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Characterisation of polysaccharides by in-source pyrolysis positive- and negative-ion direct chemical ionisation-mass spectrometry

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ABSTRACT

Series of oligosaccharide ions have been generated from a range of polysaccharides by the application of in-source pyrolysis mass spectrometry, using both ammonia positive-ion chemical ionisation and negative-ion chlorine-nucleophilic-addition ionisation. Glucans with α-(1→6), β-(1→6), α-(1→4), β-(1→4), β-(1→3), and β-(1→2) linkages were studied, together with pentosans, xyloglucans, and an arabinogalactan. The series of ions correspond to intact, desorbed oligosaccharides with a terminal anhydro-sugar unit, and to similar oligosaccharides with attached sugar ring-cleavage fragments. The ions generated are dependent on the position of the linkage and ring size, and retain significant information on the structure of the original polysaccharide.

INTRODUCTION

Levoglucosan (1,6-anhydro-β-D-glucopyranose), first identified[1] as a product of pyrolysis of cellulose in 1918, is a major product of the pyrolysis of hexosans, under some conditions[2] reaching 60%. Much work has been performed on the mechanisms of pyrolysis of polysaccharides[3–5]. Various reaction pathways (homolytic, heterolytic, and ionic) have been suggested to account for the wide and variable range of products[6]. Almost all of this work is predicated on, or limited by, an assumption that pyrolysis of hexosans produces levoglucosan as the largest primary product. When oligosaccharide products have been detected[7], it was assumed that they were formed by polymerisation of levoglucosan, which produces polymeric material on pyrolysis[8–10]. For slow-pyrolysis methods, this assumption is probably correct as the oligosaccharides produced contain a range of linkages and ring sizes[7]. However, with rapid (flash) pyrolysis, oligosaccharides retaining the configuration of the glycosidic linkages are produced[11].

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Using Curie point pyrolysis and in-source pyrolysis-m.s., considerable amounts of oligosaccharide material are produced from amylose and cellulose[12]. The condensates from pyrolysis of these polymers contain oligosaccharides which, on benzoylation, gave products equivalent to anhydro-oligosaccharides (the main products; A series of ions) and to anhydro-oligosaccharides with fragments of 42 and 60 mass units (D and F series of ions, respectively)[13], thus demonstrating that the oligosaccharides are real products of pyrolysis and not artifacts produced in the mass spectrometer. The series of ions are named using an extension of the Coates–Wilkins nomenclature[14]; the main ions found are summarised in Table I.

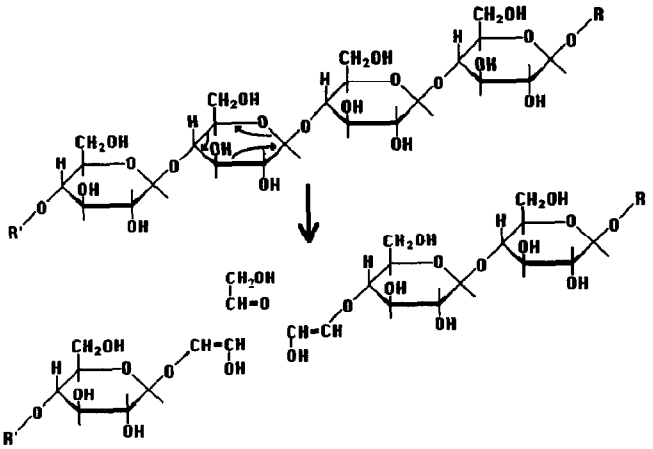
TABLE I

Nomenclature for the main ion series on in-source pyrolysis of glucans

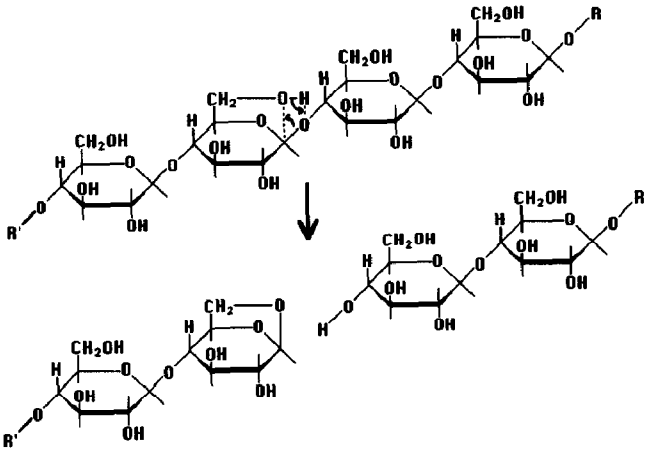
Ion series	*Mass increment on A series ion*	*Comments*
A	0	Anhydro-terminated oligosaccharide
D	42	
F	60	
L	102	
S	18	Oligosaccharide
Z	62	Found only for the (1→2)-β-D-glucan

Recent work[15] has confirmed the formation of anhydro-oligosaccharides and has identified the main ring-cleavage fragments (F series) for cellulose as oligosaccharides with attached acetaldehyde groups, *i.e.*, sugar plus 42-mass-unit fragments. The cleavage fragment was found on the reducing end (aglycon) and as a 4-substituent of the sugar. This finding suggests that "reverse aldolisation", involving disproportionation of the sugar ring (Scheme 1), is an important mechanism in the fast pyrolysis of polysaccharides. The recent realisation that hydroxyacetaldehyde, the third fragment of a disproportionating sugar, is a major product of the pyrolysis of cellulose[16] is another indicator of the importance of this mechanism. The main depolymerisation process on pyrolysis of cellulose (and other polysaccharides) is considered to take place via cleavage of the glycosidic bonds by an intramolecular transglycosylation mechanism[5,7] (Scheme 2), yielding a mixture of anhydro sugars which rearrange into the more stable 1,6-anhydro sugar. This mechanism was thought to be an "unzipping" process progressing from the non-reducing end[7,17]; however, the presence of large proportions of anhydro-oligosaccharides suggests that more random in-chain cleavages occur (Scheme 2).

We now report on the in-source pyrolysis mass spectra of a range of polysaccharides.



Scheme 1. Reverse aldolisation mechanism for the fragmentation of cellulose.



Scheme 2. Cleavage of the glycosidic bonds in cellulose by intramolecular transglycosylation.

