[^{13}C]-Enriched Tetroses and Tetrofuranosides: An Evaluation of the Relationship between NMR Parameters and Furanosyl Ring Conformation

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Proton and carbon-13 NMR spectra of D-tetroses and their methyl glycofuranosides and dimethyl acetals were obtained and interpreted. Using [^{13}C]-enrichment and coupling patterns, all proton and carbon chemical shifts were assigned, including the protons at C4. The H4*S* proton, which by definition is cis to O3, is the more shielded of the methylene protons in tetrofuranosyl rings. [^{13}C]-Enriched compounds were used to measure ^{13}C–^{1}H and ^{13}C–^{13}C couplings and ^{13}C spin-lattice relaxation times in each isomer, and these values were interpreted along with ^{1}H–^{1}H couplings in terms of ring conformation and dynamics. The α-threofuranosyl ring appears to be the most rigid and the β-erythrofuranosyl ring the most flexible. The anomeric effect plays a dominant role in determining furanosyl ring conformation, with O1 assuming a quasi-axial orientation in all isomers. Conformational comparisons are made between tetro- and pentofuranosyl rings having similar configurations. Preferred conformations of four-carbon linear hydrates and dimethyl acetals are suggested. The limitations of ^{13}C–^{1}H and ^{1}H–^{1}H vicinal couplings for evaluation of furanosyl ring conformation are discussed.

Introduction

Conformational analysis of furanosyl rings is essential to the evaluation of solution structures of many complex biological compounds such as oligo- and polynucleotides. Ring conformations have been deduced mostly from three-bond ^{1}H–^{1}H coupling constants ($^{3}J_{HH}$) using appropriate Karplus equations.[1] It is well-known, however, that the interpretation of ^{3}J in five-membered rings is complicated since ring substitution alters the form of the Karplus equation and coupling constants can be averaged by pseudorotation.[2a,b]

Furanoside derivatives have been examined by ^{1}H NMR in nonaqueous solvents,[3] but simple furanosides in aqueous solution have received less attention, possibly because of the complexity of their spectra. Angyal[4] has obtained $^{3}J_{HH}$ values for several furanosides in $^{2}H_2O$, and Cyr and Perlin[5] have reported $^{3}J_{HH}$, $^{2}J_{CH}$, and $^{3}J_{CH}$, with ^{13}C–^{1}H couplings obtained from ^{1}H-coupled ^{13}C NMR spectra. Gerlt and Youngblood[6] used ^{1}H NMR to determine the preferred conformations of methyl β-D-ribofuranoside and its 5-phosphate, and their 2-deoxy analogues. In addition to their importance to carbohydrate chemistry, however, studies of five-membered rings in H_2O ($^{2}H_2O$) are essential to defining the factors that affect protein and nucleic acid conformation. The conformational flexibility of the furanosyl ring in DNA and RNA, and of the proline ring in proteins, is important in determining the structure and dynamics of these substances in solution.[7]

Unlike solutions of most pentoses and hexoses, aqueous solutions of the tetroses at equilibrium contain α- and β-furanose, 1,1-*gem*-diol (hydrate), and aldehyde forms,[8-10] and methyl glycosidation yields both cyclic and linear acetals.[10,11] These properties make the tetroses ideal for the study of the effect of structure on conformation and on static and dynamic NMR parameters. To obtain complete NMR data for these compounds, we prepared [^{13}C]-enriched derivatives to facilitate precise determination of J_{CH} by ^{1}H NMR, J_{CC} by ^{13}C NMR, and ^{13}C relaxation times. These data are used in conjunction with J_{HH} (obtained from unenriched compounds) to examine the conformational behavior of these molecules.

Methods and Instrumentation

Materials. Lead tetraacetate and $^{2}H_2O$ (99.8 atom %) were purchased from Aldrich Chemical Company. ^{2}H-depleted water, enzymes, and resins were purchased from Sigma Chemical Company. Potassium [^{13}C]cyanide (91 and 99 atom %) was obtained from the Los Alamos Scientific Laboratory, Los Alamos, New Mexico. Potassium [^{14}C]cyanide (46 mCi/mmol) was obtained from New England Nuclear.

Instrumentation. Rapid-scan correlation ^{1}H NMR spectroscopy at 600 MHz was performed at the NMR Facility for Biomedical Research, Department of Chemistry, Carnegie-Mellon University, Pittsburgh, PA, which is partly supported by NIH Grant RR 292.

High-field ^{1}H and ^{13}C FT NMR spectroscopy was performed on a WH-180 Bruker FT-NMR spectrometer at the Department of Chemistry, Michigan State University, East Lansing, MI 48824, and on a WM-300 Bruker FT-NMR spectrometer at the Cornell NMR Facility, Department of Chemistry, Cornell University, Ithaca, NY 14853 (supported by NSF Grant CHE 79-04825). ^{13}C relaxation (20 MHz) and ^{2}H relaxation (14 MHz) measurements were made on Varian CFT-20 and JEOL FX-90Q FT (supported by NSF Grant PCM 80-18643) spectrometers, respectively. Temperature was determined by using a Fluke 2190A digital thermometer and a copper–constantan thermocouple.

^{1}H NMR spectra were simulated by using the ITRCAL program as described previously.[12]

Preparations. D-Erythrose[13] and D-threose[14] were prepared from 4,6-*O*-ethylidene-D-glucose and 4,6-*O*-ethylidene-D-galactose, respectively. D-[1-^{13}C]Erythrose, DL-[2-^{13}C]erythrose, DL-[3-^{13}C]erythrose, D-[1-^{2}H]erythrose, D-[1-^{13}C]threose, DL-[2-^{13}C]-threose, DL-[3-^{13}C]threose, and D-[1-^{2}H]threose were prepared and purified as described previously.[9,12]

Preparation of D-[4-^{13}C]Erythrose and L-[4-^{13}C]Threose. D-[4-^{13}C]Erythrose and L-[4-^{13}C]threose were prepared by treating D-[6-^{13}C]glucose and L-[6-^{13}C]idose, respectively, with lead tetraacetate ($Pb(OAc)_4$). The [6-^{13}C]hexoses were prepared by a modification of the procedure described by Schaffer and Isbell.[15] Potassium [^{13}C]cyanide containing ~10^7 cpm K^{14}CN was reacted

(1) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11.
(2) (a) Kilpatrick, J.; Pitzer, K.; Spitzer, K. *J. Am. Chem. Soc.* **1947**, *69*, 2485. (b) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
(3) Stevens, J. D.; Fletcher, H. G., Jr. *J. Org. Chem.* **1968**, *33*, 1799.
(4) Angyal, S. J. *Carbohydr. Res.* **1979**, *77*, 37.
(5) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504.
(6) Gerlt, J. A.; Youngblood, A. V. *J. Am. Chem. Soc.* **1980**, *102*, 7433.
(7) Westhof, E.; Sundaralingam, M. *J. Am. Chem. Soc.* **1983**, *105*, 970.
(8) Serianni, A. S.; Pierce, J.; Huang, S.-G.; Barker, R. *J. Am. Chem. Soc.* **1982**, *104*, 4037.
(9) Serianni, A. S.; Clark, E. L.; Barker, R. *Carbohydr. Res.* **1979**, *72*, 79.
(10) Angyal, S. J.; Wheen, R. G. *Aust. J. Chem.* **1980**, *33*, 1001.
(11) Serianni, A. S.; Barker, R., unpublished results.
(12) Serianni, A. S.; Barker, R. *Can. J. Chem.* **1979**, *57*, 3160.
(13) Perlin, A. S. *Methods Carbohydr. Chem.* **1962**, *1*, 64.
(14) Ball, D. H. *J. Org. Chem.* **1966**, *31*, 220.
(15) Schaffer, R.; Isbell, H. S. *J. Res. Natl. Bur. Stand.* **1956**, *56*, 191.

