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The conformation of 1,6-anhydrolactose and its hexa-acetate in solution

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ABSTRACT

The conformation of 1,6-anhydrolactose (**1**) has been investigated by n.m.r. spectroscopy and molecular mechanics calculations. For a solution in D_2O, the 1,6-anhydroglucopyranoid ring has a $^1C_4$ conformation, whereas there is a ~ 1:1 equilibrium between the $^1C_4$ and the $B_{O,3}$ conformations in $(CD_3)_2SO$. There is restricted flexibility with φ $-80 \pm 20°$ and ψ $-120 \pm 40°$. The hexa-acetate (**2**) of **1** shows a similar conformational behaviour.

INTRODUCTION

As a part of a project on the molecular recognition of synthetic analogues of methyl β-lactoside by ricin, a cytotoxic plant lectin[1], the existence has been suggested of a non-polar interaction of the lectin and the C-3 region of the disaccharide[2], on the basis of a marked enhancement of the binding of the 3-deoxy analogue and a six-fold decreased affinity of the 3-*O*-methyl derivative. The affinity of 1,6-anhydrolactose for the lectin was approximately two and a half times lower than that of methyl β-lactoside, which suggested the involvement in the binding of the D-glucopyranose moiety in the $^1C_4$ conformation.

In a systematic study of the affinities of receptor-active analogues of oligosaccharides[3,4], it is necessary to consider their conformations in order to assess the biological activity in terms of the size and shape of the hydrophilic and hydrophobic surfaces[5]. In this context, we now report on the conformation in solution of 1,6-anhydrolactose (**1**), using n.m.r. data and molecular mechanics calculations[6–9]. Since methyl sulfoxide has been presumed to model the behaviour of protein surfaces for several recognition processes[4,10], the n.m.r. experiments were performed on solutions in D_2O and $(CD_3)_2SO$. The conformation of the hexa-acetate (**2**) of **1** has also been studied.

EXPERIMENTAL

1,6-Anhydrolactose was obtained from deacetylation of its hexa-acetate {m.p. 104–106°, $[\alpha]_D$ $-39.8°$ (*c* 1, chloroform)}, which was prepared as previously described[11].

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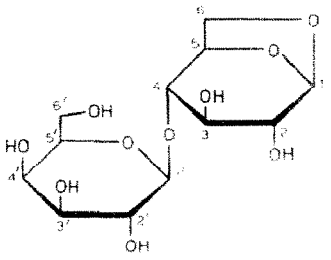

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Molecular mechanics calculations. — Hard-sphere exo-anomeric (HSEA) calculations were performed using the PFOS programme[12]. The constituent monosaccharides were assumed to be rigid entities and a value of 117° was given to the glycosidic bond angle, leaving as variables that determined the disaccharide conformation only the torsion angles φ (O-5′–C-1′–O-1′–C-4) and ψ (C-1′–O-1′–C-4–C-5). The co-ordinates for the glucose ($^1C_4$ and $B_{O,3}$ conformations) and galactose ($^4C_1$ form) residues were taken from a data bank[13] of MM2 optimised structures. Two preferred orientations were assumed for the C-4′–C-5′–C-6′–O-6′ angle of the galactose residue, namely, −175° (*gt*) or −65° (*tg*). These orientations led to similar conformational hard-sphere maps for **1** in terms of φ and ψ with a 10° grid. After lone pairs were added to the oxygen atoms, the local minima were optimised through molecular mechanics calculations, using the MM2(85) programme[13], which partially accounts for hydrogen bonding. Two different dielectric constants (ε 1 and 80) were used in order to model the effect of D_2O and $(CD_3)_2SO$ in the energy and geometry of the conformers. Similar energy values (ΔE < 0.4 kcal/mol) for the *gt* and *tg* orientations of the lateral chain were found for each conformer and only the values for the *gt* rotamers are shown.

