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N.m.r. and conformational analysis of some 2,3-disubstituted methyl α-L-rhamnopyranosides*

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ABSTRACT

Conformational studies of the branched trisaccharide glycosides X-(1→2)[Y-(1→3)]-α-L-Rha-OMe (where X and Y are residues of α-L-, β-L-, α-D-, and β-D-hexopyranoses) were based on ^{1}H- and ^{13}C-n.m.r. data (n.O.e.'s, ^{13}C chemical shifts) and theoretical calculations. In the majority of the trisaccharide glycosides, there is insignificant restriction of rotation around the glycosidic linkages in the disaccharide units as compared to the corresponding disaccharide glycosides X-(1→2)-α-L-Rha-OMe and Y-(1→3)-α-L-Rha-OMe. Differences in the conformations observed for several compounds resulted in changes of the n.O.e. patterns and in deviations from additivity of glycosylation effects in the ^{13}C-n.m.r. spectra.

INTRODUCTION

Study of conformations of oligosaccharides on the basis of n.m.r. data (n.O.e., ^{13}C chemical shifts) and theoretical calculations allows stereochemical and structural factors to be identified which determine the conformational states and their spectral features[2–4]. Knowledge of these regularities makes it possible not only to simulate and interpret the ^{13}C-n.m.r. spectra of linear oligo- and poly-saccharides but also to create the data base which is necessary for non-destructive ^{13}C-n.m.r.-based computer-assisted structural analysis of oligo- and poly-saccharides[5–7].

Studies of branched oligo- and poly-saccharides with vicinal substitution showed that their ^{13}C-n.m.r. spectra may differ significantly from those calculated by using an additivity scheme starting from the data for the constituent disaccharide units[8–12]. This fact indicated that, in branched oligo- and poly-saccharides, the same disaccharide units may have different conformations. Therefore, extension of the ^{13}C-n.m.r.-based computer-assisted method for the analysis of branched structures requires data on deviations from additivity in the effects of glycosylation in the ^{13}C-n.m.r. spectra for a wide range of branched oligosaccharides. In this context, the conformational and spectral properties of 3,4-di-*O*-glycosyl derivatives of D-galactopyranoses were studied in detail[9–11,13], and attention has been paid also to several 2,3-di-*O*-glycosyl-β-D-galactopyranosides[9,14,15] and 3,4-di-*O*-glycosyl-β-D-glucopyranosides[12]. The regularities found were

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applied in the ^{13}C-n.m.r.-based structural analysis of several bacterial branched polysaccharides[16,17].

Taking into account the practical importance of the study of conformation and n.m.r. spectra of branched oligosaccharides, we began a systematic investigation of these compounds. Since polysaccharides containing 2,3-disubstituted α-rhamnopyranosyl residues are widespread in Nature, methyl 2,3-di-*O*-glycosyl-α-L-rhamnopyranosides were studied first. In such oligosaccharides, one of two vicinal substituents is axial and the other is equatorial, with neighbouring positions 1 and 4 lacking bulky substituents, *e.g.*, a hydroxymethyl group.

In branched oligosaccharides, the absolute and anomeric configurations of the constituent monosaccharides are essential structural parameters[2,3,18] so that α-L-rhamnopyranosides glycosylated at positions 2 and 3 by α-L-, β-L-, α-D-, and β-D-hexopyranoses have to be studied. Apart from other less important structural factors, the total number of stereochemical combinations is 16 and we now report n.m.r. and conformational studies of a complete series of 2,3-di-*O*-glycosyl derivatives (**1**–**16**) of methyl α-L-rhamnopyranoside of the general formula X-(1→2)-[Y-(1→3)]-α-L-Rha-OMe.

1 X = α-D-Man, Y = α-D-Man
2 X = α-D-Man, Y = β-D-Glc
3 X = β-D-Glc, Y = α-D-Man
4 X = β-D-Glc, Y = β-D-Glc
5 X = α-D-Man, Y = α-L-Rha
6 X = α-D-Man, Y = β-L-Fuc
7 X = β-D-Glc, Y = α-L-Rha
8 X = β-D-Glc, Y = β-L-Fuc
9 X = α-L-Rha, Y = α-L-Rha
10 X = α-L-Fuc, Y = β-L-Fuc
11 X = β-L-Fuc, Y = α-L-Rha
12 X = β-L-Fuc, Y = β-L-Fuc
13 X = α-L-Fuc, Y = α-D-Man
14 X = α-L-Fuc, Y = β-D-Glc
15 X = β-L-Fuc, Y = α-D-Man
16 X = β-L-Fuc, Y = β-D-Glc

Of these oligosaccharides, **1**, **4**, **9**, and **12**, which contain two residues of α-D-Man, β-D-Glc, α-L-Rha, and β-L-Fuc, respectively, have been synthesised[19] and studied earlier[20]. In continuation of this study, 12 more oligosaccharides (**2**, **3**, **5**–**8**, **11**, **13**–**16**) were synthesised[1,21], in which the monosaccharide substituents differ in absolute and/or anomeric configuration. We now report on these oligosaccharides and summarise the conformational regularities for 2,3-di-*O*-glycosylated derivatives of α-L-rhamnopyranose.

RESULTS AND DISCUSSION

The n.O.e.'s observed on pre-irradiation of H-1′ and H-1″ in **1**–**16** are given in Table I (the primes refer to the 2-substituent, and the double-primes to the 3-substituent). For several compounds, n.O.e.'s were observed not only on the resonances of the Rha-OMe residue and the pre-irradiated residue but also on the protons of the non-bonded residue. Thus, on pre-irradiation of H-1′ in **7** and **9**, n.O.e.'s were detected on the resonances of H-3″ and H-5″.

TABLE I

N.O.e. (%) observed on pre-irradiation of anomeric protons of the trisaccharide-glycosides **1–16**

Glycoside	*Irradiation of H-1'*		*Irradiation of H-1''*	
	Observed n.O.e.		*Observed n.O.e.*	
α-D-Man-(1→2)[α-D-Man-	H-2'	7.8(1.3)	H-2'',3	19.2(2)
-(1→3)]-α-L-Rha-OMe (**1**)	H-2	5.8(1)	H-2	9.8(1)
	H-1	7.2(1.2)	H-5'	3.8(0.4)
α-D-Man-(1→2)[β-D-Glc-	H-2'	5.8(1)	H-2''	3.1(0.3)
-(1→3)]-α-L-Rha-OMe (**2**)	H-2	5.8(1)	H-3'',5''	21.2(2)
	H-1	5.8(1)	H-3	10.8(1)
β-D-Glc-(1→2)[α-D-Man-	H-2',3',5'	21.4(3)	H-2''	7.8(1.6)
-(1→3)]-α-L-Rha-OMe (**3**)	H-2	7.1(1)	H-3	4.7(1)
	H-1	0.8(0.1)	H-2	6.1(1.3)
β-D-Glc-(1→2)[β-D-Glc-	H-3',5'	17.0(1.7)	H-3'',5''	19.0(2)
-(1→3)]-α-L-Rha-OMe (**4**)	H-2	9.6(1)	H-3	9.3(1)
α-D-Man-(1→2)[α-L-Rha-	H-2'	7.6(1.2)	H-2''	10.3(1)
-(1→3)]-α-L-Rha-OMe (**5**)	H-2	6.6(1)	H-3	10.6(1)
	H-1	8.6(1.3)		
α-D-Man-(1→2)[β-L-Fuc-	H-2'	7.2(1)	H-3''	6.0(0.7)
-(1→3)]-α-L-Rha-OMe (**6**)	H-2	7.0(1)	H-5''	7.8(0.8)
	H-1	6.2(0.9)	H-3	9.1(1)
			H-2	1.5(0.2)
β-D-Glc-(1→2)[α-L-Rha-	H-2',3',5'	18.3(1.8)	H-2''	8.1(0.8)
-(1→3)]-α-L-Rha-OMe (**7**)	H-2	10.0(1)	H-3	9.5(1)
	H-3'',5''	3.5(0.3)		
β-D-Glc-(1→2)[β-L-Fuc-	H-2',3',5'	21.3(1.8)	H-2''	2.6(0.3)
-(1→3)]-α-L-Rha-OMe (**8**)	H-2	12.0(1)	H-3''	6.1(0.8)
			H-5''	7.5(1)
			H-3	7.5(1)
			H-2	4.4(0.6)
α-L-Rha-(1→2)[α-L-Rha-	H-2',2	14.5(1)	H-2''	8.0(0.8)
-(1→3)]-α-L-Rha-OMe (**9**)	H-1	0.5	H-3	10.6(1)
	H-3'',5''	5.1(0.3)	H-2	2.2(0.2)
α-L-Fuc-(1→2)[β-L-Fuc-	H-2'	11.4(1)	H-3''	5.5(0.8)
-(1→3)]-α-L-Rha-OMe (**10**)	H-2	11.4(1)	H-5''	7.4(1.1)
			H-3	6.7(1)
			H-2	3.5(0.5)
β-L-Fuc-(1→2)[α-L-Rha-	H-2'	3.2(0.4)	H-2''	8.4(0.8)
-(1→3)]-α-L-Rha-OMe (**11**)	H-3'	6.7(0.9)	H-3	10.7(1)
	H-5'	8.7(1.2)		
	H-2	7.1(1)		
	H-1	5.0(0.7)		
β-L-Fuc-(1→2)[β-L-Fuc-	H-3'	6.4(1.0)	H-3''	7.7(0.8)
-(1→3)]-α-L-Rha-OMe (**12**)	H-5'	9.5(1.5)	H-5''	8.5(0.9)
	H-2	6.4(1)	H-3	9.3(1)
	H-1	6.2(1.0)	H-2	3.2(0.3)
α-L-Fuc-(1→2)[α-D-Man-	H-2'	7.4(0.7)	H-2''	7.1(1.3)
-(1→3)]-α-L-Rha-OMe (**13**)	H-2	10.0(1)	H-3	5.7(1)
			H-2	7.6(1.4)
α-L-Fuc-(1→2)[β-D-Glc-	H-2'	11.1(1.1)	H-2''	3.0(0.3)
-(1→3)]-α-L-Rha-OMe (**14**)	H-2	10.1(1)	H-3'',5''	17.5(1.6)
			H-3	11.1(1)
			H-2	1.2(0.1)

