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Note

A simple n.m.r. method for assigning the carbonyl resonances of carbohydrate acetates

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A classical problem in carbohydrate chemistry is determining the point of attachment of substituents known to be bound to the oxygen atoms of the sugar hydroxyl groups. A number of methods that utilize n.m.r. spectroscopy have been developed for application to this general problem. Until recently, most of these methods followed the protocol of Horton and Lauterbach[1], involving the unambiguous synthesis of derivatives containing a trideuterioacetyl or trideuteriomethyl group at a predetermined position. Recently, two-dimensional n.m.r. techniques, designed to detect ^{1}H-^{13}C spin–spin coupling through two and three bonds, have been applied to carbohydrates[2].

In light of these reports, we now draw attention to an alternative technique that is well suited for addressing problems of this nature. In the work presented herein, on β-cellobiose octaacetate (**1**) and cellulose triacetate (**2**), it is shown that INAPT (Insensitive Nuclei Assigned by Polarization Transfer)[3] is a n.m.r. technique useful for assigning the resonances of carbonyl groups appended to carbohydrates. In particular, the problem of analyzing the ^{13}C-n.m.r. spectrum of the carbonyl carbon atoms of **1** and **2** is addressed.

EXPERIMENTAL

N.m.r. data (^{1}H- and ^{13}C-) were obtained with a JEOL Model GX-400 n.m.r. spectrometer operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Sample-tube sizes were 5 mm for ^{1}H, and 10 mm for ^{13}C. Sample concentrations were 0.04M for the COSY experiment, and 0.3M for the INAPT experiments. All spectra were recorded at ambient probe-temperature. Chemical shifts are reported in p.p.m. from tetramethylsilane, with $CDCl_3$ as an internal reference. For ^{1}H-n.m.r. spectra,

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residual $CHCl_3$ was taken as 7.24 p.p.m. For ^{13}C-n.m.r. spectra, the center peak of the triplet resonance of $CDCl_3$ was taken as 77.0 p.p.m.

The COSY spectrum was collected by using a 512 × 2048 data matrix size, and 64 transients were acquired for each of the 512 t_1 values. Spectral widths of 1740.9 Hz were used in both dimensions. Total measuring time for the COSY spectrum was 16 h. The COSY spectrum was processed by using a sine-bell filtering-function in both dimensions.

In the INAPT experiments, unless otherwise noted, the delays were 50 ms and the length of the soft 90° ^1H pulse was 10.7 ms. Originally, the soft pulse was determined by using a sample of 1:1 (v/v) dichloroacetic acid–$CDCl_3$, as described by Bax[4]. Later, we found that it is more efficient to find the 90° ^1H soft pulse directly by optimizing the INAPT experiment for β-cellobiose octaacetate. This was done by first arbitrarily setting the pulse length at 10.7 ms, and then, while irradiating H-1 in an INAPT experiment, the pulse voltage was adjusted to 3.5 mV. This procedure very quickly gave good spectrometer tuning.

Designation of the carbonyl groups follows the suggestions of prior workers[2a].

The preparation of β-cellobiose octaacetate (**1**) and cellulose triacetate (**2**) has been reported[5,6].

DISCUSSION

β-Cellobiose octaacetate (**1**). — In developing INAPT for use in our laboratories, we selected β-cellobiose octaacetate (**1**) for our initial experiments. Our first step was to record the ^1H–^1H COSY spectrum shown in Fig. 1. Based on its downfield shift and small homonuclear coupling, the signal of the (anomeric) H-1 atom was identified (5.68 p.p.m.). Assignment of ring A was readily made from the proton–proton connectivities. In a similar fashion, the H-1′ signal (4.54 p.p.m.) was located, and the assignments for ring B were made.