N.m.r. spectroscopy. — 300-MHz ^{1}H-n.m.r. experiments were performed with a Varian XL-300 spectrometer at 30°. A solution of **1** (~ 20 mg) in D_2O was lyophilised, the process was repeated, the residue was dissolved in 0.5 mL of 99.96% D_2O or 99.9% $(CD_3)_2SO$, and the solution was degassed in the n.m.r. tube under argon. In separate experiments, **1** and **2** were each dissolved in 99.9% $(CD_3)_2SO$ and $CDCl_3$, respectively, and the solutions were degassed. Chemical shifts (δ, p.p.m.) were measured by reference to internal residual HDO (δ 4.710) or to Me_4Si, depending on the solvent used. Double-quantum-filtered phase-sensitive COSY experiments were performed using the pulse sequence 90°–t_1–90°–90° acquisition. A 512 × 1k data matrix was obtained which was zero-filled prior to Fourier transformation. The first-order values of the chemical shifts and coupling constants were used as input parameters for the calculation of the 1D-spectrum, using the PANIC programme (Bruker software). 2-D-NOESY experiments were performed in the phase-sensitive mode with the pulse sequence 90°–t_1–*tm*–90°–acquisition, using mixing times of 0.5 and 1.0 s and a relaxation delay of 3 s. The cross-peak and diagonal-peak volumes were obtained by using standard Varian software. The estimated error was 10%. Interatomic distances were estimated from n.O.e. ratios, since the magnitude of the n.O.e. is proportional to the inverse sixth power of the internuclear distance[14]. Thus, from the n.O.e. values for a pair of protons that are

separated by a known fixed distance (*i.e.*, 2.5 Å for $r_{H\text{-}1',H\text{-}3'}$ in the galactopyranoid ring), it is possible to translate the n.O.e. value for a given proton on the glucopyranoid ring in terms of average distance $\{<r^{-3}>^2\}^{-1/6}$. Only the values from the experiment carried out with a mixing time of 500 ms were considered to give such average distances. These distances were compared to those expected for a Boltzmann distribution of the possible conformations obtained through molecular mechanics[15]. Spin–lattice relaxation times were determined for a solution of **1** in D_2O, using the inversion-recovery technique with 10 values of the variable delay. The mean values of two independent measurements are given and the estimated error was 10%.

50-MHz ^{13}C-N.m.r. experiments were performed with a Bruker AM-200 spectrometer equipped with a dual probe. Chemical shifts are expressed relative to external acetone (δ 29.8) or $CDCl_3$ (δ 77.0). The chemical shifts of the resonances of **1** in solution in D_2O or $(CD_3)_2SO$ varied by only 0.1 p.p.m. between 30° and 80°. Heteronuclear correlation experiments with F1 decoupling were performed using standard Bruker software. A 64 × 4k data matrix was obtained and processed after zero-filling. Spin–lattice relaxation times were determined by the inversion–recovery technique, using a non-linear least-squares fit procedure. At least 7 delays were used for each determination of T_1 and the estimated error was 5%. Long-range H-1′–C-4 coupling constants for solutions of **1** in D_2O and **2** in $CDCl_3$ were determined using the spin-flip method with a DANTE sequence for the selective proton pulse. A 16 × 8k data matrix was obtained and processed after zero-filling.

RESULTS AND DISCUSSION

Conformations of the monosaccharide residues in **1** *and* **2**. — The atom numbering of **1** is shown in the formula. The ^1H-n.m.r. parameters for solutions of **1** in D_2O and $(CD_3)_2SO$ and of **2** in $CDCl_3$ are given in Tables I and II. There are large and positive ^4J couplings ($J_{1,3}$, $J_{2,4}$, and $J_{3,5}$) for the D-glucopyranoid ring for solutions of **1** in D_2O and of **2** in $CDCl_3$, which indicate[16] planar arrangements of the protons involved and, therefore, a major $^1C_4$ conformation. However, these ^4J couplings were not observed for a solution of **1** in $(CD_3)_2SO$, and the $^3J_{2,3}$ and $^3J_{3,4}$ values are noticeably larger than those for a solution in D_2O. The magnitudes of the coupling constants for hydroxyl protons also differ from those reported for other 4-*O*-substituted derivatives of 1,6-anhydro-D-glucopyranose[17]. The expected values of $^3J_{H,H}$, calculated by applying Altona's equation[18] to the vicinal proton torsion angles obtained for the chair and boat forms of the glucopyranoid ring of **1** by MM2 calculations, are given in Table III. The values for solutions of **1** in D_2O and **2** in $CDCl_3$ account for a major $^1C_4$ conformation of the glucopyranoid ring, whereas those for a solution of **1** in $(CD_3)_2SO$ are in between those expected for the $^1C_4$ and $B_{0,3}$ forms[16,19]. The difference in free energy between these two conformations in 1,6-anhydro-β-D-glucopyranose is 1.4 kcal/mol[19]. Intramolecular hydrogen bonding favours the chair form. Thus, 4-substitution precludes an HO-4···O-2 hydrogen bond, so that, for a solution of **1** in $(CD_3)_2SO$, the difference in free energy between the chair and boat forms is <1.4 kcal/mol and the glucose ring exists as a $\sim$1:1

