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SOLUTION BEHAVIOR OF METHYL β-XYLOBIOSIDE: CONFORMATIONAL FLEXIBILITY REVEALED BY N.M.R. MEASUREMENTS AND THEORETICAL CALCULATIONS*

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ABSTRACT

The conformations of methyl β-xylobioside in solution have been determined by n.m.r. spectroscopy. Interglycosidic $^3J_{C,H}$ values and the chemical shifts of the ^{13}C resonances were measured at various temperatures in the range 238–378 K for solutions in 1,4-dioxane, methanol, methyl sulfoxide, and water. The temperature and solvent dependencies of the data obtained suggest conformational flexibility. Quantum-chemical PCILO calculations, with evaluation of the solvent effects, and molecular mechanics calculations revealed the existence of 7 low-energy regions for which the geometries and energies were determined. The computed abundances of conformers and averaged *J* values accord with the experimental data.

INTRODUCTION

Both theoretical and experimental approaches have been made[1,2] in order to determine the conformation of oligosaccharides in solution. N.m.r. spectroscopy is an important method[3] and n.O.e. measurements have been used widely in studies of the conformations about glycosidic bonds. However, studies of interglycosidic $^3J_{C,H}$ values have not often been carried out, because of the complicated and time-consuming measurements and the lack of a quantitative relationship between $^3J_{C,H}$ values and the dihedral angle of the C–O–C–H segment. However, 2D-n.m.r. techniques[4–6] now allow a precise determination of $^3J_{C,H}$ values in a reasonable time, and a formula[7] that relates $^3J_{C,H}$ values and dihedral angles around the glycosidic linkage permits interpretation of the data. Due to the time-averaged character of the n.m.r. data, characterization of the conformational properties in solution requires a combination of experimental and computational methods.

Experimental and theoretical investigations aimed at determining the confor-

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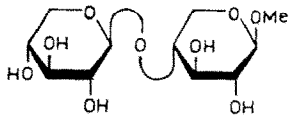

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mational behaviour of methyl 4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside (methyl β-xylobioside, **1**) in solution are now reported.

EXPERIMENTAL

Methyl β-xylobioside (**1**), prepared[8] by the Helferich method, had m.p. 376 K, $[\alpha]_D$ −74.7° (water).

N.m.r. spectroscopy. — Spectra were obtained on 0.05M solutions of **1** in water, methanol, methyl sulfoxide, and 1,4-dioxane with a Bruker AM-300 spectrometer at various temperatures (±1 K).

The ^{13}C resonances for solutions in methanol, methyl sulfoxide, and 1,4-dioxane were referenced to internal Me_4Si. Medium effects in variable temperature measurements can affect the values of the chemical shifts[9]. In order to reduce these effects, the ^{13}C resonances for solutions in D_2O were referenced to the resonance of internal acetone at 298 K (set to 30.0 p.p.m.) for measurements at 278, 318, 338, and 358 K (measured without using the deuterium lock and, thus, at a constant magnetic field B_0).

The $^3J_{C,H}$ values were determined by semi-selective *J*-resolved[4] and modified[6] 2D-INEPT experiments. In the latter method, each ^1H pulse was soft ($\gamma B_2/2\pi = 20$ Hz) and affected only the proton chosen. Selected traces in the F2 domain of the 2D matrix were zero-filled to give a digital resolution of 0.08 Hz/point. A weighted function (Gaussian) was used before Fourier transformation. In order to characterize the conformation of the glycosidic bond, the $^3J_{C,H}$ values for H-1′–C-1′–O-1′–C-4 (J^{Φ}) and H-4–C-4–O-1′–C-1′ (J^{Ψ}) were measured. Thus, after application of soft pulses on H-1′ or H-4, the corresponding long-range couplings with C-4 and C-1′, respectively, were detected.

