Preparation of Cellulose [1-^{13}C]Acetates and Determination of Monomer Composition by NMR Spectroscopy

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ABSTRACT: A series of cellulose acetates labeled at the carbonyl carbons with carbon-13 was prepared by aqueous acid hydrolysis, metal-catalyzed methanolysis, and acetic acid promoted methanolysis of 2,3,6-tri-([1-^{13}C]acetyl) cellulose. A total of 16 carbonyl carbon resonances were identified in the carbon-13 NMR spectra of the series of cellulose acetates prepared by aqueous acid hydrolysis. Fewer carbonyl carbon resonances were found in the carbon-13 NMR spectra of the cellulose acetates prepared by metal-catalyzed methanolysis while the carbon-13 NMR spectra of the cellulose acetate prepared by acetic acid promoted methanolysis was very similar to that of a cellulose acetate prepared by aqueous acid hydrolysis. Using the 2D COSY and 1D INAPT experiments, these resonances were assigned to carbonyl carbons attached to either a C2, a C3, or a C6 hydroxyl. In the case of the C2 and C3 carbonyl resonances, these resonances were assigned to specific monomers in the polymer backbone.

In recent years, a better understanding of the crucial relationship between the microstructure of polymers and their observed physical properties has evolved.[1] However, for cellulose esters, the relationship between microstructure and physical properties is poorly understood. The lack of knowledge in this area can be traced to the scarcity of analytical techniques that allow the needed depth of analysis. Historically, we have relied heavily on analysis of X-ray crystal structures, thermal analysis, chromatographic techniques, intrinsic viscosity, and various methods of wet analysis as probes of cellulose ester structure.

The advent of high-field nuclear magnetic resonance (NMR) spectrometers and the development of new NMR experimental techniques potentially provide the analytical tools needed for probing the microstructure of cellulose esters. The utility of proton (^{1}H) NMR for probing the structure of cellulose acetates (CA) was established in 1973 by the classical work of Goodlett et al.[2] The method of Goodlett et al. involves acetylation of a CA with acetyl-d_3 chloride to give a triester. Integration of the acetyl region of the ^{1}H NMR spectrum of this cellulose derivative provides a measure of the relative degree of substitution (RDS) at the three possible sites of substitution as well as the total degree of substitution (DS). In 1984, Shibata et al. demonstrated that carbon-13 (^{13}C) NMR can also be used to obtain information about the RDS via integration of the ring carbons.[3] Recently, we have described NMR techniques that can be used for probing the microscopic conformation and supramolecular structure of cellulose esters.[4] Thus, NMR techniques are now available for determining the total DS and the RDS and for probing the conformation and dynamics of cellulose esters.

The pivotal problem that remains is the development of NMR techniques for analyzing the distribution of acetyl substituents along the length of the cellulose backbone. Neglecting end groups, incomplete esterification of cellulose or hydrolysis of cellulose triacetate (CTA) should provide a cellulose acetate containing eight monomers (Figure 1). This cellulose acetate should give NMR spectra in which a maximum of 12 carbonyl carbon, methyl acetyl carbon, or methyl acetyl proton resonances are observed. This simple model assumes that there are no interactions between adjacent anhydroglucose rings or between polymer chains as well as a random distribution of acetyl substituents. The Japanese school of cellulose chemistry has long recognized the fundamental importance in the relationship between acetyl distribution along the length of the cellulose chain and observed physical properties.[5] Despite extensive work and the development of empirical rules,[6] NMR techniques that permit the unambiguous assignment of the carbonyl carbon resonances of cellulose acetates have not been reported. In the work disclosed in this report, we describe our first steps toward addressing this problem.

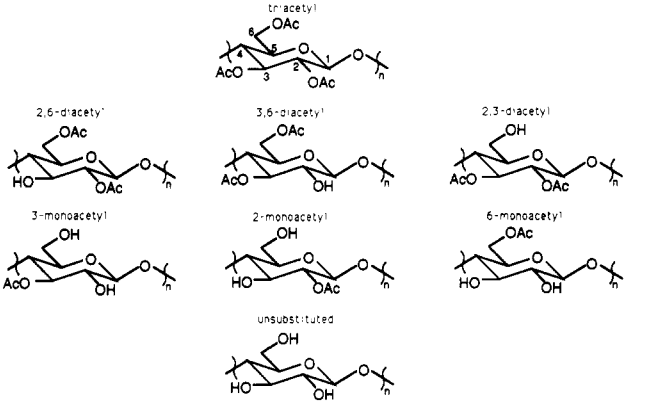

Figure 1. Eight anhydroglucose rings that can be present in a CA after hydrolysis of CTA or direct esterification of cellulose. In the absence of hydrogen bonding, these monomers can give rise to 12 distinct carbonyl carbon or methyl acetyl proton resonances.

Experimental Section

Proton NMR data were obtained on a JEOL Model GX-400 NMR spectrometer operating at 400 MHz. The sample tube size was 5 mm and the sample concentrations were ca. 10 mg/mL of DMSO-d_6. One of two drops of trifluoroacetic acid (TFA) was added to the sample to shift residual water from the spectral region of interest. Carbon-13 NMR data were obtained on either a JEOL Model GX-400 or a JEOL Model GX-270 NMR spectrometer operating at 100 or 67.9 MHz, respectively. The sample concentration was 100 mg/mL of DMSO-d_6 and the sample tube size was 10 mm. Chemical shifts are reported in parts per million from tetramethylsilane with the center peak of DMSO-d_6 as an internal reference. For ^{1}H NMR spectra, residual DMSO-d_6 was taken as 2.49 ppm. For ^{13}C NMR spectra, the

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center peak of DMSO-d_6 was taken as 39.5 ppm. All spectra were recorded at 80 °C. The proton-decoupled ^{13}C NMR spectra were collected with 32 768 points and were zero filled to 65 536 points to give a resolution of 0.52 Hz.

The spectrum shown in Figure 3 is typical of the COSY spectra collected for this work. This spectrum was collected by using a 256 × 512 data matrix size, and 32 transients were acquired for each t_1 value. The delay time between scans was 1.0 s and the total measuring time was 2.8 h. The spectrum was processed by using a 100-point-wide sine bell filtering function phase shifted by 10° in both dimensions.

In the INAPT experiments, the length of the soft pulse was 11.5 ms. The delays were calculated according to the type of proton that was irradiated: methine, $\Delta_{1,2} = (1/8)J$; methylene, $\Delta_{1,2} = (1/12)J$; methyl, $\Delta_{1,2} = (1.16)J$. A $^3J_{CH}$ value of 2.5 Hz was assumed for the long-range coupling between the ring protons and the carbonyl carbons. A $^2J_{CH}$ value of 5.7 Hz was found experimentally for the two-bond coupling between the acetyl methyl protons and the carbonyl carbons. After determining the proton irradiation frequencies, the INAPT experiments for each CA were collected and stored by using an indirect command file. Total collection time ranged from 12 to 36 h.

All of the spectra were processed by using a 8-Mbyte Mac II Macintosh Computer, with VersaTerm Pro as an emulation package and MacDraw II as a graphics package, interacting with Hare's FTNMR software[7] running on a VAX 8800 computer.

2,3,6-Triacetyl-*1*-^{13}*C* Cellulose (1). A heterogeneous mixture of 3 g (18.5 mmol) of cellulose (regenerated from a CA with a DS = 2.45 using MeOH/NaOMe), 50 mL of H_2O, and 50 mL of PyH was stirred overnight. After filtration, the cellulose was placed in a dry 300-mL round-bottom flask along with 75 mL of dry PyH. The mixture was heated at reflux for 1 h before cooling, filtering, and washing with three 50-mL portions of dry PyH. The reflux/wash procedure was repeated two additional times.

