Carbohydrate Research, 210 (1991) 13–20
Elsevier Science Publishers B.V., Amsterdam

Nuclear Overhauser effects and the flexibility of saccharides: methyl β-xylobioside

Miloš Hricovíni*, Igor Tvaroška, Ján Hirsch,
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava (Czechoslovakia)

and Anthony J. Duben
Southeast Missouri State University, Cape Girardeau, Missouri 63701 (U.S.A.)

(Received June 1st, 1990; accepted for publication, July 10th, 1990)

ABSTRACT

The behavior of methyl β-xylobioside (**1**) has been analysed by ^{1}H-n.m.r. spectroscopy for solutions in water and methanol in the range $-15°$ to $85°$. Experimental n.O.e. values did not change with temperature and solvent in contrast to the inter-glycosidic $^3J_{C,H}$ values. Experimental n.O.e. data accorded with the values calculated from time-averaged cross-relaxation terms. The tumbling times were determined from relaxation data and were in the sub-ns range.

INTRODUCTION

The important biological roles of many oligosaccharides have stimulated interest in their conformations in solution, and molecular and quantum mechanics methods[1–5], together with several experimental techniques[6–17] including ^{1}H-n.m.r. n.O.e. experiments[5–16], have been applied.

We now report quantitative n.O.e. values and spin–lattice relaxation times for methyl β-xylobioside (**1**) at different temperatures for solutions in methanol and water. The inter-glycosidic $^3J_{C,H}$ values and ^{13}C chemical shifts of **1** indicated[18] a marked dependence of the conformational equilibrium on solvent and temperature.

RESULTS AND DISCUSSION

^{1}H and ^{13}C spin–lattice relaxation times. — The ^{13}C T_1 values for solutions of **1** in D_2O at 5°, 25°, 65°, and 85° are recorded in Table I. The T_1 values at 5° were ~0.4 s for CH, and 0.19 and 0.20 s for CH_2; T_1 increased to 2.3–2.4 s at 85° (a value of 2.3 s was used to determine τ_c). The rotational correlation times calculated for aqueous solutions at 5°, 25°, 65°, and 85° were 126, 63, 25, and 21 ps, respectively. Thus, τ_c values that are in the range 10^{-10}–10^{-11} s reflect rapid re-orientation of **1** in aqueous solution and indicate the dependence of the tumbling on the temperature.

---

* Author for correspondence.

0008-6215/91/$03.50

TABLE I

^{13}C spin–lattice relaxation times (s) for **1** in aqueous solutions

Atom	*Temperature*			
	5°	*25°*	*65°*	*85°*
C-1	0.41	0.87	2.25	2.45
C-1′	0.38	0.78	1.91	2.32
C-2	0.39	0.78	2.03	2.63
C-2′	0.39	0.79	2.03	2.63
C-3	0.39	0.78	1.97	2.43
C-3′	0.39	0.76	1.96	2.23
C-4	0.38	0.78	1.98	2.23
C-4′	0.39	0.79	1.95	2.20
C-5	0.20	0.38	0.98	1.00
C-5′	0.19	0.39	1.07	1.21
OMe	0.77	1.69	3.83	4.03

The values of τ_c for solutions of **1** in methanol at different temperatures were determined from ^{1}H longitudinal relaxation times (Table II) (again assuming an exclusively dipole–dipole relaxation mechanism). For this purpose, H-5*eq* was chosen due to its large dipole–dipole relaxation with H-5*ax* as well as H-4. Both relaxation contributions are seen in the n.O.e. spectra (Fig. 1) when H-5*eq* was saturated. In addition to the n.O.e. of the signal for H-5*ax*, enhancements were observed for the signals of H-4 and H-1′. However, as already mentioned, the contribution of H-1′ was neglected due to the time-averaged character. The agreement between the τ_c values (73 and 27 ps) determined for aqueous solutions at 25° and 65°, respectively, based on ^{1}H longitudinal relaxation