Quantum-mechanical calculations. — The total Gibbs free energy ΔG_T of each conformer of **1** with the corresponding intramolecular energy ΔG_U in a given solvent can be described[11] as

$$\Delta G_T = \Delta G_U + \Delta G_{Solv} = \Delta G_U + \Delta G_{Cav} + \Delta G_{Elst} + \Delta G_{Disp},$$

where ΔG_{Cav} represents the free energy for the creation of the cavity in the solvent, and ΔG_{Elst} and ΔG_{Disp} are the free energies due to the electrostatic and dispersion interactions, respectively. The equations for individual terms of the ΔG_{Solv} have been published[10,11]. The energy calculated by quantum-mechanical semiempirical PCILO or molecular mechanics methods were used for ΔG_U. The differences in

entropy between conformers around the glycosidic linkages were neglected. All molecular parameters characterizing the individual conformers of **1** in the equations for the calculation of ΔG_{Solv} were also calculated by the PCILO method, except for the refractive index, where the value of 1.4600 was used. A 2D (Φ,Ψ) map was obtained by rotation of the monosaccharide residues in steps of 20°, using constant geometry based on that of methyl β-D-xylopyranoside[12] with a glycosidic bond angle of 117°.

The molecular geometries and energies of the local minima were determined by optimizing 61 geometrical parameters of the 114 bond lengths, bond angles, and dihedral angles. The bond lengths and dihedral angles of the side chains (OH and OMe) were included in the minimization procedure.

Theoretical $^3J_{C,H}$ of the individual conformers were determined from the dihedral angles using a Karplus-type equation[7]. Averaged $^3J_{C,H}$ values can be calculated from the formula[13]

$$\langle J^{\Phi,\Psi}\rangle = \int J(\Phi,\Psi)\,\{\exp[-\Delta G(\Phi,\Psi)/kT]\}\mathrm{d}\Phi\mathrm{d}\Psi/\int \exp[-\Delta G(\Phi,\Psi)/kT]\mathrm{d}\Phi\mathrm{d}\Psi,$$

where $\langle J\rangle$ denotes the average over the states of restricted rotation, k is the Boltzmann constant, T is the absolute temperature, $\Delta G(\Phi,\Psi)$ is the Gibbs free energy for rotation, and integration is over all hindered rotamer states. However, these functions are usually not known, especially for internal motion in more complex molecules such as oligosaccharides. Therefore, simplified approaches were used. In the first, an integration was substituted by summation through the conformational space. In the second, only minima on the potential energy surface were used to calculate the n.m.r. data.

RESULTS AND DISCUSSION

Chemical shfits. — The chemical shifts of the ^{13}C resonances of **1** measured for solutions in water, methyl sulfoxide, methanol, and 1,4-dioxane are recorded in Tables I–IV. Comparison of the values with those for the standard temperature (298 K) indicates the solvent dependence of the individual ^{13}C resonances. The chemical shifts of the resonances of C-1′ and C-4 are of interest for a study of conformations around the glycosidic linkage[14–18]. The resonance of C-1′ ranged from 101.57 p.p.m. in water to 104.02 p.p.m. in methanol, and that of C-4 from 75.28 p.p.m. in methyl sulfoxide to 78.25 p.p.m. in methanol. Differences in the chemical shifts of the remaining ^{13}C resonances are also considerable. For example, C-2′ resonated at 74.33 p.p.m. in methanol and 72.41 p.p.m. in methyl sulfoxide, and C-2 resonated at 74.65 p.p.m. in methanol and 73.05 p.p.m. in methyl sulfoxide. Considerable δ ranges were observed also for C-1 (2.33 p.p.m.) and C-4′ (2.17 p.p.m.). It is assumed that the changes in the resonance of C-1 reflect the reorientation of the OMe group[10]. Variations of chemical shift for the remaining ^{13}C resonances are probably caused by variation in the orientation of the OH

