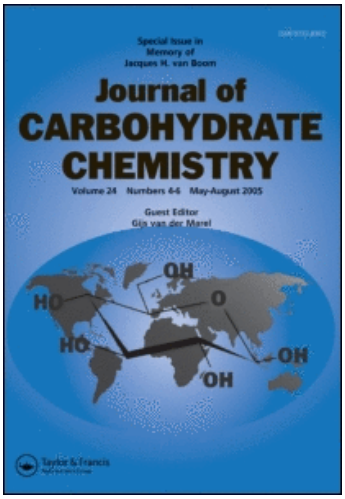

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DETERMINATION OF THE SUBSTITUTION PATTERN OF CELLULOSE ACETATES[1]

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ABSTRACT

The distribution of acetyl groups in cellulose acetates (DS 2.5) has been determined. After mild methylation with methyl triflate the acetyl groups were exchanged for higher alkyl groups (ethyl, propyl, pentyl) in a subsequent alkaline alkylation step. The mixed alkyl ethers obtained in this way were degraded by reductive cleavage. The monomeric mixtures were acetylated and analyzed by GLC and GLC-MS.

INTRODUCTION

Cellulose esters are widely used as foils, as thermoplastic materials, artificial silk or as constituents for laquers. The chemical and physical properties of these derivatives depend on the nature of the substituents, the degree of substitution (DS) and the substitution pattern. The latter is influenced by the quality of the raw material and the derivatization conditions. The determination of distribution patterns is usually achieved by methylation analysis, reductive cleavage or NMR. The localization of acetyl groups in oligo- and polysaccharides is a difficult task due to the lability of these groups to both alkaline

methylation conditions and acidic hydrolysis. Husemann et al.[2] have investigated acetylated amylose by classical chemical methods using tritylation and periodate oxidation. Later on phenylisocyanate[3] and methyl vinyl ether[4] were used to protect the free hydroxyl groups in a modified "methylation analysis". The latter method has been applied to cellulose acetates by Björndal et al.[5]. Using NMR the DS of the hydroxyl groups in positions 2, 3 and 6 of the anhydroglucose units (AGU) could be determined,[6,7] but not the molar ratios of un-, mono-, di- and trisubstituted.

Due to the very different properties (solubility, viscosity) of acetylated polysaccharides, especially with respect to the DS, no generally applicable method of analysis is available. We have already investigated esterified starch acetates of medium DS by Prehm-methylation, hydrolysis, reduction and reacetylation[8] and symmetrically substituted mixed alkyl/acyl cyclodextrin derivatives by reductive cleavage.[9] An indirect method for the determination of the substitution pattern of cellulose acetates with a DS of 2.5 is reported here.

RESULTS AND DISCUSSION

Methylation. Five commercial cellulose acetates (CA1 - CA5) all with a DS of 2.5 were used in this study. In our earlier investigations on starch esters the mild methylation procedure according to Prehm[10] using methyl triflate in the presence of 2.6-di-*tert.*-butyl-pyridine as a sterically hindered proton scavenger in trimethyl phosphate was found to be superior to other protecting methods. For comparison the less toxic trimethyloxonium tetrafluoroborate, as the strongest methylating agent,[11] has also been used but was only preferable for samples soluble in dichloromethane, for example highly substituted benzyl celluloses. Completion of the methylation was ensured by repeated methylation.

