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An n.m.r. and conformational analysis of the terminal trisaccharide from the serologically active glycolipid of *Mycobacterium leprae* in different solvents

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ABSTRACT

The ^{1}H- and ^{13}C-n.m.r. spectra of allyl 2-*O*-[4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl]-3-*O*-methyl-α-L-rhamnopyranoside (**3**), a glycoside of the terminal trisaccharide found in the phenolic glycolipid I from *Mycobacterium leprae*, and those of the two component disaccharides, allyl 4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranoside (**1**) and allyl 2-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranoside (**2**) have been assigned completely by 1D and 2D techniques. The preferred conformations, determined by chemical shift and n.O.e. studies, were different in D_2O, CD_3OD, and $CDCl_3$. The preferred conformation of **3** accorded with the results of hard-sphere exo-anomeric (HSEA) calculations.

INTRODUCTION

The structure of the phenolic glycolipid I, isolated from infected livers of the armadillo, has been determined[1,2], and synthesis studies have shown that the 3,6-di-*O*-methyl-β-D-glucopyranose unit is the major immunological determinant[3,4]. Although work has been initiated in order to develop synthetic antigens in a form suitable for enzyme-linked immunoabsorbent assay (ELISA)[5–9], few spectroscopic data are available and the conformational properties of the antigen are unknown.

We now report a complete n.m.r. spectroscopic assignment of allyl 4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranoside (**1**), allyl 2-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranoside (**2**), and allyl 2-*O*-[4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl]-3-*O*-methyl-α-L-rhamnopyranoside (**3**), together with the data from model compounds, and the results are discussed in relation to the preferred conformation inferred

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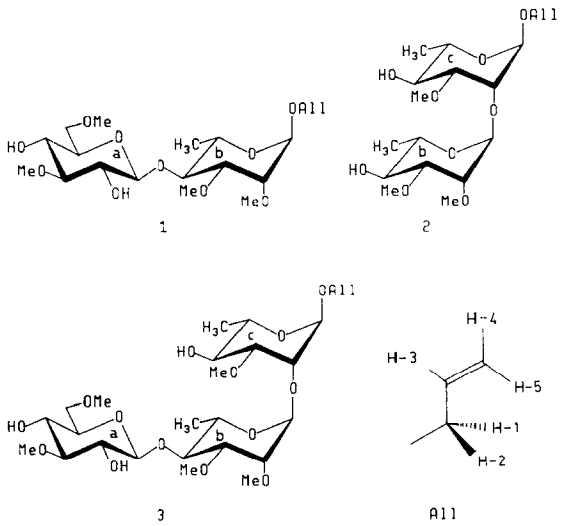


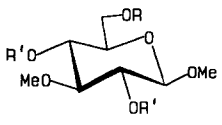
from hard-sphere exo-anomeric (HSEA) calculations. The high degree of methylation in **1–3** allowed the conformations in organic solvents and water to be investigated.

RESULTS AND DISCUSSION

The reference compounds methyl 3,6-di-*O*-methyl-β-D-glucopyranoside (**4**), methyl 2,3-di-*O*-methyl-α-L-rhamnopyranoside (**9**), and methyl 3-*O*-methyl-α-L-rhamnopyranoside (**12**) were synthesized, using standard procedures, by methylation of appropriately protected monosaccharide derivatives of methyl 3-*O*-methyl-β-D-glucopyranoside and methyl α-L-rhamnopyranoside, respectively.

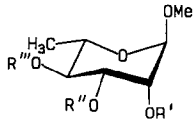
The ^{1}H- and ^{13}C-n.m.r. spectra (D_2O and $CDCl_3$) of the above compounds were assigned on the basis of H/H and C/H-correlation experiments, and $J_{H,H}$ values were obtained where second-order effects and/or spectral crowding did not impede the analysis. Normally, it is assumed that significant changes in the conformation will be reflected in the magnitude of the J values as well as in the chemical shifts of the ^{1}H and ^{13}C resonances, so that comparison with reference compounds is a valid approach in conformational analysis[10]. The J values for rigid molecules are independent of the solvent and, consequently, changes with change in solvent are a reflection of the conformation only. N.O.e. measurements also are independent of the bulk magnetic susceptibility of the solvent and constitute a good method for assignment of structures in different solvents[11]. The absolute magnitudes of the chemical shifts are dependent on the solvent, but these effects will not be discussed here.

