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NUCLEAR OVERHAUSER EFFECTS AND CONFORMATIONS OF BRANCHED TRISACCHARIDE METHYL β-GLYCOSIDES THAT CONTAIN A 2,3-DISUBSTITUTED GALACTOSE RESIDUE

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ABSTRACT

On the basis of the n.O.e. data and theoretical calculations, it was found that <90% of one conformer was present in aqueous solution for each of a series of trisaccharide methyl β-glycosides with a 2,3-disubstituted galactose residue.

INTRODUCTION

This paper reports a study of the conformations of branched oligosaccharides on the basis of the n.O.e. and theoretical calculations. In the preceding paper[1], glycosides that contained a 3,4-disubstituted Gal residue of the general formula A-(1→4)[B-(1→3)]-β-D-Gal-OMe were studied, and we now report on glycosides that contain a 2,3-disubstituted Gal residue, namely, A-(1→2)[B-(1→3)]-β-D-Gal-OMe: **1** A = α-D-Glc, B = α-D-Man; **2** A = β-D-Glc, B = α-D-Man; **3** A = α-D-Glc, B = α-L-Rha; and **4** A = β-D-Glc, B = α-L-Rha. The substituents are equatorial whereas, in the 3,4-disubstituted analogues[1], one substituent was axial and the other was equatorial.

Conformational analysis and the n.O.e. data for the glycosides **1**–**4** are absent from the literature. However, Lemieux *et al.*[2] studied the determinant oligosaccharide of the blood-group substance with B specificity, namely, α-L-Fuc-(1→2)[α-D-Gal-(1→3)]-β-D-Gal-OMe, which is related to **1**–**4**. The conformation of this trisaccharide glycoside was proposed on the basis of theoretical calculations using the HSEA method and analysis of the ^{1}H-n.m.r. data.

RESULTS AND DISCUSSION

The n.O.e.'s, observed after pre-irradiation of the anomeric protons of the glycosidic linkages, *i.e.*, H-1′ of D-Glc and H-1″ of the α-D-Man or α-L-Rha residues in **1**–**4**, are given in Table I, and those for the disaccharide glycosides[3,4] related to the disaccharide units of **1**–**4** are presented in Table II. The procedure for theoretical conformational analysis of **1**–**4** is described in the preceding paper[1].

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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.*
α-D-Glc-(1→2)[α-D-Man-(1→3)]-	H-2′	9.8 (1.3)[a]	H-2″	7.0 (2.7)
β-D-Gal-OMe (**1**)	H-2	7.7 (1)	H-3	2.6 (1)
	H-1	1.0 (0.1)	H-4	11.6 (4.5)
	OMe	1.9 (0.2)		
β-D-Glc-(1→2)[α-D-Man-(1→3)]-	H-2′	2.1 (0.2)	H-2″,3	7.7 (1)
β-D-Gal-OMe (**2**)	H-3′,5′	15.9 (1.4)	H-4	7.7 (1)
	H-2,3″	11.2 (1)		
	OMe	1 (0.1)		
α-D-Glc-(1→2)[α-L-Rha-(1→3)]-	H-2′	11.0 (2.5)	H-2″	5.5 (0.5)
β-D-Gal-OMe (**3**)	H-2	7.1 (1)	H-3	11.6 (1)
	H-1	1.1 (0.2)	H-4	1.0 (0.1)
	OMe	1.2 (0.2)	H-3′	2.3 (0.2)
			H-5′[b]	
β-D-Glc-(1→2)[α-L-Rha-(1→3)]-	H-2′	1.7 (0.2)	H-2″	6.4 (0.7)
β-D-Gal-OMe (**4**)	H-3′	5.6 (0.6)	H-3	9.6 (1)
	H-5′	7.4 (0.9)	H-4	1.1 (0.1)
	H-2	8.7 (1)		
	H-2″	3.5 (0.4)		
	OMe	1.7 (0.2)		

[a] Relative values of the n.O.e. are given in brackets. [b] N.O.e. was not measured.

Only one preponderant conformer (<90%) was found to be selected for each of **1–4**. The angles of rotation around the glycosidic bonds in the Glc-(1→2)-Gal units [ϕ_1 (C-1′–O), ψ_1 (O–C-2)] and in the Man-(1→3)-Gal or Rha-(1→3)-Gal units [ϕ_2 (C-1″–O), ψ_2 (O–C-3)] for the optimal conformers are given in Table III.

