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Carbohydrate Research 338 (2003) 733–741
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Determination of the DS and substituent distribution of cationic alkyl polyglycosides and cationic starch ethers by GLC after dealkylation with morpholine

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Received 4 November 2002; accepted 28 December 2002

Abstract

The total DS and substituent distribution of starch and alkyl polyglycosides functionalised as *O*-(2-hydroxy-3-trimethylammonium)propyl ethers were determined by GLC. To achieve volatile analytes, the samples were submitted to methanolysis, N-demethylation and O-trimethylsilylation. Alternatively hydrolysis, reduction with $NaBH_4$ and subsequent O-acetylation were performed, but suffered from intramolecular acetal formation of 2-*O*-substituted residues, preventing reduction. Morpholine as nucleophile was superior to thiophenolate with regard to quantitative dealkylation and side product formation. The ratio of un-, mono-, di-, tri-, and tetrasubstituted compounds was determined. The total DS values calculated from these mole fractions were in good agreement with those obtained from elemental analysis or NMR from standards. Regioselectivity of the cationisation reaction was determined after methanolysis, permethylation and Hofmann elimination by GLC.

Keywords: Cationic alkyl polyglycosides; Cationic starch; N-Dealkylation; Degree of substitution; Substituent distribution

1. Introduction

Alkyl polyglycosides (APG®), surfactants based on starch and fatty alcohols, have been produced in a 70,000 t/a scale since 1995. Based on the renewable resources starch and natural fats and oils, they are used in a widespread field of applications in personal care products as well as in cleaning and washing agents. In addition to their excellent washing property and base stability, they are ecotoxicologically harmless and skin and mucous membrane compliant.[1,2] Although called polyglycosides, the average DP is usually between 1 and 2. Due to transglycosylation under the Fischer-type reaction conditions, all types of interglucosidic linkages are present in the product mixture. In addition, the length of the fatty alcohol chain may vary. By functionalisation of OH groups, the application range can be expanded. So far, neutral and anionic substituents have been introduced into the sugar moieties.[1,2] Mixed ethers, esters with citric or tartaric acid, carbonates and sulfates are produced. Another possibility is the introduction of cationic groups like quaternary ammonium functions. Cationic surfactants are used as hair conditioners, as antistatics and as fabric softeners for textile products in industry and household. Cationic starches with a low degree of substitution (DS) are already well established as additives for paper manufacturing and those with higher DS are discussed as flocculants for waste water. They are produced in large scale by alkali catalysed addition of 2,3-epoxypropyl-trimethylammonium chloride (QUAB 151). Under these conditions, a pronounced regioselectivity for *O*-2-substitution is observed due to the higher acidity caused by the vicinal acetal function.[3] So far, the DS is determined by elemental analysis of nitrogen of the substituent or of chloride as the counter ion to the quaternary ammonium function. Therefore, determination of purity of the samples, which often contain side products of the cationisation reagent, is required. The reaction of APG® with 2,3-epoxypropyl-trimethylammonium chlo-

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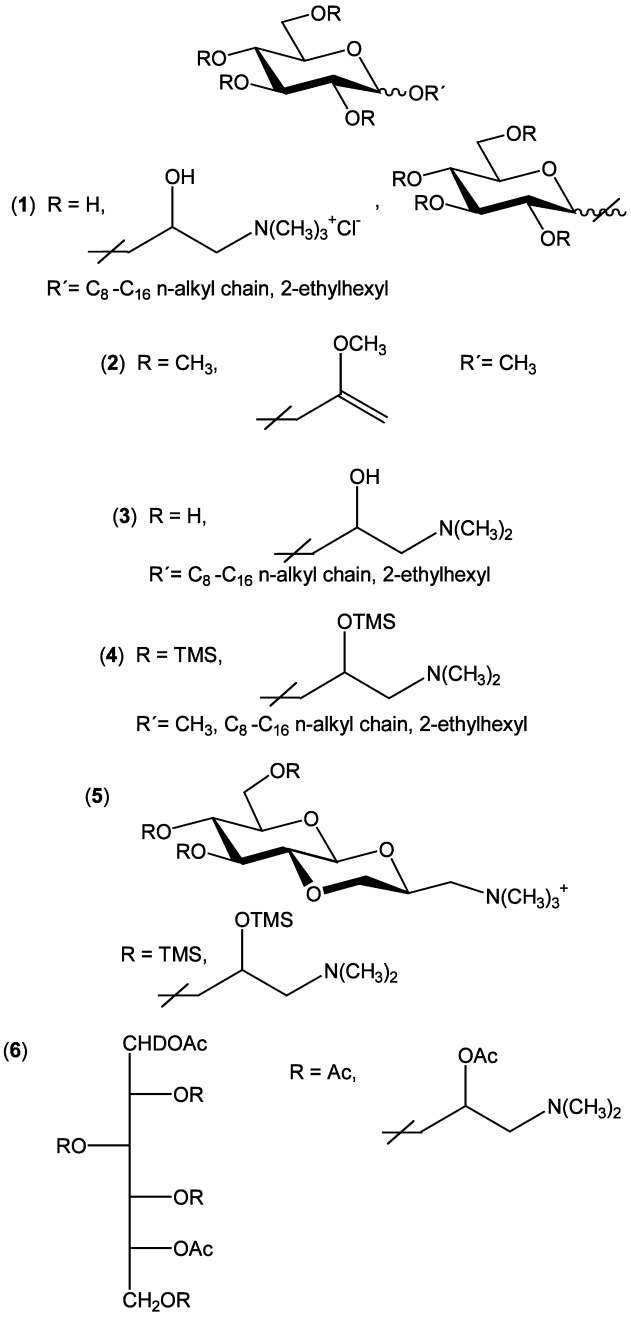
0008-6215/03/$ - see front matter
doi:10.1016/S0008-6215(03)00014-4

