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NUCLEAR OVERHAUSER EFFECTS AND CONFORMATIONS OF BRANCHED TRISACCHARIDE METHYL β-GLYCOSIDES THAT CONTAIN A 3,4-DISUBSTITUTED GALACTOSE RESIDUE

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ABSTRACT

N.O.e. data after pre-irradiation of the anomeric protons and the $^3J_{C,H}$ values associated with the glycosidic linkages for a series of methyl β-glycosides of trisaccharides that contain 3,4-disubstituted galactose residues have been measured. On the basis of the experimental results and theoretical calculations, it was shown that one preferred conformer (<90%) was present for each trisaccharide derivative.

INTRODUCTION

Branched oligo- and poly-saccharides have a widespread occurrence in Nature and their structures are closely associated with conformational features of the branch points. Non-bonded monosaccharide residues in close proximity to each other should limit the number of possible conformations, Moreover, the resonances in the n.m.r. spectra of branched oligo- and poly-saccharides exhibit chemical shifts which differ from those expected from the data for the corresponding disaccharides.

We now report the conformational analysis of methyl β-glycosides of branched trisaccharides that contain a 3,4-disubstituted D-galactose residue. The compounds had the general structure A-(1→4)[B-(1→3)]-β-D-Gal-OMe, namely, **1** A = β-D-Glc, B = α-D-Man; **2** A = β-D-Glc, B = α-L-Rha; **3** A = α-D-Glc, B = α-L-Rha; and **4** A = α-D-Glc, B = α-D-Man.

The 3,4-substituents in the galactose moieties in **1–4** occupy axial and equatorial positions. Conformational analysis of glycosides related to the disaccharide units in **1–4** has been described[1,2].

The angles of rotation around the C-1′–O and O–C-4 bonds of the Glc-(1→4)-Gal units in **1–4** are designated conventionally as φ_1 and ψ_1, respectively (see **5**). The primed numbers refer to the Glc residue and the double-primed numbers to the residue B. The angles of rotation around the C-1″–O and O–C-3 bonds of Rha-(1→3)-Gal or Man-(1→3)-Gal units are designated φ_2 and ψ_2. φ_1, ψ_1, φ_2, and ψ_2 are zero for *cis*-orientation of the corresponding C–H and C–O bonds and

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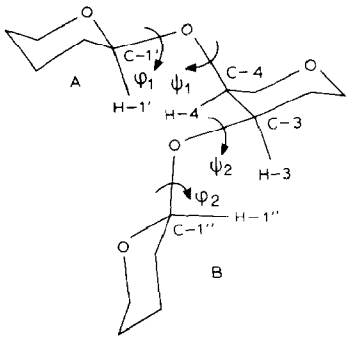

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positive when the rotation is clockwise viewing from C-1′ or C-1″. Three conformers for the hydroxymethyl group of the galactose residue[1] (*gt*, *tg*, and *gg*) are taken into account.

Most significant for the conformational analysis of carbohydrates in solution are the n.O.e. data from the ^{1}H-n.m.r. spectra and $^3J_{C,H}$ values for the H–C–O–C fragments, associated with the glycosidic linkages. Therefore, a detailed study of ^{1}H- and ^{13}C-n.m.r. spectra of **1–4** was carried out (see Experimental).

RESULTS AND DISCUSSION

The n.O.e.'s caused by pre-irradiation of H-1′ and H-1″ of residues A and B in **1–4** are given in Table I. The ^{1}H-n.m.r. spectra were measured on solutions in D_2O, and the n.O.e. parameters define the conformations. The data in Table I show that n.O.e.'s were observed not only for the resonances of protons related to the pre-irradiated monosaccharide or residue linked thereto but also for those of protons of the other monosaccharide residue (*e.g.*, B after pre-irradiation of H-1′ of A).

For purposes of comparison with the n.O.e. values for the disaccharide units in **1–4**, the corresponding data[1] for disaccharide glycosides are also included in Table II.

The $^3J_{C,H}$ values for the fragments H-1′–C-1′–O–C-4 (J^{φ_1}), C-1′–O–C-4–H-4 (J^{ψ_1}), H-1″–C-1″–O–C-3 (J^{φ_2}), and C-1″–O–C-3–H-3 (J^{ψ_2}) are presented in Table III.

Calculation of the optimal conformations of **1–4** was based on minimization of the potential energies of the molecules by variation of φ_1, ψ_1, φ_2, and ψ_2, using, as initial approximations, the data on the optimal conformers for the corresponding disaccharide glycosides (~3 for each compound). Only one low-energy conformer was found for each of **1–4**.

After determination of the lowest-energy conformer of the trisaccharide glycoside, the cross-section was drawn of its potential surface (conformational map) φ_1–ψ_1 (with φ_2 and ψ_2 at their optimal values) and φ_2–ψ_2 (with φ_1 and ψ_1 at their optimal values). Such conformational maps demonstrate the range of admissible values of φ and ψ in the trisaccharide glycoside being studied.

TABLE I

N.O.E (%) OBSERVED ON PRE-IRRADIATION OF ANOMERIC PROTONS OF **1–4**

Glycoside	*Irradiation of H-1'*		*Irradiation of H-1"*	
	Observed n.O.e.		*Observed n.O.e.*	
β-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**)	H-2'	2.8 (0.5)[a]	H-2"	6.0 (1.1)
	H-3',5'	16.4 (2.8)	H-3	5.5 (1)
	H-4	5.9 (1)	H-4	5.9 (1.1)
	H-1"	3.0 (0.5)	H-1'	3.2 (0.6)
	H-2"	3.4 (0.6)		
β-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**2**)	H-2'	1.9 (0.4)	H-2"	5.6 (1.1)
	H-3',5'	10.2 (2.1)	H-3	5.0 (1)
	H-4	4.7 (1)		
	H-3"	3.0 (0.6)		
	H-5"	3.0 (0.6)		
α-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**3**)	H-2'	7.6 (1)	H-2"	9.8 (1.3)
	H-4	7.6 (1)	H-3	7.7 (1)
	H-6,6	1.6 (0.2)		
	H-5"	1.7 (0.2)		
α-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**4**)	H-2'	9.0 (1.6)	H-2"	4.0 (1.2)
	H-4	5.5 (1)	H-3	3.4 (1)
	H-6,6	6.3 (1.1)	H-4	3.4 (1.0)
			H-1'	0.5 (0.15)
			H-5'	1.0 (0.3)

[a]The ratios of the n.O.e. value to that for the proton bonded with the carbon atom forming the glycoside linkage are given in brackets.