The results of the n.O.e. calculations f_s^d, where d and s are observed and irradiated nuclei in optimal conformations of trisaccharides, as well as the average values $\langle f_s^d \rangle$ are summarized in Table IV.

α-D-Glc-(1→2)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**). — After pre-irradiation of H-1′ and H-1″ of **1**, an n.O.e. was observed only on the protons of the irradiated residues and the galactose residue (Table I).

The preferred conformer of **1** (Table III) is depicted in Fig. 1 in which the non-admissible approach of the non-bonded α-D-Glc and α-D-Man residues will occur only when ψ_1 and ψ_2 are $>-30°$. Therefore, the corresponding regions of conformational maps of α-D-Glc-(1→2)-β-D-Gal-OMe and α-D-Man-(1→3)-β-D-Gal-OMe[3,4] on cross-sections of the potential surface of **1**, ϕ_1–ψ_1 and ϕ_2–ψ_2, are of high energy (Fig. 2). On the basis of the position of Δ1 kcal.mol^{-1} energy contours, it is concluded that the most probable angles are ψ_1 −40 to −50°, ϕ_1 −60 to −70°, ψ_2 −50 to −70°, ϕ_2 −50 to −70°.

The observed values of the n.O.e. (Table I) demonstrate that ϕ and ψ for **1** are within the above limits. Thus, H-1″ of the α-D-Man residue in the optimal con-

TABLE II

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

Glycoside	*Observed protons*		
	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-2	*H-2′*	*H-3′ + H-5′*
α-D-Glc-(1→2)-β-D-Gal-OMe	7.0 (1)	6.9 (1)	
β-D-Glc-(1→2)-β-D-Gal-OMe	7.6 (1)	2.0 (0.3)	11 (1.4)

[a] Relative n.O.e. values are in brackets.

TABLE III

OPTIMAL VALUES OF ϕ AND ψ (°) IN THE PREFERRED CONFORMERS OF **1–4**

Glycoside	ϕ_1 *(C-1′–O)*	ψ_1 *(O–C-2)*	ϕ_2 *(C-1″–O)*	ψ_2 *(O–C-3)*
1	−68.3	−40.0	−65.5	−51.9
2	57.7	15.3	−68.4	−55.2
3	−71.8	−39.7	51.7	−10.0
4	50.7	17.5	51.7	−23.4

formation of **1** (Fig. 1) in the α-D-Man-(1→3)-β-D-Gal unit is closer to H-4 than to H-3 of the β-D-Gal residue [r(H-1″–H-4) = 2.4 Å, r(H-1″–H-3) = 2.9 Å]. Hence, the calcualted value of the n.O.e. $f^{\text{H-4}}_{\text{H-1}''}$ is many times larger than that of $f^{\text{H-3}}_{\text{H-1}''}$ (Table IV). The observed n.O.e. for H-4 pre-irradiation of H-1″ is 5-fold higher than that for H-3 (Table I), which confirms the presence of the above conformation of **1** in aqueous solution. The ratio of the average values $\langle f^{\text{H-3}}_{\text{H-1}''}\rangle$ and $\langle f^{\text{H-4}}_{\text{H-1}''}\rangle$ after statistical calculation was 1:6 (Table IV).

The significant increase in statistical weight of conformers with ψ_2 −50 to −70° in the Man-(1→3)-Gal unit of **1**, as compared with that of the corresponding disaccharide glycoside, can be seen from the ratio of the n.O.e. for H-4 and H-3, which is ~5 for **1** (Table I) and only 2.1 for the disaccharide glycoside (Table II). It is noteworthy that, for α-D-Glc-(1→4)[α-D-Man-(1→3)]-β-D-Gal-OMe[1] where ψ_2 values of −40 to −70° are prohibited, the n.O.e. for H-4 and H-3 are equal. Thus, various regions of the surface ϕ–ψ of α-D-Man-(1→3)-β-D-Gal-OMe appear to be permitted for two branched trisaccharides with the same unit Man-(1→3)-Gal (*cf.* Figs. 2b and 8b in ref. 1).