ride yields a complex mixture of different products and N-containing side products, especially the 2,3-(dihydroxy)-propyl-trimethyl ammonium chloride (QUAB-diol). As a consequence, elemental analysis is not appropriate for DS determination. The method developed in our laboratory to determine the substituent distribution in the glucosyl unit[3,4] after transformation to methyl *O*-(2-methoxy-2-propenyl)-*O*-methyl-α,β-D-glucosides discriminates the unsubstituted fraction and consequently requires an independent determination of the total DS. This encouraged us to develop an alternative method for the determination of the total DS. By this method, the molar fractions of APG with no, one, two, three or more substituents are obtained.



2. Results and discussion

2.1. Samples

The cationic APG® (CAPG, **1**) were prepared in laboratory scale. The ratio of glucosyl units (AGU) to NaOH and 2,3-epoxypropyl-trimethylammonium chloride (QUAB 151) or the corresponding chlorohydrin (QUAB 188) were varied. Beside technical products, uniform standards with DP1 and a defined alkyl chain were included in method development. For comparison, two cationic starches (CS) with a DS of 0.90 and 0.88 were also investigated. During the reaction with the QUAB 151 or QUAB 188, which are in equilibrium, N-containing by-products are formed by hydrolysis, elimination and oligomerisation reactions.[5] Samples and data are listed in Table 1.

2.2. ^{1}H NMR spectroscopy for the determination of the DS

The DS of alkyl monoglycosides can be estimated from the ^{1}H NMR spectra in CD_3OD. For DS calculation, the integral of the methine proton ($\delta = 4.34$ ppm, *H-b*) of the substituent is referred to the total of the *H-1* resonances of the glucosyl units (δ [ppm]: α-H-1 = 4.76, α-H-1$_{2\text{-}O\text{-subst.}}$ = 4.83, β-H-1 = 4.25, β-H-1$_{2\text{-}O\text{-subst.}}$ = 4.32), or to a single methylene group of the alkyl chain (δ [ppm] = 1.63 for 2′-CH_2 and 1.30 for C$H_2 \geq$ C-3′).

$$DS_{H\text{-}1} = \frac{\int H_b}{\int \alpha\text{-H-1} + \int \beta\text{-H-1} + \int \beta\text{-H-1}_{2\text{-}O\text{-subst}} + \int \alpha\text{-H-1}_{2\text{-}O\text{-subst}}}$$

$$\text{with} \quad \frac{\int \alpha\text{-H-1}}{\int \beta\text{-H-1}} = \frac{\int \alpha\text{-H-1}_{2\text{-}O\text{-subst}}}{\int \beta\text{-H-1}_{2\text{-}O\text{-subst}}}$$

$$DS_{H\text{-}1} = \frac{\int H_b}{\int \alpha\text{-H-1} + \int \beta\text{-H-1} + \int \beta\text{-H-1}_{2\text{-}O\text{-subst}} + \left(\int \alpha\text{-H-1} / \int \beta\text{-H-1}\right) \int \beta\text{-H-1}_{2\text{-}O\text{-subst}}} \quad (1)$$

$$DS_{alkyl} = \frac{2 \int H_b}{\int 2'\text{-}CH_2} \quad (2)$$

By *O*-2-substitution, the H-1 signal is shifted downfield. Interference of the 2-*O*-substituted α isomer with the water signal is corrected with respect to the α/β-ratio of the non-shifted H-1-signal of the APG, which of course is only an approximation since the reactivity is influenced by the anomeric configuration. Table 2 contains the DS values gained according to Eqs (1) and (2) for CAPG 1 and 2. At higher DP, ^{1}H NMR spectra of raw products, which contain side products of the reagent, are too complex for DS estimation.

