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Determination of the substitution patterns of cellulose methyl ethers by HPLC and GLC – comparison of methods

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Summary

The substitution patterns of methyl cellulose as well as of a thexyldimethylsilyl cellulose after permethylation were determined by hydrolysis and separation of the resulting partially methylated glucoses without further derivatization by h.p.l.c.. On an amine-modified silica column the solutes get separated into glucose, 2,3,6-tri-O-methyl glucose and the groups of mono-O-methyl- and di-O-methyl glucoses. A chromatographic run on a reversed-phase column enables the identification of the single mono-O-methyl- and di-O-methyl glucoses. In this way, a determination of both the average degree of substitution and the substitution pattern of cellulose derivatives is possible. Comparison of the results with those obtained by standard methylation analysis including g.l.c.-m.s. proves the correctnes of the method employed.

Introduction

The relation of physical properties to the distribution of substituents in the anhydroglucose unit (AGU) as well as along the polymer chain of cellulose and starch derivatives has recently become a field of intensive studies. The most reliable methods for the determination of the substitution pattern of different cellulose derivatives are n.m.r. spectroscopy (without /1-5/ or after hydrolysis to the monomer units /6-9/) and methylation analysis.

Standard methylation analysis includes permethylation of the sample, hydrolysis, reduction of the partially methylated aldohexoses to the corresponding alditols, and peracetylation. The partially methylated alditol acetates obtained in this way are separated and quantified by g.l.c. and identified by g.l.c.-m.s. /10-14/.

A second type of methylation analysis, the reductive-cleavage method, introduced by Gray and coworkers /15/, yields in one step partially methylated anhydroglucitols which are separated, identified and quantified after acetylation in the same way /16-21/.

In the course of our studies on regioselectively substituted celluloses like trialkylsilyl ethers /22/ we were interested in the development of a rapid and convenient method to obtain information on the average degree of substitution (D.S.) and the substitution pattern of these derivatives by means of high performance liquid chromatography (h.p.l.c.). This method should provide preliminary information on the structure of the products to optimize synthesis. After that more detailed investigations by methylation analysis should be performed.

In contrast to g.l.c. samples for h.p.l.c. analysis do not have to be volatile, but have to be soluble in the eluent. Therefore, methylcelluloses can be directly analyzed after hydrolysis without further time-consuming

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derivatization. Cellulose derivatives with acid-labile substituents as trialkylsilyl ethers (or sulfates, acetates) can be investigated after the introduction of the inverse substitution pattern by permethylation /20/.

In the case of chemically substituted polyglucans like cellulose or starch derivatives and without consideration of anomeric configurations 8 products have to be separated. These products include glucose (glc), 2-O-methyl- (2-Me_1), 3-O-methyl- (3-Me_1), 6-O-methyl- (6-Me_1), 2,3-di-O-methyl- (2,3-Me_2), 2,6-di-O-methyl- (2,6-Me_2), 3,6-di-O-methyl- (3,6-Me_2), and 2,3,6-tri-O-methyl glucose (2,3,6-Me_3). A survey of liquid chromatographic systems proposed for the separation of partially methylated glucoses is given in Tab.1..

Table 1: Survey of liquid chromatographic systems for separations of partially methylated glucoses

glucose derivatives separated	h.p.l.c.-system, detection
Σ(2-Me_1, 3-Me_1, 6-Me_1), 2,3-Me_2, 2,3,4-Me_3 (α,β), 2,3,6-Me_3 (α,β), 2,3,4,6-Me_4 (α,β)	Waters μ-Bondapak C18, 1% ammonium acetate/ethanol (9/1, v/v), refractive index, /23/
2,3-Me_2 (α,β), 2,4-Me_2 (α,β), 2,3,4-Me_3 (α,β), 2,3,6-Me_3 (α,β), 2,4,6-Me_3 (α,β), 2,3,4,6-Me_4 (α,β), and corresponding glucitols	Waters Radial-Pak C18 or Waters Dextropak, water or water/methanol (4/1 - 49/1, v/v), refractive index in combination with polarimetric detector, /24/
2-Me_1, 3-Me_1, 3,6-Me_2, 2,3,4-Me_3, 2,3,6-Me_3, 2,3,4,6-Me_4, as borate complexes	Bio-Rad Aminex A-14, borate-buffer pH 8,8, post-column-derivatization: orcinol - H_2SO_4, VIS-detection, /25/
2,3,6-Me_3, 2,4,6-Me_3	Whatman Partisil (silica gel), acetonitrile/water (18/1, v/v), refractive index /26/

