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Synthesis of D-*erythro*-2-pentulose and D-*threo*-2-pentulose and analysis of the ^{13}C- and ^{1}H-n.m.r. spectra of the 1-^{13}C- and 2-^{13}C-substituted sugars

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ABSTRACT

The pentuloses D-*erythro*-2-pentulose (**1**) and D-*threo*-2-pentulose (**2**) and their 1-^{13}C- and 2-^{13}C-substituted derivatives were prepared by hydrogenating the corresponding isotopically normal and ^{13}C-substituted D-pentos-2-uloses with a Pd–carbon catalyst. The *threo* isomer and its labeled derivatives were alternatively prepared from isotopically normal and ^{13}C-substituted D-xyloses with immobilized D-xylose (D-glucose) isomerase (E.C. 5.3.1.5). The equilibrium compositions of **1** and **2** (furanose anomers and acyclic *keto* forms) in ^{2}H$_2$O were determined from ^{13}C-n.m.r. spectra (75 MHz) of the 2-^{13}C-labeled derivatives. The conformational properties of the cyclic and acyclic forms in ^{2}H$_2$O were assessed with the use of ^{1}H–^{1}H, ^{13}C–^{1}H, and ^{13}C–^{13}C spin-coupling constants obtained from ^{1}H-n.m.r. (620 MHz) and ^{13}C-n.m.r. (75 MHz) spectra. Compared with the structurally related aldotetrofuranoses the 2-pentulofuranoses more strongly prefer conformations in which the anomeric hydroxyl group is oriented quasi-axially. The strongly dipolarized carbonyl group in the acyclic *keto* forms of **1** and **2** appears to stabilize chain conformations having O-1 and O-3 eclipsed with the carbonyl oxygen.

INTRODUCTION

In a series of earlier papers the conformational properties of aldofuranoses and methyl aldofuranosides in aqueous (^{2}H$_2$O) solution were studied by high-resolution ^{1}H- and ^{13}C-n.m.r. spectroscopy[1-4]. These studies showed that a reliable analysis of furanose ring conformation cannot be based solely on ^{1}H–^{1}H spin-coupling constants because the number of potential conformational models exceeds the number of couplings available to test these models. While proton chemical shifts are also conformation dependent, it is not straightforward at the present time to correlate these values with conformation except when reference data obtained on related compounds of known conformations are available.

Vicinal carbon–carbon ($^{3}J_{C,C}$) and carbon–proton ($^{3}J_{C,H}$) spin-coupling constants have proven to be valuable in carbohydrate conformational analysis mainly because the number of these couplings is often greater than the number of vicinal proton–proton

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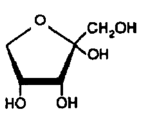
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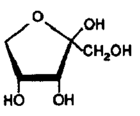
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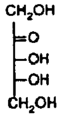
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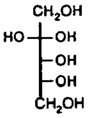
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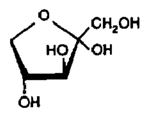
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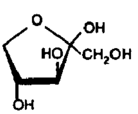
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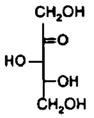
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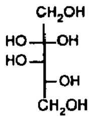
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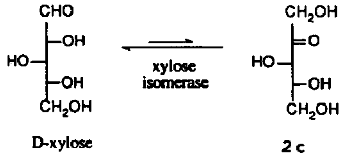
($^3J_{H,H}$) couplings in a molecule, thus providing complementary structural information. One- and two-bond ^{13}C–^{1}H and ^{13}C–^{13}C coupling constants are also conformation dependent, but the correlations between structure and vicinal coupling constants are more firmly established[5].

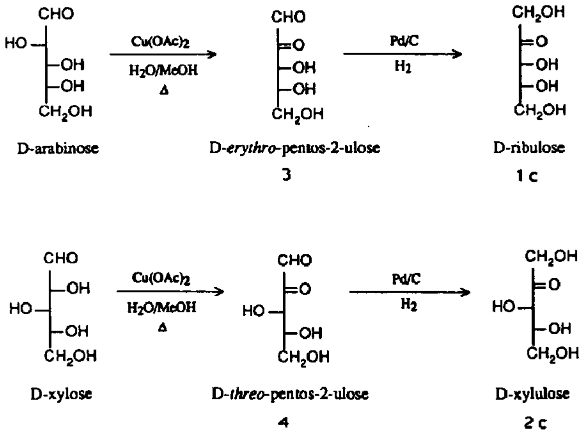
^{13}C-Substituted compounds are used frequently to determine ^{13}C–^{1}H and ^{13}C–^{13}C spin-coupling constants because of high sensitivity and ease of analysis compared with other techniques, although these advantages are tempered by the less ready availability of appropriately labeled compounds. In the latter regard, the synthetic methods used to prepare ^{13}C-labeled compounds should meet some special requirements[6a], most notably simplicity and a high yield of the labeled product.

The synthesis of the D-2-pentuloses **1** and **2** has been achieved in this study by several routes. The simplest preparation of D-*threo*-2-pentulose (**2**, D-xylulose) is *via* enzytic isomerization of D-xylose (Scheme 1). The required enzyme, D-xylose (D-glucose) isomerase (E.C. 5.3.1.5), is available commercially in an immobilized form[6b]. The method allows recovery of unreacted D-xylose, which is important when labeled compounds are being prepared. In contrast, there are no readily available enzymes that convert D-ribose directly to D-*erythro*-2-pentulose (**1**, D-ribulose), although D-ribose 5-phosphate may be treated with D-ribose 5-phosphate isomerase (E.C. 5.3.1.6), D-ribulose 5-phosphate kinase (E.C. 2.7.1.19), and ATP to generate D-ribulose 1,5-bisphosphate[7a], which may be subsequently dephosphorylated with acid phosphatase (E.C. 3.1.3.2) to generate **1**.



Scheme 1

D-Fructose has been prepared previously in high yield by hydrogenating D-*arabino*-hexos-2-ulose catalytically[7b]. In a recent study we prepared the ^{13}C-labeled pentos-2-uloses **3** and **4** and studied their solution properties by ^{1}H- and ^{13}C-n.m.r. spectroscopy[8]. It became evident that these compounds might be reduced catalytically to give the corresponding labeled 2-pentuloses (Scheme 2). In this study, we describe this method for the preparation of stable-isotopically substituted D-2-pentuloses **1** and **2**, and examine the conformational properties of the 2-pentulofuranoses and their acyclic carbonyl forms by ^{13}C- and ^{1}H-n.m.r. spectroscopy. The 2-pentuloses have not been studied extensively by n.m.r. methods. The equilibrium composition of **2** in ^2H_2O was determined[9] by ^{13}C-n.m.r. spectroscopy at 15 MHz, and the ^{1}H-n.m.r. spectra of the methyl D-pentulofuranosides were examined[10] at 80 MHz in C^2HCl_3. This study attempts to integrate ^{1}H- and ^{13}C-n.m.r. parameters to obtain a more detailed understanding of 2-ketofuranose ring structure and conformation.



Scheme 2

EXPERIMENTAL

Materials. — D-*erythro*-Pentos-2-ulose and D-*threo*-pentos-2-ulose were prepared as described elsewhere[8]. D-(1-^{13}C)Arabinose, D-(2-^{13}C)arabinose, D-(1-^{13}C)xylose, and D-(2-^{13}C)xylose were obtained as a gift from Omicron Biochemicals, Inc. D-Xylose was purchased from Nutritional Biochemicals Corporation. Immobilized D-xylose (D-glucose) isomerase (E.C. 5.3.1.5) was purchased from Miles Laboratories, Inc. (Taka-Sweet Type Q). Palladium-on-carbon (Pd–C, 10%) was purchased from Engelhard Industries. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% chlorotrimethylsilane (TMCS) was purchased from Pierce Chemical Company. Hydroxylamine hydrochloride was obtained from Sigma Chemical Company.

