PREPARATIVE ISOLATION OF OLIGOMERS WITH A TERMINAL ANHYDROSUGAR UNIT BY THERMAL DEGRADATION OF CHITIN AND CELLULOSE

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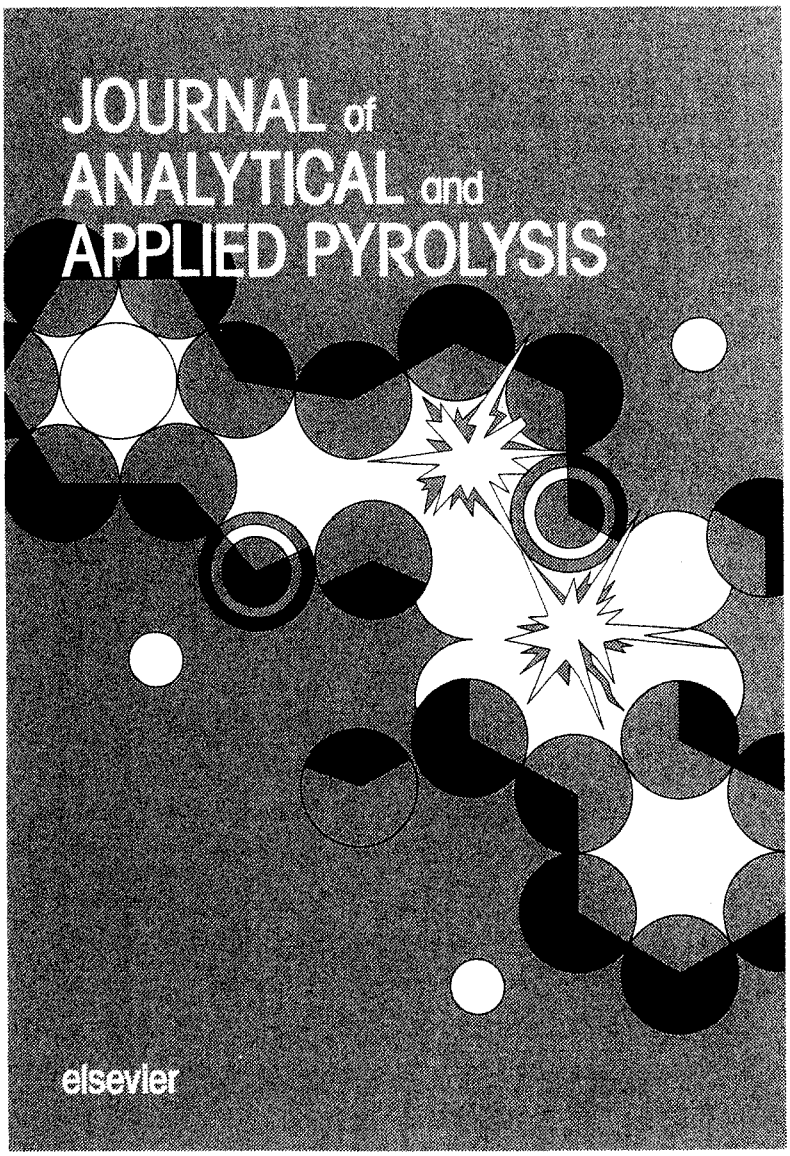

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ABSTRACT

Chitin and cellulose have been depolymerized by thermal degradation in a high boiling and inert solvent to give water-soluble oligomers with a terminal anhydrosugar unit. Pure oligomers **1** and **3** up to the pentamers have been obtained by preparative gel permeation chromatography, and characterized by ^{1}H-NMR, ^{13}C-NMR and fast atom bombardment-mass spectrometry.

Anhydrosugars; cellulose; chitin; fast atom bombardment-mass spectrometry; nuclear magnetic resonance; polysaccharides; pyrolysis.

