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Synthesis and X-ray crystal and solution structures of 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol: a locked $^4T_3$ furanose conformer *

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ABSTRACT

2,5-Anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**) was prepared from 2,5-anhydro-D-mannitol (**2**) in three steps. The fused ring system was introduced by a phase-transfer alkylation using 1,2-dibromoethane. Its conformation in solution was determined by NMR studies at 500 MHz. Variable-temperature studies showed no lineshape change from 25 to 80° in D_2O. The data indicate that the five-membered ring is locked by the *trans*-fused six-membered 1,4-dioxane ring into a twist $^4T_3$ conformation. A single-crystal X-ray study was carried out. The crystals are orthorhombic, $C222_1$, $a = 4.7252$ (6), $b = 14.0364$ (12), $c = 13.268$ (2) Å, $Z = 4$, with $R = 0.032$ for 894 observations. The molecule lies upon a crystallographic two-fold axis, and thus the five-membered ring exists in a perfect $^4T_3$ conformation with a pseudorotation angle of 0° and amplitude of 47.2°, in agreement with the NMR results. We have shown earlier that, among twenty possible conformers, phosphofructokinase acts specifically on the $^4T_3$ conformer of the β anomer of D-fructose 6-phosphate.

INTRODUCTION

During the past two decades, we have carried out studies on phosphofructokinase (EC 2.7.1.11) from rabbit muscle, which is one of the key regulatory enzymes of the glycolytic pathway[1–4]. Through the use of structurally locked analogues of the α- and β-forms of its carbohydrate substrate, we have established that the enzyme acts on the β-form. Specifically, we have found that 2,5-anhydro-D-mannitol 6-phosphate (an analogue of the β-form of D-fructose 6-phosphate) was a good alternate substrate, whereas 2,5-anhydro-D-glucitol 6-phosphate (an analogue of the α-form) was a competitive inhibitor for this enzyme[1]. In addition, we have

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* Dedicated to Professor Henry A. Lardy whose pioneering work on the utilization of sugar analogues for investigating carbohydrate metabolism has been an inspiration to many workers.

investigated the kinetic properties of the epimers of D-fructose 6-phosphate[2]. These substituted tetrahydrofuran rings may exist in twenty different conformers each with a different stability and concentration in solution. Based upon NMR data and estimated interaction energies, we have calculated the conformational composition of these systems[3]. Out of the twenty possible conformers, only three are thermodynamically favored. These three major conformers of the D-ketohexose 6-phosphates are the $^4T_3$, the $^OT_2$, and the $^OT_5$. The plot of the logarithm of the substrate efficacy index V_{max}/K_m versus the logarithm of the amount of each conformer in solution was observed to be linear only in the case of the β-$^4T_3$ conformer. Therefore, we concluded that the $^4T_3$ conformer of β-D-fructose 6-phosphate is the physiological substrate of rabbit muscle phosphofructokinase[3,4]. In order to further study this effect, we have selected 2,5-anhydro-D-mannitol (**2**) as a candidate for modification in an attempt to create a system locked as the $^4T_3$ conformer in solution. It should be noted that **2** exists, on average, as the $^4T_3$ conformer in solution and deviates slightly from the ideal $^4T_3$ in the crystalline state[5]. The *trans* disposition of the hydroxy groups on carbons 3 and 4 and their equatorial orientation in the predominant $^4T_3$ conformer should allow the formation of a *trans*-fused 1,4-dioxane ring. This paper reports a procedure for the synthesis of the twist-locked hexitol, 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**), as well as its X-ray crystal and solution structures.

EXPERIMENTAL

General methods.—NMR spectra were recorded with a Bruker AMX 500 spectrometer and were referenced to the indicated internal standard. NMR simulations were performed on an ASPECT 3000 computer using PANIC (1985 version, Bruker Instrument Co.). TLC was performed using HPTLC plates (Analtech) and 24:1 benzene–MeOH as solvent.

