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Controlled reduction of acarbose: conformational analysis of acarbose and the resulting saturated products

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ABSTRACT

Saturation of the double bond in the non-reducing terminal unit of the tetrasaccharide amylase inhibitor, acarbose (**1**), with Raney nickel as the catalyst and at pH 8, gave 57% of a ~1:1 mixture of the 5a-carba-*gluco* (**2**) and -*ido* (**3**) isomers together with cleavage products including 26% of the trisaccharide 6-deoxy-α-D-Glc*p*4N-(1→4)-α-D-Glc*p*-(1→4)-D-Glc (**4**). The saturated compounds were isolated and characterised using ^{1}H- and ^{13}C-n.m.r. spectrosopy. The preferred conformations of **1** and **2** were dependent on the state of ionisation of the bridging nitrogen atom. The inhibition by **1**–**4** of the hydrolysis of methyl β-maltoside by glucoamylase has been investigated; **1** and **2** were strong inhibitors.

INTRODUCTION

Acarbose (**1**) is a potent glycohydrolase inhibitor[1] and its efficiency has been accounted for by its similarity to a transition state analogue of a glycosyl carbocation. However, the saturated analogues, or the structurally similar compound, oligostatin, are also strong inhibitors[1,2]. Easy access to this type of compound is of considerable interest due to the expected higher chemical and physiological stability obtained by saturation of the double bond.

Investigations of the saturated analogues of acarbose have been hampered by the low yield obtained during attempts to saturate the double bond, *e.g.*, for the preparation[3] of **2** and **3**. Recently, chemical syntheses have been described of acarbose[4] (**1**), a saturated compound similar to **4**, but with one less reducing glucosyl unit[5], and of methyl oligobiosaminide[6]. However, this approach is not suitable for the synthesis of large amounts of saturated products. We now report on a reinvestigation of the hydrogenation of acarbose and the conformational analysis of the products obtained.

RESULTS AND DISCUSSION

The saturation of the double bond of acarbose catalysed by noble-metals (Pd/C

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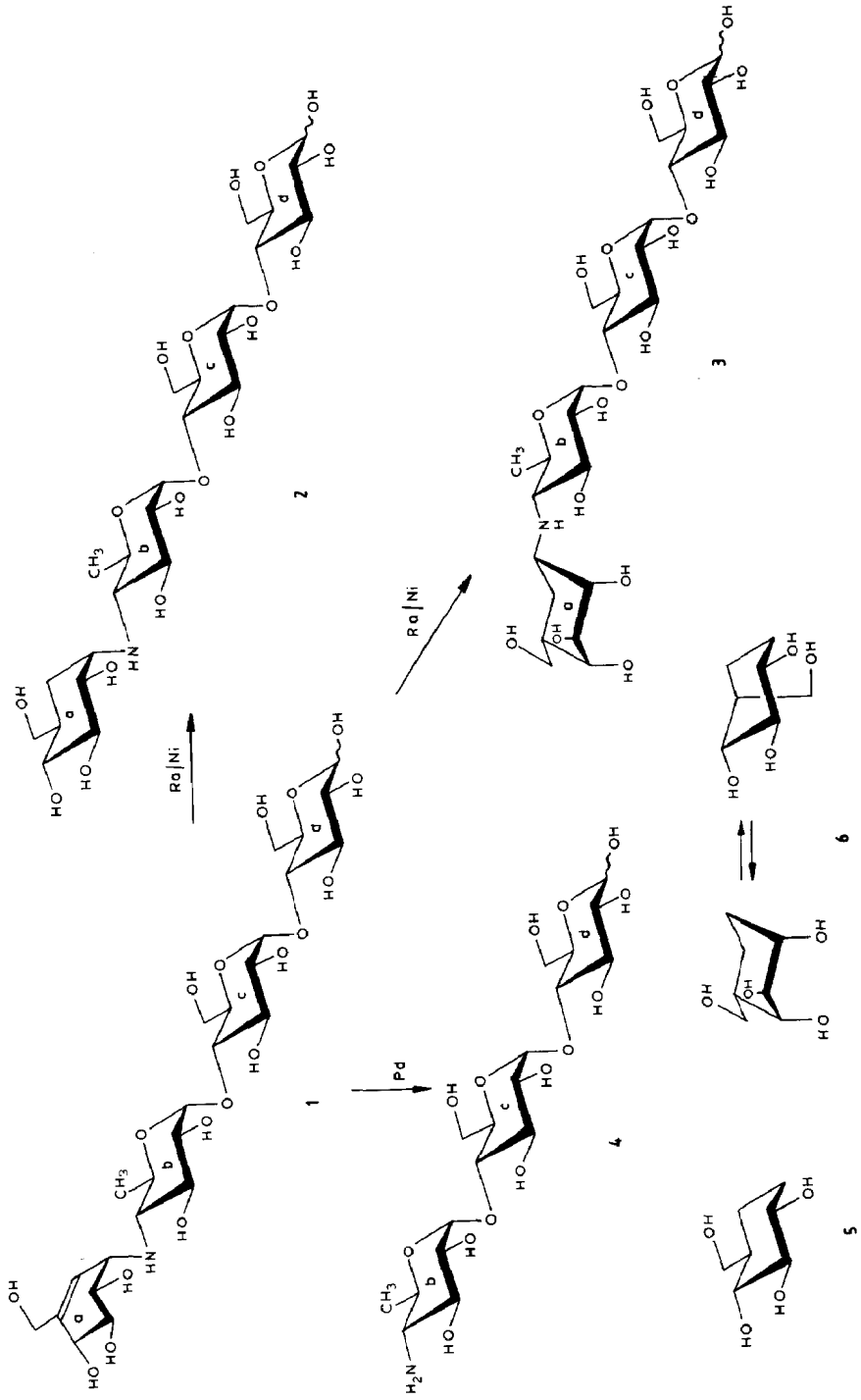

Ra/Ni
Ra/Ni
Pd
1
2
3
4
5
6

or PtO_2) yielded a complex mixture of products as reported[3]. Even with $Pd/BaSO_4$, only 25% of one major degradation product (**4**) could be isolated after amino-group protection with Fmoc, purification by h.p.l.c., and deprotection, together with small amounts of **2** and **3** and several other unidentified products.

