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FAST-ATOM-BOMBARDMENT MASS-SPECTROMETRY FOR CARBOHYDRATE-STRUCTURE DETERMINATION

ANNE DELL, HOWARD R. MORRIS,

Department of Biochemistry, Imperial College of Science and Technology, London SW7 2A2 (Great Britain)

HEINZ EGGE, HUBERTUS VON NICOLAI,

Institut für Physiologische Chemie, Universität Bonn, Nussallee 11, D-5300 Bonn (Federal Republic of Germany)

AND GÉRARD STRECKER

Laboratoire de Chimie Biologique et Laboratoire Associé au CNRS, Université des Sciences et Techniques de Lille I, F-59650, Villeneuve d'Asq (France)

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ABSTRACT

The potential of fast-atom-bombardment (f.a.b.) mass-spectrometry in the carbohydrate field was assessed with the aid of unmodified, permethylated, and peracetylated oligosaccharides and glycosphingolipids. F.a.b. spectra are presented for (D-Gal→D-GlcNAc)$_5$→(D-Man)$_3$→D-GlcNAc, permethylated (D-Gal→D-GlcNAc)$_5$→(D-Man)$_3$-D-[^{2}H]GlcNAcol, permethylated gangliotetraosylceramide, and reduced and peracetylated tetra- to hepta-saccharides from human milk. From unmodified oligosaccharides, pseudomolecular ions ($M + Na^+$) were obtained as major ions in the high-mass range. Permethylated oligosaccharides and glycosphingolipids gave pseudomolecular ions ($M + H^+$) of high intensity, together with fragment ions of high diagnostic importance. At ambient temperature, f.a.b. spectra could only be obtained for the lower homologs of per-*O*-acetylated oligosaccharides. Reduced and peracetylated penta- to hepta-saccharides from human milk gave f.a.b. spectra only after heating of the target.

INTRODUCTION

Mass spectrometry (m.s.) is one of the key techniques used in the structure determination of carbohydrates and glycolipids; for example, electron impact (e.i.) and chemical ionization (c.i.) methods are now applied routinely for the identification of low-molecular-weight compounds obtained by methylation analysis[1]. In recent years, m.s. procedures have found a wider application in the structure determination of oligosaccharides and glycolipids having much higher molecular weights[2–5]. This was made possible largely as a result of instrumental developments allowing high sensitivity at high mass[6], and improvements in sample handling enabling the acquisi-

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tion of e.i. mass spectra[2,3] from suitable derivatives that have molecular weights in excess of 3000. E.i.m.s. of these high-mass substances does, however, suffer from some very serious drawbacks, notably the loss in signal intensity at high mass, which often results in the absence of the molecular ion and high-mass, sequence ions. The method can be improved by combining e.i.m.s. data with the results of field-desorption (f.d.) studies. The latter method has recently been used with considerable success for the molecular-weight determination of several carbohydrates and glycolipids[2,5]. Field desorption mass spectrometry (f.d.m.s.) is potentially attractive because underivatized samples can be examined at the μg level. However, the technical difficulties associated with f.d.m.s. have limited its use and it is unlikely that the method will become more widely exploited. For this reason, we view the new development of fast-atom-bombardment mass-spectrometry (f.a.b.m.s.) as being of considerable importance for carbohydrate-structure elucidation[8]. F.a.b. spectra are relatively easy to acquire, and early reports have shown that f.a.b.m.s. is capable of obtaining both molecular weights and fragment data from small quantities of polar, biological substances. It is likely that f.a.b.m.s. will complement e.i.m.s. and c.i.m.s. and replace f.d.m.s. for many applications. In order to assess f.a.b.m.s. for the structural analysis of biological substances, we describe herein the results obtained from representative examples of permethylated, peracetylated, and unmodified carbohydrates and glycolipids.

EXPERIMENTAL

Materials. — Gangliotetraosylceramide (2 mg), prepared from G_{M1} ganglioside by partial acid hydrolysis[9], was permethylated essentially by the method of Hakomori[10]. The excess of dimethyl sulfoxide was removed by passage through a column (1 × 20 cm) of Sephadex LH-20 with 1:1 (v/v) acetone–methanol as eluent.

