THE DETERMINATION OF THE CONFIGURATION OF THE GLYCOSIDIC LINK IN OLIGOSACCHARIDES BY P.M.R. SPECTROSCOPY OF TRIMETHYLSILYL DERIVATIVES

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SUMMARY: The essential points of the p.m.r. spectra of the trimethylsilyl derivatives of maltose, cellobiose, α,α-trehalose, lactose, sucrose, lactulose, raffinose and melezitose are given. The protons on the anomeric carbon atoms resonate at lower field than the remaining protons. On the basis of this feature a new method for the determination of the configuration of the glycosidic link in oligosaccharides has been developed.

The use of n.m.r. spectroscopy has become an important technique in carbohydrate chemistry. For monosaccharides it has been applied for conformational analysis (1), configurational effects (2), solvent effects (3), determination of the position of free hydroxyl groups in partially acetylated sugars (4) and anomerization experiments (5). Free oligosaccharides have been studied by Van der Veen (6) and Ovodov et. al. (7). P.m.r. spectra of peracetyl derivatives of sucrose, 1-kestose and nystose have been described by Binkley et. al. (8). Recently Friebolin (9) published a 220 MHz-study of peracetyl- and perbenzoyl-polysaccharides.

In the framework of sequence studies of oligosaccharides, we are interested in methods to determine the configuration of the hydrogen atom on the anomeric carbon atom of the glycosidic link in oligosaccharides. To that purpose we investigated the trimethylsilyl derivatives of a number of di- and tri-saccharides by p.m.r. spectroscopy. In literature (5,10,11,12,13) there are a few examples of p.m.r. spectroscopy of trimethylsilyl monosaccharides and of trimethylsilyl ethers of flavonoïd glycosides.

MATERIALS AND METHODS

Preparation of trimethylsilyl (TMS) derivatives (14): 10 mg of a di- or tri-saccharide was dissolved in 1 ml of dry pyridine, subsequently 0,4 ml of hexamethyldisilazane (EGA-Chemie KG) and 0,2 ml of trimethylchlorosilane (Schuchardt) were added. The turbid mixture was shaken for 15 minutes at room temperature. After two hours 3 ml of hexane and 3 ml of water were added. The two-layer system was strongly agitated and subsequently the water layer was removed. The hexane layer was washed with 1 ml of water. Immediately thereafter anhydrous Na_2SO_4 was added to the hexane layer. After a few minutes the organic layer was decanted into another tube and evaporated. The residue was dissolved in aceton-D_6.

Carbohydrates: β-D(+)-maltose monohydrate, β-D(+)-cellobiose, α,α-D-trehalose dihydrate, α-D(+)-lactose monohydrate, D(+)-sucrose, D-lactulose, D(+)-raffinose pentahydrate, D-melezitose dihydrate were obtained from J.T. Baker Chemicals N.V.

P.m.r. spectroscopy: the spectra were recorded with an Varian HA 100 spectrometer, locked on the trimethylsilyl peaks and operating in the field sweep mode. The chemical shifts are determined relative to tetramethylsilane in the δ-scale at room temperature.

RESULTS AND DISCUSSION

By trimethylsilylation of carbohydrates, the OH groups are converted into $OSi(CH_3)_3$ groups, so that the often rather broad OH signal is eliminated. The protons of the trimethylsilyl groups resonate at high applied magnetic field ($\delta \approx 0$), in the low field area appear only the protons on C1 up to C6. The protons on the anomeric carbon atoms occupy an unique position, because they are attached to carbon atoms which bear two oxygen atoms. On the whole they resonate at a lower field than the remaining protons, taking into account that protons in equatorial position show a larger chemical shift than protons in axial position. In the case of axial-axial couplings between the protons on C1 and C2, the spin-spin coupling constants are larger than in the case of equatorial-axial, axial-equatorial or equatorial-equatorial couplings (15). We compared the spectra of TMS-derivatives of some specific anomeric forms of reducing oligosaccharides with those of oligosaccharides which have been kept in a water solution during 48 hours before silylation. After anomerization the spectra of the reducing carbohydrates have changed. In the case of (1 → 4) reducing disaccharides, we observed that the hydrogen atom on the anomeric carbon atom of the glycosidic link shows different chemical shifts in both anomeric forms. In the spectra of free oligosaccharides such differences are not observed (6). Further, we can conclude that during