The ^{13}C-n.m.r. data for **1–3** are given in Table I, and comparison between **3** and the three monosaccharide units **4**, **9** and **12** are given in Table II. The data in Table I show that, for a given solvent, there can be only small changes in the conformation of the individual units in **1–3** because of the good agreement in chemical shifts for



4 R = Me, R' = H

5 R = Me, R' = OAc

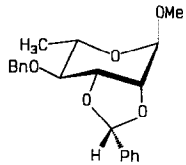


6 R', R'' = CMe_2 , R''' = H

7 R' = R'' = H , R''' = Bn

8 R' = R'' = Me , R''' = Bn

9 R' = R'' = Me , R''' = H



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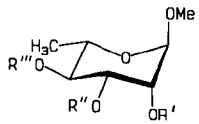

11 R' = R''' = Bn , R'' = H

12 R' = R''' = H , R'' = Me

TABLE I

^{13}C-n.m.r. data for **1–3**[a]

Atom	*Solvent D_2O*			*$CDCl_3$*			*CD_3OD*		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
3,6-Di-O-methyl-β-D-glucopyranosyl group									
C-1a	103.7		103.7	105.3		105.5	104.9		104.9
C-2a	73.7		73.7	74.8		74.9	75.5		75.5
C-3a	86.0		86.0	85.3		85.4	87.6		87.6
C-4a	69.9		69.9	71.0		71.1	71.2		71.2
C-5a	75.2		75.2	73.8		73.9	76.8		76.8
C-6a	71.6		71.6	72.6		72.7	73.0		73.0
2,3-Di-O-methyl-α-L-rhamnopyranosyl residue									
C-1b	100.2	99.2	99.1	95.8	98.4	98.1	97.6	100.3	100.2
C-2b	76.6	76.7	76.6	75.6	75.7	75.7	77.8	78.1	77.7
C-3b	80.6	79.8	80.2	81.6	80.5	81.5	82.4	81.9	82.1
C-4b	79.1	72.0	79.1	80.4	67.7	80.0	79.4	73.1	79.2
C-5b	67.9	69.8	68.3	67.4	68.6	68.0	69.2	70.4	69.1
C-6b	17.7	17.4	17.4	17.3	17.4	17.4	18.2	18.0	18.2
3-O-Methyl-α-L-rhamnopyranosyl residue									
C-1c		98.1	98.1		98.1	98.0		99.3	99.3
C-2c		74.9	74.9		71.7	71.7		75.7	75.8
C-3c		80.5	80.5		81.5	81.6		82.3	82.3
C-4c		72.0	72.0		71.5	71.8		73.4	73.4
C-5c		69.7	69.7		68.2	68.2		70.1	70.1
C-6c		17.4	17.8		17.5	17.6		18.1	18.1

[a] Measured at 125.7 MHz at 27°.

TABLE II

Chemical shift differences[a] (△) for the ^{13}C resonances of **3** and the model compounds **4**, **9**, and **12**

Atom	D_2O			$CDCl_3$		
Methyl 3,6-di-O-methyl-β-D-glucopyranoside (**4**)						
	3	**4**	△	**3**	**4**	△
C-1a	103.7	104.0	−0.3	105.5	103.7	1.8
C-2a	73.7	73.1	0.6	74.9	74.2	0.7
C-3a	86.0	86.0	0.0	85.4	85.5	−0.1
C-4a	69.9	69.9	0.0	71.1	70.8	0.3
C-5a	75.2	75.1	0.1	73.9	73.7	0.2
C-6a	71.6	71.7	−0.1	72.7	72.4	0.3
Methyl 2,3-di-O-methyl-α-L-rhamnopyranoside (**9**)						
	3	**9**	△	**3**	**9**	△
C-1b	99.1	98.4	0.7	98.1	98.2	−0.1
C-2b	76.6	76.5	0.1	75.7	75.9	−0.2
C-3b	80.2	80.2	0.0	81.5	81.1	0.4
C-4b	79.1	72.0	7.1	80.0	71.6	8.4
C-5b	68.3	69.1	−0.8	68.0	68.1	−0.1
C-6b	17.4	17.4	0.0	17.4	17.7	−0.3
Methyl 3-O-methyl-α-L-rhamnopyranoside (**12**)						
	3	**12**	△	**3**	**12**	△
C-1c	98.1	101.6	−2.5	98.0	100.6	−1.4
C-2c	74.9	66.4	8.5	71.7	66.9	4.8
C-3c	80.5	80.5	0.0	81.6	81.5	0.1
C-4c	72.0	71.7	0.3	71.8	71.4	0.4
C-5c	69.7	69.2	0.5	68.2	67.7	0.5
C-6c	17.8	17.4	0.4	17.6	17.7	−0.1

[a] Measured at 125.7 MHz at 27°.

identically substituted positions. The notion that each unit can be treated as an independent entity is substantiated further by the data presented in Table II, where the differences in chemical shifts between the resonances for the monosaccharide units in **3** and the methyl glycosides of the individual units were $\leqslant 0.7$ p.p.m. for all carbons which are not directly affected by the glycosylation. There was no solvent effect, but the downfield shift observed on glycosylation of C-2c was significantly different in D_2O (8.5 p.p.m.) and $CDCl_3$ (4.8 p.p.m.). The glycosylation shifts observed for solutions in D_2O are in fair agreement with the values reported by Lipkind *et al.*[12].