TABLE II

^{1}H spin–lattice relaxation times (s) for **1** in water and methanol

Atom	*Methanol*			*Water*	
	−15°	*25°*	*45°*	*25°*	*65°*
H-1	0.67	1.08	1.73	0.79	2.09
H-1′	0.49	0.88	1.31	0.58	1.55
H-2	1.62	2.25	2.68	1.87	3.62
H-2′	1.48	1.99	3.14	2.32	4.63
H-3	1.15	1.85	2.82	0.97	3.36
H-3′	1.58	1.57	2.12	1.18	3.36
H-4	0.60	1.06	1.55	0.88	2.08
H-4′	0.93	1.52	2.47	1.13	3.02
H-5*eq*	0.28	0.58	0.88	0.39	1.07
H-5′*eq*	0.30	0.64	0.97	0.52	1.29
H-5*ax*	0.28	0.48	0.92	0.40	1.05
H-5′*ax*	0.24	0.57	0.95	0.45	1.14
OMe	0.72	1.28	1.84	0.91	2.00

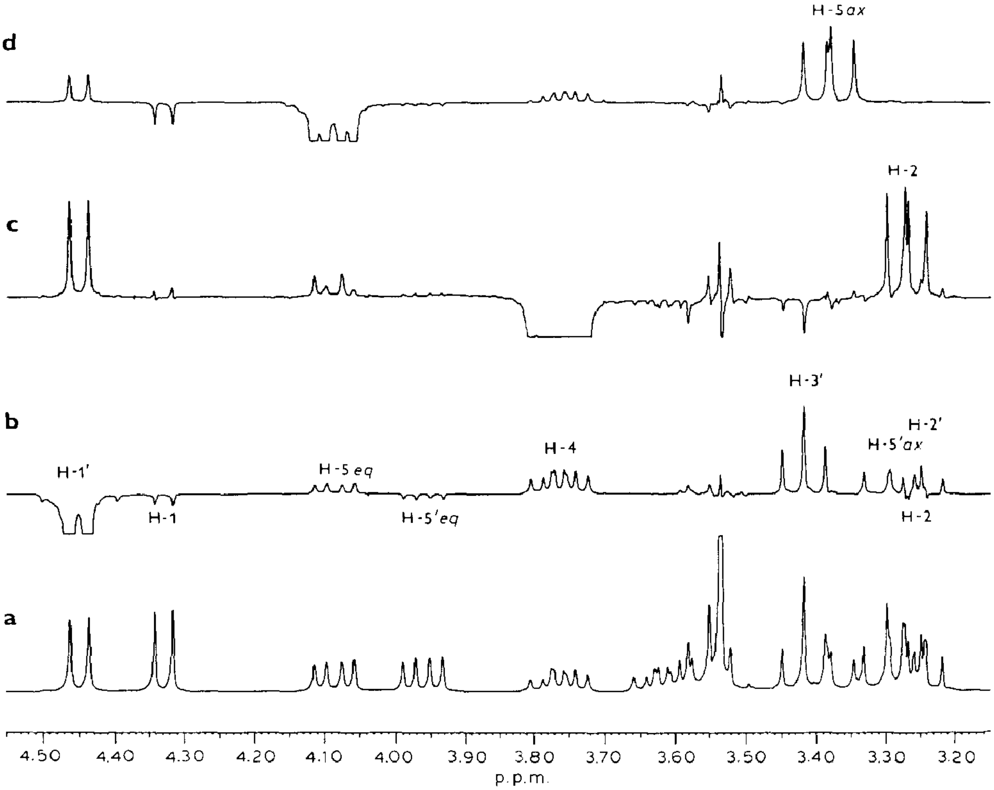
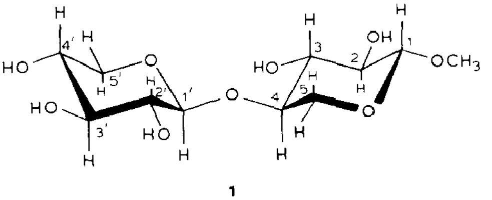


Fig. 1. ^{1}H-N.O.e. difference spectra of **1** in aqueous solution at 25°: (*a*) reference spectrum, (*b*) saturation of H-1′, (*c*) saturation of H-4, (*d*) saturation of H-5*eq*.

times of H-5*eq* and those determined previously from ^{13}C T_1 measurements, is satisfactory. The tumbling times in methanol determined from ^{1}H T_1 data at $-15°$, 25°, and 45° were 103, 50, and 33 ps, respectively.