TABLE II

N.O.E (%) OBSERVED ON PRE-IRRADIATION OF ANOMERIC PROTONS OF THE DISACCHARIDE GLYCOSIDES

Glycoside	*Observed n.O.e.*			
	H-3	*H-4*	*H-2'*	
α-D-Man-(1→3)-β-D-Gal-OMe	3.8 (1)[a]	8.1 (2.1)	7.2 (1.9)	
α-L-Rha-(1→3)-β-D-Gal-OMe	6.6 (1)	0.6 (0.1)	5.2 (0.8)	
	H-4	*H-6,6*	*H-2'*	*H-3'+H-5'*
β-D-Glc-(1→4)-β-D-Gal-OMe	6.1 (1)		2.8 (0.5)	10.3
α-D-Glc-(1→4)-β-D-Gal-OMe	8.4 (1)	3.7 (0.4)	8.0 (1.0)	

[a]The relative values of the n.O.e. are given in brackets.

The parametrisation of the force field deserves comment. Conformational analysis of (1→3)- and (1→4)-linked glycosylgalactosides[1] showed that the application of atom–atom potential functions[3] afforded the best fit with the experimental data. Therefore, these functions were used initially for the conformational analysis of **1–4**.

TABLE III

EXPERIMENTAL $^3J_{C,H}$ VALUES (Hz) FOR **1–4**

Glycoside	J_{φ_1}	J_{ψ_1}	J_{φ_2}	J_{ψ_2}
1		5.1		3.4
2	4.1	5.3	3.7	5.0
3		4.8		4.8
4	3.9	5.0	3.1	5.5

However, such calculations could not reproduce quantitatively the n.O.e.'s in **1–4**. The n.O.e. values calculated for protons of the non-bonded residues Glc and Man (or Rha) were overestimated significantly; this resulted from the use of Scott and Scheraga functions with the lowest admissible value (1.2 Å) of van der Waals radius of hydrogen atom. Therefore, atom–atom potentials for the calculation of the interactions of glycosidically linked carbohydrate moieties are inadequate for functions which should be taken into account in calculations of the interactions of the non-bonded moieties that are similar to ordinary intermolecular interactions. Therefore, it seemed expedient to apply as such functions the atom–atom potentials suggested for the calculation of molecular packing in crystals. The functions proposed by Momany *et al.*[4] and Zhurkin *et al.*[5] were tested, and similar results were obtained that fitted well with the experimental n.O.e. data.

The potentials in calculations of the conformations of oligosaccharides in aqueous solutions are those of "medium force", and the parametrisation of other minor components of the energy is also dealt with in refs. 6 and 7. The data on the structures of the anomers of Glc, Gal, Man, and Rha residues for conformational calculations were taken from neutronographic studies[8–11]. The valency angle of the glycoside bond oxygen[12] was taken as the average value (116.7°).

The conformational analysis of **1–4** allowed calculation of the n.O.e. values and comparison with the experimental values. The variation of signal intensity f_s^d for a proton d on pre-irradiation of a proton s can be calculated using the formula reported by Shirmer *et al.*[13], taking into account the influence of adjacent protons on the n.O.e. for proton d. In order to compare with the experimental data, the average values of the n.O.e. $\langle f_s^d \rangle$ (s = H-1′ and H-1″) were calculated and Boltzmann probabilities were considered for all possible conformations of each trisaccharide glycoside.

$$\langle f_s^d \rangle = \frac{\sum_{\varphi_1}\sum_{\psi_1}\sum_{\varphi_2}\sum_{\psi_2} (f_s^d)_{\varphi_1,\psi_1,\varphi_2,\psi_2} \exp(-\Delta U_{\varphi_1,\psi_1,\varphi_2,\psi_2}/kT)}{\sum_{\varphi_1}\sum_{\psi_1}\sum_{\varphi_2}\sum_{\psi_2} \exp(-\Delta U_{\varphi_1,\psi_1,\varphi_2,\psi_2}/kT)}$$

In order to minimize the time on the computer; only those values of φ and ψ that

corresponded to conformations with relative energy ≤3 kcal.mol^{-1} on cross-sections of the potential surface were considered. The step in the accepted range was 10°.

For each of the trisaccharide glycosides **1–4**, only one preferred conformer was determined on the corresponding potential surface with a statistical weight in the conformational equilibrium of <90%. The optimal values of φ and ψ in such conformers are given in Table IV. Table V contains the calculated values of the n.O.e. f_s^d for the optimal conformers as well as average values of the n.O.e. $\langle f_s^d \rangle$ and their relative values used for comparison with corresponding observed values.