INTRODUCTION

Thermal degradation of polysaccharides is a very important process and the primary degradation products are of great interest. Monomeric anhydrosugars have been isolated from pyrolytic syrups obtained from polysaccharides, e.g. levoglucosan from cellulose [1,2] and starch [3], 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose from chitin [2].

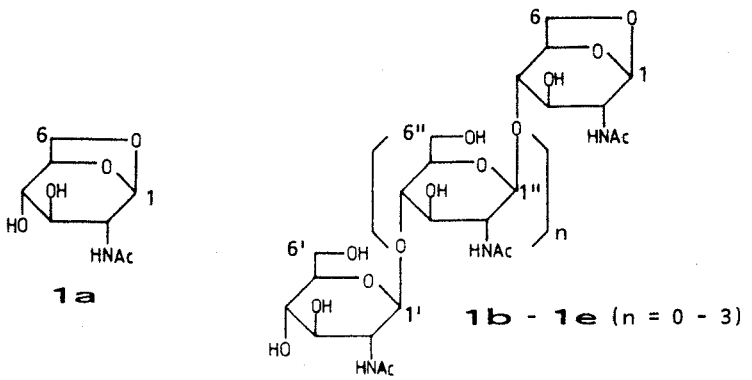
Recently, it has been reported that anhydro-oligosaccharides could also be primary degradation products. Pyrolysis field-desorption mass spectrometry [4], direct chemical ionization-mass spectrometry [5–8] and laser-desorption Fourier transform mass spectrometry [9] of polysaccharides such as cellulose [7,9], starch [4,6,8,9], dextran [6,7,9] and chitin [9,10] have provided ion mass evidence for the formation of oligomers in the pyrolysates. Cellobiosan has also been identified in the fast pyrolysis syrups of cellulose by high-performance liquid chromatography (HPLC) and gas chromatography [11]. However, unambiguous identification and characterization of the oligomers requires preparative isolation and separation. We report

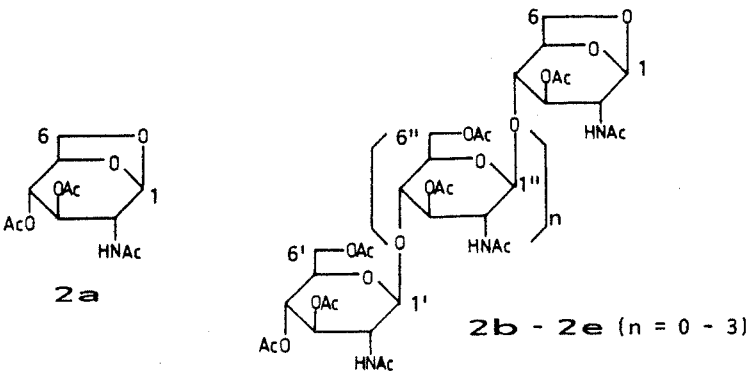
here some results on a very simple method for thermal degradation of polysaccharides on a preparative scale, and the isolation, separation and unambiguous characterization of oligomers in the degradation syrups of chitin and cellulose.

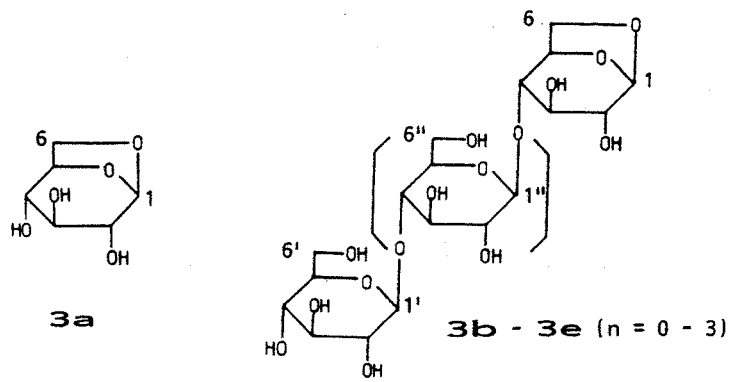
EXPERIMENTAL

Thermal degradation of chitin

1 g of chitin (Sigma, practical grade, from crab shells), pulverized and suspended in 5 ml of tetraethyleneglycol dimethyl ether, was introduced into a two-necked flask equipped with an air condenser and heated under argon