EXPERIMENTAL

Cellulose (Avicel) was purchased from Merck, amylose (from potato starch) from Janssen Chimica, the (1→3)-β-D-glucan from *Laminaria hyperborea* from Koch–Light, the (1→6)-α-D-glucan (dextran) from Pharmacia, and the (1→6)-β-D-glucan (pustulan) from Calbiochem. The (1→2)-β-D-glucan was a gift from Dr. L. P. T. M. Zevenhuizen (Agricultural University, Wageningen, The Netherlands), the xyloglucan from tamarind seeds (galactoxyloglucan) was a gift from Dr. G. Reid (University of Stirling, Great Britain), and the xyloglucan from sycamore cell walls (fucogalactoxyloglucan) was a gift from Dr. W. S. York (University of Georgia, U.S.A.). The purified xylan and arabinoxylan fractions were prepared from wheat flour[18], the arabinan was a gift from Dr. A. Voragen (Agricultural University, Wageningen), and the arabinogalactan was purchased from Sigma.

Mass spectrometry. — A JEOL DX-303 instrument was used equipped with a direct chemical ionisation (d.c.i.) probe fitted with a platinum–rhodium wire (0.1 mm diam., 10% Rh) heated to ~800° at 16°/s. Positive-ion ammonia chemical ionisation-mass spectra were recorded at a source pressure of 20 Pa and in the range 60–2000 mass units (m.u.) with a cycle time of 1 s. Negative-ion chlorine-nucleophilic-addition ionisation mass spectra were obtained by saturating a flow of Ar or He with CCl_4, using a small glass bubbler and maintaining a pressure of this gas in the ion source of 30 Pa. Mass spectra were recorded over the range 150–1800 m.u. with a cycle time of 1 s and an electron energy of 250 eV. Each mass spectrum was obtained at an acceleration voltage of 2.2 kV, with a conversion dynode voltage of 10 kV, and was processed in a JEOL JMA DA-5000 data system.

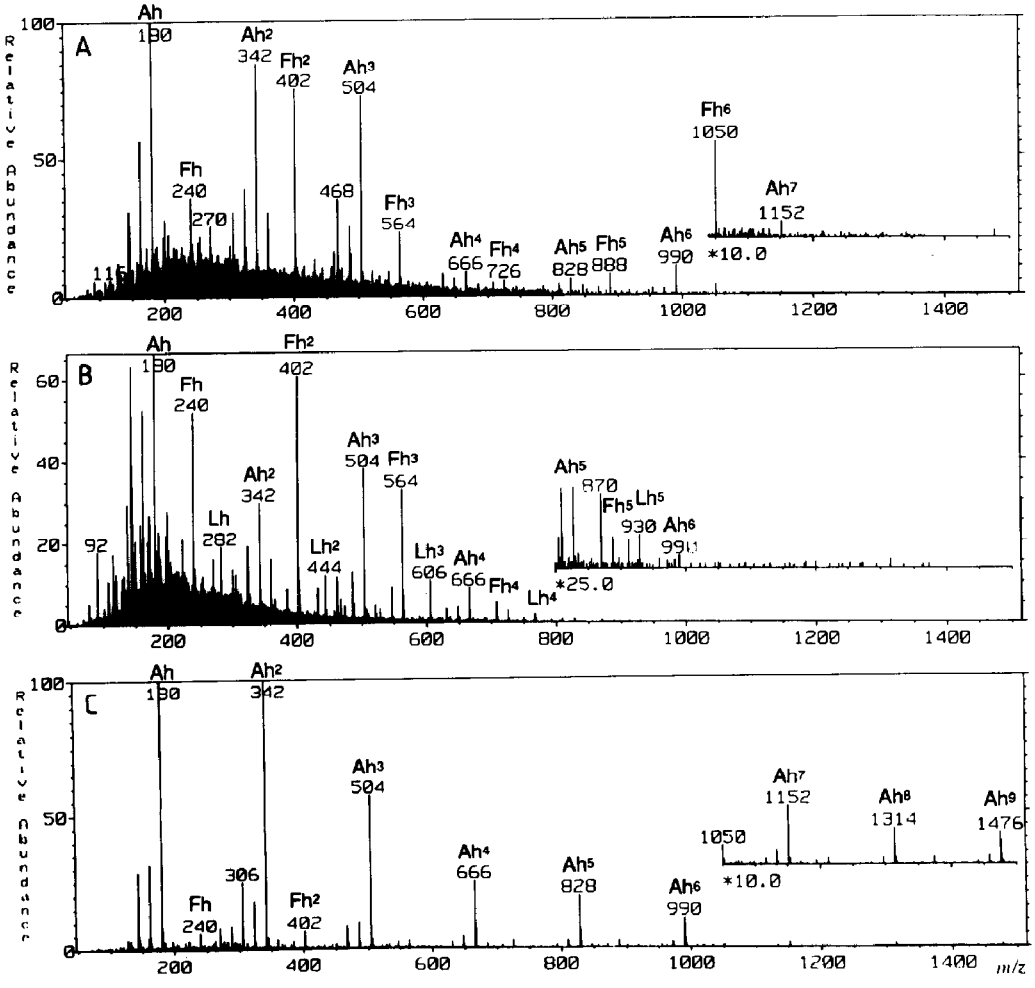
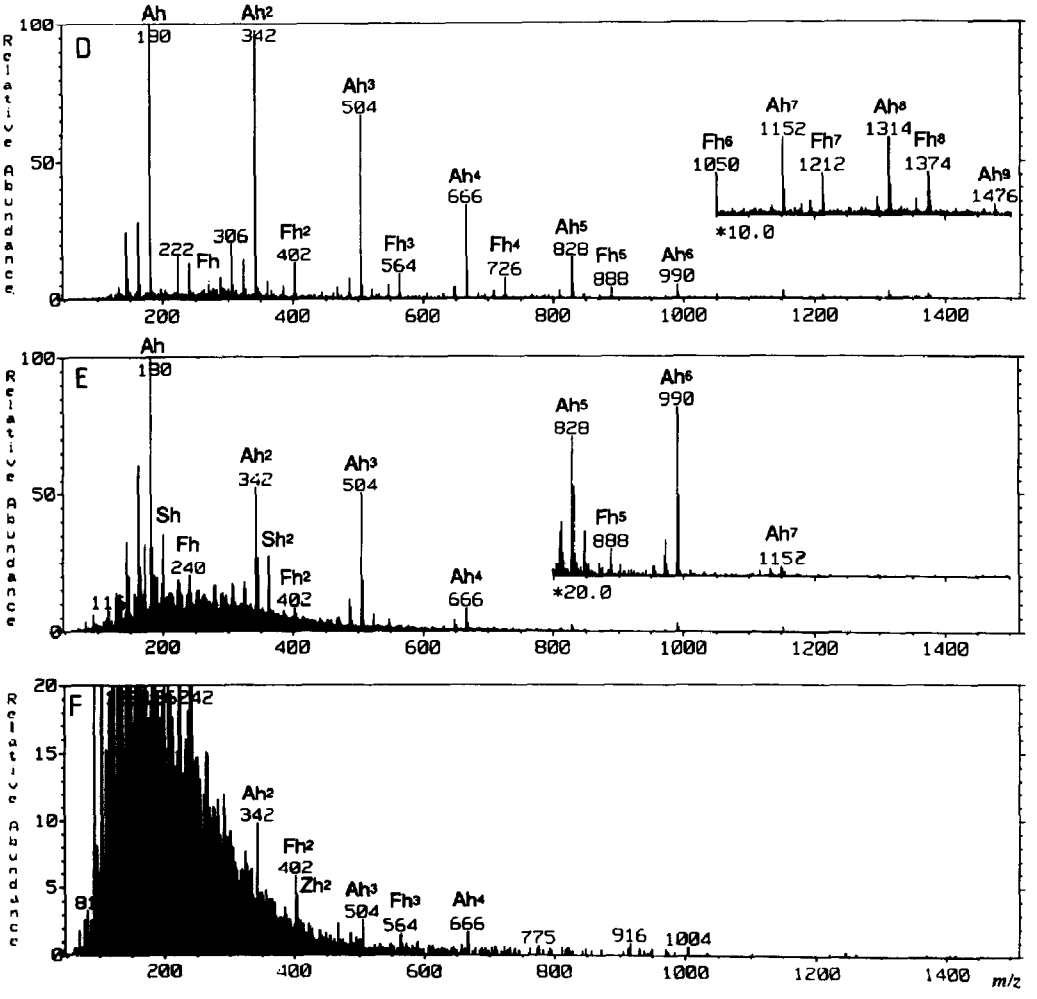