TABLE IV

OPTIMAL ANGLES OF ROTATION (°) IN THE PREFERRED CONFORMERS OF **1–4**

Glycoside	φ_1 *(C-1′–O)*	ψ_1 *(O–C-4)*	φ_2 *(C-1″–O)*	ψ_2 *(O–C-3)*
1	57.2	8.3	−58.4	−38.8
2	55.6	14.0	58.1	12.1
3	−23.2	13.9	58.0	21.1
4	−35.8	−24.3	−69.5	−43.8

TABLE V

CALCULATED AVERAGE VALUES[a] OF THE n.O.e. (%) AND THE n.O.e's (f_s^d) FOR OPTIMAL CONFORMATIONS OF **1–4**

Glycoside	*Pre-irradiation of H-1′*			*Pre-irradiation of H-1″*		
	Observed	f_s^d	$\langle f_s^d \rangle$	*Observed*	f_s^d	$\langle f_s^d \rangle$
1	H-2′	5.1	5.8 (0.5)	H-2″	13.2	10.1 (1.3)
	H-3′	15.1	15.1 (1.2)	H-3	3.5	7.5 (1)
	H-5′	16.2	16.1 (1.3)	H-4	12.5	8.5 (1.1)
	H-4	8.1	12.1 (1)	H-1′	9.1	4.0 (0.5)
	H-1″	10.1	5.8 (0.5)			
	H-2″	7.5	6.8 (0.6)			
2	H-2′	6.0	6.0 (0.5)	H-2″	17.0	15.2 (1.2)
	H-3′	14.7	15.2 (1.2)	H-3	12.5	12.1 (1)
	H-5′	15.6	16.4 (1.3)			
	H-4	8.2	12.1 (1)			
	H-3″	14.0	7.1 (0.6)			
	H-5″	6.4	7.1 (0.6)			
3	H-2′	21.8	22.0 (1)	H-2″	17.2	15.1 (1.3)
	H-4	21.8	21.8 (1)	H-3	9.1	11.4 (1)
	H-6,6	0	4.1 (0.2)			
	H-5″	3.8	5.1 (0.25)			
4	H-2′	22.1	21.8 (1.8)	H-2″	11.2	10.9 (1.4)
	H-4	11.2	12.1 (1)	H-3	2.1	8.0 (1)
	H-6,6	15.1	12.9 (1.1)	H-4	11.0	9.1 (1.1)
				H-1′	0	0.5 (0.08)
				H-5′	5.1	3.2 (0.4)

[a]See footnote to Table I.

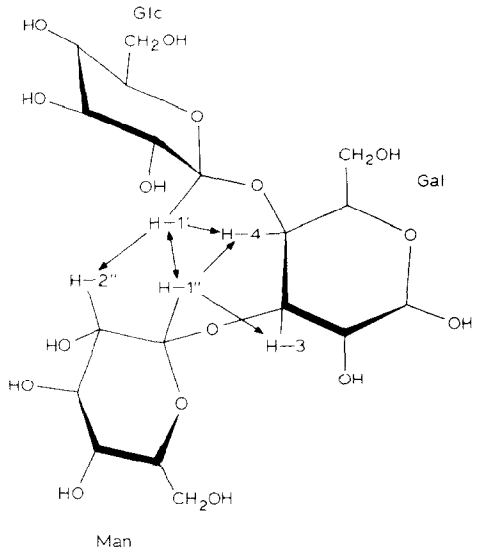

Fig. 1. Molecular model of the conformer of lowest energy for β-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**). The optimal rotation angles are given in Table III. The protons with the fixed n.O.e.'s are denoted with arrows.

The correlation between calculated and observed data is considered below for each trisaccharide glycoside.

β-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**). — Pre-irradiation of H-1′ in **1** resulted, apart from the atoms of the residue irradiated, in n.O.e.'s for the resonances of H-4 of the β-D-Gal residue and H-1″ and H-2″ of the α-D-Man residue (Table I). This result demonstrates the close proximity of the β-D-Glc and α-D-Man residues. Upon pre-irradiation of H-1″ of α-D-Man and for α-D-Man-(1→3)-β-D-Gal-OMe (Table II), n.O.e.'s for the resonances of H-3 and H-4 were observed (Table I) as well as for that of H-1′ (see results on irradiation of H-1″ in Table I).

The conformational analysis of **1** indicated one preferred conformer, with the values of φ_1, ψ_1, φ_2, and ψ_2 given in Table IV, and is shown in Fig. 1. In this conformation, apart from H-1′ and H-4 in the Glc-(1→4)-Gal unit [r(H-1′–H-4) = 2.3 Å] and H-1″, H-4, and H-3 in the Man-[1→3)-Gal unit [r(H-1″–H-4) = 2.3 Å, r(H-1″–H-3) = 3 Å], the protons of β-D-Glc (H-1′) and α-D-Man (H-1″ and H-2″) are in close proximity, the distances r(H-1′–H-1″) and r(H-1″–H-2′) being 2.35 and 2.7 Å, respectively.

The n.O.e. values for the resonances of H-1″ and H-2″ caused by pre-irradiation of H-1′ (Table I) were only half that of the n.O.e. of the resonance for H-4, which indicates the significant statistical weight of the proposed conformation of **1**.

The values calculated for $f_{H\text{-}1'}^{H\text{-}4}$ and $f_{H\text{-}1'}^{H\text{-}1''}$ (or $f_{H\text{-}1'}^{H\text{-}2''}$) were almost equal for the optimal conformation, but the experimental ratio of 1:2 for the n.O.e.'s of the resonances for H-1″ (or H-2″) and H-4 (*cf.* Table I) resulted on calculation of the average values $\langle f_{H\text{-}1'}^{H\text{-}4} \rangle$, $\langle f_{H\text{-}1'}^{H\text{-}1''} \rangle$, and $\langle f_{H\text{-}1'}^{H\text{-}2''} \rangle$ (Table V). According to the experimental