The differences in conformation of the Glc-(1→2)-Gal unit in **1** and in α-D-Glc-(1→2)-β-D-Gal-OMe can be seen easily from a comparison of the n.O.e. for H-2′ of the pre-irradiated Glc moiety and H-2 of the Gal moiety, the ratio of which is 1 for the disaccharide glycoside (Table II) and 1.3 for **1** (Table I); this is con-

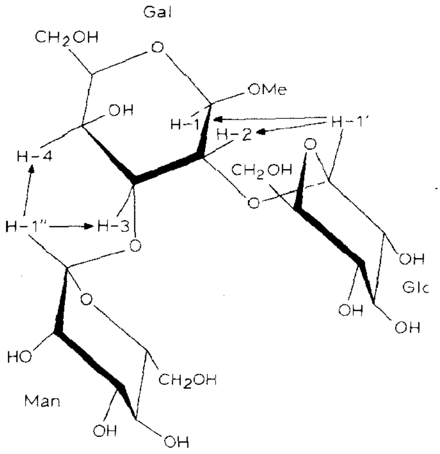

Fig. 1. Molecular model of the preferred conformer of α-D-Glc-(1→2)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**) (optimal values of ϕ and ψ are given in Table III). The protons for which n.O.e.'s are observed are indicated by arrows.

TABLE IV

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

Glycoside	*Pre-irradiation of H-1′*			*Pre-irradiation of H-1″*		
	Observed *n.O.e.*	f_s^d	$\langle f_s^d \rangle$	*Observed n.O.e.*	f_s^d	$\langle f_s^d \rangle$
1	H-2′	22	21 (1.2)[a]	H-2″	17	16 (4.2)
	H-2	7	17 (1)	H-3	1	3.8 (1)
	H-1	2.5	2.5 (0.2)	H-4	23	22 (5.8)
2	H-2′	6.1	6.1 (0.2)	H-2″	19	17.5 (0.8)
	H-3′	17.5	18.2 (0.6)	H-3	0	3.7 (0.2)
	H-5′	18.1	19.0 (0.6)	H-4	22	21.7 (1)
	H-2	25	22.6 (0.75)	H-3,2″		21.2 (1)
	H-3″	9.4	7.6 (0.25)			
	H-2,3″		30.2 (1)			
3	H-2′	24	24.0 (1.4)	H-2″	11.8	11.1 (0.6)
	H-2	5.5	17.5 (1)	H-3	17.2	19.5 (1)
	H-1	2.0	2.5 (0.15)	H-4	0	3.1 (0.15)
				H-3′	4.9	3.5 (0.2)
				H-5′	0	2.0 (0.1)
4	H-2′	6.1	6.1 (0.25)	H-2″	14.7	14.5 (0.7)
	H-3′	17.5	17.5 (0.7)	H-3	18.9	19.6 (1)
	H-5′	18.5	19.0 (0.8)	H-4	0	3.0 (0.15)
	H-2	26.6	24.6 (1)			
	H-1	2.5	0			
	H-2″	6.5	7.7 (0.3)			

[a] Relative n.O.e. values are in brackets.

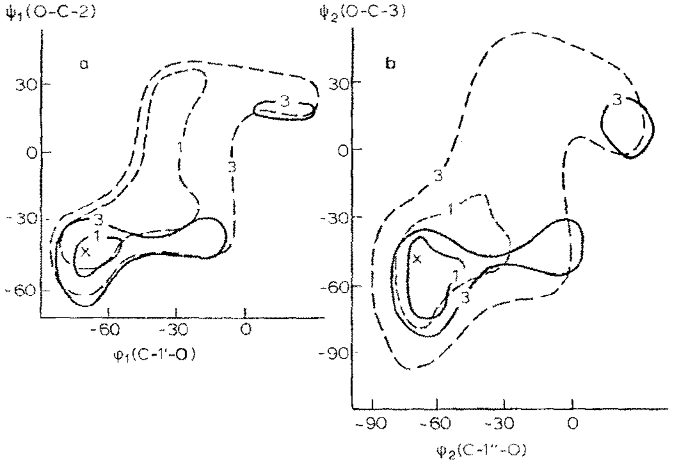

Fig. 2. Cross-sections of the potential surface of α-D-Glc-(1→2)[α-D-Man-(1→3)]-β-D-Gal-OMe (**1**): (*a*) ϕ_1–ψ_1 (ϕ_2 −65.5°, ψ_2 −51.9°) and (*b*) ϕ_2–ψ_2 (ϕ_1 −68.3°, ψ_1 −40.0°); ——, equipotentials of Δ1 and Δ3 kcal.mol^{-1}; ×, local minima; ---, energy contours for the corresponding disaccharide glycosides.