0022-3263/84/1949-3292$01.50/0

with 1,2-*O*-isopropylidene-D-*xylo*-dialdopentofuranose followed by alkaline hydrolysis of the aldononitriles to the 5-epimeric [6-^{13}C]aldonates. The alkaline mixture containing ~3.5 mmol of the aldonates was applied to a 2.2 × 56 cm column of Dowex 1 × 8 (200–400 mesh) (OAc^-) and the column was eluted with a linear gradient of acetic acid (2500 mL, 0.0–1.4 M, adjusted to pH 3.0 with NH_4OH). Two peaks, detected by radioactivity, eluted late in the gradient: peak I, L-ido epimer; peak II, D-gluco epimer. The purified [6-^{13}C]aldonic acids were lactonized and reduced with $NaBH_4$ in methanol/sodium methoxide[16] to the protected [6-^{13}C]aldoses. The isopropylidene group was removed with 0.1 N H_2SO_4 and the purified hexoses were treated with excess $Pb(OAc)_4$.[13] D-[4-^{13}C]Erythrose and D-[3-^{13}C]glyceraldehyde were produced in a 6:1 ratio from D-[6-^{13}C]glucose. The mixture was applied to a column containing Dowex 50 × 8 (200–400 mesh) (Ba^{2+})[17] and eluted with distilled water. Fractions were assayed for aldose with phenol–sulfuric acid[18] and/or radioactivity. D-[3-^{13}C]Triose eluted first followed by pure D-[4-^{13}C]erythrose.

L-[3-^{13}C]Glyceraldehyde and L-[4-^{13}C]threose were formed in similar amounts from the treatment of L-[6-^{13}C]idose with $Pb(OAc)_4$. These compounds are not easily separated by chromatography on Dowex 50 (Ba^{2+}). Therefore, the mixture was treated with glycerolkinase (EC 2.7.1.30) from *Candida mycoderma* and Mg^{2+}–ATP at pH 7.8 to convert the L-triose to L-triose 3-phosphate.[9] The mixture was applied to a column (1.3 × 27 cm) of DEAE-Sephadex A-25 (OAc^-), and the column washed with distilled water. The column effluent containing L-[4-^{13}C]threose was deionized with Dowex 1 × 8 (200–400 mesh) (OAc^-) and Dowex 50 × 8 (200–400 mesh) (H^+), concentrated at 30 °C in vacuo to ~3 mL, and applied to a column of Dowex 50 × 80 (200–400 mesh) (Ba^{2+}). Pure L-[4-^{13}C]threose was eluted with distilled water.

Purity was assessed by comparing the ^{1}H and ^{13}C NMR spectra of the [4-^{13}C] compounds with spectra of unenriched standards.

Glycosidation. Tetroses (0.05–0.1 M) were treated with anhydrous methanol containing 0.4 N H_2SO_4 for 24 h at 22 °C. Reaction mixtures were concentrated at 30 °C in vacuo to ~5 mL and applied to columns containing excess Rexyn 203 (OH^-) or Amberlite IRA-68 (OH^-). Columns were washed with methanol, followed by H_2O until all sugar had eluted. The neutral or slightly basic effluents were concentrated to ~2 mL at 30 °C in vacuo and applied to columns of Dowex 1 × 2 (200–400 mesh) (OH^-).[19] Glycosides eluted in the following order: dimethyl acetal, O1–O2 cis furanoside, O1–O2 trans furanoside. Fractions containing each compound were pooled and concentrated to dryness at 30 °C in vacuo.

Pentofuranosides were prepared as described previously: D-*arabino*,[20] D-*lyxo*,[21] D-*ribo*,[22] and D-*xylo*.[20] Acidic reaction mixtures containing pyranosides and furanosides were neutralized as described above and purified by chromatography on columns containing Dowex 1 × 2 (200–400 mesh) (OH^-) resin. Pyranosides generally eluted first, followed by furanosides. Anomers were identified by their characteristic ^{13}C chemical shifts.[23]

NMR Sample Preparation. Aqueous solutions (~10 mL) of aldoses or glycosides were treated batchwise and separately with Dowex 1 × 2 (200–400 mesh) (OAc^-) and Dowex 50 × 8 (200–400 mesh) (H^+). Glycosides were treated with Dowex 50 (H^+) at 4 °C to prevent hydrolysis. Deionized solutions were concentrated several times to dryness (glycosides) or to ~0.2 mL (aldoses) at 30 °C in vacuo. Residues were exchanged with 3 mL of ^2H_2O (3–4 times) and dissolved in ~0.7 mL (^{1}H NMR) or ~1.8 mL (^{13}C NMR) of ^2H_2O. Solutions were passed through columns containing ~1 mL of activated[24] ^2H_2O-washed Chelex 100 (Na^+ form for glycosides, H^+ form for aldoses) and collected in 5-mm (^{1}H) or 10-mm (^{13}C) NMR tubes. Final solutions were approximately 50 mM (^{1}H) and 100 mM (^{13}C) in sugar. Tubes were sealed with plastic caps.

Spin-Lattice Relaxation Time Measurements. Samples containing equal amounts of α- and β-glycosides were treated with Dowex 50 (H^+), Dowex 1 (OAc^-), and Chelex 100 (Na^+) prior to concentration at 30 °C in vacuo to syrups. Syrups were dissolved in HOAc/NaOAc buffer (pH 5.0) containing (ethylenedinitrilo)tetraacetic acid (EDTA). Final solutions were 50 mM in buffer, 3 mM in EDTA, 0.2 M in each glycoside and 50:50 (v/v) ^2H_2O:H_2O. The clear, colorless solutions were degassed for 5–10 min with Ar immediately prior to each determination.

^{13}C spin-lattice relaxation times at 30 ± 1.5 °C were measured at 20 and 75 MHz by inversion recovery.[25] T_1 values were determined from semilogarithmic plots using a linear least-squares program. ^{2}H spin-lattice relaxation times (36 ± 2 °C) were measured at 14 MHz in a similar fashion with solutions of [1-^{2}H]tetrofuranosides prepared as described above but substituting ^{2}H-depleted H_2O for ^2H_2O. For ^{2}H spectra, the spectrometer was locked on the resonance of external Li^+, and spectra were obtained with broad-band ^{1}H decoupling.

Results and Discussion

This study is concerned with the determination and interpretation of coupling constants and relaxation times in tetrofuranosyl rings. The essential first step is the unambiguous assignment of chemical shifts. In the tetroses, it is particularly important to assign resonances to the various tautomeric forms, and to the methylene protons (H4*R* and H4*S*) since their coupling to C1 provides a direct assessment of conformation about the anomeric carbon.

^{1}H and ^{13}C NMR spectra of D-erythrose and D-threose in ^2H_2O indicate the presence of four forms:[8-10] α- and β-furanose (~88%), hydrate (~10%), and aldehyde (~2%). At 180 and 300 MHz, the H2–H4 region of D-threose is complex and interpretation requires selective deuterium substitution or computer simulation.[12] At 600 MHz, however, the spectrum is easily interpreted and nearly all assignments can be made for the three most abundant forms. The ^{1}H NMR spectrum of D-erythrose can be interpreted readily at 300 MHz.[12]

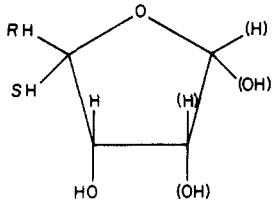
Proton Chemical Shift Assignments. Coupling patterns and/or computer simulation were used to assign ^{1}H chemical shifts (Table I).

Furanosyl ring anomeric protons resonate between 4.9–5.0 ppm (glycosides) and 5.2–5.4 ppm (aldoses). H1 of hydrates and dimethyl acetals resonate at ~5.0 and ~4.5 ppm, respectively, while H1 of aldehyde forms is found at ~9.5 ppm.[8] The remaining protons resonate between 3.6–4.4 ppm.

Glycoside formation shifts H1 of furanoses upfield by 0.37 ± 0.03 ppm, while H2 and H3 are virtually unaffected. Dimethyl acetalation shifts H1 of hydrates upfield by 0.57 ± 0.05 ppm and H2 downfield by 0.14 ± 0.02 ppm. All protons are shifted downfield when dimethyl acetals are converted to methyl furanosides (Table I).