Σ(2-Me_1, 3-Me_1, 6-Me_1): sum of the three components

(α,β): partial or complete separation of the anomers reported

Because of the partial or complete separation of the α- and β- anomers of several sugar derivatives on reversed-phase columns /23, 24/ a reduction of the partially methylated glucoses to the corresponding glucitols was carried out in some cases /24/. A further derivatization to perbenzoylated and pernitrobenzoylated /27-30/ or peracetylated sugar alcohols /31/ was proposed to increase the sensitivity of the method by replacing refractive index- (r.i.) by u.v.-detection. Other methods suggest post-column derivatization to achieve the same effect /25/.

However, no method to determine the D.S. value and the substitution pattern of cellulose derivatives by means of h.p.l.c. was published yet.

Experimental

INSTRUMENTATION

The following h.p.l.c. equipment was used: a pump with analytical head, an injection valve with 20 μl sample loop, a variable wavelength u.v.-photometer with deuterium lamp and a differential refractometer for analytical use, all manufactured by KNAUER. The data control and analysis system was a h.p.l.c. software/hardware package (KNAUER) with interface, 12 bit analog/digital converter and personal computer. Columns used were VERTEX 250x4 mm with precolumn 5x4 mm filled with LiChrospher 100 NH_2 5 μm (column A) or LiChrospher 100 RP18 5 μm (column B). The chromatographic

experiments were carried out at ambient temperature. The eluent acetonitrile was of u.v.-grade. All eluents were degassed by an ultrasonic bath.

G.l.c. analysis was carried out on a CARLO ERBA GC 6000 Vega instrument, equipped with an on-column-injector, a flame ionization detector, a 40 m capillary column with DB5 (0.25 i.d.), connected with a 2 m retention gap, and a MERCK HITACHI D-2000 integrator. Peak areas were multiplied with response factors, calculated on the basis of the e.c.r.-concept /32/.

G.l.c.-m.s. was performed with a VG/70-250S instrument. For c.i.-m.s. ammonia was used as reactant gas.

MATERIALS

Methyl cellulose and 3-O-methyl glucose were purchased from ALDRICH. 2-O-methyl glucose and 2,6-di-O-methyl glucose were synthesised by hydrolysis of the corresponding 2-O-methyl and 2,6-di-O-methyl β-D-cyclodextrins (ALDRICH). 2,3,6-tri-O-methyl glucose was obtained by hydrolysis of permethylated cellulose /33/. 6-O-methyl, 2,3-di-O-methyl and 3,6-di-O-methyl glucoses were synthesized according to /34/ and the literature cited therein.

6-O-thexyldimethylsilyl (thexyl = 2,3-dimethylbut-2-yl, ThxDMS) cellulose was prepared from AVICEL^{R} (FLUKA) in N,N-dimethylacetamide/LiCl using ThxDMS-Cl/pyridine at 25 °C according to STEIN, ERLER and KLEMM /35/. The D.S. value was determined by means of Si-elemental analysis (D.S. = 0.93) and ^{1}H- n.m.r. spectroscopy (D.S. = 0.92).

PERMETHYLATION

For permethylation 1 g (3.3 mmol) ThxDMS cellulose was dissolved in 100 ml dry tetrahydrofuran. Then 0.8 g (33 mmol) of powdered NaH was dispersed under vigorous stirring and 4.7 g (33 mmol) iodomethane was added dropwise to the mixture. Stirring was continued for 5 days at 25 °C. The precipitated NaI was filtered off and the clear solution was evaporated to a volume of 20 ml. The solution was poured into 500 ml of aqueous phosphat buffer (0.026 m NaH_2PO_4/ 0.041 m Na_2HPO_4). The product was washed with water and dried at 50 °C under vacuum (0.01 Torr).

HYDROLYSIS

Hydrolysis of methyl cellulose and permethylated ThxDMS cellulose was carried out with 2 N trifluoroacetic acid (UVASOL^{R}, MERCK) within 4 h at 120 °C. After evaporation of the acid in vacuo at max. 50 °C water was distilled from the samples ten times to remove traces of the acid. The samples were dried for 2 days in vacuo (0,01 Torr) over KOH.