nected with the relative increase of the distance between H-1′ and H-2 at negative values of ψ. Moreover, there is a response on H-1 of the Gal residue of **1** (Table I), which is absent[3] from α-D-Glc-(1→2)-β-D-Gal-OMe, that also confirms the validity of the ψ_1 values of −40 to −60° in this unit of **1**.

Thus, all the n.O.e. data demonstrate the existence of one preferred conformer of **1** in aqueous solution.

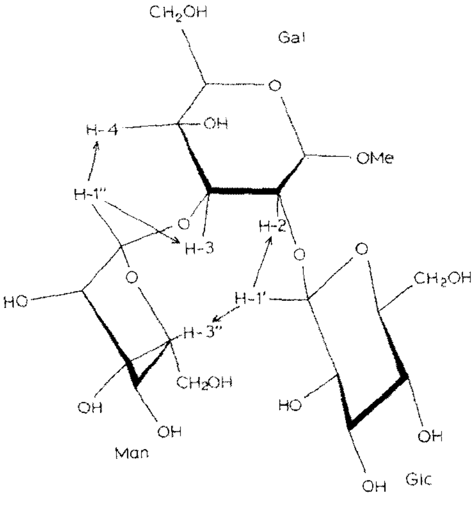

Fig. 3. Molecular model of the preferred conformer of β-D-Glc-(1→2)[α-β-Man-(1→3)]-β-D-Gal-OMe (**2**).

*β-*D*-Glc-(1→2)[α-*D*-Man-(1→3)]-β-*D*-Gal-OMe* (**2**). — Pre-irradiation of H-1′ of **2** results in an n.O.e. for H-3″ of the α-D-Man residue (Table I), which is non-bonded to the β-D-Glc residue. The distance between these protons is 2.5 Å in the optimal conformation of **2** (Table III, Fig. 3).

The freedom of rotation about the glycosidic linkage of the Glc-(1→2)-Gal unit of **2** is slightly different from that in β-D-Glc-(1→2)-β-D-Gal-OMe (see cross-section ϕ_1–ψ_1 in Fig. 4a). On the other hand, for the Man-(1→3)-Gal unit, the range (−40°–+40°) for ψ_2, which is possible for α-D-Man-(1→3)-β-D-Gal-OMe[4], is prohibited for **2** (see cross-section ϕ_2–ψ_2 in Fig. 4b). Thus, for **1** and **2**, the conformations of the Man-(1→3)-Gal unit are the same (*cf.* Figs. 2b and 4b).

This conclusion is proved also by the n.O.e. values (Table I) for **1** and **2**. The n.O.e. for H-4 caused by pre-irradiation of H-1″ is many-fold higher than that for H-3 (the precise value of the n.O.e. for H-3 of **2** was not determined due to overlapping of the signals for H-3 and H-2″, but this signal is weak according to the pattern of the spectrum). The calculated ratio of the average n.O.e. values $\langle f^{\mathrm{H}\text{-}3}_{\mathrm{H}\text{-}1''}\rangle$ and $\langle f^{\mathrm{H}\text{-}4}_{\mathrm{H}\text{-}1''}\rangle$ is ~1:6 (Table III), but it is stressed that the calculated sum of the n.O.e. for H-2″ and H-3 is equal to the n.O.e. for H-4 which agrees with the experimental data (Table I).

In the preferred conformation of **2** (Fig. 3), H-1″ of the Man-(1→3)-Gal unit is in close proximity to H-4 of the Gal residue [*r*(H-1″–H-4) = 2.3 Å and, to a lesser extent, to H-3 [*r*(H-1″–H-3) = 3 Å]. For the Glc-(1→2)-Gal unit, H-1′ and H-2 are in van der Waals contact [*r*(H-1′–H-2) = 2.4 Å]. Moreover, H-1′ and H-3″ of the Glc and Man residues are in close proximity. The observation of an n.O.e. for H-3″ caused by pre-irradiation of H-1′ is a significant argument for the proposed conformation of **2**.