2,5-Anhydro-3,4-O-(1,2-ethanediyl)-1,6-di-O-trityl-D-mannitol (**3**). — To a solution of 2,5-anhydro-1,6-di-*O*-trityl-D-mannitol (**4**)[6] (3.24 g, 5.0 mmol) in 1,2-dibromoethane (20 mL, 0.23 mol) was added 50% NaOH solution (20 mL) and benzyltriethylammonium chloride[7] (150 mg, 0.66 mmol). The flask was connected to a bubbler since the slightly exothermic reaction released a gas (probably vinyl bromide) as a by-product. The mixture was stirred rapidly for 24 h at room temperature, after which time it had turned a red–brown color with considerable suspended materials. TLC analysis indicated the formation of a new fast-running component ($R_F = 0.75$, R_F of **4** = 0.35). Additional 1,2-dibromoethane (10 mL) and 50% NaOH (10 mL) solution were added, and the reaction was allowed to proceed further for 48 h. The reaction mixture was then diluted with cold water (100 mL) and extracted with diethyl ether (100 mL). The organic layer was washed with water, dried (Na_2SO_4), and concentrated to a brown foamy residue (2.16 g). Filtration of a diethyl ether solution of the crude residue through a short column of silica gel removed the very polar components from the mixture. The product

separated, in a crystalline form, from the residual starting material upon cooling of an EtOH solution to give **3** (0.63 g, 18%): mp 170–171°; ^{1}H-NMR data (C_6D_6, Me_4Si): an AA′BB′XX′YY′ eight-spin system, and independent CC′DD′ four spin system and an aromatic system, δ 3.23 (m, CC′, 2 H, $J_{7ax,7eq} = J_{8ax,8eq} =$ 12 Hz, $J_{7ax,8ax} = 12$ Hz, $J_{7ax,8eq} = J_{7eq,8ax} = 3$ Hz, H-7*ax*,8*ax*), 3.28 (m, DD′, 2 H, $J_{7eq,8eq} = 1$ Hz, H-7*eq*,8*eq*), 3.46 (dd, AA′, 2 H, $J_{1,1'} = J_{6,6'} = 10$ Hz, $J_{1,2} = J_{5,6} =$ 5 Hz, H-1,6), 3.61 (dd, BB′, 2 H, $J_{1',2} = J_{5,6'} = 3$ Hz, H-1′,6′), 3.77 (m, YY′, 2 H, $J_{2,3} = J_{4,5} = 9.5$ Hz, $J_{3,4} = 9$ Hz, H-3,4), 4.32 (m, XX′, 2 H, H-2,5), 7.00–7.80 (m, 30 H, Ar-H); HRFABMS: Calcd for $C_{46}H_{42}O_5Na_1$ $(M + Na)^+$: 697.2930. Found: 697.2936.

*2,5-Anhydro-3,4-*O*-(1,2-ethanediyl)-*D*-mannitol* (**1**).—The addition of 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-1,6-di-*O*-trityl-D-mannitol (**3**) (0.50 g, 0.74 mmol) to 90% trifluoroacetic acid (5 mL) resulted in a bright yellow solution[8]. After 1 h at room temperature, the clear solution was diluted with water (10 mL), filtered to remove the triphenylmethanol by-product, and concentrated to dryness. The residue was suspended in water, extracted with diethyl ether three times, and the aqueous layer was concentrated to dryness. The residue was crystallized from EtOH to give **1** (0.10 g, 70%): mp 150–152°; NMR data (D_2O, TSP-d_4): ^{1}H, an AA′BB′XX′YY′ eight-spin system and an independent CC′DD′ four-spin system, Karplus equation derived torsion angle in square brackets [ ], X-ray derived torsion angle in curved brackets { }, δ 3.43 (dd, AA′, 2 H, $J_{1,1'} = J_{6,6'} = 13$ Hz, $J_{1,2} = J_{5,6} = 4$ Hz [55°] {64°}, H-1,6), 3.53 (m, YY′, 2 H, $J_{2,3} = J_{4,5} = 9.5$ Hz [155°] {165°}, $J_{3,4} = 9$ Hz [150°] {178°}, H-3,4), 3.55 (dd, BB′, 2 H, $J_{1',2} = J_{5,6'} = 3$ Hz [50°] {58°}, H-1′,6′), 3.60 (m, CC′, 2 H, $J_{7ax,7eq} = J_{8ax,8eq} = 12$ Hz, $J_{7ax,8ax} = 12$ Hz [170°] {175°}, $J_{7ax,8eq} = J_{7eq,8ax} = 3$ Hz [55°] {55°}, H-7*ax*,8*ax*), 3.69 (m, XX′, 2 H, H-2,5), 3.72 (m, DD′, 2 H, $J_{7eq,8eq} = 1$ Hz [70°] {75°}, H-7*eq*,8*eq*); ^{13}C, δ 63.8, 69.9 (C-1,6,7,8), 79.4, 80.6 (C-2,3,4,5); HRFABMS: Calcd for $C_8H_{14}O_5Na_1$ $(M + Na)^+$: 213.0740. Found: 213.0744.

