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Analysis of positions of substitution of *O*-acetyl groups in partially *O*-acetylated cellulose by the reductive-cleavage method

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Abstract

Described herein are methods for determining the degree of substitution (ds) and the positions of substitution of *O*-acetyl groups in commercial samples of partially *O*-acetylated cellulose. Sequential neutral methylation of the polymers, base-catalyzed ethylation, and reductive cleavage gives eight products that are analyzed as their *O*-acetyl derivatives by gas-liquid chromatography. Alternatively, the permethylated polymers are subjected to reductive cleavage directly, under conditions that reduce *O*-acetyl groups to *O*-ethyl groups, to give the same eight products. The ds values obtained by these two methods were in good agreement with values obtained by integration of the *O*-acetyl and *O*-trimethylsilyl resonances of the fully *O*-trimethylsilylated polymers.

Keywords: Cellulose; Reductive-cleavage analysis; Acetate position

1. Introduction

Cellulose acetate has a wide range of physical properties and commercial applications depending upon its degree of substitution (ds) as well as the distribution of *O*-acetyl groups [1]. The structural characterization of cellulose acetate is therefore important to understanding its chemical and physical properties. Several methods for performing such analyses have been reported. ^{1}H NMR spectroscopy has been employed [2–4] to determine ds, but this method has limited application due to problems of solubility and viscosity. Moreover, ^{1}H NMR spectroscopy has not been successful in establishing the relative molar proportions of the eight possible monomeric units in partially acetylated

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celluloses. ^{13}C NMR spectroscopy has been more successful in the latter case, but even a very careful study [5] of the [1-^{13}C]acetyl signals in cellulose [1-^{13}C]acetate failed to reveal the relative molar proportions of the eight monomer residues.

In contrast, chemical methods of analysis, although more laborious, appear to provide a reliable means to determine both the ds and the distribution of substituents in partially acetylated cellulose. Such analyses were initially performed by methylation analysis after either replacement of *O*-acetyl groups with *O*-methyl groups [6] or directly after methylation of free hydroxyl groups [7]. The former method, although laborious, gave ds values in excellent agreement with those determined by other methods. The latter method, however, gave low ds values, which was attributed to incomplete methylation. More recently, a strategy employing the reductive-cleavage method [8] was used for such analyses [9]. In this strategy, cellulose acetate was subjected to neutral methylation [10] followed by base-catalyzed alkylation [11] (ethylation, propylation, or pentylation) and the resulting methyl/ethyl, methyl/propyl, and methyl/pentyl anhydro-D-glucitol derivatives were analyzed as their *O*-acetyl derivatives by GLC. Based upon these results, the author preferred the acetyl–pentyl exchange strategy over the acetyl–ethyl exchange strategy since the resultant partially methylated and pentylated anhydro-D-glucitol derivatives gave better separation in GLC than did the corresponding methylated and ethylated derivatives. Unfortunately, the product mixture derived from the acetyl–pentyl exchange strategy was more complex than expected due to the presence of significant amounts of ring-contracted products [12].

As part of our continuing, comprehensive investigation of the reductive-cleavage method, we came to reexamine its applicability to the structural analysis of cellulose acetate. Described herein is a general method for such analyses involving sequential neutral methylation [10], acetyl–ethyl exchange, reductive cleavage, and acetylation. Also described herein is a new, simpler approach for such analyses involving sequential neutral methylation, reductive-cleavage under conditions known [13] to reduce esters to ethers, and acetylation. The eight positional isomers of methylated and ethylated 4-*O*-acetyl-1,5-anhydro-D-glucitol obtained by both methods were separated by GLC on a Restek RT_x-200 column, which was found to give much better resolution than the DB-5 column previously used [14].

2. Results and discussion

Determination of ds by ^1H NMR spectroscopy.—Cellulose acetates displayed quite different solubility properties depending upon their ds. Samples having high ds (2.5) formed gels upon mixing with chloroform, dioxane, dimethyl sulfoxide, *N*,*N*-dimethylformamide, or trimethyl phosphate. Samples having low ds (0.8), on the other hand, had extremely poor solubility in the aforementioned solvents. Attempts to determine ds by integration of *O*-acetyl and ring hydrogen signals were unsuccessful because consistent results could not be obtained.

