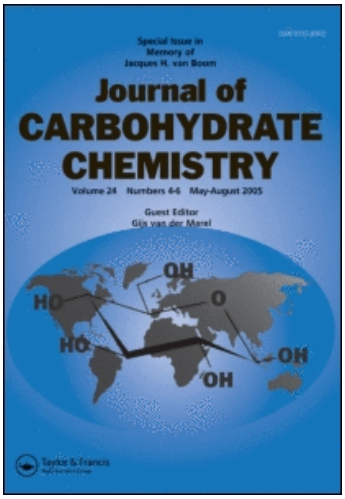

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A Study of Methyl β-Xylobioside: An Illustrative Example of Two-Dimensional NMR Methods

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A STUDY OF METHYL β-XYLOBIOSIDE: AN ILLUSTRATIVE EXAMPLE OF TWO-DIMENSIONAL NMR METHODS

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ABSTRACT

A combination of two-dimensional NMR techniques has been applied to unambiguously assign the ^{1}H and ^{13}C spectra of methyl 4-O-β-D-xylopyranosyl-β-D-xylopyranoside.

INTRODUCTION

The utility of NMR spectroscopy in elucidating the primary structures of carbohydrates[1] has long been recognized. Recently, its potential has matured, due partly to developments in the Fourier transform method.[2] This technique, dependent as it is on the mathematical transformation of the responses of the sample to radio frequency pulses, has necessitated the incorporation of the computer into the spectrometer system. Not only does the computer

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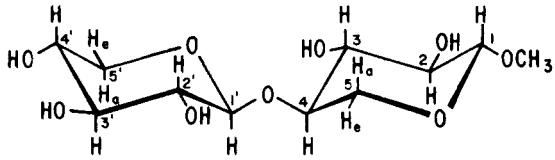
acquire and process the data, but more importantly, it controls and coordinates the functioning of those accessories required to implement a variety of experiments. This latter development resulted in the revolution in NMR spectroscopy which is now called two-dimensional (2-D) NMR.[3,4] This field, once the domain of the specialist, can now be applied routinely by anyone who has acquired the skills to obtain a good 1-D Fourier transform ^{13}C or ^{1}H spectrum.

In this paper we illustrate the use of several of the 2-D techniques in assigning the ^{1}H NMR spectrum, and confirming the ^{13}C spectrum of methyl 4-O-β-D-xylopyranosyl-β-D-xylopyranoside (1). To achieve these goals we first correlated an unambiguously



METHYL 4-O-β-D-XYLOPYRANOSYL-β-D-XYLOPYRANOSIDE

assigned ^{13}C resonance with an isolated proton resonance using a heteronuclear shift correlation experiment;[5,6] then, the scalar couplings in a homonuclear shift correlation spectrum[3,7,8] (COSY) were exploited to locate and assign the proton resonances of one pyranosyl ring, the remaining resonances being assigned to the second ring. Finally, we obtained a 2-D J-resolved spectrum[9] in order to determine the scalar couplings between protons.

RESULTS AND DISCUSSION

The principal task in a structural investigation of an oligosaccharide by NMR is to unequivocally assign the resonances. The methods developed for accomplishing this goal have been

discussed in a number of reviews.[10,11] Here we first utilized the deuterium-induced isotope shift[12] (DIS) technique to unambiguously assign a single ^{13}C resonance. Consideration of the structure of 1 indicates that six carbon atoms are void of hydroxyl groups and should therefore exhibit minimal DIS effects; these are C-1, C-1', C-4, C-5, C-5', and OCH_3. Kovác et al.[13] have found that in the β-D-xylopyranosyl residues, C-1 and C-1' resonate at greater than 102 ppm, C-5 and C-5' at less than 68 ppm, and OCH_3 at less than 60 ppm. The resonance for nuclei located at the C-4 position in the D-xyloside ring is expected to fall between these extremes, and this was confirmed in a DIS experiment for a peak occurring at 77.6 ppm.