Instrumentation. — High-resolution 620 MHz ^{1}H-n.m.r. spectra were obtained in the FT-mode at the NMR Facility for Biomedical Studies*, Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA.

High-resolution ^{13}C-n.m.r. spectra at 75 MHz were obtained on a General Electric GN-300 FT-n.m.r. spectrometer in the Department of Chemistry and Biochemistry at the University of Notre Dame. Quantitative ^{1}H-decoupled ^{13}C-n.m.r. spectra were obtained without nuclear Overhauser enhancement (nOe) by gating the decoupler on only during the acquisition interval and using a relaxation delay of 20 s between data acquisitions. ^{1}H-Coupled ^{13}C-n.m.r. spectra were obtained with nOe using the same pulse sequence but gating the ^{1}H-decoupler off during data acquisition.

To enhance the resolution of non-quantitative ^{1}H- and ^{13}C-n.m.r. spectra, a double-exponential apodization function was chosen empirically and applied to free-induction decays (FIDs) prior to Fourier transformation. In quantitative ^{13}C-n.m.r. spectra an exponential multiplication function was applied to FIDs prior to Fourier transformation in order to increase the number of data points defining the shape of the signals and thereby improve the accuracy of signal integration.

Gas-liquid chromatography (g.l.c.) was performed on a Varian Model 1400 gas chromatograph. Samples were analyzed using a glass column (6 ft.) packed with 3% OV-17 on 100/120 Chromosorb W-HP obtained from Alltech Associates.

Preparation of D-erythro-*2-pentulose* via D-erythro-*pentos-2-ulose (Scheme 2).* — Palladium-on-carbon (10%, 80 mg) and water (8 mL) were added to a 50-mL side-arm flask, and the flask was sealed with a rubber septum and purged carefully with hydrogen gas. The mixture was stirred under hydrogen at atmospheric pressure for 10 min at ambient temperature and 10 min at 80°. D-*erythro*-Pentos-2-ulose (**3**; 200 mg) in water (2 mL) was then injected *via* a syringe into the reaction flask, and the reaction was monitored by observing the consumption of hydrogen gas. When hydrogen uptake ceased (~30 min), the reaction mixture was cooled, the catalyst was removed by filtration through a 0.45 μm Millipore filter, and the supernatant was concentrated at 30° *in vacuo* to a volume of ~5 mL. Analysis of the product mixture by g.l.c. showed the yield of D-*erythro*-2-pentulose (**1**) was 70%. Byproducts of the reaction included D-ribose, D-arabinitol, and D-ribitol. The reaction mixture also contained ~10% of unreacted D-*erythro*-pentos-2-ulose.

The product D-*erythro*-2-pentulose (**1**) was purified by chromatography on a column (2.6 × 50 cm) containing Dowex 50-X8 (200–400 mesh) cation-exchange resin in the calcium form using water as the eluent. The column was eluted at a flow rate of 1 mL/min, and fractions (15 mL) were collected and assayed with phenol–sulfuric acid[11a]. The elution volume of **1** was 450 mL. D-*erythro*-2-Pentulose was well separated from the other products except for D-ribose.

The fraction containing **1** and D-ribose was concentrated to a syrup at 30° *in vacuo*, dissolved in 10 mL of 76% aqueous ethanol, and applied to a column (3.0 × 51 cm) containing Dowex 1-X8 (200–400 mesh) anion-exchange resin in the sulfate-hydrogen sulfate form. The column was eluted with 76% aqueous ethanol at a flow rate

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of 1 mL/min, and fractions (15 mL) were collected and assayed with phenol–sulfuric acid[11a]. The elution volumes of D-*erythro*-2-pentulose and D-ribose were 980 mL and 1130 mL, respectively.

Preparation of D-threo-*2-pentulose* via D-threo-*pentos-2-ulose (Scheme 2).* — The reduction of D-*threo*-pentos-2-ulose (**4**) was performed as described above for D-*erythro*-pentos-2-ulose. The yield of **2** was 60% as determined by g.l.c.. The principal byproduct was D-xylose; D-lyxose and pentitols were formed in smaller quantities.

D-*threo*-2-Pentulose (**2**) was purified by chromatography on a column (4.0 × 106 cm) containing Dowex 50-X8 (200–400 mesh) cation-exchange resin in the calcium form using water as the eluent. The column was eluted at a flow rate of 1 mL/min, and fractions (15 mL) were collected and assayed with phenol–sulfuric acid[11a]. The elution volume of **2** was 900 mL. D-*threo*-2-Pentulose was separated from the byproducts except for D-lyxose.

The fraction containing D-*threo*-2-pentulose and D-lyxose was concentrated at 30° *in vacuo* to a syrup, dissolved in 76% aqueous ethanol (10 mL), and applied to a column (3.0 × 51 cm) containing Dowex 1-X8 (200–400 mesh) anion-exchange resin in the sulfate-hydrogen sulfate form. The column was eluted with 76% aqueous ethanol at a flow rate of 1 mL/min, and fractions (15 mL) were collected and assayed with phenol–sulfuric acid[11a]; D-*threo*-2-pentulose eluted before D-lyxose (elution volume, 1030 mL).

Preparation of D-threo-*2-pentulose with immobilized* D-*xylose* (D-*glucose*) *isomerase (Scheme 1).* — Immobilized D-xylose (D-glucose) isomerase (E.C. 5.3.1.5) (50 mg) was washed twice with 5 mL of phosphate buffer (0.05M KH_2PO_4, 0.002M $MgCl_2$, 0.0001M $CoCl_2$) at 75°. The washed immobilized enzyme was added to a solution of 1.0 g of D-xylose dissolved in 4.5 mL of the same phosphate buffer. This suspension was mixed gently on a rotary evaporator at 75° for 120 min. The resulting solution was decanted and the enzyme was washed twice with water (8 mL) at 75°.

The combined aqueous fractions were eluted through a column (2.0 cm i.d.) containing anion-exchange resin (Dowex 2-X8, 6 mL) in the acetate form and cation-exchange resin (Dowex HCR-W2, 6 mL) in the H^+ form. The column was eluted with distilled water (100 mL) and the eluates were concentrated to a syrup at 30° *in vacuo*. The isomerization mixture obtained during the preparation of the 1-^{13}C-labeled sugar was assayed by ^{13}C-n.m.r. and found to contain 1% D-lyxose, 73% D-xylose, and 26% D-*threo*-2-pentulose (**2**).

The concentrated isomerization mixture was dissolved in 76% aqueous ethanol (15 mL) and applied to a column (3.0 × 51 cm) of Dowex 1-X8 (200–400 mesh) anion-exchange resin in the sulfate–hydrogen sulfate form. The column was eluted with 76% aqueous ethanol at a flow rate of 1 mL/min, and fractions (15 mL) were collected and assayed with phenol–sulfuric acid[11a]. The elution volumes of **2** and D-xylose were 1030 mL and 1380 mL, respectively. Although D-lyxose eluted after **2**, their elution profiles partially overlapped; therefore the trailing fractions of **2** were rechromatographed to remove the residual D-lyxose.

Characterization of the product 2-pentuloses. — An aqueous sample containing

1–2 mg of compound was concentrated to a syrup at 30° *in vacuo* and treated with hydroxylamine hydrochloride (5 mg), dissolved in 0.5 mL of anhydrous pyridine, for 2 h at ambient temperature. BSTFA containing 1% TMCS (0.25 mL) was added, and the solution was incubated at ambient temperature for ~20 min, then analyzed by g.l.c.[11b].

The per-O-trimethylsilylated products were separated on a glass g.l.c. column packed with 3% OV-17 on Chromosorb W-HP. Temperature was programmed at 10°/min from 100° to 200°, then held for 5 min at 200°. Retention times (in min) were as follows: pentitols, 7.3–7.4; D-*erythro*-2-pentulose, 7.95; D-*threo*-2-pentulose, 7.95. The retention times of the pentoses and pentos-2-uloses under similar experimental conditions were reported previously[8].