A. Assignment of Methylene (C4) Protons Based on ^{13}C–^{1}H Coupling and Selective Deuteration. By definition, in tetrofuranosyl rings, the C4 proton cis to O3 is H4*S*, and that trans to O3 is H4*R* (1). Complementary three-bond ^{13}C–^{1}H couplings between C4 and H1, and C1 and the C4 protons provide a basis for the assignment of the enantiotopic C4 protons in the proton spectra. All $^3J_{C4,H1}$ values lie between 3.7 and 6.0 Hz (Table II) corresponding to dihedral angles (Θ) between 0–40° or 140–180°.[26] Ring geometry prohibits Θ < 60° between C4

(16) Frush, H. L.; Isbell, H. S. *J. Am. Chem. Soc.* **1956**, *78*, 2844.
(17) Jones, J. K. N.; Wall, R. A. *Can. J. Chem.* **1960**, *38*, 2290.
(18) Hodge, J. E.; Hofreiter, B. T. *Methods Carbohydr. Chem.* **1962**, *1*, 380.
(19) Austin, P. W.; Hardy, F. E.; Buchanan, J. C.; Baddiley, J. *J. Chem. Soc.* **1963**, 5350.
(20) Augestad, I.; Berner, E. *Acta Chem. Scand.* **1954**, *8*, 251.
(21) Kjølbergs; O.; Tjeltveit, O. J. *Acta Chem. Scand.* **1963**, *17*, 1641.
(22) Barker, R.; Fletcher, H. G., Jr. *J. Org. Chem.* **1961**, *26*, 4605.
(23) Ritchie, R. G. S.; Cyr, N.; Korsch, B.; Koch, H. J.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1424.
(24) Willard, J. M.; Davis, J. J.; Wood, H. G. *Biochemistry* **1969**, *8*, 3137.
(25) Vold, R. L.; Waugh, J. S.; Klein, M. P.; Phelps, D. E. *J. Chem. Phys.* **1968**, *48*, 3831.



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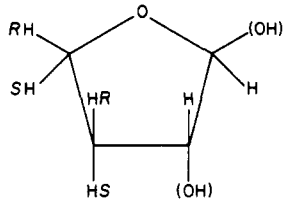

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and H1; therefore, in all cases the C4–H1 dihedral angles must lie between 140° and 180°.

For methyl α-D-threofuranoside, $^3J_{C4,H1}$ = 6.0 Hz and corresponds to a C4–H1 dihedral angle of ~165°. This arrangement should produce a larger ^{13}C–^1H coupling between C1 and H4*R* (Θ ≅ 165°) than between C1 and H4*S* (Θ ≅ 75°). The ^1H NMR spectrum of the [1-^{13}C]-enriched compound shows the larger $^3J_{C1,H4}$ coupling to the less-shielded C4 proton, identifying it as H4*R*. Similar analyses using ^{13}C–^1H couplings across the ring oxygen indicate that, in all tetrofuranosyl rings, *H4S is the more shielded C4 proton* (Table I).

Two-bond couplings (^2J) between C3–H4*R* and –H4*S* support this assignment. The magnitude and sign of $^2J_{CH}$ in carbohydrates depends on the orientation and electronegativities of substituents.[27-29] The projection rule[29] predicts that, for conformers of tetrofuranosyl rings, $^2J_{C3,H4R}$ should be in the range –2 to +2 Hz, and $^2J_{C3,H4S}$ in the range –2 to –5.5 Hz. Observed absolute values are in the ranges 0–1.3 Hz and 4.8–5.0 Hz, respectively, supporting the assignment of H4*S* as the more shielded C4 proton.

C4 proton resonance assignments were verified by treating methyl β-D-erythrofuranoside and methyl α-D-threofuranoside with deuterated Raney nickel in ^2H_2O at 90 °C, which causes ^1H–^2H exchange at H2, H3, and a single H4.[30] The exchanged C4 proton was identified by converting the trideuterated tetroses to trideuterated [1-^{13}C]pentoses and evaluating the coupling of C1 to the remaining H5 in the more conformationally rigid pyranosyl rings. These results also confirm that H4*S* is the more shielded C4 proton in tetrofuranosyl rings.

B. Assignment of Methylene (C4) Protons Based on Substituent Effects on ^1H Chemical Shifts. In pentacyclic systems, according to the "syn-upfield" rule,[31] the resonance of a proton vicinal to a substituent (–OH or CH_3) becomes deshielded by up to ~0.5 ppm as the dihedral angle between them increases from 0° (syn) to 180° (anti). In tetrofuranosyl derivatives, syn–anti designations refer to dihedral angles of 0° ± 60° and 120° ± 60°, respectively. In fact, it may be more accurate to refer to these as cis–trans arrangements. We have done so in the ensuing discussion, while recognizing the original terminology proposed by Anteunis and Danneels.[31]

The applicability of the "syn–upfield" rule to methylene proton assignment in tetrofuranosyl rings was assessed by comparing equivalent protons in epimeric pairs. For example, H1 is cis to O2 in β-erythrofuranose and trans to O2 in β-threofuranose. The "syn–upfield" rule predicts that H1 in the former will be upfield from H1 in the latter, and it is by 0.15 ppm. Similarly, H3 in β-threofuranose is cis to O2 and 0.09 ppm upfield from H3 in β-erthrofuranose, in which H3 is trans to O2. In general, tetrofuranosyl ring methine protons cis to a vicinal hydroxyl group are shielded by ~0.1 ppm compared to those that are trans. This difference is smaller than that reported in other systems, possibly due to long-range shielding by other substituents and to conformational effects.

In applying these correlations to the assignment of the C4 protons, vicinal interactions with O3 and long-range interactions with O1 and O2 were considered. In the β-erythro ring, H4*S* is cis to O3 and O2 and trans to O1, while H4R is trans to O2 and O3 and cis to O1. If 1,3-interactions with O1 and O2 cancel, the net difference is a cis interaction of H4*S* and a trans interaction of H4*R* with O3. The "syn–upfield" rule predicts H4*S* to be the more shielded proton, in agreement with conclusions based on $^2J_{CCH}$, $^3J_{COCH}$, and [^2H] substitution. The average difference in chemical shift between H4*R* and H4*S* is 0.35 ± 0.1 ppm for β-erythrofuranose, α-threofuranose, and their methyl glycosides (Table I), the only isomers in which the effects of O1 and O2 could cancel.

For β-threo compounds, H4*S* is cis to O3 and trans to O1 and O2, and H4*R* is trans to O3 and cis to O1 and O2. Differences in chemical shifts are 0.53 ppm for β-threofuranose and 0.48 ppm for methyl β-threofuranoside (Table I). If 0.35 ppm of this difference is attributed to the 1,2-interaction between the C4 protons and O3, then the 1,3-cis interactions with O1 and O2 must each cause downfield shifts of ~0.08 ppm.

For α-erythro compounds, H4*S* is cis to O1 and O2, and H4*R* is trans to O1 and O2. When the empirical shielding values for 1,2- and 1,3-interactions derived above were used, the resonance of H4*S* is predicted to be 0.2 ± 0.1 ppm downfield of H4*R*. Observed differences of 0.10 ppm for α-erythrofuranose and 0.26 ppm for methyl α-erythrofuranoside (Table I) are in fair agreement with this prediction.

These results suggest that the "syn–upfield" rule, as applied to 1,2-interactions, can be used to make an internally consistent assignment of protons in tetrofuranosyl rings and that 1,3-interactions may have smaller and opposite effects on ^1H chemical shifts. Similar arguments can be made to assign proton resonances in related structures, for example, in the trans-O1,O2 anomer of 3-deoxytetrofuranose (**2**). ^1H chemical shifts (ppm) for **2**: H1 (5.25), H2 (4.23), H3*R* (2.26), H3*S* (1.91), H4*R* (4.1), H4*S* (4.1). From the difference in the C3 methylene proton chemical shifts (0.35 ppm), the more shielded proton can be assigned to H3S. In this compound, the C4 protons have similar chemical shifts since both have one cis and one trans 1,3-interaction. Also, the chemical shift difference between the C2 protons of methyl 2-deoxy-D-ribofuranoside is larger for the α-anomer than for the β-anomer,[32] as predicted from these rules.

It is worth stressing that these correlations are made without consideration of conformational effects. The "syn–upfield" rule is, however, based on conformational considerations. The generally satisfactory fit of the data may indicate that, in the present case, configuration rather than conformation is the important corollary of chemical shift, although rapid conformational averaging could give the same result.

Assignment of the C4 protons of the linear hydrate form of D-erythrose was also accomplished by selective [^2H] substitution.[30] The more shielded C4 proton in this com-

(26) Perlin, A. S.; Hamer, G. K. "Carbon-13 NMR in Polymer Science"; Pasika, W. M., Ed.; American Chemical Society: Washington, DC, 1979; p 123.
(27) Schwarcz, J. A.; Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1872.
(28) Cyr, N.; Hamer, G. K.; Perlin, A. S. *Can. J. Chem.* **1978**, *56*, 297.
(29) Bock, K.; Pedersen, C. *Acta Chem. Scand., Ser. B* **1977**, *31*, 354.
(30) Wu, G. D.; Serianni, A. S.; Barker, R. *J. Org. Chem.* **1983**, *48*, 1750.
(31) Anteunis, M.; Danneels, D. *Org. Magn. Reson.* **1975**, *7*, 345.
(32) Serianni, A. S.; Barker, R., unpublished observation.