TABLE I

^{1}H-N.m.r. chemical shifts (δ, p.p.m.) and relaxation times (s) for 1,6-anhydrolactose (**1**) and its hexa-acetate (**2**) at 30°

Proton	*Compound*			
	1(*D_2O*)		**1**[*$(CD_3)_2SO$*]	**2**(*$CDCl_3$*)
	δ	T_1	δ	δ
H-1	5.450	1.24	5.136	5.408
H-2	3.540	1.01	3.143	4.504
H-3	3.855	1.04	3.440	5.108
H-4	3.830	0.62	3.470	3.516
H-5	4.779	0.63	4.583	4.546
H-6*endo*	4.089	0.39	3.806	3.937
H-6*exo*	3.778	—	3.521	3.763
H-1′	4.525	0.45	4.263	4.757
H-2′	3.562	1.01	3.340	5.234
H-3′	3.655	0.61	3.281	4.987
H-4′	3.911	0.55	3.619	5.334
H-5′	3.679	0.45	3.358	3.944
H-6′a	3.783	—	3.480	4.109
H-6′b	3.741	—	3.480	4.005
HO-2			4.776	
HO-3			4.935	
HO-2′			4.839	
HO-3′			4.685	
HO-4′			4.352	
HO-6′			4.534	

mixture. A similar situation has been found for 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose[20]. The coupling constants for the galactopyranoid ring account for a major $^4C_1$ conformation for both **1** and **2** that was independent of the solvent, whereas the conformation of the hydroxymethyl group can be described as 70:30 and 50:50 equilibria of the *gt* and *tg* rotamers for solutions of **1** in D_2O and **2** in $CDCl_3$, respectively[21].

Conformations of the disaccharide. — The ^{13}C-n.m.r. chemical shift data and relaxation times of **1** and **2** are given in Table IV. The T_1 value of C-4′ is slightly smaller than that of the other carbon nuclei for **1** in D_2O and $(CD_3)_2SO$; therefore, the molecule has a preferred rotation axis, parallel to the C-4′–H-4′ bond[22]. Nevertheless, the corresponding average correlation times for the glucose and galactose residues are 0.08 and 0.09 ns, respectively, whereas that about the preferred C-4′–H-4′ bond is 0.10 ns. Therefore, the molecule tumbles almost isotropically in solution and the n.O.e. data can be used to estimate the conformational behaviour of **1**. On the other hand, the average correlation times for solutions in $(CD_3)_2SO$ are 0.16 and 0.20 ns for the glucose and galactose moieties, respectively, whereas **2** in solution in $CDCl_3$ tumbles isotropically with a τ_c value of 0.11 ns.

TABLE II

Coupling constants (Hz) for 1,6-anhydrolactose (**1**) and its hexa-acetate (**2**) at 30°

Coupling constant	*Compound*		
	1 *(D_2O)*	**1**[*(CD_3)$_2$SO*]	**2**(*$CDCl_3$*)
$J_{1,2}$	1.0	0.4	1.0
$J_{1,3}$	0.9	<0.4	1.1
$J_{2,3}$	1.0	3.9	1.2
$J_{2,4}$	0.9	<0.4	1.0
$J_{3,4}$	1.0	4.0	1.3
$J_{3,5}$	1.1	<0.4	1.1
$J_{4,5}$	1.0	0.5	1.0
$J_{5,6endo}$	1.0	0.5	0.6
$J_{5,6exo}$	5.9	5.4	5.1
$J_{6endo,6exo}$	−7.7	−7.1	−7.7
$J_{1',2'}$	7.6	7.6	7.9
$J_{2',3'}$	10.4	9.8	10.2
$J_{3',4'}$	3.5	3.3	3.5
$J_{4',5'}$	0.4	0.5	0.9
$J_{5',6'a}$	4.7	N.d.[a]	6.3
$J_{5',6'b}$	7.9	N.d.	6.6
$J_{6'a,6'b}$	−12.0	N.d.	−10.9
$J_{HO-2,2}$	—	7.0	—
$J_{HO-3,3}$	—	3.9	—
$J_{HO-2',2'}$	—	3.9	—
$J_{HO-3',3'}$	—	5.1	—
$J_{HO-4',4'}$	—	4.5	—
$J_{HO-6',6'}$	—	5.3, 5.3	—

[a] Not determined.

