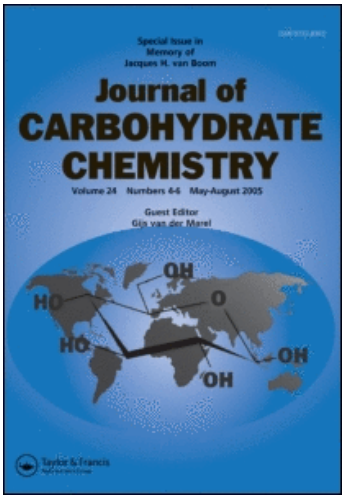

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THE SOLUTION CONFORMATION OF LACTAL AND ITS HEXA-*O*-ACETYL DERIVATIVE

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ABSTRACT

The conformation of lactal (**1**) in D_2O and DMSO-d_6 has been investigated by employing NMR techniques and molecular mechanics calculations. The glucal ring shows a $^4H_5$ conformation in deuterium oxide and DMSO-d_6. In contrast, the glucal ring of the hexa-*O*-acetyl derivative (**2**) in $CDCl_3$ can be described as an equilibrium between the $^4H_5$ and $^5H_4$ forms. The disaccharide **1** has a restricted flexibility with the ϕ angle oscillating about -105 ± 30° and the ψ angle varying about -145 ± 30°. Compound **2** shows a similar conformational behaviour.

INTRODUCTION

As a part of a project on the molecular recognition of synthetic lactose analogues by ricin, a cytotoxic plant lectin,[1] we have suggested the existence of a hydrophobic interaction between the lectin and the C-3 region of the disaccharide,[2] on the basis of a remarkable enhancement of the binding for the 3-deoxy analogue, and a six-fold

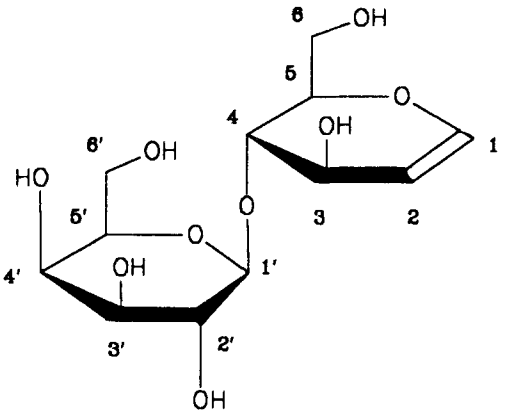


FIG. 1 View of **1** showing the atom numbering.

decreased affinity for the 3-*O*-methyl derivative. The affinity of 1,6-anhydrolactose was approximately two and a half times lower than that of methyl ß-lactoside, while the association constant for lactal was slightly better than that of the methyl ß-lactoside (0.023 μM^{-1} vs 0.013 μM^{-1}, respectively), suggesting the importance of the conformation of the D-glucopyranose moiety in the recognition and binding.

In a systematic study of the affinities of receptor-active analogues of oligosaccharides,[3,4] it is necessary to consider the conformation of the different analogues in order to assess the change in biological activity in terms of the size and shape of the hydrophilic and hydrophobic surfaces of disaccharide molecules.[5] The conformation of acetylated glycals has been previously studied,[6,7] indicating an equilibrium between the $^4H_5$ and $^5H_4$ half chair forms. The presence of these forms at the reducing end of the disaccharide may alter the global shape of the polar and nonpolar regions of the molecule and affect the process of recognition and binding.

We now report on the solution conformation of lactal (**1**) based upon NMR data and results from molecular mechanics calculations.[8-11] Since methyl sulfoxide has been presumed to model the behaviour of protein surfaces in a satisfactory way for a number of recognition processes,[4,12] the NMR experiments have been performed in D_2O and DMSO-d_6. The conformation of the hexa-*O*-acetyl derivative (**2**) has also been studied.

TABLE I. Proton-Proton Torsion Angles and Coupling Constants for the $^4H_5$ and $^5H_4$ Conformations of the Glucopyranoid Moiety of **1** According to MM2 Calculations and the Altona Equation, respectively.