2.3. Substituent distribution in the glucosyl residues by the enol ether method

For a detailed determination of the monomer composition, poly- or oligosaccharides are usually cleaved to monomers and transformed to appropriate derivatives for chromatographic separation.[6] We have developed a method for cationic starches[3,4] which was applied to the cationic APG after slight modification. Briefly: The sample is submitted to a reaction sequence including methanolysis, extraction of the fatty alcohol with chloroform, permethylation, and Hofmann elimination. The resulting methyl *O*-(2-methoxy-2-propenyl)-*O*-methyl-α,β-D-glucosides (**2**) are then analysed by GLC. By this procedure, only the relative molar ratios of the substituted monomer units are determined. Fig. 1 shows the gas chromatogram obtained from CAPG 7 with a DS of 0.52. While 2-, 3-, 4-, and 6-mono-*O*-substituted methyl glucosides could all be assigned, the 2×6 regioisomers of di-*O*-substituted methyl α- and β-D-glucosides partially overlapped and could not be completely identified. However, elimination of α and β anomers by reduction of the sugars and therefore simplification of the product mixture is not possible in this case. Reduction to alditols requires aqueous hydrolysis instead of methanolysis. However, under aqueous conditions, significant amounts of intramolecular acetals are formed from the 2-*O*-(2-hydroxy-3-trimethylammonium)propyl glucosyl moieties. In contrast, methanol as a nucleophile can compete with the hydroxy group of the substituent for the intermediate carboxonium ion. Therefore, methanolysis is performed in this case. Fig. 2 illustrates the quantitative results obtained for CAPG 3–7, and CS 1 and 2 for comparison. While for starch the reaction at *O*-2 is strongly preferred, the APG did not show any pronounced regioselective substitution. The order of reactivity for the APG with DP1 was found to be $4 > 6 > 2 > 3$. CAPG 6 containing up to pentasaccharides shows a slight preference for *O*-2-substitution (32%) followed by O-3 (26%) and O-4 (23%) substitutions, while only 19% of the substituents were located at the primary position. This reflects the preferred formation of $1 \rightarrow 6$-linked glycosides during APG synthesis and therefore blocking of the O-6 under thermodynamic control. Base concentration had much less influence on the regioselectivity in APG compared to starch (see Table 1). It must be considered that α- and β-glucosidic linkages are present in **1**, and that the configuration also influences relative reactivities as known from starch and cellulose.[6]

2.4. N-Dealkylation with thiophenolate

As a side reaction of the Hofmann elimination N-dealkylation can occur, especially if iodide from the permethylation step with methyl iodide is present as a

Table 1
Structural features and reaction conditions for the synthesis of CS and CAPG

Sample	Structural features of **1**		Molar ratio AGU:Quab:NaOH	MS_{EA} [b]
	DP [a]	Alkyl chain		
CAPG 1	1	C_{12}	1:2.5:0.05	i.n.a. [d]
CAPG 2	1	C_{12}	1:5:0.05	i.n.a.
CAPG 3	1	C_{12}	1:1:0.05	i.n.a.
CAPG 4	1	C_{12}	1:1:1.1	i.n.a.
CAPG 5	1	C_{12}	1:1:0.28	i.n.a.
CAPG 6	1–5	$C_{12/14}$	1:1.44:i.n.a. [d]	i.n.a.
CAPG 7 [c]	1–3	2-ethylhexyl	1:5:i.n.a.	i.n.a.
CS 1				0.90 (N)
CS 2				0.88 (N) 0.82 (Cl)

[a] Degree of polymerisation.
[b] Molar substitution calculated from elemental analysis of N or Cl.
[c] Purified by membrane filtration.
[d] Information not available.

Table 2
DS distribution and total DS values obtained by the morpholine method for CAPG samples

Mol fraction (%)	CAPG 1		CAPG 2		CAPG 3		CAPG 4		CAPG 4 [d]		CAPG 5	
c_0 [a]	91.00	89.71	85.50	84.92	84.72	84.61	73.29	77.94	72.80	76.45	88.67	89.78
c_1	8.37	9.32	11.72	13.37	13.95	13.51	22.39	14.55	24.80	19.01	10.43	9.70
c_2	0.58	0.93	2.78	1.70	1.28	1.82	4.07	6.77	2.40	4.54	0.90	0.52
c_3	0.04	0.03	0.00	0.00	0.03	0.06	0.21	0.66	0.00	0.00	0.00	0.00
c_4	0.00	0.00	0.00	0.00	0.02	0.00	0.04	0.08	0.00	0.00	0.00	0.00
DS	0.10	0.11	0.17	0.17	0.17	0.17	0.31	0.30	0.30	0.28	0.12	0.11
Average DS	0.11		0.17		0.17		0.31		0.29		0.12	
DS_{NMR} [b]	0.09–0.12	0.37										
DS_{NMR} [c]	0.12–0.15	0.16										
Reaction efficiency	4%		3%		17%		31%		29%		48%	

