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# Investigation of Polysaccharides by Two-Dimensional N.M.R. Spectroscopy

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## Summary

This paper is relative to a study of polysaccharides in solution by two-dimensional nuclear Overhauser enhancement (2D NOE) and correlated (SECSY) experiments. The main advantages and limitations of these techniques are described in the case of molecules in solution into dimethyl sulfoxide (d.m.s.o.). In particular only one 2D NOE experiment is capable to provide informations concerning dipolar interactions as well as chemical exchange phenomena. It also open a new route to obtain conformational data with polysaccharides.

## Introduction

Many recent papers have shown the various improvements obtained with two-dimensional (2D) J-resolved spectroscopy in the case of different macromolecules (AUE et al. 1976 ; NAGAYAMA et al. 1978) and polysaccharides (GAGNAIRE et al. 1982). The derived resolution enhancement, identification of overlapping protons and accurate determination of coupling constants lead to an easier interpretation of $^1H$ spectra and to informations capable of providing insight into structural and stereochemical details.

2D spin-echo correlated spectroscopy is another attractive technique which manifests connectivities between scalar coupled nuclei (SECSY) or protons through dipol-dipol interactions (2D NOE) providing assignments of spin systems or informations related to spatial molecular structure, respectively (NAGAYAMA et al. 1979 ; JEENER et al. 1979).

The nuclear Overhauser effect is a particularly useful technique for measurement of distances between hydrogen atoms. However its application to macromolecules at high magnetic fields is limited by spin diffusion effects and difficulties to obtain selective saturation of individual lines in crowded region of the spectra. These difficulties are largely overcome by doing a 2D NOE experiment.

In the following some recent examples of SECSY and 2D NOE experiments in the case of oligo- and polysaccharides which contain, a) only 1 → 4' linked α-D-glucose as the repeating unit : amylose and cyclohexaamylose, b) alternated 1 → 3' and 1 → 4' linked α-D-glucose residues in the repeating unit : nigeran, are discussed.

## Experimental

N.M.R. 2D NOE and SECSY experiments were recorded at 250 MHz on a Bruker WM 250 spectrometer equipped with an Aspect 2000 computer using the Bruker FT NMR 2D program version 810515. The data were obtained with the following spectrometer settings :

- for commercially available amylose sample (Nutritional Biochemical Corporation) in d.m.s.o. $d_6$ (5 % solution) at 303° K ; 2D NOE's parameters:

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F2 frequency range = 1000 Hz over 2K data size (0.98 Hz per point) ; F1 frequency range ± 500 Hz with 1K data size (0.98 Hz per point) ; mixing delay 0.5 s ; relaxation delay : 3 s ; scan number = 16 ; before Fourier Transformation the data were multiplied in both direction by exponential window functions and a line broadening factor of 0.3 Hz.

- for commercially available cyclohexaamylose sample (Sigma) in d.m.s.o. $d_6$ (5 % solution) at 303° K ; 2D NOE's parameters : F2 frequency range = 900 Hz over 2K data size (0.88 Hz per point) ; F1 frequency range ± 450 Hz with 1K data size (0.88 Hz per point) ; mixing delay = 0.6 s ; relaxation delay : 3 s ; scan number = 12 ; before Fourier Transformation the data were processed as described previously.

- For biosynthesized nigeran (gift of Dr M. VINCENDON) in d.m.s.o. $d_6$ (5 % solution) at 373° K ; 2D SECSY parameters : F2 frequency range = 800 Hz over 2K data size (0.78 Hz per point) ; F1 frequency range 400 Hz with 1K data size (0.78 per point) ; relaxation delay : 3 s ; scan number : 16 ; for 2D NOE experiment the same general parameters were used but with a mixing delay of 0.3 s. In both experiments the window functions used were of an exponantial form with a line broadening factor of 0.3 Hz.

## Results and discussion

In solution into deuterated dimethyl sulfoxide (d.m.s.o.) at 303° K the one-dimensional $^{1}$H spectrum of amylose shows proton signals of the ring, and of the hydroxyl groups, OH-2, OH-3 and OH-6, whose corresponding assignments are already published (St JACQUES et al. 1976).

With the same solvent and temperature conditions a 2D NOE experiment was performed. Dipolar connectivities are indicated on parallel diagonal lines as illustrated Fig. 1. Thus H-1 appears connected with H-2 (as expected for an α-glycosidic bond) and H-4' (the symbol ' refers to an adjacent unit). In the same way a correlation between H-1 and H-3' through space can be detected. Another interesting feature concerns strong connectivities between OH-3 and H-3, OH-6 and H-6, OH-2 and OH-3' respectively. This last NOE's effect indicates a relative closeness in space for both hydroxyl groups, in agreement with the existence of a conformation in which an intramolecular hydrogen bond between OH-2 and OH-3' could take place, i.e., 2.4 Å ⩽ O-2----O-3' distance ⩽ 3.2 Å (CASU et al. 1966).

Furthermore the correlations between OH-3' and H-3', OH-2 and H-3 could also show that OH-3' is the donor hydroxyl group in the OH-3'----OH-2 hydrogen bond.

