Carbohydrate Research, 180 (1988) c1–c7
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Structural information obtained by negative-ion laser desorption ionization Fourier transform ion cyclotron resonance (l.d.i.–F.t.–i.c.r.) mass spectrometry on bacterial capsular polysaccharides

ZAMAS LAM, GUY G. S. DUTTON*, MELVIN B. COMISAROW,
Department of Chemistry, The University of British Columbia, Vancouver, B.C., V6T 1Y6 (Canada)

DAVID A. WEIL,
Nicolet Analytical Instruments, 5225-1 Verona Road, Madison, WI 53711-0508 (U.S.A.)

AND ASGEIR BJARNASON
Science Institute of Iceland, Dunhaga 3, 107 Reykjavik (Iceland)

(Received December 8th, 1987; accepted for publication in revised form, March 17th, 1988)

Traditionally, the structural elucidation of polysaccharides has been established by using various chemical and enzymic degradation procedures with limited use of instrumental techniques[1]. This limitation is due to the high molecular weight, generally in the range of 10^6, and the involatility of these biopolymers. It is now accepted that endoglycanases associated with specific bacteriophages are capable of depolymerizing specific polysaccharides to their corresponding repeating-units[2–4]. The molecular weight of such oligosaccharide repeating units, for example, a hexasaccharide, is approximately 10^3, which is well amenable to mass spectrometric (m.s.) analysis using novel desorption–ionization techniques[5].

The most frequently used ionization techniques are desorption chemical ionization (d.c.i.)[6] and fast-atom-bombardment (f.a.b.)[7]. These desorption techniques give abundant sequence information, but provide little or no information on the positions of linkage or the anomeric configuration at the linkage. Tandem m.s. experiments, like collision-activation dissociation (c.a.d.), have been used to distinguish the position of linkage[8], and mass analyzed ion kinetic energy (m.i.k.e.s.) experiments have been used to confirm the anomeric configuration[9] with some success. The main drawback of both methods is that the oligosaccharide, unless of very low molecular weight, must be derivatized prior to analysis. Positive ion laser desorption ionization Fourier transform ion cyclotron resonance spectroscopy (l.d.i.–F.t.–i.c.r.)[10–13] has been used for the mass-spectral analysis of glycosides[14–17]. We have recently shown that positive-ion l.d.i.–F.t.–i.c.r. on *underivatized* oligosaccharides provides both sequence information and a tentative indication of some linkage positions[18]. We now report that *negative* ion l.d.i.–F.t.–

0008-6215/88/$ 03.50

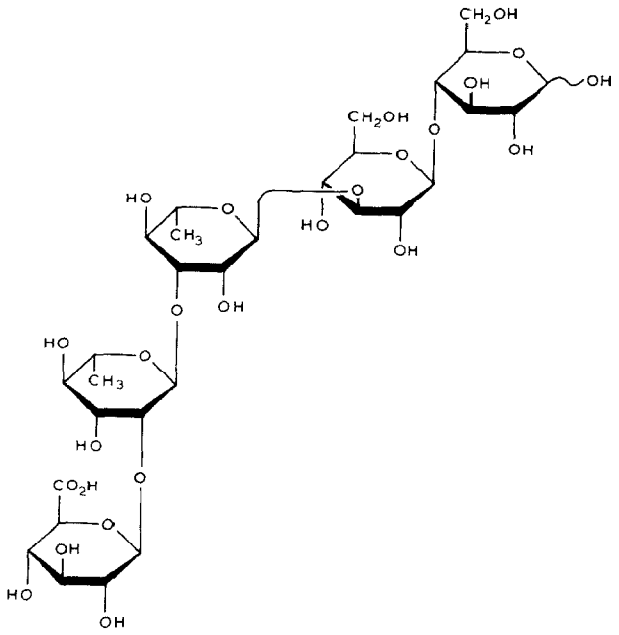

Fig. 1. Structure of *O*-deacetylated oligosaccharide obtained from *Klebsiella* K44 by bacteriophage degradation.

i.c.r. on *underivatized* oligosaccharides also provides information on the sequence, and, in addition, potential information on the anomeric configuration of the individual monosaccharides.