RESULTS AND DISCUSSION

Synthesis of the D-*2-pentuloses.* — Catalytic reduction of D-*erythro*-pentos-2-ulose (**3**) and D-*threo*-pentos-2-ulose (**4**) at ambient temperature gave D-*erythro*-2-pentulose (**1**) and D-*threo*-2-pentulose (**2**) in 50% and 25% overall yields, respectively. At 80° these yields increased to 70% and 60%, respectively. The Pd–C hydrogenation of D-*arabino*-hexos-2-ulose[7b] gives D-fructose in a somewhat better yield.

The catalytic hydrogenation of the 2-uloses **3** and **4** appears to proceed with high selectivity. At 80°, aqueous solutions of D-*threo*-pentos-2-ulose contain approximately 100 times more free ketone forms than free aldehyde forms[8], yet reduction of the latter is required to obtain the 2-pentuloses. Thus, the *aldehydo* structures must be reduced at least 150 times more rapidly than the *keto* forms in order to give **1** and **2** in the observed yields. It is also remarkable that the 2-pentuloses are relatively stable towards hydrogenation even though these compounds exist to a significant extent (~35%) in the *keto* form in aqueous solution at 80°; some reduction of **1** and **2** may occur, however, giving the observed alditol byproducts.

The effect of temperature on the reduction yields may be due to differences in the hydration enthalpies of aldehydes and ketones, and to enhanced proportions of furanoid structures at elevated temperatures[8]. The difference in the reduction selectivities of **3** and **4**, however, is not easily explained. It is clear that the D-*erythro*-pentos-2-ulopyranoses are less susceptible to hydrogenation than the D-*threo*-pentos-2-ulopyranoses, because the observed reduction rates of **3** and **4** are quite similar even though aqueous solutions of **4** contain a considerably lower proportion of free ketone forms[8].

The overall yields of **1** and **2** were 20% and 32%, respectively, when prepared from D-xylose and D-arabinose *via* the pentos-2-uloses. In comparison to the aldos-2-ulose reduction method, the enzyme-catalyzed isomerization of D-xylose to D-*threo*-2-pentulose (**2**) proceeded in lower overall yield (26%; see Fig. 1), but the unreacted D-xylose could be recovered and reused. The isomerization route is easy to implement and thus more attractive than the reductive route for the preparation of isotopically substituted **2**.

Although the chemical or enzymic conversions of pentoses to 2-pentuloses were straightforward, some difficulties were encountered in the purification of products.

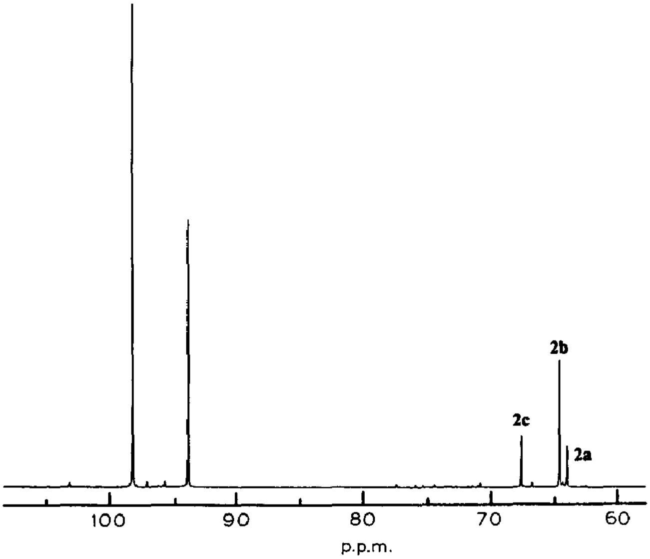


Fig. 1. The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of the reaction mixture after treatment of D-(1-^{13}C)xylose with D-xylose (D-glucose) isomerase (see Experimental). The C-1 signals at 93.7 and 98.1 p.p.m. are the α-pyranose and β-pyranose forms of the aldopentose, respectively, while those at 63.9, 64.5 and 67.5 p.p.m. arise from the three major forms of the product, D-(1-^{13}C)-*threo*-2-pentulose (**2a**, **2b**, **2c**).

Unreacted pentos-2-uloses had to be removed from the reduction mixtures (by chromatography on Dowex 50 in the Ca^{2+} form) prior to chromatography on anion-exchange resin because the pentos-2-uloses emerged with considerable tailing when aqueous ethanol was used as the eluent. Chromatography on Dowex 50 (Ca^{2+}) gave **1** and **2** contaminated with D-ribose and D-lyxose, respectively. The ketopentoses **1** and **2** were separated from these contaminants by chromatography on anion-exchange resin in the sulfate–hydrogen sulfate form, although separation was not easy to achieve. An improved separation may be possible on a resin having a higher relative content of sulfate ions[12].

^{13}C-N.m.r. spectra of the 2-pentuloses. — The ^{13}C-n.m.r. spectra of **2** substituted with ^{13}C at C-1 and C-2 are shown in Fig. 2. At ambient temperature equilibrated aqueous solutions of the 2-pentuloses contain the acyclic *keto* form and two furanose forms in a ratio of 20:80 (ref. 13a). The anomeric carbon signals (C-2) were assigned (Table I) to specific furanose anomers in several ways. The most reliable method is based on an analysis of $^3J_{\text{C-1,H-3}}$ values which were obtained from ^{1}H-n.m.r. spectra of the 1-^{13}C-labeled compounds. The value of $^3J_{\text{C-1,H-3}}$ is 2.3 Hz for the more abundant anomer of each 2-pentulose (Table II), whereas in the minor anomer $^3J_{\text{C-1,H-3}} \leqslant 0.3$ Hz. Because of geometric contraints imposed by the furanose ring, the latter small couplings are not possible when C-1 and H-3 are *cis*. Based on this reasoning, the α-furanose **1a** is the predominant furanose in aqueous solutions of **1**, and the β-furanose **2b** is the predominant furanose in aqueous solutions of **2**. These assignments are supported by the observation

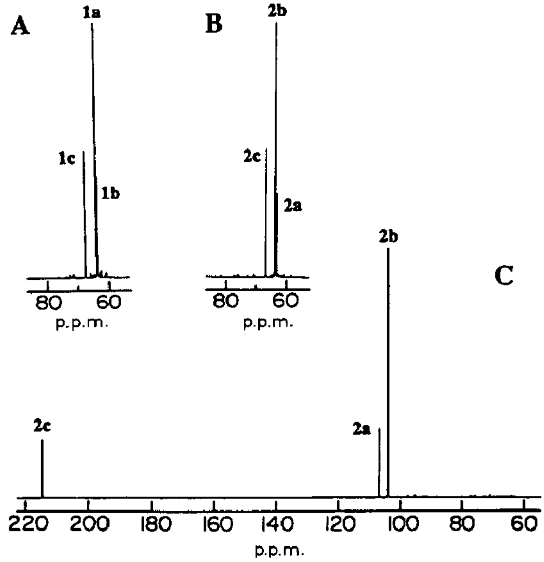


Fig. 2. A, the ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-(1-^{13}C)-*erythro*-2-pentulose, showing only the enriched carbons. The three C-1 signals are assigned to the α-furanose (**1a**; 64.3 p.p.m.), β-furanose (**1b**; 64.0 p.p.m.), and acyclic *keto* (**1c**; 67.9 p.p.m.) forms; **B**, the ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-(1-^{13}C)-*threo*-2-pentulose, showing only the enriched carbons. The three signals are assigned to the α-furanose (**2a**; 63.9 p.p.m.), β-furanose (**2b**; 64.5 p.p.m.), and acyclic *keto* (**2c**; 67.5 p.p.m.) forms; C, the ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-(2-^{13}C)-*threo*-2-pentulose, showing only the enriched carbons. The C-2 signals have been assigned to the α-furanose (**2a**; 107.2 p.p.m.), β-furanose (**2b**; 104.4 p.p.m.), and acyclic *keto* (**2c**; 214.4 p.p.m.) forms.