The one-dimensional ^1H spectrum of 1 in D_2O at 400 MHz is shown in Fig. 1. In this spectrum several of the resonances can be assigned by comparison with the shifts in the model compound, methyl β-D-xylopyranoside;[14] for example, the anomeric protons, and the protons at carbon atoms four and five. However, the

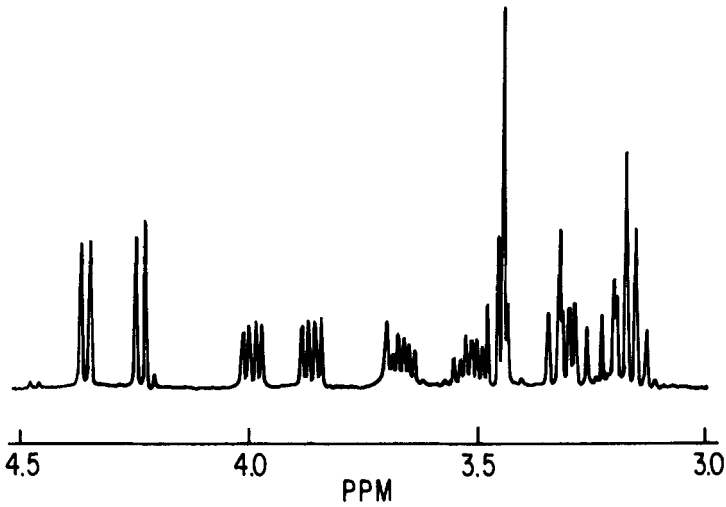


FIG. 1. 400 MHz ^1H NMR spectrum for 0.1 M methyl β-xylobioside in D_2O solution at 295°K. The peak at 3.7 ppm is an unidentified impurity.

complex pattern of multiplets between 3.1 and 3.4 ppm, while it might be simplified by homonuclear decoupling, would still be difficult to assign unequivocally. We therefore performed a heteronuclear chemical shift correlation experiment,[15] which provides a two-dimensional spectrum in which signals representing ^{13}C chemical shift information are displayed along one axis, while those representing ^{1}H chemical shifts lie along the second axis. Thus, for every covalently bonded proton-carbon pair in the molecule, a single peak appears in the 2-D spectrum, its coordinates corresponding to the proton and carbon chemical shifts. For compound 1, 13 correlations are expected, two extra being required for each number five ring position where the axial and equatorial protons are chemically nonequivalent. In Fig. 2 the results of an experiment carried out at 400 MHz for protons and 100 MHz for carbons indicate 12 correlations; thus, one must be incompletely resolved. Using this spectrum, it was easy to locate the shift of the proton bonded to C-4, assigned in the DIS analysis, at 3.67 ppm.

Formerly a series of homonuclear spin decoupling experiments would have been executed to establish the scalar connectivities between H-4 and its neighbors (H-3, H-5e, H-5a). This procedure would have been repeated for H-3 to locate H-2, etc., thus aiding in the analysis and assignment of the one-dimensional spectrum. This lengthy process has been largely supplanted by the 2-D COSY experiment, which displays the 1-D spectrum along the diagonal and cross peaks signifying connectivities between scalar coupled multiplets in the region off the diagonal. The experiment and treatment of the data by this method is briefly described in Coxon's[16] paper in this issue. The result of such an experiment performed at 400 MHz on compound 1 is illustrated in Fig. 3. Using these data it is possible to completely assign the 1-D proton spectrum of 1. Beginning on the diagonal with the resonance for H-4, the resonance of H-3 may be located by observation of its cross peaks with H-4 (e.g., drop a vertical line from H-4 to the first set of cross peaks then proceed hori-

