*Carbohydrate Research*, 132 (1984) 142–149
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

## Note

# The solution conformation of acarbose

KLAUS BOCK AND HENRIK PEDERSEN
*Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby (Denmark)*

(Received December 12th, 1983; accepted for publication, February 21st, 1984)

The pseudotetrasaccharide acarbose (**1**) is a potent inhibitor of glucosidases and saccharases in *in vitro* systems[1,2], and is of potential interest in connection with the regulation of the uptake of D-glucose in the intestine. In order to obtain a better understanding of the inhibitory activity of **1**, we have investigated its solution conformation using, particularly, $^1$H-n.m.r. spectroscopy and proton n.O.e. in combination with an empirical evaluation of the preferred conformation using the hard-spheres *exo*-anomeric effect (HSEA) calculation[3]. This method has given convincing results about the preferred conformation of oligosaccharides in solution[4–9].

The $^1$H-n.m.r. data (400 or 500 MHz) for **1** and the disaccharide glycoside **2** are presented in Table I, and a spectrum of **1** in aqueous solution is shown in Fig. 1. The assignments of the signals given in Table I are based on comparison with those of appropriate model compounds[10], homonuclear decoupling experiments, and partially relaxed spectra. The $^{13}$C-n.m.r. data (67.89 MHz) of **1** and **2** are shown in Table II. The assignments of these signals were based on comparison with those of suitable model compounds[11].

The HSEA calculations were performed as published[3,9], but excluding the *exo*-anomeric effect in the calculation of the bond between the valienamine (a-unit) and the 4-amino-4,6-dideoxy-$\alpha$-D-glucopyranose (b-unit). The coordinates for the b-unit were constructed by bond modification of the neutron diffraction data for methyl $\alpha$-D-glucopyranoside[12]. The C-4–N-4 bond-length was set at 1.48 Å along the original C-4–O-4 vector, and the C-6–O-6 bond was changed to a C-6–H-6 bond (1.10 Å). The coordinates for the valienamine unit were based on an X-ray determination of a methylcyclohexene derivative[13], using the following bond-lengths: C–O, 1.42; C–H, 1.10; and C–N, 1.48 Å. The minimisation of the interaction between the a- and b-units was calculated on the basis of a "glycosidic" C–N–C bond angle of 108°.

The minimum energy conformation ($E_{min}$ $-0.23$ kcal/mole) was found for a "$\varphi_H$" (H-1a,C-1a,N-1a,C-4b) angle of $-34°$ and a "$\psi$" (C-1a,N-1a,C-4b,H-4b) angle of $-19°$. The minimum energy conformation of acarbose is shown in Fig. 2 together with that of maltotetraose, using $\varphi_H/\psi_H$ values of $-25°/-20°$ for the maltose glycosidic bonds[8,14].

0008-6215/84/$ 03.00 © 1984 Elsevier Science Publishers B.V.

TABLE I

$^1$H-N.M.R DATA[a] FOR ACARBOSE (**1**) AND THE DISACCHARIDE-GLYCOSIDE **2**

| *Compound* | *H-1* | *H-2* | *H-3* | *H-4* | *H-5* | *H-6* | *H-6'* | *H-7* | *OMe* |
|---|---|---|---|---|---|---|---|---|---|
| *Acarbose* (**1**) | | | | | | | | | |
| a-unit | 3.62 | 3.69 | 3.77 | 4.05 | | 4.13 | 4.23 | 5.90 | |
| | (4.8) | (9.6) | (7.0) | | | (13.9) | | (4.8) | |
| b-unit | 5.29 | 3.60 | 3.60 | 2.52 | 3.78 | 1.46 | | | |
| | (3.6) | | | | (6.3) | | | | |
| c-unit | 5.38 | 3.62 | 3.94 | 3.64 | 3.84 | 3.90 | 3.78 | | |
| | (3.8) | (9.6) | (9.5) | (9.5) | | (2.2) | (5.8,12.0) | | |
| d-unit-alfa | 5.23 | 3.59 | 3.97 | 3.62 | 3.86 | 3.92 | 3.78 | | |
| | (3.6) | (9.5) | (9.5) | (9.5) | | (2.2) | (5.8,12.0) | | |
| -beta | 4.64 | 3.28 | 3.77 | 3.69 | | 3.91 | 3.81 | | |
| | (7.8) | (9.5) | (9.5) | (9.5) | | (2.0) | (5.8,12.0) | | |
| *Disaccharide-glycoside* **2** | | | | | | | | | |
| a-unit | 3.56 | 3.69 | 3.78 | 4.06 | | 4.12 | 4.25 | 5.90 | |
| | (4.8) | (9.5) | (6.7) | | | (13.2) | | (5.0) | |
| b-unit | 4.34 | 3.29 | 3.41 | 2.50 | 3.48 | 1.42 | | | 3.57 |
| | (7.8) | (9.5) | (9.5) | (9.5) | (6.0) | | | | |