The resulting solvent-exchanged cellulose was placed in a dry 300-mL round-bottom flask containing 100 mL of dry PyH. To the mixture was added $CH_3{}^{13}COCl$ in three 2-g portions. The reaction was stirred for 16.5 h under nitrogen at room temperature before heating to reflux for 1.5 h. The reaction mixture was vacuum filtered while hot and the product was isolated by precipitation into MeOH. The solids were dissolved in acetone and reprecipitated into H_2O three times. After drying under vacuum at 60 °C, 5.71 g of a white fiber was obtained. The carbon-13 NMR spectrum was that of cellulose triacetate with a 97% ^{13}C enrichment at the carbonyl carbons.

Acetyl-*1*-^{13}*C* Cellulose (2–5). A 300-mL flask was charged with 7 g of 1 and 140 mL of AcOH. The heterogeneous mixture was stirred overnight at ambient temperature to obtain solution. The solution was heated to 80 °C before dropwise addition of a solution of 168 mg of H_2SO_4 in 21 mL of H_2O. Aliquots were removed from the reaction flask at 9.5 h (**2**), 32 h (**3**), 52 h (**4**), and 69.5 h (**5**). Samples **2** and **3** were isolated by precipitation into H_2O. The white fibrous solids were washed with three portions of hot H_2O before drying overnight at 60 °C under 100-Torr vacuum. Sample **4** was isolated in a similar manner except that the solids were washed with cold H_2O. Sample **5** was isolated by precipitation into MeOH. The white powder was extensively washed with MeOH before drying. **2**, DS = 2.54; **3**, DS = 1.99; **4**, DS = 1.61; **5**, DS = 1.06.

Acetyl-*1*-^{13}*C* Cellulose (6). Three grams of 2,3,6-triacetyl-*1*-^{13}*C* cellulose (**1**), 300 mL of MeOH, and 13 mg of magnesium acetate tetrahydrate were heated at 175 °C (2 h heatup time) at 1000 psi for 45 min in an autoclave. After the solution cooled, the solvents were removed by filtration and the solid was dried under vacuum at 60 °C to provide 1.93 g of **6**, DS = 2.03.

Acetyl-*1*-^{13}*C* Cellulose (7). Diacetyl-*1*-^{13}*C* cellulose (**6**, 1.6 g), 300 mL of MeOH, and 8.4 mg of magnesium acetate tetrahydrate were heated at 175 °C (2-h heatup time) at 1000 psi for 30 min in an autoclave. After the solution cooled, the solvents were removed by filtration and the solid was dried under vacuum at 60 °C to provide 0.69 g of **7**, DS = 0.42.

Acetyl-*1*-^{13}*C* Cellulose (8). One gram of 2,3,6-triacetyl-*1*-^{13}*C* cellulose (**1**), 4.5 mL of MeOH, and 10.5 mL of acetic acid were heated at 145–155 °C (95-min heatup time) for 16 h in a stainless steel reactor. After the solution cooled, the solvents were first removed by decantation before dissolving the solids in ca. 25 mL of water. The aqueous solution was added dropwise to 200 mL of ^{i}PrOH. The resulting solid was isolated by filtration and washed with three 10-mL portions of ^{i}PrOH. Drying under vacuum at 60 °C provided 250 mg of **8**, DS = 0.67.

Results and Discussion

We have shown that a combination of the two-dimensional COSY experiment and the one-dimensional INAPT experiment can be used to assign the carbonyl carbon resonances of carbohydrate acetates.[8] We illustrated the application of this experimental protocol to polysaccharides through the assignment of the carbonyl carbon resonances of CTA in $CDCl_3$.

Cellulose acetates with a DS of less than 2.85 are not soluble in $CDCl_3$. However, DMSO-d_6 dissolves a wide range of CA (DS = 3.0–0.4), provides for good resolution, and is the solvent of choice. Addition of a small amount of trifluoroacetic acid to a ^{1}H NMR sample in DMSO-d_6 serves to shift both residual water and hydroxyl protons out of the spectral region of interest without perturbing the chemical shifts of the ring protons. For the short collection times required for ^{1}H NMR experiments, degradation of the polymer or hydrolysis of acetyl groups is not observed. Unfortunately, when we attempted the assignment of the carbonyl carbon resonances of CTA **1** in DMSO-d_6, only very weak polarization transfer was observed in the INAPT experiment. We believe that this loss in sensitivity in DMSO-d_6 occurs because the $^3J_{CH}$ values approach the T_2 values of the carbonyl carbons.[9] To overcome the problem of sensitivity, we prepared a series of cellulose acetates enriched at the carbonyl carbons with carbon-13. Thus, reaction of regenerated celluose with acetyl-*1*-^{13}*C* chloride in pyridine led to the formation of CTA (**1**) with 97% enrichment of ^{13}C at the carbonyl carbons. Aqueous acid hydrolysis of **1** with removal of aliquots gave a series of cellulose acetates **2–5**, covering the DS range 2.55–1.06. The method of preparation for CA **2–5** was selected on the basis that this method should give cellulose acetates in which the maximum number of monomers should be present at intermediate DS levels (i.e., a random distribution of substituents). It was also our expectation that the series prepared by this method would provide information on the change of chemical shifts as a function of changing DS. The DS values for this work were selected on the basis of studies involving hydrolysis of unlabeled cellulose acetates. Cellulose acetate **6** was prepared by $Mg(OAc)_2$-catalyzed methanolysis of CTA **1** at 175 °C. Cellulose acetate **7** was prepared by subjecting a portion of isolated **6** to further $Mg(OAc)_2$-catalyzed methanolysis. The method of preparation of CA **6** and **7** was selected on the basis of studies involving hydrolysis of unlabeled cellulose acetates, which showed that this method gives cellulose acetates with an acetyl distribution different from that of CA **2–5** and with different physical properties.[10] Cellulose acetate **8** was prepared by acetic acid promoted methanolysis of CTA **1** at 145–155 °C. Prior studies involving acetic acid promoted methanolysis of unlabeled cellulose acetates had shown that this method of preparation gives cellulose acetates with physical properties virtually identical with those of cellulose acetates prepared by aqueous acid hydrolysis at similar DS levels.[10] Therefore, the expectation was that CA **8** would have an acetyl distribution similar to that of CA **5**.

Cellulose Acetates 1–5. The ^{1}H NMR spectrum for **1** has been presented elsewhere.[11] Figure 2 shows the ring region of the resolution-enhanced ^{1}H NMR spectra of **2–5**. Each peak is labeled with a letter to designate its chemical shift. The proton resonances that were irradiated in the INAPT experiments are indicated by arrows (vide infra).