Fig. 1. In-source pyrolysis, ammonia positive ionisation mass spectra of dextran [(1→6)-α-D-glucan, A], pustulan [(1→6)-β-D-glucan, B], amylose [(1→4)-α-D-glucan, C], cellulose [(1→4)-β-D-glucan, D], laminarin [(1→3)-β-D-glucan, E], and (1→2)-β-D-glucan (F). Each mass spectrum was recorded at the moment of maximum production of oligosaccharides.

RESULTS AND DISCUSSION

The range of oligosaccharides detected on in-source pyrolysis seems to be dependent on the geometry of the source or other features of the design of the mass spectrometer. Thus, a Jeol DX-303 mass spectrometer has given[12,19] ions up to the equivalent of undecahexasaccharides (1962 m.u.), whereas other instruments have detected only lower oligosaccharides[20,21].

Glucans. — The ammonia chemical ionisation, positive-ion mass spectra for glucans with (1→6), (1→4), (1→3), and (1→2) linkages are shown in Fig. 1. All the major ions are ammonia adducts. Each glucan gave strong ions of the anhydro-oligosaccharide series (A), although the relative efficiency of their production varied. Each spectrum also contained additional series of ions, still with the sugar repeat unit, but with a different starting mass, which probably represent oligosaccharides with



attached ring-cleavage fragments. Each glucan gave an F series of ions (A + 60), but their relative intensity varied significantly. For the (1→6)-linked polysaccharides, and especially for the (1→6)-β-D-glucan, these ions are large compared to those of the A series. This finding is not surprising, as direct transglycosidation to produce the 1,6-anhydro sugar cannot take place when the 6-position is blocked. Ions equivalent to dehydration products from the anhydro-oligosaccharide and the F series of ions are also seen and are particularly strong for the (1→2)-β-D-glucan. Ions for the reducing sugar oligomers (S series) are also formed from each polymer except the (1→6)-β- and the (1→2)-β-D-glucans. The (1→6)-β-D-glucan is unusual in that it produced a sub-