The ^1H-n.m.r. data are given in Table III, and chemical shift differences between the resonances of **3** and the model glycosides are given in Table IV. The *J* values, where available, are similar not only for **1**–**3** but also to those of the model compounds. Moreover, there were no significant differences between the three solvents, indicating that $^4C_1$ or $^1C_4$ conformations were unpertubed in **1**–**3**. Only small differences in the chemical shifts of the resonances between the units in **1**–**3** were observed especially for the terminal units a and c (see formulae **1**–**3**).

TABLE III

^{1}H-n.m.r. data for **1–3** [a]

Atom	*Solvent D_2O*			*$CDCl_3$*			*CD_3OD*		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
3,6-Di-O-methyl-β-D-glucopyranosyl group									
H-1a	4.66		4.65	4.42		4.41	4.54		4.53
	7.5		7.5	7.7		7.5	7.8		7.8
H-2a	3.34		3.32	3.42		3.41	3.20		3.20
				9.1			9.2		9.2
H-3a	3.31		3.30	3.16		3.16	3.08		3.08
	9.1		9.0	8.4					8.7
H-4a	3.51		3.49	3.53		3.53	3.33		3.34
				9.5					
H-5a	3.51		3.49	3.42		3.41	3.31		3.31
				4.5					
H-6a(a)	3.66		3.66				3.57		3.57
							5.0, 11.1		5.1, 11.1
				3.63		3.63			
H-6b(a)	3.78		3.78				3.68		3.68
							1.9		1.9
2,3-Di-O-methyl-α-L-rhamnopyranosyl residue									
H-1b	5.06	5.15	5.15	4.89	5.08	5.06	4.88	5.03	5.03
	1.9	1.9	1.8	1.7	1.7	1.8	1.8	1.8	1.9
H-2b	3.85	3.94	3.92	3.65	3.68	3.70	3.66	3.71	3.74
	3.4	3.5	3.1		3.2	2.7		2.8	2.7
H-3b	3.71	3.52	3.72	3.68	3.42	3.59	—	3.36	3.60
	9.5	9.6	9.5		9.5				
H-4b	3.63	3.41	3.63	3.66	3.54	3.54	3.62	3.36	3.60
	9.5	9.5	9.5		9.5	8.9			
H-5b	3.80	3.73	3.77	3.69	3.67	3.69	3.61	3.63	3.68
	6.2	6.4	5.9		6.2	6.2	5.7	6.3	6.2
H-6b	1.32	1.32	1.32	1.35	1.32[b]	1.33	1.26	1.21	1.23
3-O-Methyl-α-L-rhamnopyranosyl residue									
H-1c		5.00	5.00		4.82	4.77		4.86	4.83
		1.8	1.5		1.8	1.8		1.8	1.8
H-2c		4.24	4.24		4.11	4.07		4.05	4.04
		3.1	3.0		2.9	2.8		2.6	2.3
H-3c		3.59	3.58		3.46	3.45		3.43	3.42
		9.3	9.8		9.3				
H-4c		3.52	3.52		3.55	3.52		3.42	3.41
		9.3	9.8		9.3	8.9		9.2	
H-5c		3.77	3.77		3.67	3.64		3.59	3.60
		6.4	5.9		6.2	6.2		6.2	6.3
H-6c		1.26	1.32		1.30[b]	1.31		1.25	1.25

[a] Measured at 500.0 MHz at 27°: the ^3J values given in Hz (~0.3 Hz) below the chemical shifts refer to the next higher-numbered proton. [b] Assignments may be reversed.

TABLE IV

Chemical shift differences[a] (△) for the ^1H resonances in **3** and the model compounds **4**, **9**, and **12**

Atom	D_2O			$CDCl_3$		
Methyl 3,6-di-O-methyl-β-D-glucopyranoside (**4**)						
	3	**4**	△	**3**	**4**	△
H-1a	4.65	4.39	0.26	4.41	4.18	0.23
H-2a	3.32	3.33	−0.01	3.41	3.41	0.00
H-3a	3.30	3.29	0.01	3.16	3.18	−0.02
H-4a	3.49	3.48	0.01	3.53	3.55	−0.02
H-5a	3.49	3.58	−0.09	3.41	3.43	−0.02
H-6a(a)	3.66	3.64	0.02	3.63	3.69	−0.06
H-6b(a)	3.78	3.79	−0.01			
Methyl 2,3-di-O-methyl-α-L-rhamnopyranoside (**9**)						
	3	**9**	△	**3**	**9**	△
H-1b	5.15	4.90	0.25	5.06	4.77	0.29
H-2b	3.92	3.82	0.10	3.70	3.62	0.08
H-3b	3.72	3.45	0.27	3.59	3.42	0.17
H-4b	3.63	3.40	0.23	3.54	3.57	−0.03
H-5b	3.77	3.67	0.10	3.69	3.62	0.07
H-6b	1.32	1.28	0.04	1.33	1.32	0.01
Methyl 3-O-methyl-α-L-rhamnopyranoside (**12**)						
	3	**12**	△	**3**	**12**	△
H-1c	5.00	4.75	0.25	4.77	4.72	0.05
H-2c	4.24	4.19	0.05	4.07	4.07	0.00
H-3c	3.58	3.41	0.17	3.45	3.38	0.07
H-4c	3.52	3.48	0.04	3.52	3.52	0.00
H-5c	3.77	3.70	0.07	3.64	3.65	−0.01
H-6c	1.32	1.31	0.01	1.31	1.33	−0.02

[a] Measured at 500.0 MHz at 27°.

