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Note

Combined application of two-dimensional techniques for complete, unambiguous assignments of ^{1}H- and ^{13}C-n.m.r. spectra of cellobiononitrile octa-acetate

LÁSZLÓ SZILÁGYI
Department of Organic Chemistry, L. Kossuth University, Debrecen, H-4010 (Hungary)
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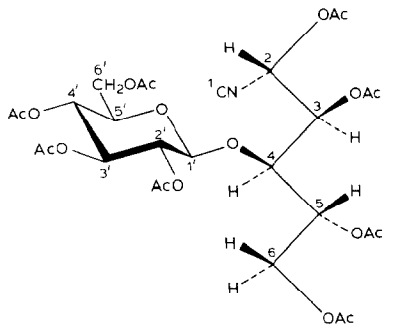
Conventional techniques for assigning n.m.r. (especially ^{13}C) spectra[1] are time-consuming, ambiguous, and often of limited scope. Recently developed, two-dimensional (2D) techniques[2], on the other hand, offer a more powerful alternative, at least for spectra whose complexity is mainly due to overlapping of signals from independent sub-systems of weakly coupled nuclear-spins rather than to strong spin–spin coupling within these systems. Such is frequently the case for polymers of moderate molecular mass, *e.g.*, oligosaccharides.



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Cellobiononitrile octa-acetate [**1**, 2,3,5,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-gluconononitrile] was chosen as a model system to illustrate the potential of the 2D technique for n.m.r. spectral assignment. Fig. 1A shows the conventional 1D ^{1}H-n.m.r. spectrum of **1**, which illustrates some of the practical difficulties commonly encountered in the analysis of n.m.r. spectra. Some of these are (*a*) distortion of the relative intensity of multiplet lines from the expected (first-order) figures, as a consequence of strong coupling; (*b*) accidentally identical spacings; phenomena (*a*) and (*b*) both occur for the H-3′ and H-4′ multiplets between 5.10 and 5.35 p.p.m.; (*c*) overlap of two (in general, several) indepen-

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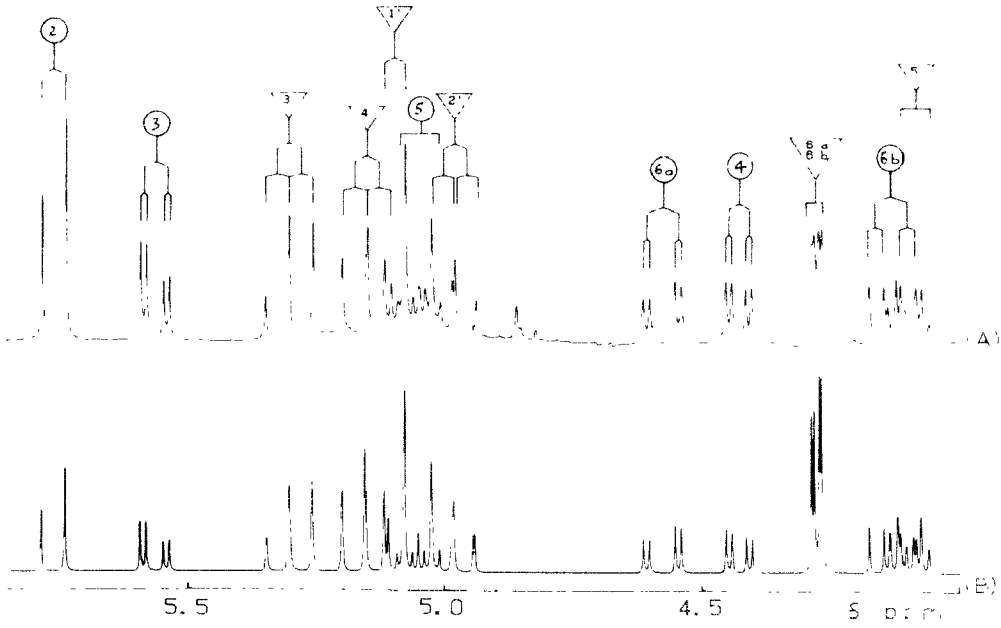