Direct reductive cleavage. For the final identification of the number and position of substituents per AGU by GLC-MS the polymer must be degraded without loss of the required information. Secondly, from an economic point of view and with respect to the accuracy of the results, a minimum number of reaction steps should be used. We have investigated several starch, cellulose and cyclodextrin derivatives by

the reductive cleavage method introduced by Gray et al.[12] By this degradation method anhydroglucitol derivatives are obtained in one step. Acetyl groups are expected to be stable under reductive cleavage conditions,[13] using a Lewis acid and triethylsilane as hydride donor. Therefore, for direct degradation of methylated acetyl derivatives this method was employed. However, it was found, that acetyl groups, especially in position 6, may easily be reduced to the corresponding ethyl groups when more than 5 equiv. of reagents are used.[8,9,14] Also hydrolysis of acetyl residues has been observed to a small extent, presumably favoured by traces of humidity. In addition the glycosidic bond is deactivated by an electron withdrawing acetyl group in position 2. Therefore, direct reductive cleavage was only applicable in the case of 3-*O*- or 6-*O*-acylated cyclodextrin derivatives bearing alkyl substituents in position 2 and 6 or 2 and 3, respectively.[9]

Direct hydrolysis (methylation analysis). Direct hydrolysis of the methylated polysaccharide acetate has given satisfactory results in the case of starch acetates and benzoates with medium DS.[8] However, side reactions increase with an increasing number of hydroxyl groups liberated under these conditions. Therefore, direct hydrolysis was not applicable to highly acetylated celluloses.

Exchange of the acetyl groups. Alternatively the labile acetyl groups were exchanged for alkyl residues higher than methyl in a subsequent alkaline etherification step. Ethyl, propyl and pentyl halides were used in order to find the most appropriate reagent with respect to the reaction yield, the GLC properties of the resulting derivatives after degradation and the interpretation of their mass spectra. The method of Ciucanu and Kerek[15] using powdered sodium hydroxide and the alkyl halide in dimethyl sulfoxide was applied. Reaction time of at least 5 days and repeated addition of reagents were necessary to ensure high yields.

Yields for acetyl exchange reactions were all comparable, showing no preference for any alkyl chain length. The products were also controlled by ^{1}H NMR. Figure 1 shows the ^{1}H NMR spectrum of a cellulose acetate after permethylation (**a**) and after subsequent acetyl-pentyl exchange (**b**). The acetyl-CH_3 signals at 1.94 ppm (3-*O*-Ac), 2.00 ppm (2-*O*-Ac) and at 2.13 ppm (6-*O*-Ac) have disappeared.

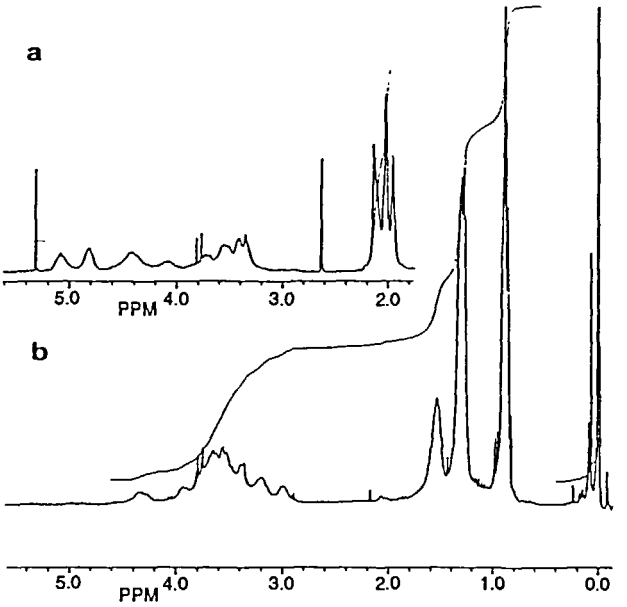

Fig.1. ^{1}H-NMR-spectra of cellulose acetate (DS 2.5) after permethylation (a) and after subsequent acetyl-pentyl exchange (b).

Reductive cleavage. The mixed cellulose alkyl ethers were subjected to reductive cleavage. This method is more convenient and faster than conventional methylation analysis, since cleavage of the glycosidic bond and reduction of the anomeric carbon atom is achieved in one step at room temperature. A reaction scheme is given in Fig. 2.

