Long Range $^{13}C-^{1}H$ Coupling in Carbohydrates by Selective 2D Heteronuclear *J*-resolved N.M.R. Spectroscopy

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A 2D n.m.r. method has been used to determine unambiguously two, three, and four bond $^{13}C-^{1}H$ coupling constants (including those across glycosidic linkages) in non-enriched carbohydrates.

The measurement of long range $^{13}C-^{1}H$ coupling constants (particularly ^{2}J and ^{3}J) in carbohydrates provides important information both on the conformation of individual sugar residues and on the relative orientation of adjacent residues.[1—3] However, because of the large number of such couplings in most carbohydrate systems, individual splittings are often obscured or unresolved in ^{1}H-coupled ^{13}C n.m.r. spectra. For this reason, coupling constant measurements have generally relied on spectral simulation[2,4,5] and/or isotopic enrichment with ^{2}H or ^{13}C.[1,6,7] We now show that the selective 2D heteronuclear *J* experiment developed by Bax and Freeman[8] provides a powerful means of directly measur-

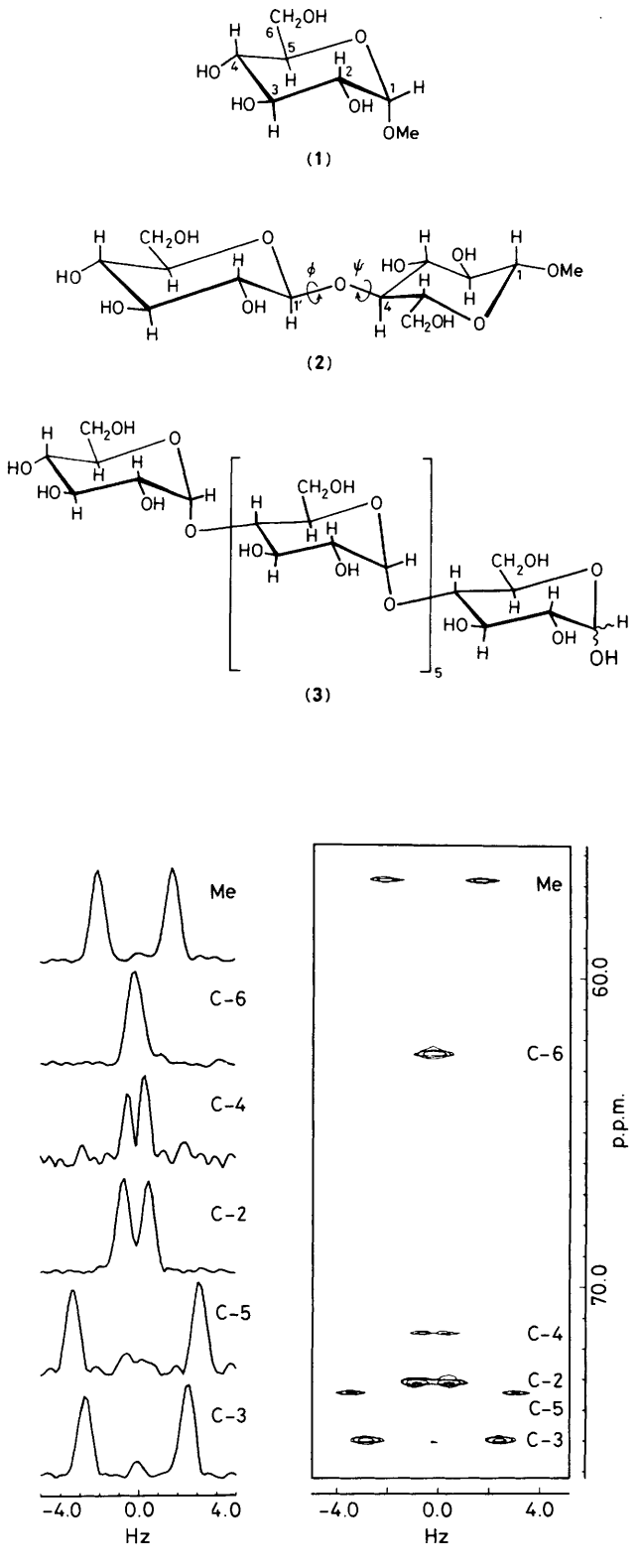

Figure 1. Partial 2D heteronuclear *J* spectrum (obtained on a Bruker CXP 300 instrument) of α-methyl glucoside (**1**) in D_2O at 40 °C following selective irradiation (using a DANTE sequence) of H-1, and cross sections at the chemical shifts of all non-anomeric carbons. 64 Experiments were carried out varying the evolution period up to 3.2 s, giving rise to a spectral width of 20 Hz in the F_1 dimension. Weak signals at $F_1 = 0$ in cross-sections showing coupling are probably due to pulse imperfections.[8]