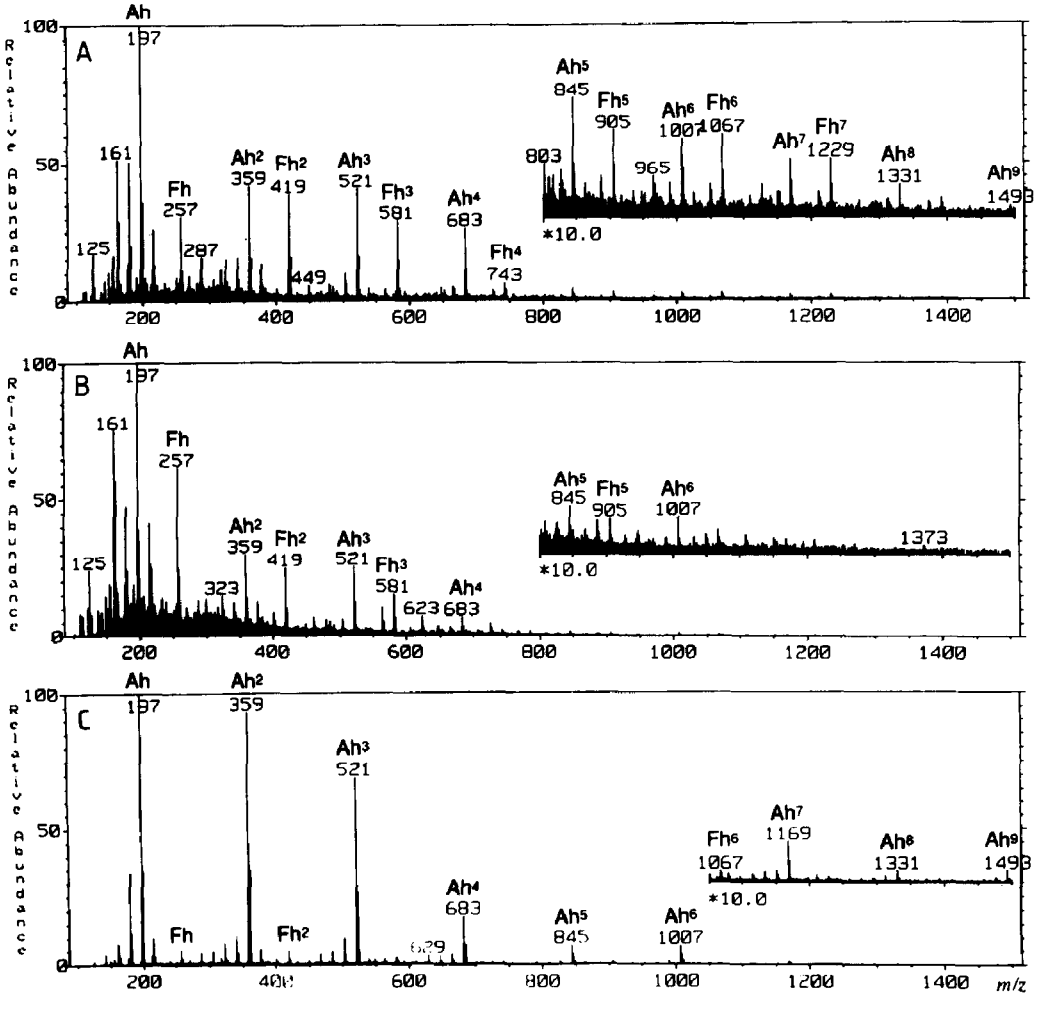
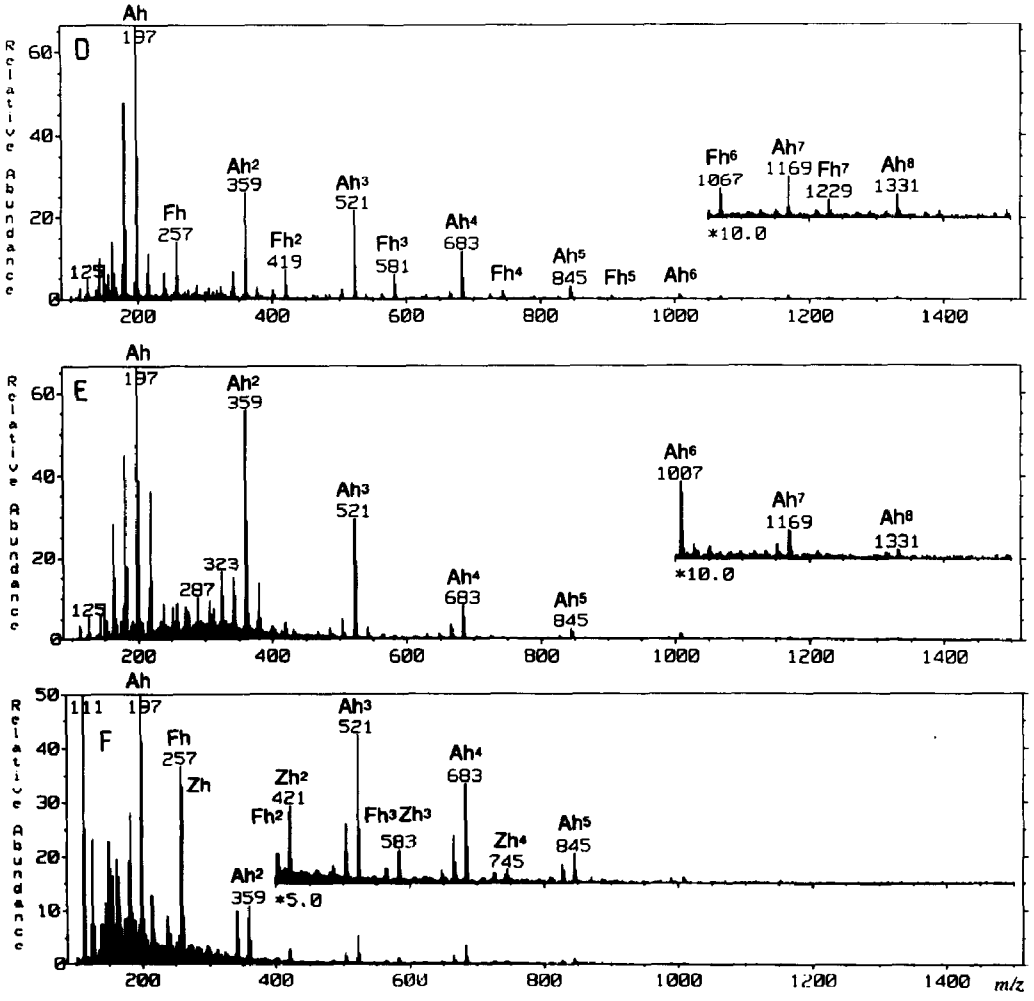


Fig. 2. In-source pyrolysis, chlorine-nucleophilic-addition negative ionisation mass spectra of dextran [(1→6)-α-D-glucan, A], pustulan [(1→6)-β-D-glucan, B], amylose [(1→4)-α-D-glucan, C], cellulose [(1→4)-β-D-glucan, D], laminarin [(1→3)-β-D-glucan, E], and (1→2)-β-D-glucan (F). Each mass spectrum was recorded at the moment of maximum production of oligosaccharides.

stantial A + 102 (L) series of ions. The (1→2)-β-D-glucan gave an additional series of ions (Z) at A + 62, which could be of value in identifying such linkages, and work is in progress to identify their structures. The difference noted for the (1→2)-β-D-glucan could be due to its cyclic structure[22], although tests with cyclomalto-oligosaccharides (cyclodextrins) showed no significant difference from the linear polymer.