Choice of the catalyst. In order to avoid isomerization with respect to ring size of the resulting anhydroglucitols (1.4-anhydro beside 1.5-anhydro) a mixture of trimethylsilyl methanesulfonate (TMS mesylate) and boron trifluoride etherate [BF_3-$O(C_2H_5)_2$] was used as the Lewis acid. While isomerization products are always observed with trimethylsilyl trifluoromethanesulfonate (TMS triflate) as Lewis acid,[16] isomerization could be avoided by using this reagent in combination with BF_3-$O(C_2H_5)_2$ (5:1) as it is known for TMS mesylate and BF_3-$O(C_2H_5)_2$[17] and the combination diborane dimethylsulfide com-

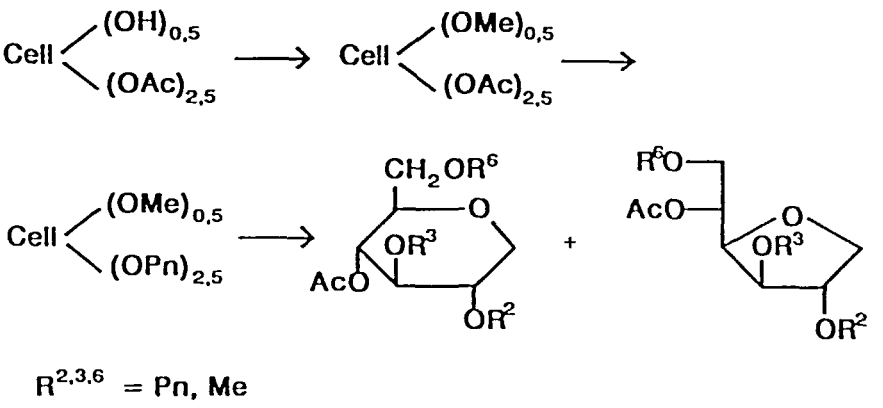

Fig.2. Reaction scheme for the analysis of the substitution pattern of cellulose acetates by reductive cleavage

plex/BF_3-O(C_2H_5)$_2$.[18] Isomerization could also be avoided when TMS trifluoroacetate/BF_3-O(C_2H_5)$_2$ was used as a catalyst, however further investigations of this system will be necessary. Therefore, it may be concluded that the combination of a Lewis acid with BF_3-O(C_2H_5)$_2$ would warrant ring size stability. However, dealkylation as another undesired side reaction was observed when the mixture of TMS triflate/BF_3-O(C_2H_5)$_2$ (>5 equiv.) was applied. Thus, the mixture of TMS mesylate/BF_3-O(C_2H_5)$_2$ was preferred. As expected, isomerization was not observed in the case of the methyl-ethyl celluloses, but could not be fully suppressed in the case of the methyl-pentyl derivatives under the same conditions.

Separation of the anhydroglucitol derivatives. All 4-*O*-acetyl-1,5-anhydro-D-glucitol derivatives investigated until now (*O*-CH_3/*O*-R, R= ethyl, hydroxyethyl, hydroxypropyl, acetyl, benzyl, propyl, pentyl) elute in the following order from a non-polar GLC column (e.g. CPSil 8 CB): 3 - 6 - 2 - 2,3 - 3,6 - 2,6 - 2,3,6 (with respect to the position of the substituent R). The 2- and 6-mono-*O*-substituted isomers as well as the 2,3- and 3,6-di-*O*-substituted derivatives are often not at all or only incompletely separated. An acceptable separation of the corresponding ethyl derivatives is accompanied by very long retention times.[19] Baseline separation was not even obtained when two-dimensional GLC[20] was used, employing two capillary columns with a non-