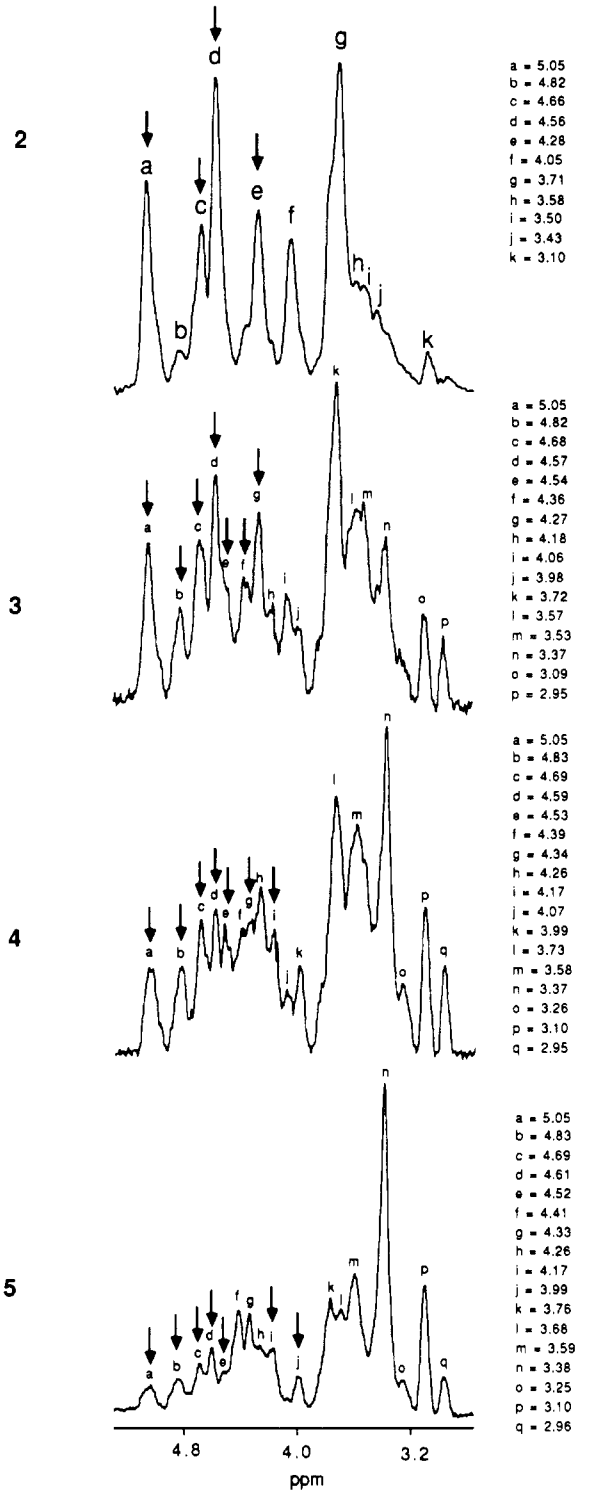

Figure 2. Resolution-enhanced proton NMR spectra of the ring proton region of cellulose acetates **2–5**. The peaks that were irradiated in the INAPT experiments are indicated with arrows. Because of the broad lines and peak overlap, only the chemical shifts of the center of the major peaks are provided.

From Figure 2 it can be seen that there is little change in the chemical shifts of the ring protons with changing level of substitution. This observation was extremely helpful in assigning the ^{1}H NMR spectra of CA **2–8** and in determining the proton resonances to be irradiated in the INAPT experiments.

The COSY spectrum for CTA **1** has been presented elsewhere.[11] Figure 3 shows the COSY spectrum of CA **2**. The COSY spectra for **3–5** are contained in the supplementary material. Assignments of the COSY spectra of

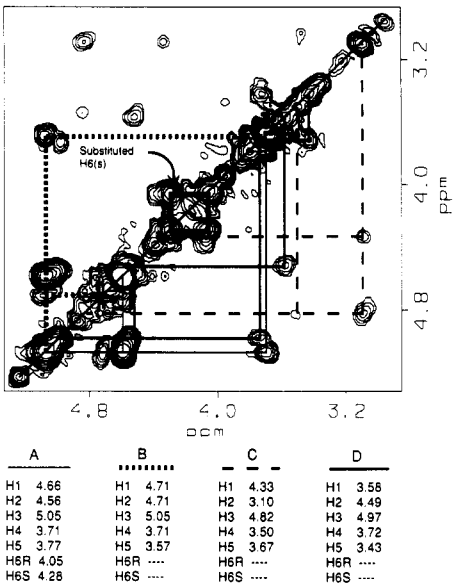

Figure 3. COSY spectrum for CA **2**. Four coupling networks can be found in this spectrum. These coupling networks are identified by the solid and dashed lines and by their chemical shifts. For clarity, the coupling network labeled B is shown in the upper diagonal.

CA **2–5** are based on two observations: H3 ring protons attached to carbons bearing an acyl substituent are the furthermost downfield peaks in the ^{1}H NMR spectra of cellulose esters[11] and, with the exception of H1, ring protons attached to carbons that do not bear an acetyl substituent will appear between 3.8 and 2.9 ppm (vide infra).[12] Hence, the H2, H3, and H6 protons of interest should appear between 5.1 and 3.9 ppm. In Figure 3, the diagonal peak centered at 5.05 ppm can be assigned to a H3 proton. The pair of connecting cross-peaks at 4.56 and 4.71 ppm are assigned as H2 ring protons, which, of course, means that there are two overlapping H3 ring protons at 5.05 ppm (coupling networks A and B). The H2 protons connect to two H1 protons at 4.66 and 4.71 ppm. Similarly, there are two overlapping H4 protons at 3.71 ppm, which connect to two H5 protons at 3.77 and 3.57 ppm. Although there are no cross-peaks connecting the H5 protons to the H6 protons, the resonances at 4.05 and 4.28 ppm can be assigned to H6 protons attached to carbons bearing an acetyl substituent on the basis of characteristic geminal coupling. In a similar manner, networks C and D can be traced out.

On the basis of the ^{1}H NMR assignments for CA **1** (CTA), network A can be assigned to the triacetyl monomer. (Each monomer will be described as a tri-, di-, or monoacetyl monomer. For the di monomers, italics indicate the resonance in question. An * will indicate a carbonyl resonance whose chemical shift is perturbed by hydrogen bonding.) Assuming preferential hydrolysis at the 1° (C6) hydroxyl[5] and that loss of C6 acetyl will only cause minor perturbations on the remaining ring protons, network B can be assigned to the 2,3-diacetyl monomer. Since H3 in network C connects to a H2 above 3.9 ppm, network C was assigned to overlapping 3,6-diacetyl and 3-monoacetyl monomers.[13] Network D is believed to be the result of hydrogen bonding between the carbonyl carbons of the triacetyl monomer and hydroxyl on adjacent anhydroglucose rings or between polymer chains (vide infra).

By making use of the assignments in each preceding COSY spectrum and the insensitivity of proton chemical shifts to DS, these same four coupling networks can be found in the COSY spectra for **3** and **4**. In some cases the

entire coupling network cannot be traced out because of peak overlap and poor signal to noise. In particular, except for CA 1 (CTA), we could not obtain correlations between any H5 and H6 protons. Overlapping coupling networks for the 2,6-diacetyl and 2-monoacetyl monomers (network E, Figures 14 and 15, supplementary material) can be located in the COSY spectra of 4 and 5. Network F (Figure 15, supplementary material, vide infra) was assigned to the unsubstituted anhydroglucose monomers in CA 5.

It should be obvious that the limitation in this analysis of the ^{1}H NMR spectra of CA 1–5 is the lack of spectral resolution due to peak overlap and the absence of H5–H6 correlations. This is also true in the INAPT experiments where peak overlap created problems in selective irradiation. For example, overlap of the 3,6-diacetyl and 3-monoacetyl coupling networks and the absence of a H5–H6 cross-peak do not permit us to directly distinguish between the corresponding carbonyl carbon resonances in the INAPT experiments. Even with H5–H6 correlations, peak overlap would have rendered selective irradiation in the INAPT experiments difficult at best.

