Carbohydrate Research, 221 (1991) 245–251
Elsevier Science Publishers B.V., Amsterdam

Note

Measurement of the $J_{C\text{-}1,H\text{-}1}$ coupling constants for the *Escherichia coli* O1A O-polysaccharide, a comparison of some n.m.r. experiments

Anne Helander
Kabi Pharmacia Therapeutics, Urology/Gynaecology, S-112 87 Stockholm (Sweden)

and Lennart Kenne
Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm (Sweden)

(Received April 12th, 1991; accepted for publication May 23rd, 1991)

N.m.r. spectroscopy is one of the most important techniques in structural analysis of oligo- and poly-saccharides. The $J_{C\text{-}1,H\text{-}1}$ coupling constants are often used to obtain information about the anomeric configuration of the pyranosyl residues as there is a 10-Hz difference between the values for α anomers (~170 Hz) and those for β anomers (~160 Hz)[1]. The use of these coupling constants is especially valuable for determination of the anomeric configuration of sugar residues with the *manno* configuration, for which the $J_{H\text{-}1,H\text{-}2}$ values are approximately the same for the α and β configurations, and no significant difference in the chemical shifts of the anomeric carbon signals from α and β residues is observed. In one-dimensional n.m.r. spectra, the $J_{C\text{-}1,H\text{-}1}$ coupling constants can be measured from ^{13}C-n.m.r. spectra recorded without proton decoupling or from the ^{13}C-satellites observed in ^{1}H-n.m.r. spectra[2]. Both of these techniques have disadvantages, as low sensitivity of the experiments and often overlap of signals occur. Another problem is the heterogeneity of, or impurities in, the sample, giving rise to signals that interfere with the small satellite signals in the ^{1}H-n.m.r. spectrum.

By use of two-dimensional n.m.r. experiments, the coupling constants can be observed despite the problems discussed above. The heteronuclear *J*-resolved experiment[3] resolves the overlap of signals caused by the coupling, but is rather time-consuming. A more sensitive two-dimensional technique is proton-detected heteronuclear multiple-quantum coherence (HMQC)[4–6], where the peak positions are dependent on both the ^{1}H- and the ^{13}C-n.m.r. chemical shifts, which also has the advantage of resolving complex spectra.

In this study, we have compared some different one- and two-dimensional experiments with respect to the possibility of obtaining the $J_{C\text{-}1,H\text{-}1}$ coupling constants for the anomeric signals from the *Escherichia coli* serotype O1A, O-polysaccharide, for which structure **1** was recently elucidated[7]. In that study, the anomeric proton and carbon signals were assigned [β-D-Man*p*NAc-(1→, δ 5.05/100.63; →2,3)-α-L-Rha*p*-

0008-6215/91/$ 03.50

TABLE I

Experiments investigated, the obtained information, and the corresponding time used for each experiment

Obs. nucleus	*Experiment*	*Time (h)*[a]	*Scans*	*Acquisition time (s)*	*P.d. (s)*	*Obtained* $J_{C\text{-}1,H\text{-}1}$ *values*	*Additional information*
^{13}C	1D, gated decoupled	12.5	15000	1.1	2.0	Three signals	
^{13}C	1D, DEPT	12.5	15000	1.0	2.0	Three signals	$CH/CH_2/CH_3$[b]
^{13}C	1D, INEPT	12.5	15000	1.0	2.0	Three signals	
^{1}H	1D, single pulse	4.2	3200	2.7	2.0	—	$^{3}J_{H,H}$ values
^{13}C	2D, *J* resolved	37	2500 (2048 × 64)	0.13	0.70	Three signals	
^{1}H	2D, HMQC	3.5[c]	64 (2048 × 128)	0.54	0.32	All signals	$^{3}J_{H,H}$ values H,C-connectivity

[a] Total time of the experiment. [b] Signals from the CH_2 carbons will appear downfield. [c] A 0.5-s delay, corresponding to the τ_{zero} signal of the fastest relaxing anomeric signal, was used in the BIRD–HMQC sequence.

(1→, δ 5.18/102.05; →3)-α-L-Rha*p*-(1→, δ 5.03/102.78; →3)-β-L-Rha*p*-(1→, δ 4.90/101.24; and →4)-β-D-Glc*p*NAc-(1→, δ 4.80/102.78], but only three of the $J_{C\text{-}1,H\text{-}1}$ values could be determined due to overlap of two anomeric carbon signals at δ_C 102.78 in the ^{13}C-n.m.r. spectrum.