The ds values of these samples were readily obtained, however, by integration of the *O*-acetyl and *O*-trimethylsilyl resonances of the fully *O*-trimethylsilylated polymers (Table 1). Silylation was carried out in Me_2NCHO in the presence of *N*,*O*-bis(trimethylsilyl)acetamide and 1-methylimidazole at room temperature, and the products were

Table 1
Comparison of the ds values for cellulose esters obtained by the silylation method with values given by the suppliers

Sample [a]	Degree of substitution (ds)		
	Method 1 [b]	Method 2 [c]	Reported [d]
A	0.81	0.76	0.8
B	1.97	2.05	2.1
C	2.28	2.50	2.5
D	2.77	3.00	3.0
E	2.81	2.96	3.0
F	1.86	2.43	2.45

[a] A, Eastman X22285-129-4; B, Eastman X23030-077-4; C, Eastman X22285-129-5; D, Eastman X23030-109-B; E, Eastman X22285-129-3; F, Aldrich 18,095-5; samples A, B, C, D, and F are cellulose acetates; sample E is a cellulose propionate.
[b] Calculated from the integrals of the ring hydrogen and *O*-acetyl resonances in the ^{1}H NMR spectra of the fully *O*-trimethylsilylated polymers.
[c] Calculated from the integrals of the *O*-acetyl and *O*-trimethylsilyl resonances in the ^{1}H NMR spectra of the fully *O*-trimethylsilylated polymers.
[d] Values given by the suppliers.

isolated by dialysis against water and subsequent lyophilization. The unreacted silylating agent was readily decomposed by the addition of water, forming water-soluble compounds, and the solvent and catalyst were also readily soluble in water, making the isolation procedure easy and effective. Analysis of the lyophilized products by IR spectroscopy showed complete absence of free hydroxyl groups. The products were readily soluble in chloroform and well-resolved NMR spectra could be obtained.

As is evident in Table 1, the ds values obtained by integration of the *O*-acetyl and *O*-trimethylsilyl resonances were in excellent agreement with values provided by the suppliers and *vide infra*, with those found by chemical analysis. The ds values obtained by integration of the ring hydrogen and *O*-acetyl resonances of the fully silylated polymers were usually lower than those determined by other methods, however. The reason for this discrepancy is not known.

Analysis of cellulose acetates by methylation, acetyl–ethyl exchange, and reductive cleavage.—Methylation of the cellulose acetate samples was carried out by the method of Prehm [10] essentially as described by Mischnick [9]. The samples were dissolved in trimethyl phosphate and reacted with methyl trifluoromethanesulfonate in the presence of 2,6-di-*tert*-butylpyridine for seven days at room temperature. Attempted workup of the reactions by extraction into chloroform was not very successful, however, due to the formation of a gel that was not readily soluble. The reactions were therefore processed by dialysis against water and subsequent lyophilization. Small amounts of the methylated products were silylated as already described and then examined by ^{1}H NMR spectroscopy. Integration of the *O*-acetyl and *O*-trimethylsilyl resonances demonstrated that $> 98\%$ of the free hydroxyl groups had been methylated in all the samples analyzed.

The fully methylated cellulose acetates were then subjected to acetyl–ethyl exchange by dissolution in dimethyl sulfoxide and treatment with iodoethane in the presence of sodium hydroxide. The procedure was repeated twice to ensure complete ethylation;

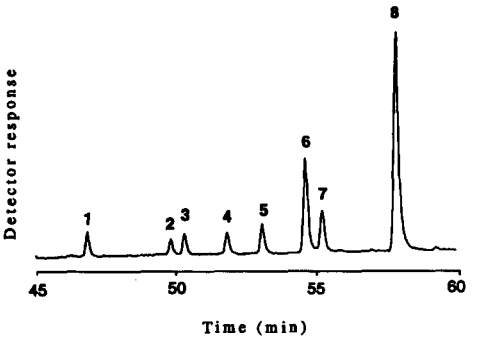
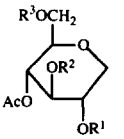