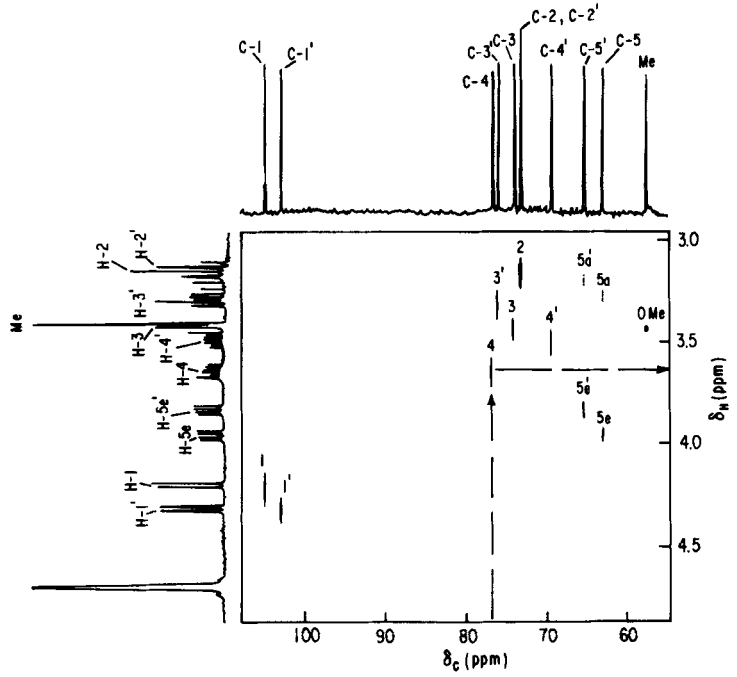


FIG. 2. 100 MHz ^{13}C-^{1}H shift-correlated 2-D NMR of 0.17 M methyl β-xylobioside in D_2O solution at 295°K.

zontally to the triplet on the diagonal at 3.45 ppm). Proceeding in a similar fashion, the connectivity pathways among the six protons on the xyloside ring may be found and assignments made.

The connectivities for the protons in the xylosyl ring were made starting with the resonance of the remaining anomeric proton. The complete ^{1}H assignments are given in Table 1.

Having assigned the proton resonances, it is now possible to reconsider the heteronuclear chemical shift correlation experiment in Fig. 2 and confirm all of the ^{13}C chemical shifts.

Because of the greater chemical shift dispersion at 100 MHz, two resonances are observed for carbons C-2 and C-2' (c.f. Ref. 13). Close examination of the shift correlation spectrum suggests that the carbon resonance correlated with the proton resonance at

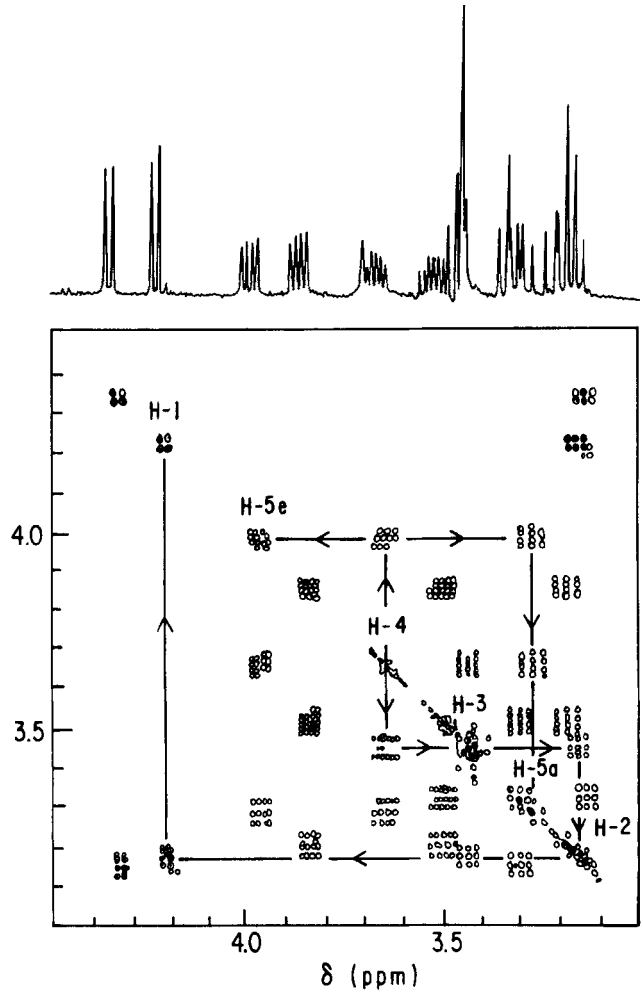


FIG. 3. 400 MHz ^1H shift-correlated 2-D NMR (COSY) experiment of 0.1 M methyl β-xylobioside in D_2O solution at 295°K.