1

Experimental and theoretical steady-state n.O.e. — N.O.e. difference spectra for solutions of **1** in D$_2$O and methanol are shown in Figs. 1 and 2. Fractional enhancements of the signals of H-4 and H-5*eq* were observed (through the glycosidic bond), and of H-5′*ax* and H-3′, when H-1′ was saturated. Saturation of H-4 caused changes of intensity of the signals of H-2, H-5*eq*, and H-1′. Negative intensities (for example, the signals of H-5′*eq* and H-2) were due to three-spin effects.

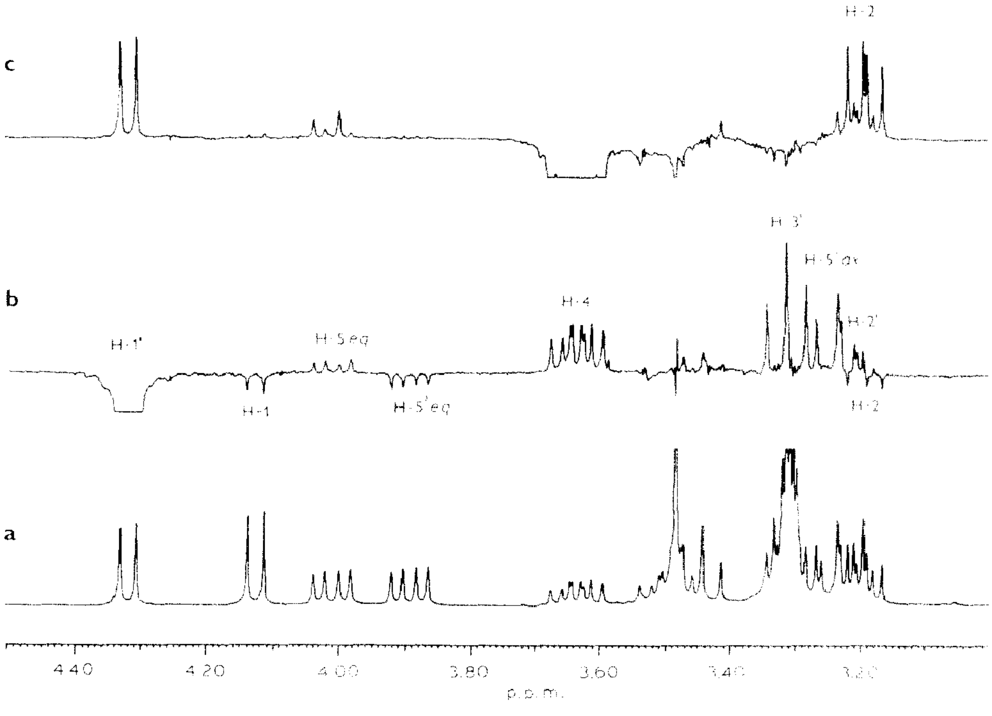


Fig. 2. ^{1}H-N.O.e. difference spectra of **1** in methanol at 25°: (*a*) reference spectrum, (*b*) saturation of H-1′, (*c*) saturation of H-4.

Integrated n.O.e. effects for solutions of **1** in water and methanol at different temperatures are given in Tables III and IV. In aqueous solutions (Table III), the n.O.e. of the signal for H-4 on saturation of H-1′ was 12 (5°), 15 (25°), 16 (65°), and 15% (85°), *i.e.*, essentially constant. Similarly, saturation of H-4 caused 11–12% enhancements of the signal for H-1′ regardless of the temperature. Thus, the n.O.e. values do not vary significantly with temperature. Comparable results were obtained for a solution of **1** in methanol (Table IV) at −15°, 25°, and 45°. The n.O.e. of the signal of H-4 was 16–17% on saturation of H-1′. The n.O.e. of the signal of H-1′ on irradiation of H-4 changed from 7% (−15°) to 12% (45°). The low value of the n.O.e. at −15° probably resulted from insufficient (80%) saturation of H-4 due to lower T_1 values.