Fig. 1. Gas–liquid chromatogram of the anhydroalditol acetates derived from cellulose acetate (*Sample C*) by sequential per-*O*-methylation, acetyl–ethyl exchange, reductive cleavage, and acetylation. Chromatography was conducted on a Restek RT_x-200 column. The peaks are numbered with the compound numbers.

subsequent examination of the products by IR spectroscopy confirmed the disappearance of the carbonyl band at 1720 cm^{-1}. The methylated and ethylated cellulose products were then subjected to reductive cleavage [15] for 24 h in the presence of 5 equiv (per equiv of acetal) of Et_3SiH, 5 equiv of Me_3SiOSO_2Me, and 1 equiv of $BF_3 \cdot Et_2O$, and the products were acetylated. Analysis of the acetylated products by GLC on a DB-5 column as previously described [14] gave the same eight products as derived by perethylation of *O*-methyl cellulose. It was discovered, however, that much better separation of these products could be achieved by GLC on a Restek RT_X-200 column (Fig. 1). The individual components in these mixtures were identified based upon a comparison of their mass spectra with those of authentic standards [16]. As an aid to those who wish to use this method, the retention indices [17] of compounds **1–8** were determined on both the DB-5 and RT_X-200 columns and are presented in Table 2.



	R^1	R^2	R^3
1	Me	Me	Me
2	Me	Et	Me
3	Me	Me	Et
4	Et	Me	Me
5	Me	Et	Et
6	Et	Et	Me
7	Et	Me	Et
8	Et	Et	Et

Table 2
Linear temperature programmed gas–liquid chromatography retention indices (LTPGLCRI) of compounds **1–8** [a]

Compound	Stationary phase	
	DB-5	RT_x-200
1	1535.79	1834.69
2	1576.32	1865.67
3	1594.56	1870.48
4	1595.61	1888.45
5	1635.53	1900.00
6	1635.08	1920.63
7	1652.98	1925.58
8	1693.85	1954.94

[a] Values were determined as described in [17].

Integration of all peaks (Fig. 1) and correction for molar response [18,19] gave the mole percent of each product (**1–8**) in each sample of cellulose acetate analyzed (see Table 3, Method 1). Using these values, the fractional degree of substitution, x_i, at each of the three positions on the D-glucopyranosyl residues of cellulose and the average degree of substitution ($ds = x_2 + x_3 + x_6$) were calculated. As is evident in Table 3, the ds values determined by reductive cleavage Method 1 and the silylation method (see

Table 3
Mole percents of products derived by reductive cleavage of *O*-ethyl-*O*-methylcellulose and *O*-acetyl-*O*-methylcellulose

Compound or parameter	Sample [a]							
	A [b] Method [d]		B [b]		C [b]		C [c]	
	1	2	1	2	1	2	1	2
1	49.00	43.30	7.46	6.67	2.89	7.11	2.37	4.54
2	11.04	10.06	4.14	4.39	3.79	4.18	2.23	2.58
3	8.86	9.07	9.20	9.84	3.27	2.92	2.95	2.60
4	12.36	12.88	9.16	8.58	6.01	3.32	3.31	3.46
5	1.84	2.46	5.83	6.75	5.78	9.76	5.30	9.37
6	12.34	14.04	19.33	19.59	24.41	26.58	19.64	23.20
7	2.14	3.16	13.68	12.81	9.21	5.08	8.31	4.62
8	2.42	5.03	31.20	31.37	44.64	41.05	55.89	49.63
x_2	0.293	0.351	0.734	0.724	0.843	0.760	0.872	0.809
x_3	0.276	0.316	0.605	0.621	0.786	0.816	0.831	0.848
x_6	0.153	0.197	0.599	0.608	0.629	0.588	0.725	0.662
ds	0.72	0.86	1.94	1.95	2.26	2.16	2.43	2.32

[a] See footnote a, Table 1.
[b] Methylation was carried out at room temperature for 7 days.
[c] Methylation was carried out at 85°C for 3 h.
[d] Method 1: sequential methylation, acetyl–ethyl exchange, reductive cleavage (Et_3SiH, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$, 24 h), and acetylation; Method 2: sequential methylation, reductive cleavage (Et_3SiH, Me_3SiOSO_2Me–$BF_3 \cdot Et_2O$, 7 days), and acetylation.