Table I. Proton Chemical Shifts[a] of Tetrofuranoses, Tetrofuranosides, Pentofuranosides, Tetrose Hydrates, and Dimethyl Acetals

compound	chemical shift, ppm							
	H1	H2	H3	H4*R*	H4*S*	H5*S*	H5*R*	OCH_3
α-erythrofuranose	5.27	4.10	4.28	4.02	3.92			
α-erythrofuranoside	4.90	4.12	4.24	4.13	3.87			3.40
β-erythrofuranose	5.25	4.02	4.39	4.20	3.79			
β-erythrofuranoside	4.90	4.04	4.35	4.11	3.81			3.39
α-threofuranose	5.24	4.05	4.21	4.20	3.95			
α-threofuranoside	4.91	4.06	4.20	4.27	3.84			3.37
β-threofuranose	5.40	4.04	4.30	4.18	3.65			
β-threofuranoside	5.00	4.09	4.28	4.15	3.67			3.42
α-arabinofuranoside	4.91	4.04	3.93	4.02		3.80	3.69	3.40
β-arabinofuranoside	4.89	4.13	3.99	3.87		3.76	3.59	3.41
α-lyxofuranoside	4.95	4.11	4.32	4.24		3.81	3.73	3.44
β-lyxofuranoside	4.91	4.19	4.24	4.15		3.83	3.72	3.40
α-ribofuranoside	4.99	4.11	4.03	4.09		3.73	3.66	3.42
β-ribofuranoside	4.88	4.02	4.14	4.00		3.78	3.58	3.38
α-xylofuranoside	4.99	4.14	4.29	4.23		3.76	3.69	3.44
β-xylofuranoside	4.89	4.12	4.21	4.35		3.83	3.73	3.39
erythrose hydrate	5.09	3.54		3.64	3.79			
erythrose dimethyl acetal	4.49	3.66	3.72	~3.61[b]	~3.74[b]			3.48, 3.45
threose hydrate	5.02	3.46	3.87	3.67[b]	3.63[b]			
threose dimethyl acetal	4.49	3.61	3.79	~3.62	~3.62			3.47, 3.46

[a] Relative to internal sodium 3-(trimethylsilyl)-1-propanesulfonate; accurate to ±0.01 ppm. [b] *R*, *S* assignment may be reversed.

Table II. ^{13}C–^{1}H Coupling Constants[a] in Tetrofuranoses and Tetrofuranosides

coupled nuclei (C, H)	α-erythro-furanose	α-erythro-furanoside	β-erythro-furanose	β-erythro-furanoside	α-threo-furanose	α-threo-furanoside	β-threo-furanose	β-threo-furanoside
1,1	173.9		171.8		172.1	173.1	172.8	173.5
1,2	1.1	br	0	2.4		br	2.0	1.3
1,3	4.6	5.5	4.0	3.9		3.1	2.6	2.1
1,4R	3.4	3.7	1.3	0.6	3.5	4.0	1.1	br
1,4S	1.9	1.2	3.8	4.8	2.7	1.6		5.6
1,OCH_3		4.5		4.4		4.5		4.5
2,1	3.1	2.1	0	br	<1	<1	4.0	2.8
2,2	148.1	147.7	148.7	149.2	152.4	153.3	149.8	148.4
2,3	0.7	1.6	0	0	2.1	~3.3	3.7	4.1
2,4R	1.7	1.4	1.7	1.6	1.9	~2.4	2.4	2.4
2,4S	3.1	2.6	3.5	3.5	1.8	1.3	1.9	1.7
3,1		~4.4	1.7	1.9	2.1	2.1	3.1	~4.1
3,2				1.0	2.3	~2.3		
3,3		155.0		152.0	152.1	152.4		150.9
3,4R			0	1.3	br	br		0
3,4S		5.0	5.0	5.0	4.9	4.8		~4.9
4,1	5.0		3.7	4.7	5.2	6.0	4.2	4.8
4,2	1.2		2.3	2.5				1.1
4,3	2.8		2.0	2.2	3.0			
4,4R	149.3		148.0	148.1		150.4		150.2
4,4S	150.4		151.0	151.2	149.2	149.3		149.8

[a] ±0.2 Hz. br = broadened.

pound is H4*R* (Table I), in contrast to the tetrofuranosyl rings in which H4*S* is the more shielded C4 proton.

Carbon-13 Chemical Shift Assignments. ^{13}C Chemical shifts for the tetroses and their glycosides (Table III) were assigned unequivocally by using [^{13}C]-enriched compounds (see Materials and Methods). One-bond ^{13}C–^{13}C coupling to unenriched carbons and spin-lattice relaxation times (T_1) (see below) support these assignments.

Anomers were assigned on the basis of C1 chemical shifts[23] and $^{2}J_{C2,H1}$. Generally, absolute $^{2}J_{C2,H1}$ values of 0–2 Hz are observed for furanose anomers having O1–O2 trans and 2–5 Hz for O1–O2 cis (Table II).

Resonances of ring carbons are affected in a predictable fashion by the configuration of hydroxyl groups.[23] A cis interaction between OH groups attached to contiguous carbons produces an upfield shift of 5.0 ± 1.1 ppm for both carbons relative to a trans relationship. The observed differences in the chemical shifts of C1 in anomeric pairs

Table III. Carbon-13 Chemical Shifts[a] of Tetrofuranoses, Tetrofuranosides, Tetrose Hydrates, and Dimethyl Acetals

compound	chemical shift, ppm				
	C1	C2	C3	C4	CH_3
α-erythrofuranose	96.8	72.4	70.6	72.9	
α-erythrofuranoside	103.7	72.9	70.2	73.8	57.0
β-erythrofuranose	102.4	77.7	71.7	72.4	
β-erythrofuranoside	109.9	76.9	72.0	73.0	57.1
α-threofuranose	103.4	82.0	76.4	74.3	
α-threofuranoside	110.3	81.4	76.6	74.6	56.3
β-threofuranose	97.9	77.5	76.2	71.8	
β-threofuranoside	104.8	78.4	76.2	72.0	57.0
erythrose hydrate	90.8	74.9	73.0	64.0	
erythrose dimethyl acetal	106.1	72.6	73.1		
threose hydrate	91.1	74.6	72.2	64.4	
threose dimethyl acetal	105.9	71.5	72.1		

[a] Referenced to the anomeric carbons of D-[1-^{13}C]glucopyranose (α, 93.6 ppm; β, 97.4 ppm); accurate to ±0.2 ppm.

Table IV. ^1H–^1H Coupling Constants in Tetrofuranoses, Tetrofuranosides, Pentofuranosides, Tetrose Hydrates, and Dimethyl Acetals

compound	^1H–^1H coupling constants, Hz[a]								
	1,2	2,3	3,4*R*	3,4*S*	4*R*,4*S*	4,5*S*	4,5*R*	5*S*,5*R*	1,3
α-erythrofuranose	4.7	5.2	5.1	3.0	−10.1				
α-erythrofuranoside	4.6	5.6	4.9	2.2	−10.3				
β-erythrofuranose	3.4	4.8	4.9	3.5	−9.8				0.6
β-erythrofuranoside	2.9	4.8	5.0	3.5	−9.8				0.3
α-threofuranose	1.2	1.9	5.6	3.3	−10.1				0.4
α-threofuranoside	0.5	1.2	5.8	3.3	−9.9				0.6
β-threofuranose	4.1	4.1	5.4	3.6	−9.6				0.5
β-threofuranoside	4.4	4.6	6.1	4.1	−9.7				0.5
α-arabinofuranoside	1.7	3.3	5.9			3.1	6.1	−12.2	0.5
β-arabinofuranoside	4.6	8.0	7.1			3.2	7.4	−12.1	
α-lyxofuranoside	3.6	4.8	4.3			4.4	6.7	−11.9	
β-lyxofuranoside	4.8	5.0	4.6			4.5	7.6	−11.9	
α-ribofuranoside	4.3	6.2	3.3			3.1	4.8	−12.4	
β-ribofuranoside	1.2	4.6	6.9			3.1	6.6	−12.2	
α-xylofuranoside	4.5	5.5	6.1			3.8	6.0	−12.2	
β-xylofuranoside	br	1.7	5.1			4.4	7.6	−11.9	
erythrose hydrate	4.1	6.7	7.6		−12.1				
erythrose dimethyl acetal	4.6	6.1	~7.3	~3.3	−12.5				
threose hydrate	6.2	2.7	5.0[b]	7.3[b]	−11.5				
threose dimethyl acetal	7.0	2.3	5.1[b]	7.3[b]					

[a] ±0.1 Hz. br = broadened. [b] Assignments could be reversed.