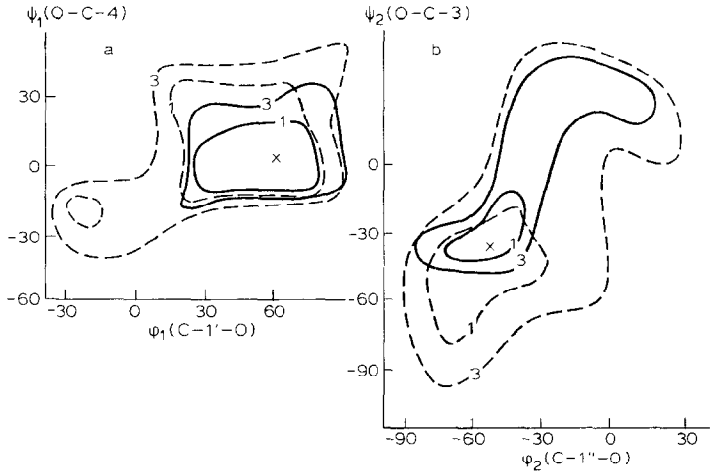

Fig. 2. Cross-sections of the potential surface of β-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**): (*a*) φ_1–ψ_1 (φ_2 −58.4°, ψ_2 −38.8°) and (*b*) φ_2–ψ_2 (φ_1 57.2°, ψ_1 8.3°); —, equipotentials of relative energy (Δ1 and Δ3 kcal.mol^{-1}); ×, local minima; - - - , energy contours for the corresponding disaccharide glycosides.

results, the average n.O.e. values of the resonances for H-3 and H-4 caused by pre-irradiation of H-1″ of the Man residue are almost equal and twice that of $\langle f^{H\text{-}1'}_{H\text{-}1''} \rangle$ respectively (Table V).

Fig. 2 shows cross-sections of the potential surface of **1**. In the cross-section for the Glc-(1→4)-Gal unit (Fig. 2a), there is a small narrowing of the Δ1 kcal. mol^{-1} energy contour as compared with that of β-D-Glc-(1→4)-β-D-Gal-OMe (denoted by the broken line). In contrast, the freedom of rotation in the Man-(1→3)-Gal unit is altered significantly. Thus, for **1**, the negative angles ψ_2 −40°–−80° (Fig. 2b), which are related to the lowest energies in α-D-Man-(1→3)-β-D-Gal-OMe[1], are prohibitively high. Within the above range of ψ_2 values, the α-D-Man residue can approach the β-D-Glc residue to the prohibited distance.

The different degrees of conformational freedom in the Man-(1→3)-Gal unit of **1** and the corresponding disaccharide glycoside are shown by the n.O.e. data. The n.O.e. for the resonance of H-4 of the disaccharide glycoside caused by pre-irradiation of H-1″ of the Man moiety is 2.1-fold higher than for the resonance of H-3 (Table II), whereas these effects for **1** are equal on the basis of both observed (Table I) and calculated data (Table V). The higher value of ψ_2 in **1** is proved also by comparison of the J^{ψ} valus for **1** (3.4 Hz, Table III) and α-D-Man-(1→3)-β-D-Gal-OMe[1] (2.7 Hz).

In contrast, the J^{ψ} values for the Glc-(1→4)-Gal unit of **1** and the corresponding disaccharide glycoside are ~5 Hz (Tables III and II of ref. 1). For each compound, the ratio of the n.O.e. for the resonances of H-2′ of β-D-Glc and H-4 of β-D-Gal is 1:2 (Tables I and II). This finding demonstrates the similarity of conformations of Glc-(1→4)-Gal units in the molecules compared.

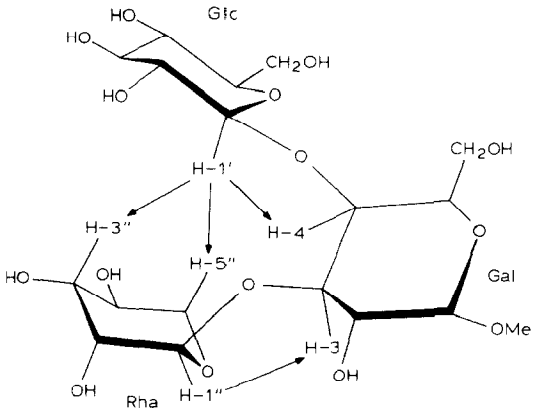

Fig. 3. Molecular model of the preferred conformer of β-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**2**) (*cf.* legend to Fig. 1).

The dimensions of the Δ1 kcal.mol^{-1} contours for the cross-sections in Fig. 2 suggest that the angles of rotation around the inter-unit glycosidic linkage are small, for example, $\psi_1 = 0 \pm 10°$ and $\psi_2 = -30 \pm 10°$.

Thus, the combination of the n.O.e. data and calculations demonstrates that there is one preferred conformer for **1** (Fig. 1).

β-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**2**). — After pre-irradiation of H-1′, the n.O.e. of the signals for H-4 of the β-D-Gal residue and for H-3″ and H-5″ of the α-L-Rha residue were observed (Table I). Thus, the carbohydrate unit in the branching point of **2** as compared with **1** is turned towards the Glc residue (with regard to the middle plane of the residue). Pre-irradiation of H-1″ resulted in

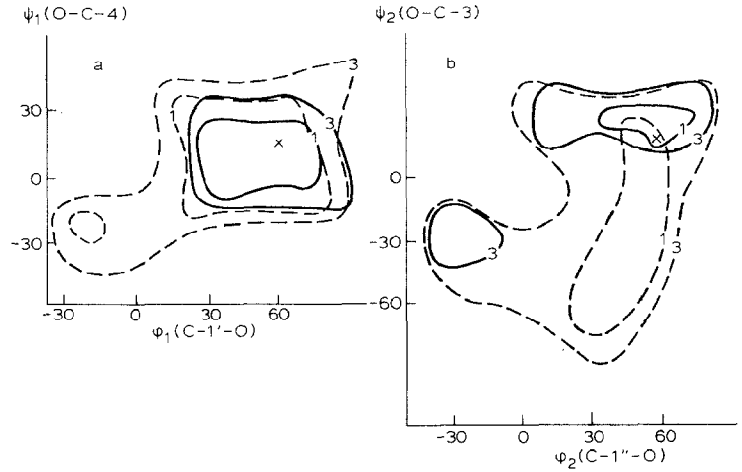

Fig. 4. Cross-sections of the potential surface of β-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**2**): (*a*) φ_1–ψ_1 (φ_2 58.2°, ψ_2 12.1°) and (*b*) φ_2–ψ_2 (φ_1 55.6°, ψ_1 14.0°) (*cf.* legend to Fig. 2).

a n.O.e. of the signal for H-3 of the β-D-Gal residue only, apart from that for H-2″ (Table I).