The structure of *Klebsiella* K44 capsular polysaccharide is made up[19,20] of a linear pentasaccharide repeating-unit consisting of one β-D-glucosyluronic acid group, two α-L-rhamnosyl residues, one β-D-glucosyl residue, and a terminal 6-*O*-acetylated-α-D-glucose residue (see Fig. 1). The negative-ion high resolution l.d.i.–F.t.–i.c.r. experiment was performed on the *O*-deacetylated oligosaccharide obtained from bacteriophage degradation. A 1-mg sample was dissolved in methanol–water, placed on the probe tip, and after evaporation of the solvent, ionized by laser desorption. Other experimental conditions were as given in ref. 18. After analysis, most of the sample could be recovered from the probe tip. Pseudo-molecular ions and fragment-ions were observed in abundance. Five distinct, fragmentation pathways were observed for the oligosaccharide, with one pathway leading, *via* glycoside-bond cleavages, to intact sugar residues. The other four pathways were assigned to ring cleavages.

Tables I and II show all peaks in the negative-ion spectrum above 2% of the base peak. Table I contains a list of all the negative ions derived from glycosidic-bond cleavages. Typically, these ions are deprotonated polyhexose structures that can be interpreted to give two possible sequences for the oligosaccharide.

TABLE I

NEGATIVE-ION L.D.I.-F.T.-I.C.R. FRAGMENT STRUCTURES ARISING FROM GLYCOSIDIC-BOND CLEAVAGE OF *Klebsiella* K44 *O*-DEACETYLATED OLIGOSACCHARIDE OBTAINED BY PHAGE DEGRADATION

Observed mass (amu)	*Calculated mass (amu)*	*Relative intensity (%)*	*Mass error (p.p.m.)*	*Proposed structure*
809.1107	809.2565	12.7	180	[(2Hex,2deoxyHex,HexA) − H]$^-$
791.1422	791.2463	23.3	132	[(2Hex,2deoxyHex,HexA) − H_2O − H]$^-$
647.0402	647.2040	13.8	25.3	[(2deoxyHex,Hex,HexA) − H]$^-$
633.2509	633.2247	9.5	−41.3	[(2Hex,2deoxyHex) − H]$^-$
629.1266	629.1935	40.0	106	[(2deoxyHex,Hex,HexA) − H_2O − H]$^-$
615.1231	615.2142	22.9	135	[(2Hex,2deoxyHex) − H_2O − H]$^-$
487.1299	487.1669	7.6	75.8	[(2Hex,deoxyHex) − H]$^-$
485.1076	485.0512	76.7	−116	[(2deoxyHex,HexA) − H]$^-$
471.0974	471.1719	19.3	158	[(2deoxyHex,Hex) − H]$^-$
469.0985	469.1563	35.3	123	[(2Hex,deoxyHex) − H_2O − H]$^-$
467.0406	467.0848	99.3	−94.6	[(2deoxyHex,HexA) − H_2O − H]$^-$
453.1006	453.1614	53.8	134	[(2deoxyHex,Hex) − H_2O − H]$^-$
339.0650	339.0933	22.2	83.6	[(deoxyHex,HexA) − H]$^-$
325.0614	325.1140	12.4	162	[(deoxyHex,Hex) − H]$^-$
323.0603	323.0984	16.7	118	[(2Hex) − H_2O − H]$^-$
321.0506	321.0827	77.5	99.9	[(deoxyHex,HexA) − H_2O − H]$^-$
309.1585	309.1191	20.4	127	[(2deoxyHex) − H]$^-$
307.0743	307.1035	68.4	94.9	[(deoxyHex,Hex) − H_2O − H]$^-$
291.0885	291.1085	100.0	68.9	[(2deoxyHex) − H_2O − H]$^-$
193.0250	193.0354	45.1	54.0	[HexA − H]$^-$
179.0445	179.0561	35.6	65.1	[Hex − H]$^-$
175.0221	175.0248	30.2	15.4	[HexA − H_2O − H]$^-$
161.0355	161.0456	72.0	62.3	[Hex − H_2O − H]$^-$

TABLE II

NEGATIVE-ION L.D.I.-F.T.-I.C.R. FRAGMENTS STRUCTURES ARISING FROM RING CLEAVAGE OF *Klebsiella* K44 *O*-DEACETYLATED OLIGOSACCHARIDE OBTAINED BY PHAGE DEGRADATION