Results and Discussion

METHYL CELLULOSE

For testing some liquid chromatographic systems with respect to their ability to separate all products of the hydrolysis of partially methylated celluloses we first investigated a commercial methyl cellulose sample yielding all substitution patterns of the AGU possible. Fig. 1. represents a typical elution pattern of this sample on an amine-modified silica column (column A) using acetonitrile/water (75/25, v/v) as the eluent. As confirmed by comparison with the standard compounds, separation of the groups of un-, mono-, di-, and tri-O-methylated glucoses was achieved. Response factors for the r.i. detector were found to be independent on the degree of methylation and therefore equal within the error limits of the chromatographic system. This result enables both the determination of the D.S. value and a rough estimation of the distribution of substituents of the sample.

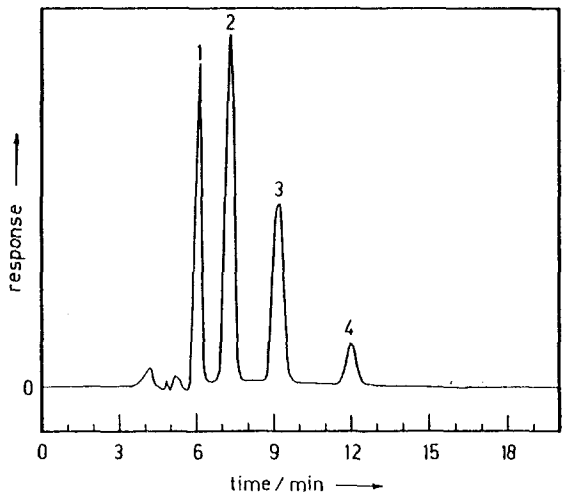

Fig.1.: H.p.l.c. analysis of a methyl cellulose sample after hydrolysis on an amine-modified silica gel column

1 = 2,3,6-Me_3; 2 = Σ(2,3-Me_2, 2,6- Me_2, 3,6-Me_2); 3 = Σ(2-Me_1, 3-Me_1, 6-Me_1); 4 = glucose; column A, acetonitrile/water = 75/25 (v/v), 0,5 ml/min, r.i. detection

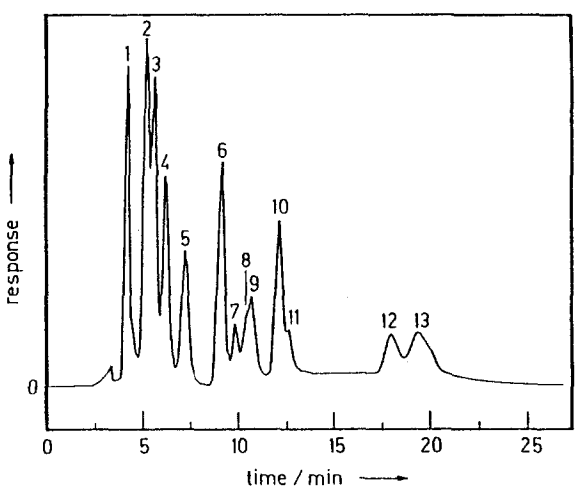

Fig.2.: H.p.l.c.- analysis of a methyl cellulose sample after hydrolysis on a reversed-phase column

1 = glucose + impurities; 2 = 3-Me_1 ($\alpha+\beta$) + 2-Me_1 (α or β); 3 = 2-Me_1 (αor β); 4, 5 = 6-Me_1 (α/β); 6, 10 = 2,6-Me_2 (α/β); 7, 8 = 3,6-Me_2 (α/β); 9 = β-2,3-Me_2; 11 = α-2,3-Me_2; 12 = β-2,3,6- Me_3; 13 = α-2,3,6-Me_3

column B, eluent: water, flow gradient: 0...9:00 min 0,5 ml/min, 9:00... 9:10 min 0,5 -> 1,0 ml/min, 9:10...12:00 min 1,0 ml/min, 12:00...12:10 min 1,0 -> 2,0 ml/min, 12:10...25:00 min 2,0 ml/min, 25:00...25:10 min 2,0 -> 0,5 ml/min, 25:10...27:00 min 0,5 ml/min; r.i. detection

We also attempted to separate all individual methyl glucoses on a reversed-phase column using water as the weakest eluent. To get a good resolution of the first eluted substances an increase of the flow rate in two steps (see legend of Fig. 2.) was programmed. The results are demonstrated in Fig. 2.. The use of the r.i.- detector was favoured again because of the good correlation between detector response and concentration of the samples. Our attempts with u.v.-detection at 190 nm resulted in a qualitative detection of all solutes but the detector response differs strongly for different sugars at the same concentration /36/.