	CAPG 6 [d]		CAPG 7	CAPG 7 [e]	CS 2	
c_0	90.60	89.80	67.41	63.48	31.46	27.91
c_1	8.70	10.20	16.52	25.34	50.19	59.79
c_2	0.70	0.00	12.59	7.89	16.29	12.04
c_3	0.00	0.00	3.34	2.81	2.06	0.26
c_4	0.00	0.00	0.13	0.48	0.00	0.00
DS	0.10	0.10	0.52	0.52	0.89	0.85
Average DS	0.10	0.52	0.52	0.87		
DS_{EA}					0.88 (N) 0.82 (Cl)	
Reaction efficiency	7%		10%	10%		

Results of twofold analysis are given.
[a] c_i = Mol fraction of glucosyl units substituted with i substituents.
[b] Calculated according to Eq. (1).
[c] Calculated according to Eq. (2).
[d] Sample was submitted to hydrolysis and reduction prior to N-demethylation.
[e] Sample was submitted to methanolysis prior to N-demethylation.

good nucleophile. Thermal N-dealkylation in the hot split injector of the gas chromatograph has been used in the analysis of simple quaternary ammonium compounds.[7] For carbohydrate derivatives, it must be performed chemically prior to GLC analysis. Various dealkylation procedures of quaternary ammonium compounds are reported in the literature.[8–15] Shamma et al.[12] used sodium thiophenolate in 2-butanone for N-dealkylation, and Jenden et al.[13] applied a modified procedure of this method to choline and acetylcholine as pharmacologically active substances. When thiophenolate was applied to the cationic APG or methyl glucosides, formation of the (2-hydroxy-3-dimethylamino)propyl groups was not complete within 3 days (ESI-MS control), and unexpected high amounts of by-products were formed. For example, intramolecular nucleophilic displacement of trimethylamine by the deprotonated 2-hydroxy function occurred. Competing substitution of the trimethylamino group by thiophenolate, which can occur directly or via the mentioned oxirane intermediate, was also observed (Scheme 1). Diphenyl disulfide was another by-product. No sufficient purification could be achieved by extraction of an acidified aqueous phase with hexane. The main problem is the detergent character of the APG. For this reason, another reagent was chosen.

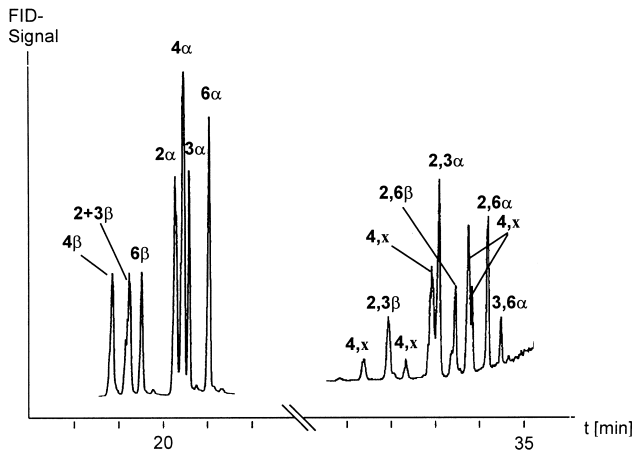


Fig. 1. Gas chromatogram of the methyl *O*-(2-methoxy-2-propenyl)-*O*-methyl-α,β-D-glucosides (**2**) obtained from CAPG 7 (DS = 0.52). Groups of mono- and disubstituted constituents are shown. Peaks are assigned according to the substituted position and the anomeric configuration.

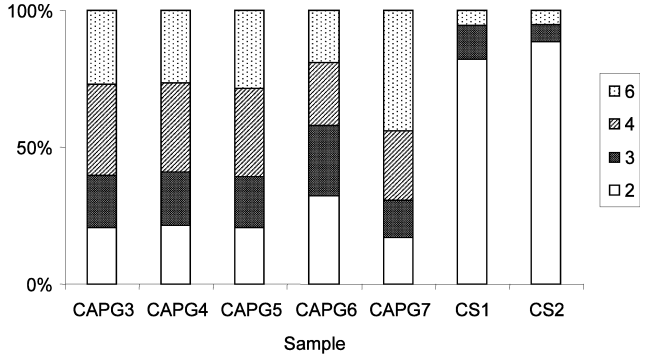
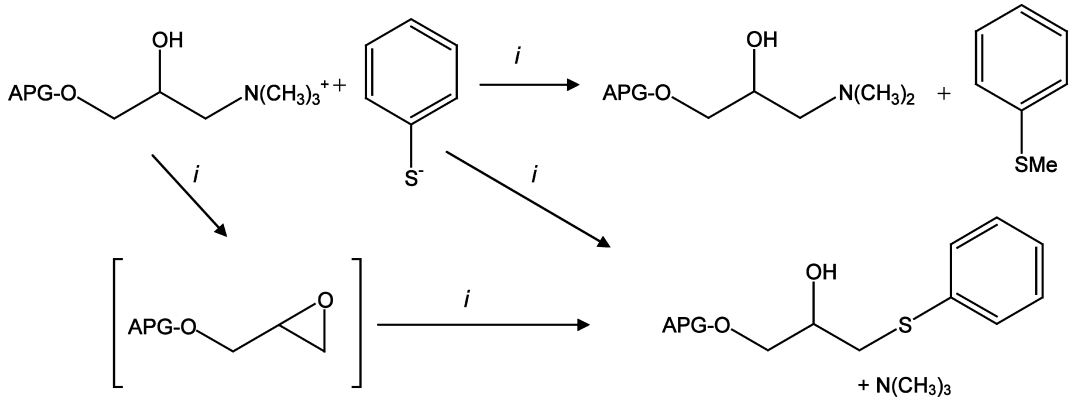