Table 1) were in good agreement for cellulose acetates having a low ds (Sample A) and a medium ds (Sample B); i.e., Sample A was found to have ds = 0.72 by the reductive cleavage method and ds = 0.76 by the silylation method whereas Sample B was found to have ds = 1.94 by the reductive cleavage method and ds = 2.05 by the silylation method. For the high-ds cellulose acetate (Sample C), the ds values obtained by reductive cleavage Method 1 (2.26) and the silylation method (2.50) were not in as good agreement. However, it was discovered that methylation of the high-ds material at high temperature for a much shorter period of time (85°C, 3 h) gave much better results than when methylation was carried out for a long period of time (7 days) at room temperature; i.e., analysis of Sample C by reductive cleavage Method 1 gave a ds of 2.43, in excellent agreement with the value (2.50) obtained by the silylation method.

Analysis of cellulose acetates by methylation and direct reductive cleavage.—The previous finding [13] that propionyl esters were reduced to (1-propyl) ethers during long term exposure to Et_3SiH in the presence of Me_3SiOSO_2Me and $BF_3 \cdot Et_2O$ suggested that it might be possible to analyze methylated cellulose acetate samples directly by the reductive cleavage method. Indeed, the methylated and ethylated 1,5-anhydro-D-glucitol derivatives (**1–8**) derived by such a strategy would be identical to those derived by acetyl–ethyl exchange. As a test of this proposal, the same permethylated cellulose acetate samples that had been used for acetyl–ethyl exchange were subjected to reductive cleavage in the presence of 20 equiv of Et_3SiH, 20 equiv of Me_3SiOSO_2Me, and 4 equiv of $BF_3 \cdot Et_2O$ for seven days, and the products were acetylated and analyzed as before. Indeed, for all three cellulose acetate samples (A, B, and C) that were analyzed, compounds **1–8** were the only products observed. Given in Table 3 (see Method 2) are the mole percents of these products and the calculated ds values derived therefrom. As is evident in Table 3, the mole percents of products derived by direct reductive cleavage (Method 2) are in quite good agreement with those derived by acetyl–ethyl exchange (Method 1). Although the values are somewhat at variance in a few cases, the ds values obtained by the two methods agree quite closely. It is concluded from these results that the method of direct reductive cleavage (Method 2) is acceptable for detemining both the ds and the distribution of ester groups in cellulose acetate samples. The satisfactory results obtained using the method suggest that it might well be the method of choice for analyzing esterified polysaccharides containing more than one type of ester substituent.

3. Experimental

Materials.—Cellulose acetates were provided by the Eastman Chemical Co., Kingsport, TN. *N,O*-Bis(trimethylsilyl)acetamide, triethylsilane, trimethylsilyl methanesulfonate, boron trifluoride etherate ($BF_3 \cdot Et_2O$), and methyl trifluoromethanesulfonate ($MeOSO_2CF_3$) were purchased from Aldrich Chemical Co. and were used without further purification. 2,6-Di-*tert*-butylpyridine and 1-methylimidazole were distilled over NaOH under vacuum prior to use. Trimethyl phosphate was distilled over Na_2CO_3 under vacuum and stored over 4A molecular sieves. Dimethyl sulfoxide (Me_2SO) was distilled over barium oxide under vacuum and stored over 4A molecular sieves.

N,*N*-Dimethylformamide (Me_2NCHO) was distilled over NaOH under vacuum and stored over 4A molecular sieves. BioRad AG501 × 8(D) was used as a mixed-bed resin.