The differences in the chemical shifts of the resonances of **1**–**3** and those of the corresponding monosaccharides are sensitive indicators of the changes in the surroundings of the glycosylated position caused by the glycosylation. Downfield shifts can be ascribed to van der Waals contact between protons and oxygens, whereas upfield shifts are caused by proton–proton interactions. These differences are interpreted most readily with the aid of hard-sphere exo-anomeric (HSEA) calculations[13–16].

The results of HSEA calculations on 4-*O*-β-D-glucopyranosyl-3-*O*-methyl-α-L-rhamnopyranose and 3-*O*-methyl-2-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranose, in which MeO-3 was given rotational freedom, are presented in Fig. 1. Calculations on 2-*O*-(4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranose gave similar results for the glycosidic linkages, indicating that the absence of OMe groups from the rhamnose residues in the latter molecule has little effect on the conformational preference. Values for the φ_H (H-1–C-1–O-1–C-X) and ψ_H (C-1–O-1–C-X–H-X) angles of the minimum energy conformation and selected inter-residue distances <3 Å are given in Table V. Methyl 4-*O*-β-D-glycopyranosyl-α-L-rhamnopyranoside has been

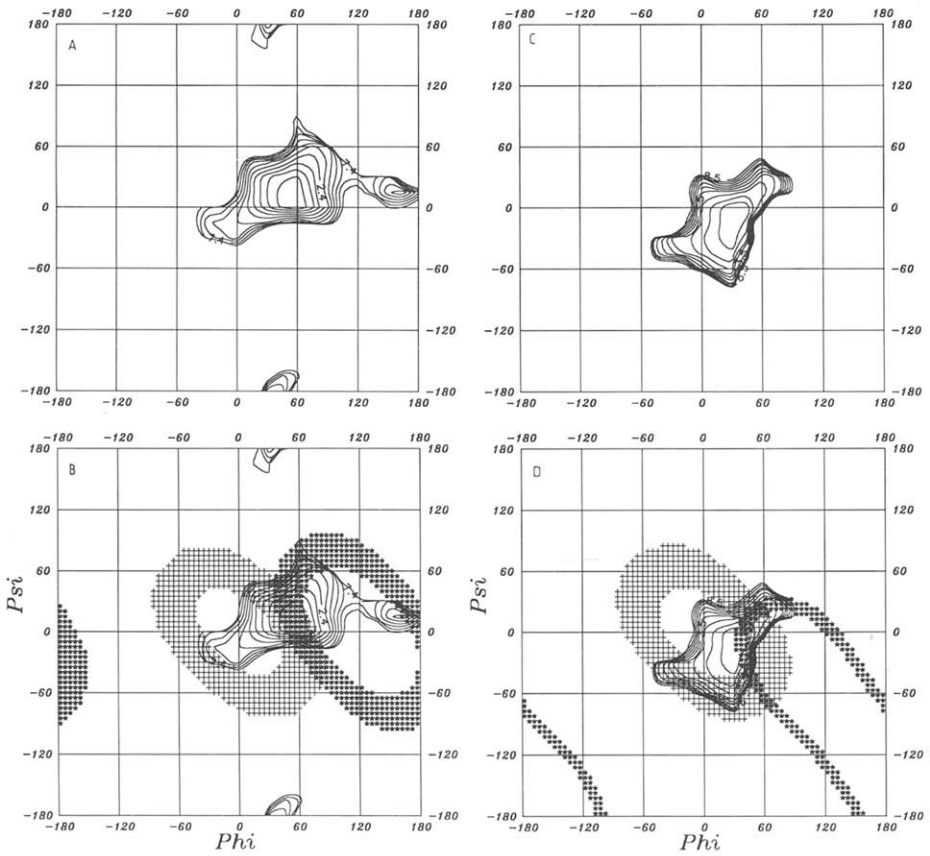

Fig. 1. β-D-Glc*p*-(1→4)-3-OMe-α-L-Rha*p*: A, Isoenergy contour map (1 kcal/mol spacing between contours) for the glycosidic linkage between the a and b units; B, distance constraints for H-1a/H-4b 2.47 +/− 0.25Å (+), and H-4b/O-5a 2.51 +/− 0.25Å (*), as a function of φ_H and ψ_H; α-L-Rha*p*-(1→2)-3-OMe-α-L-Rha*p*: C, isoenergy contour map (1 kcal.mol^{-1} spacing between contours) for the glycosidic linkage between the b and c units; D, distance constraints for H-1b/H-2c 2.45 +/− 0.25Å (+), and H-5b/H-1c 2.33 +/−0.25Å (*), as a function of φ_H and ψ_H.

