), -2.7 ± 0.9 kcal.mol^{-1}; β-D-*threo*-2-pentulofuranose (**2b**), -2.9 ± 1.3 kcal.mol^{-1}. These values are somewhat less negative than those observed for the D-erythrofuranoses (**3a**, -4.0 kcal.mol^{-1}; **3b**, -5.1 kcal.mol^{-1}) (ref. 14) and the D-threofuranoses (**4a** and **4b**, -6.0 kcal.mol^{-1}) (ref. 2). Entropies of conversion for ring-closing were as follows: α-D-*erythro*-2-pentulofuranose, -8.6 ± 2.7 cal.mol^{-1}.K^{-1}; β-D-*erythro*-2-pentulofuranose, -10 ± 2.0 cal.mol^{-1}.K^{-1}; α-D-*threo*-pentulofuranose, -9.2 ± 3.0 cal.mol^{-1}.K^{-1}; β-D-*threo*-2-pentulofuranose, -7.4 ± 4.3 cal.mol^{-1}.K^{-1}. These values are comparable to those obtained for the tetrofuranoses[2]. It would appear, therefore, that enthalpic factors, rather than entropic factors, are primarily responsible for the increased proportion of acyclic carbonyl form in solutions of 2-pentuloses compared to those of aldotetroses.

Unidirectional rate constants of 2-pentulose anomerization. — Saturation-transfer ^{13}C-n.m.r. spectroscopy[2-5] was used to determine the unidirectional ring-opening rate constants (k_{open}) for **1** and **2**. The carbonyl signal of 2-^{13}C-substituted compounds was saturated for varying times with the F3-decoupler, and the decrease in intensity of the (C-2 signals of the α- and β-furanose forms was observed and quantified. Solution

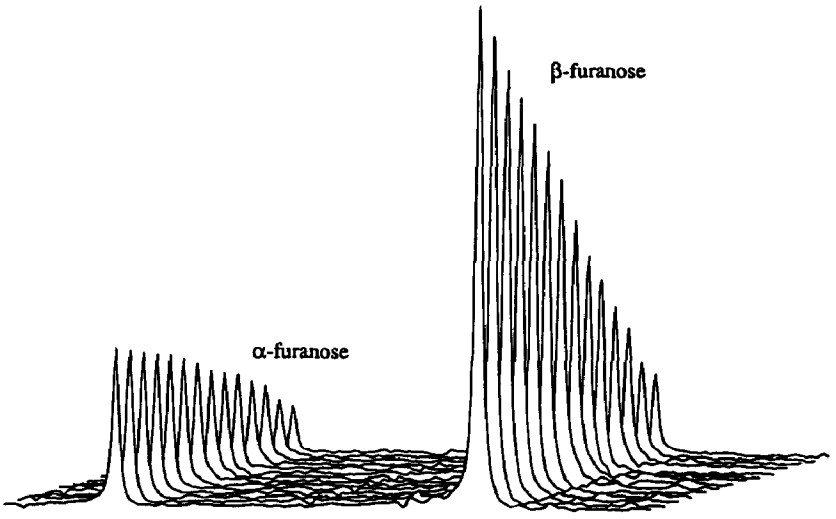


Fig. 3. The decay of intensity of the anomeric carbon (C-2) signals of D-(2-^{13}C)xylulose at 50° due to saturation of C-2 of the acyclic keto form. Saturation times were 100 ms, 500 ms, 1 s, 1.5 s, 2 s, 2.5 s, 3 s, 4 s, 5 s, 6 s, 8 s, 10 s, 15 s, and 20 s. Solution conditions are described in Fig. 1.