Because of overlapping of the signals for H-2 and H-3″, the sum of average values $\langle f^{\mathrm{H}\text{-}3''}_{\mathrm{H}\text{-}1'}\rangle + \langle f^{\mathrm{H}\text{-}2}_{\mathrm{H}\text{-}1'}\rangle$ was calculated and found to be 5-fold higher than that of

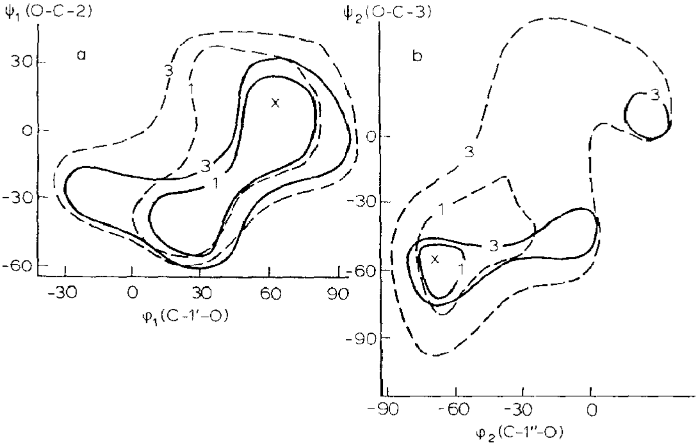

Fig. 4. Cross-sections of the potential surface of β-D-Glc-(1→2)[α-D-Man-(1→3)]-β-D-Gal-OMe (**2**): (*a*) ϕ_1–ψ_1 (ϕ_2 −68.4°, ψ_2 −55.2°) and (*b*) ϕ_2–ψ_2 (ϕ_1 57.7°, ψ_1 15.3°) (*cf.* legend to Fig. 2).

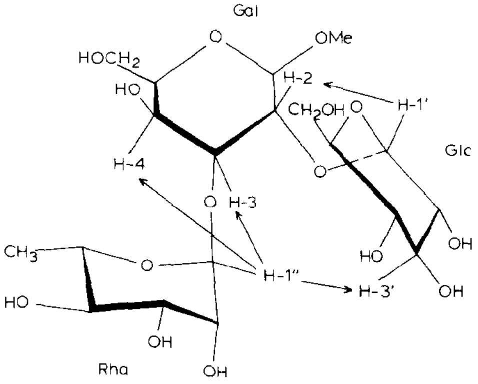

Fig. 5. Molecular model of the preferred conformer of α-D-Glc-(1→2)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**3**).

$\langle f^{H-2'}_{H-1'}\rangle$ (Table IV). The observed ratio of the corresponding n.O.e. is ~5.5 (Table I). Thus, the n.O.e. value for H-3″ caused by pre-irradiation of H-1′ is almost equal to that for H-2′ or about 3-fold lower than that for H-2.

α-D-*Glc-(1→2)*[α-L-*Rha-(1→3)*]-β-D-*Gal-OMe* (**3**). — After pre-irradiation of H-1′ and H-1″ of **3**, the n.O.e. was observed for protons of the Gal residue associated with the inter-unit linkage as well as much lower n.O.e. values for the adjacent protons (H-2 and H-1 in the former and H-3 and H-4 in the latter; Table I). Moreover, the n.O.e. observed for H-3′ on pre-irradiation of H-1″ (Table I) is to be expected on the basis of the molecular model **3** (Fig. 5).

The approach of the non-bonded α-D-Glc and α-L-Rha residues to less than the admissible distances in **3** occurs at ψ_1 >−20° in the Glc-(1→2)-Gal unit and at ψ_2 >0° in the Rha-(1→3)-Gal unit (*cf.* the cross-section in Fig. 6).