TABLE I

Carbon-13 chemical shifts of the 2-pentulofuranoses and acyclic 2-pentuloses in $^{2}H_2O$

Pentulose tautomer	*Chemical shifts (p.p.m.)*[a]				
	C-1	*C-2*	*C-3*	*C-4*	*C-5*
α-*erythro* (**1a**)	64.3	104.0	71.9	71.6	72.9
β-*erythro* (**1b**)	64.0	107.0	77.3	72.0	72.2
keto-erythro (**1c**)	67.9	213.9	76.8	74.0	62.8
α-*threo* (**2a**)	63.9	107.2	82.0	77.3	73.38[b]
β-*threo* (**2b**)	64.5	104.4	77.7	76.4	71.3
keto-threo (**2c**)	67.5	214.4	76.8	73.44[b]	63.4

[a] Reported relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS); measured from the resonance for the anomeric carbon of α-D-(1-^{13}C)mannopyranose (95.5 p.p.m.); accurate to ±0.1 p.p.m. [b] These values are given to 0.01 p.p.m. to indicate that the signals, while close in chemical shift, are nevertheless resolvable at 75 MHz.

TABLE II

^{13}C–^{1}H Coupling constants for the 2-pentulofuranoses and acyclic 2-pentuloses in $^{2}H_2O$[a]

Coupled nuclei	α-erythro (**1a**)	β-erythro (**1b**)	keto-erythro (**1c**)	α-threo (**2a**)	β-threo (**2b**)	keto-threo (**2c**)
C-1, H-1	143.6	144.9	~144.0	144.6	143.5	143.8
C-1, H-1′	143.8	144.4	143.8	143.9	143.9	143.7
C-1, H-3	2.3	0.3	0	0	2.3	0
C-2, H-1	1.9	2.6	3.4	2.6	2.3	3.5
C-2, H-1′	4.3	3.6	3.5	3.6	4.0	3.3
C-2, H-3	0	1.7	3.5	*obsc*	<0.4	3.9
C-2, H-4	4.6 (4.6)	3.0 (4.0)	2.0	2.7	1.4 (2.6)	1.4
C-2, H-5*R*	3.2 (3.4)	1.0 (1.3)	0	4.1 (3.5)	1.3 (1.1)	0
C-2, H-5*S*	1.7 (1.9)	~4.5 (3.8)	0	1.3 (2.7)	~4.6	0
C-3, H-3	149.2	150.3	145.6	151.0	147.7	141.8
C-4, H-4	154.4	150.8	*obsc*	151.7	149.9	144.0
C-5, H-5	150.7	149.5	144.0	*obsc*	149.8	143.0

[a] Values are reported in Hz and are accurate to ±0.1 Hz. Obscured signals are denoted by *obsc*. Values in parentheses are corresponding couplings in the configurationally related aldotetrofuranoses[1].

that the anomeric carbon signals of **1a** and **2b** (104.2 ± 0.3 p.p.m.) are upfield of those of **1b** and **2a** (107.1 ± 0.1 p.p.m.) (Table I), as expected for 2-ketofuranose rings having O-2 and O-3 *cis*[13b]. Finally, $^{1}J_{C\text{-}2,C\text{-}3}$ values are larger in **1b** and **2a** than in **1a** and **2b**, as expected for 2-ketofuranose rings having O-2 and O-3 *trans* (Table III)[14a]. The C-2 signals of the acyclic forms **1c** and **2c** were assigned from their characteristic chemical shifts (214.2 ± 0.4 p.p.m.).

The C-1 signals of the 2-pentuloses were clearly identified in ^{13}C spectra of the 1-^{13}C-labeled compounds and assigned to specific forms from relative signal intensities. The C-1 hydroxymethyl carbons of the acyclic forms **1c** and **2c** resonate at an unusually low field due to their proximity to the carbonyl carbon at C-2 (Fig. 2, Table I).

The large two-bond coupling between C-1 and C-3 in **1c** and **2c** (13.9 ± 0.1 Hz) enabled the assignment of C-3 in these forms (Table III). In the furanose the C-3 signals

TABLE III

^{13}C–^{13}C Coupling constants for the 2-pentulofuranoses and acyclic 2-pentuloses in $^{2}H_2O$[a]

Coupled nuclei	α-erythro (**1a**)	β-erythro (**1b**)	keto-erythro (**1c**)	α-threo (**2a**)	β-threo (**2b**)	keto-threo (**2c**)
C-1, C-2	51.8	51.3	41.2	51.8	51.2	41.3
C-1, C-3	3.2	1.4	14.0	<1.0	3.2	13.8
C-1, C-4	1.0	0	0	0	0	0
C-1, C-5	2.0	2.0	0	2.0	1.0	0
C-2, C-3	46.8 (43.2)	47.1 (46.8)	43.3	47.0 (46.1)	44.7 (42.5)	43.1
C-2, C-4	1.1 (1.2)	2.9 (3.0)	0	3.0 (2.9)	3.4 (2.7)	0
C-2, C-5	0	br[b]	<1.0	0	0	2.8

[a] Values are reported in Hz and are accurate to ±0.1 Hz. Values in parentheses are corresponding couplings in the configurationally related aldotetrofuranoses[14a]. [b] Broadened signal.

were assigned by observing the large one-bond coupling between C-3 and C-2 in 2-^{13}C-labeled compounds. It is noteworthy that $^1J_{C\text{-}1,C\text{-}2}$ is about 10 Hz smaller in the acyclic carbonyl forms **1c** and **2c** than in the cyclic furanoses; $^1J_{C\text{-}2,C\text{-}3}$ is also smaller in **1c** and **2c** but the difference is not as great ($<$4 Hz).

In **1c** and **2c**, C-1 and C-5 are separated by four bonds and no coupling is observed between these carbons in 1-^{13}C-labeled compounds. In contrast, $^3J_{C\text{-}1,C\text{-}5}$ is observed in **1a**, **1b**, **2a**, and **2b** (1.0–2.0 Hz; Table III), as C-1 and C-5 are separated by three bonds (C-1–C-2–O-5–C-5). The C-5 signals were therefore assigned on the basis of observed coupling to C-1 and relative signal intensities.

The C-4 signals were assigned by difference; the signals of the acyclic and furanose forms were assigned on the basis of relative intensities and the magnitude of $^1J_{C\text{-}4,H\text{-}4}$ (Table II). These latter coupling constants, and $^1J_{C\text{-}3,H\text{-}3}$ and $^1J_{C\text{-}5,H\text{-}5}$, were found to be larger in the furanoses than in the acyclic *keto* forms; a similar effect was observed previously between the acyclic and cyclic forms of the aldotetroses[14b].

Previous ^{13}C signal assignments for D-*threo*-2-pentulose (**2**) at 15 MHz[9] agree with those reported in Table I with the exception of the line for C-5 of **2a**, which overlaps that of C-4 of **2c** at low field.

The preparations of **1** and **2** contained minor impurities and therefore it was not possible to determine whether the acyclic hydrates **1d** and **2d** were present in aqueous solution. The C-2 signals of these forms would be expected at ~98 p.p.m., by analogy to the C-2 signal for the hydrate form of D-ribulose 1,5-bisphosphate[7a]. However, the absence of clearly discernible signals in this region indicates that the proportion of acyclic hydrate form in aqueous solutions of **1** and **2** is significantly less than found in solutions of the aldotetroses (~10%)[14b].

The chemical shifts of the non-anomeric ring carbons of the 2-pentulofuranoses are similar to those of corresponding non-anomeric ring carbons of the aldotetrofuranoses[1]. The ring-carbon chemical shifts of the 2-pentulofuranoses appear to depend mainly on *cis* interactions with neighboring hydroxyl groups[13b].