With the exception of the weak dependence of n.O.e. values on the temperature, there was little difference in the integrated intensities for the aqueous and methanolic solutions (~15% n.O.e. of the signal for H-4 on saturation of H-1′ and ~11% n.O.e. for the signal of H-1′ on saturation of H-4). The observed n.O.e.'s were essentially constant, in contrast with the results[18] obtained using $^3J_{C,H}$ and ^{13}C chemical shifts which showed a marked dependence on solvent and temperature and were supported by theoretical quantum-chemical PCILO, MM2, and MM2CARB calculations.

For more quantitative information on stereochemistry, it is necessary to utilise reliable modelling methods and calculate theoretical n.O.e. values; for this purpose, the

TABLE III

Experimental n.O.e. values (%) on pre-irradiation of H-1′, H-4, and H-5*eq* in aqueous solutions

Proton observed[a]	*Proton saturated*											
	H-1′				*H-4*				*H*-5eq			
	5°	*25°*	*65°*	*85°*	*5°*	*25°*	*65°*	*85°*	*5°*	*25°*	*65°*	*85°*
H-1′					11	12	12	12	6	5	5	4
H-2					17	20	19	19				
H-3′	10	14	15	15								
H-4	12	15	16	15					8	7	8	6
H-5*eq*	3	4	4	3	6	5	5	4				
H-5′*eq*	−2	−3	−3	−3								
H-5*ax*									25	29	30	31
H-5′*ax*	8	7	9	9								

[a] There was no effect on H-1, H-2′, H-3, and H-4′.

TABLE IV

Experimental n.O.e. values (%) on pre-irradiation of H-1′, H-4, and H-5*eq* in methanol

Proton observed[a]	*Proton saturated*								
	H-1′			*H-4*			*H*-5eq		
	−15°	*25°*	*45°*	*−15°*	*25°*	*45°*	*−15°*	*25°*	*45°*
H-1′				7	11	12	4	3	3
H-2				14	20	18			
H-3′	15	13	15						
H-4	17	16	16				13	8	7
H-5*eq*	4	3	3	3	4	5			
H-5′*eq*	−2	−3	−3						
H-5*ax*							25	28	30
H-5′*ax*	12	10	8						

[a] There was no effect on H-1, H-2′, H-3, and H-4′.

PCILO-calculated data based on the geometries and energies of conformers for each solvent and temperature were used. The values of τ_c for both solvents at various temperatures were determined from measurements of T_1. Computed-averaged n.O.e. values for solutions in water and methanol are listed in Table V. Changes in the intensity of the signal for H-4 were 16–17% when H-1′ was saturated. Thus, the n.O.e. values of H-4 are constant, regardless of temperature and solvent, which is consistent with experimental findings. The same trend was observed for enhancements of the signal for H-1′ (saturation of H-4), but the calculated values were lower than the experimental values. The difference for H-1′ may result from the same limitations, as with the $^3J_{C,H}$ values through the glycosidic bond.

TABLE V

Calculated n.O.e. values (%) on pre-irradiation of H-1′ and H-4 in water and methanol[a]

Proton observed[b]	*Proton saturated*							
	H-1′				*H-4*			
	Water			*Methanol*	*Water*			*Methanol*
	5°	*25*	*65°*	*25*	*5°*	*25*	*65°*	*25*
H-1′					4	5	5	5
H-2					3	4	4	4
H-3′	12	12	10	11				
H-4	16	17	16	17				
H-5*ax*					3	4	3	4
H-5*eq*	4	4	4	4	−2	−2	−2	−2
H-5′*ax*	9	10	10	11				

[a] Geometry of energy minima were taken from the previous study[18], tumbling times were determined from T_1 relaxation times for **1**. [b] There was no effect on H-1, H-2′, H-3, H-4′, and H-5′*eq*.