Negative ions, generated by nucleophilic attachment of chlorine ions, gave strong mass spectra with patterns similar to those of the ammonia positive-ion mass spectra, but, in general, there was a lower background and less indication of post-ionisation reactions. This finding is expected since collision-induced dissociation normally leads to neutralisation for negative ions, whereas, with positive ions, a cascade of smaller products can result[23]. Fig. 2 shows the chlorine negative-ion chemical ionisation mass spectra for the glucans. Ion masses are increased by 35 m.u. due to the attachment of chlorine, and the distinctive presence of the large M + 2 ion from the ^{37}Cl isotope helps



to identify the peaks. Again, the differences between the different linkage positions are apparent, and again the (1→2)-β-D-glucan gives the distinctive A + 62 series of ions. These high-mass negative ions could be of great value in tandem m.s. (m.s.–m.s.) where remote charge effects on high-energy collisional dissociation could produce characteristic fragmentation patterns.

The spectra in Figs. 1 and 2 are taken from the position of maximum production of oligosaccharides. The time-course of the in-source pyrolysis of cellulose with chlorine negative ionisation is shown in Fig. 3; similar results were obtained using ammonia positive ionisation. It can be seen that, with increasing temperature, small units are observed first, in order of increasing molecular weight until, in a sudden event, the full range of oligosaccharide fragments are seen together. Sometimes, expecially with larger samples, further release events occur which also produce a wide range of oligosaccha-

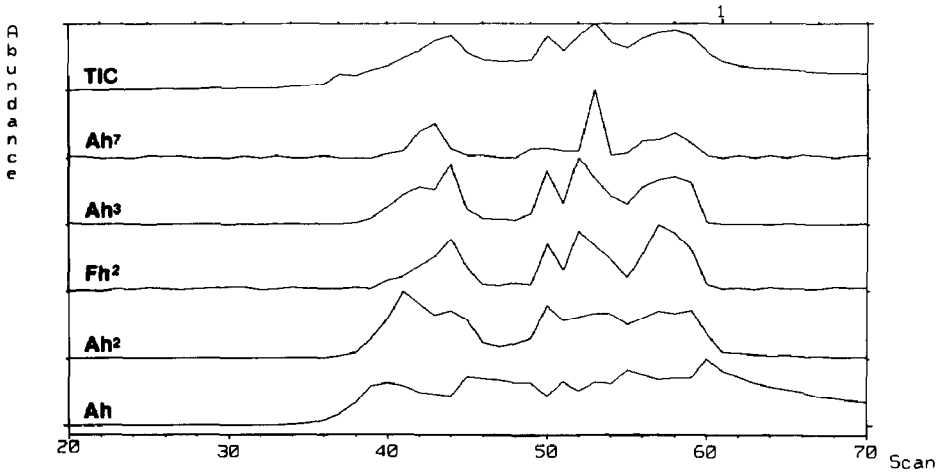


Fig. 3. Time-course of the in-source pyrolysis of cellulose.

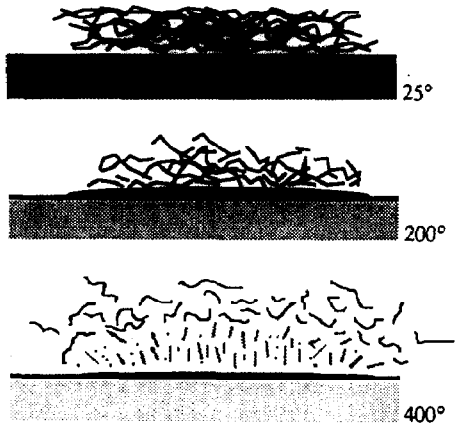


Fig. 4. Possible mechanism for the production of oligomers on rapid pyrolysis of polysaccharides.

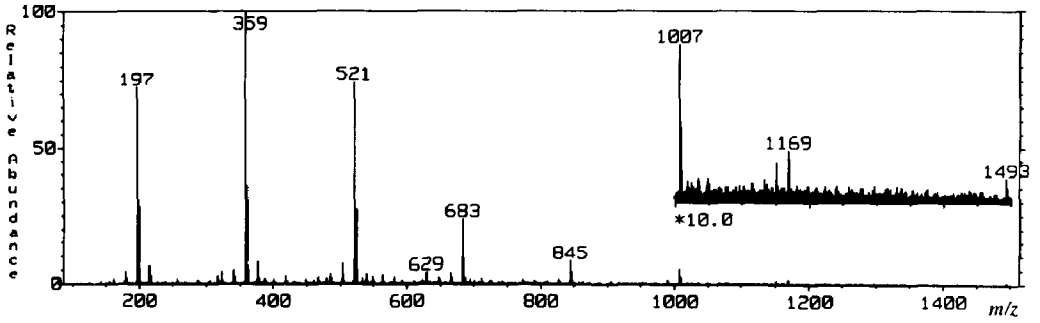


Fig. 5. In-source pyrolysis, chlorine negative ionisation mass spectrum of 120 μg of maltose.

rides. The nature of the processes involved have not been established, but there may be an almost explosive expulsion of the large fragments when the absorbed thermal energy is sufficient to volatilise a proportion of the material. Fig. 4 gives a schematic representation of this process. A minimum sample size (at least 5 μg) is necessary for the efficient production of oligosaccharides. With large samples of maltose (*e.g.* > 100 μg), a range of high molecular weight oligomers or clusters can be produced, as shown in Fig. 5. These oligosaccharides, formed by recombination, seem to be distinguished from real fragments of the polymer in that no ring-cleavage products are produced, whereas, for normal pyrolysis of polysaccharides, the profiles for specific members of the various series of ions are similar (see Fig. 3). Thus, the A series ions are never produced without the apearance of ring-cleavage fragments. Production of the ions from recombination artifacts is more pronounced in the negative mass sprectra, perhaps reflecting the high acidity and reactivity of chlorine ions.

Pentosans. — In-source pyrolysis of pentosans also gave mass spectra with clear series of ions with a sugar-residue-repeat interval (132 m.u.) and major peaks corresponding in mass to anhydro-oligosaccharides. Fig. 6 shows spectra from a (1→5)-linked arabinan[24] and an arabinoxylan consisting of a (1→4)-linked xylopyranose backbone with arabinofuranose side chains[18]. In each mass spectrum, the A, A + 42, and A + 60 series of ions are prominent. Possible dehydration products (−18 m.u.) are also significant, and ions of the sugar series (S) are more noticeable than for the glucans. Chlorine-addition negative-ion mass spectra showed no great difference from the positive-ion mass spectra, as can be seen from Figs. 6A and 6C.