Fig. 2a shows the proton-decoupled, ^{13}C-n.m.r. spectrum for the carbonyl region of **1**. The assignments for the carbonyl groups were determined by using INAPT, as shown in Figs. 2b–2i. Figs. 2j–2l demonstrate both the problems that we encountered with INAPT and the precautions that must be exercised in interpreting INAPT spectra. When H-3′ was irradiated (see Fig. 2j), transfer to the carbonyl at 169.9 p.p.m. occurred. In addition, centered at 169.1 and 169.3 p.p.m. are negative resonances corresponding to C-4′OAc and C-2OAc, respectively. In the absence of supporting evidence, it is difficult to gauge which of these peaks is the result of transfer from the irradiated proton and which is the result of spurious transfer. We have found that the peak(s) resulting from spurious transfer could be identified by using a shorter delay[3b]. That is, if the resonance(s) is spurious, in the second spectrum the peak(s) will be of opposite phase (*cf.*, Figs. 2j and 2k). From Fig. 2l, it may be seen that selective irradiation of H-1 provides the carbonyl resonance at 168.6 p.p.m. The small peak at 169.9 p.p.m. is the result of spurious transfer. However, we observed that a decrease in peak-to-peak pulse voltage (from 3.5 to 2 mV)

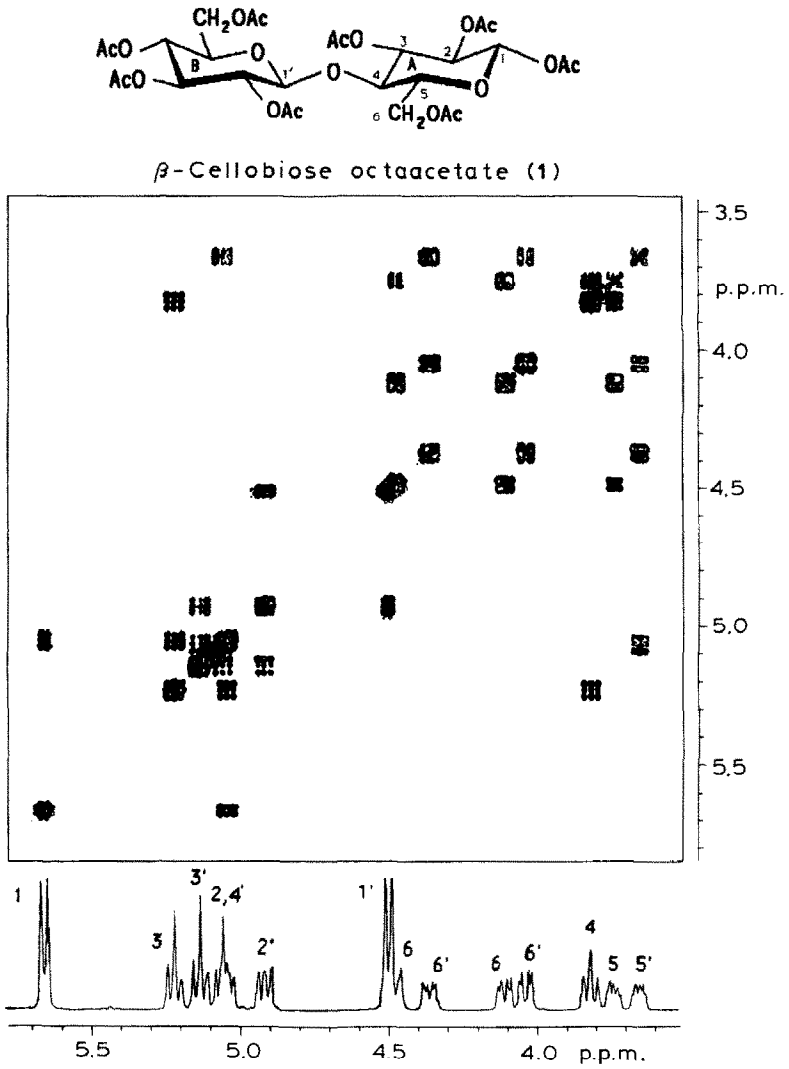


Fig. 1. Two-dimensional COSY spectrum of β-cellobiose octaacetate (**1**).

caused the spurious transfer to vanish (*cf.*, Figs. 2l and 2b). Except for H-2, assignment of these spectra presented no additional problems. Irradiation of H-2 (see Fig. 2g), separated from H-4′ by 6 Hz, gave two resonances at 169.3 and 169.1 p.p.m. In a separate experiment (see Fig. 2f), C-4′OAc could be identified as causing the resonance at 169.1 p.p.m. By default, the resonance at 169.3 p.p.m. must be that produced by C-2OAc.