In the preferred conformation of **2** (Table IV, Fig. 3), the pre-irradiated H-1′ is in close proximity to both H-4 of the β-D-Gal and H-3″ and H-5″ of the α-L-Rha residue [the distances r(H-1′–H-4), r(H-1′–H-3″), and r(H-1′–H-5″) are 2.4, 2.3, and 2.6 Å, respectively]. High n.O.e. values for the resonances of H-3″ and H-5″ were observed experimentally (the n.O.e. for H-4 was only 1.5-fold higher than that for the resonances of H-3″ and H-5″; Table I), which correlates well with the proposed conformation of **2**. Calculation of the average n.O.e. values for the above protons reproduced the observed ratios. Thus, $\langle f^{H\text{-}3''}_{H\text{-}1'}\rangle/\langle f^{H\text{-}4}_{H\text{-}1'}\rangle$ and $\langle f^{H\text{-}5''}_{H\text{-}1'}\rangle/\langle f^{H\text{-}4}_{H\text{-}1'}\rangle$ were 0.6 (Table V).

Comparison of the conformational maps φ–ψ of the disaccharide glycosides[1] related to the units of **2** (*cf.* the broken contours in Fig. 4) with the corresponding cross-sections in Fig. 4 demonstrates the significant limitation of ψ_2 for the Rha-(1→3)-Gal unit. The values $\psi_2 < 0°$ at which the α-L-Rha residue encounters the β-D-Glc residue are prohibited and the conformation similar to the preferred conformer of α-L-Rha-(1→3)-β-D-Gal-OMe (φ,ψ 40, −50°; Table III in ref. 1) was precluded. Thus, the interactions of non-bonded residues govern the rigidity of the branched oligosaccharides.

There are some other data that demonstrate the limitations in freedom of rotation in the Rha-(1→3)-Gal unit of **2**. Thus, the n.O.e. for the resonance of H-4 of the Gal moiety was lacking, whereas, although small, it was observed for α-L-Rha-(1→3)-β-D-Gal-OMe, where negative values of ψ are possible (Table II, in ref. 1). The close proximity of H-1″ and H-3 in the disaccharide glycoside was demonstrated by the ratio of the n.O.e. for the resonances of H-2″ of the α-L-Rha

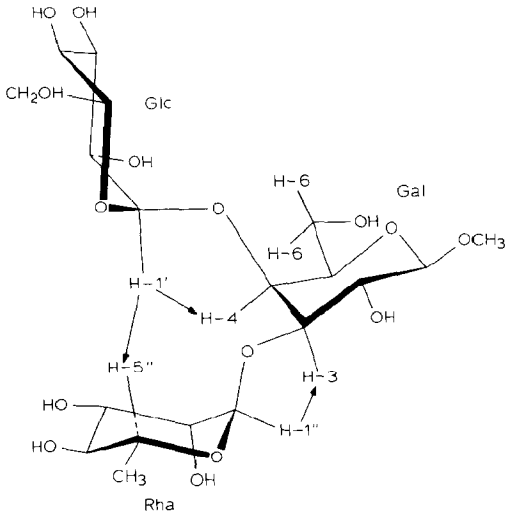

Fig. 5. Molecular model of the preferred conformer of α-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**3**) (*cf.* legend to Fig. 1).

moiety and H-3 of the β-D-Gal moiety, which is <1 (Table II), whereas, for **2**, this ratio is >1 (Table I). The J^{ψ} value for α-L-Rha-(1→3)-β-D-Gal-OMe[1] is 4.1 Hz, and 5.1 Hz for **2** (Table III), which is close to maximal value[14], and ψ_2 should be closer to 0°. According to the cross-section in Fig. 4b, the possible values of ψ_2 are 15 ±10°.

Thus, there is one preferred conformer for **2** with limited freedom of rotation around the inter-unit linkage. In the optimal conformation (Fig. 3), the non-bonded residues β-D-Glc and α-L-Rha are positioned at the distances equal to the sum of the van der Waals radii. Therefore, the energy of their interaction has a negative value (~ −2.5 kcal.mol^{-1}) and separation of the non-bonded moieties is unfavourable. Noteworthy is the presence of the hydrophobic cluster from hydrogen atoms in the center of the molecule (H-1′,4,3″,5″ as well as H-1″,3, Fig. 3).

α-D-Glc-(1→4)[α-L-Rha-(1→3)]β-D-Gal-OMe (**3**). — Pre-irradiation of H-1′ of the α-D-Glc results in an n.O.e. for the resonance H-5″ of the α-L-Rha residue (Table I). In the preferred conformation of **3** (Fig. 5), only the above protons of Glc and Rha residues are in close proximity (separating distance of 2.8 Å).

For α-D-Glc-(1→4)-β-D-Gal-OMe, two conformations are possible with φ,ψ −40°, −20° and −20°, +30°, of which the former (*A*) is favoured. However, the negative values of ψ_1 in **3** become doubtful due to the inadmissible proximity of the hydroxymethyl group of the α-D-Glc residue with the α-L-Rha residue. Therefore, conformer *B* of the Glc-(1→4)-Gal unit with ψ_1 +15° becomes that with low energy (Table IV). This conclusion is confirmed by the shape of cross-section φ_1–ψ_1 of the potential surface of **3** in Fig. 6.