Observed mass (amu)	*Calculated mass (amu)*	*Relative intensity (%)*	*Mass error (p.p.m.)*	*Proposed structure*
689.1260	689.2140	26.5	128	[(2deoxyHex,Hex,HexA) + $C_2H_2O_2$]$^-$
675.1469	675.2353	66.9	131	[(2deoxyHex,2Hex) + $C_2H_2O_2$]$^-$
671.0775	671.2035	20.7	188	[(2deoxyHex,Hex,HexA) − H_2O + $C_2H_2O_2$]$^-$
529.1347	529.1769	30.9	79.8	[(2deoxyHex,HexA) + $C_2H_4O_2$]$^-$
513.1100	513.1825	58.2	141	[(2Hex,deoxyHex) + C_2H_2O]$^-$
383.0857	383.1190	42.9	86.8	[(HexA,deoxyHex) + $C_2H_4O_2$]$^-$
367.0879	367.1246	70.5	99.9	[(2Hex) + C_2H_2O]
365.0945	365.1084	8.0	40.8	[(HexA,deoxyHex) − H_2O + $C_2H_4O_2$]$^-$
349.0812	349.1104	11.3	94.1	[(2Hex) − H_2O + C_2H_2O]$^-$
235.0294	235.0454	58.6	68.2	[HexA + $C_2H_2O_2$]$^-$
221.0473	221.0667	64.4	87.5	[Hex + $C_2H_2O_2$]$^-$
203.0461	203.0561	26.2	49.4	[Hex − H_2O + $C_2H_2O_2$]$^-$