[a] Chemical shifts and coupling constants (in parentheses) measured at 500 MHz on 0.01M solutions in $D_2O$ at 37°, using acetone ($\delta$ 2.22) as internal reference.

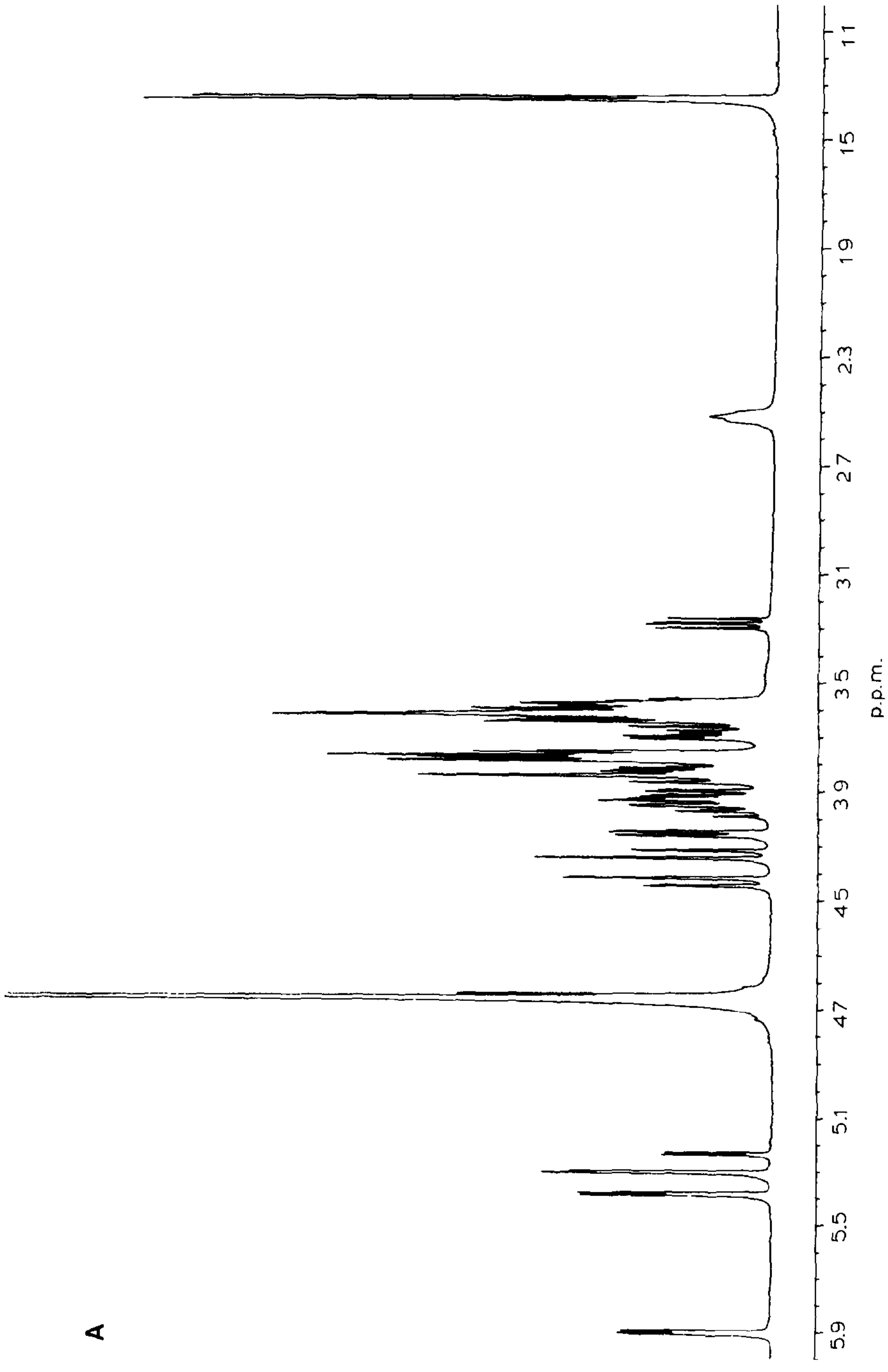
TABLE II

$^{13}$C-N M R. DATA[a] FOR ACARBOSE (**1**) AND THE DISACCHARIDE-GLYCOSIDE **2**

| *Compound* | *C-1* | *C-2* | *C-3* | *C-4* | *C-5* | *C-6* | *C-7* | *OMe* |
|---|---|---|---|---|---|---|---|---|
| *Acarbose* (**1**) | | | | | | | | |
| a-unit | 56.8 | 73.5 | 73.5 | 71.3 | 140.2 | 62.4 | 124.1 | |
| b-unit | 100.8 | 72.0 | 73.7 | 65.6 | 70.1 | 18.2 | | |
| c-unit | 100.4 | 72.4 | 74.1 | 78.1 | 72.1 | 61.4 | | |
| d-unit-beta | 96.6 | 74.8 | 77.0 | 77.9 | 75.4 | 61.4 | | |
| alfa | 92.7 | 72.1 | 74.0 | 78.1 | 70.8 | 61.6 | | |
| *Disaccharide-glycoside* **2** | | | | | | | | |
| a-unit | 56.5 | 73.6 | 73.6 | 71.8 | 139.6 | 62.2 | 124.4 | |
| b-unit | 103.7 | 76.6 | 74.6 | 65.7 | 71.3 | 18.0 | | 57.7 |

[a] Chemical shifts measured at 67.89 MHz for solutions in $D_2O$ at 37°, using 1,4-dioxane as internal reference ($\delta$ 67.4).

Several short, inter-ring, proton–proton distances were found in this minimum energy conformation: H-1a/H-4b, 2.12; H-7a/H-4b, 2.29; H-7a/H-6b, 2.44 and 2.90 Å. These short proton–proton distances were proved to be present in the preferred conformation in aqueous solution by using n.O.e. experiments (Table III; Fig. 3).


A
5.9
5.5
5.1
4.7
4.3
3.9
3.5
3.1
2.7
2.3
1.9
1.5
1.1
p.p.m.