^{1}H-N.m.r. spectra of the 2-pentuloses. — ^{1}H-N.m.r. spectra of **1** and **2** obtained at 620 MHz (Fig. 3) were well resolved and permitted a firm assignment of signals to specific forms and the complete determination of chemical shifts and ^{1}H–^{1}H spin-couplings (Tables IV and V). Spectra of ^{13}C-enriched compounds were used to evaluate ^{13}C–^{1}H spin-couplings (Fig. 4). Protons H-1 and H-1′ of the acyclic forms **1c** and **2c** were markedly deshielded, relative to H-1 and H-1′ of the cyclic forms, by their adjacent carbonyl groups and therefore the signals could be assigned easily in ^{1}H spectra obtained at 620 MHz (Fig. 3, Table IV). The H-3 protons of **1c** and **2c** are coupled only to H-4 protons, and the resulting doublets are also deshielded relative to the H-3 doublets of the cyclic forms. Protons H-5 and H-5′ of **1c** and **2c** will not be coupled to C-2 in 2-^{13}C-substituted compounds and thus these signals could be distinguished from those of the cyclic furanoses in which coupling between C-2 and the C-5 protons occurs across the ring oxygen. The H-4 signals of **1c** and **2c** were assigned on the basis of $^3J_{H\text{-}3,H\text{-}4}$, $^3J_{H\text{-}4,H\text{-}5}$, and $^3J_{H\text{-}4,H\text{-}5'}$, obtained from an analysis of the H-3, H-5, and H-5′ multiplets (Table V).

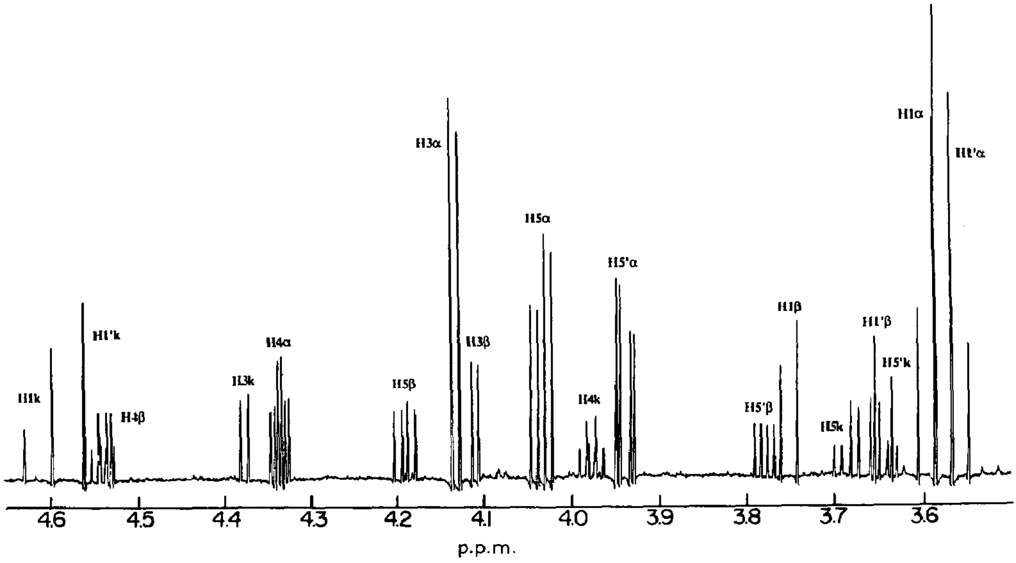


Fig. 3. The resolution-enhanced ^{1}H-n.m.r. spectrum (620 MHz) of D-*erythro*-2-pentulose, showing complete signal assignments.

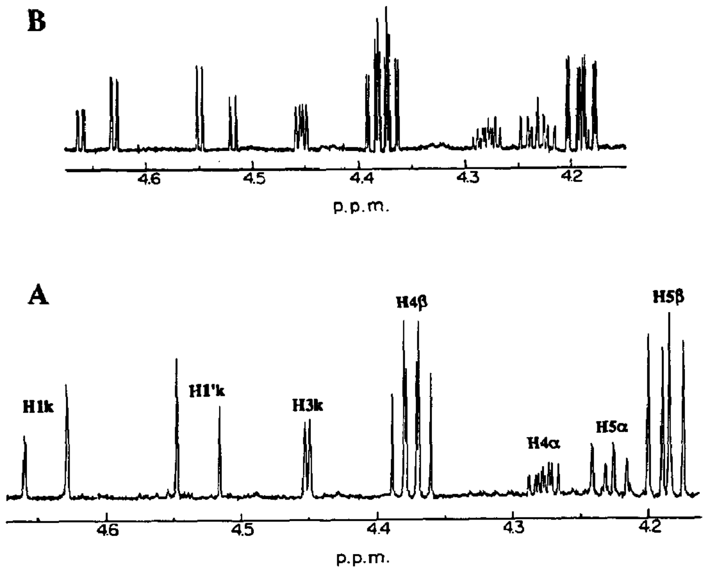


Fig. 4. A, partial ^{1}H-n.m.r. spectrum (620 MHz, resolution-enhanced) of D-*threo*-2-pentulose, showing signal assignments; B, partial ^{1}H-n.m.r. spectrum (620 MHz, resolution-enhanced) of D-(2-^{13}C)-*threo*-2-pentulose. Comparison with the spectrum in A reveals the additional signal splittings ($J_{C,H}$) caused by ^{13}C-substitution.

TABLE IV

Proton chemical shifts of the 2-pentulofuranoses and acyclic 2-pentuloses in ^2H_2O[a]

Pentulose tautomer	*Chemical shifts (p.p.m.)*					
	H-1	*H-1′*	*H-3*	*H-4*	*H-5*R	*H-5*S
α-erythro (**1a**)	3.59	3.55	4.13 (4.10)	4.34 (4.28)	4.03 (4.02)	3.93 (3.92)
β-erythro (**1b**)	3.75	3.64	4.11 (4.02)	4.54 (4.39)	4.19 (4.20)	3.77 (3.79)
keto-erythro (**1c**)	4.61	4.54	4.38	3.97	3.68[b]	3.64[b]
α-threo (**2a**)	3.70	3.65	4.08 (4.05)	4.28 (4.21)	4.23 (4.20)	3.89 (3.95)
β-threo (**2b**)	3.61	3.56	4.04 (4.04)	4.37 (4.30)	4.19 (4.18)	3.64 (3.65)
keto-threo (**2c**)	4.64	4.53	4.45	4.08	3.70[c]	3.65[c]

[a] Values are reported relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). They were measured from internal HOD (4.80 p.p.m.) and are accurate to ±0.01 p.p.m. Values in parentheses are corresponding chemical shifts in the configurationally related aldotetrofuranoses[1]. [b,c] Assignments may be reversed.

TABLE V

^1H–^1H Coupling constants in the 2-pentulofuranoses and acyclic 2-pentuloses in ^2H_2O[a]

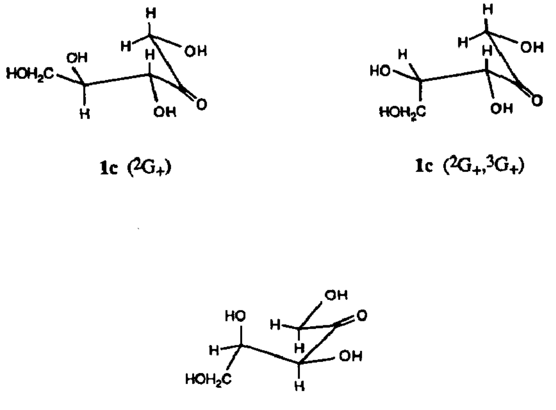
Coupled nuclei	α-erythro (**1a**)	β-erythro (**1b**)	keto-erythro (**1c**)	α-threo (**2a**)	β-threo (**2b**)	keto-threo (**2c**)
H-1*R*, H-1*S*	−12.0	−11.9	−19.5	−11.9	−12.1	−19.4
H-3, H-4	5.3 (5.2)	4.9 (4.8)	5.4	2.9 (1.9)	5.6 (4.1)	2.4
H-4, H-5*R*	4.8 (5.1)	5.9 (4.9)	6.3[b]	6.1 (5.6)	6.5 (5.4)	7.0[c]
H-4, H-5*S*	2.8 (3.0)	4.8 (3.5)	5.2[b]	4.3 (3.3)	5.3 (3.6)	5.8[c]
H-5*R*, H-5*S*	−10.1	−9.5	−11.6	−9.6	−9.5	−11.5

[a] Values are reported in Hz and are accurate to ±0.1 Hz. Values in parentheses are corresponding couplings in the configurationally related aldotetrofuranoses[1]. [b,c] Assignments may be reversed.