The INAPT spectra of 1 in DMSO-d_6 showing polarization transfer from the ring protons to the carbonyl carbons as well as from the methyl acetyl protons to the carbonyl carbons are provided in the supplementary material. The assignments for the carbonyl carbons of 1 in DMSO-d_6 (Figure 4) are the same as in $CDCl_3$,[8] demonstrating that the chemical shifts of the carbonyl carbons of 1 are not significantly perturbed by this change of solvent.

Figure 5 shows the results for the INAPT experiments for 2–5 in DMSO-d_6. Because of long collection times in these experiments and since water presents no problems in the INAPT experiment, TFA was not used since it could have caused degradation of the polymer as well as hydrolysis or migration of the acetyls. For each INAPT experiment, the peaks irradiated in the ^{1}H NMR spectra (in parts per million) are provided as well as the chemical shift(s) of the carbonyl carbons observed in the INAPT experiment. All of the major peaks from 5.1 to 3.9 ppm in each of the ^{1}H NMR spectra of 1–5 were irradiated. Except for a few cases where fully selective irradiation could not be obtained, these spectra are clean and the assignments for the carbonyl carbon resonances from the experiments are evident.

With the INAPT experiments for CA 2 as an example, irradiation of the H3 protons at 5.05 ppm returned two carbonyl carbon resonances at 169.37 and 169.05 ppm. On the basis of the analysis of the COSY spectra, the results from the INAPT spectra for CA 1, and the expectation that the triacetyl monomer will be the most abundant at this DS level, the carbonyl carbon resonance at 169.37 ppm was assigned to the 2,3-diacetyl monomer and the resonance at 169.05 ppm to the 3-triacetyl monomer. Similarly, irradiation of the H2 protons at 4.71 and 4.56 ppm returned the two carbonyl carbon resonances at 168.83 ppm (2,3-diacetyl) and 168.72 ppm (2-triacetyl), respectively. Irradiation of the H6 proton at 4.28 ppm gave polarization transfer to the carbonyl carbon resonance at 169.93 ppm (6-triacetyl). The carbonyl carbon resonance at 169.78 for 2 was not assigned in this set of experiments. We found this experience to be general; we could not assign every carbonyl carbon peak for 2–5 from the INAPT set for each CA due to insufficient sensitivity in the INAPT experiment or insufficient resolution in the ^{1}H NMR spectra. Poor sensitivity in the INAPT experiment arises in part from the exchange of labeled acetyls for unlabeled acetyls, which occurred in the reaction medium during hydrolysis. If the abundance of carbon-13-labeled carbonyl carbon attached to a given hydroxyl is small, the INAPT experiment will not be successful. However, the carbonyl carbon resonance at 169.78 ppm can be assigned on the basis of the INAPT experiments for 3.

As we noted earlier, the coupling networks for the 3,6-diacetyl monomer and the 3-monoacetyl monomer overlap in the ^{1}H NMR spectra of CA in which they appear, as do the coupling networks of the 2,6-diacetyl monomer and the 2-monoacetyl monomer. The C3 carbonyl carbon resonances for the 3,6-diacetyl and 3-monoacetyl monomers were distinguished on the basis of the INAPT experiments for CA 3–5. Irradiation of the proton resonance at 4.82 ppm in CA 3 returned two carbonyl carbon resonances at 169.49 and 169.13 ppm. The smaller resonance in 169.49 ppm was assigned to the C3 carbonyl carbon of the 3-monoacetyl monomer on the expectation that this monomer would be the least abundant at this DS (1.99) level and that the population of this monomer would increase with decreasing DS. In the case of the overlapping 2,6-diacetyl and 2-monoacetyl monomer coupling networks, irradiation of the proton resonance at 4.34 and 4.32 ppm for CA 4 and 5, respectively, returned a single resonance at 168.63 ppm, which we assigned as overlapping 2,6-diacetyl and 2-monoacetyl monomer carbonyl carbon resonances.

Thus, all of the carbonyl carbon resonances observed for the series 1–5 can be identified as either a C2, C3, or C6 carbonyl carbon resonance and were assigned to a particular monomer so far as spectral resolution and ^{1}H–^{1}H correlations would allow. The lone exception is the carbonyl carbon resonance at 169.86 ppm (CA 4). However, inspection of Figure 5 shows that the chemical shifts of the assigned C3 carbonyl carbon resonances appear from 169.56 to 169.05 ppm. All of the C6 carbonyl carbon resonances are found downfield from the C3 carbonyl carbon resonances, while all of the C2 carbonyl carbon resonances are found upfield from the C3 carbonyl carbons. On the basis of this observation, we have assigned the carbonyl carbon resonance at 169.86 ppm to a C6 carbonyl carbon.

As discussed earlier, a maximum of 12 carbonyl carbon resonances should be observed in any single CA spectrum. From Figure 4 it can be seen that a total of 15 distinct carbonyl carbon resonances are observed in the ^{13}C NMR spectrum of CA 4 and CA 5. Of these 15 carbonyl carbon resonances, eight can be assigned to C3 carbonyl carbons. We believe the origin of the additional C3 carbonyl carbons is due to interactions between adjacent anhydroglucose rings or between polymer chains in the form of hydrogen bonding between hydroxyl and C3 carbonyls. A priori, one would expect that the C6 carbonyl carbon and, in particular, the C2 carbonyl carbon would participate in hydrogen bonding. If this is occurring, within the resolution of our spectra, we could not detect it since we did not observe perturbations in the chemical shifts of the C2 or C6 carbonyl carbon resonances.[14]

In the ^{13}C NMR spectrum for CTA 1, the resonance for the C3 carbonyl carbon appears at 169.05 ppm. The chemical shift of the resonance remains unchanged for CA 2 and moves downfield by 0.02, 0.03, and 0.05 for CA 3, 4, and 5, respectively, relative to the chemical shift of the C3 carbonyl carbon resonance in CTA 1 (the CA in which this resonance first appears). For CA 4 and 5 (DS = 1.61 and 1.06) there is a resonance at 169.14 ppm, which, on the basis of the INAPT experiments where the proton resonance at 5.05 ppm was irradiated for CA 5, can also be assigned as a C3 carbonyl carbon of the triacetyl