TABLE I

^{13}C-N.M.R. DATA (δ, p.p.m.) FOR METHYL β-XYLOBIOSIDE (**1**) IN D_2O

T (K)	C-1	C-1′	C-2	C-2′	C-3	C-3′	C-4	C-4′	C-5	C-5′	OMe
278	103.37	101.42	72.49	72.32	73.28	75.12	75.86	68.75	62.45	64.78	56.83
298	103.51	101.57	72.58	72.49	73.48	75.32	76.11	68.90	62.60	64.93	56.93
318	103.66	101.73	72.70	72.67	73.67	75.51	76.35	69.07	62.77	65.08	56.99
338	103.78	101.83	72.78	72.78	73.81	75.67	76.50	69.21	62.88	65.19	56.99
358	103.83	101.88	72.85		73.91	75.76	76.60	69.31	62.95	65.26	56.99

TABLE II

^{13}C-N.M.R. DATA (δ, p.p.m.) FOR METHYL β-XYLOBIOSIDE (**1**) IN METHYL SULFOXIDE

T (K)	C-1	C-1′	C-2	C-2′	C-3	C-3′	C-4	C-4′	C-5	C-5′	OMe
298	104.33	101.85	73.05	72.41	74.08	76.17	75.28	69.37	63.05	65.69	55.87
318	104.36	101.90	73.08	72.48	74.18	76.21	75.42	69.46	63.11	65.74	55.80
338	104.39	101.95	73.11	72.55	74.26	76.25	75.56	69.54	63.15	65.76	55.73
358	104.43	102.00	73.17	72.65	74.34	76.30	75.71	69.64	63.20	65.79	55.65
378	104.48	102.07	73.26	72.77	74.43	76.36	75.87	69.76	63.26	65.83	55.65

TABLE III

^{13}C-N.M.R. DATA (δ, p.p.m.) FOR METHYL β-XYLOBIOSIDE (**1**) IN METHANOL

T (K)	C-1	C-1′	C-2	C-2′	C-3	C-3′	C-4	C-4′	C-5	C-5′	OMe
238	105.73	103.62	74.52	74.22	75.54	77.37	77.77	70.88	64.22	66.92	57.30
258	105.78	103.77	74.56	74.26	75.65	77.45	77.94	70.93	64.32	66.99	57.30
278	105.82	103.91	74.60	74.28	75.76	77.54	78.11	70.99	64.42	67.05	57.27
298	105.88	104.02	74.65	74.33	75.85	77.63	78.25	71.07	64.50	67.10	57.24
318	105.93	104.08	74.70	74.38	75.92	77.71	78.34	71.15	64.56	67.14	57.21

TABLE IV

^{13}C-N.M.R. DATA (δ, p.p.m.) FOR METHYL β-XYLOBIOSIDE (**1**) IN 1,4-DIOXANE

T (K)	C-1	C-1′	C-2	C-2′	C-3	C-3′	C-4	C-4′	C-5	C-5′	OMe
298	105.30	103.13	74.21	72.81	75.40	77.19	75.66	70.37	64.38	66.45	56.49
318	105.35	103.19	74.23	72.97	75.41	77.24	75.97	70.47	64.34	66.51	56.42
338	105.38	103.22	74.25	73.12	75.41	77.28	76.24	70.57	64.27	66.56	56.34
358	105.44	103.30	74.29	73.30	75.42	77.35	76.53	70.70	64.24	66.64	56.28

groups. In spite of difficulties in the interpretation, the observed data indicate conformational flexibility and different conformations of **1** in the various solvents.

The temperature dependence of the chemical shifts was studied next. The C-1′ resonances differed by 0.46 p.p.m. in water and methanol with a temperature difference of 80 K, and C-4 showed a larger range of differences, 0.87 p.p.m. in 1,4-dioxane (298–358 K) and 0.74 p.p.m. in D_2O (278–358 K). The latter values reflect the higher flexibility around the O-1′–C-4 bond in comparison with the C-1′–O-1′ bond. Other ^{13}C resonances exhibited more constant chemical shifts. Although the shifts observed have an averaged value due to the time scale of the n.m.r. experiment, variations indicate that the solvent and temperature influence the conformations of the glycosidic linkage. However, the effects of the medium and changes in conformations of the side chains must be taken into account also. In order to provide other independent evidence on the flexibility of **1**, the interglycosidic $^3J_{C,H}$ values were determined.