TABLE 1. ^{1}H Chemical Shifts (ppm) of Methyl β-Xylobioside[a].

Ring	H-1	H-2	H-3	H-4	H-5e	H-5a
Xylosyl	4.35	3.15	3.32	3.52	3.86	3.20
Xyloside	4.24	3.17	3.45	3.67	3.99	3.29

a. For a solution in D_2O measured at 400 MHz, with digital resolution, 244 mHz/point, referenced to OCH_3 at 3.44 ppm.

3.15 ppm is more shielded and should be assigned to the xylosyl ring. The complete assignments are given in Table 2.

In order to complete the assignment of the spectral parameters in 1, it is necessary to deduce the scalar proton-proton coupling constants. This proves to be quite difficult for this compound because of several overlapping multiplets. In this regard a third type of two-dimensional spectroscopy is useful, namely the homonuclear J-resolved method. Similar to the various shift correlation techniques in design and execution, this experiment takes advantage of the fact that spin echo amplitudes are not affected by chemical shifts but are modulated solely by the effects of nuclear spin-spin coupling constants. This fact permits the production of a two-dimensional spectrum in which all members of a resonance multiplet with chemical shift δ_i lie on a cross section which makes an angle of 45° with the F_2 axis. With appropriate data processing, it is possible to apply an additional 45° tilt so that each multiplet appears orthogonal to the F_2 axis. Figure 4 illustrates the 2-D J-resolved spectrum of compound 1 obtained at 400 MHz.

Also shown are perpendicular slices through the multiplets (the sharp spike in the centers of these multiplets arises from the extended tail of the OCH_3 resonance) comprising resonances H-2', H-4', and H-5a', demonstrating the effectiveness of this technique in alleviating the effects of overlap in a complicated spectral region. Examination of the remaining multiplets permitted the

TABLE 2. $^{13}C_a$ Chemical Shifts (ppm) of Methyl β-Xylobioside[a].

Ring	C-1	C-2	C-3	C-4	C-5
Xylosyl	103.0	74.0	76.9	70.4	66.4
Xyolside	105.0	74.1	75.0	77.6	64.0

a. For a solution in D_2O measured at 100 MHz, with digital resolution, 734 mHz/point, referenced to OCH_3 at 58.4 ppm.

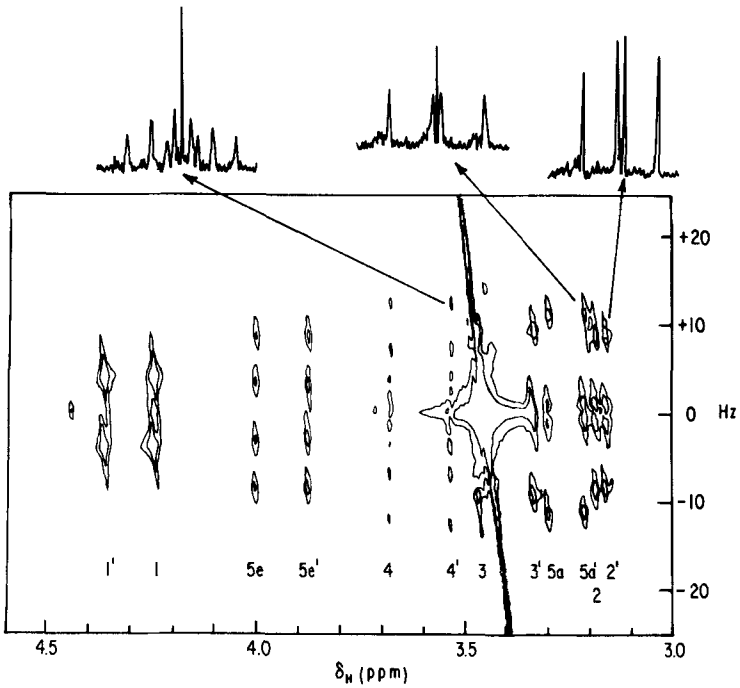


FIG. 4. 400 MHz ^1H 2-D J-resolved NMR experiment of 0.1 M methyl β-xylobioside in D_2O solution at 295°K.

measurement of scalar couplings for all nearest neighbor proton pairs, only the resonances for H-4 and H-4' constituted multiplets more complicated than double-doublets. The resonances for H-3 and H-3' were obscured by the OCH_3 peak and were not considered. Coupling constants to these protons were determined from resonances of neighboring protons. The observed coupling constants are given in Table 3. Only an average set is given, as the resolution was insufficient to establish significant differences between the two rings.