with magnetic stirring for 1 h at 210 °C. After cooling, the suspension was diluted with 50 ml of water and centrifuged at 3000 r.p.m. for 15 min. After the clear solution had been decanted, the residue was resuspended in another 50 ml of water and again centrifuged for 15 min. The combined decanted solutions were treated with activated charcoal. After filtration, the water was removed in vacuo at room temperature. A solid precipitated from the resulting solution. The precipitate was filtered off using a sintered glass funnel and, after repeated washings with diethylether, 180 mg (18%) of a brownish solid were obtained. 100 mg of the solid were dissolved in 5 ml of water and separated by preparative gel permeation chromatography (GPC) (Merck Fractogel TSK HW-40(S); 2 columns, 25 × 800 mm; 0.5 ml H_2O/min, 25 °C, RI detector, Knauer; HPLC pump, Knauer).

By five or six GPC separations it was possible to isolate, in total, 30–70 mg of the pure sugars **1a–1e**, up to the pentamer. Acetylation of the isolated sugars in acetic anhydride/pyridine at 25 °C gave the peracetylated products **2a–2d**. All the products were characterized by fast atom bombardment-mass spectrometry (FAB–MS) (Finnegan-MAT 212 with Data System SS 300 and FAB source, AMD Intektra GmbH, Xenon, 10 kV), ^{1}H-NMR (300 MHz, D_2O) and ^{13}C-NMR (75 MHz, D_2O; Bruker AM 300).

Thermal degradation of cellulose

25 g of cellulose (native, Merck) were suspended in 70 ml of tetraethyleneglycol dimethyl ether and heated under argon with magnetic stirring for 4 h at 250 °C. After cooling, the suspension was diluted with 200 ml of water. Work-up as described for chitin gave 1.1 g (4.5%) of a white, hygroscopic solid. 110 mg of the solid were dissolved in 5 ml of water, separated by chromatography, and characterized by the method described for chitin.

RESULTS

Thermal degradation of chitin suspended in tetraethyleneglycol dimethyl ether gave a maximum yield of non-volatile water-soluble products at 210 °C (Table 1), besides some volatile products, i.e. acetamide [2,12]. ^{1}H-NMR spectroscopy revealed that the water-soluble products were

TABLE 1

Yield of water-soluble and non-volatile degradation products of chitin by degradation in tetraethyleneglycol dimethyl ether for 1 h

T (°C)	155	175	200	210	215	230
Yield (%) *	1.8	6.1	10.5	18.0	14.8	3.9

* Based on weighted chitin.