In contrast to the other pentosans, the results for a linear (1→4)-linked xylopyranan[25] (Fig. 6D) look quite different. Although a basic regularity is still visible, many possible dehydration products obscure the sugar-unit-mass periodicity. The A, A + 42, and A + 60 series of ions are present and can be reconciled as the parents for ions formed by multiple losses of water. The configuration and position of the linkage in this sample make the direct production of any unstrained bicyclic anhydro-product unlikely, thus ruling out transglycosidation as a significant mechanism. A reverse-aldolisation mechanism, as found for cellulose[13], could operate and would produce the A + 42 and A + 60 series of ions. The relatively low intensity of these ions and the

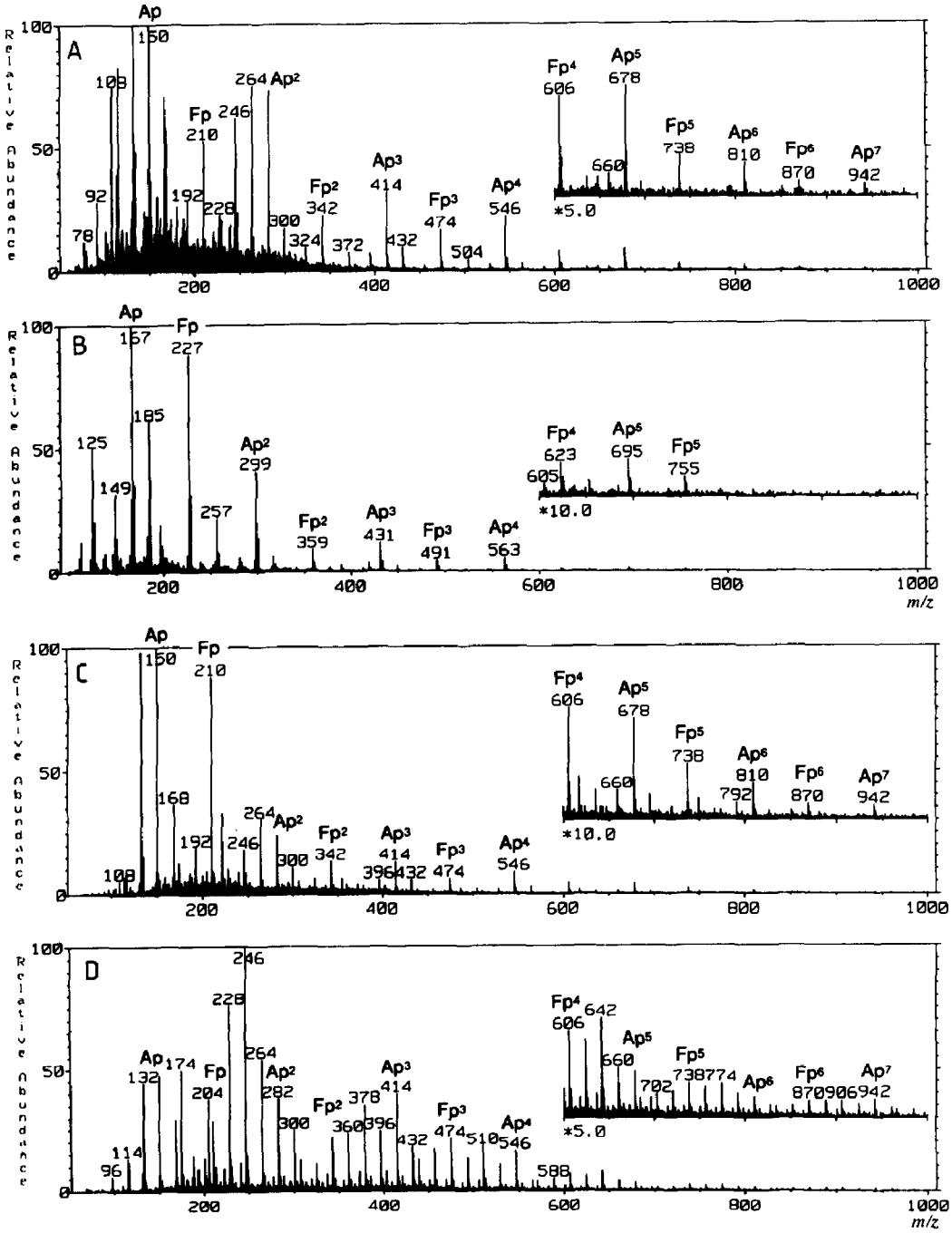


Fig. 6. In-source pyrolysis mass spectra of arabinan (A, ammonia positive ionisation; B, chlorine negative ionisation), arabinoxylan (C, ammonia positive ionisation), and xylan (D, ammonia positive ionisation).