TABLE III

Torsion angles (°) and coupling constants (Hz) for the $^1C_4$ and $B_{O,3}$ conformations of the 1,6-anhydro-β-D-glucopyranose moiety in **1** according to MM2 calculations and the Altona equation, respectively

Protons	*Conformer*			
	$^1C_4$		$B_{O,3}$	
	Torsion angle	J	*Torsion angle*	J
H-1,2	58	2.9	109	1.2
H-2,3	−77	1.9	−162	6.4
H-3,4	81	1.6	153	5.2
H-4,5	−61	2.0	−91	1.0

TABLE IV

^{13}C-N.m.r. chemical shifts (δ, p.p.m.) and relaxation times[a] (s) for **1** and **2** at 30

Carbon atom	*Compound*					
	1 (D_2O)		**1**[(CD_3)$_2$SO]		**2** ($CDCl_3$)	
	δ	T_1	δ	T_1	δ	T_1
C-1	100.8	0.62	103.0	0.32	98.6	0.45
C-2	69.3	0.62	72.6	0.30	70.8	0.46
C-3	70.8	0.68	72.7	0.37	70.5	0.46
C-4	77.0	0.66	79.9	0.29	76.2	0.42
C-5	73.5	0.60	74.1	0.28	72.6	0.42
C-6	64.5	0.37	65.6	0.17	66.5	0.37
C-1′	101.5	0.59	102.2	0.27	100.9	0.45
C-2′	70.1	0.60	70.5	0.24	70.5	0.46
C-3′	71.9	0.60	73.3	0.22	71.3	0.45
C-4′	68.1	0.47	68.2	0.20	68.9	0.45
C-5′	74.7	0.56	75.5	0.28	72.6	0.42
C-6′	60.5	0.40	60.6	0.24	60.7	0.36

[a] Accurate to 5%.

When two hard-sphere energy maps that corresponded to the two allowed conformations[16,19,20], $^1C_4$ and $B_{O,3}$, of the glucose ring were computed for **1**, the general shape of these potential energy surfaces was similar. Table V shows the values of torsion angles and of relative steric energy for the stable conformers of **1**, obtained by single-point MM2 optimisation of the hard-sphere local minima. There were only slight variations of φ and ψ with the form of the glucose ring in conformers A–E and the dielectric constant used in the calculations. Three (A–C, Fig. 1) out of the five conformers are included in a region with $\Delta E < 3$ kcal/mol, which describes ~3% of the total potential energy surface. The predicted distribution of conformers, estimated from the relative energy values according to a Boltzmann distribution at 30°, are also given in Table IV. Although the energy values provided by the MM2 programme are only approximate, the predicted contribution of conformers that have a boat conformation of the glucopyranoid ring decreases when the bulk dielectric constant increases from 1 to 80 debyes, in accord with the experimental results. According to the relative energy values and ignoring entropic factors, the conformational behaviour of **1** involves a major conformer C (49–63%), with contributions of conformers A (7–10%) and B (39–27%). For $\varepsilon = 1$, 5% of conformer D would also be present. Although the n.m.r. parameters are time-averaged among all those corresponding to the states that contribute to the conformational equilibrium[6], n.m.r. spectroscopy can be used to distinguish the different geometries of these conformers[5] and to estimate their populations[15]. Thus, conformers A–C show short distances (see Table VI) between H-1′ and H-4, whereas B and C have additional close proximities of H-1′ and H-3, and of H-1′ and H-5, respectively; H-2′ is close to H-4 in conformer D, and close to H-3 in conformer E.