(continued)

TABLE I (continued)

Glycoside	*Irradiation of H-1'*		*Irradiation of H-1''*	
	Observed n.O.e.		*Observed n.O.e.*	
β-L-Fuc-(1→2)[α-D-Man-	H-2'	5.7(1.1)	H-2''	7.7(1.2)
-(1→3)]-α-L-Rha-OMe (**15**)	H-3'	8.1(1.4)	H-3	6.5(1)
	H-5'	11.0(2.2)	H-2	9.0(1.4)
	H-2	5.0(1)		
	H-1	5.0(1)		
β-L-Fuc-(1→2)[β-D-Fuc-	H-2'	5.3(0.6)	H-2'',3'',5''	15.3(1.9)
-(1→3)]-α-L-Rha-OMe (**16**)	H-3'	9.1(1.0)	H-3	8.1(1)
	H-5'	11.0(1.3)		
	H-2	8.7(1)		
	H-1	5.1(0.6)		

TABLE II

N.O.e. (%) observed on pre-irradiation of H-1' of the disaccharide glycosides **17–25**

Glycoside	*Observed n.O.e.*						
	H-1	*H-2*	*H-2,2'*	*H-2'*	*H-2',5'*	*H-3'*	*H-5'*
α-L-Rha-(1→2)-α-L-Rha-OMe (**17**)		8.6		5.6			
α-L-Fuc-(1→2)-α-L-Rha-OMe (**18**)	0.6	14.9		11.9			
β-L-Fuc-(1→2)-α-L-Rha-OMe (**19**)	4.1	8.1				4.9	6.7
α-D-Man-(1→2)-α-L-Rha-OMe (**20**)	7.1		15.9				
β-D-Glc-(1→2)-α-L-Rha-OMe (**21**)	1.0	10.5			11.5	8.9	
	H-2	*H-3*	*H-2'*	*H-3'*	*H-5'*	*H-3',5'*	
α-L-Rha-(1→3)-α-L-Rha-OMe (**22**)		11.0	9.1				
β-L-Fuc-(1→3)-α-L-Rha-OMe (**23**)	4.9	9.1		6.0	7.8		
α-D-Man-(1→3)-α-L-Rha-OMe (**24**)	10.0	5.7	7.5				
β-D-Glc-(1→3)-α-L-Rha-OMe (**25**)		4.6	1.8			7.5	

In order to compare the conformations of the disaccharide units in the branched trisaccharide glycosides with those of the corresponding disaccharide glycosides, the values of the n.O.e.'s observed[8,20,22,23] on pre-irradiation of the anomeric protons in the glycosylrhamnopyranosides **17–25** are given in Table II. The data for fucopyranosyl-rhamnopyranoside **22** are novel.

As mentioned above, the conformational features of branched trisaccharides may affect considerably the ^{13}C-n.m.r. spectra. In order to estimate the deviations from additivity for **1–16**, Δ values were calculated which represent the differences between the observed and calculated ^{13}C chemical shifts, *i.e.*, $\Delta = \delta_{exp} - \delta_{calc}$, where $\delta_{calc} = \delta_{X\text{-}(1\rightarrow2)\text{-}\alpha\text{-L-Rha-OMe}} + \delta_{Y\text{-}(1\rightarrow3)\text{-}\alpha\text{-L-Rha-OMe}} - \delta_{\alpha\text{-L-Rha-OMe}}$.

The deviation from additivity of the chemical shifts of the ^{13}C signals for the substituted residue of methyl rhamnopyranoside, as well as for C-1 of the substituents,

TABLE III

Deviations from additivity Δ (p.p.m.) in ^{13}C-n.m.r. spectra of the trisaccharide glycosides **1–16** for C-1–C-6, C-1′, and C-1″ atoms

Glycoside	Δ							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1′*	*C-1″*
1	0.2	0.5	0.2	0.1	0.0	0.1	0.0	0.4
2	0.6	0.5	−1.3	0.2	0.0	−0.3	−0.1	0.5
3	0.3	0.0	0.2	−0.2	−0.1	0.0	0.0	0.4
4	0.6	−0.5	−0.2	−0.4	0.1	0.0	−0.2	0.1
5	0.3	0.3	−2.0	1.2	0.3	0.2	−0.5	−0.3
6	0.6	+1.5	0.7	0.3	−0.1	−0.1	0.4	1.1
7	0.8	−0.2	−0.6	0.6	0.4	0.2	0.0	0.4
8	0.4	−0.8	−0.9	−0.4	−0.1	−0.1	−0.5	−0.5
9	0.6	−0.2	0.2	0.5	0.3	0.6	−0.4	0.3
10	0.5	−1.0	−0.4	−0.3	0.0	0.0	−0.7	−0.2
11	0.2	0.4	−1.0	0.2	0.2	0.5	0.1	0.2
12	0.1	−0.8	0.4	−0.6	−0.2	0.0	−0.3	0.6
13	0.4	−0.4	0.4	−0.2	−0.1	0.0	−0.2	0.5
14	0.5	−1.7	−0.1	−0.5	0.0	−0.2	−1.0	0.2
15	0.4	0.0	−0.8	−0.6	−0.1	0.0	0.8	−0.5
16	0.3	0.0	−1.1	−0.5	0.0	−0.2	0.2	0.2

are given in Table III. The deviations for other ^{13}C resonances were close to zero. The ^{13}C-n.m.r. data for **1–16** and the corresponding disaccharides which were used for the calculation are given in the Experimental section. The data in Table III show that, for the majority of the compounds, the deviations were mostly in the range −1 to +1 p.p.m., *i.e.*, they are comparable to the sum of the experimental errors (0.3–0.5 p.p.m.) in the measurement of the ^{13}C chemical shifts of the disaccharide glycosides. However, for several compounds, the deviations from additivity considerably exceeded the errors, *e.g.*, for **5** and **6** (Table III).

Theoretical conformational analysis of **1–16** was carried out on the basis of molecular mechanics, using atom–atom potential functions. Calculation of the optimal conformations was based on minimisation of the potential energies of the molecules by variation of the angles of rotation around the glycosidic bonds, namely, φ_1 and ψ_1 in the X-(1→2)-α-L-Rha units and φ_2 and ψ_2 in the Y-(1→3)-α-L-Rha units (Fig. 1). These angles are zero for *cis*-orientation of the bonds in the fragments H-1′–C-1′–O–C-2, C-1′–O–C-2–H-2, H-1″–C-1″–O–C-3, and C-1″–O–C-3–H-3. For the angles φ_1, φ_2, ψ_1, and ψ_2, the data on the optimal conformations for the corresponding disaccharides were used as initial approximations (Table IV, see also refs. 22 and 23).

After determination of the lowest-energy conformer (or set of conformers) of **1–16**, the cross-section was drawn of the potential energy 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.

The parametrisation of the force field for calculations of conformations of

TABLE IV

Parameters [angles φ and ψ (°), and energy U (kcal/mol)] of optimal conformations *A*, *B*, and *C* of the disaccharide glycosides **17–25**

Glycoside	*Data*	A	B	C
17	φ, ψ	50, 20	40, −30	−40, −20
	U	−3.4	−3.7	−2.5
18	φ, ψ	60, 30	40, −20	−40, −20
	U	−3.5	−3.7	−2.5
19	φ, ψ	−50, −30	−10, 40	50, 10
	U	−2.7	−2.4	−1.8
20	φ, ψ	−60, −40	−30, 20	20, 20
	U	−4.1	−3.6	−2.0
21	φ, ψ	50, 20	30, −40	−20, −30
	U	−2.8	−2.8	−1.7
22	φ, ψ	60, 20	50, −20	−40, −20
	U	−3.5	−3.6	−2.0
23	φ, ψ	−60, −50	−20, 40	50, 0
	U	−3.1	−2.5	−1.2
24	φ, ψ	−60, −30	−30, 30	30, 20
	U	−4.1	−3.2	−1.4
25	φ, ψ	50, 30	30, −40	−20, −30
	U	−2.7	−2.8	−1.2

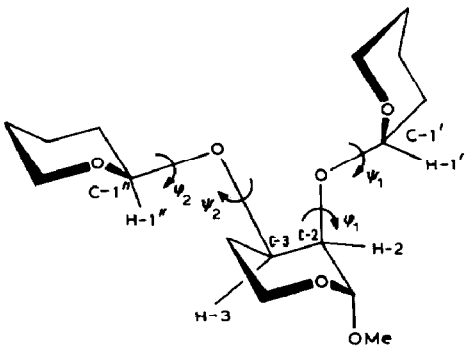

Fig. 1. Models of the trisaccharides X-(1→2)[Y-(1→3)]-α-L-Rha-OMe with designation of the angles of rotation φ_1(C-1′–O) and ψ_1(O–C-2) in the 1,2-linked unit and φ_2(C-1″–O) and ψ_2(O–C-3) in the 1,3-linked unit.

branched trisaccharides has been described[13,14]. The energy of the non-bonded interactions for the disaccharide units of trisaccharide glycosides were calculated using the Scott and Scheraga function[24], whereas those of the non-bonded sugar residues were calculated using the procedures Momany *et al.*[25] or Zhurkin *et al.*[26]. The atom–atom potentials in calculations[27] of the conformations of oligosaccharides in aqueous solutions are those of "medium force". Other details of the calculations of conformations of glycosides of 2,3-di-*O*-glycosyl-α-L-rhamnopyranoses have been described[20].