However, with Raney nickel as the catalyst, it was possible to saturate the double bond in **1** in high yield and to produce mainly the two 5a-carba compounds, **2** (*gluco* isomer) and **3** (*ido* isomer), in a pH-dependent ratio as seen from Table I. The products were isolated easily by preparative h.p.l.c. in an almost quantitative yield. The data in Table I show that at low pH there is more allylic cleavage of the C–N bond with formation of the trisaccharide **4** and the inositol derivatives **5** and **6**, and the 5a-carba *ido* isomer (**3**) preponderates over the *gluco* isomer (**2**). The optimal pH for the production of the 5a-carba *gluco* isomer (**2**) was ~8, which resulted in a compromise between stereoselectivity and the formation of **4**. The addition of an organic base, such as triethylamine, suppressed the formation of the products of C–N bond cleavage. However, the catalyst plays an important role and further optimisation may be possible.

Minor products, formed during the reductions and isolated by h.p.l.c., were shown by ^{1}H- or ^{13}C-n.m.r. data (not presented) to be alditol derivatives of **2** and **3**, but further characterisation was not attempted due to their low yield. 5a-Carba-1,5-anhydro-D-glucitol (**5**) or -L-iditol (**6**) were isolated by preparative h.p.l.c., and characterised by spectroscopy and comparison with the published structures[3]. The results in Table I show that, in accord with published data[3], the *gluco* isomer (**5**) was formed mainly when Raney nickel was the catalyst, and a higher proportion of the *ido* isomer (**6**) was produced when Pd was the catalyst.

The products **1**–**6** were characterised by ^{1}H- and ^{13}C-n.m.r. spectroscopy (Tables II–IV), and the structures were confirmed by comparison with assigned[7] or partially assigned n.m.r. data[3]. The ^{1}H-n.m.r. data were obtained at 500 MHz for solutions in

TABLE I

Saturation of the double bond in acarbose (**1**)

Conditions	*Products*					
	1	**2**	**3**	**4**	**5**	**6**
Raney Ni, pH 3	11[a]	5	20	33	30	3
Raney Ni, pH 8	0	27	30	26	18	2
Raney Ni + Et_3N[b]	0	27	58	7	6	2
Raney Ni + Et_3N[c]	0	50	50	0	0	0
Raney Ni, pH 9	0	28	28	22	16	4
Raney Ni, pH 6.9	20	20	20	20	15	5
Pd/C	0	7	5	40	14	26
Pd/C + Et_3N	0	42	21	17	6	11
$Pd/BaSO_4$	0	11	17	36	18	18

[a] Yields in % (±10%) measured from the ^{13}C-n.m.r. spectra. [b] Reduction performed with commercial, preactivated Raney nickel. [c] Experiment carried out with freshly prepared Raney nickel, washed neutral.

TABLE II

N.m.r. data for acarbose and saturated analogs[a]

Atom	**1**		**2**			**3**			**4**			**5**	**6**
	pH 3	*pH 9*	*pH 3*	*pH 9*	*Ref. 2*	*pH 3*	*pH 9*	*Ref. 2*	*pH 3*	*pH 9*	*Ref. 2*		
Unit a[b]													
H-1	4.21	3.62	3.90	3.31		3.68	2.90	2.78				1.95	1.80
												1.34	1.45
H-2	4.08	3.69	3.78	3.52		4.15	3.98					3.42	3.47
H-3	3.82	3.77	3.52	3.62		4.12	4.09					3.23	3.36
H-4	4.15	4.05	3.38	3.27		3.95	3.88					3.21	3.64
H-5			1.85	1.80	1.65	2.12	1.98	1.88				1.54	2.17
H-6a	4.28	4.13	3.65	3.65		3.58	3.52					3.57	3.60
H-6b	4.28	4.23	3.75	3.73		3.71	3.64					3.74	3.83
H-7*eq*	5.91	5.90	2.22	1.19	1.77	1.91	1.54	1.45				1.77	1.78
H-7*ax*			1.76	1.38	1.23	1.82	1.40	1.29				1.15	1.48
Unit b													
H-1	5.39	5.29	5.39	5.30	5.16	5.42	5.28	5.20	5.40	5.34	5.33		
H-2	3.73	3.60	3.64	3.57		3.68	3.61		3.66	3.60			
H-3	4.10	3.60	4.10	3.64		3.88	3.50		3.78	3.50			
H-4	3.29	2.52	3.22	2.41	2.26	3.13	2.32	2.22	2.97	2.48	2.48		
H-5	4.25	3.78	4.20	3.78		4.22	3.75		4.08	3.74			
H-6	1.44	1.46	1.43	1.30	1.16	1.42	1.27	1.16	1.32	1.25	1.23		
Unit c													
H-1	5.38	5.38	5.39	5.39	5.26	5.39	5.35	5.27	5.38	5.40	5.39		
H-2	3.63	3.62	3.60	3.63		3.60	3.60		3.60	3.63			
H-3	3.94	3.94	3.94	3.94		3.94	3.95		3.94	3.95			
H-4	3.64	3.64	3.62	3.61		3.62	3.60		3.63	3.62			
H-5	3.84	3.84	3.82	3.82		3.85	3.85		3.92	3.84			
H-6a	3.92	3.90	3.92	3.92		3.92	3.90						
H-6b	3.78	3.78	3.80	3.79		3.80	3.74						