X-ray crystal structure determination.—Slow evaporation of an EtOH solution of **1** yielded needles suitable for X-ray study. A colorless needle fragment was used for data collection on an Enraf-Nonius CAD-4 diffractometer with Cu-$K\alpha$ radiation and a graphite monochromator; cell dimensions were obtained from setting angles of 25 reflections having $25 < \theta < 30°$. The unit-cell constants are shown in Table I. Data collection was by ω–2θ scans; one hemisphere of data having $2 < \theta < 75°$, $h = 0$ to 5, $k = -17$ to 17, $l = -16$ to 16, was measured and corrected for background, Lorentz, and polarization effects. Absorption corrections were based on ψ scans. Standard reflections 200, 080, and 004 exhibited no decrease in intensity. The space group was determined from systematic absences *hkl* with $h + k$ odd and 00*l* with l odd. Redundant data were averaged to yield 907 unique data. All but 13 had $I > 3\sigma(I)$ and were used in the refinement.

The structure was solved using direct methods and refined by full-matrix least squares based on F with weights $w = 4F_0^2\,[\sigma^2(I) + (0.02\ F_0^2)^2]^{-1}$. The atomic scattering factors were taken from International Tables[9]. Non-hydrogen atoms

TABLE I

Crystal data for 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**)

Formula	$C_8H_{14}O_5$
Formula weight	190.2
Cell Constants	
a (Å)	4.7252 (6)
b (Å)	14.0364 (12)
c (Å)	13.268 (2)
Volume (Å^3)	880.0 (3)
Z (molecules/cell)	4
Density calculated (g/cm^3)	1.435
$\mu_{CuK\alpha}$	9.8 cm^{-1}
Space group	$C222_1$
Crystal size (mm)	$0.20 \times 0.25 \times 0.35$
λ(Cu $K\alpha$) (Å)	1.54184
Minimum transmission	0.9016
Reflections measured	2185
Unique data	907
$I > 3\sigma(I)$	894
R	0.0319
R_w	0.0446
S (89 variables)	2.785

were refined anisotropically, and H-atoms were located from difference maps and refined isotropically. The largest Δ/σ was 0.01 on the final cycle, and the maximum residual electron density was 0.50 e/Å^3 on the C-3–C-3′ bond, extinction coefficient $5.1(2) \times 10^{-5}$. The programs used were MULTAN80[10], SDP/VAX[11], PLUTO78[12], and ORTEP[13]. Atomic coordinates and equivalent isotropic

TABLE II

Atomic coordinates for 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**) [a,b]