The conformational analysis of **1–16** allowed the n.O.e. values to be calculated and compared with those observed. The variation of the signal intensity f_s^d for a pro-

ton *d* caused by pre-irradiation of a proton *s* can be calculated using the formula of Shirmer *et al.*[28], taking into account the influence of n.O.e.'s for adjacent protons on the n.O.e. for the proton *d*. In order to compare the calculated and experimental data, the average values $\langle f_s^d \rangle$ of the n.O.e.'s (where s = H-1, H-1', H-1'') were calculated and the Boltzmann probabilities were considered for all possible conformations (for more details, see ref. 20):

$$\langle f_s^d \rangle^* = \left(\frac{\Sigma\Sigma\Sigma\Sigma (f_s^d)_{\varphi_1,\psi_1,\varphi_2,\psi_2} \exp(-\Delta U_{\varphi_1,\psi_1,\varphi_2,\psi_1}/RT)}{\Sigma\Sigma\Sigma\Sigma \exp(-\Delta U_{\varphi_1,\psi_1,\varphi_2,\psi_2}/RT)} \right)_{\phi_1\psi_1\phi_2\psi_2}$$

The optimal values for φ_1, ψ_1, φ_2, and ψ_2 in the lowest-energy conformations for **1–16** are listed in Table V. Table VI contains the calculated average values of the n.O.e.'s.

The trisaccharide glycosides **1–16** can be divided into four groups depending on absolute configurations of the 2- and 3-substituents, *i.e.*, DD, DL, LL, and LD, and, within each group, on their anomeric configurations (αα, αβ, ββ, βα).

TABLE V

Optimal values of φ and ψ (°) in the low-energy conformations of **1–16**

Glycoside	*Conformer*	φ_1(C-1'–O)	ψ_1(O–C-2)	φ_2(C-1''–O)	ψ_2(O–C-3)
1	*AA*	−59.0	−37.6	−61.5	−35.1
2	*AB*	−59.4	−35.0	61.2	−23.3
3	*AA*[a]	57.1	19.7	−61.5	−40.2
4	*AB*[a]	57.7	19.0	53.9	−26.8
5	*AA*	−65.6	−41.0	50.9	29.7
6	*AA*	−61.1	−41.7	−50.1	−17.2
7	*AA*	50.0	20.3	56.2	18.6
	BB	37.9	−34.7	51.5	−26.1
8	*AA*[a]	57.0	15.5	−54.1	−50.3
9	*AA*	55.0	13.5	60.3	15.1
	BB	39.0	−37.1	49.2	−25.1
10	*BA*[a]	42.5	−25.5	−55.7	−48.7
11	*AA*[a]	−57.1	−20.0	60.0	20.1
12	*AA*	−56.9	−25.1	−54.1	−39.0
13	*BA*[a]	42.6	−39.7	−60.9	−40.1
14	*AB*	61.0	18.4	51.0	−37.7
15	*AA*[a]	−57.8	−20.1	−59.7	−39.8
16	*AA*[a]	−51.3	−27.2	50.1	28.8

[a] Energies of conformations *AA*, *AB*, *BA*, and *BB* of trisaccharides are the sums of the energies of the corresponding conformations of the disaccharides (Table IV).

* $\langle f_s^d \rangle$ can also be calculated[29] using $\langle r^{-6} \rangle$ or $\langle r^{-3} \rangle^2$, but, for the inter-residue glycosidic linkages, the three methods gave similar results with deviations less than the errors (±20%) in the determination of n.O.e.'s (see also refs. 30 and 31).

TABLE VI

Calculated ratios of average values of n.O.e.'s

Glycoside	*Proton*	$\frac{\langle f^{a}_{H\text{-}1'} \rangle}{\langle f^{H\text{-}2}_{H\text{-}1'} \rangle}$	*Proton*	$\frac{\langle f^{a}_{H\text{-}1''} \rangle}{\langle f^{H\text{-}3}_{H\text{-}1''} \rangle}$
2	H-2′	1.1	H-2″	0.3
	H-2	1	H-3″,5″	2.0
	H-1	1.2	H-3	1
3	H-2′	0.5	H-2″	1.4
	H-3′,5′	2.2	H-3	1
	H-2	1	H-2	1.3
	H-1	0.1		
5	H-2′	1.1	H-2″	1.0
	H-2	1	H-3	1.0
	H-1	1.4		
7	H-2′	0.4	H-2″	0.9
	H-3′,5′	1.7	H-3	1.0
	H-2	1		
	H-1	0.15		
	H-3″,5″	0.3		
13	H-2′	0.9	H-2″	1.5
	H-2	1	H-3	1
	H-1	0.1	H-2	1.4
	H-1″,2″	0.3		
14	H-2′	1	H-3′,5′	1.8
	H-2	1	H-3	1
			H-2	0.2

[a] Values of $\langle f^{a}_{s} \rangle$ for **1**, **4**, **9**, and **12** are given in ref. 20.

Trisaccharide glycosides (**1–4**) *with* DD *substituents.* — The conformational features of **1** and **4** with two α-D-Man and β-D-Glc substituents, respectively, have been studied previously[20]. Comparison of the values of the n.O.e.'s for the disaccharide units in **1–4** with those of methyl α-D-mannopyranosyl- (**20** and **24**) and β-D-glucopyranosyl-L-rhamnopyranosides (**21** and **25**) (see Tables I and II), and the calculated conformations, indicate that the degrees of conformational freedom are similar. Hence, the ^{13}C chemical shift data for **1** and **4** almost coincided with the values calculated by the additivity scheme (Table III).

The situation is similar in β-D-Glc-(1→2)[α-D-Man-(1→3)]-α-L-Rha-OMe (**3**), where the Glc and Man substituents are remote and do not interact. The similarity of the conformational states of the disaccharide units in **3** and of the disaccharide glycosides **21** and **24** was confirmed by the experimental values of the n.O.e.'s. Thus, in a consideration[22] of conformational properties of β-D-Glc-(1→2)-α-L-Rha-OMe (**21**), it was concluded that the small n.O.e. (1%, Table II) on the resonance of H-1 of the α-L-Rha residue, together with that (10.5%) on H-2 after pre-irradiation of H-1′, is indicative of a wide range of admissible values of ψ from $-60°$ to $+60°$. On pre-irradiation of H-1′ in **3**, n.O.e.'s of the resonances of H-1 and H-2 (0.8 and 7.1%, respectively, Table I) were also detected. Therefore, the conformational situation in the glycosyl-rhamnoside unit

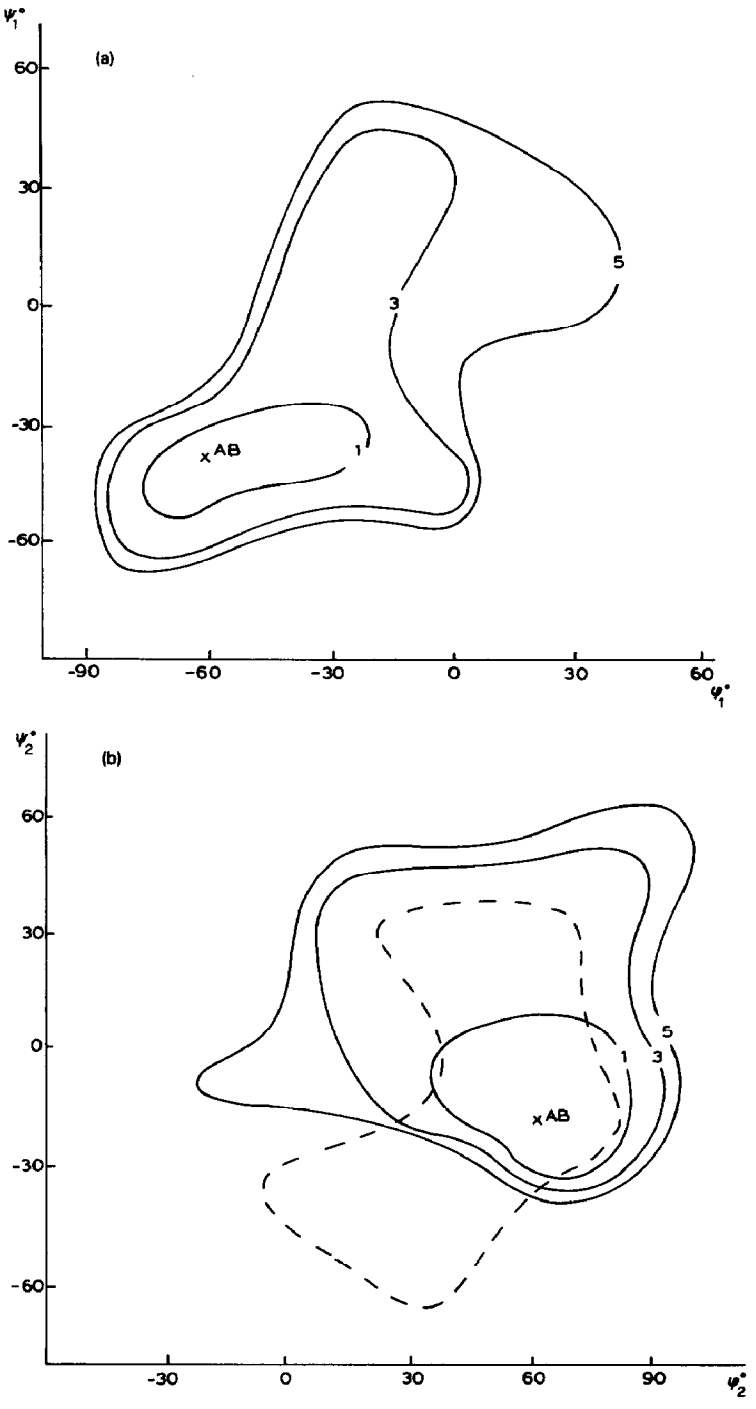

Fig. 2. Cross-sections of the potential surface of α-D-Man-(1→2)[β-D-Glc-(1→3)]-α-L-Rha-OMe (**2**): (*a*) φ_1–ψ_1 (φ_2 61.2°, ψ_2 −23.3°) and (*b*) φ_2–ψ_2 (φ_1 −59.4°, ψ_1 −35.0°) (Table V). The equipotentials of relative energy $\Delta 1$, $\Delta 3$, and $\Delta 5$ kcal/mol are shown: x, local minima; – – –, energy contour $\Delta 1$ kcal/mol for β-D-Glc-(1→3)-α-L-Rha-OMe (**25**, ref. 22).

does not change on going from disaccharide glycoside **21** to the trisaccharide glycoside **3**. Consequently, no marked deviations of the additivity of the ^{13}C chemical shifts were observed for **3** (the Δ values are close to zero, Table III).