According to the calculated Boltzmann distributions, the average $r_{H\text{-}1',H\text{-}4}$, $r_{H\text{-}1',H\text{-}3}$, and $r_{H\text{-}1',H\text{-}5}$ distances should be 2.39, 3.73, and 2.74 Å, respectively, for $\varepsilon = 1$, and 2.43, 3.30, and 2.61 Å, for $\varepsilon = 80$, respectively. On the other hand, the H-2′–H-4 and H-2′–H-3 distances would be >4 Å. These predicted interatomic distances can be correlated with the experimental n.O.e.s. The ratios between the observed inter- and intra-residue n.O.e.s for **1** and **2** are given in Table VII, which also shows the corresponding average distances, estimated from the n.O.e. ratios, according to the r^{-6} dependence. The inter-residue H-1′–H-4, H-1′–H-3, and H-1′–H-5 n.O.e.s are observed for both **1** and **2**, although with different intensities, which indicate that the three predicted conformers contribute to the conformational equilibrium. The data for a solution of **1** in D_2O agree with a ~65:5:30 distribution of the conformers A–C, whereas those for a solution in $(CD_3)_2SO$ reflect ratios of ~30:50:20. The ratios of conformers A–C for **2** are ~60:15:25. The relaxation times have a similar dependence on the inter-proton distances[23]. The values for **1** (Table I) also indicate a minor contribution of conformer B for a solution in D_2O, since T_1 of H-3 is similar to that of H-2 and, therefore, has a similar disposition, surrounded only by H-2 and H-4, with little influence of H-1′. On the other hand, H-1′ is affected by the glucose ring, since its relaxation time is noticeably smaller than that of H-3′.

TABLE V

Relative steric energies and populations at 30° of the stable conformers of **1**[a]

Conformers	*Conformation of the 1,6-anhydro-D-glucose moiety*	*φ/ψ* (°)	*Dielectric constant* (ε)			
			1		*80*	
			ΔE (kcal/mol)	*Population (%)*	*ΔE (kcal/mol)*	*Population (%)*
A	$^1C_4$	−63/−137	1.11	5.8	1.08	9.4
	$B_{O,3}$	−72/−119	2.16	1.0	2.92	0.4
B	$^1C_4$	−108/−166	1.85	1.7	0.50	24.7
	$B_{O,3}$	−94/−168	0.00	37.1	1.83	2.7
C	$^1C_4$	−80/−83	0.02	35.9	0.00	57.1
	$B_{O,3}$	−87/−80	0.63	12.9	1.40	5.5
D	$^1C_4$	52/−107	3.53	0.1	3.40	0.2
	$B_{O,3}$	55/−107	1.15	5.4	4.67	0.0
E	$^1C_4$	17/−168	3.34	0.1	5.30	0.0
	$B_{O,3}$	32/−167	5.64	0.0	6.95	0.0

[a] Calculated by the MM2 programme, at dielectric constants (ε) of 1 and 80.

The chemical shifts for the H-1′ resonance in the spectra of **1** in solution in D_2O or $(CD_3)_2SO$ are 0.22 and 0.30 p.p.m., respectively, larger than that of the H-1 resonance of methyl β-D-galactopyranoside. The deshielding observed for solutions in methyl sulfoxide can be explained by an important contribution of conformer B, with the 1,6-anhydroglucose moiety in the boat form, which has a short H-1′···O-3 distance. However, no satisfactory explanation can be given for the smaller but still noticeable deshielding observed for solutions in water. The C-1′ resonances of **1** are shifted ~2 p.p.m. upfield in comparison with the data for methyl β-D-galactopyranoside [103.4 and 104.6 p.p.m. in D_2O and $(CD_3)_2SO$, respectively]. This fact indicates the proximity of C-1′ to C-3, O-3, or C-5, in agreement with the important proportions of conformers B or C. The observed $J_{C\text{-}4,H\text{-}1'}$ values for solutions of **1** in D_2O (4.7 ± 0.6 Hz) and **2** (5.0 ± 0.6 Hz) indicate similar average conformations around the glycosidic linkage[24,25].

Thus, the experimental data indicate similar average conformations for **1** and **2** in the solvents studied, which may be described satisfactorily by different contributions of conformers A–C (Figs. 1 and 2). These conformers account for ~3% of the total potential energy surface, which indicates that the conformation of each compound is fairly well defined. Conformer A and, partially, conformer C are favoured by the exo-anomeric effect[26]. Conformers A–C are shown in Fig. 1 and superpositions in Fig. 2. A similar distribution has been reported for hexa-*O*-acetyl-1,6-anhydro-β-cellobiose[27]. On changing the solvent from water to methyl sulfoxide, the average conformation of the 1,6-anhydroglucose moiety changes, although the overall shape of the disaccharide molecule is not modified substantially. There is no intramolecular hydrogen bonding between the pyranoid rings in **1**, in contrast with that observed between HO-3 and O-5′