Fig. 1 (A) 200-MHz, ^{1}H-N m r spectrum of **1** (0.02M in acetone-d_6), showing assignments, (B) spectrum calculated by addition of the simulated[7] 6-spin (glucononitrile part) and 7-spin (glucosyl part) sub-spectra

dent sub-spectra; (*d*) occurrence of lines not belonging to the spin systems (in this case, lines between 4.8 and 4.9 p.p.m. arising from impurities).

The majority of these ambiguities can be removed with the aid of the 2D *J*-resolved[3] ^{1}H-spectrum (Fig. 2). For the 13 backbone protons, 12 multiplets can be clearly distinguished [H-6′a and H-6′b being virtually equivalent]. This is remarkable in view of the strong coupling between the pairs H-2–H-3, H-3′–H-4′, and H-1′–H-2′ ($\Delta\nu/J$: 4.32, 3.01, and 2.53, respectively). Extra peaks show up, as predicted theoretically[3], between the multiplets of the strongly coupled protons but, in this case, they can easily be distinguished from the pure *J*-peaks on the basis of their reduced intensities and characteristic frequencies in both dimensions. The doublet for H-1′ can be located unequivocally at 5.09 p.p.m. whereas, in the 1D spectrum, it is difficult to identify in the cluster of lines spaced almost equally between 4.9 and 5.4 p.p.m.

Useful though the 2D *J*-resolved spectrum may be, the *connectivity* of the coupled spins cannot be conveniently deduced therefrom. In fact, it has no advantage over the conventional 1D-spectrum in this respect. This essential information is classically acquired through a series of double-resonance experiments (decoupling, tickling, or INDOR). However, 2D homonuclear-correlated ^{1}H-spectroscopy (COSY[4] or SECSY[5]) is a superior approach. Fig. 3 shows the COSY-45[6] 2D ^{1}H-spectrum of **1**. Starting from the two doublets at 5.76 and 5.09 p.p.m., respectively, it is straightforward to trace the connectivities in the two sub-spectra, as indicated.