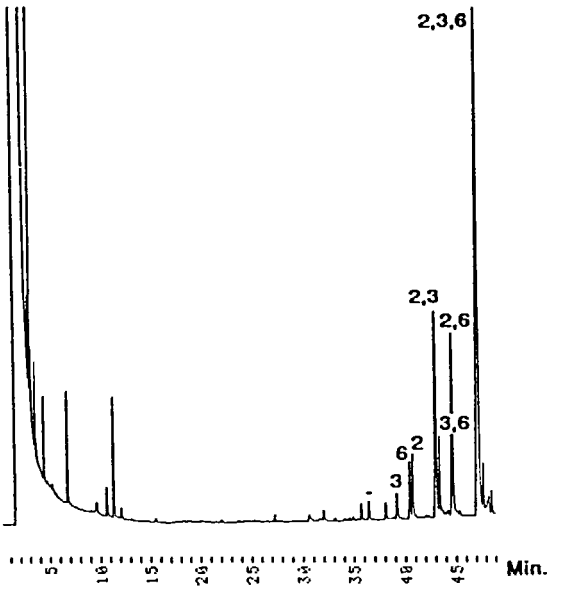

Fig.3. Separation of 4-*O*-acetyl-1,5-anhydro-*O*-ethyl-*O*-methyl-D-glucitol derivatives obtained from cellulose acetate by methylation, acetyl-ethyl exchange, reductive cleavage, and acetylation (reaction conditions see Experimental) on a 25m capillary column with hexakis-(2,3,6-tri-*O*-pentyl)α-cyclodextrin. (H_2 at 0.6 bar, temp.program: 90°C, 2°C/min. to 180°C). The numbers of the peaks indicate the positions of ethoxyl groups.

polar and a polar stationary phase. However, on cyclodextrin derivatives, e.g. hexakis-(2,3,6-tri-*O*-pentyl)α-cyclodextrin[21] as introduced by König et al. as chiral stationary phases for enantiomer separation,[22] these isomeric compounds could be separated (Figure 3). Separation of all 4-*O*-acetyl-1.5-anhydro-D-glucitol derivatives bearing methyl and pentyl substituents was readily achieved (Figure 4).

Identification of the degradation products by GLC-MS. Although small proportions of the corresponding 5-*O*-acetyl-1,4-anhydro-D-glucitols were formed in the case of the methyl-pentyl derivatives, the molar ratios of the 8 possible differently substituted AGUs could be determined by integration of the GLC peaks, while the structures of the compounds could be derived from their EI- and CI-mass spectra.

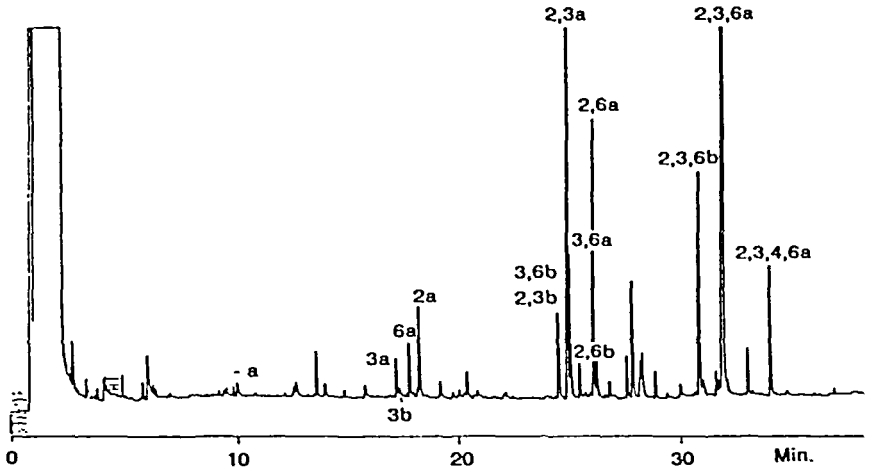

Fig.4. Gas chromatogram of the 4-*O*-acetyl-1,5-anhydro- (a) and 5-*O*-acetyl-1,4-anhydro- (b) -*O*-methyl-*O*-pentyl-D-glucitol derivatives obtained from cellulose acetate by methylation, acetyl-pentyl exchange, reductive cleavage, and acetylation (reaction conditions see Experimental). (Column: 25m CPSil 8 CB, carrier gas: H_2 at 0.8 bar, on-column injection, temp. program: 70°C (1 min.), 20°C/min. to 130°C, then 4°C/min. to 290°C. The numbers of the peaks indicate the position of pentyl groups.