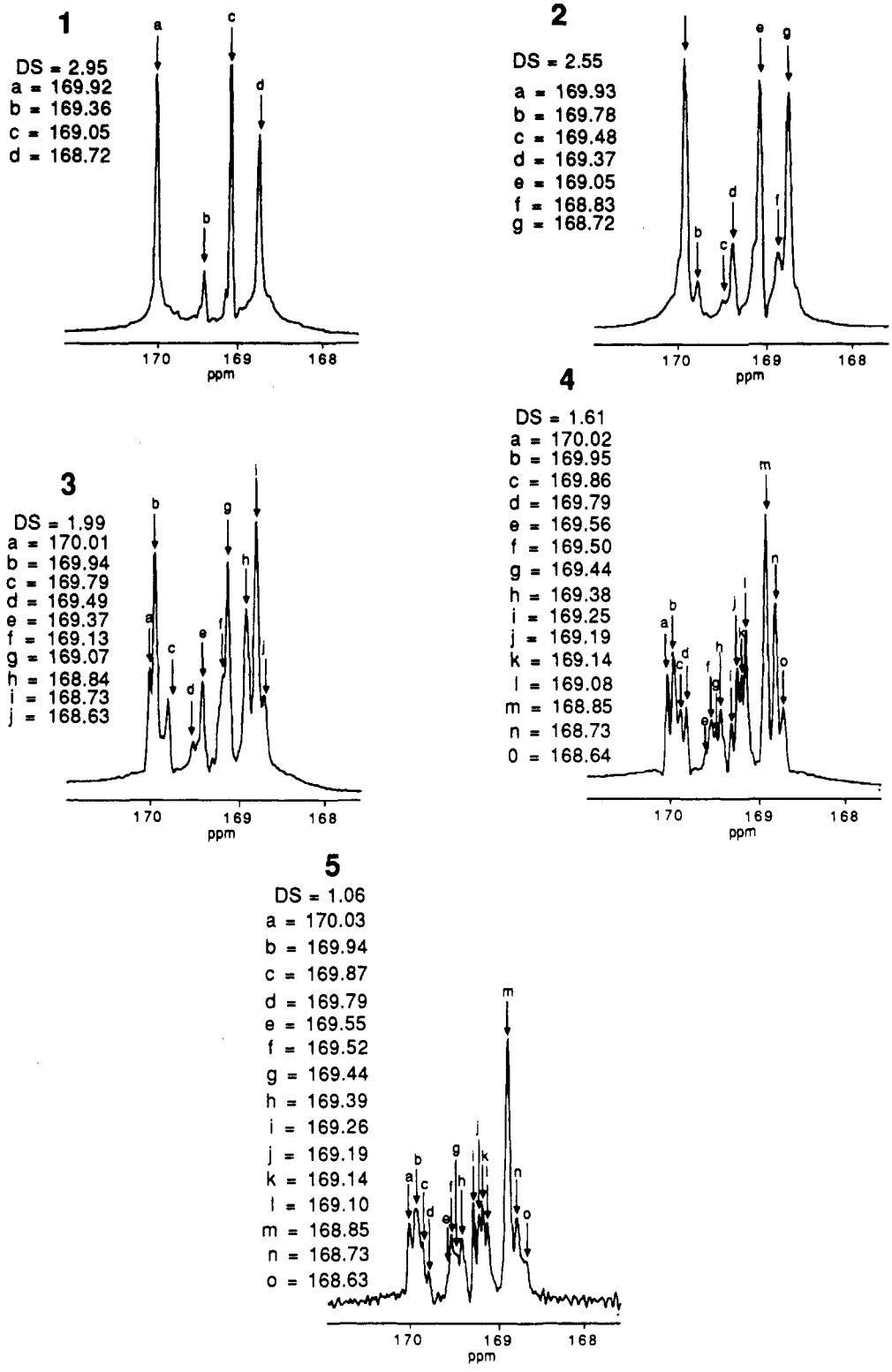

Figure 4. Resolution-enhanced carbon-13 NMR spectra (67.9 MHz) of the carbonyl carbon region for cellulose acetates 1–5.

monomer. Since the resonance at 169.14 ppm does not appear until a high hydroxyl level has been obtained and since it appears simultaneously with a resonance also assigned to a C3 carbonyl carbon of the triacetyl monomer at low hydroxyl level, we attribute the 169.14 ppm resonance to a C3 carbonyl carbon of the triacetyl monomer that is hydrogen bound to hydroxyl(s). A similar pattern is observed with the resonances of the 2,3-diacetyl, 3,6-diacetyl, and 3-monoacetyl monomers. For example, a carbonyl carbon resonance for the 3,6-diacetyl monomer first appears at 169.13 ppm in the ^{13}C NMR spectrum of CA 3. In the ^{13}C NMR spectra of CA 4 and 5, this resonance appears at 169.19 ppm. Also appearing in the same spectra for CA 4 and 5 is a 3,6-diacetyl resonance at 169.25 and 169.26 ppm, respectively, the multiplicity of which we attribute to hydrogen bonding. For the 2,3-diacetyl monomer, the non-hydrogen-bonded carbonyl carbon resonance first appears at 169.36 ppm in the ^{13}C NMR spectrum of CA 1. This resonance is shifted downfield by 0.01 ppm for CA 2 and 3, by 0.02 for CA 4, and by 0.03 ppm for CA 5. The hydrogen-bonding 2,3-diacetyl monomer resonance appears in the spectrum of CA 4 and 5 at 169.44 ppm. The non-hydrogen-bonding carbonyl carbon resonance for the 3-monoacetyl monomer first appears at

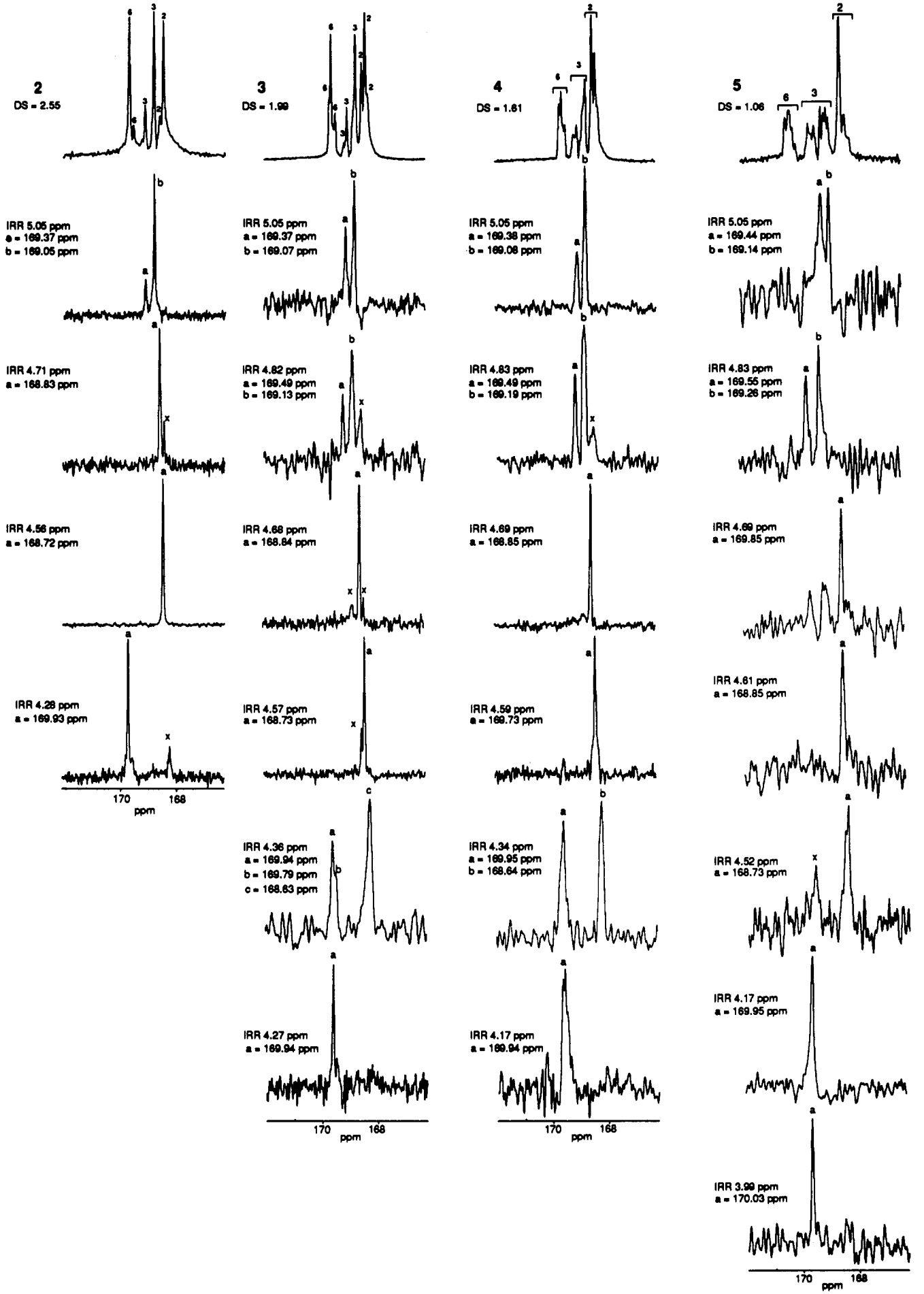

Figure 5. INAPT spectra (100 MHz) for **2**–**5**. For each CA, the top figure is the proton-decoupling carbon-13 NMR spectrum of the carbonyl region. IRR refers to the proton that was irradiated. An x indicates peaks due to spurious transfer or nonselective irradiation.