Unit d (α)											
H-1	5.22	5.23	5.22	5.22	5.08	5.22	5.21	5.10	5.20	5.22	5.21
H-2	3.57	3.59	3.52	3.57		3.52	3.56		3.55	3.58	
H-3	3.95	3.97	3.94	3.94		3.94	3.95		3.95	3.95	
H-4	3.64	3.62	3.62	3.63		3.62	3.62		3.63	3.62	
H-5	3.92	3.86	3.92	3.92		3.92	3.95		3.97	3.95	
H-6a											
H-6b											
Unit d (β)											
H-1	4.64	4.64	4.63	4.65	4.50	4.63	4.62	4.52	4.64	4.66	4.63
H-2	3.28	3.28	3.22	3.28		3.28	3.27	3.14	3.26	3.27	3.25
H-3	3.78	3.77	3.74	3.75		3.74	3.75		3.75	3.78	
H-4	3.64	3.69	3.63	3.62		3.63	3.62		3.62	3.62	
H-5	3.58	3.58	3.58	3.58		3.58	3.58		3.57	3.60	
H-6a									3.94		
H-6b									3.73		

[a] Measured at 500 MHz in D_2O at 27°, using acetone (2.225 p.p.m.) as the reference. [b] See formulae.

TABLE III

J Values[a] for acarbose and saturated analogs

Atom	**1**		**2**			**3**			**4**			**5**		**6**
	pH 3	*pH 9*	*pH 3*	*pH 9*	*Ref. 2*	*pH 3*	*pH 9*	*Ref. 2*	*pH 3*	*pH 9*	*Ref. 2*			
Unit a[b]														
$J_{1,2}$	4.8	4.5		3.4			1.0					$J_{1ax,2}$	11.5	10.2
$J_{2,3}$	9.6	8.8	9.5	9.8		3.0	3.2	3.0				$J_{1eq,2}$	4.7	4.6
$J_{3,4}$	7.0	7.4	9.5	9.5		3.0	3.2	3.0				$J_{2,3}$	—	8.7
$J_{4,5}$			9.5	9.3		3.0	3.2					$J_{3,4}$	—	9.4
$J_{5,6a}$				6.0								$J_{4,5}$	—	5.3
$J_{5,6b}$				3.6								$J_{5,6a}$	5.4	10.0
$J_{6a,6b}$	13.9			11.4								$J_{5,6b}$	3.7	4.9
$J_{5,7ax}$			13.0	13.0	13.0	12.0	12.5	13.0				$J_{6a,6b}$	11.1	11.1
$J_{5,7eq}$			2.5	3.2	3.0	3.8	3.4	3.5				$J_{5,7ax}$	12.3	—
$J_{7ax,7eq}$			15.0	14.8	14.0	13.0	12.5	13.0				$J_{5,7eq}$	3.6	—
$J_{7ax,1}$	4.8	4.5	3.0	3.4	3.0	13.0	12.5	13.0				$J_{7ax,7eq}$	13.7	—
$J_{7eq,1}$			2.8	3.4	3.0	3.8	3.4	3.5				$J_{1ax,7ax}$	11.7	—
												$J_{1ax,7eq}$	3.7	—
												$J_{1eq,7ax}$	3.5	—
												$J_{1eq,7eq}$	3.5	—
Unit b														
$J_{1,2}$	3.6	3.6	3.6	3.6	4.0	3.9	4.0	3.5	3.8	3.8	3.6			
$J_{2,3}$		9.5	10.0	10.0		9.8	9.8		10.0	9.8				
$J_{3,4}$		10.2	10.0	10.0	9.0	9.8	9.8	9.5	10.0	9.8	9.5			
$J_{4,5}$		10.2	10.0	10.0	9.0	9.8	9.8	9.5	10.0	9.8	9.5			
$J_{5,6}$	6.3	6.3	6.4	6.3	6.3	6.4	6.3	6.3	6.4	6.3	6.3			
Unit c														
$J_{1,2}$	3.8	3.6	3.7	3.6	3.6	3.8	3.8	3.5	3.6	3.8				
$J_{2,3}$	9.6	9.5	9.8	9.8		9.8	9.8		9.8	9.8				
$J_{3,4}$	9.5	9.6	9.8	9.8		9.8	9.8		9.8	9.8				
$J_{4,5}$	9.5	9.8	9.8	9.8		9.8	9.8		9.8	9.8				

$J_{5,6a}$	2.2										
$J_{5,6b}$	5.8										
$J_{6a,6b}$	12.0										
Unit d (α)											
$J_{1,2}$	3.6	3.6	3.6	3.6	3.5	3.6	3.8	3.5	3.6	3.7	3.8
$J_{2,3}$	9.5	9.5	9.8	9.8		9.8	9.8		9.8	9.8	
$J_{3,4}$	9.5	9.5	9.8	9.8		9.8	9.8		9.8	9.8	
$J_{4,5}$	9.5	9.6	9.8	9.8		9.8	9.8		9.8	9.8	
$J_{5,6a}$	2.2										
$J_{5,6b}$	5.8										
$J_{6a,6b}$	12.0										
Unit d (β)											
$J_{1,2}$	7.8	7.9	7.9	8.0	8.0	7.9	7.9	8.0	8.0	7.9	8.0
$J_{2,3}$	9.5	9.6	9.5	9.8		9.5	9.8	9.0	9.8	9.5	9.0
$J_{3,4}$	9.5	9.8		9.8		9.6	9.8		9.8	9.8	
$J_{4,5}$	9.5	9.8		9.8		9.6	9.8			9.8	
$J_{5,6a}$	2.0										
$J_{5,6b}$	5.8										
$J_{6a,6b}$	12.0										

[a] Measured at 500 MHz in D_2O at 27°; first-order values ($\pm$0.3 Hz). [b] See formulae.