→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*NAc-(1→
2
↑ 1
1
β-D-Man*p*NAc

In this study, all spectra were recorded at 70°, for a solution containing polysaccharide (20 mg) in deuterium oxide (0.5 mL). A JEOL GX-400 spectrometer was used to record ^{1}H-n.m.r. spectra, whereas the ^{13}C-n.m.r. spectra were obtained with a JEOL EX-270 spectrometer. All experiments were run with the pulse sequences avail-

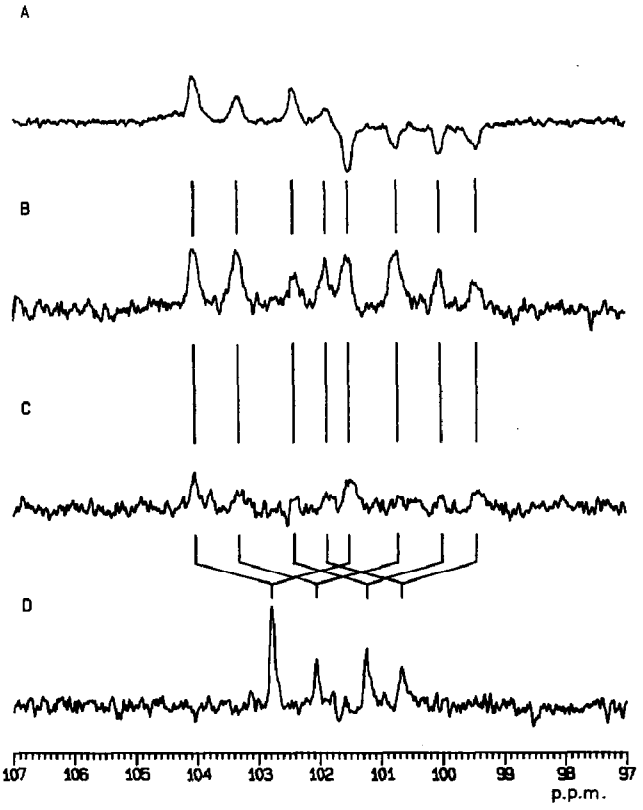

Fig. 1. The anomeric region in different ^{13}C-n.m.r. spectra (67 MHz): A, INEPT; B, DEPT (135°); C, gated decoupled; and D, complete proton decoupled. All spectra were recorded with a resolution of 1.0 Hz/datapoint and the f.i.d.'s exponentially multiplied with a line-broadening factor of 2 Hz before the Fourier transformation.

able in the JEOL standard softwares. The proton-detected heteronuclear multiple-quantum coherence (HMQC) experiments[4-6] were performed together with a bilinear rotation decoupling (BIRD) sequence[8], in order to suppress the ^{1}H–^{12}C signals more effectively. The experiments investigated and the corresponding experimental parameters are summarised in Table I, and the parameters used for processing the f.i.d.'s are written in the figure legends.

Three different one-dimensional ^{13}C-n.m.r. experiments (Fig. 1) were investigated, using the same number of transients. The more sensitive DEPT and INEPT experiments, utilising polarisation transfer, gave spectra with better signal-to-noise ratios than the gated-decoupled experiment, which is only enhanced by the n.O.e. From these experiments, the $J_{C\text{-}1,H\text{-}1}$ values could be determined for only three of the five anomeric carbons, as no resolution was obtained for the two pairs of overlapping signals centered at δ_C 102.78. It is sometimes more convenient to use INEPT instead of DEPT, as the different signs of the related signals in the INEPT experiment can facilitate