The significant difference in the conformations of the Glc-(1→4)-Gal unit in **3** and the corresponding disaccharide glycoside follows from the pronounced difference in the ratios of the n.O.e. for the resonances of H-6,6 and H-4 of the β-D-Gal

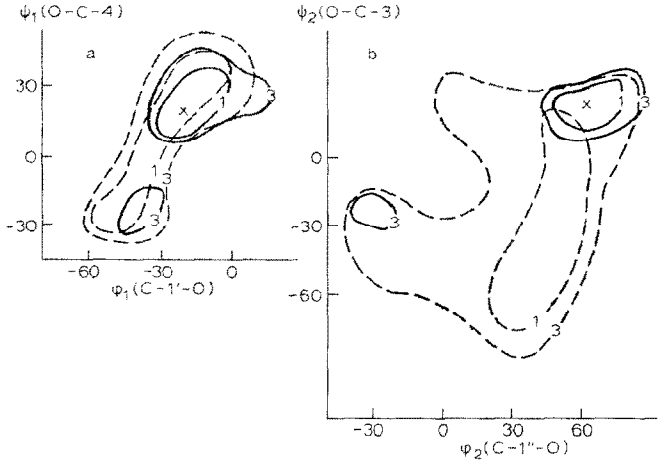

Fig. 6. Cross-sections of the potential surface of α-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**3**): (*a*) φ_1–ψ_1 (φ_2 58.1°, ψ_2 21.1°) and (*b*) φ_2–ψ_2 (φ_1 −23.2°, ψ_1 13.9°) (*cf.* legend to Fig. 2).

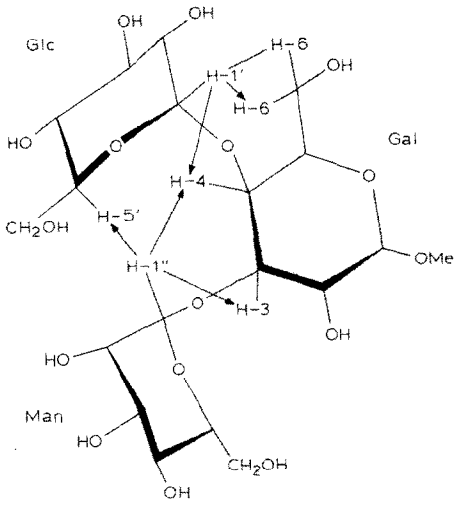

Fig. 7. Molecular model of the preferred conformer of α-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**4**) (*cf.* legend to Fig. 1).

residue caused by pre-irradiation of H-1′. Whereas, for α-D-Glc-(1→4)-β-D-Gal-OMe, the n.O.e. for the resonances of H-6,6 are half that for the resonance of H-4 (Table II), the effect for **3** is 5-fold lower (Table I). Thus, the conformer *B* with φ,ψ −20°, +15° is present in this unit of **3** with a preponderant statistical weight, whereas the proportion of conformer *A*, responsible for the n.O.e. for the resonances of H-6,6, is ~10%.

There is a wide range of admissible values of φ,ψ for α-L-Rha-(1→3)-β-D-Gal-OMe, whereas the admissible values of ψ_2 in **3** are in the range 20 ±10° and those of ψ_2 are −60 ±10° (Fig. 6b). Thus, in the disaccharide units of **3**, the variations of φ_1, ψ_1, φ_2, and ψ_2 from the optimal values do not exceed 10°, which confirms conformational rigidity.

The n.O.e. values observed for the resonances of H-4 and H-2′, caused by pre-irradiation of H-1′ of **3**, are the same as those calculated (Table I and V). The ratios of the observed n.O.e. values for the resonancers of H-5″ and H-4 are 0.2 (Table I). The calculated ratio is 0.25 (Table V). After pre-irradiation of H-1″, the ratio of averager values $\langle f^{H\text{-}2''}_{H\text{-}1''}\rangle$ and $\langle f^{H\text{-}2}_{H\text{-}1''}\rangle$ is 1.3 (Table V), as is the experimental ratio (Table I).

Of the two admissible optimal conformers for each of the disaccharide units[1], Glc-(1→4)-Gal and Rha-(1→3)-Gal, the high-energy conformer is present in **3**.

α-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe (**4**). — On pre-irradiation of H-1′ of **4**, an n.O.e. was observed only for protons of the Glc-(1→4)-Gal unit, whereas, after pre-irradiation of H-1″ of the Man residue, n.O.e.'s were observed for protons of the β-D-Gal residue and for H-5′ and H-1′ of the α-D-Glc residue (Table I). The n.O.e. for H-5′ was twice that for H-1′.

The preferred conformation of **4** (the corresponding angles of rotation are

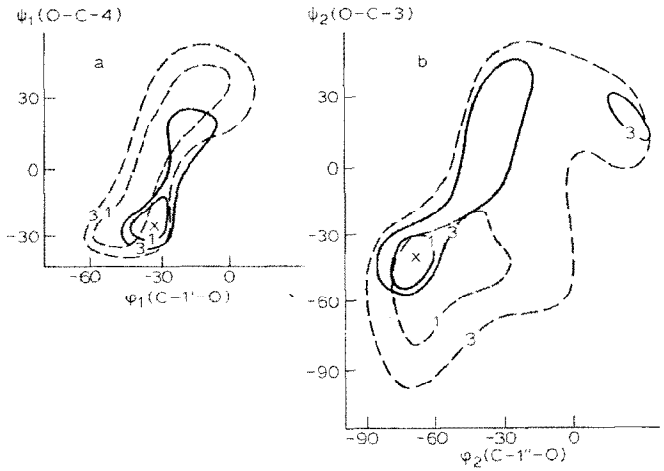

Fig. 8. Cross-sections of the potential surface of α-D-Glc-(1→4)[α-D-Man-(1→3)]β-D-Gal-OMe (**4**): (*a*) φ_1–ψ_1 (φ_2 −69.5°, ψ_2 −43.8°) and (*b*) φ_2–ψ_2 (φ_1 −35.8°, ψ_1 −24.3°) (*cf.* legend to Fig. 2).