In considering α-D-Man-(1→2)-[β-D-Glc(1→3)]-α-L-Rha-OMe (**2**), it may be noted that the φ–ψ conformation map of disaccharide α-D-Man-(1→2)-α-L-Rha-OMe (**20**) contains the optimal conformations *A* (φ, ψ −60, −40°), *B* (−30, 20°), and *C* (20, 20°) with the energies −4.1, −3.5, and −2.0 kcal/mol, respectively]. (Table IV). For β-D-Glc-(1→3)-α-L-Rha-OMe (**25**), there are optimal conformations *A* (50, 20°), *B* (30, −40°), and *C* (−20, 30°) with the energies −2.7, −2.8, and −1.7 kcal/mol, respectively (Table IV). For **2**, four conformers are admissible, namely, *AA*, *AB*, *BA*, and *BB* [the symbols refer to the (1→2)- and (1→3)-linked units, respectively]. Of the four conformers, *AB* (Table V) has the lowest energy and is stabilised additionally by non-bonded interactions of the non-bonded Man and Glc residues. The φ_1–ψ_1 and φ_2–ψ_2 cross-sections of the potential energy surface of **2** are given in Fig. 2. For the (1→2)-linked unit, the lowest-energy region A is in the range ψ_1 −20 to −60° (Fig. 2a) and, for the (1→3)-linked unit, the admissible range is ψ_2 −30 to 0° (Fig. 2b). The ψ_2 range −70 to −30°, which is admissible for the disaccharide glycoside **25** (see the cross-section in Fig. 2b), is prohibited in **2** due to the proximity of the non-bonded β-D-Glc and α-D-Man residues. Therefore, the optimal value of ψ for **25** (−40°) changes to −23° in **2** (Tables IV and V).

The n.O.e.'s on the resonances of H-1, H-2, and H-2′ caused by pre-irradiation of H-1′ in **2** were similar (5.8%, Table I, Fig. 3). The total n.O.e.'s on resonances of H-2 and

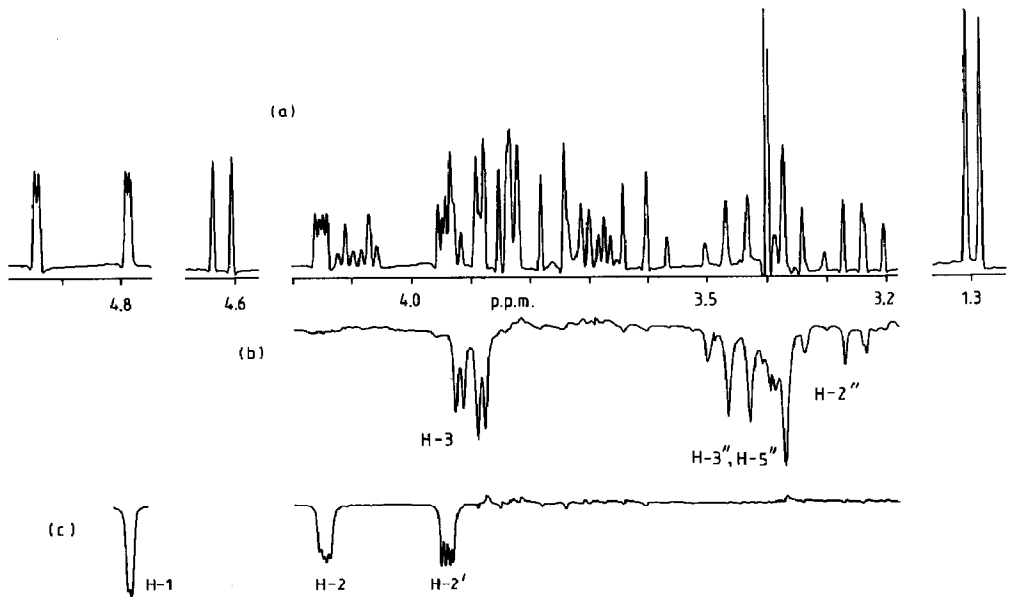

Fig. 3. ^{1}H-N.m.r. spectrum of α-D-Man-(1→2)[β-D-Glc-(1→3)]-α-L-Rha-OMe (**2**) (*a*), and the n.O.e. spectra arising on pre-irradiation of H-1″ (*b*) and H-1′ (*c*).

H-2′ in the disaccharide glycoside **20** were twice as intense as that on the resonance of H-1 (Table II). For **2**, the ratio of these n.O.e. values is also 2:1. Moreover, the average values of the n.O.e.'s arising on pre-irradiation of H-1′ in **2**, calculated on the basis of the φ_1–ψ_1 and φ_2–ψ_2 cross-sections (Fig. 2), are in good agreement with the experimental data. Like the observed values, the calculated average values $\langle f^{H\text{-}2'}_{H\text{-}1'}\rangle$, $\langle f^{H\text{-}1}_{H\text{-}1'}\rangle$, and $\langle f^{H\text{-}2}_{H\text{-}1'}\rangle$ are similar (Table VI).

The pattern of the n.O.e.'s caused by pre-irradiation of H-1″ in **2** also can be accounted for by calculation. The observed n.O.e.'s of the resonances of H-3″ and H-5″ are twice that of the resonance of H-3 (Table I). The ratio of the average values $(\langle f^{H\text{-}3''}_{H\text{-}1''}\rangle + \langle f^{H\text{-}5''}_{H\text{-}1''}\rangle)/\langle f^{H\text{-}3}_{H\text{-}1''}\rangle$ is also 2:1 (Table VI). There are small differences in the n.O.e. values in the (1→3)-linked unit of **2** and in **25**, for which the ratio of the aforementioned n.O.e.'s is smaller (1.6:1, Table II). Such a change showed that, in **25**, H-1″ and H-3 are in closer proximity than in the corresponding unit of **2**. In fact, for **2**, the conformations with ψ_2 −70 to −30° (Fig. 2b), which are unfavourable, correspond to the closest proximity of H-1″ and H-3 [*r*(H-1″–H-3) = 2.3–2.35 Å]. As a result, the interaction of H-1″ and H-3 is weakened and results in a decrease of the α-effects of glycosylation on the corresponding ^{13}C resonances (see ref. 3) and, consequently, a marked displacement of the signal for C-3 in **2** (Δ −1.3 p.p.m., Table III).

Trisaccharide glycosides (**5–8**) *with* DL *substituents.* — In **5–8**, the spatial proximity of the monosaccharide substituents considerably restricts the freedom of rotation of two disaccharide units and is most strongly pronounced in α-D-Man-(1→2)-[α-L-Rha-(1→3)]-α-L-Rha-OMe (**5**). Nevertheless, minimisation of the potential energy of **5** showed that the optimal packing of two of the sugar substituents is possible and is realised in conformation *AA* (Table V, the corresponding molecular model is given in Fig. 4). This is the sole preferred conformation, since minimisation of the energy starting from other initial approximations, which correspond to conformation *AB*, *BA*, *BB*, *AC*, *etc.*, leads to conformer *AA*. In spite of the conformational rigidity of **5**, the

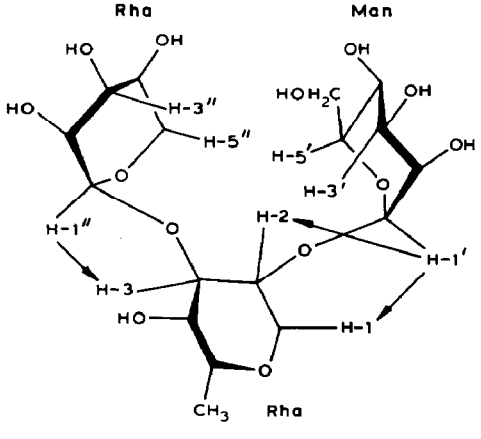

Fig. 4. Molecular model of the preferred conformer *AA* for α-D-Man-(1→2)[α-L-Rha-(1→3)]-α-L-Rha-OMe (**5**). The optimal rotation angles are given in Table III. The protons with the detected n.O.e.'s are denoted with arrows.

angles φ and ψ in its disaccharide units are close to their optimal values in the disaccharide glycosides **20** and **22** (Table IV). In the conformation *AA* of **5**, H-3′ and H-5′ of α-D-Man, and H-3″ and H-5″ of α-L-Rha, are in van der Waals contact (Fig. 4) and the energy of dispersional interactions of these two residues is −2.5 kcal/mol.

The φ_1–ψ_1 and φ_2–ψ_2 cross-sections demonstrate for **5** that, for the α-D-Man-(1→2)-α-L-Rha unit, the lowest-energy region has ψ_1 −40 to −50°, and the same is true for the disaccharide glycoside **20** (Fig. 2, see ref. 22). In the α-L-Rha-(1→3)-α-L-Rha unit, only a ψ_2 value of 30° is admissible and all of the possible conformations of the disaccharide glycoside **22** with ψ_2 values in the range from −70° to 20° are prohibited for **5** (Fig. 5).