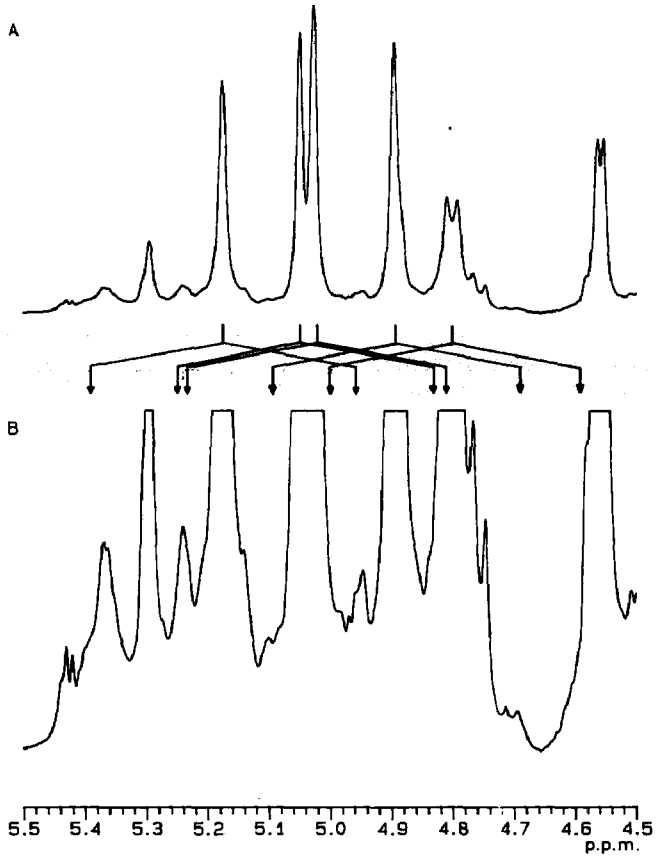

Fig. 2. A, The anomeric region in the ^{1}H-n.m.r. spectrum (400 MHz) with the expected positions for the ^{13}C satellites indicated by arrows; B, a vertical expansion of the same spectrum as A. The spectrum was recorded with a resolution of 0.37 Hz/datapoint and zero-filled once. The f.i.d. was exponentially multiplied before the Fourier transformation with a line-broadening factor of 0.2 Hz.

the interpretation of the spectrum and it is more sensitive due to shorter delay times in the pulse sequence. This is especially noticeable in spectra from polysaccharides with short T_2 values.

In the one-dimensional ^{1}H-n.m.r. spectrum, the $J_{C\text{-}1,H\text{-}1}$ coupling constants can be obtained from the ^{13}C satellites[2]. However, O-polysaccharides also consist of the core oligosaccharide in addition to the repeating units and often have some heterogeneity in their structure; this results in the occurrence of several small signals in the anomeric region and these interfere with the ^{13}C-satellite signals. Another problem is the broad lines normally obtained in spectra from polysaccharides. As can be seen from the ^{1}H-n.m.r. spectrum (Fig. 2), it was not possible to find the ^{13}C satellites due to several overlapping signals, including the large ^{1}H–^{12}C signals. The use of an inversion–recovery pulse, which decreases the intensities of the ^{1}H–^{12}C signals due to longer relaxation times than for the protons connected to ^{13}C, did not improve the spectrum. This technique, however, increased the relative height of the ^{13}C satellite signals for lower-molecular-weight carbohydrates. However, for this polysaccharide, the coupling constants could not be obtained by this experiment.

The two-dimensional heteronuclear J-resolved spectrum (Fig. 3) shows less overlap of signals, as the couplings appear in the f_1 dimension. The $J_{C\text{-}1,H\text{-}1}$ values were easily obtained, except for the two anomeric signals at δ_C 102.78, which still appeared unresolved.

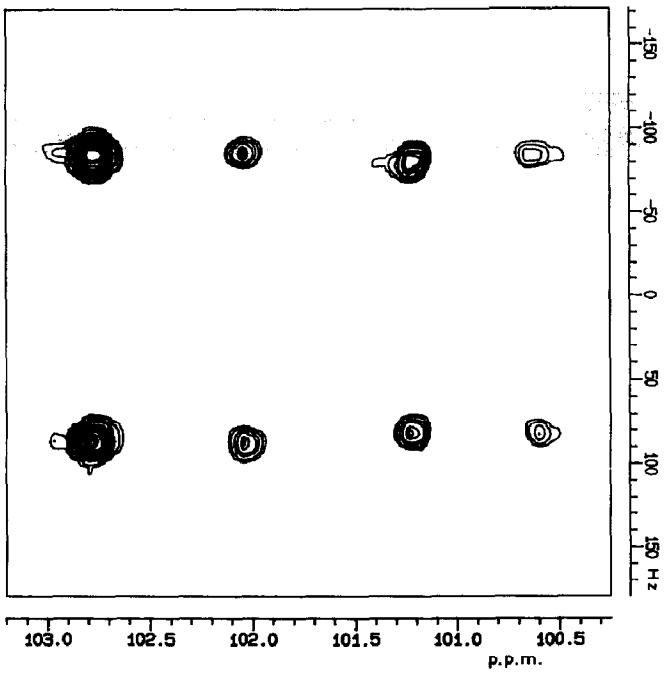

Fig. 3. The anomeric region in a 2D heteronuclear J-resolved spectrum. Exponential and sine-bell filtering in t_2 and t_1, respectively, after zero-filling to 128 datapoints in t_1 were performed.