The proton signals of the furanose anomers of **1** and **2** were assigned based on internal consistency in coupling patterns for vicinal protons, and on relative signal intensities. In the furanoses **1a**, **1b**, **2a**, and **2b**, a significant difference was observed between the chemical shifts of H-5 and H-5′ within each structure (0.10–0.55 p.p.m.; Table IV). Based on the "*syn*-upfield" rule[15a], the C-5 proton *cis* to O-4 is expected to be more shielded than the C-5 proton *trans* to O-4; this rule has been found to be reliable in assigning prochiral protons in aldotetrofuranose rings[1]. Therefore, the more shielded C-5 proton in **1a**, **1b**, **2a**, and **2b** may be assigned as H-5*S* (Table IV).

Conformation of the acyclic forms **1c** *and* **2c**. — Acyclic carbohydrate derivatives are generally considered to be conformationally flexible, and n.m.r. parameters such as chemical shifts (δ) and spin–spin coupling constants (J) will reflect this dynamic behavior. However, the rotational barriers for the interconversion of acyclic conformers are relatively large compared to those for the interconversion of nonplanar forms of furanoses (see below), allowing for some meaningful conformational conclusions to be drawn from these parameters.

In **1c** and **2c**, ${}^2J_{\text{H-1,H-1}'}$ is large (-19.5 ± 0.1 Hz). The magnitude of this coupling has been shown to depend on the relative orientation of O-1 and the carbonyl group, with maximal coupling when the methylene protons are symmetrically oriented with respect to the carbonyl group[15b,c,d]. The large ${}^2J_{\text{H-1,H-1}'}$ in **1c** and **2c** is consistent with an eclipsed or antiperiplanar geometry of the O-1–C-1–C-2–O-2 fragment. The latter geometry may, however, be excluded as abnormal for carbonyl compounds[15e]. The symmetrical orientation of H-1 and H-1′ (Chart 1) is further supported by similar values of ${}^2J_{\text{C-2,H-1}}$ and ${}^2J_{\text{C-2,H-1}'}$ (3.4 ± 0.1 Hz; Table II).



1c ($^2G_+$)

1c ($^2G_+,{}^3G_+$)

Chart 1

2c ($^2G_-$)

The absence of coupling between C-1 and C-4, and between C-1 and H-3, in **1c** and **2c** (Tables II and III) indicates that these substituents are *gauche* or near *gauche*. Therefore, the C-3–O-3 and C-2–O-2 bonds in **1c** and **2c** appear to be eclipsed or near eclipsed, a conformation further supported by the similar magnitudes of ${}^2J_{\text{C-2,H-3}}$, ${}^2J_{\text{C-2,H-1}}$, and ${}^2J_{\text{C-2,H-1}'}$ (Table II). It is noted that C-1 and C-4, and C-1 and H-3, are also *gauche* in the antiperiplanar conformation but the different orientation of H-3 with respect to the carbonyl group would result in a different value[15f] of ${}^2J_{\text{C-2,H-3}}$. The eclipsed C–O orientations adjacent to the carbonyl group (Chart 1) may be caused by intramolecular hydrogen bonding, although stereoelectronic factors may also play a role. A similar conformational effect has been observed recently[8] in unhydrated pentos-2-ulopyranoses in ${}^2H_2O$.

In **2c**, the relatively large coupling between C-2 and C-5 (2.8 Hz) suggests that these atoms are mainly antiperiplanar. This conformation is further supported by the magnitude of ${}^3J_{\text{H-3,H-4}}$ (2.4 Hz); H-3 and H-4 are gauche when C-2 and C-5 are antiperiplanar. A similar ${}^3J_{\text{H,H}}$ is found in the 1,6-anhydro-β-D-hexopyranoses (${}^3J_{\text{H-4,H-5}} = 2.2$ Hz), which have a similar configuration and conformation about the C-4–C-5 bond as **2c** has about the C-3–C-4 bond in its $^2G_-$ conformation[16] (Chart 1). The $^2G_-$ conformation of **2c** is also stabilized by an electrostatic interaction between C-2 and O-4; a similar stabilizing interaction has been observed in unhydrated D-*erythro*-pentos-2-ulopyranoses[8].

In **1c**, the C-3–C-4 rotamer having C-2 and C-5 antiperiplanar ($^2G_+$; Chart 1) orients H-3 and H-4 antiperiplanar, giving a value of $^3J_{H\text{-}3,H\text{-}4}$ of ~9.5 Hz (*cf.* aldopyranoses[17]). In the alternative stable rotamer ($^2G_+$; $^3G_+$, Chart 1), $^3J_{H\text{-}3,H\text{-}4}$ will be ~3.5 Hz. The $^2G_+$, $^3G_-$ conformation contains an unfavorable *syn* interaction between C-1 and C-5 and is probably present in low abundance. From the observed $^3J_{H\text{-}3,H\text{-}4}$ in **1c** (5.4 Hz; Table V), therefore, the relative populations of $^2G_+$ and $^2G_+$, $^3G_+$ may be estimated as ~32% and ~68%, respectively. This distribution of rotamers would generate an averaged value of $^3J_{C\text{-}2,C\text{-}5}$ of ~0.9 Hz, which is consistent with the observed value in **1c** (<1.0 Hz; Table III).

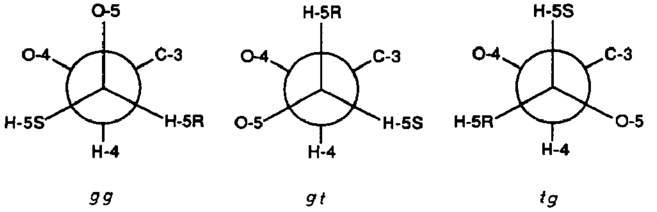


Chart 2

Based on *gauche* and *syn* interaction energies, the relative populations of the C-4–C-5 rotamers of **2c** may be estimated as 8% *gg*, 50% *gt*, and 42% *tg* (Chart 2) (ref. 18). Standard values of $^3J_{H\text{-}4,H\text{-}5}$ in *gg* and *tg* rotamers were obtained from the conformationally rigid α-D-arabinopyranose and β-D-fructopyranose (*gg*)[17,19], and β-D-xylopyranose and β-D-*threo*-pentos-2-ulopyranose hydrate (*tg*)[8,17]. General equations are also available to calculate rotamer populations of hydroxymethyl groups in carbohydrates[20,25]. The observed $^3J_{H\text{-}4,H\text{-}5}$ and $^3J_{H\text{-}4,H\text{-}5'}$ values are relatively large and of similar magnitude (7.0 and 5.8 Hz, respectively), and are consistent with the calculated rotamer populations given above. Thus, the *gt* and *tg* rotamers appear to be similar in stability, whereas *gg* is destabilized by a *syn* interaction between O-3 and O-5.