PROTON PAIR	CONFORMER			
	$^4H_5$		$^5H_4$	
	Torsion	$^3J_{H,H}$	Torsion	$^3J_{H,H}$
H-2 / H-3	-74	2.3	-44	5.7
H-3 / H-4	165	6.8	79	1.8
H-4 / H-5	175	8.5	-67	0.8

RESULTS AND DISCUSSION

Monosaccharide conformations. The expected values of $^3J_{H,H}$, calculated by applying Altona's equation[18] to vicinal proton torsion angles obtained for the two possible forms of the glucal ring of **1** by MM2 calculations are given in Table I. The ^{1}H NMR parameters for **1** in D_2O and DMSO-d_6, and for **2** in $CDCl_3$ are given in Tables II and III. The observed values for **1** in both solvents account for a major $^4H_5$ conformation of the glucal ring. In contrast, the values for **2** in $CDCl_3$ are in between those expected for the $^4H_5$ and $^5H_4$ conformations, in agreement with a 60:40 equilibrium between $^4H_5$ and $^5H_4$ forms, respectively. A similar distribution has been published for tri-*O*-acetyl-D-glucal.[6,7] Although the steric energy values provided by the MM2 programme are only approximated, the predicted distribution of conformers for $\varepsilon = 1$ is ca. 70:30 between the $^4H_5$ and $^5H_4$ forms, respectively, in agreement with the experimental results for **2** (see Table IV). On the other hand, the steric energy values for $\varepsilon = 78$ indicated a predominant contribution of the $^4H_5$ conformer, also in agreement with the NMR results for **1** in D_2O and DMSO-d_6.

TABLE II. ^{1}H NMR Chemical Shift (δ, ppm) for Lactal (**1**) and its Acetate (**2**) in Several Solvents at 30 °C.

PROTON	COMPOUND		
	1 [a]	**1** [b]	**2** [c]
H-1	6.452	6.333	6.413
H-2	4.824	4.616	4.842
H-3	4.383	4.108	5.408
H-4	3.850	3.52	3.999
H-5	4.026	3.71-3.74	4.18
H-6a	3.926	3.71-3.74	4.437
H-6b	3.926	3.71-3.74	4.200
H-1'	4.526	4.255	4.665
H-2'	3.554	3.32	5.194
H-3'	3.668	3.31	5.009
H-4'	3.926	3.601	5.367
H-5'	3.72-3.77	3.44	3.910
H-6'a	3.72-3.77	3.49-3.51	4.164
H-6'b	3.72-3.77	3.49-3.51	4.084
OH-3	---	4.659	---
OH-6	---	4.602	---
OH-2'	---	5.011	---
OH-3'	---	4.722	---
OH-4'	---	4.440	---
OH-6'	---	4.617	---

a. D_2O ; b. DMSO-d_6 ; c. $CDCl_3$

The $^3J_{H5,H6}$ values support a >70% population of the *gg* rotamer of the glucal unit of **1** in D_2O, and a ca. 1:1 equilibrium between the *gg* and *gt* rotamers of **2**.[7] On the same basis, the distribution of rotamers for the galactose moiety seems to be ca. 60:40 between the *gt* and *tg* rotamers, respectively.[19]

TABLE III. Coupling Constants (Hz) for Lactal (**1**) and its Acetate (**2**) in Several Solvents at 30 °C.

COUPLING CONSTANT	COMPOUND		
	1 [a]	**1** [b]	**2** [c]
$J_{1,2}$	6.1	6.1	6.1
$J_{1,3}$	1.6	1.7	1.2
$J_{2,3}$	2.6	2.5	3.4
$J_{3,4}$	6.5	6.6	5.4
$J_{4,5}$	9.1	N.D.[d]	7.4
$J_{5,6a}$	3.7	N.D.	2.6
$J_{5,6b}$	3.7	N.D.	6.1
$J_{6a,6b}$	N.D.	N.D.	N.D.
$J_{1',2'}$	7.7	7.5	7.9
$J_{2',3'}$	9.9	N.D.	10.5
$J_{3',4'}$	3.4	3.2	3.4
$J_{4',5'}$	0.9	0.7	1.1
$J_{5',6'a}$	N.D.	6.5	6.4
$J_{5',6'b}$	N.D.	6.5	7.2
$J_{6'a,6'b}$	N.D.	N.D.	-11.6
J_{OH-3}	---	2.8	---
J_{OH-6}	---	5.2	---
$J_{OH-2'}$	---	4.3	---
$J_{OH-3'}$	---	5.3	---
$J_{OH-4'}$	---	4.6	---
$J_{OH-6'}$	---	5.8	---

a, D_2O ; b, DMSO-d_6 ; c, $CDCl_3$; d, not determined

Disaccharide conformations. The ^{13}C NMR chemical shifts and T_1 relaxation times of **1** and **2** are given in Table V. It can be observed that T_1 of C-4' is slightly smaller than that of the other carbon nuclei for both **1** and **2**, and, therefore, both molecules seem to have a preferred rotation axis parallel to the C-4'-H-4' bond.[20] On