The oligosaccharides from the urine of a patient having G_{M1} gangliosidosis were prepared by a combination of methods essentially as previously described[11]. Neutral, human-milk oligosaccharides were separated from the sialic acid-containing compounds by ion-exchange chromatography on DEAE-Sephadex A-25 as previously described[12]. After reduction with sodium borohydride and per-*O*-acetylation, the peracetylated oligosaccharides were separated by chromatography on Iatrobeads, followed by reversed-phase h.p.l.c.[13].

Mass spectrometry. — Fast-atom-bombardment, mass spectrometry was performed with a VG Analytical ZAB 1F, reverse-geometry, mass-spectrometer fitted with an f.a.b. source and an Ion-Tech atom gun. Spectra of samples of molecular weight >2000 were obtained with a ZAB 1 F mass spectrometer fitted with a high-field magnet giving a mass range of 3000 mass units (m.u.) at 8 kV. The samples were dissolved in a suitable solvent (water, methanol, and chloroform for unmodified, acetylated, and permethylated samples, respectively) and the sample (~5 μg in 0.5 μL) was added to a drop of glycerol on the stainless-steel target. The target was bombarded with argon or xenon atoms having 8 keV energy. The spectra were ob-

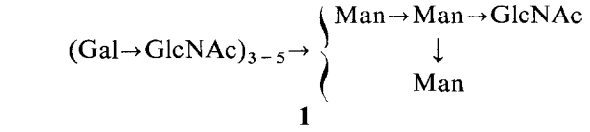
tained with a Hall-probe, controlled-linear-mass scan of 500 s duration for a full scan from 3000 to 12 m.u. The spectrum of the permethylated tetradecasaccharide obtained from G_{M1} gangliosidosis urine was recorded at 7-kV acceleration voltage. The spectra were recorded on u.v.-sensitive chart paper and calibrated manually by counting. All data on unmodified and permethylated samples were acquired with the sample and ion-source at ambient temperature. F.a.b. spectra were obtained for several of the sugar acetates after supplying a 2-A heating current to the target with the standard ZAB direct-chemical-ionisation heating unit. Under these conditions, the glycerol matrix evaporated rapidly, and several additions of glycerol to the dried sample on the target were normally required in order to obtain a complete spectrum. No significant thermal decomposition of the sample was observed under these conditions.

Field-desorption, mass-spectrometry of the sugar acetates was performed with a Kratos MS 50 mass-spectrometer fitted with a high-field magnet[6]. The spectra were obtained on u.v.-sensitive chart paper and calibrated with a crystal time-marker by comparison with the e.i. spectrum of Fomblin oil. The samples were loaded by dipping the high-temperature-activated, carbon emitter into one drop of a solution of the sample placed on a strip of aluminium foil.

Electron-impact, mass spectrometry[14] of the permethylated gangliotetraosylceramide was performed with an LKB 9000 mass spectrometer.

RESULTS AND DISCUSSION

Unmodified oligosaccharides. — A series of unmodified homologous or isomeric oligosaccharides having the general structure **1** gave f.a.b. spectra that contained the



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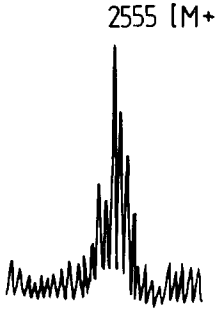
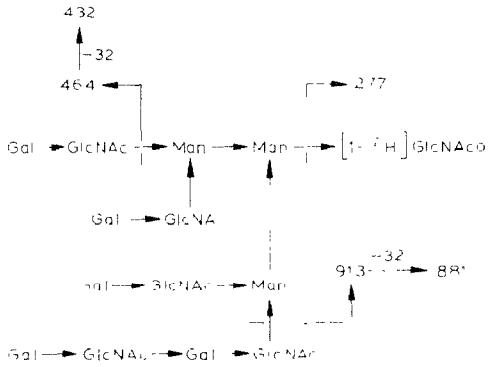


Fig. 1. Partial f.a.b. mass spectrum of the region of the molecular ion of the unmodified tetradecasaccharide isolated from the G_{M1} gangliosidosis urine. For $(M + Na)^+$, the nominal mass is given.