Table V. ^{13}C–^{13}C Coupling Constants[a] in Tetrofuranosides

compound	coupling constant, Hz						
	1,2	1,3	1,4	1,OCH_3	2,3	2,4	2,OCH_3
β-erythrofuranoside	47.6	2.1	0	1.5	36.6	1.8	3.1
α-threofuranoside	47.3	2.1	0	1.8	40.0	1.5	3.4
β-threofuranoside	43.9	2.8	0	2.1	40.6	2.8	2.1

[a] ±0.2 Hz.

and of C3 between erythro and threo isomers are predicted by this effect. The C2 resonances of α-erythro isomers are ~9 ppm upfield of those for α-threo isomers, the former having two cis interactions, the latter none. The C2 resonances of the β-anomers are similar; each has both a cis and a trans interaction.

Methyl glycosidation of the tetrofuranoses causes C1 to shift downfield by 7.1 ± 0.3 ppm while the remaining resonances are virtually unchanged. Dimethyl acetalation shifts C1 of the tetrose hydrates downfield by 15.1 ± 0.4 ppm and C2 upfield by 2.7 ± 0.6 ppm.

Cyclization shifts C1 of the tetrose hydrates downfield by 9.2 ± 3.2 ppm and C4 downfield by 8.7 ± 1.0 ppm. The large downfield shift of carbon resonances caused by ring closure can be used to monitor chemical conversions and characterize reaction intermediates.[33]

Coupling Constants. ^{13}C–^1H and ^1H–^1H coupling constants are listed in Tables II and IV. ^{13}C–^1H couplings were measured in ^1H NMR spectra (600 MHz) of specifically [^{13}C]-enriched compounds. $^3J_{CH}$ couplings accurate to ±0.2 Hz can be measured by this approach in contrast to measurements made on ^1H-coupled ^{13}C NMR spectra where only couplings >2 Hz can be resolved and where second-order perturbations are common.[5,34]

^{13}C–^{13}C coupling constants (Table V) were obtained from spectra of singly [^{13}C]-enriched tetrofuranosides.

Conformations of Tetrofuranoses and Tetrofuranosides. A. ^{13}C and ^2H Spin-Lattice Relaxation Times. ^{13}C spin-lattice relaxation times reflect the relative mobilities of carbon atoms if other factors, such as C–H bond lengths, are similar for the atoms being compared. In rigid structures, all carbon atoms will have similar relaxation times if overall molecular motion is isotropic, as expected for small molecules such as tetrofuranoses. In flexible structures with uniform internal and overall isotropic motion, relaxation times should also be similar, whereas segmental motions (libration) may produce differences in relaxation times.

Table VI. ^{13}C Spin-Lattice Relaxation Times (T_1) in Methyl D-Furanosides

compound	carbon T_1, s[c]				
	C1	C2	C3	C4	C5
α-erythrofuranoside[a]	3.7	3.9	3.5	2.4 (1.9)[d]	
β-erythrofuranoside[a]	4.1	4.0	4.4	2.5 (2.2)	
α-threofuranoside[a]	3.4	3.6	3.6	2.5 (1.8)	
β-threofuranoside[a]	4.2	3.9	4.4	2.4 (2.2)	
α-arabinofuranoside[b]	2.5	2.4	2.4	2.4	1.4 (1.2)[d]
β-arabinofuranoside[b]	2.3	2.4	2.4	2.2	1.4 (1.2)
α-lyxofuranoside[b]	2.6	2.6	2.5	2.6	1.5 (1.3)
α-ribofuranoside[b]	2.5	2.5	2.2	2.5	1.3 (1.2)
β-ribofuranoside[b]	2.3	2.2	2.3	2.2	1.2 (1.1)
α-xylofuranoside[b]	2.5	2.4	2.4	2.5	1.4 (1.2)
β-xylofuranoside[b]	2.3	2.2	2.4	2.4	1.4 (1.2)

[a] 30 ± 1 °C. [b] 36 ± 1 °C. Values taken from ref 30. [c] Accurate to within ±10%. [d] NT_1 Values predicted from average value of other carbons are shown in parenthesis.

The relaxation of carbon nuclei in furanosides is predominantly dipolar;[35,36] nuclear Overhauser enhancements (NOE) of 2.9 ± 0.2 were obtained for each carbon. T_1

(33) Serianni, A. S.; Nunez, H. A.; Barker, R. *J. Org. Chem.* **1980**, *45*, 3329.
(34) Cyr, N.; Ritchie, R. G. S.; Spotswood, T. M.; Perlin, A. S. *Can. J. Spectrosc.* **1974**, *19*, 190.
(35) Berry, J. M.; Hall, L. D.; Wong, K. F. *Carbohydr. Res.* **1977**, *56*, C16.
(36) Serianni, A. S.; Barker, R. *J. Magn. Reson.* **1982**, *49*, 335.

Table VII. Correlation between Θ and ^3J

Θ[a]	$^3J_{H1,H2}$[b]	$^3J_{H2,H3}$[b]	$^3J_{H3,H4R}$[b]	$^3J_{H3,H4S}$[b]	$^3J_{CCCH}$[c]	$^3J_{COCH}$[d]
0	6.1	8.2	8.7	8.1		
12	5.8	7.8	7.8	7.8		
24	5.0	6.9	5.2	7.2		
32	4.5	6.2	4.0	6.5		
38	4.1	5.3	3.3	5.9		
40	3.8	4.9	2.9	5.6		
80	1.0	0.7	0.6	1.3	0.6	0.3
82	1.0	0.7	0.7	1.1	0.5	0.2
88	1.0	0.7	1.2	1.0	0.3	0
96	1.2	0.9	2.0	1.1	0.2	0.2
108	1.9	1.8	4.0	1.8	0.2	0.5
120	3.0	3.2	6.0	2.9	0.4	1.4
132	4.2	5.3	8.2	4.5	0.8	2.2
144	5.8	7.2	9.6	6.9	2.3	3.9
152	6.6	8.0	9.9	7.7	3.6	4.6
158	7.0	8.6	10.1	8.4	4.6	5.1
160	7.1	8.9	10.2	8.6	5.1	5.2

[a] Dihedral angles appropriate for $\tau_m = 40°$. [b] Values calculated as described in ref 37. [c,d] Values determined from correlations drawn in ref 38(c) and ref 26(d).

values of C1, C2, and C3 in the α-anomers of tetrofuranosides are significantly smaller than those of β-anomers (Table VI). T_1 values of C4 are larger than one-half of the average T_1 for C1–C3, suggesting internal motion in the region of these nuclei. Differences in T_1 between carbons in the tetrofuranosyl rings are reproducible. These results contrast sharply with the essentially identical ^{13}C T_1 values found for ring carbons in pentofuranosides[30] and suggest that exocyclic hydroxymethyl groups stabilize furanosyl ring conformation. Because of their larger molecular weight, C_5 glycosides are expected to tumble more slowly in solution than C_4 glycosides, and to have smaller T_1 values, as is observed.[36]

Whether differences between C1 T_1 values of tetrofuranoside anomers are due to differences in C1–H1 bond lengths can be evaluated from ^{2}H spin-lattice relaxation, which is predominantly quadrupolar and essentially unaffected by bond lengths. Deuterium T_1 values of [1-^{2}H]-enriched compounds at 36 ± 2 °C: α-threofuranoside, 150 ± 9 ms; β-threofuranoside, 201 ± 10 ms; β-erythrofuranoside, 192 ± 3 ms. The essentially constant ratio of ^{13}C T_1 (Table VI) to ^{2}H T_1 values (21.6 ± 0.9) indicates that differences in ^{13}C T_1 between isomers are not due to differences in C–H bond lengths.