Cellulose triacetate (**2**). — Spectral assignment of the carbonyl groups of cellulose carboxylic esters has long been an area of intense interest[7]. We have found

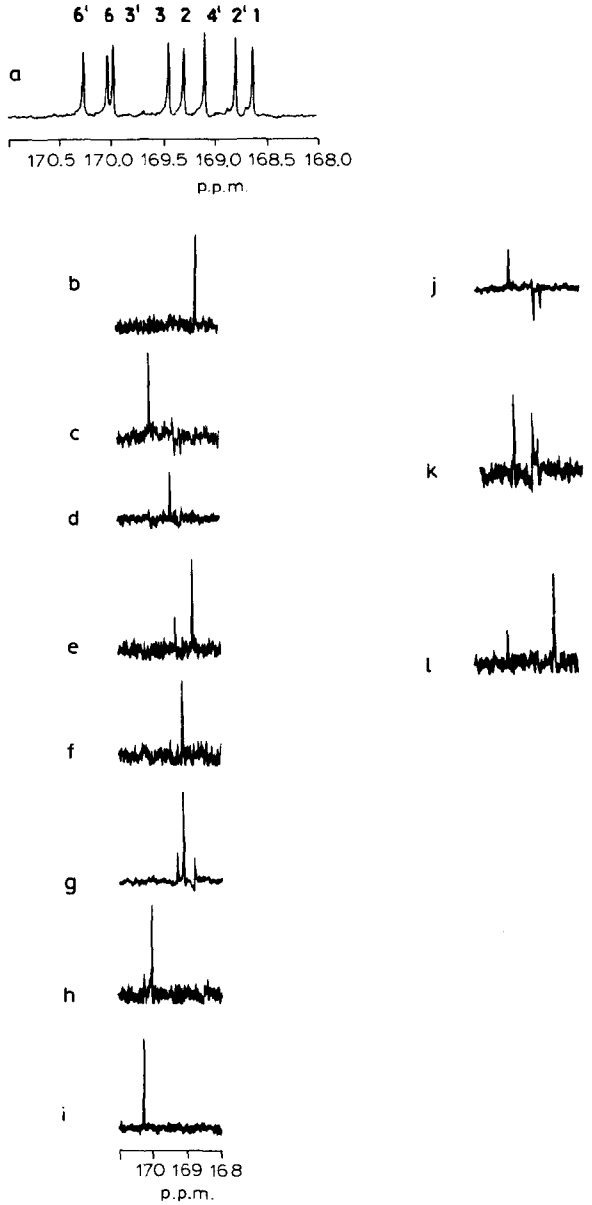
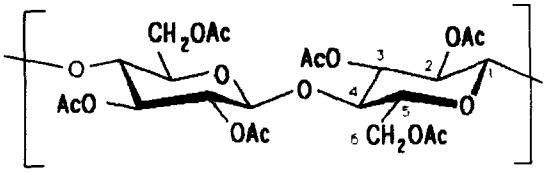


Fig. 2. (a) Proton-decoupled ^{13}C-n.m.r. spectrum of the carbonyl region of **1**. (b–i) INAPT spectra of 1. Transfer from H-1, H-3′, H-3, H-2′, H-4′, H-2, H-6, and H-6′, respectively. Each of the INAPT spectra is the result of 256 scans, and each took 19 min to record. (j) transfer from H-3′, when $\Delta 1 = \Delta 1 = 50$ ms. (k) Transfer from H-3′ when $\Delta 2 = \Delta 2 = 30$ ms. (l) Transfer from H-1 with a soft pulse voltage of 3.5 mV.