$^3J_{C,H}$ values. — The $^3J_{C,H}$ values corresponding to H-1′–C-1′–O-1′-C-4 (J^{Φ}) and H-4–C-4–O-1′–C-1′ (J^{Ψ}) are presented in Table V. For a solution of **1** in water, the J^{Φ} value changed from 4.8 Hz at 278 K to 4.1 Hz at 358 K, and the corresponding values for J^{Ψ} were 5.6 Hz and 4.2 Hz. Thus, the changes of the conformational equilibrium implied by the $\Delta\delta$ values are confirmed by the variations in the interglycosidic $^3J_{C,H}$ values. Similar trends were observed for a solution of **1** in methanol; J^{Φ} was 5.3–5.0 Hz at low temperatures and 4.5 Hz at 298 and 318 K, and J^{Ψ} decreased from 5.8 Hz at 238 K to 4.5 Hz at 318 K. It follows from these results that the O-1′–C-4 bond (J^{Ψ}) is more flexible than the C-1′–O-1′ bond (J^{Φ}). This fact accords with an analysis of crystal structures of saccharides which showed[19] that the variation of Ψ is considerably larger than that of Φ.

For a solution of **1** in methyl sulfoxide, J^{Φ} and J^{Ψ} at 298 K were 5.7 and 5.6 Hz, respectively, *i.e.*, ~1 Hz larger than the values observed for solutions in water or methanol. This difference is greater than for the corresponding values found for maltose[20]. On the other hand, the temperature dependence was less pronounced.

TABLE V

J^{Φ} AND J^{Ψ} VALUES (Hz) FOR METHYL β-XYLOBIOSIDE (**1**) IN DIFFERENT SOLVENTS AND AT VARIOUS TEMPERATURES

Temp. (K)	*Water*		*Methanol*		*Methyl sulfoxide*		*1,4-Dioxane*	
	J^{Φ}	J^{Ψ}	J^{Φ}	J^{Ψ}	J^{Φ}	J^{Ψ}	J^{Φ}	J^{Ψ}
238			5.0	5.8				
258			5.0	5.4				
278	4.8	5.6	5.3	5.2				
298	4.7	5.1	4.5	4.7	5.7	5.6	5.6	5.2
318	4.4	4.7	4.5	4.5	5.3	5.6	5.3	5.0
338	4.3	4.4			5.3	5.3	5.5	5.2
358	4.1	4.2			5.2	5.2	5.5	5.2
378					5.1	5.0		

The maximal change for J^{Φ} and J^{Ψ} was 0.6 Hz for a difference of 80 K. In studies[21–24] of solutions of mono- and oligo-saccharides in methyl sulfoxide, it was concluded that hydroxyl groups can be involved in inter- and intra-molecular hydrogen bonding. However, it has been found[25] that **1** does not form intra-molecular hydrogen bonds. Therefore, the less pronounced temperature dependence of J^{Φ} and J^{Ψ} for the solution of **1** in methyl sulfoxide probably reflects lower flexibility around the glycosidic bond due to the solvation effects.

For a solution of **1** in 1,4-dioxane, the chemical shifts of the ^{13}C resonances, but not J^{Φ} and J^{Ψ}, showed marked temperature dependence. The constant J^{Φ} and J^{Ψ} values can be explained either by the formation of a strong hydrogen bond in only one conformer or the presence of conformers with Φ and Ψ angles such that the corresponding averages J^{Φ} and J^{Ψ} values are similar. The temperature dependence of the chemical shift of the C-4 resonance supports the latter interpretation.

Quantum-chemical calculations. — Interpretation of the observed n.m.r.