In addition weak connectivities of H-1 with OH-2 and OH-3' are also visible. All these informations if well distinguishable from residual water exchange could lead to a good representation of the amylose chain in solution. However more studies are needed and are actually in progress to express in quantitative terms the 2D NOE's data, and to developp from them, conformational calculations in order to restrict the range of acceptable torsion angles $\phi$ and $\psi$ around the glycosidic bonds.

The case of the torus-shaped cyclic hexasaccharide consisting of (1 → 4') linked glucopyranose residues or cyclohexaamylose is more tricky. 2D NOE's between H-1 and H-2, H-1 and H-4', OH-3 and H-3, OH-2 and H-2, OH-6 and H-6, OH-2 and OH-3' give rise to strong cross-peaks. Weak connectivities between H-1 and OH-2, OH-3', OH-6' respectively, and between OH-2 and OH-6, OH-3 and OH-6 are also observable. The preceding general characteristics found for amylose are still valid (for example the existence of a strong connectivity between OH-2 and OH-3', H-1 and H-2, H-1 and H-4'), however cautions must be taken before to conclude, because of the hydrate character of this molecule. As reported by MANOR and SAENGER (1974) and by TABUSHI et al. (1978), in the solid state, four of the six hydration water molecules are located outside the cyclohexaamylose and are part of

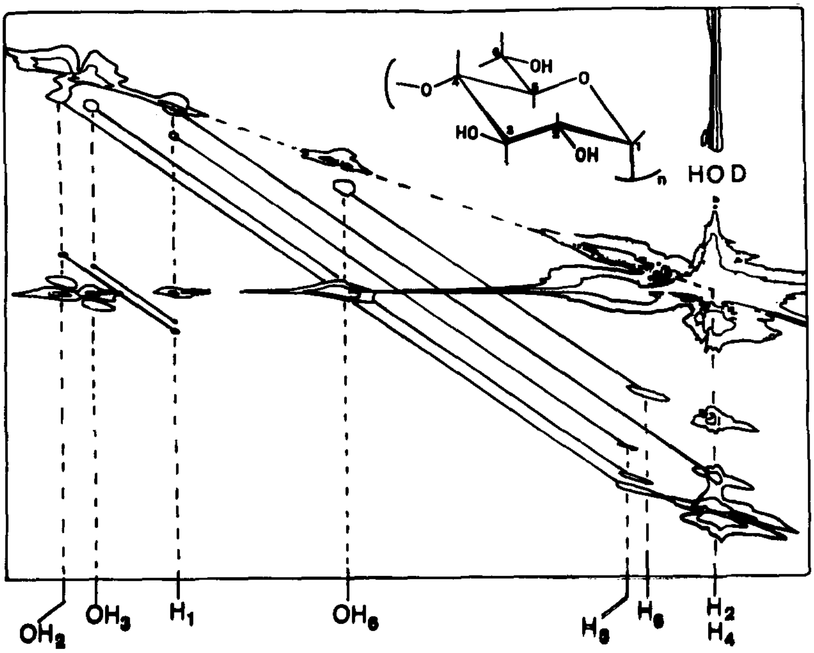

Figure 1 - Contour plot of proton 2D NOE spectrum at 250 MHz of Amylose in d.m.s.o. solution. Dipolar connectivities are indicated on parallel diagonal lines. (See text for spectrometer settings).

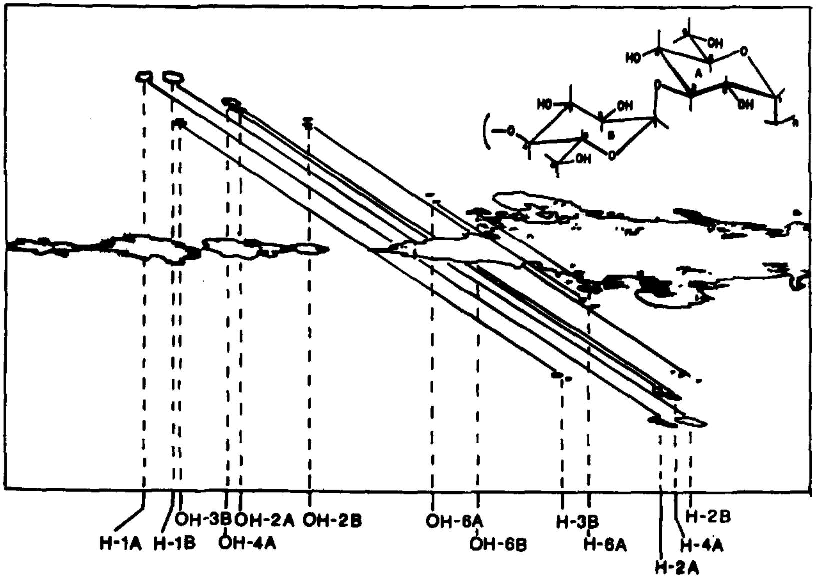

Figure 2 - Contour plot of proton 2D SECSY spectrum at 250 MHz of Nigeran in d.m.s.o. solution. Scalar connectivities are indicated on parallel diagonal lines, in particular between hydroxyl protons and the corresponding ring protons. (See text for spectrometer settings).

an extensive hydrogen bonding network, while the two remaining water molecules are located in the cavity, hydrogen bonded to each other and to two glucopyranose rings. In this example a slow chemical exchange between water and the hydroxyl groups is clearly shown by connectivities between OH-2 and OH-6, OH-3 and OH-6 and between all the hydroxyl groups with the water peak. But in this case it is difficult to distinguish dipolar relaxation from chemical exchange.