TABLE IV. Relative Steric Energy Values and Populations[a] at 30 °C of the Stable Conformers of **1**, Calculated by the MM2 Programme.

CONFORMER	GLUCAL CONFORM-ATION	ϕ / ψ	RELATIVE STERIC ENERGY OF THE ROTAMERS (Kcal/mol)							
			gg/gt		*gg/tg*		*gt/gt*		*gt/tg*	
			ΔE	Pop. (%)	ΔE	Pop. (%)	ΔE	Pop. (%)	ΔE	Pop. (%)
A	$^4H_5$	-74/-120	2.76	0.2	0.20	16.1	0.82	5.7	2.10	0.7
	$^5H_4$	-72/-119	2.11	0.6	2.24	0.5	0.39	11.7	0.73	6.6
B	$^4H_5$	-99/-172	1.64	1.4	0.00	22.5	0.42	11.1	1.15	3.3
	$^5H_4$	-99/-173	2.34	0.4	2.64	0.3	0.52	9.4	1.22	2.9
C	$^4H_5$	-134/-151	1.45	2.0	1.41	2.1	1.32	2.5	b	---
	$^5H_4$	b	b	---	b	---	b	---	b	---
D	$^4H_5$	60/-121	4.53	---	3.83	---	3.50	---	5.36	---
	$^5H_4$	55/-115	4.47	---	4.65	---	4.14	---	4.04	---
E	$^4H_5$	-91/61	5.37	---	5.66	---	3.19	---	6.76	---
	$^5H_4$	-92/62	3.68	---	3.73	---	4.12	---	3.75	---

a. Ignoring entropic effects ; b. It is not a local minimum, but converges to conformer B.

the other hand, the small values for C-1 may be due to a different C-H bond length. The C-6 relaxation times reflect the additional degree of freedom due to the rotation of the hydroxymethyl groups. The correlation times (τ_c) for the glucal moiety (0.07, 0.16, and 0.11 ns for **1** in D_2O, in DMSO-d_6, and **2**, respectively) are always smaller than those for the galactose unit (0.08, 0.18, and 0.12 ns, respectively). These values could indicate a higher degree of mobility for the glucal residue than for the galactose unit. Nevertheless, the range of τ_c values for each compound is very narrow

TABLE V. ^{13}C NMR Chemical Shift (δ, ppm) and Relaxation Times (s) for **1** and **2** in Several Solvents at 30 °C.

CARBON ATOM	COMPOUND					
	1 [a]		**1** [b]		**2** [c]	
	δ	T_1	δ	T_1	δ	T_1
C-1	143.5	0.51	143.5	0.23	145.4	0.43
C-2	101.4	0.69	103.1	0.31	99.0	0.49
C-3	66.8	0.71	67.0	0.33	68.8	0.43
C-4	77.1	0.71	79.3	0.33	74.7	0.46
C-5	76.3	0.69	77.3	0.36	74.2	0.48
C-6	59.2	0.37	60.2	0.18	61.8	0.26
C-1'	102.5	0.64	104.0	0.27	101.0	0.43
C-2'	70.5	0.62	70.8	0.26	78.8	0.43
C-3'	72.1	0.64	73.4	0.31	70.8	0.43
C-4'	68.1	0.53	68.3	0.25	66.8	0.35
C-5'	74.8	0.59	75.6	0.28	70.7	0.42
C-6'	70.5	0.41	60.6	0.22	61.1	0.30

a. D_2O ; b. DMSO-d_6 ; c. $CDCl_3$.

and it can be assumed that **1** and **2** tumble almost isotropically in solution, and the NOE data can be used for estimating the conformational behaviour of **1** and **2**.