TABLE V

Values for φ and ψ of the minimum energy conformation and selected inter-residue atomic distances <3 Å obtained by HSEA calculations

Unit	φ_H	ψ_H	
β-D-Glc-(1→4)-3-OMe-α-L-Rha	56	7	H-1a–H-4b (2.47), H-1a–O-3b (2.94), O-5a–H-4b (2.51), O-5a–H-6b (2.73)
α-L-Rha-(1→2)-3-OMe-α-L-Rha	48	17	H-1b–H-2c (2.45), H-1b–O-3c (2.59), H-5b–H-1c (2.33), O-5b–H-2c (2.59)
Allyl α-L-Rha	52	180[a]	

[a] ψ is here defined as the angle C-1–O-1–C-1all–C-2all (all = allyl).

investigated by two research groups. Backman *et al.*[16] found a global minimum (φ/ψ 55/10) similar to the present results (56/7), and the shielding effects observed in D_2O at 85° were also similar to most of the data presented here, the major difference being a more pronounced upfield shift of the resonance of H-5a of the reducing unit. This deviation, as well as those of the resonances for the anomeric protons, are most likely due to differences in the aglycons. The contour map published by Lipkind *et al.*[17] is in fair agreement with the present data; however, the HSEA calculations do not indicate that the conformer having $\varphi/\psi \sim 180/35$ should carry a statistical weight of ~30%. The present experimental data suggest that this minimum is not populated to any significant degree. In the study by Lipkind *et al.*[19], an n.O.e. of ~3% was found for H-3b and H-5b on irradiation of H-1a; however, in the present study, only a small effect (0.9%) was observed for H-3b in **1** and none was observed for H-5b.

No significant glycosylation shift for H-2c was observed for solutions of **2** and **3** in D_2O, although a normal shielding on the adjacent proton was observed. The HSEA calculations indicate that a small glycosylation shift should be expected when comparing the distances from H-2c to the nearest proton and oxygen atoms, to the appropriate distances for H-4b. However, a shift of the observed magnitude was not expected based on these calculations. The resonance of H-2 of the "reducing unit" of 8-methoxycarbonyloctyl 2-*O*-*α*-L-rhamnopyranosyl-*α*-L-rhamnopyranoside was found 0.03 p.p.m. upfield compared with that of H-2 in methyl *α*-L-rhamnopyranoside, whereas the resonance of H-2 in the terminal unit was shifted downfield by 0.17 p.p.m.[15]. A small downfield shift (0.05 p.p.m.) was found for the resonance of H-2c, whereas a downfield shift (0.10 p.p.m.) was observed for the resonance of H-2b in **3**. The glycosylation shifts for solutions in $CDCl_3$ are generally smaller than those observed in D_2O and, most notably, no significant effect on glycosylation was observed for the resonances of the protons (H-4b and H-2c) attached to the glycosylated carbons but glycosylation effects were observed for the resonances of H-3b, H-1c, and H-3c. The upfield position of the resonance of H-1c relative to that of H-1b can be explained by the van der Waals contact between H-1c and H-5b, which was indicated by the HSEA calculations and proved by n.O.e. experiments.

The ^{13}C chemical shift of the resonance C-1b in **1** exhibited unusual variations with change of the solvent. In D_2O, a downfield shift of ~1.0 p.p.m. was observed relative to the resonances of C-1b in **2** and **3**, whereas an upfield shift of ~2.5 p.p.m. was observed in $CDCl_3$ and CD_3OD. Such differences could be caused by changes in conformation at the glycosidic linkage[16], but n.O.e. experiments (Table VI) were not sufficiently accurate to detect these small changes in torsion angles. However, the experimentally determined conformation of the allyl group was in good accordance with the HSEA calculations.

As HSEA calculations alone might not be able to distinguish accurately between more energetically favored conformations, introduction of some experimentally determined constraints on the allowed φ–ψ space is necessary in order to assess the preferred conformers. These constraints can be established by n.O.e. experiments and through observation of shielding effects caused by van der Waals contact between protons and oxygen[15,19].

TABLE VI

N.O.e. data for **1–3**[a]