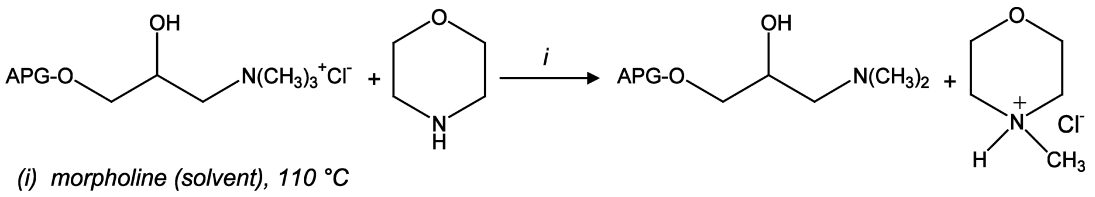
Fig. 2. Distribution of cationic substituents on the positions 2, 3, 4 and 6 in the monosubstituted fraction of CAPG and CS (see Table 1).

2.5. N-Dealkylation with morpholine

Barber et al.[15] used morpholine for N-demethylation in good yields and without formation of by-products. Therefore, this nucleophile was applied to cationic APG (Scheme 2). A solution of the sample was treated with pure morpholine at 110 °C. The high excess of the reagent is necessary to shift the equilibrium in the desired direction. In acetone as the solvent, reaction rate was not sufficient. The progress of the dealkylation was followed by ESI-MS (Fig. 3). Side-products, as mentioned for the thiophenolate reaction, were no longer detected. After a reaction time of 24 h demethylation was complete. Separation from morpholine was performed by size exclusion chromatography on Sephadex LH-20 with methanol as eluent. After O-trimethylsilylation, the samples were analysed by GLC. Fig. 4 shows the gas chromatogram of the products obtained from CAPG 4, an APG with DP1 and uniform C_{12} alkyl chain. In this case the methanolysis step was omitted. Groups of dodecyl α,β-D-glycosides with increasing number of substituents (0–3) can be recognised. The groups of isomers are much more complex than for the enol ether derivatives **2** described above, since the chirality of C-2 of the substituent is retained now, while it is lost by Hofmann elimination. Each of the four monosubstituted regioisomers occurs in four diastereomeric configurations, and the six disubstituted regioisomers produce eight stereoisomers, each. However, the fractions of isomers can be summed and from their molar ratios, the DS can be calculated after correction of the peak areas with respect to the e.c.r. concept.[16] In Table 2, the DS distribution (mol fractions c_0, c_1, c_2, c_3, and c_4) and the total DS values obtained by this procedure for several CAPG in a DS range of 0.1–0.67 and one cationic starch (DS 0.88), are listed. Oligomeric products from cationic APG with higher DP overlap with the di- and trisubstituted monomers. Therefore, samples with DP > 1 are methanolysed prior to N-demethylation as well as are the cationic starches. Methanolysis was chosen since it



Scheme 1.



Scheme 2.

prevents intramolecular acetal formation which occurs during acid hydrolysis with the 2-hydroxy group of *O*-2-substituted glucosyl units. The methyl glucosides are strongly preferred in the thermodynamically controlled acetal formation,[17] while significant amounts of **5** are formed in competition with the free sugar during aqueous hydrolysis. The methyl glucosides are then treated as described above. Fig. 5 shows the gas chromatogram of the methyl *O*-(2-trimethylsilyloxy-3-dimethylamino)propyl-*O*-trimethylsilyl-α,β-D-glucosides (**4**) obtained from CS 2. The high *O*-2-selectivity of oxirane addition to starch, as mentioned above, is obvious from the peak pattern of the monosubstituted glucosides. Even tetrasubstituted glucosyl units could be detected for this starch with a DS of 0.88 (Table 2), indicating that tandem-reaction had occurred and that the molar substitution (MS) is higher than the DS.