The only experiment that unambiguously gave the values for all the $J_{C\text{-}1,H\text{-}1}$ coupling constants was the two-dimensional HMQC experiment (Fig. 4). In less than 4 h, a spectrum was recorded which, in addition to the wanted $J_{C\text{-}1,H\text{-}1}$ values, also contained information on chemical shift values for both proton and carbon signals, heteronuclear connectivities, and, in some cases, the splitting pattern of the proton signals. The observed $J_{C\text{-}1,H\text{-}1}$ values obtained in this experiment are given in Fig. 4. From this experiment, it is also evident that the signal at δ_H 4.56 does not derive from an anomeric proton, as these peaks are observed at δ_C 53.9 and have a $^1J_{C,H}$ value of 145 Hz.

From this practical example of measurement of the $J_{C\text{-}1,H\text{-}1}$ coupling constants for a polysaccharide, it is concluded that only some of the J values are obtained from the 1D ^{13}C-n.m.r. spectrum and the 2D J-resolved experiments, and no value from the 1D ^{1}H-n.m.r. experiments. The HMQC experiment, on the other hand, gives the coupling constants and also information on chemical shifts and C,H-connectivities. It is thus concluded that 2D-HMQC is the most attractive experiment to obtain the $J_{C\text{-}1,H\text{-}1}$ coupling constants, especially when signals occur at the same frequency.

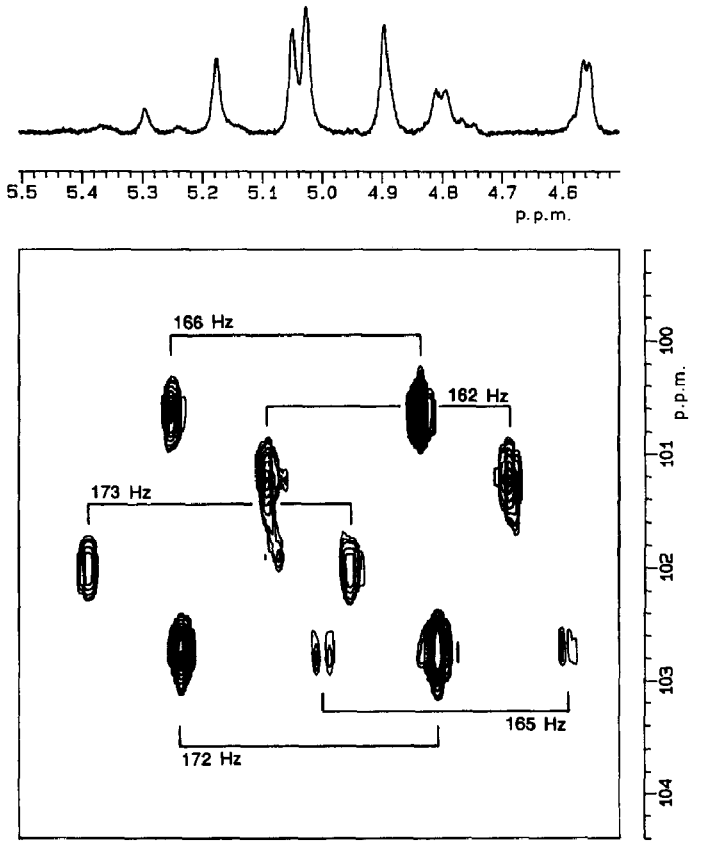

Fig. 4. The anomeric region in a 2D HMQC spectrum. After zero-filling in t_1, the data matrix was exponentially filtered with 0.5 Hz and 5 Hz in t_2 and t_1, respectively.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Natural Research Council and the Swedish National Board for Technical Development.

REFERENCES

1 K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
2 K. Bock and C. Pedersen, *Carbohydr. Res.*, 145 (1985) 135–140.
3 G. Bodenhausen, R. Freeman, and D. L. Turner, *J. Chem. Phys.*, 65 (1976) 839–840.
4 L. Müller, *J. Am. Chem. Soc.*, 101 (1979) 4481–4484.
5 A. Bax and S. Subramanian, *J. Magn. Reson.*, 67 (1986) 565–569.
6 R. A. Byrd, W. Egan, and M. F. Summers, *Carbohydr. Res.*, 166 (1987) 47–58.
7 H. Baumann, P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 211 (1991) 183–190.
8 J. R. Garbow, D. P. Weitekamp, and A. Pines, *Chem. Phys. Lett.*, 93 (1982) 504–509.