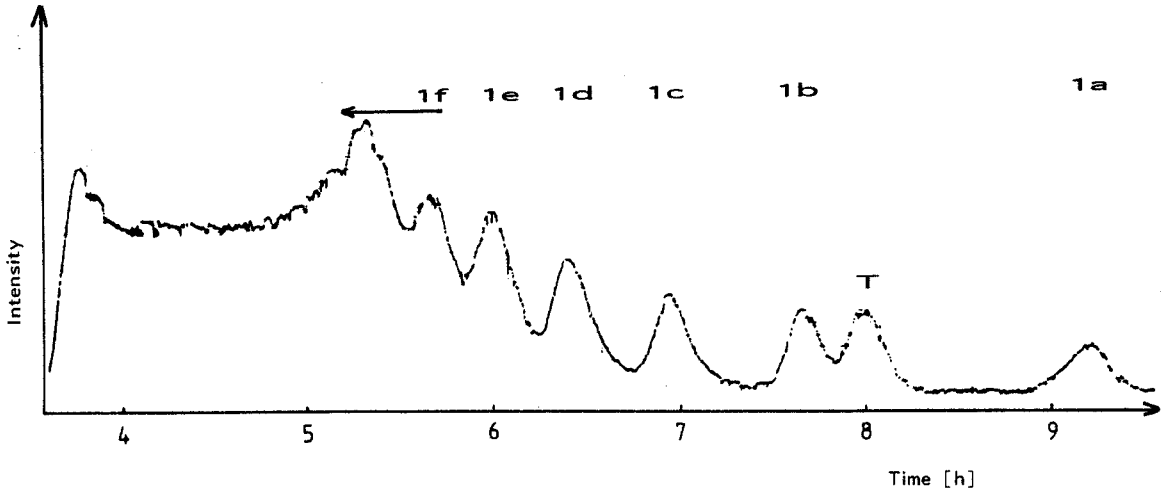


Fig. 1. GPC separation of the water-soluble *N*-acetyl-1,6-anhydro-glucosamine oligomers **1**. (Peak T = tetraethyleneglycol dimethyl ether.)

TABLE 2

Composition of water-soluble degradation products of chitin and cellulose determined by analytical GPC

	Chitin *		Cellulose **	
	Product	Relative yield (wt.%)	Product	Relative yield (wt.%)
Monomer	**1a**	3	**3a**	15
Dimer	**1b**	4	**3b**	24
Trimer	**1c**	5	**3c**	7
Tetramer	**1d**	7	**3d**	4
Pentamer	**1e**	9	**3e**	5
Higher oligomers	**1f**	72	**3f**	45

* 210 °C, 1 h.
** 250 °C, 4 h.

TABLE 3

Characteristic ^{1}H-NMR and ^{13}C-NMR data for the chitin degradation products **1a–1e** *

	1a	**1b**	**1c**	**1d**	**1e**	
H-1	5.31(s)	5.25(s)	5.25(s)	5.25(s)	5.25(s)	
H-1′		4.49(s)	4.49(s)	4.49(s)	4.49(s)	
H-1″			4.43(s)	4.43(s)	4.43(s)	
H-6	4.06(d)	4.06(d)	4.06(d)	4.06(d)	4.06(d)	J = 7.7 Hz
C-1	100.5	100.4	100.4	100.4	100.4	
C-1′		100.2	100.0	100.0	100.0	
C-1″			101.7	101.5; 101.6	101.4; 101.5	
C-6	65.6	65.0	65.0	65.0	65.0	
C-6′		60.9	60.7	60.7	60.7	
C-6″			60.3	60.2	60.2	

* Chemical shifts relative to TMS.