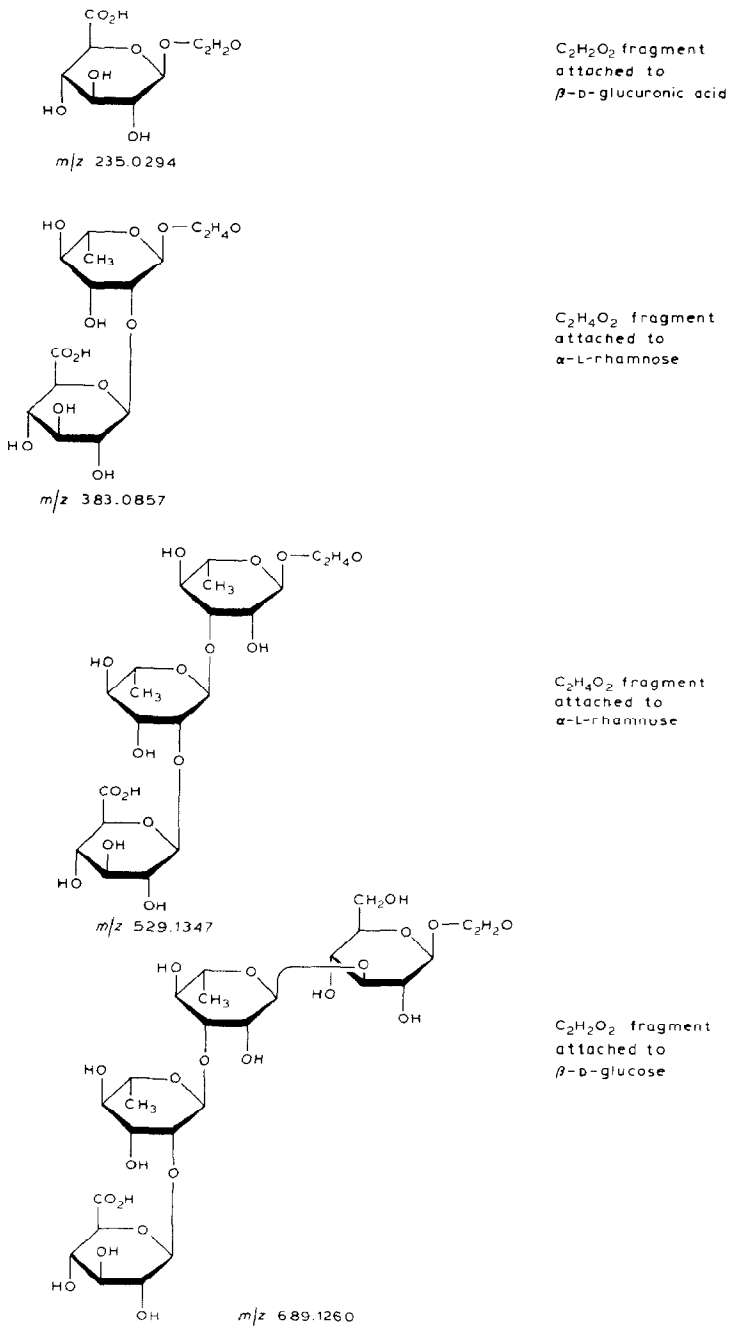

Fig. 2. Structures of nonreducing-end ring-cleavage fragments obtained from negative-ion l.d.i.–F.t.–i.c.r. of *Klebsiella* K44 *O*-deacetylated oligosaccharide.

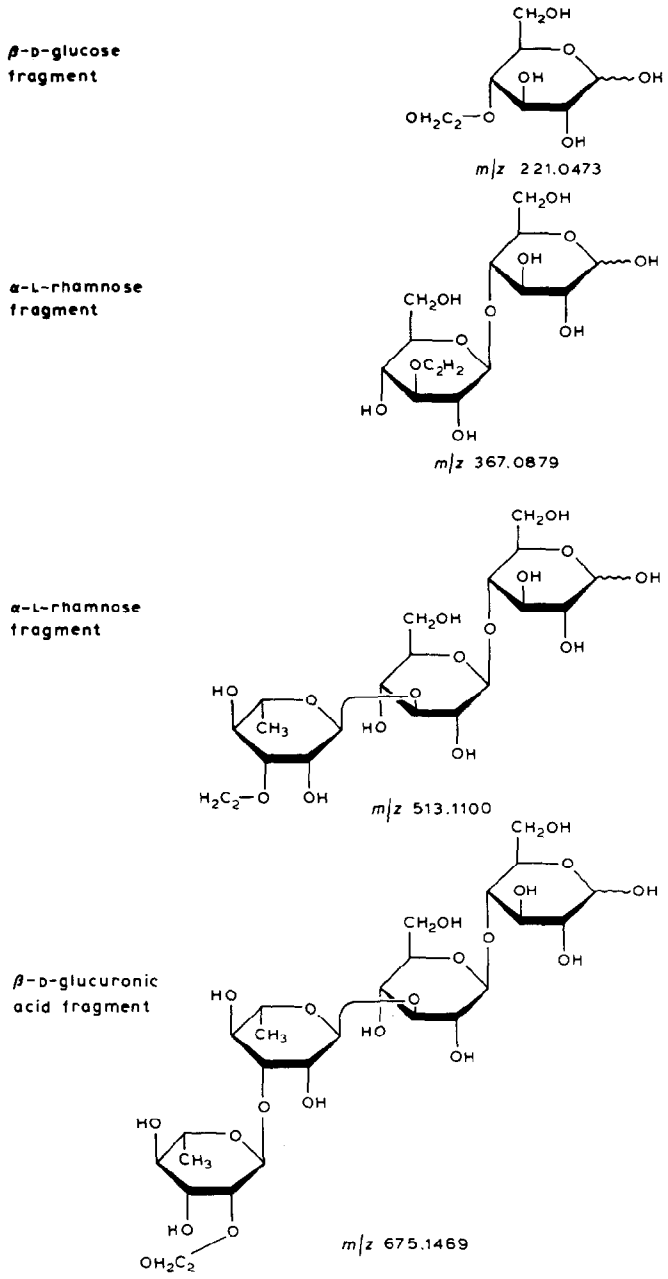

Fig. 3. Structures of reducing-end ring-cleavage fragments obtained from negative-ion l.d.i.–F.t.–i.c.r. of *Klebsiella* K44 *O*-deacetylated oligosaccharide.

(i) HexA --- deoxyHex --- deoxyHex --- Hex --- Hex
(ii) Hex --- Hex --- deoxyHex --- deoxyHex --- HexA

Table II contains a list of all the negative ions derived from ring cleavages. Four different fragmentation pathways were observed, including cleavages from both the reducing and the nonreducing end of the oligosaccharide. The presence of both ring-cleavage ions and glycosidic-bond-cleavage ions allows determination of the true sequence of the oligosaccharide.