TABLE VI

Relevant interatomic distances (< 4 Å) for the stable conformers of **1** (see Fig. 1)

Conformer	*Conformation of the 1,6-anhydro-D-glucose moiety*	*φ/ψ* (°)	*Distance (Å)*					
			H-1′,4	*H-1′,3*	*H-1′,5*	*H-2,4*	*H-2′,3*	*H-1′,O-3*
A	$^1C_4$	−63/−137	2.30	3.77	3.50		3.99	
	B_{O3}	−72/−119	2.25	–	3.15	–	–	
B	$^1C_4$	−108/−166	2.27	2.42	–	3.71		3.84
	B_{O3}	−94/−168	2.22	3.42		–	–	2.73
C	$^1C_4$	−80/−83	2.55	–	2.31	–	–	
	B_{O3}	−87/−80	2.44	–	2.27	–		
D	$^1C_4$	52/−107	3.56	–	3.70	2.33	3.13	–
	B_{O3}	55/−107	3.55	–	3.75	2.15		–
E	$^1C_4$	17/−168	3.68	3.92	–	3.51	1.84	
	B_{O3}	32/−167	3.45	–	–	3.72	2.26	

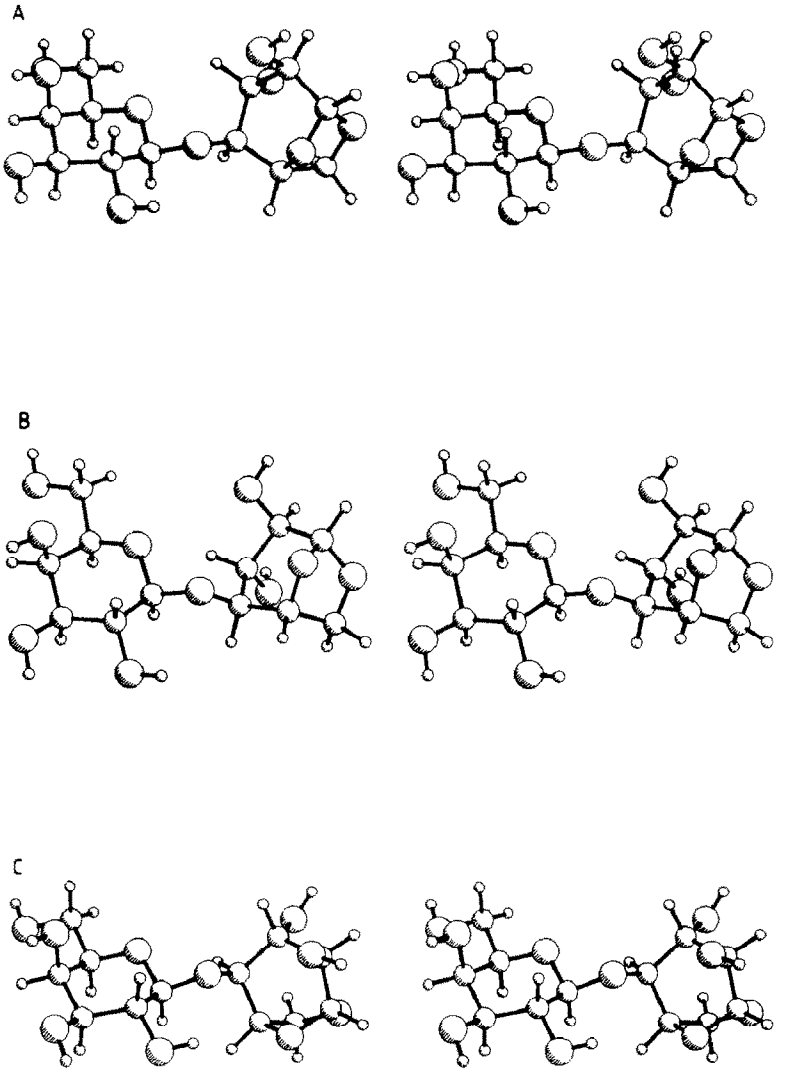

Fig. 1. Stereoscopic view of the conformations of **1** calculated using the MM2 programme: A, φ/ψ $-63°/-137°$; B, φ/ψ $-108°/-166°$; C, φ/ψ $-80°/-83°$.