Two different potential energy maps, corresponding to the two allowed conformations, $^4H_5$ and $^5H_4$, of the glucal ring were computed for **1** (Figure 2). Table IV 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 potential energy maps. For every stable conformer (A-E) there are only slight variations of ϕ and ψ torsion angles with the form of the glucal ring and the dielectric constant used in the calculations. Three out of the five conformers (A, B, and C) are included

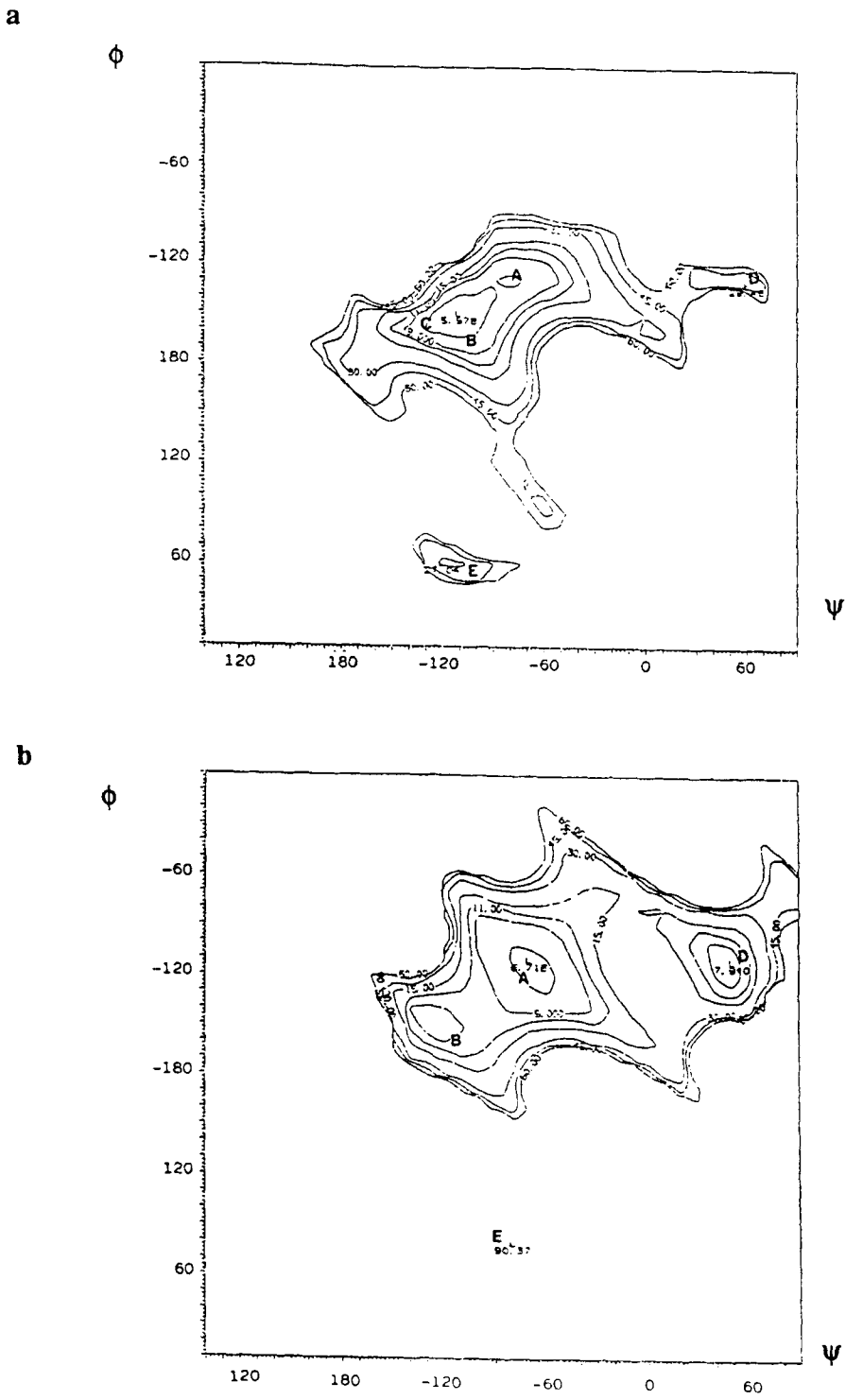
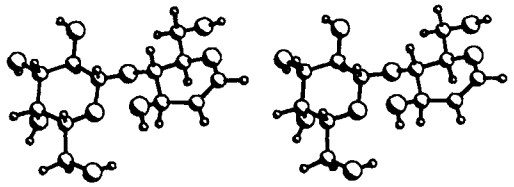
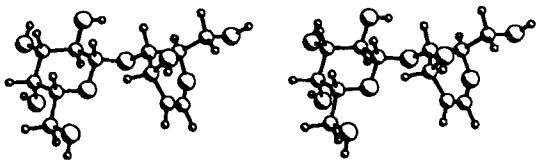