TABLE IV

^{13}N.m.r. data for acarbose and saturated analogs[a]

Atom	**1**		**2**		**3**		**4**			**5**	**6**
	pH 3	*pH 9*	*pH 3*	*pH 9*	*pH 3*	*pH 9*	*pH 3*	*pH 9*	*Ref. 2*	*pH 3*	*pH 9*
Unit a[b]											
C-1	57.3	56.8	59.5	57.7	58.4	54.7				31.6	21.2
C-2	67.2	73.5	71.0	75.3	67.7	69.8				73.3	74.2
C-3	71.9	73.5	74.5	75.4	70.6	71.2				80.1	75.7
C-4	71.1	71.3	72.5	73.9	69.6	70.1				74.1	74.2
C-5	147.7	140.2	39.1	39.1	37.8	38.6				46.7	41.8
C-6	61.9	62.4	62.2	63.1	62.9	62.3				63.3	59.3
C-7	115.1	124.1	25.6	29.6	21.9	24.8				24.0	27.5
Unit b											
C-1	100.5	100.8	100.3	100.8	100.2	100.6	100.5	100.4	101.3		
C-2	73.1	72.0	73.1	73.5	72.9	73.0	72.8	73.1			
C-3	69.0	73.7	69.0	74.6	69.3	71.9.	69.7	73.5			
C-4	63.6	65.6	64.5	65.0	63.1	62.3	57.6	58.5	58.9		
C-5	65.2	70.1	65.1	70.2	65.8	70.4	66.3	70.2			
C-6	18.2	18.2	18.0	18.1	18.0	18.0	17.4	17.7	18.2		
Unit c											
C-1	100.4	100.4	100.4	100.3	100.3	100.1	100.3	101.0	100.7		
C-2	73.1	72.4	72.4	72.1	72.4	72.1	72.4	72.3			
C-3	74.0	74.1	74.0	73.9	74.1	73.9	74.1	74.2			
C-4	78.4	78.1	78.1	78.3	77.7	77.8	77.8	78.0			
C-5	72.3	72.1	71.8	71.9	71.8	71.8	71.8	72.0			
C-6	61.3	61.4	61.3	61.2	61.3	61.2	61.2	61.4			
Unit d (α)											
C-1	92.7	92.7	92.7	92.6	92.7	92.6	92.7	92.7	93.1		
C-2	72.1	72.1	72.1	72.2	72.1	72.1	72.0	72.1			
C-3	73.9	74.0	74.0	73.8	74.0	73.8	74.0	74.0			
C-4	78.3	78.1	78.0	78.0	78.0	77.8	78.1	78.0			
C-5	70.7	70.8	70.8	70.6	70.8	70.5	70.7	70.7			
C-6	61.4	61.6	61.4	61.3	61.4	61.4	61.3	61.5			

Unit d (*β*)									
C-1	96.6	96.6	96.6	96.6	96.6	96.6	96.6	96.6	97.0
C-2	74.8	74.8	74.8	74.7	74.8	74.7	74.8	74.8	
C-3	76.9	77.0	77.0	76.7	76.9	76.8	76.9	77.0	
C-4	78.0	77.9	77.7	78.2	77.6	77.7	77.8	77.9	
C-5	75.4	75.4	75.4	75.2	75.3	75.2	75.3	75.4	
C-6	61.5	61.4	61.5	61.2	61.5	61.2	61.4	61.4	

[a] Measured at 125.77 MHz in D_2O at 27°, using 1,4-dioxane (67.4 p.p.m.) as the reference. [b] See formulae.

D_2O at 27°, and the assignments were based on COSY[8], relayed[9], and double-relayed COSY experiments together with phase-sensitive double-quantum-filtered (DQF) COSY experiments[10]. The ^{13}C-n.m.r. data were measured at 125.77 MHz and the assignments were based on heteronuclear correlation spectroscopy[11] with the assigned proton signals and by comparison with data for model compounds. N.O.e. experiments were