presented in Table IV) is shown in Fig. 7, in which H-1′ of the Glc residue is in van der Waals contact with H-6,6 (*gt*-orientation of the hydroxymethyl group) and H-4 of the Gal residue [*r*(H-1′–H-6a) = 2.3 Å, *r*(H-1′–H-6b) = 2.3 Å, *r*(H-1′–H-4) = 2.4 Å]. Also, H-1″ of the α-D-Man residue is in close proximity to H-4 of the Gal residue [*r*(H-1″–H-4) = 2.3 Å] and H-5′ of the Glc residue [*r*(H-1″–H-5′) = 2.7 Å]. The observed n.O.e. between H-1″ and H-5′ of the non-bonded residues α-D-Man and α-D-Glc (Table I) proves the proposed conformation of **4**.

The cross-sections of the potential surfaces φ_1–ψ_1 and φ_2–ψ_2 of **4** are shown in Fig. 8. The freedom of rotation[1] around the inter-glycosidic linkage in α-D-Glc-(1→4)-β-D-Gal-OMe is the lowest among the disaccharide units. The presence of α-D-Man as a substituent of the branched Gal residue results in even greater limitation of the admissible values of φ_1 and ψ_1 (Fig. 8a). Thus, of the conformers *A* (φ,ψ −40°, −20°) and *B* (−20°, +30°) of α-D-Glc-(1→4)-β-D-Gal-OMe[1], only the former is present in **4**.

The low-energy contours of the cross-section φ_1–ψ_1 of **4** (Fig. 8a) are shifted to the higher values of φ_1. The negative values of φ_1 in the range −50–−60°, permitted for α-D-Glc-(1→4)-β-D-Gal-OMe, are prohibited in **4** due to the inadmissible proximity of the hydroxymethyl group of the α-D-Glc residue with the α-D-Man residue. On the basis of the position of the Δ1 kcal.mol^{-1} iso-energy contour in Fig. 8a, it is concluded that the admissible values of φ_1 and ψ_1 are in the narrow ranges −30 to −40° and −10 to −30°, respectively. This conclusion correlates with observed J^{φ} and J^{ψ} values of 3.9 and 5.0 Hz, respectively (Table III).

There are significant changes also in the conformational freedom of the Man-(1→3)-Gal unit of **4** in comparison with that in α-D-Man-(1→3)-β-D-Gal-OMe[1] (Fig. 8b). Thus, the values of ψ_2 −60°–−90° become prohibited, which results in a larger value of J^{ψ} for the Man-(1→3)-Gal unit of **4** as compared with that for the

disaccharide glycoside (3.5 and 2.7 Hz, respectively; Tables III and II of ref. 1). Thus, the freedom of rotation is low also for the Man-(1→3)-Gal unit, the most probable values of φ_2 and ψ_2 being -60 to $-70°$ and -30 to $-50°$, respectively. It should be borne in mind that all conformers within the limits of the $\Delta 3$ kcal.mol^{-1} contour on the cross-section φ_2–ψ_2 (Fig. 8b) may contribute to the statistical sum for **4**.

For example, the value $f^{H\text{-}4}_{H\text{-}1''}$ is markedly higher than that of $f^{H\text{-}3}_{H\text{-}1''}$ in the optimal conformation of **4** (Table V). However, the calculated average values $\langle f^{H\text{-}4}_{H\text{-}1''}\rangle$ and $\langle f^{H\text{-}3}_{H\text{-}1''}\rangle$ were similar, which corresponds to the observed data (see Tables I and V). The n.O.e. for H-4 of α-D-Man-(1→3)-β-D-Gal-OMe is twice that for H-3 (Table II), which is related to the presence in equilibrium of conformers with ψ values $-50°$–$-90°$ which are unreal for **4**. Such a situation occurs in **1** also, where the n.O.e. values for H-3 and H-4, caused by pre-irradiation of H-1″, are equal (Table I).

Another important effect observed for **4** after pre-irradiation of H-1″ was the n.O.e. for H-5′ of the α-D-Glc residue, which was reproduced on calculation of the average values. Thus, the calculated (Table V) and observed (Table I) ratio of the values $\langle f^{H\text{-}5'}_{H\text{-}1''}\rangle$ and $\langle f^{H\text{-}4}_{H\text{-}1''}\rangle$ was 1:3.

On pre-irradiation of H-1′ of **4**, n.O.e.'s were observed for H-4 and H-6,6 (Table I), the latter being the greater. There were significant differences in the n.O.e. values for **4** and α-D-Glc-(1→4)-β-D-Gal-OMe, where the effect for H-6,6 was half that for H-4 (Table II).

Thus, for the disaccharide glycoside, the conformers with H-1′ and H-6,6 being in close proximity and also remote from each other participated in the equilibrium but, for the Glc-(1→4)-Gal unit of **4**, only the conformation with H-1′ and H-6,6 in van der Waals contact is present, as shown in Fig. 7. In this conformation, the calculated values of $f^{H\text{-}6,6}_{H\text{-}1'}$ are 15% (*gt*-orientation of the hydroxymethyl group of the β-D-Gal residue) andf 9% (*tg*-orientation). Taking into account the fact that the ratios of the *gt* and *tg* conformers of the Gal residue[1] are ~60 and ~40%, respectively, the average values $\langle f^{H\text{-}6,6}_{H\text{-}1'}\rangle$ and $\langle f^{H\text{-}4}_{H\text{-}1'}\rangle$ were estimated as 12.6 and 12% (Table V). Thus, the ratio of these average values is equal to that (1.1) of the observed n.O.e. values for H-6,6 and H-4 (Table I).