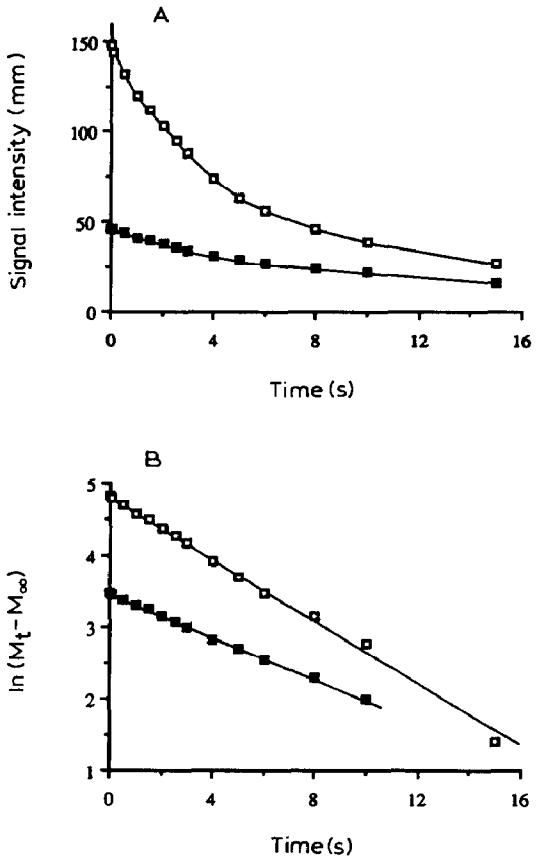


Fig. 4. A, Time course of the decrease in intensity of the anomeric (C-2) signals of D-(2-^{13}C)xylulose (data from Fig. 3); B, semilog plot of the data, used to extract k_{open} for each ring form. Symbols: ■, α-furanose; □, β-furanose; M_t and M_∞ are the magnetization at time t and at equilibrium, respectively.

conditions were chosen [50mM sodium acetate buffer, pH 4.0, 15% (v/v)^2H_2O, 50° and 55°] such that rate constants were sufficient to effect a significant loss (> 30%) of signal intensity at the furanose sites (Figs. 3 and 4A). Ring-opening rate constants (k_{open}) were extracted from semi-log plots of signal intensity data as described previously[2] (Fig. 4B), and are reported for **1** and **2** in Table III. Using k_{open} and K_{eq} values measured at the same temperature, ring-closing rate constants (k_{close}) were obtained and are reported in Table III. Data were also collected for the aldotetrofuranoses **3** and **4** under similar solution conditions to permit a valid comparison (Table III). It should be noted that the error in k_{close} of aldotetrofuranoses is greater than that in k_{close} of 2-pentulofuranoses; the lower abundance of acyclic carbonyl form in solutions of the former makes quantitation less accurate, thereby reducing the accuracy of K_{eq} and k_{close}.

D-*erythro*-2-Pentulose (**1**) and D-*threo*-2-pentulose (**2**) are related structurally to D-erythrose (**3**) and D-threose (**4**), respectively, and valid comparisons may be made within these groups. In **2** and **4**, anomers having the anomeric hydroxyl group and the

TABLE III

Unidirectional rate constants of anomerization for 2-pentulofuranoses[a] and aldotetrofuranoses

Compound	*Rate constants in s^{-1} (±5%)*			
	50°		*55°*	
	k_{open}	k_{close}	k_{open}	k_{close}
α-D-*erythro*-Pentulofuranose (**1a**)	0.076	0.17	0.18	0.38
β-D-*erythro*-Pentulofuranose (**1b**)	0.093	0.070	0.18	0.13
α-D-*threo*-Pentulofuranose (**2a**)	0.10	0.070	0.20	0.13
β-D-*threo*-Pentulofuranose (**2b**)	0.19	0.46	0.35	0.71
α-D-Erythrofuranose (**3a**)	0.38	6.2	0.51	9.9
β-D-Erythrofuranose (**3b**)	0.32	12	0.37	15
α-D-Threofuranose (**4a**)	0.20	4.8	0.25	6.2
β-D-Threofuranose (**4b**)	0.52	9.9	0.65	12

[a]Conditions: 0.3M pentulose, 15% (v/v) ^2H_2O, 50 mM acetate buffer, pH 4.0.