TABLE V

N.O.e. data for acarbose and saturated analogs

Compound	*pH*	*Proton saturated*	*N.O.e. observed (%)*					
			Intra-ring			*Inter-ring*		
Acarbose (**1**)		H-7*a*	H-6*a*	H-1*a*		H-3*b*	H-6*b*	H-4*b*
	3	—	1.6	1.7		1.7	0.2	0
	9	—	2.7	2.9		0	1.5	0.7
		H-6*b*	H-4*b*	H-5*b*		H-1*a*	H-7*a*	
	3	—	2.6	2.8		1.6	1.2	
	9	—	2.9	7.2		1.2	3.5	
		H-4*b*	H-6*b*	H-5*b*	H-2*b*	H-1*a*	H-7*a*	
	3	—	0.9	vw[a]	0	0.6	0	
	9	—	2.0	vw	1.7	3.2	1.3	
		H-1*a*	H-7*a*	H-2*a*		H-4*b*	H-6*b*	
	3	—	1.8	o.r.s.[b]		0	0.9	
	9	—	4.9	o.r.s.		4.4	1.6	
gluco-Isomer (**2**)		H-7*eq,a*	H-5*a*	H-1*a*	H-7*ax,a*	H-3*b*	H-6*b*	H-4*b*
	3	—	o.r.s.	1.8	o.r.s.	2.3	0	0
	9[c]	—	o.r.s.	1.9	o.r.s.	0	o.r.s.	0.3
		H-6*b*	H-4*b*	H-5*b*		H-1*a*	H-7*eq,a*	
	3	—	3.4	4.9		1.3	o.r.s.	
	9[d]	—	1.6	3.0		1.0	o.r.s.	
		H-4*b*	H-6*b*	H-5*b*	H-2*b*	H-1*a*		
	3	—	1.5	0.8	0.6	0		
	9	—	1.5	0.6	1.3	3.1		
		H-1*a*	H-7*eq,a*	H-7*ax,a*	H-2*a*	H-4*b*	H-6*b*	
	3	[f]						
	9[e]	—	0.4	0.5	o.r.s.	1.7	0.3	
ido-Isomer (**3**)		H-7*eq,a*	H-5*a*	H-1*a*	H-7*ax,a*	H-3*b*	H-6*b*	H-4*b*
	3	—	o.r.s.	1.8	o.r.s.	0	o.r.s.	0.1
	9	[f]						
		H-6*b*	H-4*b*	H-5*b*		H-1*a*	H-7*eq,a*	H-2*a*
	3	—	1.9	3.7		2.6	o.r.s.	0.7
	9	[g]						
		H-4*b*	H-6*b*	H-5*b*	H-2*b*	H-1*a*	H-2*a*	
	3	—	1.1	−0.3	−2.9	−1.5		
		[h]	0	0	4.0	1.8	6.8	
		H-1*a*	H-5*a*	H-2*a*		H-4*b*	H-6*b*	
	3	[f]						
	9	[h]	3.2	1.0		2.7	0.4	

[a] Very weak signals. [b] Off resonance saturation (o.r.s.). [c] O.r.s. H-5*a*. [d] O.r.s. H-7*eq,a*. [e] O.r.s. H-3*b*. [f] Not possible to saturate selectively. [g] Zero values due to correlation time. [h] Negative n.O.e. values.

carried out in the difference mode, and the results are presented in Table V. Rotating-frame n.O.e. spectroscopy (ROESY) experiments[12] confirmed the inter- and intra-residue n.O.e. interactions and the assignments, and supported the structures proposed.

The $^1J_{H,H}$ values (Table IV) show that the units *b–d* adopt $^4C_1$ conformations (see formulae) in **1–6**, as does unit *a* in **2** and **4–6**. However, for the *ido* isomer (**3**), the $^1C_4$ conformation preponderates with the OH groups axial and the CH_2OH and N–R substituents equatorial, whereas the $^4C_1$ conformation preponderates in **6**. For acetylated **6** in $CDCl_3$, $J_{2,3} = J_{3,4} = 8.0$ Hz (*cf.* $J_{2,3}$ 8.7 and $J_{3,4}$ 9.4 Hz for **6** in D_2O), which indicates a greater proportion of the $^1C_4$ conformation but with the $^4C_1$ form still preponderating. This observation is supported by the ^{13}C resonance in D_2O at 59.3 p.p.m. for the CH_2OH group in **6** compared to that at 61.3 p.p.m. in **3**.

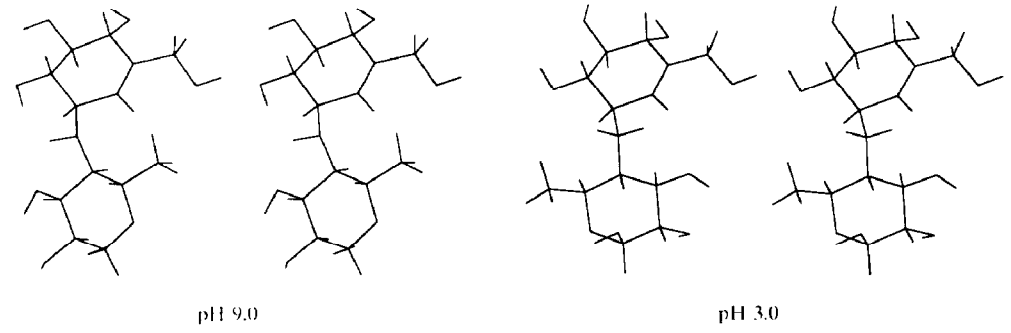
The remaining n.m.r. data, especially the n.O.e. data (Table V), correlate well with the short distances calculated for the preferred orientation around the amino linkage between units *a* and *b* calculated using the force-field option in the software package "Discover" from "BIOSYM"[13,14]. The minimum energy conformations in Table VI indicate that the conformational preferences around the amino linkage are strongly dependent on the pH, particularly for acarbose (**1**) and the *gluco* isomer (**2**). For **1** and **2**, it appears (Fig. 1) that two conformers exist in equilibrium in solution with one (ϕ_H, ψ_H −51, −10 for **1** and −44, −27 for **2**) preponderating at basic pH in agreement with earlier observations[7]. However, another minimum appears to be populated to a significant degree (ϕ_H, ψ_H −26, −176 for **1** and −22,179 for **2**) at acidic pH. These observations are based primarily on the n.O.e. effects (Table V) between H-7*a* and H-3*b* or H-4*b*, and H-1*a* and H-4*b* for **1** and **2**. In situations where the analyses were complicated by overlapping signals, the results were confirmed by ROESY experiments. The data indicate that, for **1** and **2**, there is an equilibrium in which the conformation with ψ_H −176 or 179 preponderates at low pH and that with ψ_H −10 or −27 at a high