TABLE VII

N.O.e.s and corresponding internuclear distances[a] for selected protons of **1** and **2** at 30°

Diagonal peak	*Cross peak*	*Compound*					
		1 (D_2O)		**1**[(CD_3)$_2$SO]		**2** ($CDCl_3$)	
		Observed intensity	*r* (Å)	*Observed intensity*	*r* (Å)	*Observed intensity*	*r* (Å)
H-3′	H-1′	10.1	2.5	8.4	2.5	11.7	2.5
H-5′	H-1′	10.0	2.5	8.3	2.5	11.6	2.5
H-4	H-1′	11.2	2.4	9.0	2.4	12.0	2.4
H-3	H-1′	1.6	3.3	3.0	2.9	5.0	3.1
H-5	H-1′	3.7	2.9	3.0	2.9	4.0	3.0
H-2′	H-1′	2.0	3.1	N.d.[b]		3.5	3.0

[a] Estimated on the basis of $r_{H_5'-H_6'} = 2.5$ Å. [b] Not determined.

Fig. 2. Stereoscopic view of a superposition of the conformers calculated according to the MM2 programme and supported by n.m.r. data: (*a*) conformers A and B, (*b*) conformers A and C.

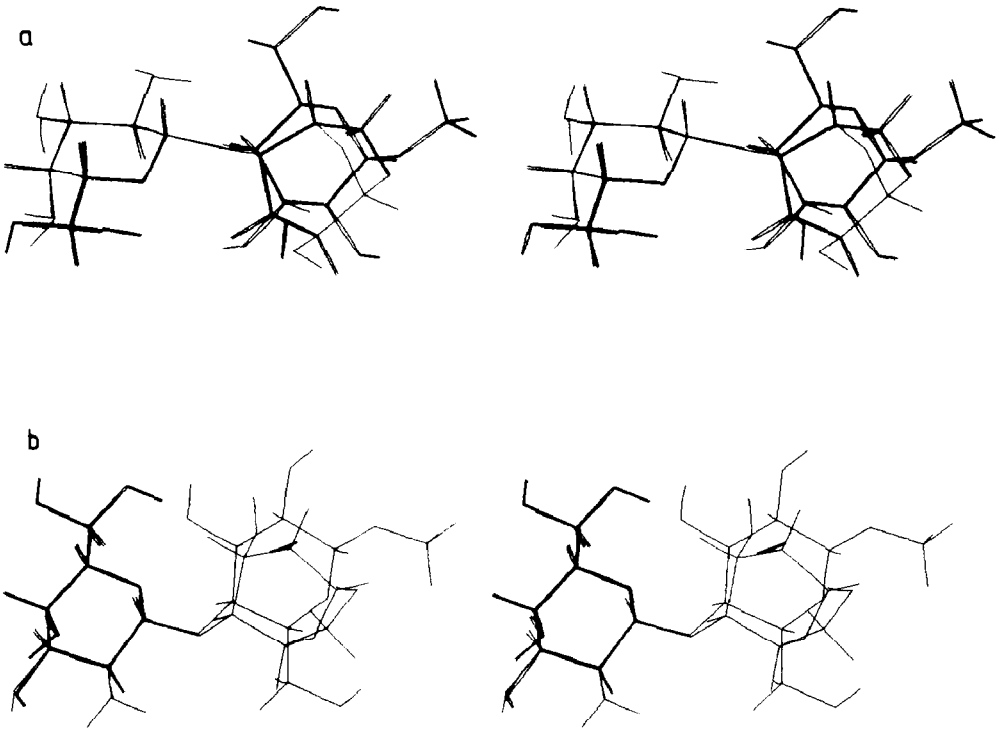

Fig. 3. Stereoscopic view of a superposition of the majore conformers of 1,6-anhydrolactose and methyl β-lactoside[2] calculated according to the MM2 programme: (*a*) conformers A, (*b*) conformers B.

in methyl β-lactoside in the solid state[28] and in solution[2,22]. However, in spite of the change of the conformation of the glucose moiety from $^4C_1$ in methyl β-lactoside to $^1C_4$ in 1,6-anhydrolactose, the overall shape of the molecule is not affected significantly. Fig. 3 shows a superposition of conformers A and B with the major conformers of methyl β-lactoside, showing H-3 of **1** in the same region of O-3 of methyl β-lactoside, which is hydrogen-bonded to O-5′. A similar arrangement has been invoked to render a polar region more lipophilic in character[29]. The C-5–H-5 bond in **1** occupies the place of the C-5–C-6 bond in methyl β-lactoside. Therefore, 1,6-anhydrolactose and methyl β-lactoside may have similar polar and non-polar regions in their interactions with ricin or other lectins.