B. Relationship of Vicinal ^{1}H–^{1}H and ^{13}C–^{1}H Coupling to Furanose Conformation. Three-bond (vicinal) couplings (^3J) can be used to determine conformation only when "Karplus" curves that relate ^3J to dihedral angle (Θ) can be established. In this study, we have used the empirical equation of Altona and co-workers[37] to interpret $^3J_{HH}$, a treatment which accounts for substituent orientation and electronegativity effects (Table VII). $^3J_{COCH}$ and $^3J_{CCCH}$ values were related to Θ as described by Perlin and co-workers (Table VII).[26,38]

The interpretation of spin–spin coupling in furanosyl rings is complicated by their known conformational flexibility in solution. Pseudorotation of furanose rings can be described with two parameters:[2b] τ_m, the puckering amplitude, and P, the phase angle of pseudorotation. These parameters can be used to calculate the five internal ring torsion angles. The twist (T) conformation where C2 and C3 are displaced below and above the plane defined by the remaining ring atoms (^3_2T) is the standard puckered state. If P is expressed in radians, then the standard state

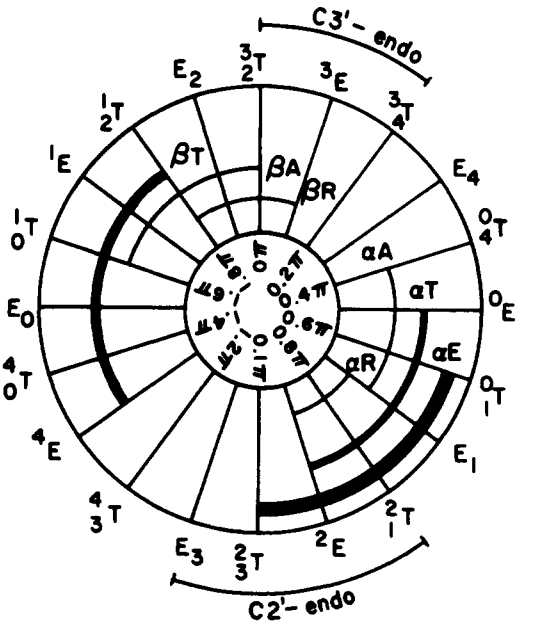


Figure 1. The pseudorotational itinerary, with preferred regions shown for α-D-erythro, α-D-threo, β-D-threo, α-D-ribo, β-D-ribo, α-D-arabino, and β-D-arabino configurations. The radius at a preferred P is not meant to imply a preferred τ_m. The preferred conformations of furanosyl rings in nucleic acids (C3′-endo and C2′-endo) are also indicated.

has $P = 0\pi$, even increments (0.2π, 0.4π,.....2π) correspond to 10 symmetrical twist (T) forms, and odd increments correspond to 10 envelope (E) forms (Figure 1).

Many furanosyl rings are flexible so that conformers can interconvert rapidly through pseudorotation, and, in addition, the puckering amplitude of conformers may vary. Jardetsky[39] has drawn attention to the general problem of making conformational assignments from NMR parameters in such systems. Nevertheless, several investigators have used two-, or, more often, three-bond coupling constants to make conformational assignments in pentofuranosyl derivatives.[3-5]

The complexity of the problem of making conformational assignments from three-bond couplings is illustrated in Figure 2. Here the equation of Altona and co-workers[37] for ^{1}H–^{1}H coupling and those of Perlin and co-workers[26,38] for ^{13}C–^{1}H coupling are used to show how these couplings vary through the pseudorotational itinerary. The relationship between P/π, used as the abscissa in Figure 2, and the usual conformational designations is shown in Figure 1. In each case illustrated in Figure 2, the couplings expected with puckering amplitudes of 40° and 60° are compared. Clearly, a single ^3J value can correspond to several different pseudorotamers and, further, the assignment is very sensitive to puckering amplitude. It is obvious also that, in conformationally stable systems, if an appropriate "Karplus" relationship is established, $^3J_{HH}$ (trans) and $^3J_{CH}$ values are more useful in making conformational assignments than are $^3J_{HH}$ (cis) values.

When conformers are mobile with time constants of interconversion that are in the NMR domain, the interpretation of coupling constants is complex. Rapidly interconverting conformers will generate couplings that reflect a weighted average of the contributing states. The number of significant conformers and the kinetics and pathway of their interconversion all contribute to the complexity of the analysis. Recent calculations[40,41] have

(37) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; de Leeuw H. P. M.; Altona, C. *Org. Magn. Reson.* **1981**, *15*, 43.
(38) Schwarcz, J. A.; Perlin, A. S. *Can. J. Chem.* **1972**, *50*, 3667.
(39) Jardetzky, O. *Biochem. Biophys. Acta* **1980**, *621*, 227.
(40) Detar, D. F.; Luthra, N. P. *J. Am. Chem. Soc.* **1977**, *99*, 1232.

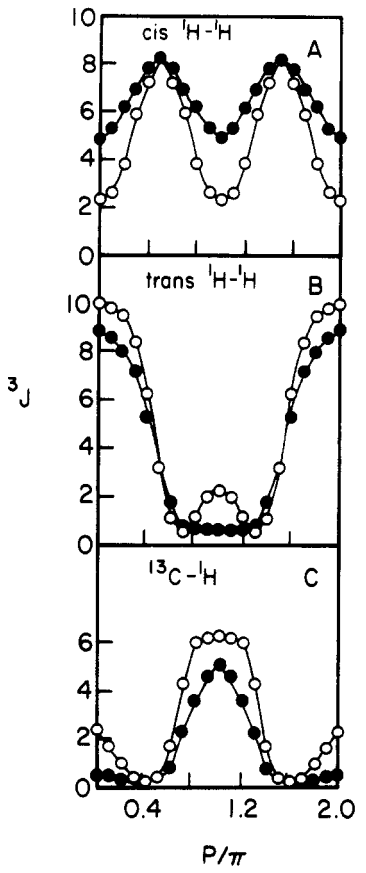


Figure 2. The effect of phase angle (P) and puckering amplitude (τ_m) on vicinal coupling in furanosyl rings. A. cis $^3J_{H2,H3}$ (erythro). B. trans $^3J_{H2,H3}$ (threo). C. $^3H_{C1,H3}$ (erythro, threo). Puckering amplitude = 40° (●) and 60° (○).

shown that barriers to pseudorotation for furanose rings are lower than those for inversion (interconversion via the planar state) and are in the range 2–4 kcal/mol, favoring conformational instability.

It is clear that conformational conclusions based on ^3J in conformationally mobile molecules, where little is known about the stabilities and lifetimes of the possible conformers, may be meaningless. Even so, conformational assignments of pentofuranosyl rings have been suggested based on as few as three $^3J_{HH}$ values. The validity of these earlier assignments can be tested by using the often more numerous $^3J_{CH}$ values, several of which correspond to the same ring dihedral angle (Θ). In some cases, the presence of significant conformational averaging will be detected in a sensitive fashion as noncomplementarity in $^3J_{CH}$ values. The observation of both $^3J_{CH}$ and $^3J_{HH}$ should allow firmer conclusions to be drawn, whether or not limited or free pseudorotational behavior occurs.

C. Determinants of Furanose Ring Conformation. Five effects determine furanosyl conformation: (a) the anomeric effect,[42-44] (b) preferred quasi-equatorial orientation of side chains,[45] (c) staggered orientation of ring substituents,[4] (d) the gauche effect,[46] and (e) a preference for tetrahedral carbon angles in the ring.[47] Only four of these effects operate in tetrofuranosyl rings, which lack side chain substituents.

Conformation at the anomeric carbon of tetrofuranosyl rings can be assessed from couplings between C1 and H4R, H4S and between C4 and H1. $^3J_{C4,H1}$ values are independent of configuration (Table II), suggesting that the anomeric effect (preferred quasi-axial orientation of O1) is strong in all four configurations. Coupling magnitudes also suggest that the quasi-axial orientation of O1 is more pronounced in tetrofuranosides than in tetrofuranoses, since $^3J_{C4,H1}$ (glycoside) > $^3J_{C4,H1}$ (aldose) and the difference between $^3J_{C1,H4R}$ and $^3J_{C1,H4S}$ (glycoside) > the difference between $^3J_{C1,H4R}$ and $^3J_{C1,H4S}$ (aldose). This observation is consistent with the relative magnitudes of anomeric effects in pyranosyl rings where OMe > OH.[48] The virtual invariance of $^1J_{C1,H1}$ with anomeric configuration (Table II) supports the conclusion that O1 assumes a similar (quasi-axial) orientation in tetrofuranose rings,[5] in contrast to the observed dependence of $^1J_{C1,H1}$ on anomeric configuration in pyranosyl rings.[49] The large $^3J_{C4,H1}$ values and the differential couplings between C1 and the C4 protons suggest that torsions about the C4–O4 and O4–C1 bonds are constrained and that pseudorotational mobility is restricted in this region.