$M + Na^+$ ions. Thus, for the corresponding deca-, dodeca-, and tetradeca-saccharides, pseudomolecular ions were obtained at *m/z* 1825, 2190, and 2555, respectively. The region of the molecular ion of the tetradecasaccharide isolated from G_{M1} gangliosidosis urine is shown in Fig. 1. No fragment ions pertinent to the carbohydrate sequence could be observed.

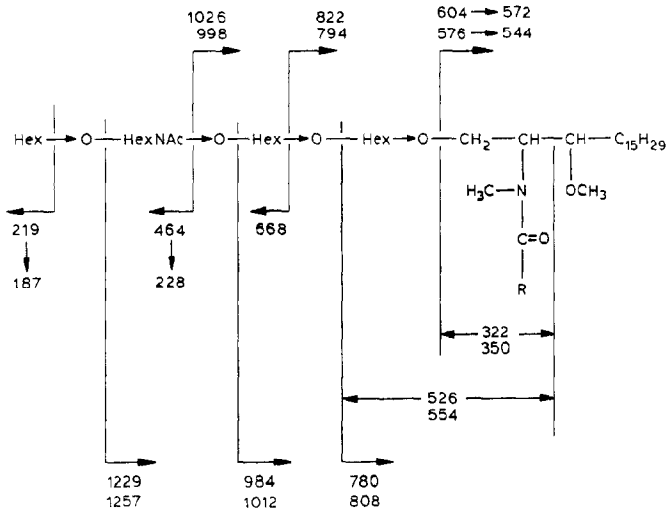
Permethylated oligosaccharides and glycolipids. — All of the permethylated samples that were examined yielded spectra containing abundant molecular-ions and



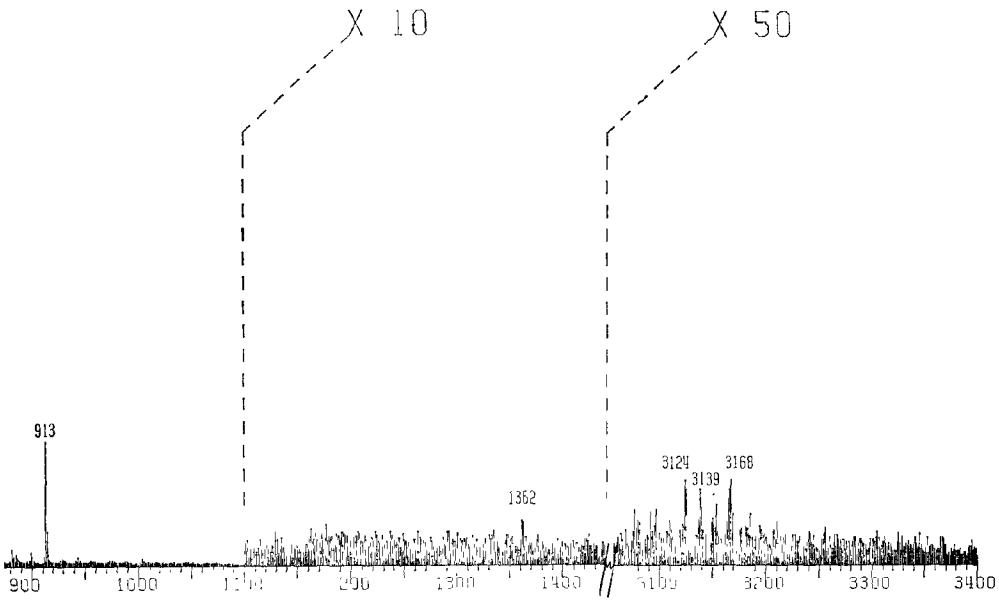
Scheme 1. F.a.b. fragments of a reduced, permethylated tetradecasaccharide isolated from a G_{M1} gangliosidosis urine

Fig. 2. F.a.b. mass spectrum of a reduced, permethylated urinary tetradecasaccharide. In the