The change in the conformation in the α-L-Rha-(1→3)-α-L-Rha unit of **5** resulted in spatial remoteness of H-1″ and H-3 as compared to the situation in **22**. Whereas, in the optimal conformation of this unit in **5**, the distance *r*(H-1″–H-3) is 2.7 Å, at the ψ values −40 to −60°, which are admissible in **22**, this distance is as short as 2.3–2.4 Å, which explains why the average distance ⟨*r*(H-1″–H-3)⟩ in **22** is rather small (2.35 Å). Increase in the distance of separation of H-1″ and H-3 should cause a decrease of the α-effect of glycosylation on the resonance of the carbon of the substituted sugar residue which forms the glycosidic linkage[3] and, as a result, an additional upfield displacement of this signal. The effect is observed for **5** and the deviation from additivity for the

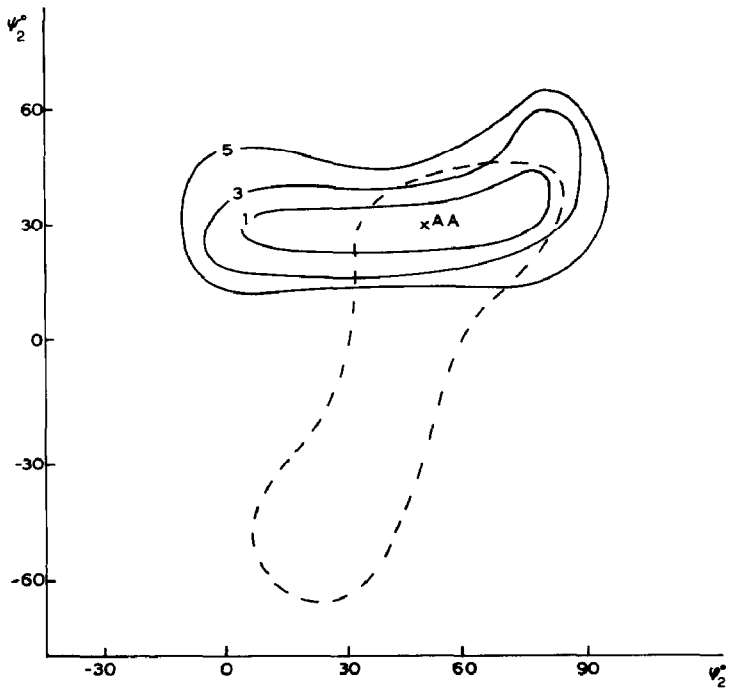

Fig. 5. Cross-section φ_2–ψ_2 of the potential surface of α-D-Man-(1→2)[α-L-Rha-(1→3)]-α-L-Rha-OMe (**5**) (φ_1 −65.6°, ψ_1 −41.0°). See the legend to Fig. 2; – – –, energy contour Δ1 kcal/mol for α-L-Rha-(1→3)-α-L-Rha-OMe (**22**, ref. 23).

resonance of C-3 of the Rha residue at the branching point is −2 p.p.m. (Table III). In contrast, in the α-D-Man-(1→2)-α-L-Rha unit of **5**, according to its φ_1–ψ_1 cross-section, the Δ value for C-2 is close to zero (Table III).

The n.O.e. values (Table I) observed for the resonances of H-2″ and H-3, caused by pre-irradiation of H-1″, indicated the relative remoteness of H-1″ and H-3 as compared to their position in disaccharide glycoside **22**, for which the n.O.e. of the resonance of H-3 is higher than that of the resonance of H-2′ (Table II). Calculation of the average values of the n.O.e.'s for **5** also led to equal values of $\langle f_{H\text{-}1''}^{H\text{-}2''} \rangle$ and $\langle f_{H\text{-}1''}^{H\text{-}3} \rangle$ (Table VI). It is noteworthy that no n.O.e. was observed on the resonance of H-2 of **5**. In contrast, for the trisaccharide **9**, in which the preferred conformer of the α-L-Rha-(1→3)-α-L-Rha unit is *B* (φ, ψ 49, −25°) (Table V), pre-irradiation of H-1″ caused the n.O.e. of the resonance for H-2 (Table I).

α-D-Man-(1→2)-[β-L-Fuc(1→3)]-α-L-Rha-OMe (**6**) is distinguished by the considerable positive deviation of the α-effect of glycosylation on the additivity for C-2 (+1.5 p.p.m., Table III). Such a downfield shift of the signal for C-2 could be rationalised by the spatial proximity of H-1′ and H-2 in the α-D-Man-(1→2)-α-L-Rha unit that results in the increase of α-effect of glycosylation on the resonance of C-2. However, most probably, this is not the explanation since the ratio of the sum of the n.O.e. values for the resonances of H-2 and H-2′ and that of the resonance of H-1 (Table I) is 2.2 in both **20** and **6** (Table I and II). In addition, the φ_1–ψ_1 cross-section for **6** is practically identical to that[22] for **20**.

The chemical shift of the resonance for C-2 in **6** is determined not only by the interaction of H-1′ and H-2, but also by that of H-1″ and H-2. It is well known[3] that, in (1→3)-linked glycosylrhamnosides, an increase of the "γ-gauche" interaction of H-1″ with the equatorial H-2 at the β-position of the aglycon leads to an upfield shift of the signal for C-2. In contrast, a decrease of "γ-gauche" interaction should make the β-effect of glycosylation positive[3], as observed for **6**.

That the positive deviation from the additivity Δ for the resonance of C-2 in **6** is associated with the change of "γ-gauche" interaction in the (1→3)-linked unit followed from the n.O.e. data. Whereas for the disaccharide glycoside **23**, the values of the n.O.e.'s of the resonances of H-3 and H-2 are 9.1% and 5%, respectively (Table II), the corresponding n.O.e. values for **6** are 9% and 1.5% (Table I). The considerable decrease of the n.O.e. of the resonance for H-2 indicated a weakened "γ-gauche" interaction of H-1″ and H-2, and, as a result, the Δ values for the resonance of C-2 as well as that for C-1″ are positive (+1.5 and +1.1 p.p.m., respectively, Table III).

The results of theoretical conformational analysis of **6** are consistent with the n.O.e. and Δ values. A considerable part of region A of the φ–ψ conformational map of **23** was prohibited in **6** due to the non-admissible closeness of the α-D-Man and β-L-Fuc residues. In the φ_2–ψ_2 cross-section of **6** (Fig. 6), ψ_2 values in the range −30 to −60° correspond to high-energy conformations, but these are conformations in which H-1″ and H-2 can be in close proximity up to the distance 2.35–2.4 Å.

The spatial proximity of the monosaccharide substituents in β-D-Glc-(1→2)[α-L-Rha-(1→3)]-α-L-Rha-OMe (**7**) followed unambigously from the n.O.e. data: pre-irradi-

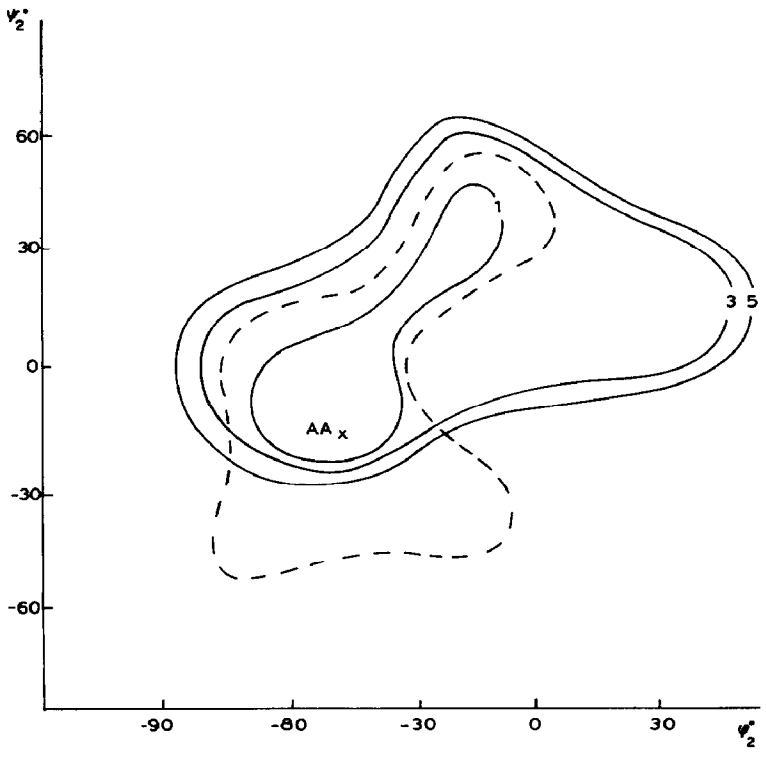

Fig. 6. Cross-section φ_2–ψ_2 of the potential surface of α-D-Man-(1→2)[β-L-Fuc-(1→3)]-α-L-Rha-OMe (**6**) (φ_1 −61°, ψ_1 −41.7°). See the legend to Fig. 2; – – –, energy contour Δ1 kcal/mol for β-L-Fuc-(1→3)-α-L-Rha-OMe (**23**).

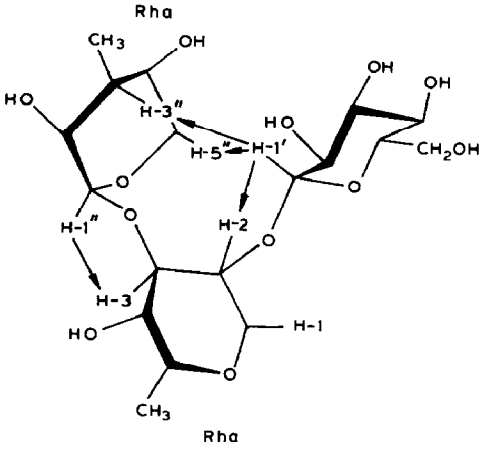

Fig. 7. Molecular model of the conformer *BB* of lowest energy for β-D-Glc-(1→2)[α-L-Rha-(1→3)]-α-L-Rha-OMe (**7**). See the legend to Fig. 4.

ation of H-1′ caused marked n.O.e.'s of the resonances of H-3″ and H-5″ (3.5% in sum, Table I).

Minimisation of the potential energy of **7** revealed the optimal low-energy conformers *AA* and *BB* (Table V). In conformation *AA*, H-1′ and H-3″ are in close proximity, as are H-1′ and H-5″ in conformation *BB* (Fig. 7). The observation of the n.O.e.'s on the resonances of both H-3″ and H-5″, caused by pre-irradiation of H-1′, is a result of an equilibrium of these two conformers. Calculation of the average values of the n.O.e.'s showed that, in accordance with the experimental data, the sum $\langle f_{H\text{-}1'}^{H\text{-}3''} \rangle + \langle f_{H\text{-}1'}^{H\text{-}5''} \rangle$ is three times less than $\langle f_{H\text{-}1'}^{H\text{-}2} \rangle$ (Table VI).