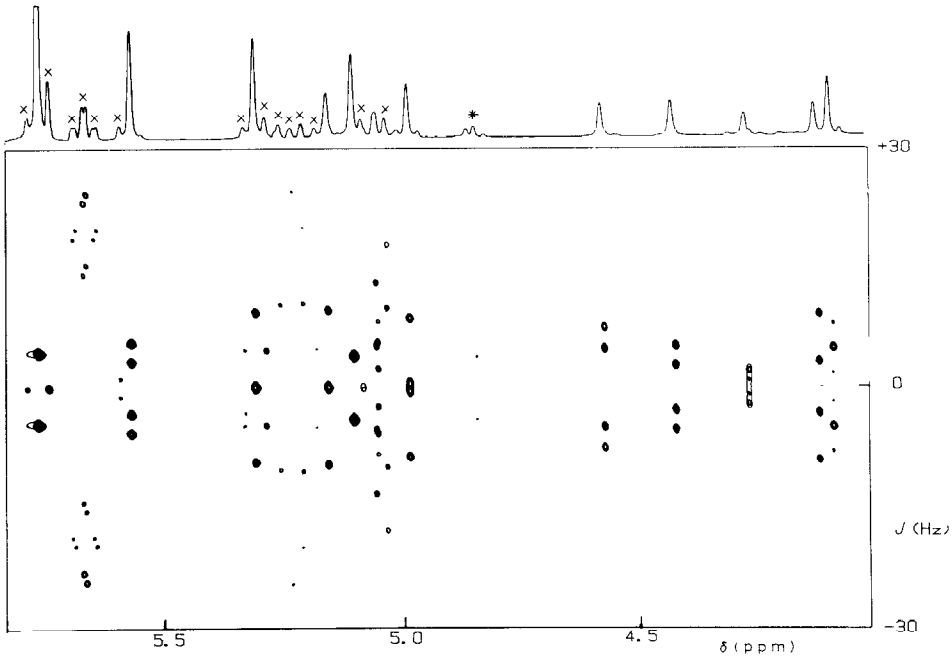

Fig.2. ^{1}H-N.m.r. spectrum (2D *J*-resolved) of **1** and the "broadband proton-decoupled" proton spectrum (above) obtained by projection[10] of the peaks onto the δ-axis. W1 = ±31.25, W2 = 1000 Hz; X1 = 128, X2 = 4k; N1 = 256, N2 = 4k. Sinebell apodisation was applied in both directions prior to Fourier transformation. After 45°-tilt[10], the Fourier-transformed data were subjected to a symmetrisation procedure[11] in order to reduce non-symmetric (with respect to the F1 = 0 axis) noise and the tails of the 2D peaks. Peaks marked with x are due to strong coupling, * marks an impurity peak.

The following points deserve emphasis. (*a*) Diagonal peaks without cross-peak counterparts arise either from "isolated" protons (or groups of magnetically equivalent protons, *e.g.*, the folded acetyl-methyl peaks between 5.35 and 5.45 p.p.m.) or impurities (at 4.85 p.p.m.). (*b*) Although the signals from H-5 and H-5′ are difficult to locate on the main diagonal, their cross-peaks identify them unambiguously. (*c*) Since the numbers of nuclei in the two sub-systems are unequal (6 and 7), the connectivity information contained in the 2D homonuclear-correlated spectrum allows clear-cut separation and unambiguous assignment of the two subspectra. Using the spectral parameters from the above two experiments (see Table I), a Laocoon-type spectrum simulation[7] gave the calculated ^{1}H-spectrum shown in Fig. 1 B.

The ^{1}H connectivity-information was also of key importance for the assignment of the ^{13}C-chemical shifts (see Table I). The correlation between ^{1}H- and ^{13}C-chemical shifts was obtained through a 2D ^{1}H/^{13}C heteronuclear-correlation experiment[8]. The map shown in Fig. 4 displays responses for each protonated carbon atom at its chemical-shift frequency in one dimension (^{13}C shift-axis) displaced in the second dimension (^{1}H shift-axis) according to the chemical-shift frequency of the directly attached proton. Carbon atoms bearing two anisochronous protons

TABLE I

NMR (^{1}H AND ^{13}C) SPECTRAL PARAMETERS[a] FOR **I**

H	2	3	4	5	6a	6b	1′	2′	3′	4′	5′	6′a	6′b
δ	5.76	5.57	4.43	5.05	4.57	4.13	5.09	4.99	5.30	5.16	4.10	4.29	4.26
C	2	3	4	5	6		1′	2′	3′	4′	5′	6′	
δ	61.71	68.94	76.34	69.74	62.33		101.55	72.13	73.41	68.41	72.55	61.95	
H,H	2,3	3,4	4,5	5,6a	5,6b	6a,6b	1′,2′	2′,3′	3′,4′	4′,5′	5′,6′a	5′,6′b	6′a,6′b
J(Hz)	8.8	2.4	8.1	2.4	5.9	−12.4	7.9	9.2	9.3	9.2	3.2	2.9	−12.2

[a]All chemical shifts are referenced to internal Me_4Si. The chemical-shift and coupling-constant values are those obtained from the PANIC simulation procedure