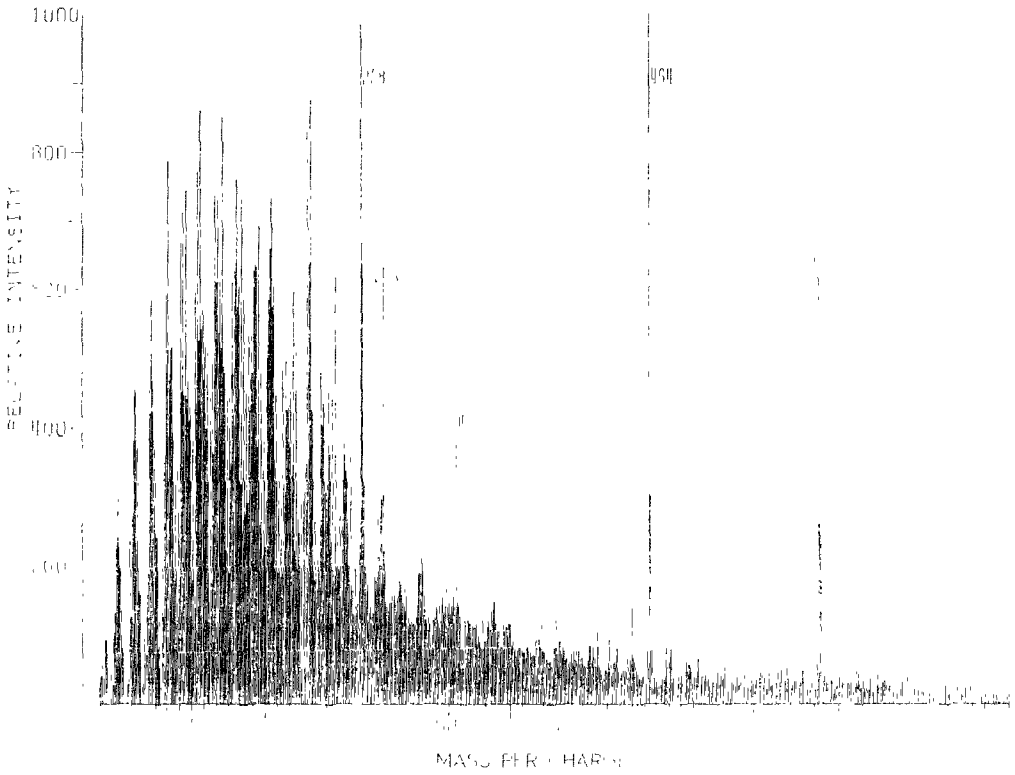
a considerable number of fragment ions. The f.a.b. mass spectrum of the reduced, permethylated tetradecasaccharide (Scheme 1) is shown in Fig. 2. In accordance with the calculated M_r 3166.63 for $^{12}C_{141}{}^{2}H_{11}H_{251}{}^{14}N_6{}^{16}O_{71}$, a pseudomolecular ion was found at M + 1 3168. Among the fragment ions, the lactosamine residues