The conformational states of two disaccharide units in **7** are interdependent, as reflected in the φ_1–ψ_1 cross-sections of the potential energy surface on the assumption that the α-L-Rha-(1→3)-α-L-Rha unit has the conformation *A* or *B* (Fig. 8). In each situation, only one local minimum, namely, *AA* (Fig. 8a) or *BB* (Fig. 8b), is revealed. In each of the disaccharide units of **7**, both low-energy conformations *A* and *B* of the disaccharide glycosides **21** and **23** are possible and, hence, their conformational distributions are similar.

This conclusion is proved by the n.O.e. data. Thus, in the α-Rha-(1→3)-α-L-Rha unit of **7** and in the disaccharide glycoside **22**, the ratios of the n.O.e.'s on the resonances H-2″ and H-3 are 0.8 (Tables I and II). Analogously, in the β-Glc-(1→2)-α-L-Rha unit and in the disaccharide glycoside **21**, the ratios of the sum of the n.O.e.'s on the resonances of H-2′, H-3′, and H-5′ and the n.O.e. of the resonance of H-2 are equal (~2).

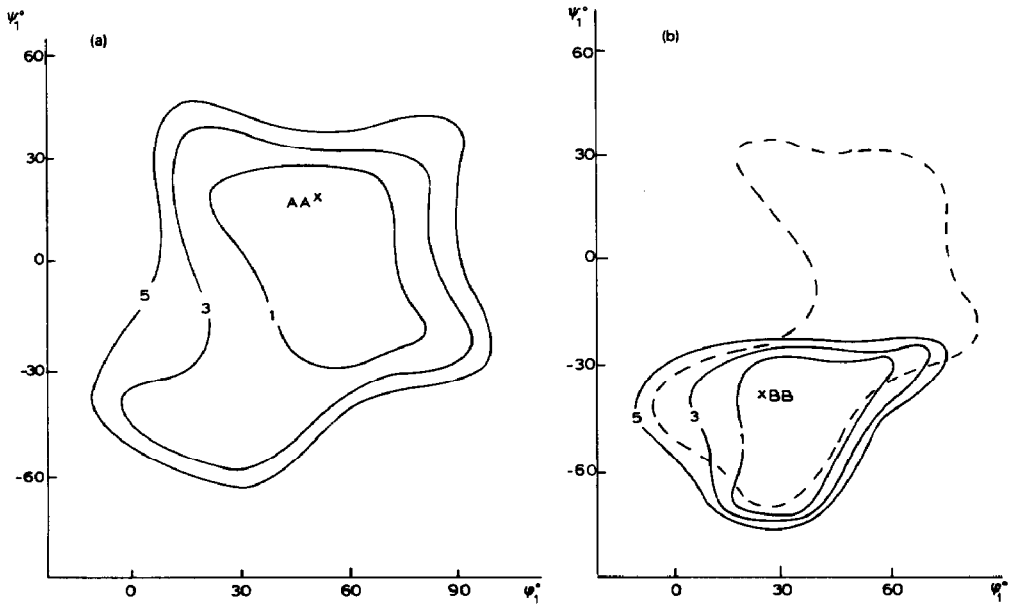

Fig. 8. Cross-section φ_1–ψ_1 of the potential surface of β-D-Glc-(1→2)[α-L-Rha-(1→3)]-α-L-Rha-OMe (**7**) [φ_2 56.2°, ψ_2 18.6° (*a*); φ_2 51.5°, ψ_2 −26.1° (*b*)]. See the legend to Fig. 2; – – –, energy contour Δ1 kcal/mol for β-D-Glc-(1→2)-α-L-Rha-OMe (**21**, ref. 22).

For this reason, the deviations of Δ in **7** are small (< 1 p.p.m., Table III). Calculation of the average values of the n.O.e.'s (Table VI) led to similar ratios.

Because both substituents in **8** are β, there are no van der Waals contacts between the non-bonded residues. Consequently, the regularities in the n.O.e. pattern are similar to those in the disaccharide glycosides **21** and **23** (Tables I and II). The Δ values in the ^{13}C-n.m.r. spectrum of **8** are also small (Table III).

Trisaccharide glycosides (**9–12**) *with* LL *substituents.* — Deviations from the additivity in the ^{13}C-n.m.r. spectra of **9–12** are small (< 1 p.p.m., Table III), which indicates the conformations of the disaccharide units and the corresponding disaccharide glycosides to be similar. This situation has been exemplified in a detailed consideration[20] of **9** and **12**. An analogous situation occurs in **10** and **11**. For example, in the β-L-Fuc-(1→3)-α-L-Rha unit of **10**, as in **23**, pre-irradiation of H-1″ caused n.O.e.'s of the resonances of H-2 and H-3, the latter being almost half that of the former (Tables I and II). Likewise for **11**, the conformational state of the α-L-Rha-(1→3)-α-L-Rha unit can be judged on the basis of the ratio (0.8) of the n.O.e.'s for resonances of H-2″ and H-3 (Table I). The same ratio is observed for **22** (Table II).

Trisaccharide glycosides (**13–16**) *with* LD *substituents.* — The pyranose rings of the sugar substituents in **13–16** and the vectors of the O–C′ and O–C″ bonds are in opposite directions with respect to the disubstituted α-L-Rha residue, and the substituents do not interact significantly. As a result, the energies of the optimal conformations of **13–16** are the sums of the energies of the disaccharide units. For example, the energies of conformers *A* (φ, ψ 50, 20°) and *B* (40, −30°) for α-L-Fuc-(1→2)-α-L-Rha-OMe (**18**) are −3.4 and −3.7 kcal/mol, respectively (Table IV). The difference in the energies of optimal conformations *AA* and *BA* for α-L-Fuc-(1→2)-[α-D-Man-(1→3)]-α-L-Rha-OMe (**13**) is also 0.3 kcal/mol. The absence of any interdependence of the conformational states of the two disaccharide units results in the φ_1–ψ_1 and φ_2–ψ_2 cross-sections of **13–16** being similar to those of the corresponding disaccharide glycosides. As a consequence, the relative n.O.e. values are also similar. For example, the ratio of the n.O.e. values for the resonances of H-2′ and H-2 is 0.7 in **13** (Tables I and IV) and 0.8 in **18** (Table II). In addition, in the α-D-Man-(1→3)-α-L-Rha unit of **13**, as in the disaccharide glycoside **24**, the n.O.e. on the resonance of H-2 is much higher than that of the resonance of H-3 (Tables I and II). As expected, the Δ values in the ^{13}C-n.m.r. spectrum of **13** are close to zero (Table III). Similar Δ values are also observed for **15** and **16**.

For **13–16**, the only anomaly which deserves consideration is a deviation from additivity of −1.7 p.p.m. for the resonance of C-2 in α-L-Fuc-(1→2)-[β-D-Glc-(1→3)]-α-L-Rha-OMe (**14**) (Table III). However, despite the high Δ value for the resonance of C-2, **14** is not an exception in this series since there is no restriction of rotation in the (1→2)-linked disaccharide unit.

The φ_1–ψ_1 cross-section contains a minimum in the region *A* of the conformational map and the φ_2–ψ_2 cross-section contains one in the region *B*. Evidently, attraction of the non-bonded residues deepened the minimum *AB* on the potential surface of **14**. After minimisation of potential energy of **14**, it was found that the conformer *AB* was preferred (Table V) due to the effective dispersional interactions of

the α-L-Fuc and β-D-Glc residues. The total energy of these interactions is −1.5 kcal/mol. This value also represents the relative gain in energy of conformation *AB* as compared to conformations *AA*, *BA*, and *BB*.

The molecular model of the preferred conformation *AB* of **14** is shown in Fig. 9. In conformation *A* (φ_1, ψ_1 61, 18.4°, Table V) of the α-L-Fuc-(1→2)-α-L-Rha unit, the distance between H-1′ and H-2 is 2.7 Å, which exceeds the average distance $\langle r$(H-1′–H-2)$\rangle$ of 2.4 Å in **18**. Evidently, the resulting decrease of the α-effect of glycosylation in this unit of **14** leads to marked deviations from additivity for both the resonances of C-2 and C-1′ (−1.7 and −1.0 p.p.m., respectively, Table III). The n.O.e. data also confirmed the relative remoteness of H-1′ and H-2 in **14**. Whereas in the disaccharide glycoside **18**, the n.O.e. of the resonance of H-2 is higher than that of the resonance of H-2′ (Table II), in **14**, the reverse pattern is observed, *i.e.*, the n.O.e. on the resonance of H-2 is lower than that on the resonance of H-2′ (Table I).

The stabilisation of conformer *B* (φ, ψ 51, −37.7°, Table V) in the (1→3)-linked unit of **14** is also shown by the n.O.e. results. Whereas in the disaccharide glycoside **25**, there was no n.O.e. of the resonance of H-2 (Table II), pre-irradiation of H-1″ in **14** caused n.O.e.'s of the resonances of both H-3 and H-2 (Table I). The appearance of the response of the resonance of H-2 can be recognised easily in the n.O.e. spectrum of **14** (Fig. 10). Since the close spatial proximity of H-1″ and H-2 and the n.O.e. of the resonance of H-2 can occur only in conformations of the region *B* of φ–ψ conformational map of the disaccharide glycoside **25** (ψ −30 to −60°), the significant statistical weight of conformer *B* in the (1→3)-linked unit of **14** is beyond doubt. Statistical calculation of the average values $\langle f^{H\text{-}2}_{H\text{-}1''}\rangle$ and $\langle f^{H\text{-}3}_{H\text{-}1''}\rangle$ (Table VI) reproduced the observed ratio (1:10) of the n.O.e.'s of the resonances of these protons in **14**. Conformer *B* is of high energy according to calculation using the HSEA method[32].