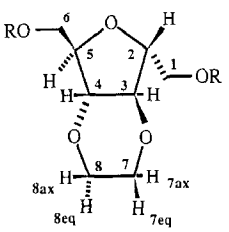
Atom	x	y	z	B_{eq} (Å^2)
O-1	0.2640 (2)	0.86444 (6)	0.44458 (4)	3.54 (2)
O-2	0	0.88193 (7)	1/4	3.06 (2)
O-3	−0.1307 (2)	0.64611 (5)	0.34845 (4)	2.97 (1)
C-1	−0.0325 (2)	0.86487 (7)	0.43221 (6)	2.76 (2)
C-2	−0.1110 (2)	0.82298 (8)	0.33060 (6)	2.42 (1)
C-3	0.0105 (2)	0.72628 (7)	0.30689 (5)	2.12 (1)
C-7	0.0047 (3)	0.56222 (7)	0.30726 (6)	3.52 (2)
H-1	0.291 (4)	0.866 (1)	0.509 (1)	4.5 (3) *
H-1	−0.129 (2)	0.821 (1)	0.4837 (7)	2.2 (2) *
H-1	−0.100 (2)	0.932 (1)	0.4349 (9)	3.4 (2) *
H-2	−0.323 (3)	0.824 (1)	0.3305 (9)	3.8 (3) *
H-3	0.206 (2)	0.728 (1)	0.3255 (8)	2.6 (2) *
H-7	−0.113 (2)	0.509 (1)	0.333 (1)	5.4 (4) *
H-7	0.211 (3)	0.565 (1)	0.3284 (9)	3.5 (2) *

[a] Starred atoms were refined isotropically. [b] $B_{eq} = (8\pi^2/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$

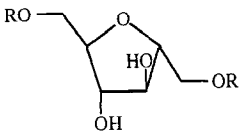
thermal parameters are given in Table II *. Refinement of the mirror-image structure under identical conditions yielded $R = 0.0321$, $R_w = 0.0452$, $S = 2.823$.

RESULTS AND DISCUSSION

2,5-Anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**) was prepared from 2,5-anhydro-D-mannitol (**2**) by a three-step procedure. The 2,5-anhydrohexitol was first di-*O*-tritylated to give the amorphous 2,5-anhydro-1,6-di-*O*-trityl-D-mannitol (**4**)[6], which was then di-*O*-alkylated utilizing 1,2-dibromoethane and sodium hydroxide under phase transfer conditions in a manner similar to the reported use of 1,2-dichloroethane[7]. However, an attempt to use 1,2-dichloroethane instead of 1,2-dibromoethane produced none of the desired product as evidenced by TLC. The resulting 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-1,6-di-*O*-trityl-D-mannitol (**3**) was detritylated using aqueous trifluoroacetic acid[8] to produce crystalline **1**.



1 (R = H)
3 (R = Tr)



2 (R = H)
4 (R = Tr)

In order to verify the structure of this substituted, strained, *trans*-fused 2,5,8-trioxabicyclo[4.3.0]nonane system, a single-crystal X-ray diffraction study was performed on **1**. The molecular structure and atomic numbering are illustrated in Fig. 1. The D form of this compound was assumed, based on the configuration of the starting material. Refinement of the mirror-image structure yielded slightly higher R values, thus supporting the overall D assignment. The crystal structure data confirm that the molecule has the D-*manno* configuration, with all nearest neighbor dispositions being *trans*. A perspective view from the 1,4-dioxane ring side of the molecule is shown in Fig. 2. The network of intermolecular hydrogen bonds linking the molecules is indicated in the crystal packing diagram (Fig. 3). Bond lengths are given in Table III and bond angles are listed in Table IV. Selected torsion angles are listed in Table V.

The molecule lies on a crystallographic two-fold axis, and all the bond lengths and angles are symmetrical about the C_2 molecular symmetry axis (through O-2,

* Lists of the torsion angles, the anisotropic thermal parameters, and the observed and calculated structure-amplitudes have been deposited with, and can be obtained from Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/501/*Carbohydr. Res.*, 230 (1992) 213–222.

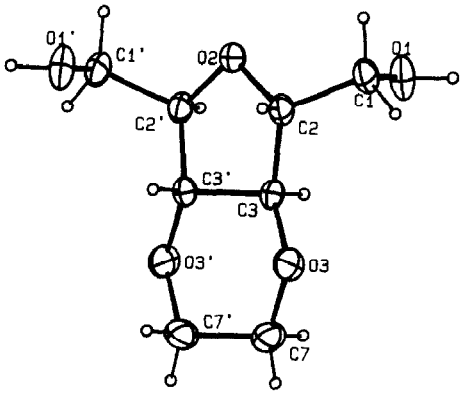


Fig. 1. Molecular structure and atomic numbering of 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**).