TABLE A
δ-values and coupling constants J_{12} of the anomeric hydrogen atoms

TMS-(D-maltose)	$H_G^{\alpha \to \beta}$	H^{β}	$H_G^{\alpha \to \alpha}/H^{\alpha}$
O-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranose	5,31 (3,2Hz)	4,66 (7,1Hz)	-
O-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose	5,32 (3,3Hz)	4,67 (7,3Hz)	5,20 (3,5Hz) and 5,09 (3,4Hz)
TMS-(D-cellobiose)	$H_G^{\beta \to \alpha}$	H^{α}	$H_G^{\beta \to \beta}/H^{\beta}$
O-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose	4,48 (7,0Hz)	5,02 (3,5Hz)	4,55 (7,0Hz) and 4,54 (7,0Hz)
TMS-(α,α-D-trehalose)	H_G^{α}		
O-α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside	4,94 (3,0Hz)		
TMS-(D-lactose)	$H_G^{\beta \to \alpha}$	H^{α}	$H_G^{\beta \to \beta}/H^{\beta}$
O-β-D-galactopyranosyl-(1 → 4)-α-D-glucopyranose	4,40 (7,5Hz)	5,00 (3,2Hz)	-
O-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose	4,40 (7,4Hz)	5,01 (3,4Hz)	4,54 (7,4Hz) and 4,44 (7,4Hz)
TMS-(D-sucrose)	H_G^{α}		
O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside	5,30 (3,4Hz)		
TMS-(D-lactulose)			
O-β-D-galactopyranosyl-(1 → 4)-D-fructose	no H_G^{α} detected		
TMS-(D-raffinose)	H_G^{α} (1)	H_G^{α} (2)	
O-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside	5,25 (3,2Hz)	5,14 (0,0Hz)	
TMS-(D-melezitose)	H_G^{α} (1)	H_G^{α} (2)	
O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(3 → 1)-α-D-glucopyranoside	5,49 (3,4Hz)	4,93 (3,1Hz)	

Legend to table A:

Table A.
Coupling constants J_{12} are given in Hz with an accuracy of ± 0,2 Hz.
Explanation of the signs used in table A:

H^{α} means: equatorial hydrogen atom on C1 of the reducing unit of the saccharide

H^{β} means: axial hydrogen atom on C1 of the reducing unit of the saccharide

$H_G^{\alpha \rightarrow \alpha}$ means: equatorial hydrogen atom on C1 of the nonreducing unit of the saccharide. The reducing unit has also an equatorial hydrogen atom on C1

$H_G^{\alpha \rightarrow \beta}$ means: equatorial hydrogen atom on C1 of the nonreducing unit of the saccharide. The reducing unit has an axial hydrogen atom on C1

$H_G^{\beta \rightarrow \beta}$ means: axial hydrogen atom on C1 of the nonreducing unit of the saccharide. The reducing unit has also an axial hydrogen atom on C1

$H_G^{\beta \rightarrow \alpha}$ means: axial hydrogen atom on C1 of the nonreducing unit of the saccharide. The reducing unit has an equatorial hydrogen atom on C1

H_G^{α} means: equatorial hydrogen atom on C1

H_G^{β} means: axial hydrogen atom on C1

trimethylsilylation no detectable anomerization takes place.

For the interpretation of the spectra of the TMS-derivatives it is essential to know the sequence of the monosaccharides in the oligosaccharides. The significant features of the p.m.r. spectra are summarized in table A.