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REFERENCES

1 S. Olsnes and A. Phil, in P. Cohen and S. Van Heyningen (Eds.), *The Molecular Actions of Toxins and Viruses*, Elsevier Biomedical Press, New York, 1982, pp. 52–105.
2 A. Rivera-Sagredo, D. Solís, T. Díaz-Mauriño, J. Jiménez-Barbero, and M. Martín-Lomas, *Eur. J. Biochem.*, submitted.
3 R. U. Lemieux, *Chem. Soc. Rev.*, 18 (1989) 347–374.
4 J. Kihlberg, S. J. Hultgren, S. Normark, and G. Magnusson, *J. Am. Chem. Soc.*, 111 (1989) 6364–6368.
5 R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto, and V. S. Rao, *Can. J. Chem.*, 58 (1980) 631–653.
6 D. A. Cumming and J. P. Carver, *Biochemistry*, 26 (1987) 6664–6676.
7 J. Breg, L. M. J. Kroon-Batenburg, G. Strecker, J. Montreuil, and J. F. G. Vliegenthart, *Eur. J. Biochem.*, 178 (1989) 727–739.
8 C. A. Bush, Z.-Y. Yan, and B. N. N. Rao, *J. Am. Chem. Soc.*, 108 (1986) 6168–6173.
9 I. Tvaroska and S. Perez, *Carbohydr. Res.*, 149 (1986) 389–410.
10 D. L. Hughes, J. J. Bergan, and E. J. J. Grabowski, *J. Org. Chem.*, 51 (1986) 2579–2585.
11 E. M. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, 65 (1943) 1848–1854.
12 S. Perez, These de Doctorat d'Etat, University of Grenoble, 1978.
13 U. Burkert and N. L. Allinger, *Molecular Mechanics*, ACS Monograph 177, 1982.
14 D. Neuhaus and M. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH, New York, 1989.
15 A. Imberty, V. Tran, and S. Perez, *J. Comput. Chem.*, 10 (1990) 205–216.
16 M. Budesinsky, I. Trnka, and M. Cerny, *Collect. Czech. Chem. Commun.*, 44 (1979) 1949–1964.
17 T. B. Grindley and R. Thangarasa, *Carbohydr. Res.*, 194 (1989) 296–299.
18 C. A. G. Haasnoot, F. A. A. M. de Leeuw, and C. Altona, *Tetrahedron*, 36 (1980) 2783–2792.
19 A. J. J. Straathof, A. van Estrik, A. P. G. Kieboom, J. M. A. Baas, and B. van de Graaf, *Carbohydr. Res.*, 194 (1989) 296–299.
20 T. Trnka, M. Cerny, M. Budesinsky, and J. Pacak, *Collect. Czech. Chem. Commun.*, 40 (1975) 3038–3045.
21 D. M. Mackie, A. Maradufu, and A. S. Perlin, *Carbohydr. Res.*, 150 (1986) 23–33.
22 M. L. Hayes, A. S. Serianni, and R. Barker, *Carbohydr. Res.*, 100 (1982) 87–101.
23 P. Dais and A. S. Perlin, *Adv. Carbohydr. Chem. Biochem.*, 45 (1987) 125–168.
24 I. Tvaroska, M. Hricovini, and E. Petrakova, *Carbohydr. Res.*, 189 (1989) 359–362.
25 B. Mulloy, T. A. Frenkiel, and D. B. Davies, *Carbohydr. Res.*, 184 (1988) 39–44.
26 J. P. Praly and R. U. Lemieux, *Can. J. Chem.*, 65 (1987) 213–225.
27 P. Dais, T. K. M. Shing, and A. S. Perlin, *J. Am. Chem. Soc.*, 106 (1984) 3082–3089.
28 K. Hirotsu and A. Shimada, *Bull. Chem. Soc. Jpn.*, 47 (1974) 1872–1879.
29 R. U. Lemieux, T. C. Wong, J. Liao, and E. A. Kabat, *Mol. Immunology*, 21 (1984) 751–759.