TABLE VI

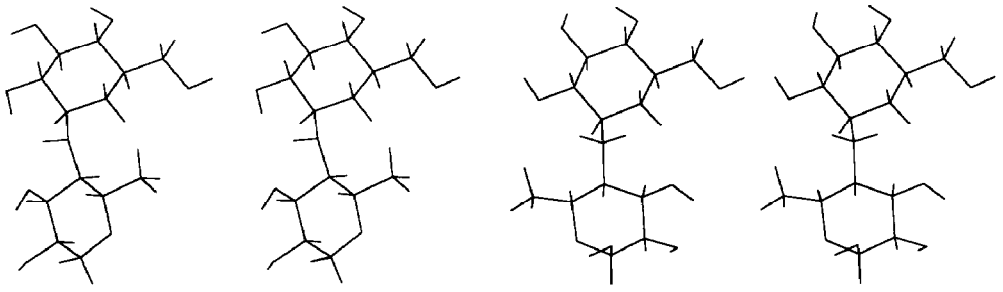
Minimum energy conformation of **1–3** as calculated by the force field in "Discover"

Compound	*pH*	*Minimum*				*ΔE (II−I) (kcal/mol)*
		I		*II*		
		ϕ_H	ψ_H	ϕ_H	ψ_H	
1	9	−51	−10	−30	178	4.5
	3	−41	−21	−26	176	1.1
2	9	−44	−27	−20	176	3.7
	3	−42	−25	−22	179	0.6
3	9	−46	−32	−18	180	1.1
		+22[a]	+22[a]			0.2[b]
	3	−48	−20	−13	174	−0.4
		+22[a]	+21[a]			1.3[b]

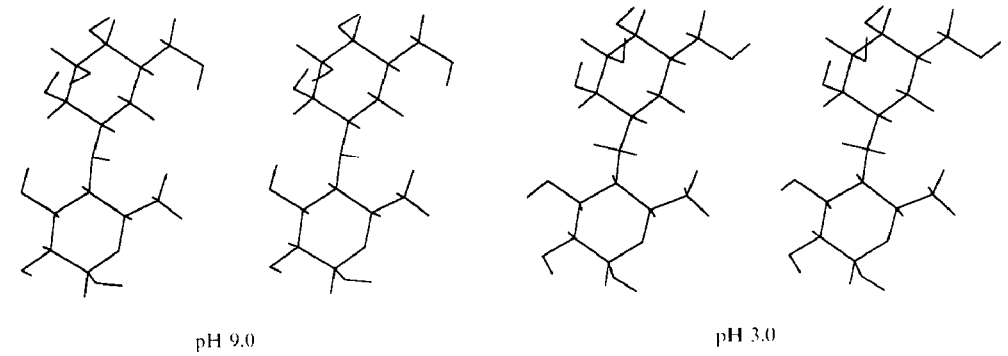
[a] φ,ψ angles for conformation III. [b] ΔE (III − I).



Acarbose (1)



gluco isomer (2)



ido isomer (3)

Fig. 1. Minimum energy conformations of the disaccharide non-reducing units of **1–3**, calculated using the force field in "Discover"[13,14], in the conformers that preponderate in solution as inferred from n.m.r. data.

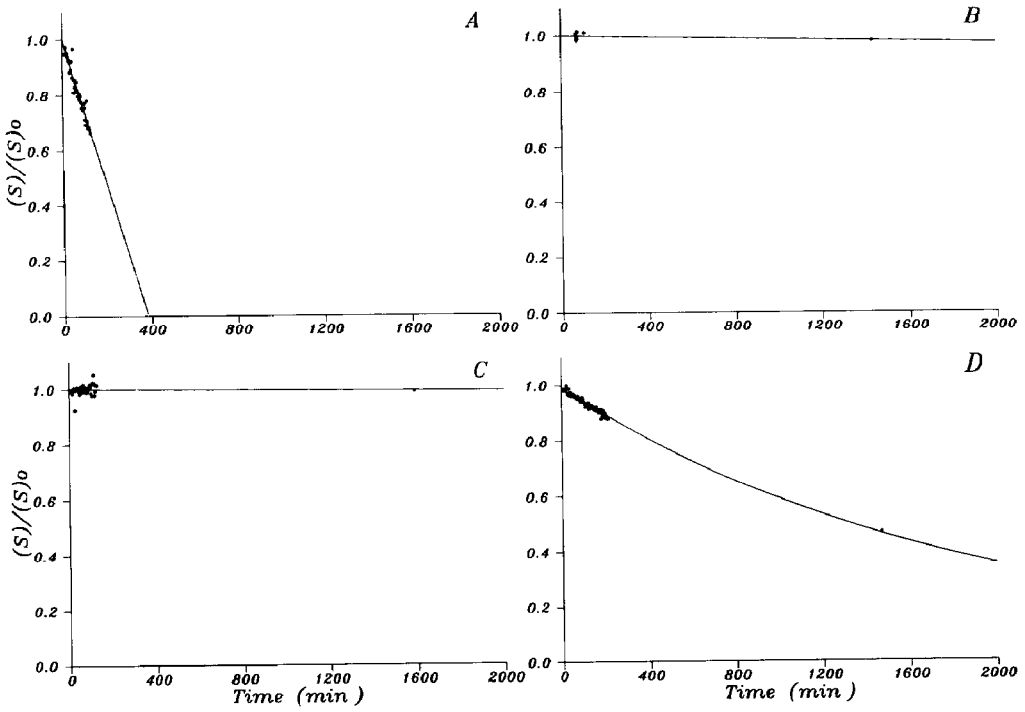


Fig. 2. Hydrolysis of methyl β-maltoside (0.055M) in the presence of 0.055mM inhibitor: *A*, **4**; *B*, **2**; *C*, **1**; and *D*, **3**.

pH. This conformational change is supported by the pH-dependent large changes in the ^{13}C chemical shift data for units *a* and *b* in **1** and **2**. Apparently, this is the first example of a strong dependence on pH of the conformations of oligosaccharides.