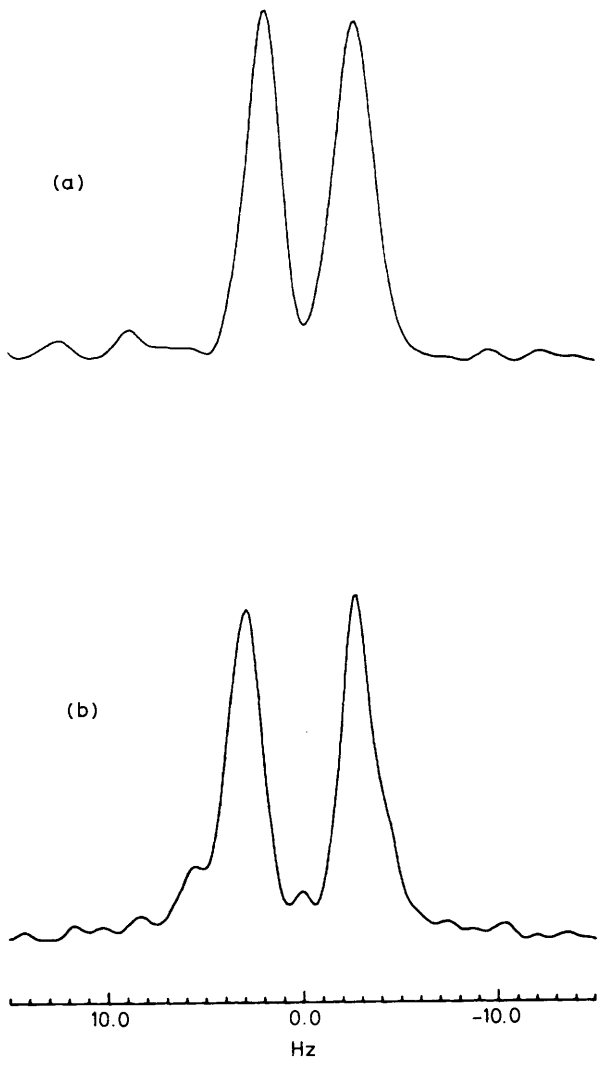

Figure 2. (a) Cross-section of the selective 2D heteronuclear *J* spectrum (CXP 300) of 10% maltoheptaose (**3**) in D_2O at 40 °C at an average chemical shift of C-4 sites in residues 1—6 following selective irradiation of H-1 in residues 2—7 (5.40 p.p.m.[9]). 128 Experiments were carried out, varying the evolution period up to 3.2 s, leading to a spectral width of 40 Hz in the F_1 dimension. The total acquisition time was approximately 9 h. (b) Cross-section showing $^3J_{H\text{-}4\text{-}C\text{-}1}$ in maltoheptaose (**3**) following selective irradiation of H-4 in residues 1—6 (3.65 p.p.m.[9]) and observation at an average C-1 for residues 2—7 in an analogous experiment to (a).

ing and assigning long range ^{13}C–^1H couplings in non-enriched carbohydrates.

In the Bax and Freeman method, the use of a selective 180° proton pulse causes only those couplings from the irradiated proton(s) to be observed in cross-sections at ^{13}C chemical shift frequencies in the heteronuclear 2D *J* spectrum.[8] Further major advantages of the technique are that the large one-bond ^{13}C–^1H couplings are not observed (because of the proton frequency selectivity) and that the frequency range in the F_1 (*J*) dimension can be made very narrow (10—40 Hz) allowing precise coupling constant measurements.

As an example of the method on a simple monosaccharide, the heteronuclear couplings to H-1 of α-methyl glucoside (**1**) have been determined (see Figure 1). Significant coupling occurs to every non-anomeric carbon atom except C-6 and the splitting of 0.85 Hz observed for C-4 is the first measurement

of a four bond ^{13}C–^{1}H coupling constant in a carbohydrate that we are aware of. By contrast, only the 3.8 Hz coupling to the methyl carbon atom could be directly measured from the ^{1}H-coupled ^{13}C n.m.r. spectrum.

The most informative long range ^{13}C–^{1}H couplings in disaccharides and higher oligomers are those across the glycosidic linkage as these coupling constants can be used to deduce torsional angles *via* a Karplus-type relationship.[2—5] Thus for a 1 → 4 linked disaccharide such as β-methyl cellobioside (**2**), $^{3}J_{C\text{-}4\text{–}H\text{-}1'}$ and $^{3}J_{C\text{-}1'\text{–}H\text{-}4}$ are related to the angles φ and ψ respectively.

Irradiation of H-1′ of (**2**) during the selective 2D *J* experiment led to the direct observation of a coupling of 4.2 Hz to C-4, identical to the value obtained by computer simulation of the ^{1}H-coupled ^{13}C n.m.r. spectrum of a partially deuteriated analogue.[5]

Inter-residue coupling in higher oligomers is generally very difficult to assess from ^{1}H-coupled ^{13}C n.m.r. spectra because of signal overlap. This problem is particularly acute for homo-oligomers such as maltoheptaose (**3**) because chemical shift differences between analogous carbon sites along the seven unit chain are of the same order as the expected long range couplings. The selective 2D *J* experiment, however, readily provides the desired information. Figure 2(a) shows the cross-section of the 2D *J* spectrum of (**3**) in D_2O at an average C-4 for residues 1—6 after selective irradiation at H-1 for residues 2—7, and Figure 2(b) shows the cross-section at an average C-1 for residues 2—7 following selective irradiation at H-4 for residues 1—6.

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