The n.O.e. for H-6,6 of **3** was almost 5-fold lower than that for H-4 of the Gal residue (Table I), which indicates the difference in the conformations of the Glc-(1→4)-Gal unit in **3** and **4**. In fact, conformer *A* of the Glc-(1→4)-Gal unit was present in **4**, whereas conformer *B* was present in **3** (Table IV).

The preference of conformer *A* (φ,ψ $-40°$, $-20°$) in the Glc-(1→4)-Gal unit of **4** follows from the ratio of the n.O.e. for H-2′ of the irradiated Glc residue and H-4, namely, 1.6 for **4** (Table I) and <1 for α-D-Glc-(1→4)-β-D-Gal-OMe (Table II) where conformers *A* and *B* were present in the equilibrium[1].

However, the conformational rigidity of this unit in **4** should not be exaggerated. Thus, after pre-irradiation of H-1″, a slight effect was observed for H-1′ of the Glc residue (Table I), the value of which was an order of magnitude lower than that

for H-4 of the β-D-Gal residue. It may be caused only by conformations with ψ_1 10–20° (Fig. 8a), where H-1″ and H-1′ are situated at a distance of ~3Å. Thus, despite the fact that all available data confirm the predominant statistical weight of one conformer (Table IV) in **4**, the variations of φ_1 and ψ_1 in the Glc-(1→4)-Gal unit may occur within the limits of all the areas of low energy in the conformational map (Fig. 8a).

Thus, **4** exists in one preferred conformation with limited freedom of rotation around the inter-unit glycosidic linkage. The energy of interaction of the non-bonded residues is ~ -2 kcal.mol^{-1}. There is an internal non-polar cluster in **4** involving H-1′,5′ (Glc), H-3,4,6,6 (Gal), and H-1″ (Man) (Fig. 7).

The conformational freedom of the disaccharide glycosides is markedly greater than that of the corresponding disaccharide units in **1–4**. The structure of the disaccharide glycosides cannot be described satisfactorily by any one rigid conformation. However, for **1–4**, in which the Gal residue is 3,4-substituted, there is one preferred conformer.

EXPERIMENTAL

The ^{1}H- (300 MHz) and ^{13}C-n.m.r. (75 MHz) spectra were recorded with a Bruker AM-300 instrument on solutions in D_2O at 30°. The reference for the ^{1}H-n.m.r. spectra was sodium 4,4-dimethyl-4-silapentanesulfonate (Tables VI and VII). The key signals were identified by selective homonuclear resonance. The n.O.e. values were measured within the t.O.e. technique[15], with D_1 (pre-irradiation time) 0.5 s and D_2 (the relaxation delay) 0.8 s. N.O.e. values (%) are expressed as the ratio of the integrated intensities of the observed and presaturated proton resonances in the differential spectrum. The J^{φ} and J^{ψ} values were determined by 2D spectroscopy[16,17]. The synthesis of **1–4** has been described[18].

TABLE VI

CHEMICAL SHIFT DATA[a] (P.P.M.) AND MULTIPLICITY IN ^{1}H-N.M.R. SPECTRA OF **1–4**

Glycoside	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
1 β-D-Gal	4.43d	3.76dd	3.90dd	4.45dd	3.81m	—	—
β-D-Glc	4.54d	3.33dd	3.55t	3.43m	3.51m	3.96dd	—
α-D-Man	5.11d	4.07dd	3.93dd	3.75t	—	—	—
2 β-D-Gal	4.42d	3.82dd	3.88dd	4.29dd	3.78m	—	—
β-D-Glc	4.75d	3.38dd	3.56t	3.44m	3.53m	3.95dd	—
α-L-Rha	5.15d	4.11dd	3.86dd	3.54t	3.80dq	1.35d	—
3 β-D-Gal	4.42d	3.71dd	3.79dd	4.10dd	3.79ddd	3.93dd	3.85dd
α-D-Glc	5.02d	3.53dd	3.79dd	3.53t	3.84m	—	—
α-L-Rha	5.15d	4.07dd	3.83dd	3.48t	3.79dq	1.31d	—
4 β-D-Gal	4.45d	3.68dd	3.82dd	4.33dd	3.80ddd	3.79dd	3.88dd
α-D-Glc	4.99d	3.54dd	3.80dd	3.55dd	3.87m	—	—
α-D-Man	5.11d	3.97dd	3.91dd	3.77t	—	—	—

[a]OMe groups at 3.57–3.63 p.p.m.

TABLE VII

J VALUES (Hz) FROM THE ^{1}H-N.M.R. SPECTRA OF **1–4**

Glycoside	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6,6}$
1 β-D-Gal	7.4	9.7	2.6	1.0	—	—	—
β-D-Glc	7.6	9.1	9.1	—	2.0	—	12.0
α-D-Man	1.8	3.4	9.5	9.5	—	—	—
2 β-D-Gal	7.1	9.8	2.5	1.0	—	—	—
β-D-Glc	7.5	9.0	9.0	—	2.1	—	11.8
α-L-Rha	1.9	3.2	9.4	9.4	6.1	—	—
3 β-D-Gal	7.0	9.9	2.4	1.0	7.0	5.4	11.0
α-D-Glc	3.5	9.6	9.4	9.4	—	—	—
α-L-Rha	1.9	3.2	9.1	9.1	6.0	—	—
4 β-D-Gal	7.7	10.1	2.5	1.0	7.5	4.6	11.4
α-D-Glc	3.6	10.0	9.2	9.8	—	—	—
α-D-Man	1.7	3.1	9.2	9.2	—	—	—

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