Irradiated proton	*Observed proton*	D_2O[b]			CD_3OD			$CDCl_3$		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
H-1a	H-3a	6.3	—	+	6.0	—	3.4	6.8	—	5.9
	H-5a	10.1	—	+	n[c]	—	4.5	10.9	—	9.4
	H-3b	0.9	—	n	n	—	n	n	—	n
	H-4b	8.3	—	0.6	16.3	—	4.7	13.5	—	11.4
H-1b	H-2b	4.3	3.2	0.7	8.8	3.5	1.9	6.8	8.8	10.3
	H-2c	—	5.3	1.6	—	6.0	3.7	—	3.7	10.9
	H-1all[d]	1.8	—	—	2.7	—	—	1.3	—	—
	H-2all	4.3	—	—	10.3	—	—	5.8	—	—
H-1c	H-2c	—	8.0	0.4	—	5.8	6.3	—	6.2	4.1
	H-5b	—	2.9	0.4	—	6.0	—	6.5	5.1	
	H-2all	—	2.8	0.3	—	6.9	5.3	—	7.3	4.8
H-2c	H-1b	—	n	n	—	8.8	11.2	—	16.1	n
H-3all[e]	H-5all	2.8	1.4				1.2			
	H-1all	1.0	1.6				1.0			
	H-2all	0.6	0.9				0.9			
H-5all[e]	H-3all	4.1	2.8				2.5			
H-1all[e]	H-3all	3.6	2.2				2.2			
	H-2all	8.3	10.4		8.6		15.5			
	H-1b	1.5	n		2.0		1.2			
	H-5b	0.8			n		3.1			
	H-1c	1.9								
	H-5c	1.3								
H-2all[e]	H-3all	2.8	1.3		1.6		0.9			
	H-4all	1.7	1.4				1.1			
	H-1all	12.5	11.9		9.6		13.4			
	H-1b	4.8	n		3.2		4.2			
	H-1c	3.5								
	H-6c	0.8								

[a] Performed in the difference mode. [b] Spectrometer was locked on internal acetone-d_6 ($\sim$ 10% v/v). [c] Not detected or available due to crowding. [d] all = allyl group. [e] These experiments were performed using the procedure of Kinns and Saunders[29].

The relevant results of the n.O.e. experiments are shown in Table VI. The n.O.e. observed between H-1a and H-3a and H-5a indicates that the 3,6-di-*O*-methyl-β-D-glucopyranosyl group adopts the $^4C_1$(D) conformation, corroborating the $^3J_{H,H}$ data. Data for the rhamnose residues are more scarce, but the observed n.O.e. values are in full accord with a $^1C_4$(L) conformation. Irradiation of each anomeric proton caused a strong inter-unit n.O.e. This interaction can be taken as a rough indication that the

inter-proton distance is ~2.5 Å. A significant n.O.e. between H-1c and H-5b was observed for the rhamnose residues in **2** and **3**. As the distance between these protons is sensitive to changes around the glycosidic linkage, this interaction provides a serious restraint on the allowed φ–ψ space. In Fig 1b, the calculated contour map for 4-*O*-β-D-glucopyranosyl-3-*O*-methyl-α-L-rhamnopyranose has superimposed a plot of the φ/ψ values for which H-1a and H-4b are within the distance of 2.47 ±0.25 Å (+) and a plot depicting the angles corresponding to a distance between H-4b and O-5a of 2.51 ±0.25 Å (*). It is seen that the HSEA-calculated minima is found within the region in which both conditions are met simultaneously. Likewise, a contour map of the glycosidic linkage of 3-*O*-methyl-2-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranose is shown in Fig 1d, with a superimposed plot depicting the φ/ψ values corresponding to a distance between H-1b and H-2c of 2.45 ±0.25 Å (+) and a distance between H-5b and H-1c of 2.33 ±0.25 Å (*), which also showed good agreement between calculations and experiments. Models of **3** in the minimum energy conformation are shown in Fig. 2.