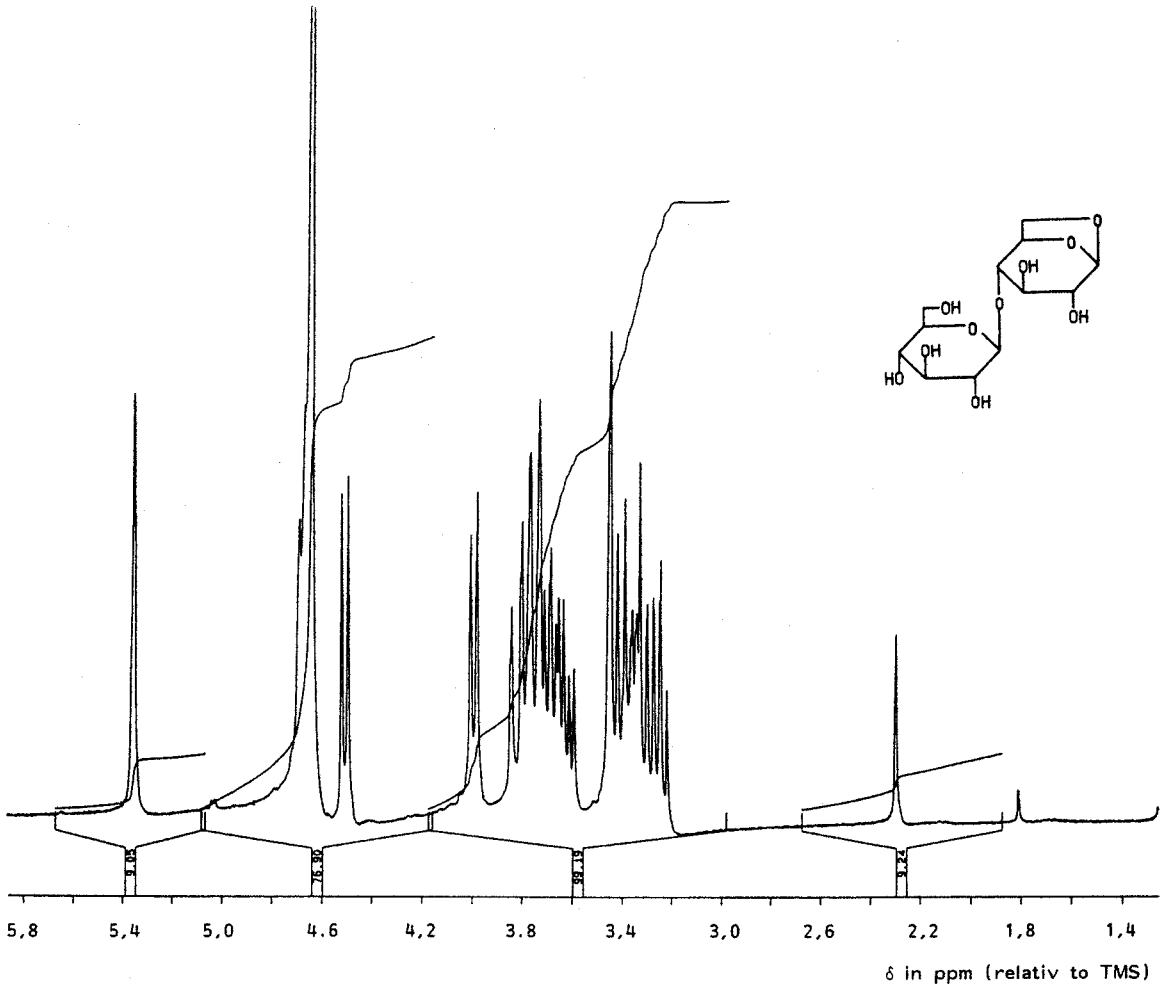


Fig. 2. ^{1}H-NMR spectrum of cellobiosan **3b** in D_2O at 25 °C. (Characteristic signals, see Table 5.)

N-acetyl-1,6-anhydro-glucosamine oligomers, and the relative intensity of proton H-1 allowed the estimation of an average polymer grade of DP ≈ 16. GPC showed the monomer and oligomers up to hexamers, well resolved, and higher unresolved oligomers (Fig. 1, Table 2). Preparative GPC yielded 30–70 mg of the monomeric and oligomeric degradation products **1a–1e**, which could be unambiguously characterized by ^{1}H-NMR [13] and ^{13}C-NMR [14]. The characteristic peaks of the ^{1}H-NMR and ^{13}C-NMR spectra of **1a–1e** are given in Table 3. As examples, the ^{1}H-NMR spectrum of cellobiosan **3b** and the ^{13}C-NMR spectrum of the oligomer **1d** are shown in Figs. 2 and 3, respectively. Intense H^+- and Na^+-attached quasi-molecular ions of the free sugars **1a–1d** and the acetylated sugars **2a–2d** were also observed, by FAB-MS (Table 4). Fragmentation of **1a–1d** occurred preferentially at the glycosidic bonds, and ion series of $[(203)_n + H]^+$ were observed. As an example, the FAB-mass spectrum of **1d** is shown in Fig. 4. The acetylated products **2a–2d** gave ion series of $[(287)_n + H]^+$.