One disadvantage of this system is the short retention time of glucose which is eluted together with some impurities. Comparison with standard compounds showed that all methylated sugars get separated into their anomers except for 3-Me_1. The assignment of the peaks to the corresponding α- and β- anomers was carried out by means of simultaneous r.i.- and polarimetric detection for 2,3,6-Me_3 and 2,3-Me_2 by HEYRAUD et al. /24/ under similar chromatographic conditions. Their results were used for the peak assignment of Fig. 2.. However, the insufficient separation of all components together with the separation of the anomers for most of the substances as well as the short retention time of glucose make it impossible to determine all hydrolysis products quantitatively within this chromatographic system. On the other hand, main products (e.g., 2,6-Me_2 among the disubstituted AGU in the commercial methyl cellulose sample) can be identified. In addition the presence or absence of individual hydrolysis products can be detected.

To verify the results obtained by h.p.l.c. we determined the substitution pattern of the same methyl cellulose by standard methylation analysis. As demonstrated in Tab. 2. the results of both methods are in good agreement.

Table 2: Comparison of the the substitution patterns of a methyl cellulose sample obtained by hydrolyis and h.p.l.c. (method a) or standard methylation analysis (method b)

	method a		method b	
	1	2	3	4
	Mol%			
unsubst.	5.51	5.67	5.58	5.74
2-Me_1	++	++	14.33	14.31
3-Me_1	+	+	2.01	1.87
6-Me_1	++	++	10.42	10.02
Σ Me_1	25.40	25.50	26.76	26.20
2,3-Me_2	+	+	9.98	9.58
2,6-Me_2	++	++	26.47	26.68
3,6-Me_2	+	+	5.36	5.15
Σ Me_2	41.79	41.16	41.81	41.41
2,3,6-Me_3	27.32	27.68	25.85	26.64
D.S. (2)			0.766	0.772
D.S. (3)			0.432	0.432
D.S. (6)			0.681	0.685
D.S.	1.91	1.90	1.88	1.89

1,2: two different hydrolyses, 3,4: two different standard methylation analyses, ++: main products of the group, +: detected

THEXYLDIMETHYLSILYL CELLULOSE

As determined by ^{13}C-n.m.r. spectroscopy thexyldimethylsilylation of cellulose proceeds regioselectively in the C-6 position of the AGU /35/. Our interest was both to confirm this result by chromatography and to apply the h.p.l.c. method proposed.

To determine the substitution pattern by chromatography permethylation of the silylated cellulose is necessary due to the lability of the silyl groups toward acidic hydrolysis. By this procedure silylated hydroxyl groups of the AGU result in free hydroxyl groups while originally free hydroxyl groups are converted to methoxy groups (inverse substitution pattern). The results of h.p.l.c. (after permethylation and hydrolysis) and g.l.c. (standard methylation analysis) are represented in Fig. 3, 4 and Tab. 3.. The D.S. value and the predominant substitution in position 6 are in good agreement with the n.m.r.-results, however, small amounts of substitution in position 2 (D.S. 0.12) and traces in position 3 (D.S. 0.01-0.02) are not recognized by this less sensitive method. By the h.p.l.c. method all components were detected which could be identified by standard methylation analysis. Some minor amounts as 2,6-Me_2, 2-Me_1, and free glucose, which seem to indicate 3-O-ThxDMS-substitution, may at least partially be caused by undermethylation of the 3-hydroxy group, which is the less reactive in etherification and additionally sterically hindered by the neighboured ThxDMS-group.

Conclusions

The determination of the substitution pattern of a methyl cellulose is achieved by h.p.l.c. analysis of the products of hydrolysis without further derivatization in two chromatographic steps. As shown for thexyldimethylsilyl cellulose the determination of the substitution pattern of starch and cellulose derivatives bearing acid labile substituents, e.g.