Durette[17] et al. conducted a variable temperature NMR study of β-D-xylopyranose tetraacetate in acetone-d_6. At -85°C they observed values of 8.1 Hz and 10.5 Hz for $J_{1,2}$ and $J_{4,5a}$, respectively, and concluded that these values represented those of the pure $^4C_1$ conformer below the temperature of "conformational freeze out." Our values of 7.8 Hz and 10.6 Hz for $J_{1,2}$ and $J_{4,5a}$, respectively, suggest that both rings in 1 are in the $^4C_1$ conformation in D_2O solution at room temperature.

In conclusion, it has been demonstrated that for a moderate sized disaccharide a coordinated application of the 2-D heteronuclear shift correlated, 2-D COSY, and 2-D J-resolved spectroscopic techniques can provide complete assignments of ^1H and ^{13}C spectra with only minimal recourse to comparisons with model compounds.

EXPERIMENTAL

Sample Preparation. Compound 1 was synthesized as has been previously described.[18]

TABLE 3. Scalar Coupling Constants[a] (Hz) of Methyl β-Xylobioside

$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5e}$	$J_{4,5a}$	$J_{5a,5e}$
7.8	9.6	9.1	5.4	10.6	11.8

a. Average observed couplings (±0.2 Hz).

All ^{1}H NMR measurements were performed on a sample of the methyl β-xylobioside in D_2O at 295°K as a ca. 0.1 M solution.

The ^{13}C deuterium-induced differential isotope shift was measured using a coaxial cell containing ca. 0.17 M solutions of the xylobioside dissolved in H_2O and D_2O as previously described.[12] These two samples were subsequently combined and used in the heteronuclear shift correlation experiment.

NMR Spectroscopy. All spectra were measured on a JEOL Model GX-400[19] NMR spectrometer system, operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The one-dimensional resolution enhanced spectrum was obtained by Fourier transformation of 2048 accumulated scans, consisting of 8192 data points in a 1 kHz spectral width, using a 0.5 Hz negative broadening factor. Data were acquired with a 90° pulse (6 μs) and a total pulse recycle delay of 10.1 s.

The ^{13}C DIS spectrum was obtained by accumulating 1000 scans, with a 12 kHz window and 32,768 data points, using a 90° pulse (20 μs) and a recycle delay of 2.4 s. An exponential filtering of 0.3 Hz was used to improve sensitivity.

The 2-D heteronuclear correlation experiment was carried out using the JEOL PLEXUS NMR acquisition and processing software. An initial data matrix (t_1 x t_2) of 512 x 1024 points represented spectral widths (F_1 x F_2) of 1 kHz x 6 kHz. The $\pi/2$ pulse widths were 19.7 μs for ^{13}C and 45 μs for ^{1}H, respectively, while the fixed delays Δ_1 and Δ_2 (selected to optimize the experiment for carbons having a single proton) were 3.33 ms. An overall recycle delay of 2.1 s was used to acquire 128 scans for each value of the incremented delay t_1.

The 2-D COSY ^{1}H NMR spectrum was obtained using a data matrix (t_1 x t_2) of 512 x 1024 points which represented 1 kHz spectral widths in each dimension. The $\pi/2$ pulse widths were 6 μs, and the overall recycle delay was 3.5 s. For each value of t_1, 16 scans were acquired.

The 2-D J-resolved spectrum was obtained using an initial data matrix (t_1 x t_2) of 128 x 2048 points that was zero filled

to 256 x 2048 points and represented a (F_1 x F_2) matrix of 50 Hz x 1000 Hz. The recycle delay was 5 s for each of 64 acquisitions taken at each separate t_1 value.

All data sets were double Fourier transformed using the TDNMR program supplied by JEOL. In all cases a trapezoidal window function was used to approximate the sine-bell function frequently used to improve the appearance of two-dimensional contour plots.

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