Their identity was proved by comparison with reference compounds from our earlier investigations of pentylated cyclodextrins.[23] The methyl-ethyl-derivatives could also be assigned by comparing their mass spectra. In some cases synthetic reference compounds were available. Additional prove was obtained by comparison with the results of Gray et al.[19] Identification of the propyl derivatives was more difficult due to the fact that both acetyl and propyl groups have the same nominal mass and therefore products of incomplete acetyl-alkyl exchange can not easily be differentiated.

Quantification of the degradation products was based on the peak areas of the GC-runs. On-column injection was used to avoid discrimination of the less volatile components. Peak areas were corrected by molar response factors (see Experimental) calculated on the basis of the

Table 1. Distribution of substituents of cellulose acetates CA1 - CA5. (Acetyl groups were exchanged for pentyl residues.)

Cellulose acetate	*1*	*2*	*3*	*4*	*5*
Position substituted			*Mol %*		
-	1.5	0.3	n.n.	2.0	3.2
2	3.6	3.5	3.6	4.7	3.4
3	2.0	1.8	2.0	5.7	4.0
6	2.4	2.1	4.9	4.3	4.4
2,3	17.1	18.6	21.0	13.4	14.4
3,6	4.2	5.1		4.1	3.7
2,6	8.3	9.8	14.1	11.7	11.6
2,3,6	58.1	57.1	53.1	52.4	52.4
2,3,4,6	2.8	1.7	1.3	1.7	2.9
unsubstituted	1.5	0.3	n.n.	2.0	3.2
monosubstituted	8.0	7.4	10.5	14.7	11.8
disubstituted	29.6	33.5	35.1	29.2	29.7
trisubstituted	60.9	58.8	54.4	54.1	55.3
DS	**2.50**	**2.51**	**2.44**	**2.35**	**2.37**
position (%)					
2	36.0	36.2		35.6	35.7
3	33.7	33.6		32.8	32.6
6	30.3	30.2		31.5	31.6

effective-carbon-response (e.c.r.) concept.[24] In Table 1 the quantitative results for the distribution pattern of the five cellulose acetates are listed.

Calculated overall DS values of between 2.35 (CA4) and 2.51 (CA2) were found. There are no significant differences for the 5 samples, except for apparent similarities of CA1 and CA2 and CA4 and CA5, respectively. 54-61% of the AGUs are trisubstituted while only a small portion of unsubstituted AGUs (0-3%) could be detected. About 36% of the acetyl groups were found in position 2, 32-33% in position 3 and 30-32% in position 6. The 2,3-di-*O*-substituted derivative was the

main product of the 3 possible isomers of this group, although the primary hydroxyl in position 6 is known to be the most reactive in acylation reactions, followed by the hydroxyl group in position 2. However, cellulose acetates with a DS of 2.5 are usually prepared from cellulose triacetate by partial saponification. Husemann and Kafka[2] deduced from their experiments with amylose acetates that there is no preferred position for either acidic or alkaline saponification. Björndal et al.[5] also found the 2,3-di-*O*-acetyl-glucose to be the main disubstituted product in a cellulose acetate of DS 2.4. The results obtained here can be explained by different rates of acetyl cleavage decreasing in the following order: 6 > 3 > 2. For further and more detailed characterization the distribution of the substituents along the cellulose chain should be investigated.

EXPERIMENTAL

Materials. Cellulose acetates were commercial products. Reagents and solvents were purchased from Merck, Fluka and Aldrich. Trimethyl phosphate was distilled before use.