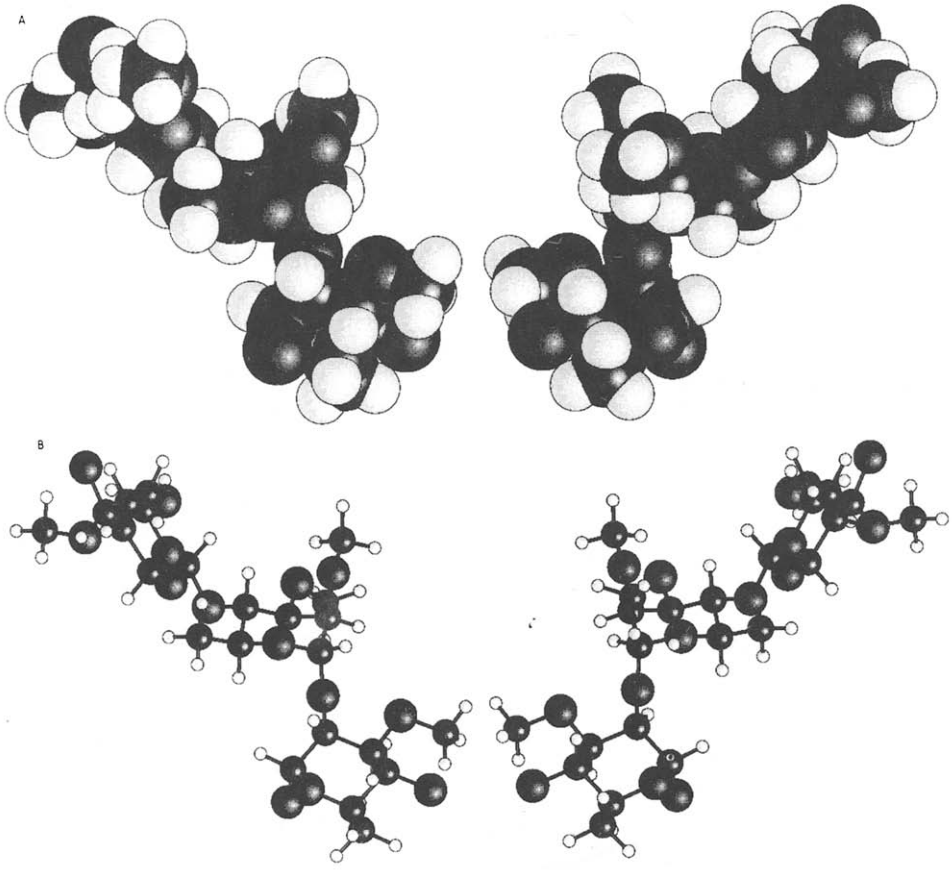

Fig. 2. A, CPK model of minimum energy conformation of **3**, using the φ_H/ψ_H angles as given in Table V; B, stick and ball model of the same molecule.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations are measured with a Perkin–Elmer 241 polarimeter. T.l.c. was performed on Silica Gel F_{254} (Merck).

N.m.r. spectra were obtained with Bruker AC-250 and AM-500 instruments. ^{1}H-N.m.r. (500 MHz) spectra were measured on solutions in D_2O at neutral pH and 300 K (internal acetone 2.22 p.p.m., DOH at 4.75 p.p.m.). Spectra recorded for solutions in $CDCl_3$ and CD_3OD were referenced to the solvent peaks at 7.24 and 3.33 p.p.m., respectively. A spectral width of 5 kHz, using 32k of computer memory giving a digital resolution of 0.3 Hz/point, was used together with pulse angles of 10 μs (60°). COSY experiments were made using Bruker standard software, and the n.O.e. experiments were performed in the difference mode.

The ^{13}C-n.m.r. (125.77 MHz) spectra were recorded at 300 K (internal 1,4-dioxane, 67.4 p.p.m.). Spectra recorded for solutions in $CDCl_3$ and CD_3OD were referenced to the solvent peaks at 77.00 and 49.00 p.p.m., respectively. A spectral width of 25 kHz, using a computer memory of 64k giving a digital resolution of 0.8 Hz/point, was used together with a pulse angle of 5 μs (60°)

*Methyl 3,6-di-*O*-methyl-β-*D*-glucopyranoside* (**4**). — Methylation[7] of methyl 3-*O*-methyl-β-D-glucopyranoside gave **4** as an oil, $[\alpha]_D^{20}$ –27.0° (*c* 1.8, chloroform), which was characterized as the 2,4-diacetate **5**, m.p. 97–98.5°, $[\alpha]_D^{20}$ –30.8° (*c* 0.6, chloroform); lit.[5] m.p. 100–101°, $[\alpha]_D^{20}$ –30.5° (chloroform). The n.m.r. data are given in Table VII.

*Methyl 4-*O*-benzyl-α-*L*-rhamnopyranoside* (**7**). — Methyl α-L-rhamnopyranoside (0.5 g, 2.8 mmol) was added to a solution of *p*-toluenesulphonic acid monohydrate (50 mg) in acetone (10 mL). After 23 h at 20°, triethylamine (0.1 mL) was added, the solution was concentrated, and a solution of the residue in dichloromethane was washed with saturated aq. sodium hydrogencarbonate, dried, and concentrated. Methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**6**; 0.528 g, 86%) was obtained as a chromatographically homogeneous syrup, which was characterized by its n.m.r. parameters (see Table VII), and used directly in the next step. To a solution of **6** (0.5 g, 2.3 mmol) in dry *N,N*-dimethylformamide (9 mL) was added sodium hydride (0.3 g, 6.3 mmol) followed by benzyl bromide (0.38 mL, 3.2 mmol). After 3 h, the mixture was poured into water, extracted with dichloromethane, and then concentrated. A solution of the crude product in aq. 90% trifluoroacetic acid (10 mL) was kept for 10 min at room temperature, then concentrated, diluted with water, and extracted with hexane. Concentration of the aqueous solution afforded crystalline **7**. Recrystallization from ethyl acetate–hexane gave **7** (0.429 g, 70%), m.p. 105–107°, $[\alpha]_D$ –68.3° (*c* 1.8, chloroform); lit.[20] m.p. 107–109°, $[\alpha]_D^{20}$ –68.3° (chloroform). The n.m.r. data are given in Table VII.