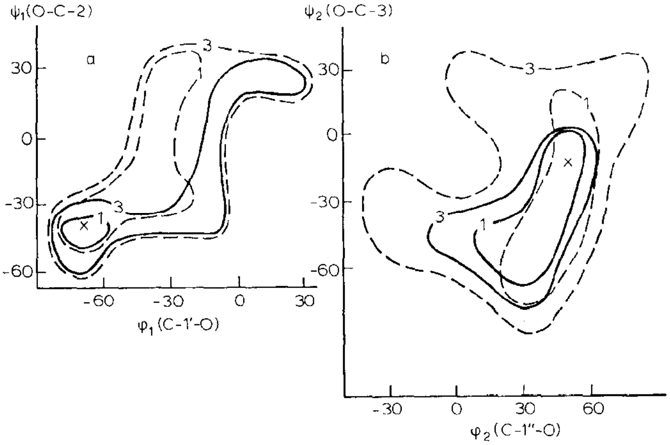

Fig. 6. Cross-sections of the potential surface of α-D-Glc-(1→2)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**3**): (*a*) ϕ_1-ψ_1 (ϕ_2 51.7°, ψ_2 10.0°) and (*b*) ϕ_2-ψ_2 (ϕ_1 −71.8°, ψ_1 −39.7°).

The differences in conformation of the disaccharide units **3** and the corresponding disaccharide glycosides are confirmed by the n.O.e. data (Table I). Thus, the n.O.e. values for H-2′ and H-2 in α-D-Glc-(1→2)-β-D-Gal-OMe, caused by pre-irradiation of H-1′, are each 7% (Table II) whereas, for the Glc-(1→2)-Gal unit of **3**, the n.O.e. for H-2 is much lower than for H-2′ (~7 and 11%, respectively, Table I). This effect is caused by significant increase for **3** of the proportion of conformers with ψ_1 in the range −40°–−50° (Fig. 6a), where H-1′ and H-2 are remote from each other. In contrast, the relative increase of the n.O.e. for H-3 occurs in the Rha-(1→3)-Gal unit. Whereas the ratio of the n.O.e. for H-2″ and H-3 of this unit is 0.5 (Table I), it is close to unity for α-L-Rha-(1→3)-β-D-Gal-OMe (Table II). Such a redistribution of the n.O.e. value shows that, in the Rha-(1→3)-Gal unit of **3**, there is significantly increased probability for negative values of ψ_2, at which H-1″ and H-3 are in closer proximity than when ψ_2 is positive. Attention should also be paid to the responses of H-1 in the Glc-(1→2)-Gal unit and H-4 in the Rha-(1→3)-Gal unit (Table I), which are absent from the disaccharide glycosides[3,4].

The data in Tables I and IV show that the n.O.e. values for H-2 and H-1, observed after pre-irradiation of H-1′, are ~1.5- and ~10-fold lower than those for H-2′. The calculated value agrees well with the above ratios of the n.O.e. (Table IV). The observed ratio of the n.O.e. for H-4 and H-3 caused by pre-irradiation of H-1″ is 0.1 and that calculated is 0.15 (Table IV). The calculated values also accord with the n.O.e. for the non-bonded α-D-Glc and α-L-Rha residues, *i.e.*, for H-1″ and H-3′ [the distance r(H-1″–H-3′) is 3 Å in the preferred conformation (Fig. 5)]. According to experimental and calculated data, the ratio of the n.O.e. for H-3′ and H-3 caused by pre-irradiation of H-1″ is 0.2. Moreover, the n.O.e. spectrum of **3** exhibits enhancement of the signal for H-5′ caused by pre-irradiation of H-1″. This effect was not quantified due to overlapping of the signals for H-5′ and H-4′. Actually, the approach of H-1″ and H-5′ and small deviations of ϕ_1 and ψ_1 from the optimal values (Table III) for **3** are also possible. The ratio of the average values $\langle f^{\text{H-5}'}_{\text{H-1}''}\rangle/\langle f^{\text{H-3}}_{\text{H-1}''}\rangle$ is 0.1 (Table IV), *i.e.*, the n.O.e. for H-5′ is twice that for H-3′.