Methylation of cellulose acetates. Cellulose acetate (10 mg, 0.037 mmol) was dissolved in trimethylphosphate (1 mL) under heating to 100°C. Methyl trifluoromethanesulfonate (42 µL, 20 equiv. per *O*H) and 2,6-di-*tert.*-butyl-pyridine (62 µL, 15 equiv. per *O*H) were added and the mixture was stirred at room temperature for 3-5 days. The methylated cellulose acetate was isolated either by extraction with chloroform/dichloromethane or by dialysis. The crude product was purified by washing with diethyl ether and dried in high vacuum. Yield (theor. 10.3 mg) : CA1 10.6/9.1/12.1 mg, CA2 6.4/11.9/10.5 mg, CA3 12.0/17.3 mg, CA4 10.9/11.5 mg, CA5 13.3/14.0 mg. For control of completion of methylation a part of methylated CA1 was subjected to a second methylation step: methylated CA1 (17 mg) was dissolved in dichloromethane (1 mL), excess of trimethyloxonium tetrafluoroborate and 2,6-di-*tert.*-butyl-pyridine were added and the mixture was stirred for 3 days at room temperature. The product was isolated as described above (yield: 17.5 mg). After acetyl exchange and reductive cleavage no increase in methyl content was found.

Exchange of acetyl against alkyl residues. The methylated cellulose acetate was dissolved in dimethyl sulfoxide (1 mL). Powdered sodium hydroxide (3 equiv. per acetyl group) and alkyl halide (iodoethane, 1-iodopropane and 1-bromo-*n*-pentane, respectively, 3 equiv. per acetyl group) were added and the suspension stirred at room temperature. After one day additional 1.5 equiv. of the reagents were added. The addition was repeated after 2 days. After 5-7 days the products were extracted with chloroform, washed several times with water and dried at high vacuum.

Reductive cleavage. The mixed methyl-alkyl cellulose ethers (2-3 mg) were dissolved in dichloromethane (200 - 300 µL), triethylsilane (5 equiv.), trimethylsilyl trifluoromethanesulfonate (5 equiv.) and boron-trifluoride etherate (1 equiv.) were added, and the mixture was stirred at room temparature for about 20 h. The reaction was quenched with methanol, deionized with Bio-Rad AG 501-X8 D resin, and the products were acetylated with acetic anhydride and N-methylimidazole.[25]

Nuclear magnetic resonance spectroscopy (NMR). ^{1}H-NMR spectra were recorded on a 250 MHz Bruker AC 250 instrument, operating in the pulsed Fourier transform mode. Samples were dissolved in $CDCl_3$. Chemical shifts are related to internal tetramethylsilane.

Gas-liquid chromatography (GLC). Quantification of the anhydroglucitol derivatives was carried out on a Carlo Erba GC 6000 Vega instrument equipped with an on-column injector, a flame ionization detector, a 25m capillary column with CPSil 8 CB (0.25 i.d.) connected with a 2m retention gap, and a Hewlett Packard 3390A integrator. Peak areas were multiplied with response factors (F), calculated on the basis of the e.c.r. concept:[24] 4-*O*-acetyl-1.5-anhydro- and 5-*O*-acetyl-1,4-anhydro-D-glucitols: tri-*O*-methyl: e.c.r. = 545, F ≡ 1.000; di-*O*-methyl-mono-*O*-pentyl: e.c.r. = 945, F = 0.577; mono-*O*-methyl-di-*O*-pentyl: e.c.r. = 1345, F = 0.405; tri-*O*-pentyl: e.c.r. = 1745, F = 0.312; 1,5-anhydro-2,3,4,6-tetra-*O*-pentyl-D-glucitol: e.c.r. = 2100, F = 0.260.

Two-dimensional GLC was performed with a Sichromat 2 (Siemens) equipped with two 25m capillary columns with CPSil 5 CB (first column) and CPSil 19 CB (second column), hydrogen as carrier gas and two flame ionization detectors.

Gas-liquid chromatography-mass spectrometry (GLC-MS). GLC-MS was performed with a VG/70-250S instrument. For CI-MS, ammonia was used as reactant gas. The EI ionization potential was 70eV.

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