FIG. 2 Potential energy maps for the two allowed conformations of the glucal ring: a) $^{4}H_{5}$, b) $^{5}H_{4}$

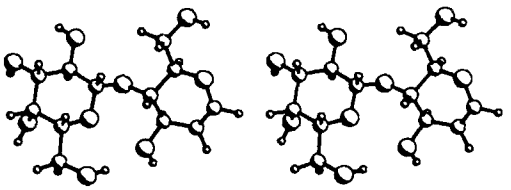


Conformer A ($^4H_5$)

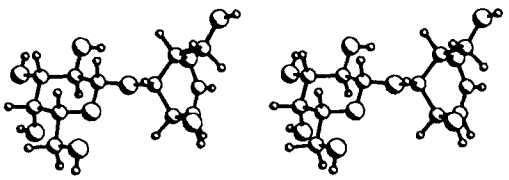


Conformer A ($^5H_4$)

FIG. 3a Stereoscopic view of conformers A

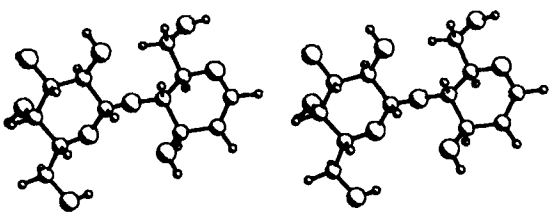


Conformer B ($^4H_5$)



Conformer B ($^5H_4$)

FIG. 3b Stereoscopic view of conformers B



Conformer C ($^4H_5$)

FIG. 3c Stereoscopic view of conformer C

TABLE VI. Relevant Interatomic Distances for the Stable Conformers of **1**.

CONFORMER	GLUCAL CONFORMATION	ϕ / ψ	DISTANCE								
			O-2'/H-6	H-1'/H-6	H-1'/H-4	H-1'/H-3	H-2'/H-4	H-1'/O-3	H-1'/O-6	O-2'/O-6	O-5'/O-3
A	$^4H_5$	-74/-120	2.80[b]	2.79[b,c]	2.25	---	---	---	2.50[b]	---	2.90
	$^5H_4$	-72/-119	---	---	2.25	---	---	---	---	---	---
B	$^4H_5$	-99/-172	3.79[b]	---	2.28	---	---	2.53	---	3.74[b]	2.69
	$^5H_4$	-99/-173	---	---	2.29	2.44	3.73	3.54	---	---	---
C	$^4H_5$	-134/-151	3.21[b,c]	---	2.25	---	---	2.48	---	2.77[b]	3.49
	$^5H_4$	a	a	a	a	a	a	a	a	a	a
D	$^4H_5$	60/-121	---	---	3.56	---	2.22	2.54[d]	---	---	---
	$^5H_4$	55/-115	---	---	---	---	2.22	---	---	---	---
E	$^4H_5$	-91/61	---	---	---	2.04	---	---	---	---	---
	$^5H_4$	-92/62	---	---	---	---	---	---	---	---	---

a. It is not a local minimum, but converges to conformer B ; b. gauche-gauche rotamer ; c. gauche-trans rotamer ; d. O-3/H-2' distance

in a region with $\Delta E < 3$ Kcal/mol, which describes ca. 3% of the complete potential energy surface. The predicted distribution of conformers, estimated from the relative energy values ($\varepsilon = 1$) according to a Boltzmann distribution at 30 °C are also given in Table IV. For $\varepsilon = 78$, the contribution of $^5H_4$ conformers is negligible, and the conformational behaviour of **1** could be described as consisting of a major conformer B (58%), with contributions of conformer A (37%) and C (5%). Although NMR parameters are time-averaged among all the corresponding to the states contributing to the conformational equilibrium,[8] NMR spectroscopy can be used to distinguish among the different geometries of these conformers[5] and to estimate their different populations.[21] Thus, conformers A, B, and C show short distances (see Table VI) between H-1' and H-4, while conformer A shows additional spatial proximity between H-1' and the H-6 protons; H-1' stands close to H-3 in conformer E, and H-2' is close to H-4 in conformer D.