β-D-Glc-(1→2)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**4**). — After pre-irradiation of H-1′, apart from the n.O.e. in the Glc-(1→2)-Gal unit, an n.O.e. was observed for H-2″ of the Rha residue, which was only 2.5 times lower than that for H-2 (Table I). Such an effect proves the close proximity of the β-D-Glc and α-L-Rha residues, mainly of H-1′ and H-2″, the separating distance of which is 2.8 Å in the preferred conformation (Fig. 7). Theoretical calculation reproduces the n.O.e. for H-2″ caused by pre-irradiation of H-1′. Thus, the ratio of the calculated average values $\langle f^{\text{H-2}''}_{\text{H-1}'}\rangle$ and $\langle f^{\text{H-2}}_{\text{H-1}'}\rangle$ is 0.3 (Table IV), and the experimental ratio is 0.4 (Table I).

The cross-sections of the potential surface of **4** are given in Fig. 8. It follows from those results that the angles ϕ_1 and ψ_1 in the Glc-(1→2)-Gal unit practically coincide with those of β-D-Glc-(1→2)-β-D-Gal-OMe[3], whereas $\psi_2 < 0°$ is prohibited in the Rha-(1→3)-Gal unit but is admissible in α-L-Rha-(1→3)-β-D-Gal-OMe[4]. The

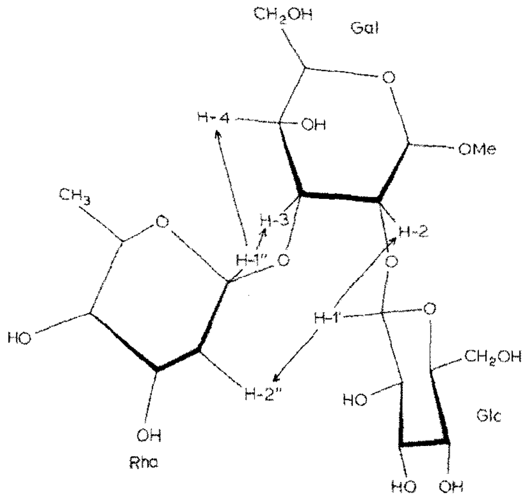

Fig. 7. Molecular model of the preferred conformer of the trisaccharide β-D-Glc-(1→2)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**4**).

first conclusion is confirmed by the constancy of the ratio of the n.O.e. for H-2 and H-2′ caused by pre-irradiation of H-1′ in the Glc-(1→2)-Gal unit (Table I) as compared with the corresponding ratio in the disaccharide glycoside (Table II). In contrast, the n.O.e. for H-3 of the Rha-(1→3)-Gal unit caused by pre-irradiation of H-1″ is 1.5-fold higher than that for H-2″ (Table I), whereas, for the disaccharide glycoside, these effects are almost equal (Table II). It follows from such redistribution of the n.O.e. values that the values $\psi_2 > 0°$ become doubtful in **4**. A similar situation occurs in the unit Rha-(1→3)-Gal of **3**.

It is interesting to compare **4** with β-D-Glc-(1→4)[α-L-Rha-(1→3)]-β-D-Gal-OMe[1]. If $\psi_2 < 0°$ is permitted for the Rha-(1→3)-Gal unit of **4** (Fig. 8b), $\psi_2 > 0°$ is permitted only in the other trisaccharide glycoside (Fig. 4 in ref. 1). These conformational differences are clearly realized in the ratio of the n.O.e. for H-2″ and H-3 [0.7 in the former (Table I) and 1.2 in the latter[1]]. Thus, for disaccharide units of trisaccharide glycosides, the admissible values of ϕ and ψ may correspond to various regions of the conformational map ϕ–ψ of the corresponding disaccharide units. This example shows that disaccharides are not conformationally rigid.

Thus, conformational analysis of **1**-**4** indicates that one conformer is preferred, as found for the 3,4-disubstituted analogues[1]. It follows that the points of branching with vicinal substitution possess unique conformations due to non-bonded interactions of the substituents. The recognition of this phenomenon allows the conformational analysis of branched oligo- and poly-saccharides to be simplified.