Thermal degradation of cellulose for 4 h at 250 °C gave water-soluble oligomers in 4% yield. GPC separation revealed a higher percentage of the

TABLE 4

Observed quasi-molecular ions by FAB-MS of chitin degradation products **1a–1d** and **2a–2d** and of cellulose degradation products **3a–3e**

Product	*m/z*	Quasi-molecular ion *	Product	*m/z*	Quasi-molecular ion **	Product	*m/z*	Quasi-molecular ion *
1a	226	[**1a**·Na]$^+$	**2a**	310	[**2a**·Na]$^+$	**3a**	163	[**3a**·H]$^+$
	204	[**1a**·H]$^+$		288	[**2a**·H]$^+$			
1b	429	[**1b**·Na]$^+$	**2b**	597	[**2b**·Na]$^+$	**3b**	347	[**3b**·Na]$^+$
				575	[**2b**·H]$^+$		325	[**3b**·H]$^+$
1c	632	[**1c**·Na]$^+$	**2c**	884	[**2c**·Na]$^+$	**3c**	509	[**3c**·Na]$^+$
	610	[**1c**·H]$^+$		862	[**2c**·H]$^+$		487	[**3c**·H]$^+$
1d	835	[**1d**·Na]$^+$	**2d**	1171	[**2d**·Na]$^+$	**3d**	671	[**3d**·Na]$^+$
				1159	[**2d**·H]$^+$			
						3e	833	[**3e**·Na]$^+$
							811	[**3e**·H]$^+$

* Matrix glycerol.
** Matrix triethanolamine.