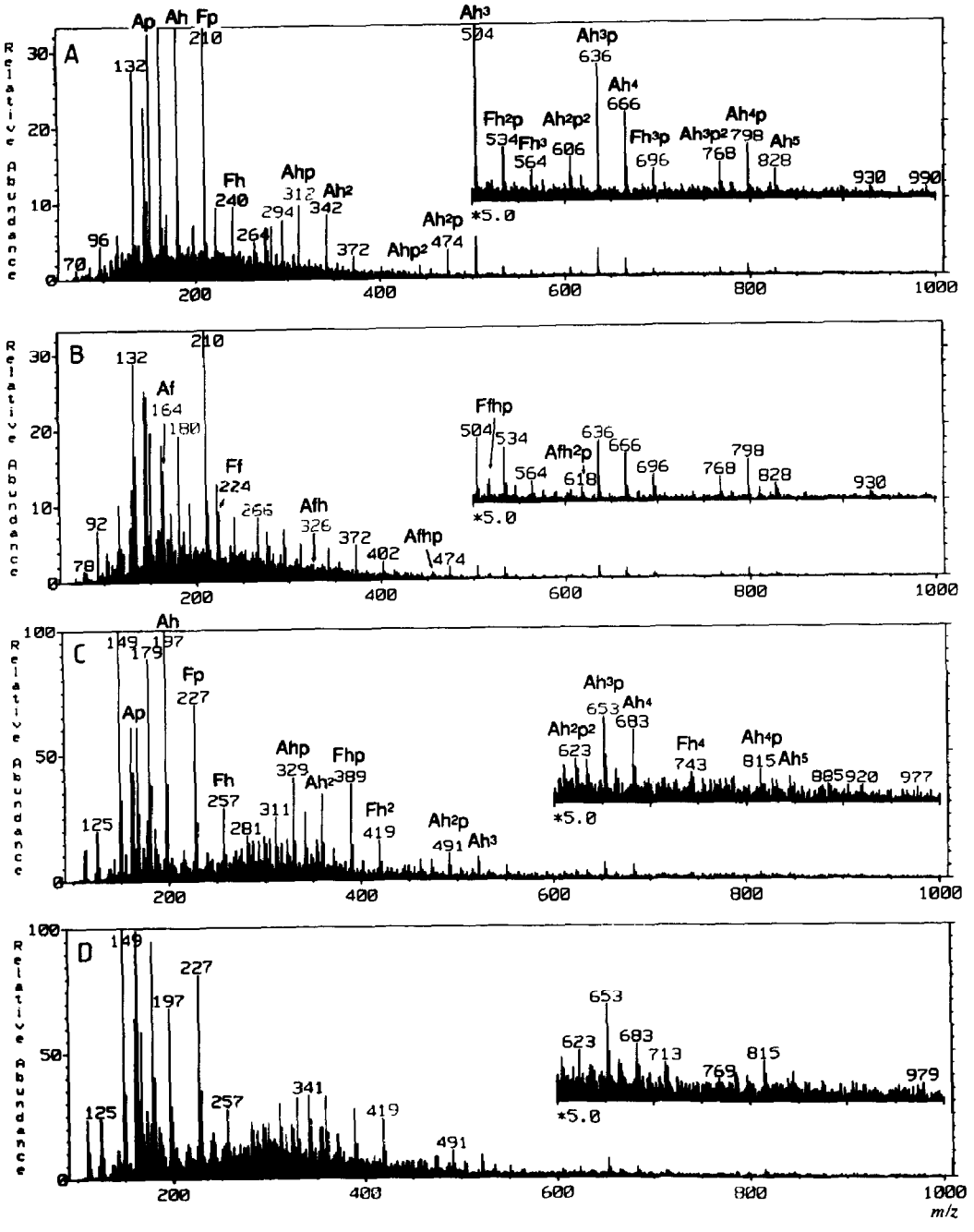


Fig. 7. In-source pyrolysis mass spectra of tamarind (A and B) and sycamore (C and D) xyloglucans, using ammonia positive (A and C) and chlorine negative (B and D) ionisation.

strength and range of the ions formed by dehydration suggest that the availability of a facile mechanism for cleavage of glycosidic bonds is necessary for the production of mass spectra containing limited numbers of structurally significant primary ions.

Heteropolysaccharides- — In-source pyrolysis mass spectra of heteropolysaccharides may provide considerably more information than for homopolysaccharides, since one of the basic limitations of mass spectrometry, namely, its inability to discriminate between isomeric ions, can be overcome if the different components of the polysaccha-

TABLE II

Possible structures for the main ions found in the ammonia positive ionisation, in-source pyrolysis mass spectrum of tamarind xyloglucan (Fig. 7A)

Compound[a]	*Mass*[b]	*Compound*	*Mass*	*Compound*	*Mass*
H	180 (240)				
HH	342 (402)				
HHH	504 (564)				
HHHH	666				
P	150 (210)				
P	312 (372)	**PP**	444 (504)		
H		**H**			
P	474 (534)	**PP**	606 (666)		
HH		**HH**			
P	636	**PP**	768 (828)	**PPP**	900 (960)
HHH		**HHH**		**HHH**	
P	798	**PP**	930	**PPP**	1062 (1122)
HHHH		**HHHH**		**HHHH**	
P	960	**PP**	1092		
HHHHH		**HHHHH**			
L	312 (372)				
P					
L	474 (534)				
P					
H					
L	636 (696)	**L**	768 (828)	**LL**	930 (990)
P		**PP**		**PP**	
HH		**HH**		**HH**	
L	798	**L**	930 (960)	**LL**	1092 (1152)
P		**PP**		**PP**	
HHH		**HHH**		**HHH**	
L	960				
P					
HHHH					
F	164 (224)	**F** 326	**F** 458 (518)	**F** 620 (680)	
		L	**L**	**L**	
			P	**P**	
				H	

[a] **H**, Glucose; **P**, xylose; **L**, galactose; **F**, fucose. [b] Masses are given for the A series and, in parenthesis, for the D series. All mass values are for ammonia addition products.

ride have different masses. However, this extra information adds greatly to the complexity of the mass spectra produced and makes unambiguous assignment of the ions much more difficult. Fig. 7 shows positive- and negative-ion mass spectra produced from two xyloglucans, namely, a storage polysaccharide from tamarind seeds which contains glucose, xylose, and galactose[26], and a cell-wall xyloglucan from sycamore[27], which contains fucose in addition to the sugars found in the storage polymer. For the simpler storage xyloglucan, it is possible to assign most of the major ions, although often multiple sources are possible: Table II shows the likely structures of the main ions. The cell-wall xyloglucan gave a more complicated mass spectrum with a higher background, which makes analysis more difficult. Even so, several new peaks, indicative of the fucose, can be identified as shown in Fig. 7. Monitoring of the pyrolysis for selected ions (Fig. 8) shows that most of the xylose residues are lost before the appearance of larger oligosaccharides, which are dominated by hexose-derived ions. This finding suggests that the outer portions of the polysaccharide are stripped off before the main explosion leading to larger oligosaccharides. Whether long sequences of unsubstituted glucose residues exist in the core of native xyloglucans, as is suggested by these results, is an open question since structural work is usually carried out on fragments produced by glucosidases which would remove such structures.