2.6. Formation of glucitol acetates

In spite of the already mentioned problems with intramolecular acetal formation during aqueous acid hydrolysis, glucitol derivatives were prepared for a qualitative investigation. The resulting chromatogram (not shown) is much simpler as the α and β anomers are eliminated and a single compound, the glucitol, is produced. The diastereoisomers caused by the racemic nature of the substituent are no longer separated, a phenomenon which is often observed for the flexible open chain alditol derivatives compared to the more rigid ring structures.[18] Now, the position of substitution could be easily assigned from the EI mass spectra. The problem that prevents us from using this procedure in general is the intramolecular acetal formation of 2-*O*-substituted constituents to form **5** as already mentioned. For quantitative evaluation, the acetals **5** must also be considered. The order of elution of the monosubstituted glucitols **6** is $3 > 4 > 2 > 6$. At higher DS values compounds, **5** from 2,*x*-di-*O*-substituted glucosyl residues interfere with the monosubstituted **6**. Investigations to avoid acetal formation are in progress.

A comparison with the results from elemental analysis of the cationic starch ether CS 2 shows very good correlation. The results for CAPG 1 and 2 are compared to the values gained from NMR and were also in good agreement (Table 2).

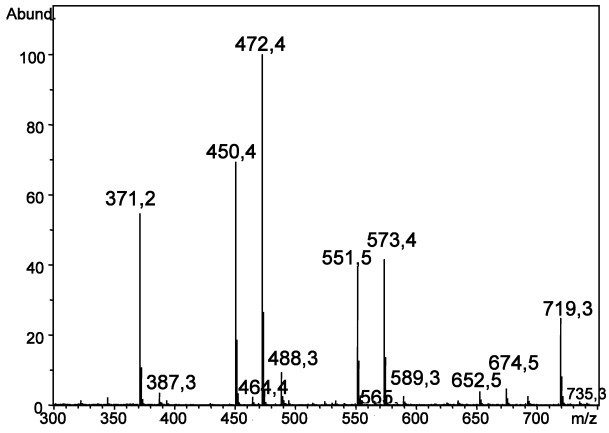
3. Experimental

3.1. General

Cationic APG were obtained from Cognis Deutschland GmbH, Düsseldorf, Germany. The cationic starch CS 1 was obtained from Dr Waltraud Vorwerg, FhG IAP, Golm, Germany, CS 2 was obtained from Professor Dr Thomas Heinze and Dr Vera Haack, Universität Jena, Germany. All reagents were of highest purity available and purchased from Fluka, Aldrich or E. Merck.

3.2. GLC

GLC separations were carried out on a Carlo–Erba GC 6000 Vega Series 2 instrument equipped with an



m/z	*Compound*	*n*[a]	*Cation*
371	**1**	0	Na^+
387	**1**	0	K^+
450	**3**	1	H^+
464	**1**	1	
472	**3**	1	Na^+
488	**3**	1	K^+
551	**3**	2	H^+
565	**1**[b]	2	
573	**3**	2	Na^+
589	**3**	2	K^+
652	**3**	3	H^+
674	**3**	3	Na^+
719	2 x **1**	0	Na^+

[a] number of substituents
[b] only one group dealkylated

Fig. 3. ESI-MS of CAPG 5 in morpholine after 16 h at 130 °C (**1** → **3**).