The interpretation of $^3J_{H\text{-}4,H\text{-}5}$ in **1c** is more complicated because two C-3–C-4 rotamers ($^2G_+$ and $^2G_+$, $^3G_+$) are present. In $^2G_+$, the calculated rotamer distribution is 26% *gg*, 57% *gt*, and 17% *tg*, which requires that $^3J_{H\text{-}4,H\text{-}5R}$ be greater than $^3J_{H\text{-}4,H\text{-}5S}$. A similar conformational situation is found in 1,5-anhydro-2,3-dideoxy-D-*erythro*-hexitol, where $^3J_{H\text{-}5,H\text{-}6R}$ = 6.5 Hz and $^3J_{H\text{-}5,H\text{-}6S}$ = 2.5 Hz (ref. 21). In the $^2G_+$, $^3G_+$ conformer of **1c**, the *gt* rotamer is relatively stable, *gg* is destabilized by a *syn* interaction between O-3 and O-5, and *tg* is destabilized by a similar interaction between C-2 and O-5. Therefore, $^3J_{H\text{-}4,H\text{-}5R}$ should be larger than $^3J_{H\text{-}4,H\text{-}5S}$ because H-4 and H-5*R* are antiperiplanar in the *gt* rotamer. The values of $^3J_{H\text{-}4,H\text{-}5}$ for **1c**, however, are relatively similar in magnitude (6.3 and 5.2 Hz). Thus, the energy of the *syn* interaction between C-2 and O-5 may be lower than expected, perhaps due to a stabilizing electrostatic interaction between C-2 and O-5.

2-Pentulofuranose ring conformation. — The conformational behavior of furanose rings has been explained by two mechanisms, namely, pseudorotation and inversion. Pseudorotation[22a,b] describes a circular pathway[22b] for the continuous interconversion of nonplanar envelope (*E*) and twist (*T*) conformers that does not involve the planar form. The alternative pathway, inversion, describes nonplanar conformer interconversion *via* the planar form. Both pathways may be adopted by furanose rings in solution, although it is generally believed that pseudorotation is preferred and that the activation barriers to conformer interconversion are low. Because of the inherent flexibility of the furanose ring, and the considerable number of conformational options available to the molecule, the conformational analysis of furanoses is complicated, as discussed in some detail in earlier studies[1,22c].

Measurements of $^3J_{\text{H-4,H-5}R}$, $^3J_{\text{H-4,H-5}S}$, $^3J_{\text{C-2,H-5}R}$, and $^3J_{\text{C-2,H-5}S}$ are very useful in the conformational analysis of **1a**, **1b**, **2a**, and **2b**, because these couplings are not affected by configuration at C-2 and C-3 and depend strongly on conformation. Standard $^3J_{\text{C,H}}$ values for the ^{13}C–O–C–^{1}H pathway have been determined across the *O*-glycosidic bonds of disaccharides[26a], and were recently determined in furanoid components of rigid bi- and tri-cyclic monosaccharide derivatives[26b]. These published standard couplings are appropriate in the conformational analysis of furanoses, whereas $^3J_{\text{C,H}}$ across the ring oxygen of pyranoses may exhibit considerably different behavior[8,22d–25].

Haasnoot *et al.*[27,28] have developed a semiempirical equation to relate $^3J_{\text{H,H}}$ to torsion angles in furanose rings. Because these rings are conformationally flexible with respect to phase angle and puckering amplitude[22b], there is always some uncertainty in applying empirical or semi-empirical standard coupling data in studies of their preferred conformations. Thus, while the following discussion attempts to interpret the available couplings in terms of preferred ring conformations, it is realized that the conclusions drawn are tentative and will require further scrutiny by both experimental and computational methods.

(a) α-D-erythro-*Pentulofuranose* (**1a**). The ^{1}H–^{1}H and ^{13}C–^{1}H spin-coupling constants (Tables II and V) suggest that **1a** prefers an E_2–^3E conformation. In these conformations, the dihedral angles between H-3–H-4, H-4–H-5*R*, and H-4–H-5*S* are ~30°, 10°, and 110°, respectively, giving rise to the observed couplings of 5.3, 4.8, and 2.8 Hz, respectively. In E_2–^3E, the dihedral angle between C-2 and H-5*R* is greater than that between C-2 and H-5*S*, and $^3J_{\text{C-2,H-5}R}$ (3.2 Hz) and $^3J_{\text{C-2,H-5}S}$ (1.7 Hz) are consistent with these angles. The values of $^3J_{\text{C-2,H-4}}$ (4.6 Hz) and $^3J_{\text{C-1,H-3}}$ (2.3 Hz) are consistent with dihedral angles of ~160° and ~40°, respectively. The observed 2.0 Hz coupling between C-1 and C-5 is consistent with the ~150° dihedral angle between these atoms. In the E_2–^3E conformations of **1a**, the C-2–O-2 bond is quasi-axial, thereby optimizing the "anomeric effect" (ref. 29).

(b) β-D-erythro-*Pentulofuranose* (**1b**). Analysis of the ^{1}H–^{1}H couplings suggests that **1b** prefers a conformation near ^2E–E_3. In these conformers, average values of the dihedral angles between H-3–H-4, H-4–H-5*R* and H-4–H-5*S* are ~30°, ~10°, and ~130°, respectively, and couplings of 4.9, 5.9, and 4.8 Hz are consistent with these angles. The values of $^3J_{\text{C-2,H-5}R}$ (1.0 Hz), $^3J_{\text{C-2,H-5}S}$ (~4.5 Hz), and $^3J_{\text{C-1,H-3}}$ (0.3 Hz) are also consistent with this assignment. However, in these conformers, the dihedral angle between C-2 and H-4 is close to 90°, which is not consistent with the observed coupling between these sites of 3.0 Hz. Unless the Karplus relationship for this pathway is

unusual, other conformers may contribute to the observed couplings, possibly ^{5}E and E_O. Inclusion of these conformers would increase the average dihedral angle between C-2 and H-4 and thus explain $^{3}J_{C\text{-}2,H\text{-}4}$; the remaining couplings are also consistent with this more extended conformational domain. As observed in **1a**, the preferred conformers of **1b** align the C-2–O-2 bond quasi-axially or near quasi-axially. The fluxional behavior of **1b** may be induced by the "gauche effect" (ref. 30), which is optimized for the C-5–O-5 and C-4–O-4 bonds in ^{5}E, and the C-2–O-5 and C-3–O-3 bonds in E_3.

(c) α-D-threo-*Pentulofuranose* (**2a**). Coupling data suggest a preferred conformation near ^{O}E–E_2 for **2a**. In these conformers, the dihedral angles H-3–H-4, H-4–H-5*R*, and H-4–H-5*S* are ~110°, ~10°, and ~130°, respectively, and are consistent with the observed couplings of 2.9, 6.1, and 4.3 Hz, respectively. Likewise, in these conformers, $^{3}J_{C\text{-}2,H\text{-}5R} > {}^{3}J_{C\text{-}2,H\text{-}5S}$ since the dihedral angle affecting the former is larger; couplings of 4.1 and 1.3 Hz, respectively, are observed. The values of $^{3}J_{C\text{-}1,H\text{-}3}$ (0 Hz) and $^{3}J_{C\text{-}2,H\text{-}4}$ (2.7 Hz) are consistent with the ~90° and ~130° dihedral angles, respectively, found in ^{O}E–E_2 conformers. Again, the C-2–O-2 bond is oriented quasi-axially in the preferred conformers of **2a**.

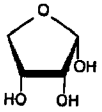
(d) β-D-threo-*Pentulofuranose* (**2b**). The values of $^{3}J_{H\text{-}3,H\text{-}4}$, $^{3}J_{H\text{-}4,H\text{-}5R}$, and $^{3}J_{H\text{-}4,H\text{-}5S}$ indicate a preferred conformation near ^{2}E. The coupling $^{3}J_{C\text{-}2,H\text{-}5R}$ (1.3 Hz) is smaller than $^{3}J_{C\text{-}2,H\text{-}5S}$ (~4.6 Hz), which is consistent with the larger dihedral angle for the latter coupling pathway. The value of $^{3}J_{C\text{-}1,H\text{-}3}$ is 2.3 Hz, as expected for a dihedral angle of ~40° in ^{2}E. Likewise, the small value of $^{3}J_{C\text{-}2,H\text{-}4}$ (1.4 Hz) is consistent with a dihedral angle of ~100°.