The ring cleavages also seem to indicate the anomeric configuration of the monosaccharide residues. For ring-cleavage fragments, including the nonreducing-terminal residue, the sub-monosaccharide unit correlates with the anomeric configuration of the monosaccharide residue adjacent to the ring-cleaved residue (see Fig. 2). In this case, α anomers gave a $C_2H_4O_2$ fragment, whereas β anomers gave a $C_2H_2O_2$ fragment. For ring-cleavage fragments which include the reducing-terminal residue, the sub-monosaccharide unit correlates with the anomeric configuration of the ring-cleaved residue itself (see Fig. 3). In this case, α anomers gave a C_2H_2O fragment, whereas β anomers gave a $C_2H_2O_2$ fragment.

It could be argued that the two-carbon fragments shown in Figs. 2 and 3 may indicate the configuration of the highest-numbered chiral carbon atom, *i.e.*, a D or L series sugar, rather than the configuration of the anomeric carbon atom. The fragmentation of a monosaccharide ring is, however, more likely to manifest itself as either a neighboring-group effect (see Fig. 2), or as a representation of the stereochemistry of the fragment center (see Fig. 3), rather than a reflection of the stereochemistry of a relatively distant group, *i.e.*, C-5 in this case.

ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1 L. KENNE AND B. LINDBERG, in G. O. ASPINALL (Ed.), *The Polysaccharides*, Vol. 2, Academic Press, New York, 1983, pp. 287–363.
2 G. G. S. DUTTON, J. L. DI FABIO, D. M. LEEK, E. H. MERRIFIELD, J. R. NUNN, AND A. M. STEPHEN, *Carbohydr. Res.*, 97 (1981) 127–138.
3 H. GEYER, K. HIMMELSPACH, B. KWIATKOWSKI, S. SCHLECHT, AND S. STIRM, *Pure Appl. Chem.*, 55 (1983) 637–653.
4 D. RIEGER-HUG AND S. STIRM, *Virology*, 113 (1981) 363–378.
5 G. G. S. DUTTON, G. K. EIGENDORF, Z. LAM, AND A. V. S. LIM, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 459–460.
6 V. N. REINHOLD, *Methods Enzymol.*, 138 (1978) 59–84.
7 H. R. MORRIS, A. DELL, M. PANICO, AND R. A. MCDOWELL, in A. L. BURLINGAME AND N. CASTAGNOLI, JR. (Eds.), *Mass Spectrometry in the Health and Life Sciences*, Elsevier, Amsterdam, 1985, pp. 363–399.
8 E. G. DE JONG, W. HEERMA, AND G. DIJKATRA, *Biomed. Mass Spectrom.*, 7 (1980) 127–131.
9 G. PUZO, J.-C. PROME, AND J.-J. FOURNIE, *Carbohydr. Res.*, 140 (1985) 131–134.

10 D. A. McCrery, E. B. Ledford, Jr., and M. L. Gross, *Anal. Chem.*, 54 (1982) 1435–1437.
11 R. B. Cody, J. Kinsinger, S. Ghaderi, J. L. Amster, F. McLafferty, and F. W. Brown, *Anal. Chim. Acta*, 178 (1985) 43–66.
12 M. L. Coates and C. L. Wilkins, *Anal. Chem.*, 59 (1987) 197–200.
13 C. L. Wilkins, D. A. Weil, C. L. P. Yang, and C. F. Ijames, *Anal. Chem.*, 57 (1985) 520–524.
14 R. E. Shomo, II, A. G. Marshall, and C. R. Weisberger, *Anal. Chem.*, 57 (1985) 2940–2944.
15 R. E. Shomo, II, A. G. Marshall, and R. P. Lattimer, *Int. J. Mass Spec. Ion Proc.*, 72 (1986) 209–217.
16 R. E. Shomo, II, A. Chandrasekaran, A. G. Marshall, R. H. Reuning, and L. W. Roberson, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 295–302.
17 D. A. McCrery and M. L. Gross, *Anal. Chim. Acta*, 178 (1985) 91–103.
18 Z. Lam, M. B. Comisarow, G. G. S. Dutton, D. A. Weil, and A. Bjarnason, *Rapid Commun. Mass Spectrom.*, 1 (1987) 83–86.
19 G. G. S. Dutton and T. E. Folkman, *Carbohydr. Res.*, 78 (1980) 305–315.
20 G. G. S. Dutton and D. N. Karunaratne, *Carbohydr. Res.*, 138 (1985) 277–291.