through the midpoint of the C-3–C-3′ bond, and through the midpoint of the C-7–C-7′ bond). Crystallographic symmetry requires that a different numbering system be utilized for the X-ray results as compared to the usual numbering. The corresponding set of labels are: C-3′ = C-4, C-2′ = C-5, C-1′ = C-6, C-7′ = C-8, O-3′ = O-4, O-1′ = O-6 and similarly for the attached hydrogen atoms. In general, the bond angles of **1** follow the trends of those of **2** except for the smaller C-3–C-3′–O-3′ and C-3′–C-3–O-3 angles in **1** (110.9°) as compared to **2** (113.6° and 114.7°) and the larger C-2–C-3–O-3 and C-2′–C-3′–O-3′ angles in **1** (117.0°) as compared to **2** (111.6 and 110.5°). This is a result of the fusion of the six-membered ring to this edge of the five-membered ring. The bond lengths of **1** are similar to the corresponding ones of **2**.

As expected, **1** has a five-membered ring conformation in the same region of the pseudorotation itinerary as the parent compound **2**, which was shown to exist in a slightly distorted $^4T_3$ conformation with pseudorotation angle of $-11.7°$ and

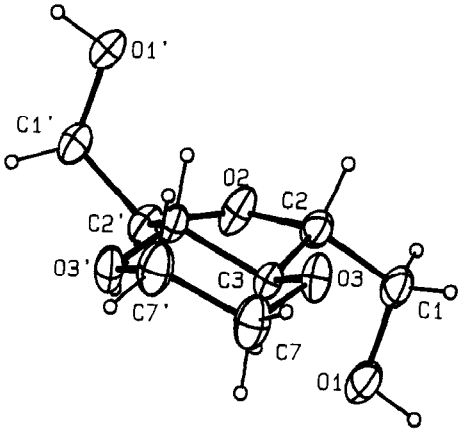


Fig. 2. Perspective view of 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**).

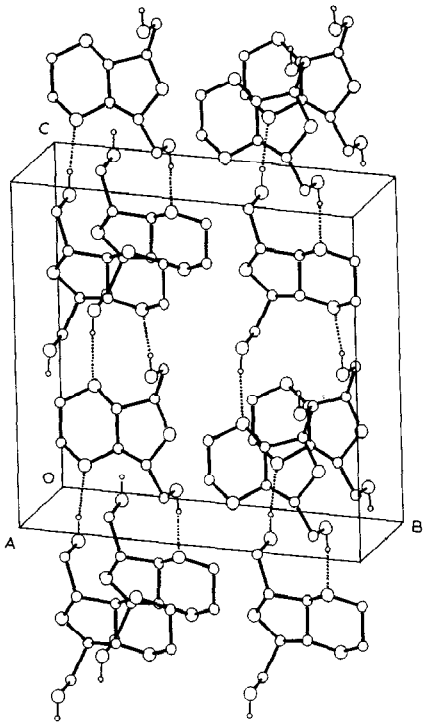


Fig. 3. Crystal packing diagram of 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**).

amplitude of 38.8° (ref. 5). The addition of the six-membered 1,4-dioxane ring in a *trans* disposition, as dictated by the *manno* stereochemistry of the parent compound, forces the five-membered ring into an ideal $^4T_3$ conformation in the crystal state with a pseudorotation angle of 0.0° and amplitude of 47.2° (ref. 14). Each of the atoms C-3 and C-3′ of **1** is located 0.3771 (8) Å out of the plane defined by atoms C-2, O-2, and C-2′. The exocyclic hydroxymethyl groups adopt the (+)-

TABLE III

Bond lengths (Å) for 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**)

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
O-1	C-1	1.411 (1)	C-1	H-1b	0.99 (1)
O-1	H-1O	0.86 (1)	C-2	C-3	1.507 (1)
O-2	C-2	1.450 (1)	C-2	H-2	1.00 (1)
O-2	C-2′	1.450 (1)	C-3	C-3′	1.513 (1)
O-3	C-3	1.420 (1)	C-3	H-3	0.96 (1)
O-3	C-7	1.447 (1)	C-7	C-7′	1.520 (1)
C-1	C-2	1.517 (1)	C-7	H-7a	0.99 (1)
C-1	H-1a	1.03 (1)	C-7	H-7b	1.01 (1)