Table I
Chemical Shifts for the Carbonyl Resonances of CA 1–5

carbonyl chemical shift, ppm[a]	monomer
170.01 (0.01, 0.02)	6-mono- or diacetyl
169.92 (0.01, 0.02, 0.03)	6-triacetyl
169.83 (0.01, 0.02, 0.03)	6-mono- or diacetyl
169.78 (0.01)	6-mono- or diacetyl
169.56	3-monoacetyl[b]
169.48 (0.01, 0.02, 0.04)	3-monoacetyl
169.44	2,3-diacetyl[b]
169.36 (0.01, 0.02, 0.03)	2,3-diacetyl
169.25	3,6-diacetyl[b]
169.14	3-triacetyl[b]
169.13 (0.06)	3,6-diacetyl
169.05 (0.02, 0.03, 0.05)	3-triacetyl
168.83 (0.01, 0.02)	2,3-diacetyl
168.72 (0.01)	2-triacetyl
168.63 (0.01)	2-monoacetyl
	2,6-diacetyl

[a] The chemical shifts that are given are for the first observed resonance(s) for that monomer. The numbers given in the parentheses give the downfield shift for that resonance with increasing hydroxyl level. [b] This resonance has been shifted by hydrogen bonding.

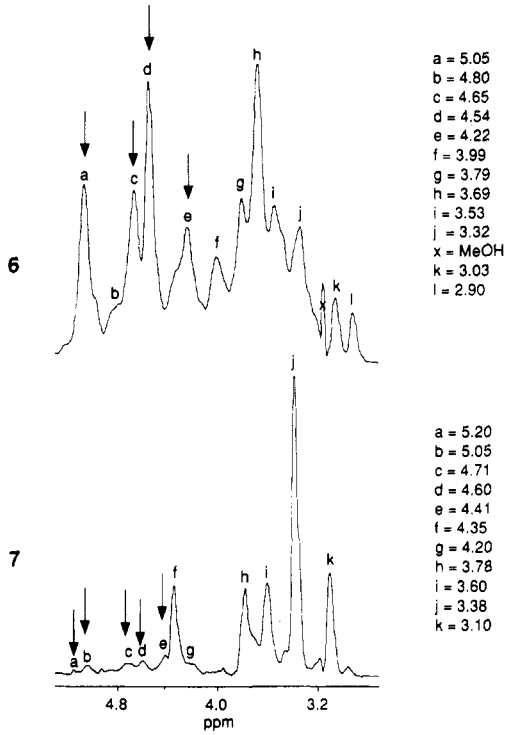

Figure 6. Resolution-enhanced proton NMR spectra (400 MHz) of CA 6 and 7 prepared by Mg-catalyzed methanolysis. The resonances irradiated in the INAPT experiments are indicated by arrows.

169.48 ppm in the spectrum of CA 2 and moves downfield by 0.01, 0.02, and 0.04 ppm for CA 3, 4, and 5. The hydrogen-bonded carbonyl carbon resonance for the 3-monoacetyl monomer can be found in the ^{13}C NMR spectrum of CA 4 at 169.56 ppm and at 169.55 ppm for CA 5.

Table I gives the chemical shifts for the carbonyl carbon resonances of cellulose acetates 1–5. Several clear trends emerge from the analysis of the chemical shifts of these carbonyl carbon resonances. In every case, the carbonyl carbon resonances move downfield with decreasing DS. The effect of hydrogen bonding on a C3 carbonyl carbon resonance is a downfield shift of ca. 0.06 ppm.

Cellulose Acetates 6 and 7. Figures 6 and 7 show the ^1H and ^{13}C NMR spectra of CA 6 and 7. The assigned

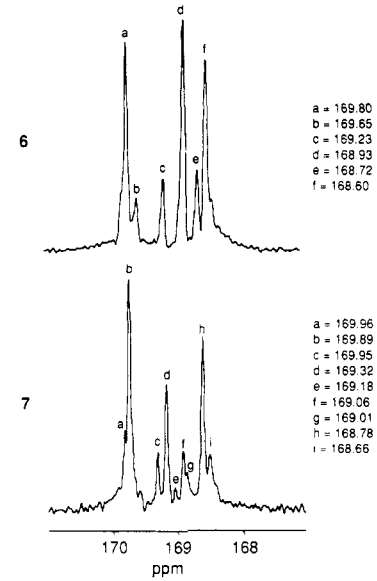

Figure 7. Resolution-enhanced carbon-13 NMR spectra (100 MHz) of CA 6 (DS = 2.03) and 7 (DS = 0.42) prepared by Mg-catalyzed methanolysis.

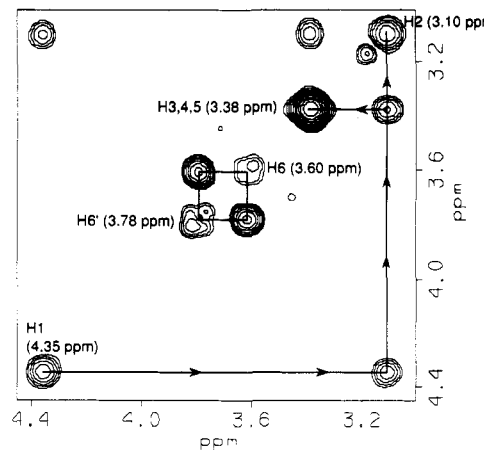

Figure 8. COSY spectrum of unsubstituted anhydroglucose monomers (cellulose) at 400 MHz. The spectrum was obtained from the COSY spectrum of CA 7 (DS = 0.42) at high threshold values.

COSY spectrum for CA 6 is contained in the supplementary material. Assignment of this spectrum was based on the same observations and assumptions used in assigning the COSY spectra of CA 1–5. The COSY spectrum of CA 7 was of little value in assigning the resonances of ring protons attached to carbons bearing an acetyl substituent since the spectrum was dominated by the proton resonances of unsubstituted anhydroglucose monomers. Assignment of the ^1H spectrum and determination of the peaks to be irradiated for CA 7 were based on extrapolation from the ^1H NMR spectra of CA 1–6 and upon the observations previously noted (vide supra). At high threshold values the COSY spectrum shown in Figure 8 was obtained from the COSY experiment for CA 7. This COSY spectrum, which is nearly identical with the COSY spectrum for cellulose in DMAC/LiCl reported by Nardin and Vincendon,[12] can be considered to be that of cellulose in DMSO-d_6.