Instruments.—^1H NMR spectra were recorded on a Varian 500 VXR-FT NMR spectrometer in $CDCl_3$ as the solvent and were referenced to internal $CHCl_3$ at δ 7.24. Infrared (IR) spectra were recorded on a Perkin–Elmer Model 1600 FT IR spectrophotometer. GLC analyses were performed using a Hewlett–Packard 5890A gas–liquid chromatograph equipped with an on-column injector connected to a Restek RT_x-200 capillary column (0.25 mm × 30 m, 0.25-μm film thickness), a flame-ionization detector, and a Hewlett–Packard 3392A integrator. For purpose of comparison, a J&W Scientific DB-5 capillary column (0.25 mm × 30 m, 0.25-μm film thickness) was also used. The temperature of the column was programmed from 80 to 300°C at 1°C/min.

Methylation of cellulose acetates.—Methylations were performed by the method of Prehm [10] by modifications of the procedure described by Mischnick [9]. For cellulose acetates having a high ds (2.5), the sample (33.0 mg) and trimethyl phosphate (5 mL) were sonicated in a bath sonicator for 15 h, then 2,6-di-*tert*-butylpyridine (0.2 mL) and $MeOSO_2CF_3$ (0.13 mL) were added to the clear solution. After stirring at 85°C for 3 h (oil bath), the tarry-colored solution was dialyzed against distilled water and the nondialyzable portion was lyophilized to give 22.2 mg (66%) of permethylated product. For cellulose acetates having a low or medium ds, the sample (101.2 mg, ds 0.8) and trimethyl phosphate (10 mL) were sonicated for 5 h, 2,6-di-*tert*-butylpyridine (0.5 mL) and $MeOSO_2CF_3$ (0.3 mL) were added, and the mixture was stirred at room temperature for 7 days. The mixture was processed as before to yield 97.5 mg (94%) of permethylated product.

Acetyl–ethyl exchange reaction.—Permethylated cellulose monoacetate (ds 0.8, 54.3 mg, 0.24 mmol) was dissolved in Me_2SO (1.5 mL) by sonicating for 2 h, then NaOH (63.1 mg, 8 equiv for each ester group) was added, and the mixture was stirred for 6 h. Iodoethane (0.12 mL, 8 equiv for each ester group) was then added and the mixture was stirred at room temperature for 40 h. NaOH (17.3 mg, 2 equiv) was again added and, after stirring for 7 h, iodoethane (0.3 mL, 20 equiv) was added and stirring was continued for 24 h. The mixture was dialyzed against deionized water, and the product was lyophilized to give 43.3 mg (86%) of a white fluffy residue.

Reductive-cleavage reaction.—Following the procedure of Jun and Gray [15], 1–9 mg of *O*-ethyl-*O*-methylcellulose was treated with Et_3SiH (5 equiv), Me_3SiOSO_2Me (5 equiv), and $BF_3 \cdot Et_2O$ (1 equiv) in CH_2Cl_2 for 24 h. The reaction was quenched by the addition of MeOH, and the solution was treated with mixed-bed resin. After evaporation, the residue was redissolved in CH_2Cl_2 (0.5 mL), and the products were acetylated with Ac_2O (5 equiv) and 1-methylimidazole (5 equiv) at room temperature for 12–24 h [20]. The mixture was then extracted with satd aq $NaHCO_3$, and the organic layer was filtered through a Spartan 13 filter and examined by GLC.

With *O*-acetyl-*O*-methylcellulose as the substrate, reductive cleavage was carried out with Et_3SiH (20 equiv per equiv of acetal), Me_3SiOSO_2Me (20 equiv) [21], and $BF_3 \cdot Et_2O$ (4 equiv) in CH_2Cl_2 for 7 days, followed by processing as described above.

Silylation of cellulose acetate.—In a representative procedure, cellulose monoacetate (ds 0.8, 36.9 mg, 0.189 mmol) was dissolved in Me_2NCHO (2.0 mL) by sonicating for 2 h. 1-Methylimidazole (45 μL, 0.551 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide

(150 μL, 0.607 mmol) were then added, and the solution was stirred at room temperature for 8 h. The solution was dialyzed against deionized water and the product was lyophilized to yield 59.6 mg (89%) of a white foamy residue.

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