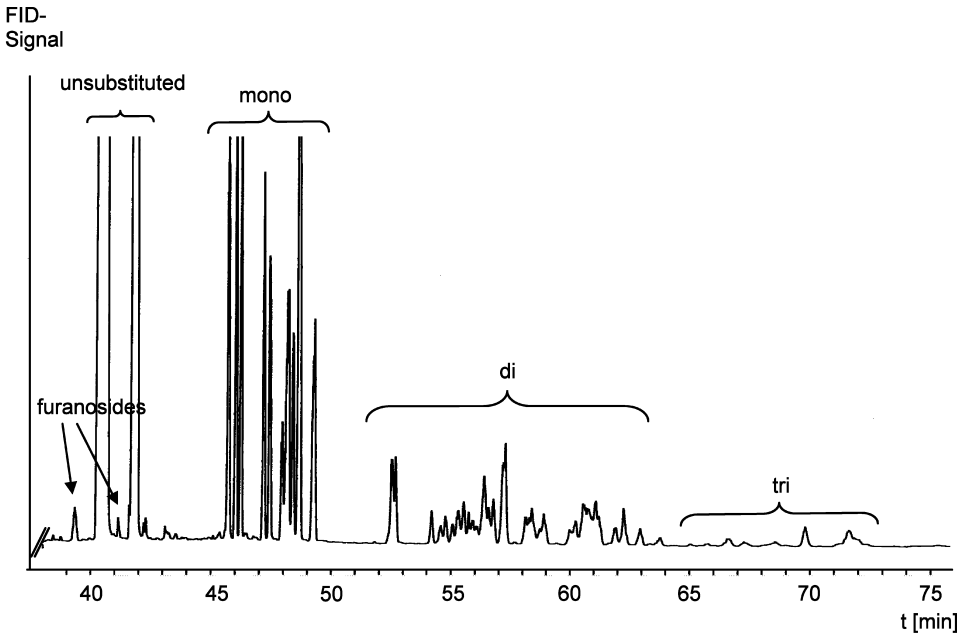


Fig. 4. Gas chromatogram of dodecyl glucosides **4** obtained from CAPG 4 (DP1, C_{12}) after N-demethylation with morpholine and subsequent O-trimethylsilylation. Fractions of un-, mono-, di-, and trisubstituted glucosides are shown.

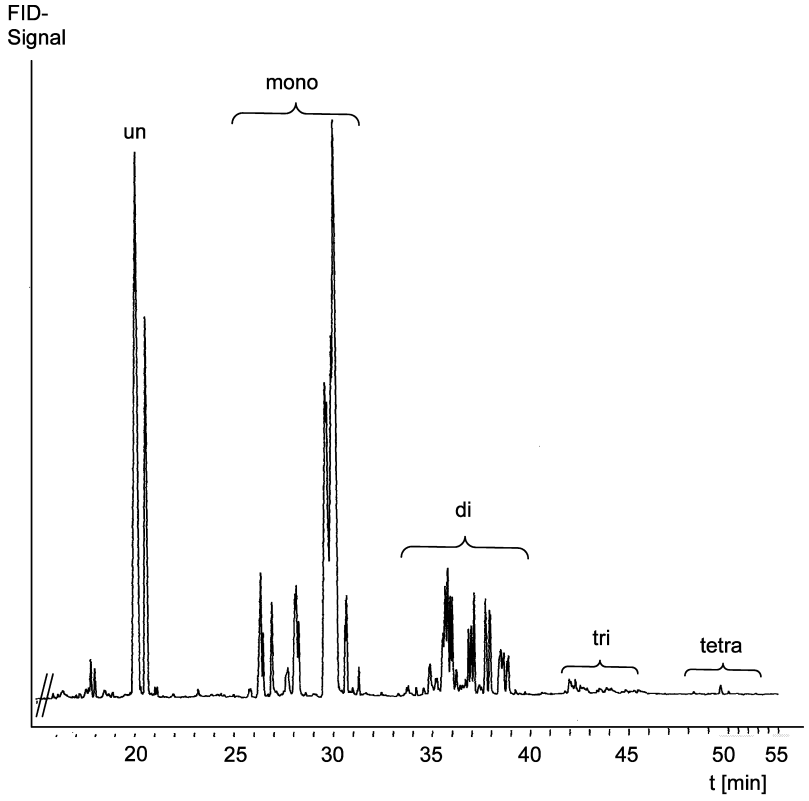


Fig. 5. Gas chromatogram of methyl *O*-(2-trimethylsilyloxy-3-dimethylamino)propyl-*O*-trimethylsilyl-α,β-D-glucosides (**4**) obtained from CS 2. Fractions of un-, mono-, di-, tri-, and tetrasubstituted glucosides are assigned.

on-column injector, a flame ionisation detector (FID), a 25 m capillary column CPSil 8 CB (Chrompack) connected with a retention gap (2 m), and a Merck Hitachi D-2500 integrator. Hydrogen was used as carrier gas (80 kPa), Temperature program 1 (for methyl *O*-(2-methoxy-2-propenyl)-*O*-methyl-α,β-D-glucosides (**2**)): 60 °C (1 min isotherm), with 20 °C/min to 160 °C (12 min isotherm), with 10 °C/min to

190 °C (10 min isotherm), with 10 °C/min to 290 °C (hold).

Temperature program 2 (for methyl *O*-(2-trimethylsilyloxy-3-dimethylamino)propyl-*O*-trimethylsilyl-α,β-D-glucosides (**4**), and glucitol acetates **6**): 60 °C (1 min isotherm), with 20 °C/min to 130 °C, with 4 °C/min to 290 °C (hold).

3.3. GLC MS

EI mass spectra (70 eV) were recorded on a Finnigan MAT SSQ 710. GC separations were carried out on a gas chromatograph Varian 3400 with a 30 m capillary column ZB-5 (Phenomenex) with splitless injection using the same temperature program as above (GLC).

3.4. NMR spectroscopy

^{1}H NMR spectra were recorded with a Bruker AMX 300 instrument (300 MHz, CD_3OD). Chemical shifts were related to HDO at 4.8 ppm.