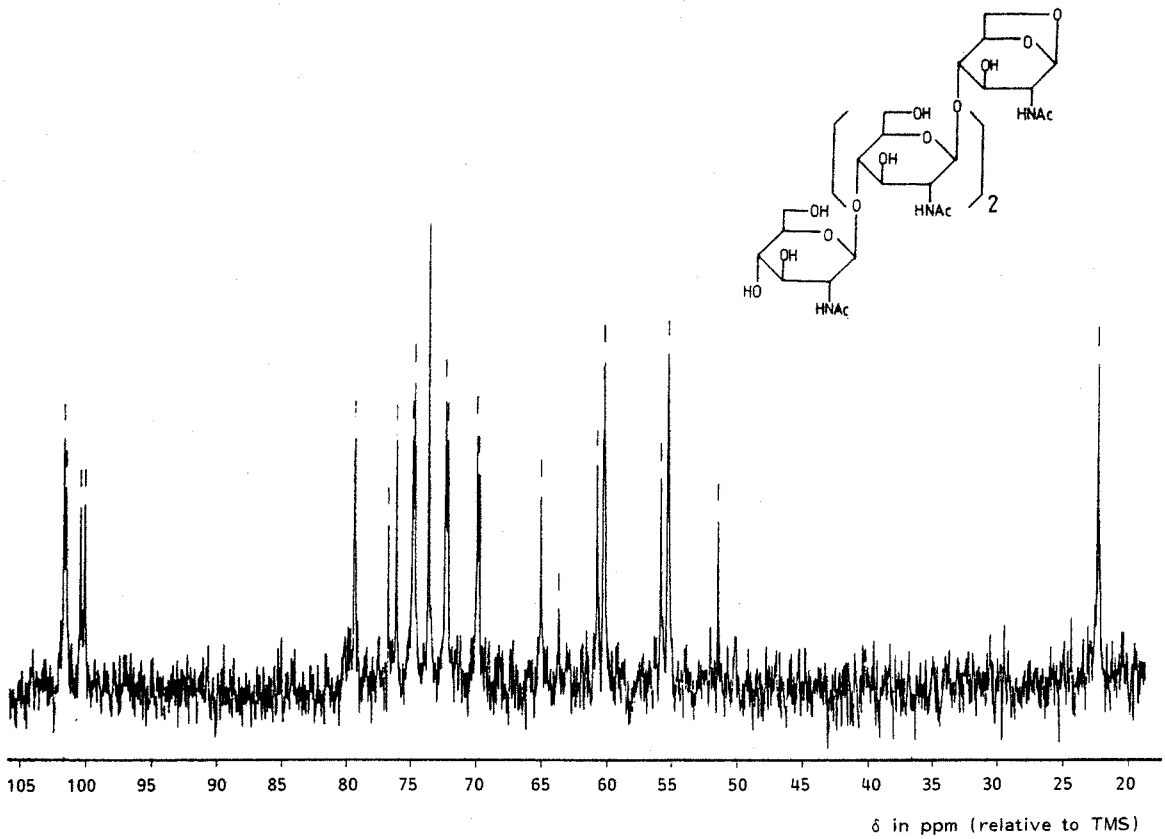


Fig. 3. ^{13}C-NMR spectrum of *N*-acetyl-1,6-anhydro-glucosamine oligomer **1d** in D_2O at 25 °C. (Characteristic signals, see Table 3.)

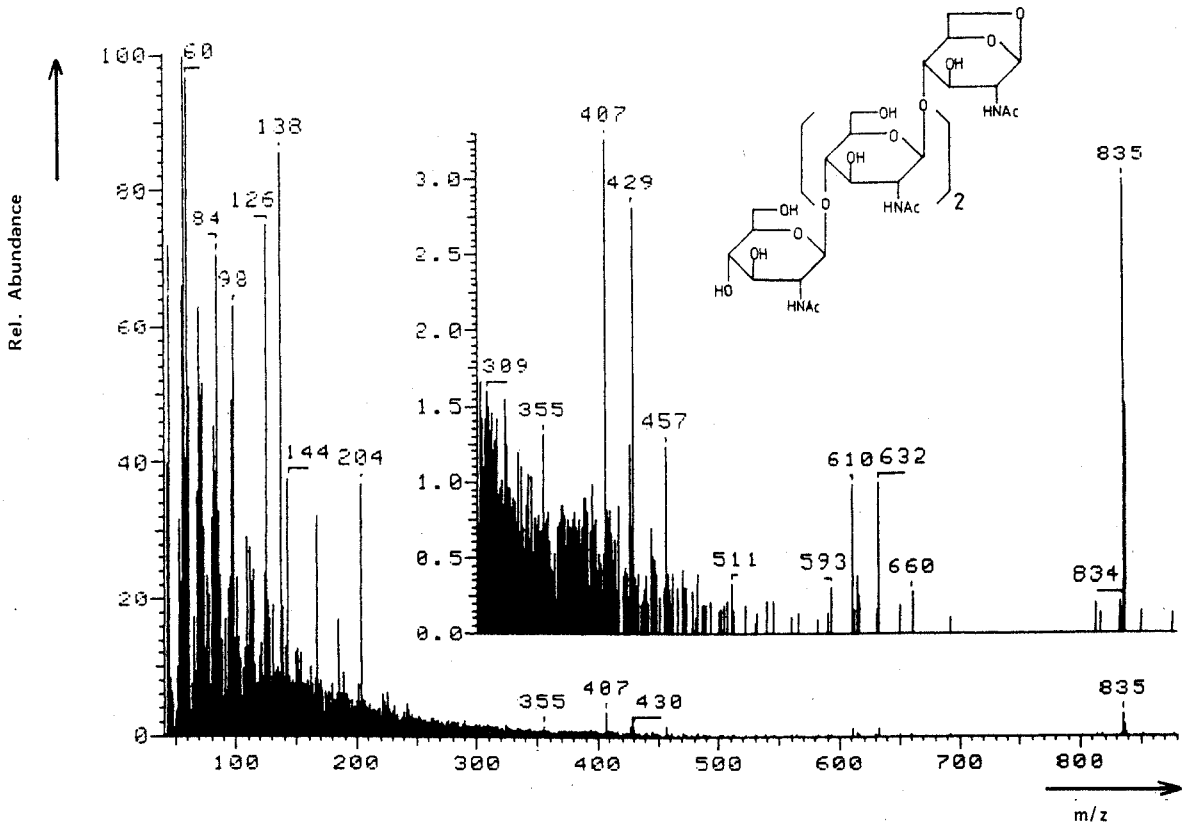


Fig. 4. FAB-mass spectrum of *N*-acetyl-1,6-anhydro-glucosamine oligomer **1d**. (See Table 4.)