Thus, conformational analysis of the series of 2,3-disubstituted rhamnose trisaccharide glycosides revealed the conformational origin of the observed deviations

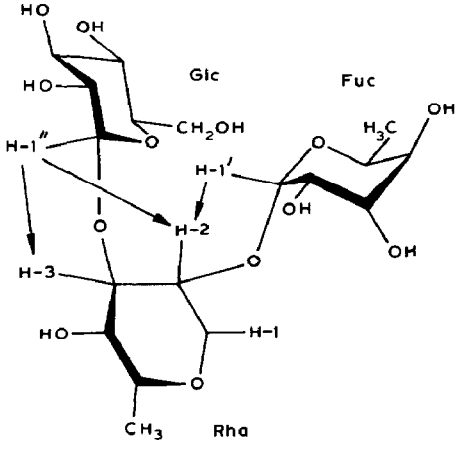

Fig. 9. Molecular model of the preferred conformer *AB* for α-L-Fuc-(1→2)[β-D-Glc-(1→3)]-α-L-Rha-OMe (**14**). See the legend to Fig. 4.

TABLE VII

^{1}H-N.m.r. data (δ in p.p.m., J in Hz) for trisaccharides **1**–**16** (D_2O)

Compound	*Residue*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*OMe*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1	α-D-Man-(1→2)	4.97	3.94	3.84	3.70	3.69	3.82	3.82		1.8	3.4	9.9	9.9			
	α-D-Man-(1→3)	5.01	3.92	3.86	3.68	3.84	3.82	3.82		1.6	3.5	9.9	9.9			
	α-L-Rha-OMe	4.86	4.19	3.89	3.54	3.71	1.30		3.39	1.7	3.5	9.6	9.6	6.3		
2	α-D-Man-(1→2)	4.94	3.94	3.87	3.74	4.10	3.83	3.83		1.6	3.1	9.5	9.5	3.0	3.0	0
	β-D-Glc-(1→3)	4.62	3.23	3.47	3.28—3.42		3.69	3.91		7.6	8.0	8.0		5.0	1.5	12.1
	α-L-Rha-OMe	4.79	4.15	3.91	3.61	3.71	1.30		3.40	1.5	3.2	9.5	9.5	5.7		
3	β-D-Glc-(1→2)	4.53	3.33	3.45	3.38—3.47		3.72	3.90		7.5	8.7	8.7		5.0	3.2	12.0
	α-D-Man-(1→3)	5.02	3.97	3.88	3.71	3.77————		3.87		1.7	3.2	9.2	9.2			
	α-L-Rha-OMe	4.97	4.23	3.90	3.57	3.70	1.32		3.40	1.6	3.1	9.0	9.0	5.8		
4	β-D-Glc-(1→2)	4.67	3.36	3.52	3.41—3.48		3.92	3.75		7.9	9.1	9.1		1.7	4.4	12.9
	β-D-Glc-(1→3)	4.71	3.33	3.46	3.41—3.48		3.89	3.72		8.0	9.1	9.1		1.7	4.5	12.8
	α-L-Rha-OMe	4.94	4.30	3.96	3.66	3.74	1.32		3.41	1.7	3.1	9.4	9.4	6.0		
5	α-D-Man-(1→2)	4.98	3.92	3.87	3.66	3.86	3.81	3.92		1.6	3.1	9.0	9.0	3.6	2.0	
	α-L-Rha-(1→3)	5.13	4.04	3.73	3.43	3.78	1.28			1.5	3.1	9.5	9.5	6.0		
	α-L-Rha-OMe	4.85	4.02	3.91	3.62	3.72	1.29		3.40	1.6	2.8	9.1	9.1	6.0		
6	α-D-Man-(1→2)	4.98	3.94	3.87	3.63	3.99	3.75	3.88		1.6	3.2	9.6	9.6	6.0	2.4	12.5
	β-L-Fuc-(1→3)	4.45	3.53	3.64	3.74	3.79	1.24			7.5	9.7	3.3	<1	6.2		
	α-L-Rha-OMe	4.86	4.15	3.95	3.58	3.70	1.32		3.40	1.7	3.1	9.5	9.5	6.0		
7	β-D-Glc-(1→2)	4.58	3.32	3.46	3.38—3.48		3.71	3.88		7.5	9.0	9.0		3.6	1.7	12.4
	α-L-Rha-(1→3)	5.05	4.04	3.77	3.46	3.75	1.30			1.5	3.2	9.7	9.7	6.0		
	α-L-Rha-OMe	4.96	4.07	3.90	3.61	3.72	1.30		3.39	1.5	3.0	9.4	9.4	6.0		
8	β-D-Glc-(1→2)	4.67	3.33	3.47	3.36—3.47		3.71	3.89		7.5	8.6	8.6		5.4	1.4	12.0
	β-L-Fuc-(1→3)	4.48	3.54	3.66	3.75	3.78	1.25			7.5	9.7	3.4	<1	6.3		
	α-L-Rha-OMe	4.96	4.23	4.00	3.61	3.71	1.32		3.40	1.6	3.0	9.0	9.0	5.8		

9	α-L-Rha-(1→2)	5.01	4.01	3.80	3.45	3.75	1.29			1.6	3.4	9.6	9.6	6.2		
	α-L-Rha-(1→3)	5.06	4.06	3.73	3.48	3.71	1.32			1.7	3.4	9.6	9.6	6.2		
	α-L-Rha-OMe	4.80	4.01	3.90	3.59	3.73	1.32		3.42	1.6	3.4	9.6	9.6	6.2		
10	α-L-Fuc-(1→2)	5.13	3.76	3.91	3.82	4.13	1.21			3.8	10.6	3.4	<1	6.8		
	β-L-Fuc-(1→3)	4.47	3.53	3.67	3.76	3.79	1.26			7.8	10.8	3.3	<1	6.5		
	α-L-Rha-OMe	4.84	4.07	4.01	3.65	3.73	1.34		3.41	1.8	3.2	9.5	9.5	5.8		
11	β-L-Fuc-(1→2)	4.39	3.47	3.62	3.72	3.72	1.27			7.5	8.6	3.5	<1	6.5		
	α-L-Rha-(1→3)	5.08	4.04	3.86	3.45	3.88	1.28			1.5	3.2	9.3	9.3	6.0		
	α-L-Rha-OMe	4.81	4.08	3.87	3.56	3.72	1.30		3.41	1.5	3.2	9.3	9.3	6.0		
12	β-L-Fuc-(1→2)	4.45	3.51	3.62	3.74	3.76	1.26			7.9	9.8	3.4	1.0	6.6		
	β-L-Fuc-(1→3)	4.50	3.54	3.65	3.73	3.78	1.25			7.9	9.8	3.4	1.0	6.4		
	α-L-Rha-OMe	4.85	4.23	3.92	3.55	3.71	1.32		3.41	1.8	3.6	9.7	9.7	6.2		
13	α-L-Fuc-(1→2)	5.03	3.75	3.89	3.80	4.09	1.21			3.8	10.0	3.0	1.0	6.6		
	α-D-Man-(1→3)	4.99	3.96	3.87	3.70	3.86	3.75—3.82			1.7	3.0	9.1	9.1			
	α-L-Rha-OMe	4.82	4.11	3.92	3.61	3.73	1.33		3.42	1.8	3.0	9.4	9.4	6.0		
14	α-L-Fuc-(1→2)	5.20	3.73	3.89	3.80	4.09	1.19			3.8	10.1	3.1	<1	6.5		
	β-D-Glc-(1→3)	4.65	3.32	3.50	3.40—3.47		3.73	3.90		7.6	8.5	8.5		3.6	1.4	11.5
	α-L-Rha-OMe	4.77	4.18	3.95	3.68	3.75	1.32		3.41	1.6	3.0	9.2	9.2	5.6		
15	β-L-Fuc-(1→2)	4.34	3.48	3.61	3.70	3.70	1.25			7.5	8.6	3.3	<1	6.3		
	α-D-Man-(1→3)	5.11	3.98	3.86	3.69	3.70—3.84				1.5	3.1	8.8	8.8			
	α-L-Rha-OMe	4.81	4.25	3.88	3.47	3.73	1.32		3.40	1.5	3.1	9.6	9.6			
16	β-L-Fuc-(1→2)	4.47	3.51	3.61	3.72	3.74	1.25			7.4	9.7	3.5	<1	6.4		
	β-D-Glc-(1→3)	4.69	3.34	3.49	3.35	3.41	3.70	3.91		7.5	9.0	9.0	9.0	3.5	1.9	12.0
	α-L-Rha-OMe	4.82	4.27	3.93	3.72	3.72	1.31		3.40	1.2	3.0	9.1	9.1	6.1		

TABLE VIII

^{13}C-N.m.r. data for trisaccharides **1**–**16** (D_2O; δ in p.p.m.)

Compound	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OMe*
1	α-D-Man-(1→2)	99.5	71.65	71.65	67.85	74.5	62.2	
	α-D-Man-(1→3)	97.95	71.75	71.9	67.8	74.0	62.2	
	α-L-Rha-OMe	98.9	72.15	74.7	71.75	70.1	18.15	56.35
2	α-D-Man-(1→2)	99.4	71.5	71.7	67.6	73.6	61.8	
	β-D-Glc-(1→3)	105.5	75.0	77.0	71.1	77.2	62.2	
	α-L-Rha-OMe	99.2	76.1	79.2	72.8	70.1	17.9	56.1
3	β-D-Glc-(1→2)	105.45	74.5	77.0	70.8	77.2	62.0	
	α-D-Man-(1→3)	97.9	71.5	71.8	67.9	74.1	62.15	
	α-L-Rha-OMe	101.1	76.6	75.4	71.8	69.7	18.0	56.2
4	β-D-Glc-(1→2)	105.3	74.75	77.0	70.8	77.2	62.0	
	β-D-Glc-(1→3)	105.1	74.8	77.0	70.9	77.2	62.0	
	α-L-Rha-OMe	101.3	80.0	81.0	72.5	69.8	18.1	56.25
5	α-D-Man-(1→2)	103.0	71.6	71.5	68.0	74.5	62.4	
	α-L-Rha-(1→3)	99.0	71.2	71.5	73.4	70.2	17.8[a]	
	α-L-Rha-OMe	98.7	75.5	76.0	73.7	70.2	17.9[a]	56.2
6	α-D-Man-(1→2)	99.9	71.7	71.6	68.1	74.2	62.4	
	β-L-Fuc-(1→3)	103.5	71.7	74.2	72.6	72.5	16.7	
	α-L-Rha-OMe	99.2	75.1	79.4	72.3	70.0	18.1	56.2
7	β-D-Glc-(1→2)	105.5	74.7	77.0	70.9	77.2	62.0	
	α-L-Rha-(1→3)	103.7	71.5	71.5	73.4	70.75	17.9[b]	
	α-L-Rha-OMe	101.3	80.4	78.1	73.4	70.05	18.05[b]	56.3
8	β-D-Glc-(1→2)	105.0	74.6	76.9	70.85	77.15	62.0	
	β-L-Fuc-(1→3)	101.8	71.9	74.2	72.6	72.2	16.6	
	α-L-Rha-OMe	101.1	77.8	78.5	71.9	69.6	18.0	56.15
9	α-L-Rha-(1→2)	103.05	71.6	71.45	73.4	70.75	18.1	
	α-L-Rha-(1→3)	103.6	71.45	71.6	73.3	70.55	18.0	
	α-L-Rha-OMe	100.9	78.8	78.8	73.3	70.2	18.1	56.3