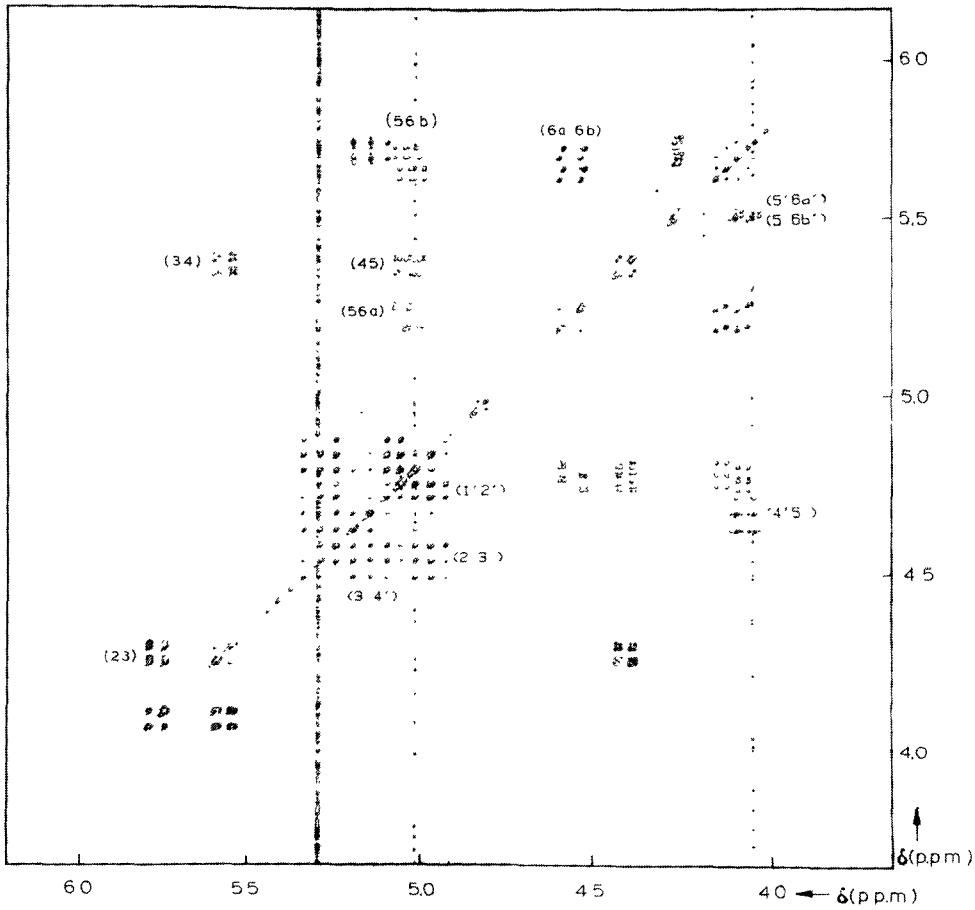

Fig. 3. ^{1}H-N.m.r. spectrum [2D homonuclear-correlated (COSY-45)] of **1**(M in acetone-d_6) showing the *J*-connectivities between the protons indicated. A 16-step phase-cycling, as described in ref. 9, was used with appropriate data-routing to suppress unwanted peaks and to ensure "quadrature detection in F1" (*cf.* ref. 4). W1 = ±250, W2 = 500 Hz, X1 = 512, X2 = 1k; N1 = 1k, N2 = 1k. Sinebell apodisation was applied in both dimensions before Fourier transformation. The acetyl-methyl protons were saturated in order to reduce aliasing, but the residual signals folded back nevertheless (three peaks between 5.35–5.45 p.p.m.), as did the "spike" arising from the decoupling field [strong, sharp response parallel with the F1 (vertical) axis at 5.3 p.p.m.]. The peaks around 4.85 p.p.m. arose from an impurity.

give rise to two ^{13}C peaks parallel with the ^{1}H shift-axis (see the C-6 peaks in Fig. 4). Knowledge of the ^{1}H-chemical shifts permitted assignment of the ^{13}C spectrum for all protonated carbons by simple inspection from the correlation map in Fig. 4. This would probably have been difficult to achieve by heteronuclear double-resonance experiments, in view of the overlap and strong coupling[2] in the ^{1}H spectrum.