According to the calculated Boltzmann distribution, the averaged $r_{H\text{-}1'\text{-}H\text{-}4}$ distance should be 2.27 Å, while $r_{H\text{-}1'\text{-}H\text{-}6}$ would range between 3.0 to 3.5 Å. On the other hand, both H-2'-H-4 and H-1'-H-3 would be higher than 4 Å. The above predicted interatomic distances can be correlated with the experimental NOE data.

The ratios between the observed interresidue to intraresidue NOE values for **1** and **2** are given in Table VII, which also shows the corresponding average distances, estimated from these NOE ratios, according to the r^{-6} dependence. The strong NOE between H-1' and H-4, and the absence of NOE between H-1' and H-3 or H-2' and H-4, support a conformational equilibrium in the region of conformers A, B, and C. The NOE between H-1' and one H-6 proton is observable for **1** in D_2O and DMSO-d_6, indicating the contribution of conformer A. The lack of other NOE cross-peaks does not allow us at this stage to distinguish among these three conformers. The NOE values for **2** in $CDCl_3$ are very similar, excepting that observed between H-1' and H-3. Since the glucal ring of **2** exists in an equilibrium between the $^4H_5$ and $^5H_4$ forms, this NOE seems to indicate an important contribution of conformer B, with the glucal moiety as $^5H_4$.

The chemical shift values for H-1' in the spectra of **1** in D_2O and DMSO-d_6 are 0.22 and 0.29 ppm, respectively, larger than that of the anomeric proton of the parent compound, methyl ß-D-galactopyranoside. This deshielding of H-1' may be caused by either O-3, in the case of conformers B and C, or O-6 in the case of the *gg* rotamer of conformer A.

The temperature dependence of hydroxylic protons of lactal in DMSO-d_6 is shown in Table VIII. It can be observed that the variation of chemical shift of the HO-3 proton is much smaller than those of the other protons.[22] This fact can be explained by the existence of HO-3 intramolecularly hydrogen bonded to O-5'. There is a short distance between these two oxygen atoms in conformers A and B.

The observed values of chemical shift for C-1' in the ^{13}C NMR spectra of **1** in both solvents are quite similar to those observed for methyl ß-D-galacto- pyranoside ($\Delta\delta$ = -0.9 and -0.6 ppm, in D_2O and DMSO-d_6, respectively), although the small upfield shift may be taken as indication of proximity of O-3 or C-6 to C-1'.

In conclusion, the experimental data are in agreement with a similar average conformation for **1** in D_2O and DMSO-d_6, which may be satisfactorily described by

TABLE VII. NOE Values and Corresponding Internuclear Distances Between Selected Protons of **1** and **2** in Several Solvents at 30 °C.

DIAGONAL PEAK	CROSS PEAK	COMPOUND					
		1[a]		**1**[b]		**2**[c]	
		Observed Intensity	r[d] (Å)	Observed Intensity	r[d] (Å)	Observed Intensity	r[d] (Å)
H-3'	H-1'	10.0	2.5	8.5	2.5	11.7	2.5
H-5'	H-1'	10.1	2.5	8.6	2.5	11.6	2.5
H-4	H-1'	13.2	2.3	10.1	2.4	13.2	2.4
H-3	H-1'	---	---	---	---	2.2	3.1
H-6	H-1'	1.6[e]	3.3	1.4[e]	3.3	1.5	3.4
H-2'	H-1'	2.0	3.1	---	---	2.2	3.1

a, in D_2O solution ; b. in DMSO-d_6 ; c, in $CDCl_3$; d. estimated distances considering $r_{H\text{-}1',H\text{-}3'}$ = 2.5 Å ; e, overlapped signal, but only this effect is possible.

TABLE VIII. Dependence of Chemical Shifts (δ, ppm) with Temperature of Hydroxylic Protons of **1** in DMSO-d_6 Solution.