TABLE IV

Bond angles (°) for 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**)

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C-1	O-1	H-1O	105 (1)	C-3	C-2	H-2	113.1 (8)
C-2	O-2	C-2′	110.43 (8)	O-3	C-3	C-2	117.00 (7)
C-3	O-3	C-7	106.90 (7)	O-3	C-3	C-3′	110.91 (7)
O-1	C-1	C-2	110.16 (8)	O-3	C-3	H-3	111.6 (8)
O-1	C-1	H-1a	111.1 (6)	C-2	C-3	C-3′	100.57 (7)
O-1	C-1	H-1b	108.5 (6)	C-2	C-3	H-3	107.3 (8)
C-2	C-1	H-1a	104.5 (6)	C-3	C-3	H-3	108.7 (7)
C-2	C-1	H-1b	108.7 (7)	O-3	C-7	C-7′	111.39 (8)
H-1	C-1	H-1b	113.7 (9)	O-3	C-7	H-7a	103.4 (8)
O-2	C-2	C-1	110.23 (8)	O-3	C-7	H-7b	106.4 (9)
O-2	C-2	C-3	102.83 (7)	C-7′	C-7	H-7a	109.3 (9)
O-2	C-2	H-2	110.7 (8)	C-7′	C-7	H-7b	107.7 (7)
C-1	C-2	C-3	116.19 (7)	H-7a	C-7	H-7b	118 (1)
C-1	C-2	H-2	103.9 (7)				

gauche disposition which results in the placement of H-2 between the hydrogens on C-1 in the crystalline state.

The solution structure of **1** was also examined by ^{1}H-NMR spectroscopy in D_2O. While the expected six sets of signals were observed, and some of the coupling constants were obtainable by inspection, a simulation (see Experimental) of the molecule was required to obtain the remaining coupling constants. The system was treated as an eight-spin system consisting of H-1, H-1′, H-2, H-3, H-4, H-5, H-6, and H-6′, and separate four-spin system consisting of H-7*ax*, H-7*eq*, H-8*ax*, and H-8*eq*. The coupling constants thus derived were used in a modified Karplus equation to calculate the hydrogen–hydrogen torsion angles[15]. These values, rounded to five-degree increments, are given in the Experimental section in square bracket notation. For ease of comparison, the X-ray values are also given in curved bracket notation.

TABLE V

Selected torsion angles (°) for 2,5-anhydro-3,4-*O*-(1,2-ethanediyl)-D-mannitol (**1**)

Atom 1	Atom 2	Atom 3	Atom 4	Angle
C-2	O-2	C-2′	C-3′	14.86 (7)
C-3	O-3	C-7	C-7′	−57.35 (11)
O-1	C-1	C-2	O-2	−63.70 (10)
O-1	C-1	C-2	C-3	52.72 (11)
O-2	C-2	C-3	C-3′	−38.24 (8)
O-3	C-3	C-3′	O-3′	−63.88 (9)
C-2	C-3	C-3′	C-2′	47.23 (8)
O-3	C-7	C-7′	O-3′	58.18 (13)
C-3	C-3′	O-3′	C-7′	59.64 (9)
H-1O	O-1	C-1	C-2	−157 (1)

Agreement between the X-ray torsion angles and the NMR-derived torsion angles of **1** is excellent. The only major deviation is in the value for the H-3–C-3–C-4–H-4 torsion angle. This may be due to an inaccuracy in the Karplus equation parameters utilized. In any event, the agreement between the solid-state data and the solution data at room temperature indicates that in both case the molecule is locked in the $^4T_3$ conformation. Concerning the exocyclic hydroxymethyl group hydrogens, they have coupling constants to the ring hydrogen which indicate rotation around the connecting bond. However, the major conformation is the same (+)-*gauche* disposition as observed in the X-ray results.