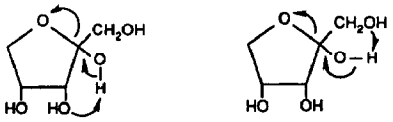


Fig. 5. Possible modes of anchimeric assistance in the ring-opening reaction.

adjacent hydroxyl group (O-2 in aldoses, O-3 in 2-ketoses) *cis* undergo ring-opening more rapidly than anomers having these substituents *trans*. Thus, **4b** opens more rapidly than **4a**, and **2b** opens more rapidly than **2a**, although the difference in rate constants is smaller in the latter case. A mechanism (Fig. 5) involving anchimeric assistance by O-3 in proton abstraction at O-2 during ring-opening of 2-pentulofuranoses may explain these results. A similar mechanism has been proposed recently[15] to explain k_{open} in aldopentofuranoses. While this mechanism[15] implies the direct participation of O-3 (O-2 in aldofuranoses) in proton abstraction at O-2 (O-1 in aldofuranoses), the possibility that an intervening water molecule is the actual proton acceptor cannot be excluded. At a more fundamental level, this mechanism provides a facile means of proton abstraction and may affect the pK_a values of anomeric hydroxyl-proton ionization; the predicted lower pK_as of 2-ketofuranose anomers having O-2 and O-3 *cis* (and of aldofuranose anomers having O-1 and O-2 *cis*), relative to the corresponding *trans* anomers, would thus be responsible for stimulating ring-opening.

Hydroxymethylation at the anomeric carbon appears to reduce ring-opening rate constants (*e.g.*, 0.52 s^{-1} for **4b** and 0.19 s^{-1} for **2b** at 50°, Table III). While **2b** and **4a** are the predominant anomers of **2** and **4**, respectively, **2b** opens more rapidly than **2a** while **4a** opens more slowly than **4b**. These observations provide further evidence that thermodynamic and kinetic stabilities are not correlated in furanose anomerization[15].

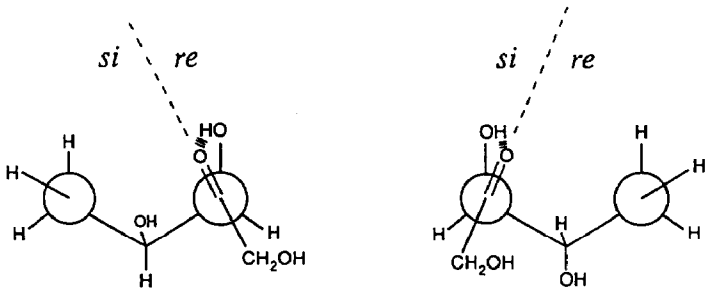
The above-mentioned relationship between relative hydroxyl configuration near

the anomeric center and the magnitude of k_{open} is not as strong in **1** and **3** as in **2** and **4**. While **3a** opens more rapidly than **3b**, the difference is small compared to that found in the threose–xylulose series. Furthermore, for **1a** and **1b** the k_{open} values are essentially the same. Thus, it appears that a *cis* hydroxyl-configuration at C-2 and C-3 of aldofuranoses, and at C-3 and C-4 of 2-pentulofuranoses, reduces the effect of furanose anomeric configuration on k_{open}. This effect has been noted in previous studies of the anomerization of 5-*O*-methylpentoses and 5-deoxypentoses[15], and attributed to possible intramolecular hydrogen bonding that reduces the potency of the proposed anchimeric assistance mechanism[15].

It should be noted that O-1 of 2-pentulofuranoses could participate in the abstraction of O2-H during ring-opening in the same manner as O-3 when the latter is *cis* to O-2 (Fig. 5). However, conformational effects about the C-1–C-2 bond in 2-pentulofuranoses[1] may reduce the potency of this mechanism, since O-1 and O-2 appear to prefer an antiperiplanar geometry. The role of anchimeric assistance, however, in the ring-opening of furanoses has not been firmly established, and the above-noted, O,O-*cis* effect in enhancing k_{open} may be caused by other structural factors, not currently appreciated.

Hydroxymethylation at the anomeric carbon reduces k_{close} more significantly than k_{open} (Table III), thus explaining the larger proportion of acyclic carbonyl form in solutions of **1** and **2** compared to that for **3** and **4**. For example, while conversion of **4b** to **2b** causes a 2.7-fold decrease in k_{open} at 50°, the same conversion causes a 21.5-fold reduction in k_{close}. Thus, the anomerization rates of 2-ketofuranoses are slower than those of structurally-related aldotetrofuranoses owing to reduced reactivity with respect to both ring-opening and ring-closing, which explains previous observations that overall rates of ketose tautomer interconversion are slower than those for aldose tautomer interconversion[16].