Figure 9 gives the results of the INAPT experiments for CA 6 and 7. As with CA 1–5, the proton resonances irradiated in the ^1H NMR spectra (in parts per million) are provided as well as the chemical shift(s) of the carbonyl carbon resonance(s) observed in the INAPT experiment. For CA 6, all six carbonyl carbon resonances

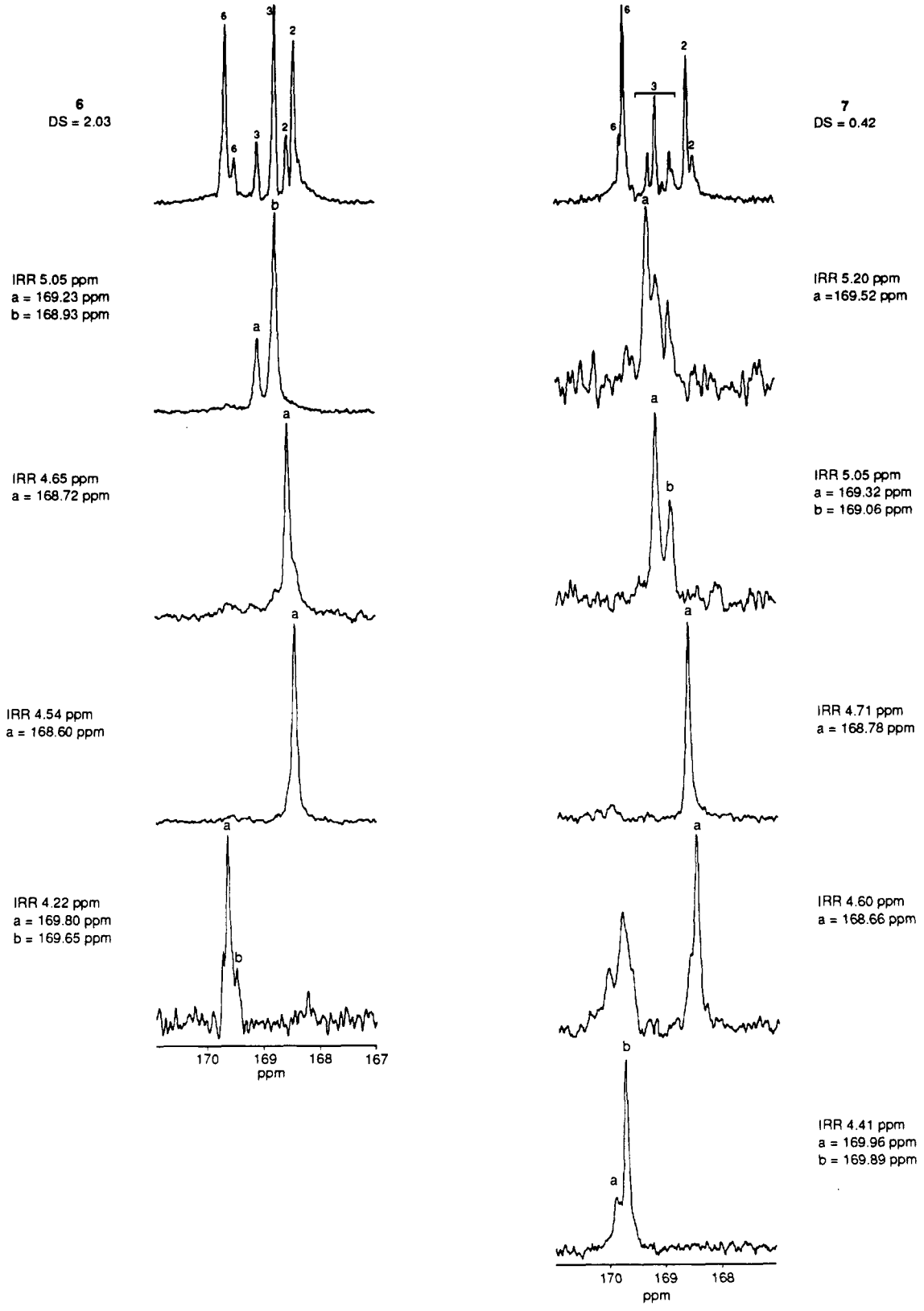

Figure 9. INAPT spectra (100 MHz) for 6 and 7. For each CA, the top figure is the proton-decoupled carbon-13 NMR spectrum of the carbonyl region. IRR refers to the proton resonance that was irradiated.

were assigned as a C6, C3, or a C2 carbonyl and, except for the C6 carbonyl resonance at 169.65 ppm, each resonance was assigned to a particular monomer. Comparison of the ^{13}C NMR spectra of the carbonyl carbons of CA **6** to those of CA **3** reveals that every carbonyl resonance of CA **6** is shifted upfield by 0.12–0.14 ppm relative to the corresponding resonance in CA **3**. The origin of this chemical shift is certainly not clear. However, we have observed that this type of chemical shift only occurs when we use CTA as the starting material for methanolysis. When CDA is the starting material for the methanolysis reaction, significant perturbations in chemical shifts are not observed.[10] If a +0.13 adjustment is applied to the chemical shifts of the carbonyl carbon resonances of CA **6**, we can directly compare CA **6** and CA **3**. We find that CA **6** differs from CA **3** by the absence of resonances

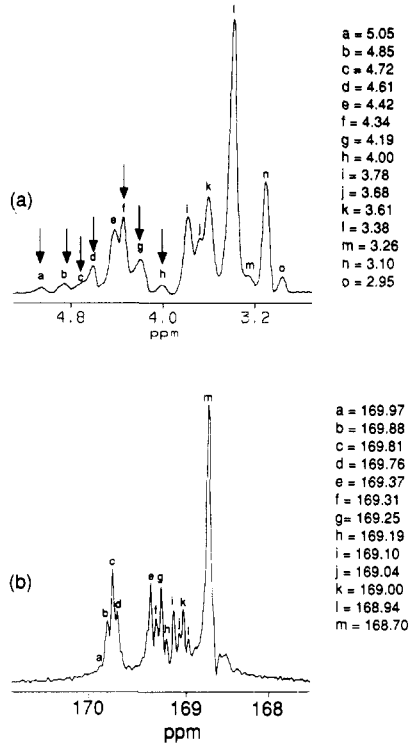

Figure 10. (a) Proton NMR (400 MHz) and (b) carbon-13 NMR (100 MHz) of CA 8 (DS = 0.67) prepared by AcOH/MeOH hydrolysis. The resonances irradiated in the INAPT experiments are indicated by arrows.

for C6, 3-monoacetyl, 3,6-diacetyl, 2-monoacetyl, and 2,6-diacetyl carbonyl carbons.[15] Similarly for CA 7, all nine carbonyl carbon resonances were assigned as C6, C3, or C2 carbonyl carbons. From the INAPT experiments for CA 5 and CA 7 in which the proton resonances at 5.05 ppm were irradiated, we find that the carbonyl resonances of CA 7 are shifted upfield by 0.07 ppm relative to those of CA 5. If a +0.07 adjustment is made, we find that CA 7 differs from CA 5 by the absence of resonances for two C6 carbonyls, a 3-monoacetyl*, a 2,3-diacetyl*, a 3,6-diacetyl, a 2-monoacetyl, and a 2,6-diacetyl carbonyl carbons.