PROTON	TEMPERATURE (°C)			
	30	50	70	90
HO-3	4.66	4.63	4.57	4.51
HO-6	4.60	4.50	4.42	4.33
HO-2'	5.01	4.91	4.80	4.69
HO-3'	4.72	4.59	4.47	4.35
HO-4'	4.44	4.34	4.23	4.13
HO-6'	4.62	4.52	4.44	4.34

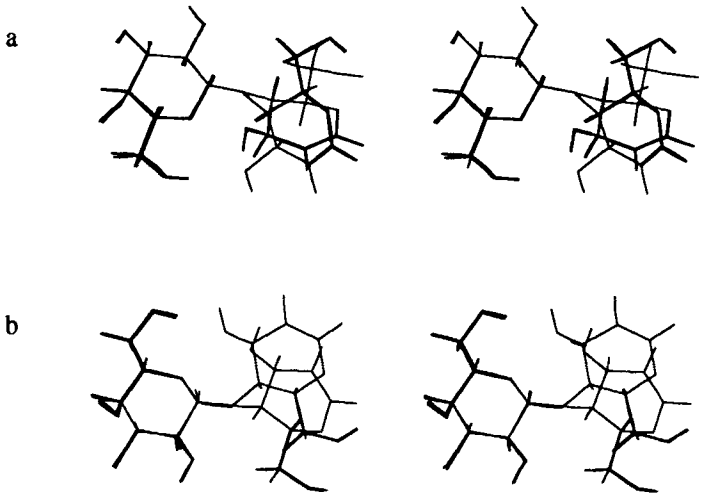


FIG. 4 Stereoscopic superimposition drawings of conformers: a) A and B, b) A and C

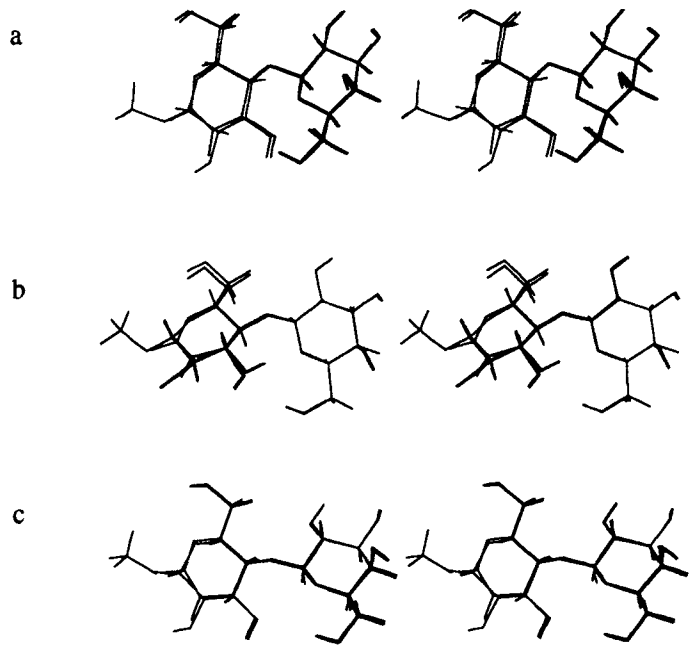


FIG. 5 Stereoscopic superimposition drawings of conformers of ß-methyl lactoside and lactal: a) A, b) B, c) C

conformers A and B, with certain contribution of conformer C. The glucal ring adopts a $^4H_5$ conformation in both solvents. The hexa-*O*-acetyl derivative shows a similar distribution of conformers, although the glucal ring adopts the $^5H_4$ conformation to the extent of ca. 30%, mainly in conformer B. Conformer A is favored by the exo-anomeric effect.[23] These conformers account for ca. 3% of the total potential energy surface, indicating that the conformation of the disaccharide is fairly well defined. A view of conformers A, B, and C is shown in Figure 3, while Figure 4 presents superimposition drawings of conformers A and B, and A and C, respectively. The global shape of **1** resembles that of methyl ß-lactoside, both in solution[2,20] and in the solid state.[24] A superimposition of conformers A, B and C with their corresponding conformers of methyl ß-lactoside is shown in Figure 5. In fact, both molecules are stabilised by intramolecular hydrogen bonding between O-5' and O-3, similar to that arrangement which has been previously invoked to render a polar region more lipophilic in character.[25] Therefore, **1** and methyl ß-lactoside may display similar polar and non polar regions to interact with ricin or other lectins.