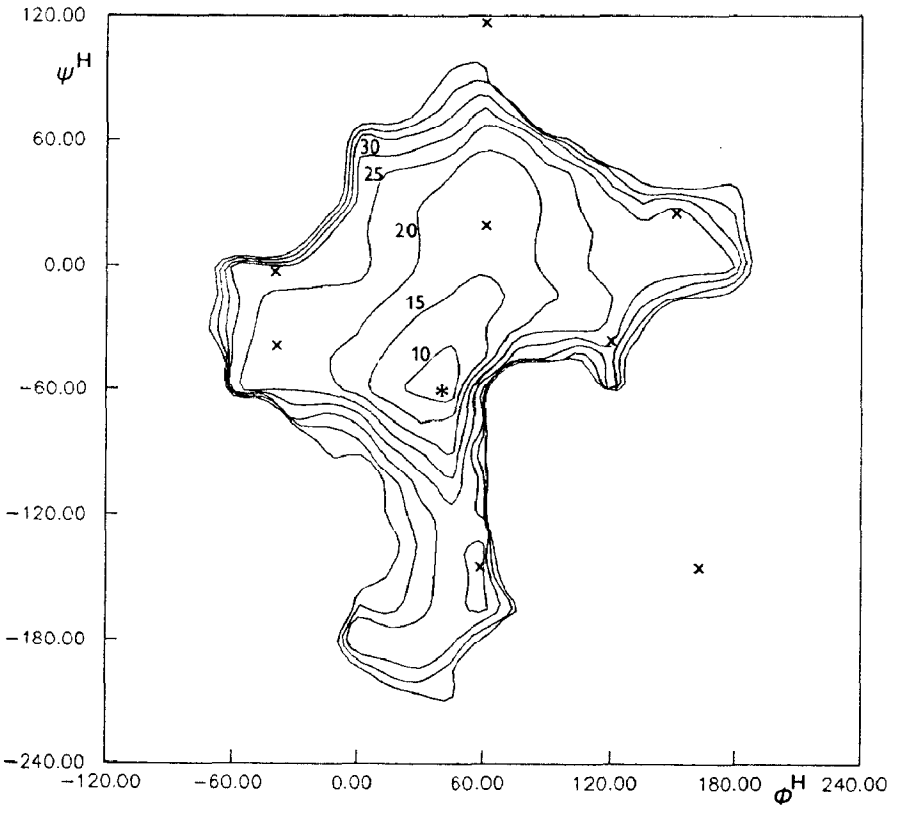

Fig. 1. Energy surface (kJ.mol^{-1}) for methyl β-xylobioside (**1**) as a function of the torsion angles Φ and Ψ: ⋆, lowest energy minimum; x, local minima.

parameters requires the computation of conformations involved in the averaging process during the n.m.r. experiments[26].

Fig. 1 shows the PCILO-calculated energy surface for the isolated molecule of **1**. Inspection of the (Φ, Ψ) map reveals nine energy minima (40,−60), (60,20), (60,−140), (160,−140), (−40,−40), (−40,0), (120,−30), (150,30), and (60,120). These minima were used as starting points for PCILO and molecular mechanics (MM2 and MM2CARB) minimizations. The conformation of the pyranoside ring was $^4C_1$ in each minimum and accords with crystal structures of xylan diacetate[27] and (1→4)-β-xylobiose hexa-acetate[28]. Minimization of the geometry affected considerably the values of Φ and Ψ as well as remaining optimized parameters in comparison with the starting geometry. This situation is shown clearly for the two most stable conformers X1 and X2. The optimized values for X1 (starting value 40,−60) were Φ^H 39.6° and Ψ^H −67.4°; for X2 (60,20), they were Φ^H 60.4° and Ψ^H 48.4°. Although the geometries of both minima differ significantly, the calculated energies are similar to that of X2, being 0.03 kJ.mol^{-1} higher than that of X1. The energy difference before optimization was 15.5 kJ.mol^{-1}.

