Convenient Mass Spectral Technique for Structural Studies on Oligosaccharides

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Summary Negative chemical ionization mass spectra of carbohydrates have been recorded and their utility in structure elucidation of underivatized oligosaccharides demonstrated.

A MAJOR problem associated with structural studies on complex oligosaccharides[1] is the reliable determination of the molecular weight of these compounds and their degradation products. Recently, we[2] developed a new method for the accurate determination of molecular weight that involves the preparation of radioactive derivatives. We report now on the use of negative chemical ionization[3] (N.C.I.) techniques for obtaining valuable structural information on very small samples of underivatized oligosaccharides.

Electrons with a wide range of energy are present in the ion chamber of a chemical ionization mass spectrometer. This greatly increases the possibility of resonance capture of electrons by molecules of a sample compound to form M^- ions in abundance.[4] Because of the low energy (0—10 eV) of the electrons captured, fragment ions are few; the M^- ions are therefore intense and may even correspond to the base peak in the mass spectrum.

We have employed a modified Biospect† mass spectrometer for observing N.C.I. spectra. In this instrument electrons of 500 eV energy are used to generate ions from the reagent gas (for example, methane); a quadrupole is utilized as a mass filter and an electron multiplier for detecting the ions. To operate in the negative ion mode, the electron multiplier detection system was modified in a manner very similar to that described by Field *et al.*[5] A gas manifold was attached to the spectrometer for conveniently switching from one reagent gas to another.

Using methane as the reagent gas and direct probe insertion of samples into the ion source,[5]† we have recorded the N.C.I. mass spectra of several oligosaccharides without any derivatization. Fairly intense molecular ions M^- were observed for mono-, di-, tri-, and tetra-saccharides, *etc.* A small number of fragment ions were observed which were somewhat difficult to interpret. Penta-saccharides gave only fragment ions but no molecular ion.

Recently, we[6] have reported that when CF_2Cl_2 (Freon 12) is used as the reagent gas for chemical ionization mass spectrometry (C.I.M.S.), Cl^- ions are the only anions generated at about 1 Torr pressure and that compounds of a wide variety when introduced into the ion chamber

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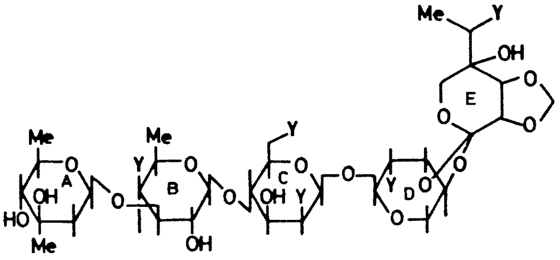
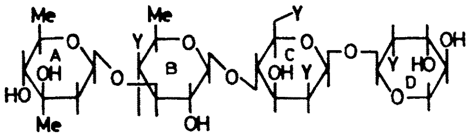
† Manufactured by Scientific Research Instruments Company, Baltimore, Maryland. The ion source was maintained at about 1 Torr pressure and a temperature of 140—250 °C. Samples (10—100 ng) were introduced through an unheated direct probe. We have not made a direct comparison between our N.C.I. spectra and the spectra on a commercially available field desorption mass spectrometer (F.D.M.S.) for spectral sensitivity. In general, samples of mg size are needed for F.D.M.S. and spectra often show multiple pseudomolecular ions corresponding to attachment with sodium, potassium, and other cations.

produce abundant anions by attachment of Cl^- to their functional groups.[7] Such chloride ion attachment appears to be a low energy process since very few fragment ions are produced. Because of these considerations, N.C.I. (CF_2Cl_2) mass spectrometry proves to be very valuable for studying oligosaccharides, especially those with a number of free hydroxy groups. Apparently, the larger the number of sites for Cl^- attachment, the higher the probability of the formation of an anion. Larger fragments are thus more prominent than the smaller fragments in the N.C.I. mass spectra. Not unexpectedly, permethylated sugars lacking free hydroxy groups give comparatively poor N.C.I.M.S.



(1)



(2)

Y = -OMe

Table. N.C.I.(CF_2Cl_2) mass spectra of oligosaccharides

N.C.I.(CF_2Cl_2) peaks *m/e*	Relative intensity	Cl^- adduct ions corresponding to
Olgose (**1**)		
893	100	*M*
748	30	*M*–A
675	18	*M*–E
588	30	*M*–A–B
530	70	*M*–A–E
Evertetrose (**2**)		
693	100	*M*
675	5	*M*–H_2O
548	80	*M*–A
401	10	*M*–A–D
388	10	*M*–A–B

The Table shows the spectra obtained by the N.C.I. (CF_2Cl_2) technique from two related compounds. Olgose (**1**) a penta-saccharide with five free hydroxy groups, produced an intense pseudomolecular ion with no fragment ions due to loss of H_2O. Evertetrose (**2**) also showed a strong molecular ion and a weak fragment ion $(M - H_2O + Cl)^-$. The remaining fragment ions for both sugars corresponded to the loss of one or more discrete sugar units from either end of the oligosaccharide chain. All cleavages involved glycosidic linkages and only Cl^- adduct ions were intense enough to be recorded. A comparison of the spectra of olgose and its degradation product, evertetrose, confirmed the molecular weight of the parent compound as well as the component sugar units. More importantly, the sequence of the sugar units could be confirmed just from the spectra of the two underivatized sugars. The sample size required was so small that analytical t.l.c. spots could be adequate for obtaining mass spectral data.

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[1] Sugar-containing antibiotics occupy an important place in clinical therapy. One of us (A.K.G.) has been involved in the structure elucidation of oligosaccharide antibiotics, *e.g.* everninomicins; see A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Amer. Chem. Soc.*, 1975, **97**, 1982.

[2] H. P. Faro, A. K. Ganguly, and D. H. R. Barton, *Chem. Comm.*, 1971, 823.

[3] R. C. Dougherty, J. Dalton, and F. J. Biros, *Org. Mass. Spec.*, 1972, **6**, 1171; R. C. Dougherty, J. D. Roberts, and F. J. Biros, *Analyt. Chem.*, 1975, **47**, 54; D. F. Hunt, G. C. Stafford, F. W. Crow, and J. W. Russell, *ibid.*, 1976, **48**, 2098.

[4] *Cf.* A. L. C. Smit and F. H. Field, *J. Amer. Chem. Soc.*, 1977, **99**, 6471, and references cited therein.

[5] A. L. C. Smit, M. A. J. Rossetto, and F. H. Field, *Analyt. Chem.*, 1976, **48**, 2042.

[6] H. Fujiwara, B. N. Pramanik, and A. K. Bose, paper presented at the 26th International Congress Pure and Applied Chem., Tokyo, September 1977.

[7] Chloride ion attachment negative ions were first studied by Dougherty *et al.* (H. P. Tannenbaum, J. D. Roberts, and R. C. Dougherty *Analyt. Chem.*, 1975, **47**, 49) using CH_2Cl_2 as the reagent gas. Under the usual C.I.M.S. conditions HCl_2^- and $CH_2Cl_3^-$ ions are also produced besides Cl^- ions.