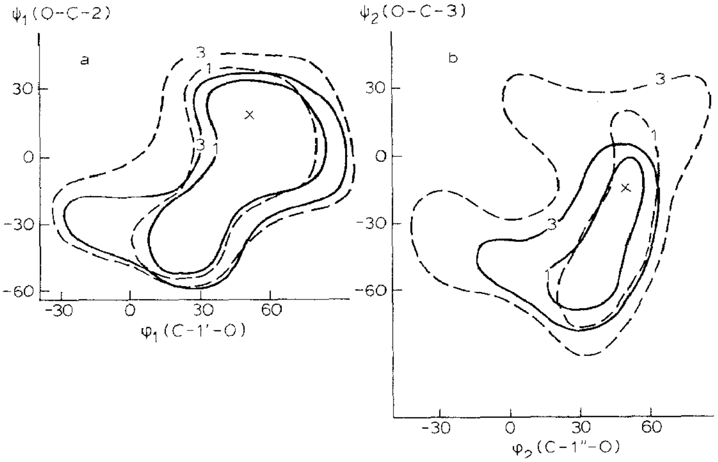

Fig. 8. Cross-sections of the potential surface of β-D-Glc-(1→2)[α-L-Rha-(1→3)]-β-D-Gal-OMe (**4**): (*a*) ϕ_1–ψ_1 (ϕ_2 51.7°, ψ_2 −23.4°) and (*b*) ϕ_2–ψ_2 (ϕ_1 50.7°, ψ_1 17.5°).

EXPERIMENTAL

The ^{1}H-n.m.r. spectra (Tables V and VI) were recorded with a Bruker AM-300 instrument for solutions in D_2O at 30°. The chemical shift data in Table V are given relative to sodium 4,4-dimethyl-4-silapentanesulfonate. The n.O.e. values were measured within the t.O.e. technique[5], with D_1 (pre-irradiation time) 0.5 s and D_2 (the relaxation delay) 0.8 s. The synthesis of **1–4** has been described[6].

TABLE V

CHEMICAL SHIFT DATA[a] AND MULTIPLICITIES IN THE ^{1}H-N.M.R. SPECTRA OF **1–4**

Glycoside	*Unit*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
1	β-D-Gal	4.50d	3.82dd	3.92dd	4.28dd	3.68m	—	—
	α-D-Glc	5.54d	3.51dd	3.69t	3.40dd	3.99ddd	—	—
	α-D-Man	5.09d	3.97dd	3.85dd	3.63t	3.70m	—	—
2	β-D-Gal	4.46d	3.89dd	3.97dd	4.25dd	3.69m	—	—
	β-D-Glc	4.71d	3.27dd	3.48dd	3.40m	3.40m	—	—
	α-D-Man	5.08d	3.98dd	3.91dd	3.66t	3.72m	—	—
3	β-D-Gal	4.48d	3.75dd	3.81dd	3.97dd	—	—	—
	α-D-Glc	5.42d	3.50dd	3.64dd	3.42dd	—	—	—
	α-L-Rha	4.98d	4.03dd	3.78dd	3.43t	3.79dq	1.25d	—
4	β-D-Gal	4.44d	3.82dd	3.89dd	3.97dd	—	—	—
	β-D-Glc	4.67d	3.27dd	3.49t	3.35dd	3.43ddd	3.89dd	3.69dd
	α-L-Rha	5.02d	4.12dd	3.81dd	3.44t	3.81dq	1.26d	—

[a] OMe, 3.57–3.63 p.p.m.

TABLE VI

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

Glycoside	*Unit*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6,6}$
1	β-D-Gal	7.5	9.8	2.9	~1	—	—	—
	α-D-Glc	3.7	9.9	9.9	9.0	2.5	5.5	—
	α-D-Man	1.6	3.5	9.6	9.6	6.2	—	—
2	β-D-Gal	7.6	9.7	2.7	~1	—	—	—
	β-D-Glc	8.1	9.5	9.1	—	—	—	—
	α-D-Man	1.7	3.5	9.5	9.5	6.1	—	—
3	β-D-Gal	7.2	9.8	3.5	~1	—	—	—
	α-D-Glc	3.6	9.6	9.0	9.6	—	—	—
	α-L-Rha	1.7	3.4	9.5	9.5	—	—	—
4	β-D-Gal	7.4	10.0	2.6	~1	—	—	—
	β-D-Glc	7.9	9.1	9.1	8.6	1.8	5.5	12.2
	α-L-Rha	1.7	3.4	9.8	9.8	6.1	—	—

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