PCILO-calculated relative energies, selected geometrical parameters, dipole moments, linkage rotations, and J^Φ and J^Ψ for all optimized minima are listed in Table VI. The geometry of the glycosidic linkage of X1 is closer to the X-ray-determined conformation of xylan diacetate[27] (42,−21) than to the conformation of (1→4)-β-xylobiose hexa-acetate[28] (20.4,−15.2). The minimum X2 is near to the left-handed conformation of (1→4)-β-D-xylan (63,25), which resulted from theoretical calculation[29]. Energies of the minima X3 (62.4,−152.4), X5 (−35.9,−49.5), X6 (118.9,−50.7), and X7 (153.5,29.9) were within 6 kJ.mol^{-1}, and only the conformer X4 (Φ^H 160.8°, Ψ^H −128.9°) had a relative energy value (20.75 kJ.mol^{-1} higher than that of X1. The starting minima (−40.0) and

TABLE VI

PCILO-CALCULATED ENERGIES ΔE (kJ.mol^{-1}), SELECTED GEOMETRICAL PARAMETERS [BOND LENGTHS (pm) AND ANGLES (°)], DIPOLE MOMENTS μ (D), LINKAGE ROTATION Λ (°), AND J^Φ AND J^Ψ (Hz) FOR THE OPTIMIZED CONFORMERS OF THE METHYL β-XYLOBIOSIDE (**1**)

	X1	*X2*	*X3*	*X4*	*X5*	*X6*	*X7*
C-1′–O-1′	139.3	139.2	139.1	139.2	139.4	139.5	139.5
O-5′–C-1′	139.7	139.8	139.8	139.6	139.8	139.6	139.7
O-1′–C-4	139.9	140.0	140.1	139.9	139.9	139.9	140.2
O-5′–C-1′–O-1′	106.7	107.4	107.6	108.6	102.6	109.4	108.8
C-1′–O-1′–C-4	113.3	112.3	114.0	118.0	113.2	113.3	112.1
O-1′–C-4–C-5	107.0	113.7	112.1	109.4	106.4	107.2	111.5
Φ^H	39.6	60.4	62.4	160.8	−35.9	118.9	153.5
Ψ^H	−67.4	48.4	−152.4	−128.9	−49.5	−50.7	29.9
ΔE	0.0	0.03	6.20	20.72	6.16	4.09	5.92
μ	4.56	4.08	6.18	2.95	3.29	3.76	5.43
Λ	70.7	−89.1	54.3	158.8	266.6	92.8	−8.4
J^Φ	3.4	1.6	1.5	6.1	3.8	2.1	5.6
J^Ψ	1.1	2.6	5.5	3.1	2.5	2.4	4.3

(−40,−40) as well as (60,120) and (60,20) merged during the optimization into the X5 and X2 minima, respectively. Geometrical parameters (Table VI) present the variation of bond angles, depending upon the stereochemistry of the linkage. In addition to changes of the glycosidic linkage bond-angle C-1′–O-1′–C-4, the angle O-5′–C-1′–O-1′ also varies and reflects the exo-anomeric effect. These results support the need to calculate (Φ,Ψ) conformational maps with optimization of all geometry parameters, including side chains.

The PCILO calculations showed that, in solution, there was a mixture of seven conformers (Table VII). The distribution of conformers for the isolated molecule was X1:X2:X3:X4:X5:X6:X7 = 41:41:3:0:3:8:4, and these ratios changed with changes in the polarity of the solvent and temperature. For solutions in methanol and methyl sulfoxide, four conformers (X1, X2, X3, and X7) were in equilibrium and in similar proportions. In methyl sulfoxide, the equilibrium did not change noticeably with change in temperature. With water as the solvent, the most preponderant conformer (~50%) was X3. Similar variations in abundances between aqueous and non-aqueous solutions have been observed for maltose and cellobiose[11,30]. Moreover, the distribution of conformers in water was strongly dependent on temperature, and the proportion of X3 changed from 58% at 283 K to 46% at 343 K.