Cellulose triacetate (2)

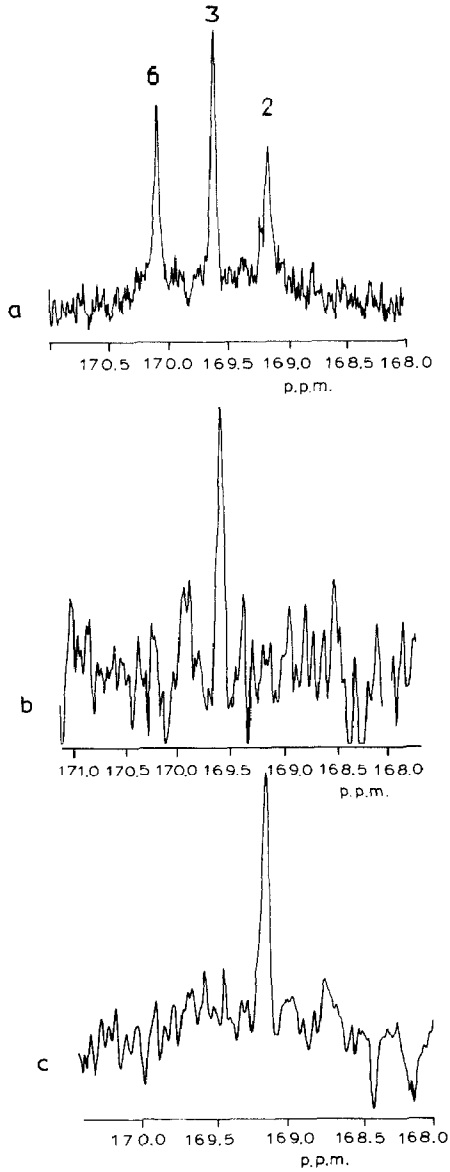


Fig. 3. (a) Proton-decoupled ^{13}C-n.m.r. spectrum of the carbonyl region of **2**. (b) Polarization transfer from H-3 (2048 scans). (c) Polarization transfer from H-2 (12,000 scans).

that the experimental procedure already outlined for the carbonyl groups of β-cellobiose octaacetate is directly applicable to cellulose triacetate (**2**).

The ^1H–^1H COSY spectrum of cellulose triacetate had been presented[6]. Fig. 3a shows the proton-decoupled spectrum for the carbonyl region of **2**. Irradiation of H-3 led to polarization transfer to the carbonyl-group resonance centered at 169.6 p.p.m. (see Fig. 3b). Similarly, irradiation of H-2 led to polarization transfer to the carbonyl signal located at 169.1 p.p.m. (see Fig. 3c). By default, the carbonyl signal at 170.1 p.p.m. must be that of the group attached to C-6.

CONCLUSION

During the preparation of this Note, Goux and Unkefer[2a] disclosed their work with peracetylated mono- and oligo-saccharides, in which they used long-range ^{13}C–^1H heteronuclear shift-correlated spectroscopy to assign the carbonyl groups of **1**. It is instructive to compare the technique employed by them to that used herein. The two techniques are similar in that they require a COSY experiment to establish ^1H–^1H connectivities. In addition, both require favorable T_2 values. With long-range ^{13}C–^1H heteronuclear shift-correlated spectroscopy, assignments of all of the carbonyl and acetyl groups can often be made in a single experiment. However, this method can suffer from lack of sensitivity, and long collection times sometimes make it necessary to utilize ^{13}C enrichment. In the case of cellulose triacetate (**2**), we could not obtain a three-bond correlation by using long-range, two-dimensional n.m.r. techniques.

With INAPT, the lack of sensitivity that is associated with two-dimensional n.m.r. techniques is generally avoided. The increased sensitivity circumvents the need for ^{13}C enrichment, and decreases the demand on instrument time. However, it must be pointed out that each INAPT experiment establishes the assignment of only a single resonance. With β-cellobiose octaacetate (**1**), a minimum of seven experiments is required, and assignment of the acetyl methyl resonances must be made in a separate experiment.

We have demonstrated that INAPT is a one-dimensional n.m.r. technique well suited for rapidly determining the point of attachment of substituents in carbohydrates. In principle, INAPT is not limited to the assignment of carbonyl groups in carbohydrates. This technique should be applicable to any carbohydrate derivative in which the substituent has a long-range, scalar interaction with the ring proton on the carbon atom to which it is attached[3b].

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