Cellulose Acetate 8. Figure 10 shows the ^{1}H and ^{13}C NMR spectra of CA 8 while Figures 11 and 12 give the COSY spectrum and the INAPT spectra, respectively. Assignment of these spectra follow the same protocol used for CA 1–7. Furthermore, the results are fully consistent with that obtained for CA 1–7. By applying a +0.14 correction to the chemical shifts of the carbonyl resonances of CA 8, direct comparison to CA 5 is possible. Relative to CA 5, we find that resonances for a C6 carbonyl, a 3-monoacetyl*, a 2-monoacetyl, and a 2,6-diacetyl carbonyl carbons are absent. In addition, we find two additional resonances in CA 8 at 170.11 and 169.33 ppm, which were not observed for CA 5.

Conclusions

In this work, we have described the synthesis of cellulose acetates labeled at the carbonyl carbons with carbon-13. Aqueous acid hydrolysis of labeled CTA provides for a random distribution of acetyls along the polymer backbone, while the range of DS values permits the observation of nearly all of the carbonyl carbon resonances for the anhydroglucose monomers. Methanolysis of labeled CTA using two different catalysts provided labeled cellulose acetates prepared by different methods at similar levels of substitution to those prepared by aqueous acid hydrolysis. Using this series of compounds, we have identified

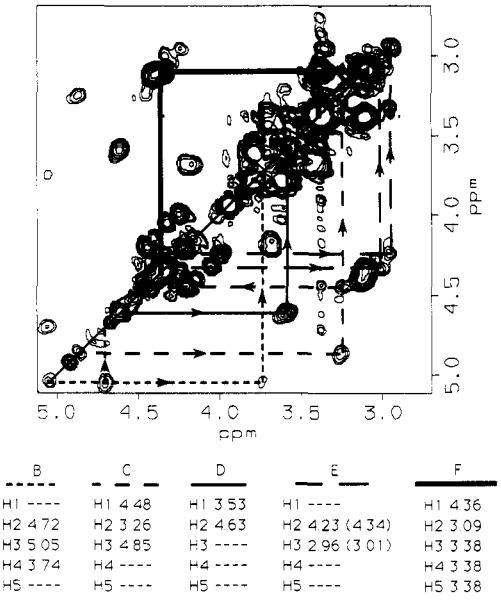

Figure 11. COSY NMR spectrum (400 MHz) for CA 8 (DS = 0.67). Five coupling networks are found in this spectrum. For clarity, the coupling network labeled F is shown in the upper diagonal.

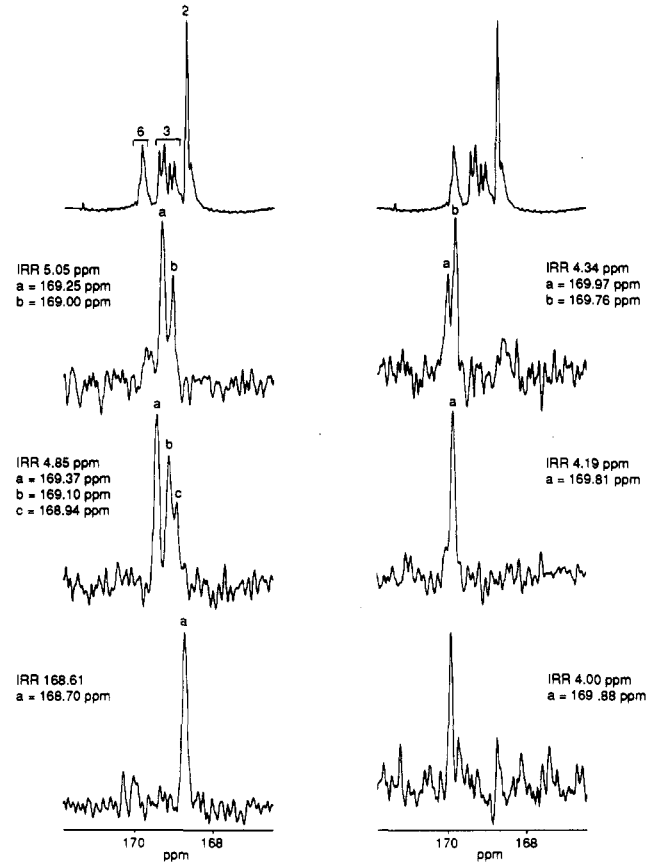

Figure 12. INAPT spectra showing polarization transfer from the ring protons to the carbonyl carbons of CA 8. The top spectrum in each column is the proton-decoupled carbon-13 NMR spectra of CA 8. IRR refers to the chemical shift of the proton resonance that was irradiated in the INAPT experiment.

16 carbonyl carbon resonances in the ^{13}C NMR spectra of these cellulose acetates. These resonances have been assigned to carbonyl carbons attached to either a C2, a C3, or a C6 hydroxyl. In the case of the C2 and C3 carbonyl carbon resonances, we were able to assign these resonances to specific monomers. We have also shown that these carbonyl carbon resonances shift downfield with increasing

hydroxyl level. We have demonstrated that, within the resolution of our experiments, only the C3 carbonyl carbons participate in hydrogen bonding and that the effect of hydrogen bonding on the C3 carbonyl carbon resonance is a downfield shift of ca. 0.06 ppm.

Since we could not assign every resonance to a specific monomer and because of spectral overlap and inherent difficulties in integrating carbon-13 NMR spectra, we view this method for analyzing monomer composition to be only semiquantitative at best. The method of analysis presented in this paper should not be viewed as a method for replacing classical methods, e.g., methylation analysis. We believe the principle value of the method described in this paper is in the comparison of closely related cellulose acetates in a nondestructive fashion.[10] As such, we believe the information provided in this paper will provide an extremely useful tool for probing the relationships between acetyl distribution and physical properties. The principle deficiency in this study is our inability to correlate all of the C6 carbonyl carbons to their respective monomers and our limited spectral resolution. We hope to address these problems by regiospecific synthesis of specifically substituted cellulose esters and by application of 3D NMR spectroscopy.

Acknowledgment. We are very grateful to Dr. Douglas W. Lowman for his technical support and helpful comments.

Supplementary Material Available: COSY spectra for CA **3**, **4**, **5**, and **6** and INAPT spectra for CA **1** (CTA) in DMSO-d_6 (6 pages). Ordering information is given on any current masthead page.

References and Notes

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(11) Buchanan, C. M.; Hyatt, J. A.; Lowman, D. W. *Macromolecules* **1987**, *20*, 2750.

(12) The proton chemical shifts observed for unsubstituted anhydroglucose rings were virtually identical with those reported for cellulose in DMAc/LiCl. Cf.: Nardin, R.; Vincendon, M. *Macromolecules* **1986**, *19*, 2452.

(13) At lower DS levels, this overlap is partially removed. However, the close proximity of the H3 proton resonances for these monomers does not permit resolution of the carbonyl resonances in the following INAPT experiments.

(14) We have experimental evidence that peak overlap is occurring in the C6 and C2 carbonyl carbon region.[10]

(15) The distinctive and characteristic resonance at 168.85 for CA **5** is observed in every cellulose acetate that we have examined. Hence, this resonance may well serve as an internal reference in examining cellulose acetates whose carbonyl carbon chemical shifts differ from that found in Table I.

Registry No. 1-^{13}C, 132883-93-3; **1**, 9012-09-3; 2-^{13}C, 132883-92-2; **2**, 9004-35-7; cellulose, 9004-34-6; magnesium acetate tetrahydrate, 142-72-3; diacetyl cellulose, 9035-69-2; acetic acid, 64-19-7.