In order to compare the results of molecular mechanics with PCILO calculations, calculations using MM2[31] and MM2CARB[32] methods were performed. Dihedral angles and bond angles calculated by MM2CARB or MM2 methods accorded with those obtained from the PCILO optimization. On the other hand, energy profiles computed by both molecular mechanics methods did not correspond with results from PCILO quantum-chemical calculations. It follows from the relative energies that seven conformers were present in solution. The distribution obtained for aqueous solution at 298 K by the MM2CARB method for X1:X2:X3:X4:X5:X6:X7 was 10:48:17:0:3:0:22 and by the MM2 method was 41:26:1:0:29:0:3. Although the different minimization procedures used may lead

TABLE VII

PCILO-CALCULATED MOLAR FRACTIONS FOR OPTIMAL CONFORMERS X1–X7 OF METHYL β-XYLOBIOSIDE (**1**) FOR THE ISOLATED STATE, AND IN DILUTE SOLUTIONS AT VARIOUS TEMPERATURES

	X1	*X2*	*X3*	*X4*	*X5*	*X6*	*X7*
PCILO	40.6	41.1	3.3	0	3.4	7.8	3.7
1,4-Dioxane	41.4	37.8	6.5	0	2.6	6.2	5.5
Methanol	37.8	24.5	22.7	0	1.4	3.6	9.8
Methyl sulfoxide 298 K	40.5	29.7	15.4	0	1.6	4.5	8.3
323 K	38.6	29.1	15.4	0	2.8	5.1	8.9
373 K	36.5	28.6	15.8	0	2.8	6.5	10.0
Water 283 K	20.4	9.2	58.1	0	0.2	0.9	11.2
298 K	21.6	10.3	54.7	0	0.3	1.2	11.8
323 K	23.4	12.1	49.6	0	0.5	1.7	12.7
343 K	24.3	13.4	46.3	0	0.7	2.1	13.2

to different final geometries, this discrepancy hints at the problem of refinement of potential functions for carbohydrates. Since calculated energies are needed in order to assess the conformational flexibility for any oligosaccharide, the establishment of an adequate calculation method is decisive.

Based on the abundances of conformers, their geometry, and the Karplus relationship, averaged $\langle {}^3J_{C,H} \rangle$ were calculated (Table VIII). Calculated $\langle J^{\Phi} \rangle$ and $\langle J^{\Psi} \rangle$ values depended on the solvent and the temperature. The most pronounced dependence was obtained for J^{Ψ} in aqueous solution, where it varied from 4.2 Hz at 283 K to 3.8 Hz at 343 K. The computed $\langle J^{\Phi} \rangle$ and $\langle J^{\Psi} \rangle$ values were lower than the experimental values, and the calculated dependencies were smaller than those observed. These differences are probably due to two reasons. First, theoretical $\langle J^{\Phi} \rangle$ and $\langle J^{\Psi} \rangle$ values were calculated from the minimized geometry of *selected* conformers. Since the (Φ,Ψ) map was obtained as a rigid-body-rotation of the monosaccharide residues without optimization of the side chains, some of the energy minima could be neglected[33]. Moreover, a simplified approach was used for the calculation of $\langle {}^3J_{C,H} \rangle$ where only energy minima were taken into account. Since this approach neglects contributions of other rotamers, the agreement with the experimental data can be only qualitative. A better agreement with the experimental data should be obtained when $\langle J^{\Phi} \rangle$ and $\langle J^{\Psi} \rangle$ values are calculated from the *whole optimized* conformational (Φ,Ψ) map. However, with the present use of the PCILO method, it is not possible to envisage such a procedure because of the CPU time required. However, the calculated results indicate changes of conformational equilibrium with changes in both the solvent polarity and temperature.