In the aldotetrofuranoses, k_{open} decreases in the order **4b** > **3a** > **3b** > **4a** (Table III). On the basis of structural relationships, k_{open} would be expected to decrease in 2-pentulofuranoses in the order **2b** > **1a** > **1b** > **2a**, but **2b** > **2a** ≈ **1a** ≈ **1b** is observed. In the aldotetrofuranoses, ring-closure is fastest to form β-D-erythrofuranose (**3b**) and slowest to form α-D-threofuranose (**4a**), whereas, in the 2-pentulofuranoses, ring-closure is fastest to form β-D-*threo*-2-pentulofuranose (**2b**) and slowest to form β-D-*erythro*-2-pentulofuranose (**1b**) and α-D-*threo*-2-pentulofuranose (**2a**). Clearly, relationships between ring configuration and relative ring-opening and -closing rate constants derived from aldotetrofuranoses do not apply to 2-pentulofuranoses. In **1b** and **2a**, steric factors involving the exocyclic CH_2OH group and O-3 may inhibit ring-closing; steric repulsion of these *cis*-oriented substituents may induce a less-than-optimal attack trajectory by O-5 during ring-closure, thus reducing k_{close}. Furthermore, the preferred acyclic conformations[1] of **1c** and **2c** (Fig. 6) are predisposed towards attack on one face of the carbonyl carbon, producing **1a** and **2b**, respectively. The relatively rigid conformation about the C-2–C-3 bond in **1c** and **2c** (ref. 1) determines the favored face of the carbonyl group. In **1c**, O-5 prefers attack on the *si* face, thus favoring the formation of **1a**. In **2c**, attack by O-5 on the *re* face is preferred, giving **2b** as the product.



1c ($^2G_+$,$^3G_+$) 2c ($^2G_-$,$^3G_-$)

Fig. 6. Preferred conformations of the acyclic keto forms of the 2-pentuloses.

Overall rate constants of conversion, $k_{\alpha\beta}$ and $k_{\beta\alpha}$, may be calculated from the unidirectional rate constants (Table III) using the following equations[2]: $k_{\alpha\beta} = (k_{\alpha o}k_{o\beta})/(k_{o\alpha} + k_{o\beta})$ and $k_{\beta\alpha} = (k_{\beta o}k_{o\alpha})/(k_{o\alpha} + k_{o\beta})$. Thus, $k_{\alpha\beta}$ and $k_{\beta\alpha}$ in s^{-1}, determined from data obtained at 50° (Table III), are as follows: **1**, 0.02, 0.07; **2**, 0.09, 0.03; **3**, 0.25, 0.11; **4**, 0.14, 0.17. As expected, the overall rate constants for the 2-pentulofuranoses are smaller than those for aldotetrofuranoses, but a notably greater difference occurs for $k_{\alpha\beta}$ in **1** and **3**, and for $k_{\beta\alpha}$ in **2** and **4**. Thus, it appears that substitution of CH_2OH for H at the anomeric carbon of aldotetrofuranoses significantly reduces (by a factor of 5–10) the overall rate of conversion of the O-1,O-2-*trans* anomer to the O-1,O-2-*cis* anomer, while the reverse conversion is much less affected (less than a factor of 2). In the aldotetrofuranoses **3** and **4**, the rate-determining step in the overall conversion of cyclic forms is ring-opening (Table III). In contrast, $k_{\alpha\beta}$ in **1** and $k_{\beta\alpha}$ in **2** are limited by ring-closing (Table III). Thus, hydroxymethylation at C-1 of aldotetrofuranoses may result in a transfer of the rate-determining step in overall anomer interconversion to the ring-closing reaction. These results show that, in certain instances, ring-closing may be rate-determining in monosaccharide anomerization.

Hydroxymethylation at C-1 of the aldotetrofuranoses **3** and **4** appears to affect furanose anomerization differently than hydroxymethylation at other sites on the aldotetrofuranose ring. Previous studies[11,15] have shown that hydroxymethylation at C-4 (producing aldopentofuranoses) or at C-3 (producing apiose) significantly *enhances* k_{close} and has only a small effect on k_{open}. In contrast, this study has shown that alkylation at C-1 (producing 2-ketofuranoses) significantly *reduces* k_{close}, with k_{open} less affected.

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