In conclusion, it was shown that combined and synergistic application of the 2D *J*-resolved, 2D homonuclear-correlated ^{1}H, and heteronuclear-correlated

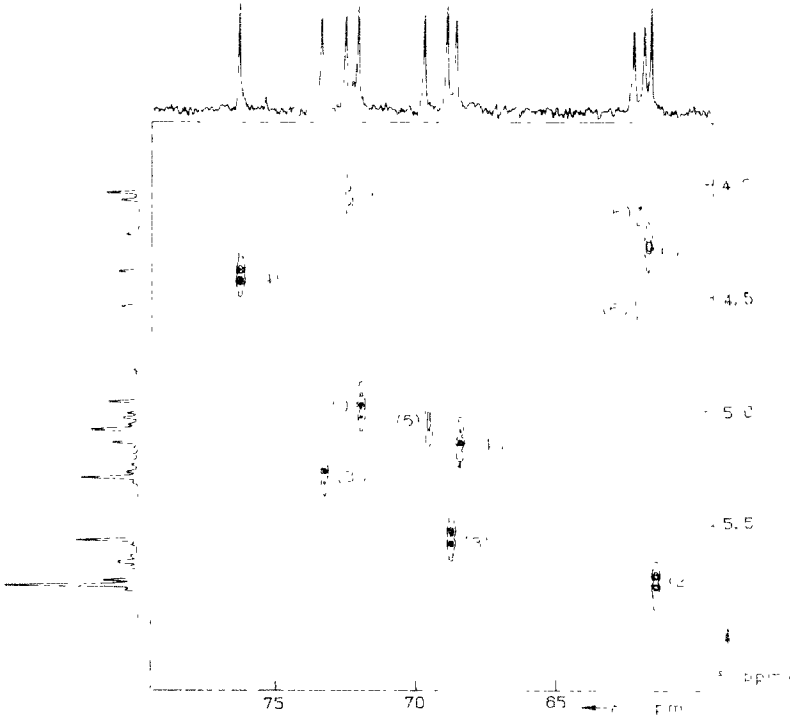

Fig. 4. $^1H/^{13}C$-N.m.r. spectrum (2D heteronuclear-correlated) of **1** (M in acetone-d_6) together with the broadband, proton-decoupled 1D ^{13}C-spectrum (above) and the projection of the 2D *J*-resolved ^1H spectrum (left). The C-1′/H-1′ peak is omitted. The pulse sequence was that described in ref. 8, the proton, carbon-13, and the receiver reference phases being cycled[9] through an 8-cycle sequence. $\tau_1 = (2J_{C,H})^{-1} = 3.3$ ms, $\tau_2 = (4J_{C,H})^{-1} = 1.67$ ms; W1 = ±500, W2 = 3500 Hz, X1 = 512, X2 = 2k, N1 = 1k, N2 = 2k. Apodisation: $\pi/4$-shifted sinebell in the t_1 dimension and $\pi/6$-shifted sinebell in the t_2 dimension. 64 scans were accumulated for each t_1 value.

$^1H/^{13}C$ experiments can lead to self-consistent assignments of the ^1H and ^{13}C spectra of such moderately sized molecules as **1**. *Complete* assignments were possible using the information contained in the 2D spectra alone, *i.e.*, without recourse to such "external" aids as empirical chemical-shift arguments or comparison with model compounds. Strong coupling (in the $\Delta\nu/J$ sense) did not interfere severely in the 2D *J*-resolved or homonuclear-correlated ^1H spectra.

EXPERIMENTAL

The title disaccharide was synthesised as described by Zemplén[12].

Measurements were carried out on a Bruker WP 200 SY spectrometer, using standard software supplied by the manufacturer[9]. Details are given with the captions to Figs. 1–4, using the following conventions: W1 (W2), spectral width in the F1 (F2) direction; X1 (X2), number of data points in the t_1 (number of FIDs) and the t_2 directions (size of FIDs), respectively; N1 (N2), number of data points (after eventual zero-filling) submitted to Fourier transformation in the appropriate dimensions.

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