This simple approach, where only the energy minima were included in the calculation of $^3J_{C,H}$ values, also resulted in computed values that were lower than those observed experimentally[18]. It is probable that better agreement may be obtained by using the whole relaxed conformational (φ,ψ) map that will take into account contributions of all energy states of the molecule. Where the shape of energy surface is shallow, the time of transition between individual rotamers may not be negligible. The discrepancy may be connected also with the change of the tumbling time with change in conformation. However, the values of τ_c for individual conformers have not been included in the present analysis of **1**.

Other factors which require attention are connected with the dynamics of the molecule itself[19,20]. In small flexible molecules, the conformers interconvert rapidly on the n.m.r. time-scale and time-averaged values of n.m.r. parameters do not reflect the statistical weight of the structural parameter. The averaging of geometrical parameters due to thermal motion is linear, which can be very different in comparison to those determinated from experimental n.O.e. data and interpreted by calculation, which are based on $(\langle r^{-6}\rangle)^{-1/6}$. For correct interpretation of experimental data, it is necessary to account for fluctuations of the magnitude of the relaxation vector and include these in the autocorrelation function.

In relaxation experiments[21] for DNA, proton T_1 values, calculated using the time-dependent internuclear distance, were not identical with those calculated using the standard expression $\langle r^{-6}\rangle$ for a wide range of rates of internal motion. Similar results were obtained only for very slow internal motion comparable to overall tumbling.

In accord with this Monte Carlo situation of aromatic protons in oligonucleotides on the ns time-scale, it was shown[22] that internuclear distances in flexible molecules can be under- or over-estimated depending on the amplitude of the motion and the internal diffusion constant. Moreover, various n.m.r. parameters, such as n.O.e. and coupling constants, average differently, and structures deduced from coupling constants can differ from those deduced from n.O.e. data. Thus, in the flexible molecules, the averaged value $\langle$n.O.e.$\rangle$ can be constant although other parameters, for example $\langle J \rangle$, may exhibit a marked change. Observed constant n.O.e. values do not necessarily reflect the presence of a single conformation and this may be a consequence of the internal dynamics of the molecule. Such a discrepancy between "*J*-structure" and "n.O.e. structure" has been reported[23] for the cyclic decapeptide antamanide.

In the analysis of the conformations of small flexible molecules in solution, appropriate model and computational procedures must be used for the interpretation of experimental results. Direct calculation from non-linear averaged n.m.r. parameters may not yield exact results. Interpretation using only one n.m.r. parameter can be misleading, and combination of experimental data (such as n.O.e. and $^{3}J_{C,H}$) will increase the chance of determining more properly the conformational properties of oligosaccharides in solution.

EXPERIMENTAL

The ^{1}H- and ^{13}C-n.m.r. spectra were obtained at 300.13 and 75.45 MHz, respectively, on 0.05M solutions of **1** with a Bruker AM-300 spectrometer, at 5°, 25°, 65°, and 85° for water, and −15°, 25°, and 45° for methanol. The ^{1}H-n.m.r. spectra were referenced to internal Me_4Si or acetone (2.225 p.p.m.). Argon was bubbled through samples in order to remove oxygen and the tubes were then sealed.

The ^{1}H and ^{13}C spin–lattice relaxation times were obtained by the inversion-recovery method with 12–14 τ values (broad-band-decoupled spectra were collected for the determination of the ^{13}C T_1 values). The T_1 values were determined from a three-parameter fit of the variation of integrated intensities; standard deviations were $<1\%$.

Experimental n.O.e. values were determined by difference spectroscopy[24] where each resonance in the multiplet was irradiated 50–100-fold with a soft pulse of duration 200–300 ms (180° pulse at the chosen decoupler power). The total time of irradiation was 10–11 s and the acquisition time was 8 s. Zero filling to give a final resolution of 0.05 Hz was used before Fourier transformation. In order to obtain good thermal equilibrium, each sample was stored for 2–3 h at each temperature before measurement in the spectrometer. Two dummy scans preceded each of 128 accumulated transitions.