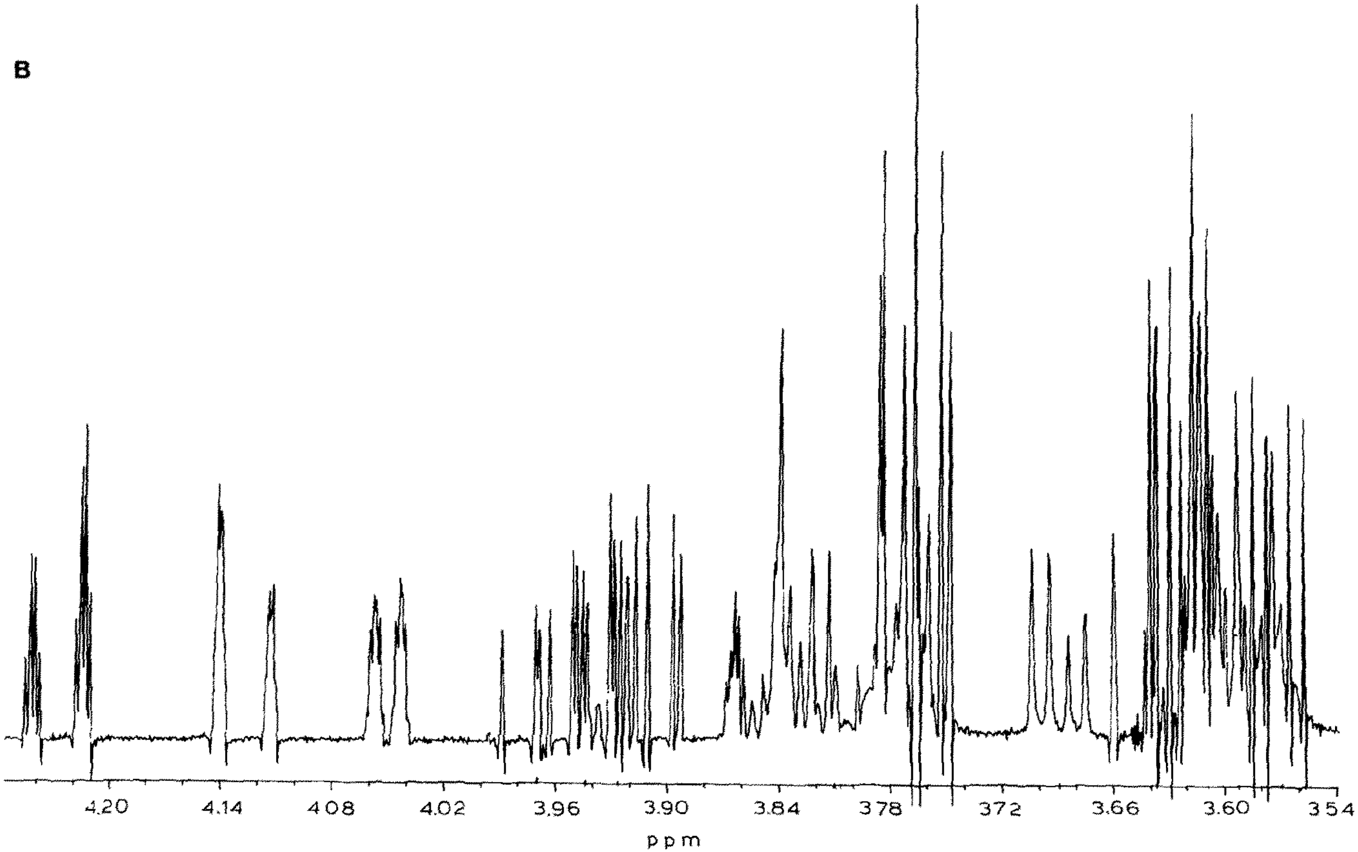
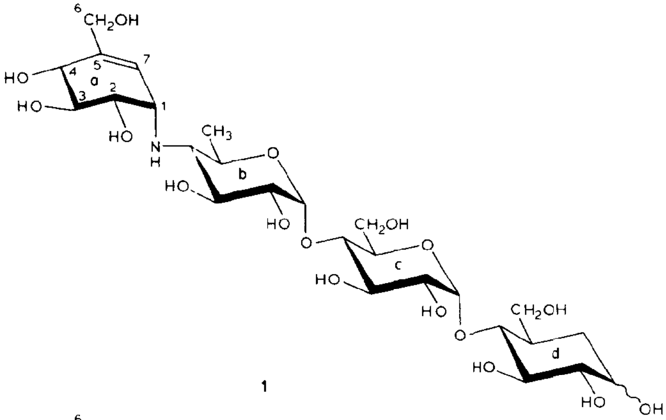
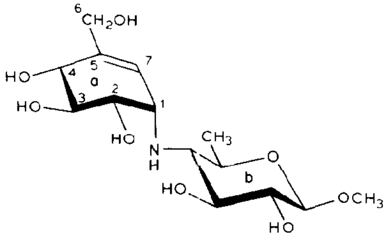


Fig. 1. $^1$H-N.m.r. spectrum (500 MHz) of acarbose (**1**) in $D_2O$ at 37°: A, normal spectrum; B, expansion of segment $\delta$ 3.5-4.3.