10	α-L-Fuc-(1→2)	102.0	69.9	70.9	73.2	68.6	16.8	
	β-L-Fuc-(1→3)	102.2	72.0	74.2	72.7	72.4	16.7	
	α-L-Rha-OMe	101.1	77.7	79.1	72.0	69.9	18.2	56.3
11	β-L-Fuc-(1→2)	103.8	71.8	74.2	72.8	72.2	17.2	
	α-L-Rha-(1→3)	103.5	71.4	71.4	73.3	70.4	17.4[c]	
	α-L-Rha-OMe	100.0	78.8	77.0	73.3	70.1	18.0[c]	56.2
12	β-L-Fuc-(1→2)	103.45	71.85	74.2	72.7	72.6	16.9	
	β-L-Fuc-(1→3)	103.0	71.65	74.2	72.7	72.5	16.8	
	α-L-Rha-OMe	100.0	76.0	79.05	71.95	69.95	18.05	56.1
13	α-L-Fuc-(1→2)	102.5	69.8	70.8	73.1	68.6	16.7	
	α-D-Man-(1→3)	97.9	71.5	71.8	67.9	74.1	62.1	
	α-L-Rha-OMe	101.1	76.2	75.7	71.8	69.9	18.1	56.4
14	α-L-Fuc-(1→2)	101.7	69.8	70.8	73.1	68.5	16.6	
	β-D-Glc-(1→3)	105.2	74.8	76.9	70.7	77.0	61.9	
	α-L-Rha-OMe	101.1	79.1	81.2	72.4	69.9	18.0	56.2
15	β-L-Fuc-(1→2)	104.5	71.7	74.2	72.8	72.0	17.0	
	α-D-Man-(1→3)	96.9	71.4	72.0	68.0	73.9	62.2	
	α-L-Rha-OMe	100.55	74.8	73.7	71.7	69.85	18.0	56.3
16	β-L-Fuc-(1→2)	103.9	71.7	74.1	72.7	72.3	16.8	
	β-D-Glc-(1→3)	105.2	74.8	76.9	71.1	77.3	62.3	
	α-L-Rha-OMe	100.35	78.8	79.4	72.7	69.9	17.9	56.1

[a-c] Assignments may be interchanged.

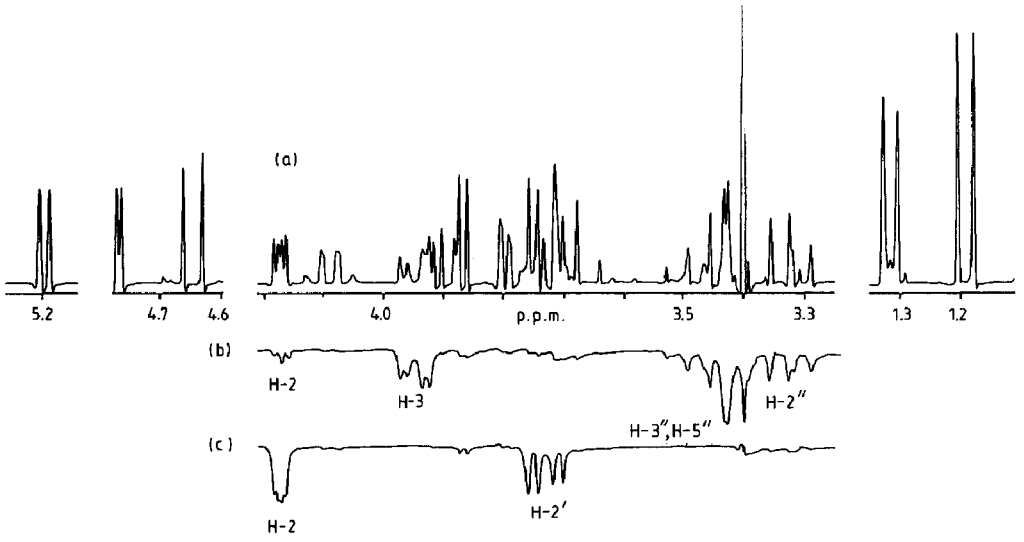

Fig. 10. ^{1}H-N.m.r. spectrum of α-L-Fuc-(1→2)[β-D-Glc-(1→3)]-α-L-Rha-OMe (**14**) (*a*), and the n.O.e. spectra arising on pre-irradiation of H-1'' (*b*) and H-1' (*c*).

from the additivity of the α-effects of glycosylation in the ^{13}C-n.m.r. spectra and the regularities in the n.O.e. spectra. The results allow computer-assisted structural analysis of regular branched polysaccharides with rhamnose or mannose residues at the branch points.

EXPERIMENTAL

The 1D- and 2D-n.m.r. experiments were performed on solutions in D_2O at 40–45°, using Bruker WM-250 (for ^{1}H) and AM-300 (for ^{13}C) spectrometers with acetone as the reference (^{1}H, 2.225 p.p.m.; ^{13}C, 31.45 p.p.m.). Assignments of the ^{1}H-n.m.r. spectra (Table VII) were accomplished using a combination of COSY and RCT 2D experiments (COSYRCT of the standard Bruker software for ASPECT-2000). The HDO signal was supressed upon the relaxation delay (1 s). D2 was 33 ms (0.25J; J 7.5 Hz). The spectral windows were ~500 Hz (the region for the pyranose ring protons only). Data sets consisting of 256 t_1 experiments each with 512 data points were zero-filled to 512 data points. Prior to Fourier transformation, the FIDs were multiplied with a sine-bell window function (not shifted).

The n.O.e. experiments were performed in the difference mode with the t.O.e. technique[33] with D_1 0.1 s and an irradiation time $C_X(D_2 + D_5) = 10 \times (0.1 + 0.005)$ s, using the NOEMULT-programme of the standard Bruker software. The pre-irradiated signal area in the difference spectra was taken as 100%. The n.O.e.'s are expressed as the ratio of integrated intensities of the observed and saturated proton resonances.

The assignments of the ^{13}C-n.m.r. spectra (Table VIII) were made by using 2D ^{1}H–^{13}C correlated spectroscopy (XHCORRD programm of the Bruker software for

TABLE IX

^{13}C-N.m.r. data for disaccharides **17–25** and methyl α-L-rhamnopyranoside (**26**) (D_2O; δ in p.p.m.)

Compound	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OMe*
17	α-L-Rha-(1→2)	103.5	71.5	71.6	73.5	70.5	17.9	
	α-L-Rha-OMe	101.0	79.8	71.5	73.6	70.0	17.9	56.1
18	α-L-Fuc-(1→2)	102.7	69.9	70.8	73.2	68.6	16.7	
	α-L-Rha-OMe	101.1	81.2	71.7	73.7	69.9	18.0	56.3
19	β-L-Fuc-(1→2)	103.7	71.7	74.05	72.65	72.3	16.8	
	α-L-Rha-OMe	100.45	79.2	70.9	73.95	69.9	17.9	56.2
20	α-D-Man-(1→2)	99.5	71.7	71.7	68.1	74.2	62.3	
	α-L-Rha-OMe	99.1	76.0	70.9	73.4	70.05	18.0	56.25
21	β-D-Glc-(1→2)	105.5	74.8	77.0	70.7	77.2	62.1	
	α-L-Rha-OMe	101.2	81.0	71.6	73.7	69.7	17.9	56.1
22	α-L-Rha-(1→3)	103.3	71.1	71.1	73.0	70.0	17.6	
	α-L-Rha-OMe	101.8	70.8	79.0	72.4	69.6	17.6	56.1
23	β-L-Fuc-(1→3)	102.35	71.9	74.2	72.7	72.4	16.8	
	α-L-Rha-OMe	101.95	69.2	79.7	71.9	69.65	18.15	56.15
24	α-D-Man-(1→3)	97.5	71.6	71.75	67.85	73.9	62.1	
	α-L-Rha-OMe	102.05	67.2	75.45	71.6	69.75	18.05	56.1
25	β-D-Glc-(1→3)	105.0	74.9	77.3	71.1	77.2	62.2	
	α-L-Rha-OMe	102.0	71.2	81.5	72.5	69.7	18.2	56.1
26	α-L-Rha-OMe	102.5	71.6	71.9	73.3	69.7	18.0	56.0

ASPECT-3000). The typical spectral window was 500 Hz for ^{1}H and 5000 for ^{13}C (the region of pyranose ring protons or carbon atoms only, respectively). Data sets consisting of 256 t_1 experiments each with 2K data points were zero-filled to 512 data points. For detection of ^1J correlations, O3 was set to 3.2 ms and D4 to 1.6 ms (0.5J and 0.25J, respectively; J 155 Hz). FIDs were multiplied with a square sine-bell window function (shifted at $\pi/2$) in both directions, prior to Fourier transformation. The ^{13}C-n.m.r. data for the disaccharide glycosides **17–25**, used to calculate the Δ values, are given in Table IX. The syntheses of **1–6** and **11–14** are reported elsewhere[1,21].

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