*Methyl 2,3-di-*O*-methyl-α-*L*-rhamnopyranoside* (**9**). — To a solution of **7** (0.160 g, 0.6 mmol) in *N,N*-dimethylformamide (5 mL) was added sodium hydride (0.17 g, 3.6 mmol), followed, after 5 min, by methyl iodide (0.22 mL, 3.6 mmol). After 3 h, the mixture was poured into water and the product was extracted with dichloromethane. Preparative t.l.c. (ether–hexane 1:1) afforded methyl 4-*O*-benzyl-2,3-di-*O*-methyl-α-L-rhamnopyranoside (**8**; 0.166 g, 94%), m.p. 91–92.5°, $[\alpha]_D^{20}$ –64.4° (*c* 1.5, chloroform); lit.[21]

TABLE VII

^{1}H- and ^{13}C-n.m.r. data[a] for **4**–**12**

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*OMe*	*OMe*
4	4.18	3.41	3.18	3.55	3.43	3.69	3.42	3.55 3.67
	7.7	9.3	9.1	9.3	4.0	4.6, 10.3		
5	4.34	4.95	—	4.97	3.47	— 3.53	3.36	3.39 3.49
	7.8	9.5	—	9.5	—			
6	4.83	4.11	4.09	3.40	3.67	1.31	3.39	
	< 1.5			9.5	6.0			
7	4.67	3.94	3.89	3.36	3.71	1.36	3.36	
				9.5	6.1			
8	4.75	.....				1.32	3.36	3.51 3.51
9	4.77	3.62	3.42	3.57	3.62	1.32	3.39	3.47 3.49
				8.8	5.8			
10	5.00	4.22	3.28	4.41	3.74	1.29	3.39	
11	4.72	3.72	3.93	3.33	3.67	1.36	3.33	
	1.8	4.0	9.0	9.5	6.0			
12	4.72	4.07	3.38	3.52	3.65	1.33	3.39	3.49
	1.8	3.2	9.0	9.0	6.0			

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OMe*		
4	103.7	74.2	85.5	70.7	73.7	72.4	57.1	59.5	60.5
5	101.7	73.6	81.2	69.9	71.8	72.0	56.7	58.3	59.6
6	98.1	75.5	78.3	74.4	65.8	17.5	54.9		
7	100.3	71.5	71.1	81.7	67.0	18.0	54.9		
8	97.9	77.3	81.5	80.4	67.6	17.9	54.7	57.7	59.1
9	98.2	75.9	81.1	71.6	68.1	17.7	54.8	56.9	59.0
10	97.9	78.3	78.3	81.2	64.3	17.9	54.8		
11	97.9	78.3	71.6	82.1	67.0	18.0	54.6	57.0	
12	100.6	66.9	81.5	71.4	67.7	17.7	54.9	57.0	

[a] All spectra recorded at 250 (^{1}H) or 62.9 MHz (^{13}C) in $CDCl_3$.

m.p. 91–93°, $[\alpha]_D^{20}$ –59.6° (chloroform). A solution of **8** (105 mg, 0.35 mmol) in ethyl acetate (5 mL) was hydrogenolysed for 24 h over 5% Pd–C (16 mg) to afford **9** (0.069 g, 95%), isolated as a syrup, $[\alpha]_D^{20}$ –28.4° (*c* 3.9, chloroform); lit.[22] $[\alpha]_D^{20}$ –27.8° (chloroform). The n.m.r. data are given in Table VII.

Methyl 3-O-methyl-α-L-rhamnopyranoside (**12**). — A solution of **7** (1.5 g, 5.6 mmol) in α,α-dimethoxytoluene (10 mL) containing a catalytic amount of *p*-toluenesulfonic acid was kept for 1 h at 55° then 1 h at 20°. Triethylamine (1 mL) was added, and the volatile components were evaporated at 60°/1 mmHg. Flash chromatography (ether–hexane, 1:6) of the residue afforded pure (*endo*) methyl 4-*O*-benzyl-2,3-*O*-(*R*)-benzylidene-α-L-rhamnopyranoside (**10**; 0.901 g, 45%), isolated as a syrup, $[\alpha]_D^{20}$ –30.3° (*c* 2.8, chloroform); lit.[23] $[\alpha]_D^{20}$ –34° (chloroform). The n.m.r. data are given in Table VII. Reductive cleavage[24] of **10** gave methyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**11**, 60%), $[\alpha]_D^{20}$ –17.1° (*c* 1.7, chloroform); lit.[24] $[\alpha]_D^{20}$ –17.7°(chloroform). The n.m.r. data are given in Table VII.

Methylation of **11** as described for **7**, and debenzylation as described for **8**, gave **12** in quantitative yield as a syrup, $[\alpha]_D^{20}$ –64.5° (*c* 3.7, chloroform); lit.[25] $[\alpha]_D^{20}$ –61° (chloroform). The n.m.r. data are given in Table VII.

Compounds **1–3** were available from previously published work[8].

The HSEA calculations were performed on an IBM PS/2 system model 80 with a 387 math-coprocessor as described earlier[13]. The torsion angles φ and ψ refer to H-1–C-1–O-1–C-X and C-1–O-1–C-X–H-X, respectively. The coordinates for the two units were taken from the neutron diffraction studies of α-L-rhamnopyranoside[26] and the non-reducing end of the X-ray structure of gentiobiose[27], and the *O*-methyl groups were attached with the program Alchemy[28].

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