1



2

The enhancement of the signal for H-7a (1.2% and 2.3% for **1** and **2**, respectively) on saturation of the C-5 methyl group of the b-unit clearly shows that this methyl group must be close to H-7a, the vinylic proton of the valienamine ring. This result was confirmed by saturation of H-7a, which causes a 2.4% enhancement of the signal of H-6b, but an enhancement of 0.8% for **1** and 2.4% for **2** of the signal of H-4b was also observed. This is further confirmed by the enhancements observed (1.0% and 2.9% for the signal of H-7b of **1** and **2**, respectively) on saturation of H-4b. All these results accord with the calculated distance between H-4b and H-7a (2.29 Å).

TABLE III

N.O.e. DATA[a] OF ACARBOSE (**1**) AND THE DISACCHARIDE-GLYCOSIDE **2**

| *Proton saturated* | *N.O.e. observed (%)* |
|---|---|
| **1**; H-7a | 3.2 (H-1a), 2.4 (H-6a), 2.4 (H-6b), 0.8 (H-4b) |
| **2**; H-7a | 9.0 (H-1a), 6.3 (H-6a), 4.5 (H-6b), 2.0 (H-4b) |
| **1**; H-4b | 2.5 (H-6b), 1.0 (H-7a), 5.0 (H-1a + H-2b) |
| **2**; H-4b | 3.8 (H-6b), 2.9 (H-7a), 11.0 (H-1a), 4.7 (H-2b) |
| **1**; H-6b | 1.2 (H-7a), 1.4 (H-4b), 2.0 (H-5b) |
| **2**; H-6b | 2.3 (H-7a), 2.3 (H-4b), 4.6 (H-5b) |

[a] Performed in the difference mode; accuracy, ±10%.

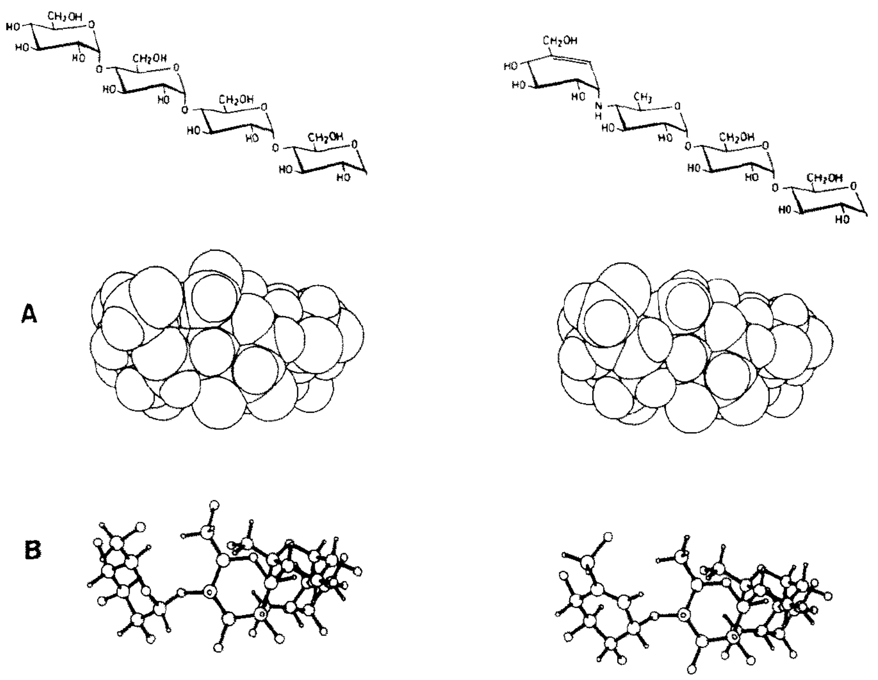


Fig. 2. Minimum energy conformation of maltotetraose and acarbose (**1**) calculated using the HSEA method: A, CPK models; B, stick and ball models. The $\varphi/\psi$ values used for the maltose glycosidic bonds are −25°/ −20°, and the $\varphi/\psi$ angles for the pseudoglycosidic bond between the valienamine unit and the 4-amino-4,6-dideoxy-α-D-glucopyranose unit are −39°/ −19°.