The preceding results show that it is sometimes difficult to precise if a connectivity observed affects two hydrogen atoms from the same glucose residue or from two neighboring glucose units. The case of nigeran, which is an alternated 1 → 3' and 1 → 4' α-D-glucan, is much clearer from this point of view. The proton analysis of this polysaccharide in d.m.s.o. is already known (BOCK *et al.*, unpublished results). However in order to assign the hydroxyl protons both 2D-J-resolved and SECSY experiments have been performed. Fig. 2 shows the scalar connectivities found between the hydroxyl protons and the corresponding protons of the rings. The following $^3J$(HCOH) coupling constants are determined at 373° K : OH-3B (3.5 Hz), OH-4A (4,5 Hz), OH-2A (6,5 Hz), OH-2B (6,5 Hz), OH-6A (5,5 Hz), OH-6B (5,5 Hz), respectively, according to their chemical shift from low to high field. Temperature dependence of the chemical shifts of these hydroxyl protons has also been studied and is reported Fig. 3.

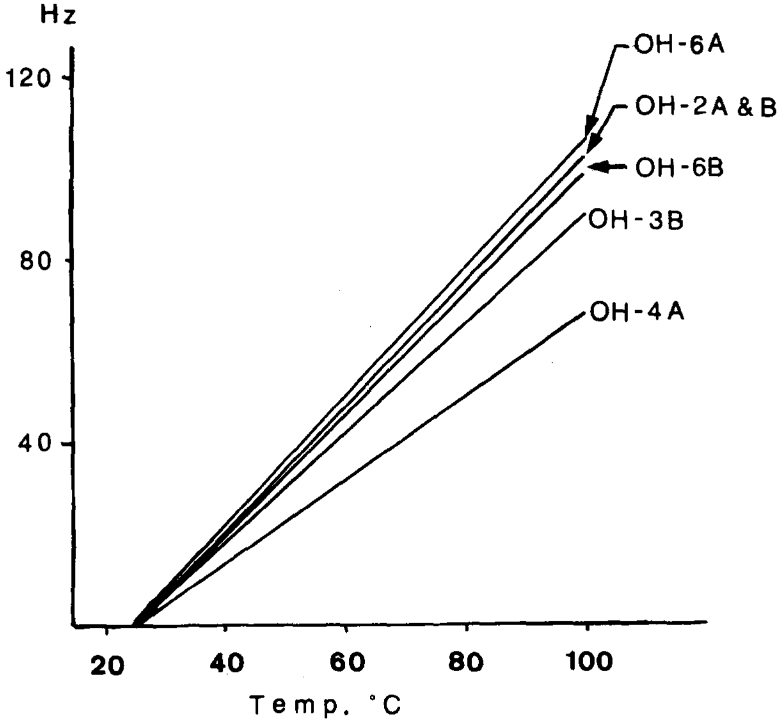

Figure 3 - Temperature dependence of the chemical shifts of the hydroxyl protons in Nigeran. The ordinate refers to the magnitude of the upfield shift Δν, measured in Hz, relative to the chemical shift at ambient temperature.

At 303° K all the hydroxyl protons are well individualized, however the residual water peak is overlapping with H-2A, H-2B, H-4A and H-4B. For this reason a 2D-NOE experiment has been performed at 373° K. Chemical exchange connectivities between hydroxyl protons and HOD are easily observed (except in the case of OH-2A). Furthermore H-1A and H-1B are strongly connected with H-2A and H-2B respectively. Strong correlations are also detected between H-1A and H-4B and between OH-4A and OH-2B, while H-1B appears weakly connected with H-3A and OH-2B. All these observations are in agreement with conventional one-dimensional experiments already performed on anomeric protons measurements of selective $^1H-\{^1H\}$ NOE's in nigerane (BOCK et al.).

From our findings a hydrogen bond between OH-2B and OH-4A could take place. However this is not confirmed by the temperature dependence of the chemical shifts of the hydroxyl protons. As shown on Fig. 3, OH-3B and OH-4A behave in a different manner than the other hydroxyl groups suggesting that an additional factor contributes in this case to the solvent-solute interaction i.e., hydrogen bonding, as discussed by St JACQUES et al. (1976). An intramolecular hydrogen bond between OH-3B and OH-4A would exist but is not detected by 2D NOE experiment. On the contrary a connectivity between OH-2B and OH-4A is observed. In order to solve this discrepancy further evidences are needed, taking into account all the established connectivities as well as conformational parameters from experimental data and theoretical calculations.

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