For the *ido* isomer (**3**), a bent conformation with ϕ_H, ψ_H $-46, -32$ or $+20, +20$, appears to satisfy the experimental observations most accurately at both basic and acid pH.

Compounds **1–4** were tested as inhibitors of the hydrolysis of methyl β-maltoside by glucoamylase, using the reported methodology[15,16], and the results for equimolar concentrations of **1–4** are shown in Fig. 2. Acarbose (**1**) and the *gluco* isomer (**2**) caused almost identical inhibition, whereas **3** and **4** were inferior, but still quite powerful inhibitors. More detailed experiments will be published elsewhere.

EXPERIMENTAL

H.p.l.c. was performed on a Waters system with a 600 controller, a 410 differential refractometer equipped with a preparative flow cell, and a model 600 pump with modified 80 mL/min pump heads. The system was fitted with switchable analytical RCM (8 × 10) and Deltapak (19 × 300) columns and a preparative radial pack module for columns (50 × 300 mm) packed with reversed phase C_{18}. Elution with an aqueous 0.1% trifluoroacetic acid buffer was employed for both analytical (1 mL/min) and preparative (20 mL/min) separations.

Fractionation of the mixture of products from the reduction of acarbose (200 mg) in the presence of Raney nickel and triethylamine yielded, after lyophilisation of the products, **2** (91 mg) and **3** (101 mg) as the trifluoroaceate salts. The two products were eluted as four components due to partial separation of the α and β anomers, and only minor by-products were observed.

Catalytic hydrogenation. — Acarbose (0.5 g) was dissolved in water (10 mL) and freshly prepared Raney Ni or Pd catalyst was added after the pH had been adjusted to the values noted in Table I or triethylamine (0.2 mL) had been added. Hydrogenation at 25° and 1000 mbar was carried out for 20 h, the catalyst was collected and washed with water, and the solvent was evaporated from the combined filtrate and washings. Before ^{13}C-n.m.r. analysis of the crude product, the pH was adjusted to 3. Integrals were measured from peak heights and are accurate within $\pm 10\%$. The results are presented in Table I.

Acetates of **5** *and* **6**. — The appropriate h.p.l.c. fractions were acetylated under standard conditions (Ac_2O/Pyr), and the products isolated in the usual way as a non-crystalline, unresolvable mixture. ^{1}H-N.m.r. data ($CDCl_3$): acetylated **5**, δ 4.89 (t, 1 H, $J_{1,2}$ 10 Hz, H-2), 5.10 (t, 1 H, $J_{2,3}$ 10 Hz, H-3), 5.00 (m, 1 H, H-4), 4.08 (dd, 1 H, $J_{5,6a}$ 3, $J_{6a,6b}$ 12 Hz, H-6a), 3.95 (dd, 1 H, $J_{5,6a}$ 5, $J_{6a,6b}$ 12 Hz, H-6b), 1.99 (m, 1 H, H-5), 1.49 (m, 1 H, H-1*ax*), 2.15 (m, 1 H, H-1*eq*), 1.46 (m, 1 H, H-7*ax*), 1.88 (m, 1 H, H-7*eq*); acetylated **6**, δ 5.20 (t, 1 H, $J_{3,4}$ 8 Hz, H-3), 5.02 (dd, 1 H, $J_{4,5}$ 4.8 Hz, H-4), 4.89 (dt, 1 H, $J_{1ax,2}$ 8.0, $J_{2,3}$ 8.0, $J_{1eq,2}$ 4.5 Hz, H-2), 4.22 (dd, 1 H, $J_{5,6a}$ 6, $J_{6,6b}$ 12 Hz, H-6a), 4.20 (dd, 1 H, $J_{5,6b}$ 8, $J_{6a,6b}$ 12 Hz, H-6b), 2.50 (m, 1 H, H-5), 1.74 (m, 1 H, H-1*ax*), 1.94 (m, 1 H, H-1*eq*), 1.57 (m, 1 H, H-7*ax*), 1.84 (m, 1 H, H-7*eq*). The data for **5** are in good agreement with the results published[3].

N.m.r. spectroscopy. — Solutions (20 mg/0.5 mL) in D_2O were used. Spectra were recorded in 5-mm tubes at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C with a Bruker AM-500 spectrometer and at 27°. The ^{1}H resonances were measured relative to that of internal acetone (2.225 p.p.m., DOH at 4.75 p.p.m.) and determined on a first-order basis. The ^{13}C resonances are relative to that of internal 1,4-dioxane (67.4 p.p.m.). The pH was adjusted with potassium carbonate for high pH values, and with trifluoroacetic acid for low pH values.

Homonuclear 2D-n.m.r. spectroscopy was performed with Bruker DISNMRP software, except for the ROESY (see below).

Relayed COSY experiments[9] were made with fixed delays of 30 ms and double-relayed COSY experiments with both delays of 30 ms in order to optimise coherence transfer for large couplings[17]. These experiments were performed with quadrature detection in the F_1 dimension, and a total of 256 t_1 increments of 16 scans each (32 for double-relayed) were recorded with a minimum delay between pulses of 0.1 s and a sweep width of 2500 Hz. The time-domain data matrix was zero-filled in the t_1 direction to 512 × 1024 points, treated with a non-shifted sine-bell function in both dimensions, and processed to give magnitude spectra.