The signals for the axial and equatorial hydrogens of **1** were separated by 0.12 ppm at room temperature. A study of the temperature dependency of the spectra was also performed. Spectra at 25, 40, 60, and 80° were obtained. All were identical in lineshape and signal patterns indicating that no change in conformation occurred over this temperature range. The only effect of the change in temperature was a differential change in relative chemical shifts of the six sets of signals such that the best resolution of the signals was obtained at 60°. The expected results for a system in which conformational equilibrium is occurring would be some coalescing of the signals. In this analysis the C-7 and C-8 protons are the most sensitive indicators of conformational change. In fact, no change in their relative chemical shift occurred over this temperature range. The 1,4-dioxane ring can exist in the chair conformation shown and in a slightly higher energy twist (or skew boat) conformation. It should be noted that an interconversion between these two conformers does not affect the five-membered ring conformation. The axial hydrogens of the chair conformation have torsion angles of 170 and 55°, while those of the twist conformation have torsion angles of 170 and 70° based upon molecular models. The NMR-derived torsion angles indicate that the 1,4-dioxane ring exists in the chair conformation in solution, the same as observed in the X-ray crystal structure. The couplings between the hydrogens on the exocyclic hydroxymethyl group and the ring hydrogen do not change. This was expected since they are already in free rotation. Thus, we conclude that the five-membered ring of **1** is in fact locked in a twisted conformation ($^4T_3$) in solution, in the same conformation as that observed in the X-ray crystal structure.

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REFERENCES

1 T.A.W. Koerner, Jr., E.S. Younathan, A.E. Ashour, and R.J. Voll, *J. Biol. Chem.*, 249 (1974) 5749–5754.

2 T.A.W. Koerner, Jr., R.J. Voll, A.E. Ashour, and E.S. Younathan, *J. Biol. Chem.*, 251 (1976) 2983–2986.
3 T.A.W. Koerner, Jr., R.J. Voll, L.W. Cary, and E.S. Younathan, *Biochemistry*, 19 (1980) 2795–2801.
4 E.S. Younathan, R.J. Voll, and T.A.W. Koerner, Jr., in C.M. Veneziale (Ed.), *The Regulation of Carbohydrate Formation and Utilization in Mammals*, University Park Press, Baltimore, Maryland, 1981, pp. 69–98.
5 S.F. Watkins, K.A. Abboud, R.J. Voll, T.A.W. Koerner, Jr., and E.S. Younathan, *Carbohydr. Res.*, 119 (1983) 49–55.
6 G.O. Aspinall, E. Przybylski, R.G.S. Ritchie, and C.O. Wong, *Carbohydr. Res.*, 66 (1978) 225–243.
7 P. Di Cesare and B. Gross, *Carbohydr. Res.*, 48 (1976) 271–276.
8 J.E. Christensen and L. Goodman, *Carbohydr. Res.*, 7 (1968) 510–512.
9 *International Tables for X-ray Crystallography*, Vol. IV, The Kynoch Press (Current distributor Kluwer Academic Publishers, Dordrecht), Birmingham, UK, 1974.
10 P. Main, S.F. Fiske, S.E. Hull, L. Lessinger, G. Germain, J.P. Declercq, and M.M. Woolfson, *MULTAN-80. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*, Universities of York, UK and Louvain, Belgium.
11 B.A. Frenz, in H. Schenk, R. Olthof-Hazekamp, H. van Konigsveld, and G.C. Bassi (Eds.), *Computing in Crystallography*, Delft University Press, Delft, The Netherlands, 1978, pp. 64–71.
12 W.D.S. Motherwell and W. Clegg, *PLUTO78. Program for Plotting Molecular and Crystal Structures*, University of Cambridge, UK, 1978.
13 C.K. Johnson, *ORTEP: A Fortran Thermal-ellipsoid Plot Program for Crystal Structure Illustrations*, Oak Ridge National Laboratory Report ORNL-3794, 1965.
14 C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 94 (1972) 8205–8212.
15 R.D. Guthrie, I.D. Jenkins, and R. Yamasaki, *Aust. J. Chem.*, 35 (1982) 1019–1029.