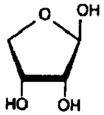
The preferred conformations of **1a**, **1b**, **2a**, and **2b** identified above should be considered "average conformations"; in at least one case (**1b**), the available data indicate considerable conformational heterogeneity. In all isomers, however, a strong conformational factor appears to be the quasi- or near quasi-axial orientation of the C-2–O-2 bond. This orientation is probably reinforced by the preferred quasi- or near quasi-equatorial orientation of the C-1–C-2 bond.

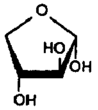
Comparison of the ring conformations of the 2-pentulofuranoses and aldotetrofuranoses. — Related coupling constants for **1a** and α-D-erythrofuranose (**5a**) (Tables II, III, and V) are very similar, indicating that these compounds assume similar ring conformations[1].

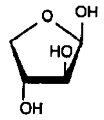
The conformational behaviors of **1b** and β-D-erythrofuranose (**5b**), on the other hand, are quite different. In both structures the anomeric carbon is strongly coupled to H-5*S* (or H-4*S*), whereas couplings between the anomeric carbon and H-5*R* (or H-4*R*) are weak[1]. This observation suggests that the preferred conformational domains are in the "western" region of the pseudorotational itinerary, preferably ^{2}E–E_3 for **1b** and ^{1}E–E_2 for **5b**. In **5b**, $^{3}J_{H\text{-}3,H\text{-}4R}$ and $^{3}J_{H\text{-}3,H\text{-}4S}$ are smaller but $^{3}J_{C\text{-}1,H\text{-}3}$ is larger than the corresponding coupling constants in **1b**, which suggests that alternative conformations make a larger contribution to **1b**.

The H-2 and H-3 signals of **5b** (ref. 1) are upfield of those for **1b** (Table IV), which indicates a significant contribution of E_3 in the conformational equilibrium of **5b**. In E_3, H-3 (of **5b**) will be less deshielded by O-1 than it is in ^{1}E. The torsion angle between O-1 and H-2 is smaller in E_3 than in ^1E and therefore H-2 will be more shielded in E_3 than in ^1E. The significant contribution of E_3 to the conformational equilibrium of **5b** is also supported by the smaller value of $^1J_{\text{C-2,H-2}}$ and larger value of $^1J_{\text{C-3,H-3}}$ in **5b** (ref. 1) than for the corresponding couplings in **1b**. In general, **1b** and **5b** are relatively conformationally flexible, with the former confined to a more limited region of the pseudorotational itinerary.


5a


5b


6a


6b

Significant differences in coupling data exist between **2a** and α-D-threofuranose[1] (**6a**). In **6a**, the couplings $^3J_{\text{C-1,H-4}R}$, $^3J_{\text{H-2,H-3}}$, $^3J_{\text{H-3,H-4}R}$, and $^3J_{\text{H-3,H-4}S}$ are smaller than the corresponding couplings in **2a**. These differences are consistent with a shift away from the ^2E conformation when the hydroxymethyl group is substituted for H-1 in **6a**. Puckering at C-2 in **6a** is further supported by the larger values of $^1J_{\text{C-2,H-2}}$ and $^1J_{\text{C-3,H-3}}$ in **6a** relative to the corresponding couplings in **2a**.

In β-D-threofuranose (**6b**), C-1 is strongly coupled to H-4*S*, whereas weak coupling is observed between C-1 and H-4*R* (ref. 1). These observations suggest that **6b** prefers a conformation near E_O–^1E. The coupling constants $^3J_{\text{H-2,H-3}}$, $^3J_{\text{H-3,H-4}R}$, and $^3J_{\text{H-3,H-4}S}$ in **6b** are smaller than the corresponding constants in **2b**, suggesting a larger phase angle for **2b**. The larger value of $^3J_{\text{C-1,H-3}}$ for **6b** also supports this difference in ring conformations.

In conclusion, the available coupling data suggest that the C-1 hydroxymethyl group of the 2-pentulofuranoses affects ring conformation by strongly favoring a quasi-equatorial orientation, and thus dictating a quasi-axial orientation for the C-2–O-2 bond. The "anomeric effect" (ref. 29) thus appears to be accentuated in the 2-pentulofuranoses (**1a**, **1b**, **2a**, **2b**) relative to the structurally related aldotetrofuranoses (**5a**, **5b**, **6a**, **6b**).

Conformation of the C-1 exocyclic hydroxymethyl group in the 2-pentulofuranoses. — Measurements of $^2J_{\text{C-2,H-1}}$ and $^2J_{\text{C-2,H-1'}}$ may be used to evaluate C-1–C-2 rotamer populations in 2-pentulofuranoses. The value of $^2J_{\text{C-2,H}}$ is ~6 Hz when one H-1 is *gauche* to O-2 and the ring oxygen, while little or no coupling is observed between the remaining H-1 and C-2 (refs. 8, 22d, 24, 25). When C-3 and O-1 are antiperiplanar, $^2J_{\text{C-2,H}} \approx 2$ Hz for both C-1 protons[8].

The sum of $^2J_{\text{C-2,H-1}}$ and $^2J_{\text{C-2,H-1'}}$ is 6.2 ±0.1 Hz in each 2-pentulofuranose, which may indicate that the rotamer having O-1 and O-2, and O-1 and O-5, *gauche* (i.e., C-3 and O-1 antiperiplanar) is present in least abundance. The different magnitudes of $^2J_{C\text{-}2,H\text{-}1}$ and $^2J_{C\text{-}2,H\text{-}1'}$ further suggest unequal populations of the remaining two rotamers. Further analysis will require the assignment of the prochiral C-1 protons by stereospecific substitution of H-1*R* or H-1*S* by ^{2}H. Measurement of $^3J_{C\text{-}3,H\text{-}1}$ in (3-^{13}C)2-pentulofuranoses would also yield useful information about C-1–C-2 rotamer populations.

CONCLUSIONS

Convenient routes for the conversion of ^{13}C-substituted pentoses into ^{13}C-substituted 2-pentuloses have been described in this study. The catalytic reduction of aldos-2-uloses to 2-ketoses is a general method that may have a greater impact on the synthesis of ^{13}C-substituted 2-ketoses than the more specific enzymic route. The chemical route may also be adapted to prepare 1-^{2}H-substituted 2-ketoses by performing the reduction in ^2H_2O with $^2H_2$ gas.

The ^{1}H–^{1}H, ^{13}C–^{1}H, and ^{13}C–^{13}C spin-coupling constants obtained in this study enabled a conformational assessment of the acyclic 2-pentuloses. The strongly dipolarized carbonyl group appears to have a significant effect on limiting the C-1–C-2 and C-2–C-3 bond conformations in these structures. Related observations have been made recently in unhydrated pentos-2-ulopyranoses[8]. Conformation in the vicinity of the carbonyl group may be determined by intramolecular hydrogen bonding, but stereoelectronic factors cannot be excluded at the present time. It is certainly true that intra- and inter-molecular hydrogen bonding are competitive processes, and their relative importance will depend on the nature of the solvent; intramolecular hydrogen bonding is possible in aqueous solution provided that steric and orientational factors are favorable.

While the use of ^{13}C–^{1}H and ^{13}C–^{13}C spin-couplings assists in evaluating the conformational properties of 2-ketofuranose rings, considerable work remains to be done in the conformational analysis of these compounds in solution. The available data suggest that the exocyclic hydroxymethyl group of the 2-pentulofuranoses confers greater conformational homogeneity on these rings than is found in the aldotetrofuranoses; in effect the C-1 exocyclic hydroxymethyl group appears to serve as a conformational "anchor". Computational studies will be needed to test this and other conclusions regarding the conformational behavior of the 2-ketofuranose ring system. Despite its limitations, however, this study of the parent 2-ketofuranose rings provides a foundation for future conformational investigations of other 2-ketoses, especially phosphorylated analogues having important biological functions.

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