TABLE 5

Characteristic ^{1}H-NMR and ^{13}C-NMR data for the cellulose degradation products **3a–3d** *

	3a	**3b**	**3c**	**3d**	
H-1	5.53(s)	5.36(s)	5.36(s)	5.36(s)	
H-1′	—	4.51(d)	4.54(d)	4.54(d)	$J = 7.8$ Hz
H-1″	—	—	4.41(d)	4.40; 4.43(d)	$J = 7.8$ Hz
H-6	3.99(d)	3.99(d)	4.00(d)	4.00(d)	$J = 7.8$ Hz
C-1	101.7	101.6	101.6	101.6	
C-1′	—	101.6	101.5	101.5	
C-1″	—	—	102.8	102.6; 102.8	
C-6	65.5	65.3	65.3	65.3	
C-6′	—	61.0	61.0	60.8	
C-6″	—	—	60.3	60.1	

* Chemical shift ppm relative to TMS.

monomeric and dimeric degradation products than was obtained for chitin, probably because of the higher degradation temperature (Table 2). The oligomers up to the pentamer could be separated and isolated by preparative GPC. The characteristic peaks of the ^{1}H-NMR and ^{13}C-NMR spectra of **3a–3e** are given in Table 5. The quasi-molecular ions of the free sugars **3a–3e** are given in Table 4.

CONCLUSION

Oligomeric anhydrosugars are primary degradation products of chitin and cellulose. This has been demonstrated by preparative isolation of the oligomers up to the pentamer from the water-soluble degradation syrups. We assume that our method can also be applied to other polysaccharides, allowing the isolation and separation of other oligomeric sugars with a terminal anhydropyranose unit. The yields have so far been relatively modest, but the polymeric starting materials are usually readily available and cheap.

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REFERENCES

1 F. Shafizadeh, Adv. Carbohydr. Res., 23 (1968) 419.
2 P. Köll and J.O. Metzger, Angew. Chem., 90 (1978) 802; Angew. Chem. Int. Ed. Engl., 17 (1978) 754.

3 M. Černy and T. Trnka, Carbohydr. Res., 174 (1988) 349.
4 H.-R. Schulten, Int. J. Mass Spectrom. Ion Phys., 32 (1979) 97.
5 J.O. Metzger, Fresenius Z. Anal. Chem., 308 (1981) 29.
6 A.C. Tas, A. Kerkenaar, G.F. La Vos and J. van der Greef, J. Anal. Appl. Pyrolysis, 15 (1989) 55.
7 A.D. Pouwels, G.B. Eijkel, P.W. Arisz and J.J. Boon, J. Anal. Appl. Pyrolysis, 15 (1989) 71.
8 R.J. Helleur and R. Guevremont, J. Anal. Appl. Pyrolysis, 15 (1989) 85.
9 M.L. Coates and C.L. Wilkins, Anal. Chem., 59 (1987) 197.
10 H.-R. Schulten and J.M. Halket, Org. Mass Spectrom., 21 (1986) 613.
11 D.St.A.G. Radlein, A. Grinshpun, J. Piskorz and D.S. Scott, J. Anal. Appl. Pyrolysis, 12 (1987) 39.
12 P. Köll and J.O. Metzger, Z. Lebensm. Unters. Forsch., 169 (1979) 111.
13 M. Černy and J. Staněk, Jr., Adv. Carbohydr. Chem. Biochem., 34 (1977) 23.
14 K. Bock and C. Pedersen, Adv. Carbohydr. Chem. Biochem., 41 (1983) 27.

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