The results discussed above assume that the preferred conformation of the valienamine ring is the half-chair $^2H_5$; the $J_{2a,3a}$ and $J_{3a,4a}$ values of 9.6 and 7.0 Hz confirm that this assumption is to a large extent fulfilled.

Thus, it is concluded that the preferred conformation of acarbose (**1**) in solution is as indicated in Fig. 2, which is very similar to that of maltotetraose[8,14], *i.e.*, with a hydrophobic side determined by H-7a, H-6b, and O-5b, and a hydrophilic side which includes all the hydroxyl groups. In agreement with experimental observations[1,2], acarbose can therefore be expected to serve as a good inhibitor for amylose-degrading enzymes since, conformationally, it is similar to maltotetraose. Hence, acarbose will fit into the active site of the enzymes, and binding is enhanced by interaction of the amine function with acid groups in the enzyme protein.

## EXPERIMENTAL

$^1$H-N.m.r. spectra were measured on 0.01M solutions in $D_2O$ (internal acetone, δ 2.22) at 37°, and at 500 or 400 MHz with a Bruker AM or WM instrument, respectively. The sweep width used was 5 kHz, which, with a computer

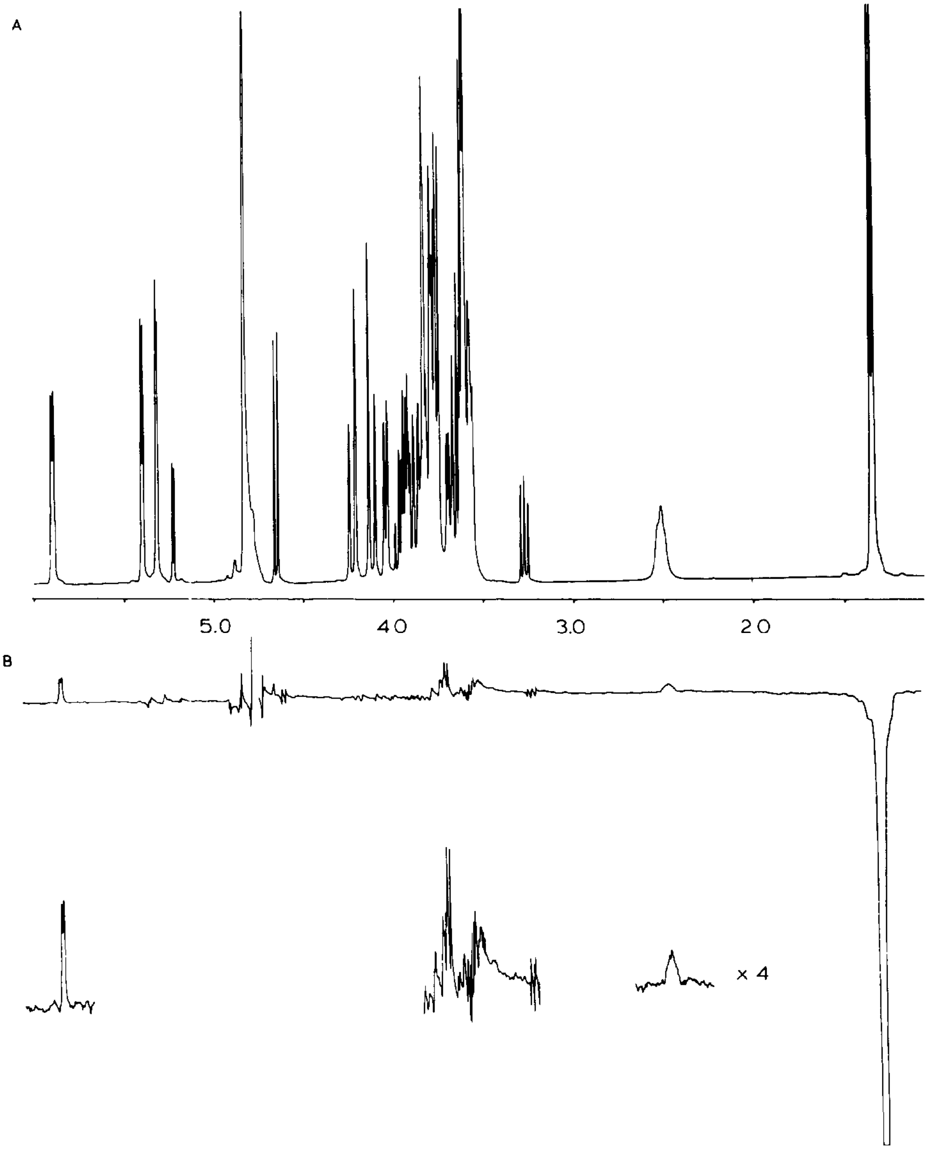


Fig. 3. $^1$H-N.m.r. spectrum (400 MHz) of acarbose (1) in $D_2O$: A, normal spectrum; B, n.O.e. experiment performed in the difference mode with saturation of H-6b.

memory of 32 k, gave a digital resolution of ±0.3 Hz, and 90° pulses (10 $\mu$s) were used. The n.O.e. experiments were performed in the difference mode[15].

$^{13}$C-N.m.r. spectra (67.89 MHz) were measured on 0.2M solutions in $D_2O$ (internal 1,4-dioxane, $\delta$ 67.4) at 37° with a Bruker HX-270 instrument. The sweep width used was 10 kHz, which, with a computer memory of 32 k, gave a digital resolution of ±0.6 Hz, and 90° pulses (13 $\mu$s) were used.