Calculated averaged dihedral angles, linkage rotations, and dipole moments are given in Table VIII. The averaged dipole moment $\langle \mu \rangle$ varied from 4.4 D *in vacuo* to 5.6 D in D_2O at 278 K, and the averaged linkage rotation $\langle \Lambda \rangle$ ranged from 9.8° to 38.3° in aqueous solution at 278 K. Thus, as for $\langle J^{\Phi,\Psi} \rangle$, both calculated $\langle \mu \rangle$

TABLE VIII

COMPUTED AVERAGED VALUES OF DIHEDRAL ANGLES $\langle \Phi^H \rangle$ AND $\langle \Psi^H \rangle$ (°) INTERGLYCOSIDIC $^3J_{C,H}$ VALUES $\langle J^{\Phi} \rangle$ AND $\langle J^{\Psi} \rangle$ (Hz), DIPOLE MOMENTS $\langle \mu \rangle$ (D), AND LINKAGE ROTATION $\langle \Lambda \rangle$ (°) FOR DIFFERENT SOLVENTS AT VARIOUS TEMPERATURES

	$\langle \Phi^H \rangle$	$\langle \Psi^H \rangle$	$\langle J^{\Phi} \rangle$	$\langle J^{\Psi} \rangle$	$\langle \mu \rangle$	$\langle \Lambda \rangle$
PCILO	69.0	181.3	2.6	2.1	4.37	9.8
1,4-Dioxane	67.5	181.8	2.6	2.2	4.49	11.4
Methanol	67.2	187.3	2.7	2.8	4.92	22.3
Methyl sulfoxide 298 K	66.8	186.1	2.7	2.6	4.74	18.2
323 K	71.4	186.4	2.7	2.6	4.74	21.4
373 K	73.6	184.5	2.7	2.7	4.74	21.4
Water 283 K	68.8	191.6	2.4	4.2	5.60	38.3
298 K	69.5	190.1	2.4	4.1	5.55	36.7
293 K	70.6	188.0	2.5	3.9	5.47	34.6
343 K	71.6	186.3	2.5	3.8	5.41	33.1

and $\langle\Lambda\rangle$ indicate a dependence of conformational equilibrium on temperature and solvent.

The values of $\langle\Phi\rangle$ and $\langle\Psi\rangle$ were in the ranges 66–73° and 181–191°. These ranges are surprisingly narrow, particularly in comparison with the remarkable dependence of conformer abundances on the solvent and temperature (see Table VII). These results imply that multiple conformers, the abundances of which change with change in the solvent, might be described by constant, averaged geometrical or n.m.r. parameters. Moreover, since the J and n.O.e. data depend, in a non-linear way, on their associated geometrical parameters (dihedral angles, distances), they cannot be obtained from averaged values of structural parameters of the individual conformers involved in averaging process.

On the basis of n.O.e. effects and spin–lattice relaxation times, it has been found that single well-defined conformations are much more common in solution[34–37]. It was concluded that the exo-anomeric effect has a dominant influence, whereas the solvent and temperature do not markedly influence the conformation of the glycosidic linkage. The interpretation was based on calculations that used exclusively potential functions with constant geometry. Since the relative energies calculated depended to a large extent upon the choice of the initial molecular geometry[38], the interpretation might be misleading. In order to examine this possibility, the conformational analysis of **1** by the HSEA method was carried out. In contrast to the results of other methods, the calculation of the energy surface of **1** revealed one energy minimum (−100,−70); the energies of two other minima were higher than 15 kJ.mol^{-1}. The corresponding J^{Φ} and J^{Ψ} at minimum HSEA are 0.8 Hz and 1.0 Hz, respectively. These values are considerably different from those observed. Thus, the result where only one minimum on the energy surface is found does not explain the observed conformational flexibility of **1**.

It is concluded that the variable temperature measurements of interglycosidic $^3J_{C,H}$ values, together with their interpretation using an adequate method of calculation with solvent-effect evaluation, could be a useful approach for the characterization of the conformation of oligosaccharides in solution. However, the results suggest that the set of conformers calculated by semiempirical quantum chemical PCILO or molecular mechanics methods is not sufficiently refined at this stage to describe quantitatively the temperature and solvent dependent properties of **1** in solution.

In order to obtain more quantitative agreement between experimental and theoretical results, it is necessary to calculate the whole optimized 2D map and evaluate all orientations of the side chains. The procedure which will allow such a complex calculation is now being developed[33].

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