EXPERIMENTAL

Molecular Modeling. Potential energy surfaces calculations were performed using the PFOS programme.[13] The constituent monosaccharides were assumed as rigid entities and a value of 117° was given to the glycosidic bond angle, leaving as variables only the torsion angles ϕ and ψ. ϕ Angle is defined by O-5'-C-1'-O-1'-C-4 and ψ by C-1'-O-1'-C-4-C-5. The coordinates for glucal (both $^4H_5$ and $^5H_4$ half chair conformations) and galactose ($^4C_1$ form) residues were taken from a data bank with MM2[14] optimised structures. Four different orientations of the lateral chain were assumed for **1**, i.e. *gg* and *gt* for the glucal residue and *gt* and *tg* for the galactose moiety.[15] The first letter refers to the O-5-C-5-C-6-O-6 torsion angle and the second one to the C-4-C-5-C-6-O-6 torsion angle.

These orientations lead to very similar conformational hard-sphere maps in terms of ϕ and ψ, using a 10° grid, for **1**. After lone pairs were added to oxygen atoms, the local minima of these maps were optimised through molecular mechanics using

the MM2 programm,[14] modified for carbohydrates using the acetal-segment parameters proposed by Jeffrey and Taylor.[16] Two different dielectric constants $\varepsilon = 1$ and $\varepsilon = 78$ were used. The steric energy differences ($\varepsilon = 1$) for the stable conformers of **1** calculated by this methodology are given in Table IV. The corresponding values for $\varepsilon = 78$ were at least 2 Kcal/mol higher for the $^5H_4$ conformers than for the $^4H_5$ forms. These values of ε gave relative steric energies which satisfactorily fit the experimental data.

NMR Spectroscopy. 300 MHz ^{1}H NMR experiments were performed with a Varian XL-300 spectrometer at 30 °C. Compound **1** (ca. 20 mg) was treated twice with D_2O, the sample was lyophilized and then dissolved in 0.5 mL 99.96% D_2O or 99.9% $(CD_3)_2SO$, and degassed in a NMR tube under argon. In separated experiments, **1** and **2** were directly dissolved in 99.9% $(CD_3)_2SO$ and $CDCl_3$, respectively, and degassed. Chemical shifts (δ, ppm) were measured by reference to internal residual HDO (δ 4.710 ppm) or tetramethylsilane (δ 0.000 ppm), depending on the solvent used. Double-quantum-filtered phase-sensitive COSY experiments were performed using the pulse sequence 90°-t_1-90°-90°-acq. RELAY experiments were performed using a mixing-time of 30 ms. A 512*1K data matrix was obtained, which was zero-filled prior to Fourier transformation. The first order values of chemical shifts and coupling constants were used as input parameters for the calculation of the 1D-spectrum, using the PANIC programme (Bruker software). 2D-NOESY experiments were performed in the phase sensitive mode by using the pulse sequence 90°-t_1-90°-tm-90°-acq., using mixing times of 0.5 and 1.0 s and relaxation delay of 3 s. The cross-peak and diagonal-peak volumes were obtained by using standard Varian software. The estimated error is ±10%. Interatomic distances were estimated from NOE ratios, since the magnitude of the NOE is proportional to the inverse sixth power of the internuclear distance.[17] Thus, considering the observed NOE values for a pair of protons at a known fixed distance (i.e., 2.5 Å for $r_{H\text{-}1'\text{-}H\text{-}3'}$ in the galactopyranoid ring), it is possible to correlate the NOE value for a given proton on the glucopyranoid ring with average distance $\langle r^{-6}\rangle^{-1/6}$. 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 $\langle r^{-6}\rangle^{-1/6}$ distances expected for a Boltzmann distribution of the possible conformations obtained through molecular mechanics.[14]

50 MHz ^{13}C NMR experiments were performed with a Bruker AM-200 spectrometer equipped with a dual probe. Chemical shifts are expressed in ppm relative to external acetone at δ = 29.8 ppm or $CDCl_3$ at 77.0 ppm. The chemical shifts of **1** in D_2O or DMSO-d_6 only varied ±0.1 ppm between 30 °C and 80 °C. Heteronuclear correlation with F1-decoupling and COLOC experiments 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 seven delays were used in each T_1 determination. The estimated error is ±5%.

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