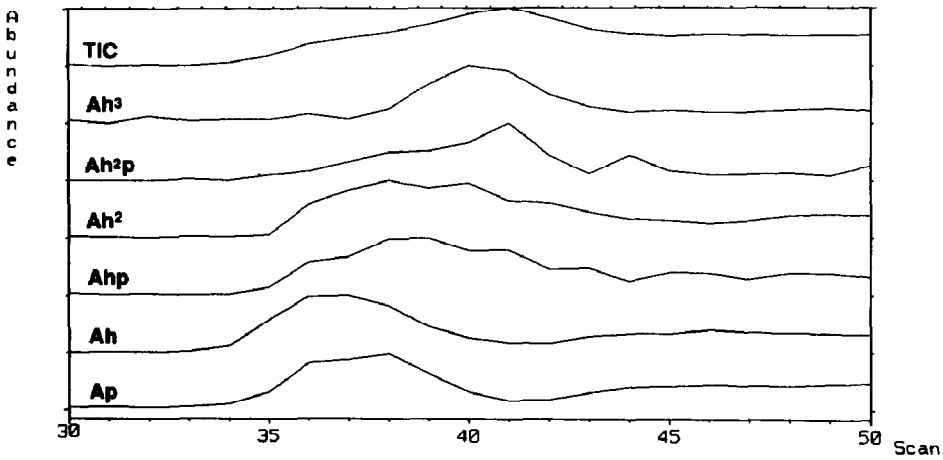


Fig. 8. Time-course of the in-source pyrolysis of tamarind xyloglucan.

Monitoring of the pyrolysis of the pectin-derived arabinan (Fig. 9; see spectrum of scans 34–41 in Fig. 6B) indicates the presence of some hexose residues (masses 197, 257 in the Cl^- negative-ion mass spectra). The early release of these ions, before the main production of oligosaccharide ions, and the limited range of ions in the hexose series, suggests that the hexose residues are side-chain or peripheral substituents. In contrast, the pyrolysis of an arabinogalactan shows early release of the arabinose side chains, well before the production of oligosaccharide fragments (Fig. 10), which are

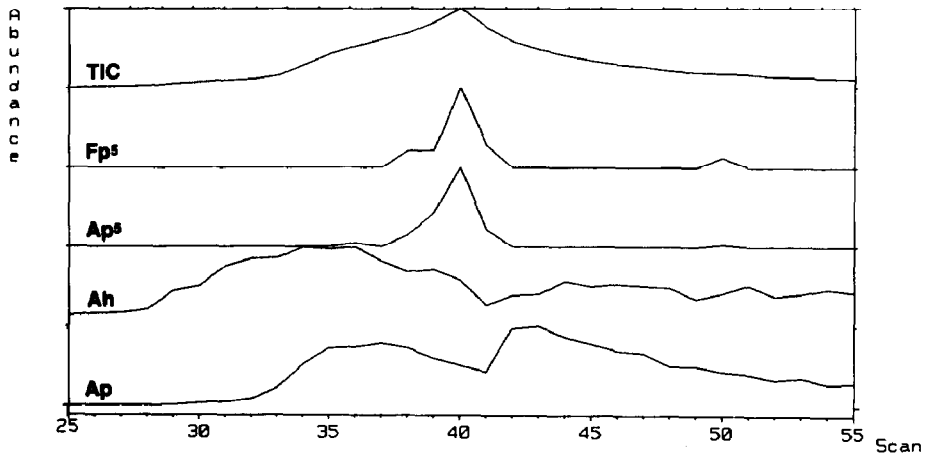


Fig. 9. Time-course of the in-source pyrolysis of the arabinan shown in Fig. 6A.

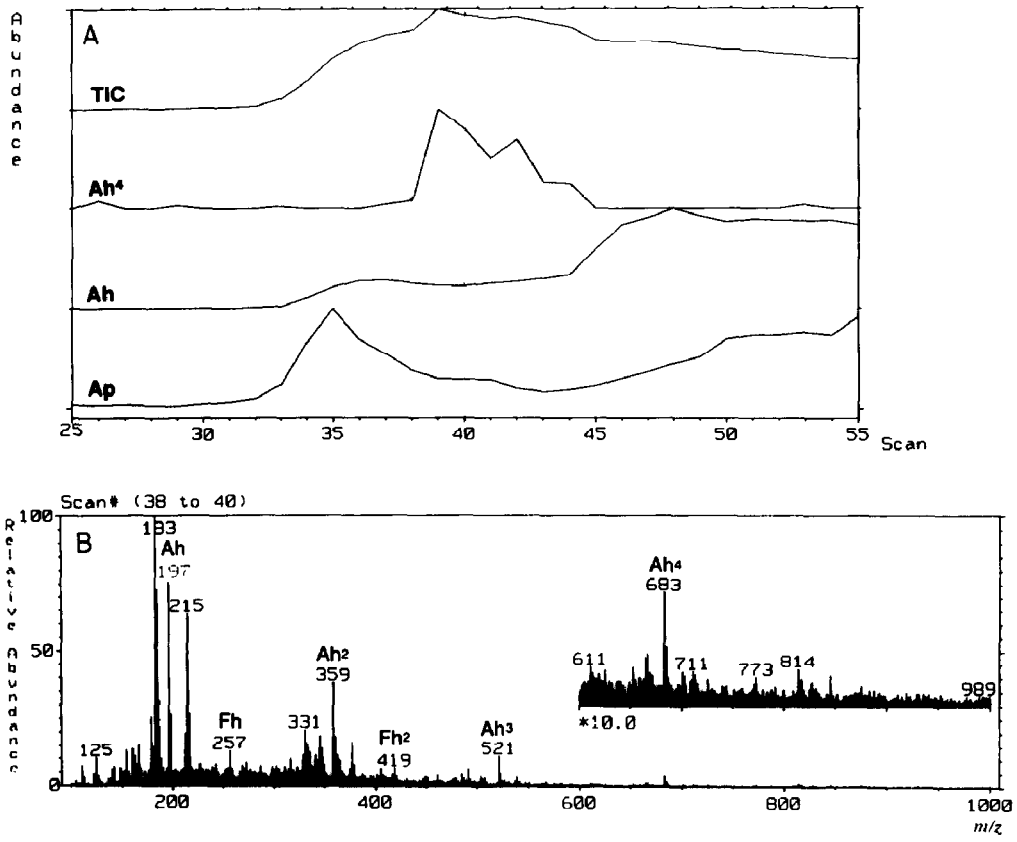


Fig. 10. Time-course of the in-source pyrolysis of an arabinogalactan (A) and the mass spectrum at the time of maximum production of oligosaccharides (B).

dominated by hexose ions derived from the linear galactose core. This finding again suggests that in-source pyrolysis mass spectra of polysaccharides are dominated by the sugars making up the backbone.

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