Table 3: Indirect determination of the substitution pattern of a thexyldimethylsilyl cellulose by hydrolysis and h.p.l.c. (method a) or standard methylation analysis (method b) of the permethylated sample

	method a		method b	
	1	2	3	4
	Mol%			
unsubst.	13.56	12.02	12.30	10.94
2-$ThxDMS_1$	+	+	2.40	2.57
3-$ThxDMS_1$	(+)	(+)	-	0.69
6-$ThxDMS_1$	++	++	72.69	74.59
6-terminal	-	-	0.94	0.61
Σ $ThxDMS_1$	75.13	76.90	76.03	78.45
2,3-$ThxDMS_2$	-	-	-	-
2,6-$ThxDMS_2$	++	++	10.31	9.11
3,6-$ThxDMS_2$	+	+	1.18	1.51
Σ $ThxDMS_2$	11.16	11.02	11.49	10.62
2,3,6-$ThxDMS_3$	-	-	0.26	(+)
D.S. (2)			0.130	0.117
D.S. (3)			0.011	0.022
D.S. (6)			0.854	0.858
D.S.	0.975	0.989	0.995	0.997

1,2: two different hydrolyses, 3,4: two different methylation analyses, ++ main product of the group, + detected, (+) traces detected, - not detected

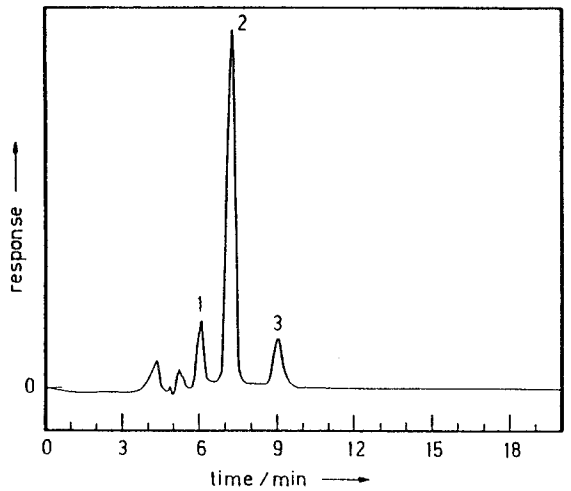

Fig.3.: H.p.l.c.- analysis of a permethylated thexyldimethylsilyl cellulose sample after hydrolysis on an amine-modified silica gel column

1 = 2,3,6-Me_3; 2 = Σ (Me_2); 3 = Σ (Me_1); glucose not detected
column A, acetonitrile/water = 75/25 (v/v), 0,5 ml/min, r.i. detection

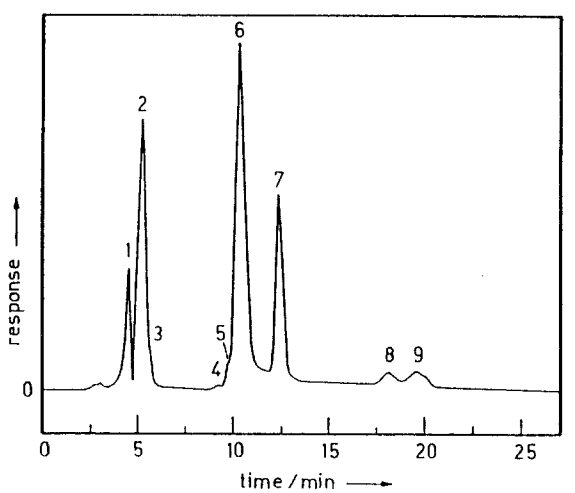

Fig.4.: H.p.l.c.- analysis of a permethylated thexyldimethylsilyl cellulose sample after hydrolysis on a reversed-phase column

1 = impurities; 2 = 3-Me_1 ($\alpha+\beta$) + 2-Me_1 (α or β); 3 = 2-Me_1 (α or β); 4 = 2,6-Me_2 traces (α or β), 5 = 3,6-Me_2 (α or β); 6 = 3,6-Me_2 (α or β) + β-2,3-Me_2; 7 = 2,6-Me_2 traces (α or β) + α-2,3-Me_2; 8 = β-2,3,6-Me_3; 9 = α-2,3,6-Me_3, glucose and 6-Me_1 not detected,
column B, water, flow gradient same as in Fig. 2, r.i. detection

acetates or sulfates should be possible. In contrast to ^{13}C-n.m.r. analysis differentiation between un-, mono- , di-, and trisubstituted anhydro-glucoses is possible, while the substituted hydroxyl positions cannot be calculated due to incomplete separation of all mono- and di-O-methylated α- and β-anomers, respectively. Quantitative results of the h.p.l.c. method are in good agreement with those obtained by the more time-consuming methylation analysis, which, however, yields the most differentiated results combined with highest sensitivity.

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