3.5. ESI mass spectrometry

ESI mass spectra were recorded with a Bruker Esquire LC 0081, Bruker Daltonik GmbH, Bremen, Germany. Samples were dissolved in MeOH (*c* 0.05–0.1 mg/mL) and filtrated (PTFE-filter CHROMATOFIL® O-45/25, *Macherey-Nagel*, Düren). All measurements were performed in the positive ion mode using the following conditions: flow rate (syringe pump) 240 μL/h dry temperature 325 °C, dry gas 4 L/min, nebulizer 10.0 psi, capillary −3500 V, end plate offset −500 V, cap exit 120 V, cap exit offset 90 V, skim I 30 V, skim II 10 V, scan range 100–1000 *m*/*z*, accumulation of 8–50 single spectra.

3.6. Determination of the substituent distribution by formation of 2

Formation of the methyl *O*-(2-methoxy-2-propenyl)-α,β-D-glucosides (**2**) was performed as described earlier.[3,4]

3.7. Methanolysis

To the cationic APG or starch (approx 30 mg) in a 3 mL-V-vial 1.5 M MeOH–HCl (3 mL) was added. After stirring for 3 h at 100 °C, the excess of acid was neutralised with NaOH and the solvent was evaporated in a stream of nitrogen. Residual water was removed by co-distillation with $C_6H_5CH_3$. The dry residue was submitted to dealkylation.

3.8. Hydrolysis

To the cationic APG or starch (approx 30 mg) in a 3 mL-V-vial, 2 M TFA (3 mL) was added. After stirring at 80 °C for at least 16 h, the solvent was evaporated in a stream of nitrogen. The residual acid was removed by co-distillation with $C_6H_5CH_3$.

3.9. N-Demethylation

CAPG (0.2 mmol glucosyl units), directly or after cleavage of glycosidic linkages, was suspended in 4 mL morpholine and stirred at 110 °C for about 24 h. A clear yellow, high viscous solution resulted. Separation of the products from the reagent was achieved by means of SEC on a Sephadex LH-20 column with MeOH as the eluent. Products were detected on silica TLC-plates with ethanolic H_2SO_4 (10%) and Dragendorff reagent after heating for 10 min at 115 °C (solution I: 0.085 g basic bismuth nitrate in a solution of 1 mL glacial AcOH in 4 mL water, solution II: 2 g KI in 5 mL water; reagent: 1 mL solution I, 1 mL solution II in 4 mL glacial AcOH and 20 mL water). The residual morpholine (about 10 mg/mL sample) did not interfere with further analysis.

3.10. Trimethylsilylation

The carbohydrate-containing fractions of LH-20 chromatography were combined. After evaporation of MeOH and co-distillation with $C_6H_5CH_3$, BSTFA (100 μL) and Py (10 μL) were added to the dry residue, the mixture was treated at 60 °C for 1 h, diluted with dry CH_2Cl_2 and analysed by GLC (temperature program 2).

3.11. Reduction

To the third part of the hydrolysed CS or CAPG sample, 0.5 M $NaBH_4$ (0.5 mL) in 2 M NH_3 was added. The mixture was stirred for 1 h at 60 °C. The excess of $NaBH_4$ was destroyed with glacial AcOH. Borate was removed as its methyl ester by repeated (× 5) evaporation with 85/15 MeOH–AcOH.

3.12. Acetylation

Acetylation of the reduced sample was carried out with Ac_2O (200 μL) and Py (50 μL) for 3 h at 90 °C. The excess of Ac_2O was hydrolysed by addition of satd $NaHCO_3$ solution. After CO_2 release had ceased, the aq phase was extracted with CH_2Cl_2 (× 3). The combined organic phases were washed with 0.1 M HCl (about 4 °C), satd $NaHCO_3$ solution and with water (× 2). Afterwards, the organic phase was dried with $CaCl_2$, filtrated and concentrated.

4. Conclusion

Molar fractions of un-, mono-, di-, tri-, and even higher substituted glucosyl units of cationic APG and therefore the molar degree of substitution (MS) can be determined by GLC after N-demethylation with morpholine. The procedure was also successfully applied to cationic starch. The advantage, compared to elemental analysis, is the specificity of this method allowing the analysis of impure samples. In contrast, the already established analysis via preparation of enol ether derivatives by methanolysis, permethylation, and Hofmann elimination of the cationic glucans requires an independent determination of the MS, but is still the method of choice to determine the relative ratios of the regioisomers. Although reduction to glucitols eliminates α and β anomers and therefore simplifies the mixture of analytes, it suffers from the formation of intramolecular acetal compounds during aqueous hydrolysis. Therefore, methanolysis is the method of choice.

Acknowledgements

Support of this work by the Cognis Deutschland GmbH is gratefully acknowledged.

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