## ACKNOWLEDGMENTS

The authors thank Dr. F. Heiker (Bayer A/G) for samples of acarbose and the methyl glycoside **2**. The project was supported by Novo A/S and The Danish Council for Scientific and Industrial Research.

## REFERENCES

1 W. Puls, U. Kemp, H. P. Krause, G. Thomas, and F. Hoffmeister, *Naturwissenschaften*, 64 (1977) 536; E. Truscheit, W. Frommer, B. Junge, L. Muller, D. D. Schmidt, and W. Wingender, *Angew. Chem.*, 93 (1981) 738; B. Junge, H. Boshagen, J. Stoftefuss, and L. Muller, *Enzyme Inhibitors, Proc. Meet.*, 1980, 109–122; *Chem. Abstr.*, 94 (1981) 204413; L. Muller, B. Junge, W. Frommer, D. Schmidt, and E. Truscheit, *ibid.*, 1980, 123–137; *Chem Abstr.*, 94 (1981) 187620.
2 K. Fukuhara, H. Murai, and S. Murao, *Agric. Biol. Chem.*, 46 (1982) 2021–2030.
3 H. Thøgersen, R. U. Lemieux, K. Bock, and B. Meyer, *Can. J. Chem.*, 60 (1982) 44–57.
4 K. Bock, S. Josephson, and D. Bundle, *J. Chem. Soc., Perkin Trans. 2*, (1982) 59–70.
5 K. Bock, J. Arnarp, and J. Lönngren, *Eur. J. Biochem.*, 129 (1982) 171–178.
6 K. Bock, J. Defaye, H. Driguez, and E. Bar-Guilloux *Eur. J. Biochem.*, 131 (1983) 595–600.
7 P. Luger, K. Vangehr, K. Bock, and H. Paulsen, *Carbohydr. Res.*, 117 (1983) 23–38.
8 R. U. Lemieux and K. Bock, *Arch. Biochem. Biophys.*, 221 (1983) 125–134.
9 K. Bock, *Pure Appl. Chem.*, 55 (1983) 605–622.
10 G. A. Morris and L. D. Hall, *Can. J. Chem.*, 60 (1982) 2431–2441.
11 A. Heyraūd, M. Rinaudo, M. Vignon, and M. Vincendon, *Biopolymers*, 18 (1979) 167–185.
12 G. A. Jeffrey, R. K. McMullan, and S. Takagi, *Acta Crystallogr., Sect. B*, 33 (1977) 728–737.
13 B. Kitschke and H. J. Lindner, *Chem. Ber.*, 111 (1978) 3780–3789.
14 R. U. Lemieux, E. Alvarado, K. Bock, and H. Pedersen, unpublished data.
15 K. Bock and H. Thøgersen, *Annu. Rep. NMR Spectrosc.*, 13 (1982) 1–57.