Conformation of the C2–C3–C4 fragment is determined by the relative strengths of 1,2- and 1,3-interactions and the gauche effect. This latter effect[46] refers to the strong preference for gauche C–O–C–O and O–C–C–O arrangements rather than trans arrangements. If these effects have similar strengths and favor different C4–C3–C2–C1 torsion angles, pseudorotation (torsional libration) may occur in this portion of the molecule.

From these considerations, a preliminary model of tetrofuranosyl ring conformation can be proposed: a relatively rigid C4–O4–C1–O1 segment due to the strong preference for a quasi-axial O1 (anomeric effect), and a relatively flexible C2–C3–C4 segment with conformation of this portion determined by several competing nonbonded interactions. This model is evaluated below by examining coupling data for eight tetrofuranosyl compounds.

D. Specific Tetrofuranosyl Conformations. α-Threo Isomers. Quasi-axial orientation of O1 is attained in conformations ^0E (0.5π) to ^2E (0.9π). The ^2E conformation is probably less stable than ^0E or E_1 due to the presence of an unfavorable 1,3-interaction between O1 and O3. Gauche effects cancel.

In assessing conformational preferences, couplings between C1 or C2 and the C4 protons are particularly useful. Coupling between C1 and C4 protons is consistent with ^2_1T and ^3_2T, that between C2 and the C4 protons is consistent with E_1 and ^1E, while coupling between C4 and H1 is consistent only with the ^0_1T conformation. $^3J_{H1,H2}$, $^3J_{H2,H3}$, $^3J_{H3,H4R}$, $^3J_{H3,H4S}$, and $^3J_{C1,H3}$ are consistent with conformations within the E_1 region. Only $^3J_{C3,H1}$ with an observed value of 2.1 Hz and an expected value of ~4–5 Hz is inconsistent with a conformation in the ^0E–^2E region of the pseudorotational itinerary. This discrepancy points to the possibility that radically different "Karplus" relationships exist for certain ring segments.

The α-threo isomer is probably the most conformationally stable tetrofuranosyl isomer, and apart from $^3J_{C3,H1}$, the coupling constants reflect a conformation in the E_1 segment of the itinerary (Figure 1), as expected on

(41) Levitt, M.; Warshel, A. *J. Am. Chem. Soc.* **1978**, *100*, 2607.
(42) Lemieux, R. U. "Molecular Rearrangements"; de Mayo, P., Ed.; Wiley-Interscience: New York, 1963; p 713.
(43) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. "Conformational Analysis"; Interscience: New York, 1965; p 375.
(44) Kirby, A. J. In "Reactivity and Structure Concepts in Organic Chemistry"; Springer-Verlag: New York, 1983; Vol. 15.
(45) Pitzer, K. S.; Donath, W. E. *J. Am. Chem. Soc.* **1959**, *81*, 3213.
(46) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102.
(47) Abraham, R. J.; McLauchlan, K. A. *Mol. Phys.* **1962**, *5*, 195.
(48) Stoddart, J. F. "Stereochemistry of Carbohydrates"; Wiley-Interscience: New York, 1971; p 72.
(49) Bock K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.

Table VIII. ^{13}C–^{1}H Coupling Constants[a] in Methyl D-Pentofuranosides

	arabino-furanoside		ribofuranoside	
J (C,H), Hz	α	β	α	β
1,1	173.0	174.7	173.3	174.2
1,2	1.7	0	~0.6	0
1,3	1.8	0	4.7	0.9
1,4	0	3.2		3.0
1,OCH_3	4.3	4.5	4.5	4.3
2,1			2.3	br
2,2				
2,3			1.1	1.6
2,4			1.0	0.6

[a] ±0.2 Hz. br = broadened.

the basis of the anomeric effect and other nonbonded interactions.

β-Threo Isomers. The anomeric effect favors conformations in the E_0–E_2 region. Maximal separation of O1 and O2 is attained in ^{1}E–E_2 while O2 and O3 are maximally separated in ^{4}E–E_0. The ^{4}E conformation is destabilized by an interaction between O1 and O2. Gauche effects may slightly favor E_2. There are no 1,3-interactions present in any of these conformers.

^{13}C–^{1}H coupling across the ring oxygen suggests a $^{4}_{0}T$ conformation and those between C2 and C4 protons are consistent with conformations near ^{1}E or E_1. The $^{1}_{0}T$ conformer is consistent with both sets of couplings and is supported by the large C3–H1 (~4.1 Hz) and C4–H1 (4.8 Hz) couplings ($\Theta_{C3,H1} \simeq \Theta_{C4,H1} \simeq 150°$). $^{3}J_{HH}$ values are consistent with conformations near ^{0}E or ^{1}E, but the former region is inconsistent with ^{13}C–^{1}H couplings. This case shows that conformational conclusions often cannot be based on $^{3}J_{HH}$ data alone, and illustrates the value of ^{13}C–^{1}H coupling data in the analysis.

α-Erythro Isomers. The anomeric effect favors the ^{0}E–^{2}E region. The presence of a 1,2-interaction in ^{0}E and a 1,3-interaction in ^{2}E may favor the E_1 conformer (Figure 1).

$^{3}J_{CH}$ values across the ring oxygen are consistent with E_1 and $^{3}_{2}T$, whereas C2 coupling to the C4 protons favors E_1 and ^{1}E. The observed C4–H1 coupling (5.0 Hz), C3–H1 coupling (~4.4 Hz), and C1–H3 coupling (5.5 Hz) are consistent with conformations near E_1 ($\Theta \cong 140°$) and inconsistent with ^{1}E ($\Theta \cong 90°$). $^{3}J_{HH}$ values are consistent with two broad pseudorotational segments centered at $^{2}_{1}T$ or $^{1}_{2}T$. Only the former is consistent with $^{3}J_{CH}$ values.

β-Erythro Isomers. The anomeric effect favors E_0–E_2 conformations. The E_0 conformer is destabilized by a 1,2-interaction. Substituents are maximally staggered and gauche effects cancel in the E_2 conformer.

In this isomer, the complementarity of ^{3}J values is poor, and conformation cannot be assigned. The data suggest rapid pseudorotation so that observed couplings are averaged in a complex fashion. Angyal[4] has suggested that β-erythrofuranoside exists in both $^{3}T_2$ and $^{2}T_3$ conformations (on opposite sides of the pseudorotational itinerary) based on $^{3}J_{HH}$.

Conformations of Pentofuranosides. ^{1}H–^{1}H coupling constants (Table IV) and several ^{13}C–^{1}H couplings (Table VIII) were measured in methyl D-pentofuranosides. Conformations for α- and β-ribofuranosides compare favorably with those reported previously,[4-6] although data supporting the assignment of the β-anomer is weak. ^{1}H–^{1}H coupling data for α-D-arabinofuranoside fit the ^{0}E–E$_1$ conformation suggested by Angyal[4] rather than the E$_0$–^{4}E conformation suggested by Cyr and Perlin.[5] ^{1}H–^{1}H and ^{13}C–^{1}H couplings support an ^{1}E–E_2 conformation for β-D-arabinofuranoside rather than the E_3–^{4}E conformation suggested by Cyr and Perlin.[5] We cannot specify preferred conformations for lyxo- or xylofuranosides from ^{1}H–^{1}H coupling data alone.

Table IX. ^{13}C–^{1}H Coupling Constants[a] for Hydrates and Dimethyl Acetals

coupled nuclei	hydrate		dimethyl acetal	
(C, H)	erythro	threo	erythro	threo
1,1	163.7	163.7	162.5	
1,2	2.9	5.1	3.5	
1,3	~2.4	1.6		
1,OCH_3			4.5, 4.5	
2,1	2.0	0	1.1	br
2,2		141.1	141.5	141.3
2,3		br		
2,4*R*		2.3		
2,4*S*		2.4		
3,1	0.9	1.4	0.8	1.3
3,2		0		
3,3				~141.6
4,2	3.6	2.0		
4,3		3.9		

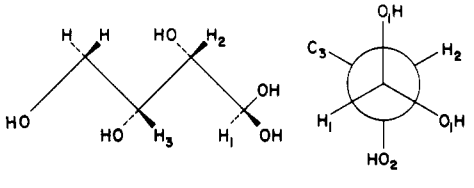
[a] ±0.2 Hz. br = broadened.