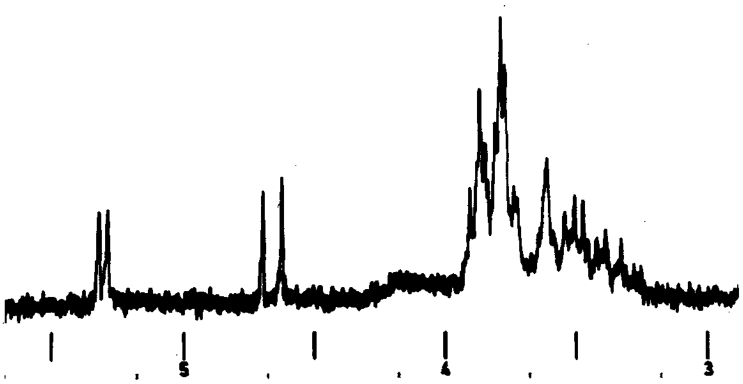

Fig. 1. P.m.r. spectrum at 100 MHz in aceton-D_6 of TMS-(β-D-maltose). The protonsignals of the trimethylsilylgroups are not given.

The spectrum of TMS-(O-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranose) (figure 1) shows a doublet with J = 3,2 Hz at δ = 5,31 and a doublet with J = 7,1 Hz at δ = 4,66. The doublet at δ = 5,31 must relate to an equatorial hydrogen atom and the doublet at δ = 4,66 to an axial hydrogen atom. The spectrum of TMS-(O-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose) shows three doublets with J ≈ 3,4 Hz at δ = 5,32 , δ = 5,20 and δ = 5,09 and further a doublet with J = 7,3 Hz at δ = 4,67. The three doublets with J ≈ 3,4 Hz must relate to equatorial hydrogen atoms and the doublet with J = 7,3 Hz to an axial hydrogen atom. The two doublets at δ = 5,20 and δ = 5,09 relate to two equatorial anomeric hydrogen atoms of TMS-(O-α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranose). However, we were unable to identify the δ-value which corresponds to one of the two α-hydrogen atoms. In the spectrum of TMS-(D-maltose), the ratio of equatorial anomeric hydrogen atoms to axial anomeric hydrogen atoms is 3:1. From this spectrum we can conclude that the configuration of the hydrogen atom on the carbon atom of the glycosidic link must be the α-configuration.

In the same manner we can conclude from the spectra of TMS-(D-cellobiose) and TMS-(D-lactose) that the configuration must be the β-configuration.

The spectrum of TMS-(α,α-D-trehalose) shows only a doublet with J = 3,0 Hz at δ = 4,94. This indicates the α,α-configuration in TMS-(D-trehalose).

The spectrum of TMS-(D-lactulose) shows, that the anomeric hydrogen atom is not attached in equatorial position, but in this case we cannot exactly detect the chemical shift of the axial proton. Other hydrogen atoms have about the same chemical shift and the same coupling constant.

The spectrum of TMS-(D-sucrose) shows a hydrogen atom in equatorial position, but also a hydrogen atom (doublet) with a coupling constant of 7,9 Hz. This "axial" hydrogen atom must be the hydrogen atom on C3 of the fructofuranose unit.

The spectrum of TMS-(D-raffinose) shows a doublet with J = 3,2 Hz at δ = 5,25 and a singlet at δ = 5,14. Probably, the peak at δ = 5,14 relates to the hydrogen atom on C1 of the galactopyranose unit, because also in the spectrum of TMS-(α-D-galactopyranose) we observed a peak with coupling constant near zero (compare ref. 13). The spectra of TMS-(D-raffinose) and TMS-(D-melezitose) show clearly that the configurations of both glycosidic links are α-configurations.

The results presented above, demonstrate the suitability of p.m.r. spectroscopy to trimethylsilyl di- and tri-saccharides for the determination of the configuration of the glycosidic link. The interpretation of the spectrum of the remaining protons gives still rise to severe problems. The separation of anomeric forms by gas-liquid chromatography seems advisable, as the availability of the pure components can be of great help for the interpretation. The recording of 220 MHz spectra, however, looks very promising to obtain an ultimate answer in the identification of the remaining protons.

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