The phase-sensitive COSY experiments were performed using double-quantum filtering[10,18] with the Bruker COSYPHDQ microprogram using fixed delays of 30 ms.

These experiments were performed using 512 t_1 increments and a sweep width of 2500 Hz, giving an acquisition time in t_1 of 0.205 s. In the F_2 dimension, 2048 data points were collected, giving an acquisition time of 0.819 s. The data matrix was zero-filled in the F_1 dimension to give a matrix of 2048 × 2048 points and was resolution-enhanced in both dimensions by a shifted sine-bell function before Fourier transformation.

The ^{13}C–^1H correlation experiments[11] were performed with the XHCORRD microprogram, using decoupling in the ^1H dimension; 128 t_1 increments of 1200 scans and a size of 2048 points were accumulated. The data matrix was zero-filled in the F_1 dimension to 256 × 2048 points before Fourier tranformation in the absolute mode, giving a digital resolution of 9.8 Hz in the ^{13}C dimension and 11.7 Hz in the ^1H dimension.

The ROESY experiments[12,19] were performed using the procedure of Griesinger and Ernst[20] with the spin-lock field surrounded by 2 hard 90° pulses in order to avoid frequency-dependent effects[21]. The transmitter was used for all hard pulses and the spin-lock field was delivered by the decoupler. The spin-lock field was placed in the middle of the spectrum. Quadrature in t_1 was obtained by the hypercomplex method of States *et al.*[22]. 512 t_1 values were recorded with 80 scans each and 2 dummy scans giving an acquisition time in t_1 of 0.223 s (SW 2300 Hz) and 0.890 s in t_2. The data sets were resolution-enhanced in the t_1 dimension by a shifted sine-bell function and zero-filled to 2048 × 2048 data points prior to Fourier transformation, thus giving a resolution of 1.1 Hz/point.

Conformational analysis. — The torsion angles are defined as follows: ϕ_H H-1a–C-1a–N-1a–C-4b and ψ_H C-1a–N-1a–C-4b–H-4b. The conformational analysis was performed with a Silicon Graphics Irish workstation 4D 25, using the software package "Discover"[13,14]. The energies given in Table VI are calculated for a vacuum and are to be considered as qualitative only, to support the experimental observations which strongly suggest a conformational dependence of the ionisation state of the nitrogen atom.

Inhibition experiments. — A solution of methyl β-maltoside (0.055M) in acetate buffer (0.52 mL, 0.1M, pH 4.3), prepared from anhydrous sodium acetate, acetic acid, and D_2O, was thermostatted to 27°, and glucoamylase (20 μL, 4.96 mg/per mL of buffer) in the D_2O buffer was added together with the inhibitor **1**–**4** (to 0.055 mM). The sample was transferred to a 5-mm n.m.r. tube, and ^1H-n.m.r. spectra were recorded at suitable time intervals as described[15,16].

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REFERENCES

1 E. Truscheit, W. Frommer, B. Junge, L. Muller, D. D. Schmidt, and W. Wingender, *Angew. Chem. Int. Ed. Engl.*, 20 (1981) 744–761.
2 B. Junge and L. Muller, Ger. Pat. DE 3123520 A1 (1982).
3 B. Junge, F.-R. Heiker, J. Kurz, L. Muller, D. D. Schmidt, and C. Wunsche, *Carbohydr. Res.*, 128 (1984) 235–268.
4 S. Ogawa and Y. Shibata, *J. Chem. Soc., Chem. Commun.*, (1988) 605–606.
5 M. Hayshida, N. Sakairi, H. Kuzuhara, and M. Yajima, *Carbohydr. Res.*, 194 (1989) 233–246.
6 Y. Shibita, Y. Kosuge, and S. Ogawa, *Carbohydr. Res.*, 199 (1990) 37–54.
7 K. Bock and H. Pedersen, *Carbohydr. Res.*, 132 (1984) 142–149.
8 W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
9 G. Wagner, *J. Magn. Reson.*, 55 (1983) 151–156.
10 M. Rance, O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 117 (1983) 479–485.
11 A. Bax and G. A. Morris, *J. Magn. Reson.*, 42 (1981) 501–505.
12 A. A. Bothner-By, R. L. Stephens, C. D. Warren, J.-M. Lee, and R. W. Jeanloz, *J. Am. Chem. Soc.*, 106 (1984) 811–813.
13 J. Maple, U. Dinur, and A. T. Hagler, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 5350–5354.
14 A. T. Hagler, S. Lifson, and P. Dauber, *J. Am. Chem. Soc.*, 101 (1979) 5122–5130.
15 K. Bock and S. Refn, *Acta Chem. Scand., Ser. B*, 42 (1988) 75–85.
16 K. Bock and B. W. Sigurskjold, *Eur. J. Biochem.*, 178 (1989) 711–720.
17 A. Bax and G. Drobny, *J. Magn. Reson.*, 61 (1985) 306–320.
18 U. Piantini, O. W. Sørensen, and R. R. Ernst, *J. Am. Chem. Soc.*, 104 (1982) 6800–6801.
19 A. Bax and D. G. Davies, *J. Magn. Reson.*, 63 (1985) 207–213.
20 C. Griesinger and R. R. Ernst, *J. Magn. Reson.*, 75 (1987) 261–271.
21 A. Bax, *J. Magn. Reson.*, 77 (1988) 134–147.
22 D. J. States, R. A. Haberkorn, and D. J. Ruben, *J. Magn. Reson.*, 48 (1982) 286–292.