Tetro- and pentofuranosides having similar configurations appear to occupy similar regions of the pseudorotational itinerary (Figure 1). The anomeric effect appears to significantly limit the conformational preferences of both tetro- and pentofuranosides. Differences in conformations can be explained by the effect of the hydroxymethyl side chain, which prefers a quasi-equatorial orientation. The quasi-axial orientation of O1 in β-anomers of pentofuranosides produces a strong 1,3-interaction nwith the hydroxymethyl group that is relieved by slight twisting of the ring. In the structurally related tetrofuranosyl ring, the 1,3-interaction is absent, permitting O1 to assume this orientation. This difference can be seen in the preferred conformations of β-threo and β-arabino isomers (Figure 1).

The effect of ring configuration on the preferred orientation of exocyclic hydroxymethyl groups has been discussed elsewhere.[30]

Conformations of Hydrates and Dimethyl Acetals. ^{1}H–^{1}H and ^{13}C–^{1}H couplings for linear hydrates and dimethyl acetals are given in Tables IV and IX.

For the threo configuration, ^{1}H–^{1}H couplings suggest that H1–H2 are anti and H2–H3 are gauche. $^{3}J_{C2,H4R} \cong {}^{3}J_{C2,H4S} \cong 2.3$ Hz, indicating that both dihedral angles are ~60°. $^{3}J_{C3,H1} = 1.4$ Hz, and $^{3}J_{C4,H2} = 2.0$ Hz and suggest 60° dihedral angles in these fragments. These data are consistent with a planar, zig–zig conformation with O4 anti to C2, as shown in **3a** and **3b**. This is the lowest energy conformation deduced from inspection by staggering substituents and minimizing 1,3-interactions.



3a **3b**

For the erythro configuration, $^{3}J_{H2,H3}$, $^{3}J_{C1,H3}$, and $^{3}J_{C4,H2}$ support a planar, zig–zag conformation for the carbon skeleton. $^{3}J_{H3,H4R}$ and $^{3}J_{H3,H4S}$ indicate that O4 and C2 are antiperiplanar. $^{3}J_{H1,H2}$ and $^{3}J_{C3,H1}$ indicate that the C1–C2 bond does not assume a fully staggered conformation; models show that two of the staggered rotamers are destabilized by 1,3-interactions with O3, and the third is

destabilized by two gauche O–O interactions. Two conformers are consistent with $^3J_{C3,H1}$ ($\theta \simeq 90°$) and neither can be distinguished by $^3J_{H1,H2}$. The data, therefore, are insufficient to assign a preferred C1–C2 bond conformation in this isomer.

Hydrates and dimethyl acetals appear to adopt similar conformations. Orientation of the methyl groups of dimethyl acetals (C1–O1 bond torsions) was not examined.

Conclusions

The uncertainties that arise in the conformational analysis of furanosyl rings have been discussed in this and other reports. The assignment of conformations of these structures based on only three ^3J coupling values is tenuous at best, although, as shown here, additional information is available from ^{13}C–^1H couplings.

Unequivocal chemical shift assignment is a prerequisite for conformational analysis. Assignment of the individual methylene (C4) protons of the tetrofuranosyl ring is particularly important because their coupling to C1 is a sensitive indicator of the conformation in the region of the anomeric center. Assignment of the resonances to individual C4 protons based on substituent effects, magnitudes of $^2J_{CH}$ and $^3J_{CH}$, and selective [^{2}H] substitution, all demonstrate that H4*S* is the more shielded methylene proton in tetrofuranosyl rings. The "syn–upfield" rule for 1,2-interactions is upheld in these systems.

Three ^{13}C–^1H couplings across the ring oxygen reflect the preferred orientation of O1. Conformations with O1 quasi-axial (or near quasi-axial) are preferred for all ring configurations, a manifestation of the anomeric effect which appears to dominate all other factors in determining ring conformation.

Ring flexibility appears to increase as 1,2- and 1,3-interactions increase, such that, in the tetroses, the α-threo isomer appears to be most stable and the β-erythro isomer least. We are currently testing this conclusion with the use of ab initio molecular orbital calculations.

While this study has shown that a first-order analysis of ^1H–^1H and ^{13}C–^1H coupling constants and ^{13}C relaxation times does not provide a complete understanding of tetrofuranosyl ring conformation, it has produced an extensive body of data that must be accommodated in future models. The use of ^{13}C–^1H coupling data in conformational studies of five-membered rings provides a better basis upon which conformational inferences can be made. Internuclear ^1H–^1H distances, determined in solution by DESERT[50] and/or dynamic NOE methods,[51] as well as more recent two-dimensional NMR approaches, may provide complementary information on the conformational properties of these ring systems.

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Registry No. D-[4-^{13}C]Erythrose, 90913-08-9; L-[4-^{13}C]threose, 90913-09-0; D-[6-^{13}C]glucose, 70491-70-2; L-[6-^{13}C]idose, 90913-10-3; potassium [^{13}C]cyanide, 25909-68-6; 1,2-*O*-isopropylidene-D-*xylo*-dialdopentofuranose, 53167-11-6; α-D-erythrofuranose, 72599-80-5; methyl α-D-erythrofuranoside, 52613-15-7; β-D-erythrofuranose, 72599-81-6; methyl β-D-erythrofuranoside, 53109-84-5; α-D-threofuranose, 80877-72-1; methyl α-D-threofuranoside, 64609-20-7; β-D-threofuranose, 80877-73-2; methyl β-D-threofuranoside, 25158-74-1; methyl α-D-arabinofuranoside, 56607-40-0; methyl β-D-arabinofuranoside, 25129-51-5; methyl α-D-lyxofuranoside, 22416-73-5; methyl β-D-lyxofuranoside, 22861-09-2; methyl α-D-ribofuranoside, 52485-92-4; methyl β-D-ribofuranoside, 7473-45-2; methyl α-D-xylofuranoside, 1824-96-0; methyl β-D-xylofuranoside, 1824-97-1; D-erythrose dimethyl acetal, 74761-31-2; D-threose dimethyl acetal, 90913-11-4.

Supplementary Material Available: ^1H NMR spectra of D-threose in D_2O at 180, 300, and 600 MHz (Figure 3); 300 MHz ^1H NMR spectrum of D-erythrose in D_2O (Figure 4); 600 MHz ^1H NMR spectra of methyl β-D-[1-^{13}C]-, [2-^{13}C]-, and [4-^{13}C]-erythrofuranosides (Figure 5) (3 pages). Ordering information is given on any current masthead page.

(50) Akasaka, K.; Imoto, T.; Shibata, S.; Hatano, H. J. *Magn. Reson.* **1975**, *18*, 328.
(51) Tropp, J. *J. Chem. Phys.* **1980**, *72*, 6035.

Spectral Properties and Basicity of Stilbazolium Betaines Containing Bulky Substituents on the Quinoid Ring

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Several derivatives of 4-[(4-oxocyclohexa-2,5-dienylidene)ethylidene]-1,4-dihydropyridine were synthesized. The effect of bulky substituents in the immediate vicinity of carbonyl on the basicity and visible spectra of these compounds is described. The basicity was established spectroscopically in 52% methanol–water. A phenol-like effect of substituents on the basicity was observed. Since this effect was much less pronounced than that in the phenolate ion, it may be concluded that the contribution of quinoid resonance structure is important in this kind of compound. A fine structure pattern was observed in the visible spectra of the studied compounds. Variation in the fine structure was investigated in a number of protic and aprotic solvents and solvent mixtures. When the relative absorption intensities at 540, 585, and 625 nm were expressed as peak ratios, a sensitive indicator of solvent polarity was obtained for some regions of the E_T polarity scale.

Spectral properties of merocyanine dyes are known to be strongly affected by the medium. Among the merocyanines, dyes of the stilbazolium betaine type (Scheme I) have aroused much interest because of their extreme solvatochromic properties.[1,2] Recently, Donchi and coworkers have observed drastic changes in the UV–vis absorption spectra of a surfactant merocyanine, 1-hexa-

(1) Brooker, L. G. S.; Keyes, G. H.; Heseltine, D. W. *J. Am. Chem. Soc.* **1951**, *73*, 5350–5356.

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