N.O.e. values were computed from the inter-atomic distances in **1**, using the expression[19]

$$f_d(s) = \sum_s \frac{\langle \sigma_{ds} \rangle}{\langle R_d \rangle} - \sum_s \frac{\langle \sigma_{dj} \rangle}{\langle R_d \rangle} f_j(s)$$

where σ_{ds} and σ_{dj} are cross-relaxation terms, R_d is the term for dipole–dipole relaxation; σ_{ds} and R_d were calculated for each of six present conformers in solution. The ensemble averaged $\langle\sigma_{ds}\rangle$ and $\langle R_d\rangle$ were calculated[25] according to Boltzmann distribution with energy of individual conformers taken from the previous study[18]. The tumbling times (τ_c) in different solvents at various temperatures necessary for calculation were determined from ^{1}H and ^{13}C T_1 relaxation times[26].

REFERENCES

1 I. Tvaroška, *Pure Appl. Chem.*, 61 (1989) 1201–1216.
2 I. Tvaroška and S. Perez, *Carbohydr. Res.*, 149 (1986) 389–410.
3 I. Tvaroška, *Biopolymers*, 21 (1982) 1887–1897.
4 G. M. Lipkind, V. E. Verovsky, and N. K. Kochetkov, *Carbohydr. Res.*, 133 (1984) 1–13.
5 M. Ragazzi, D. R. Ferro, and A. Provasoli, *J. Comput. Chem.*, 7 (1986) 105–112.
6 C. A. Bush, Z. Y. Yan, and B. N. N. Rao, *J. Am. Chem. Soc.*, 108 (1986) 6168–6173.
7 Z. Y. Yan, B. N. N. Rao, and C. A. Bush, *J. Am. Chem. Soc.*, 109 (1987) 7763–7769.
8 H. Thogersen, R. U. Lemieux, K. Bock, and B. Meyer, *Can. J. Chem.*, 60 (1982) 44–57.
9 R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto, and V. S. R. Rao, *Can. J. Chem.*, 58 (1980) 631–653
10 J. R. Brisson and J. P. Carver *Biochemistry*, 22 (1983) 3671–3680.
11 J. R. Brisson and J. P. Carver, *Biochemistry*, 22 (1983) 3680–3686.
12 S. Sabesian, K. Bock, and R. U. Lemieux, *Can. J. Chem.*, 62 (1984) 1034–1045.
13 H. Paulsen, T. Peters, V. Sinnwell, R. Lebuhn, and B. Meyer, *Liebigs Ann. Chem.*, (1984) 951–976.
14 H. Paulsen, T. Peters, V. Sinnwell, R. Lebuhn, and B. Meyer, *Liebigs Ann. Chem.*, (1985) 489–509.
15 K. Bock, S. Josephson, and D. R. Bundle, *J. Chem. Soc., Perkin Trans. 2*, (1982) 59–70.
16 S. W. Homans, R. A. Dwek, J. Boyd, M. Mahmoudian, W. G. Richards, and T. W. Rademacher, *Biochemistry*, 25 (1986) 6342–6350.
17 S. Perez, F. Taravel, and C. Vergelati, *Nouv. J. Chim.*, 9 (1985) 561–564.
18 M. Hricovini, I. Tvaroška, and J. Hirsch, *Carbohydr. Res.*, 198 (1990) 193–203.
19 J. H. Nogle and R. E. Shirmer, *The Nuclear Overhauser Effect*, Academic Press, 1971, p. 90.
20 O. Jardetzky, *Biochim. Biophys. Acta*, 621 (1980) 227–232.
21 J. W. Keepers and T. L. James, *J. Am. Chem. Soc.*, 104 (1982) 929–939.
22 D. Genest, *Biopolymers*, 28 (1989) 1903–1911.
23 H. Kessler, Ch. Griesinger, J. Lautz, A. Muller, W. F. van Gunsteren, and H. J. C. Berendsen, *J. Am. Chem. Soc.*, 110 (1988) 3393–3396.
24 D. Neuhaus, *J. Magn. Reson.*, 53 (1983) 109–114.
25 A. J. Duben, unpublished results.
26 D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, 56 (1972) 3683–3689.