Scheme 2 Structure and e.i. fragmentation pattern of permethylated glycolipid 2



molecular-ion region, the nearest round numbers of the exact masses are indicated.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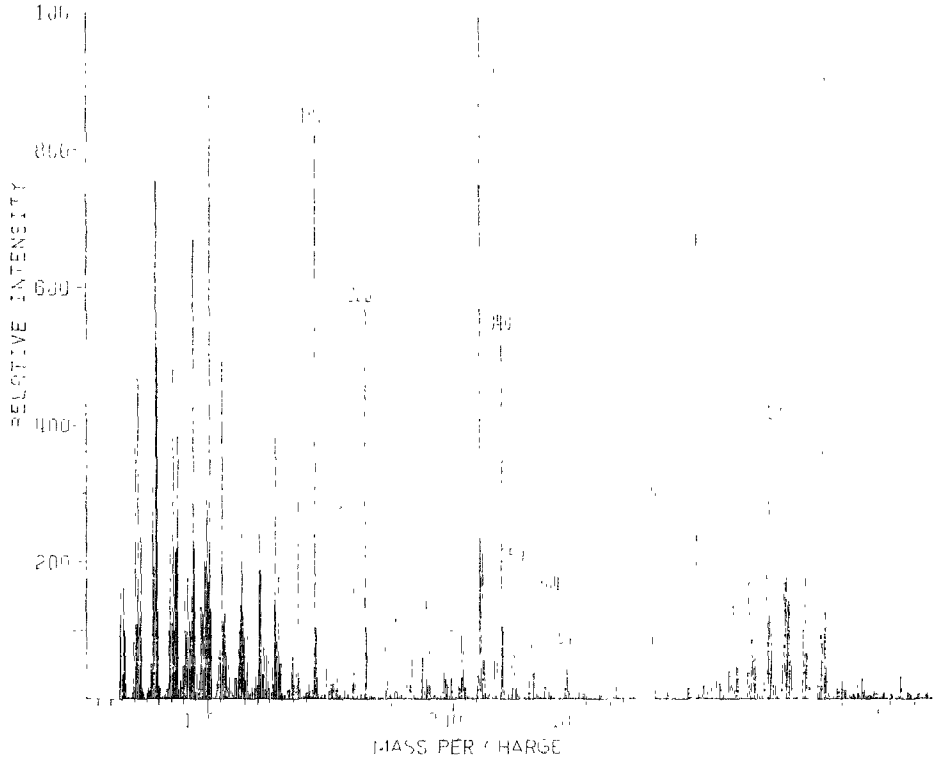
MASS PER CHARGE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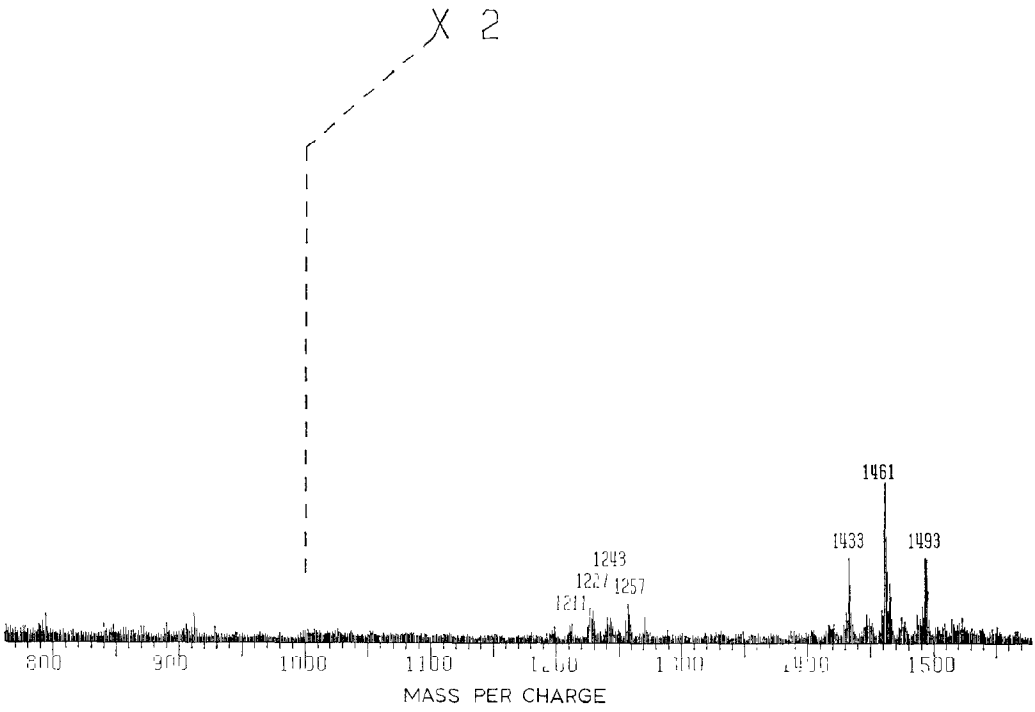
MASS PER CHARGE


X 2
1461
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1243
1227
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1211
800
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1000
1100
1200
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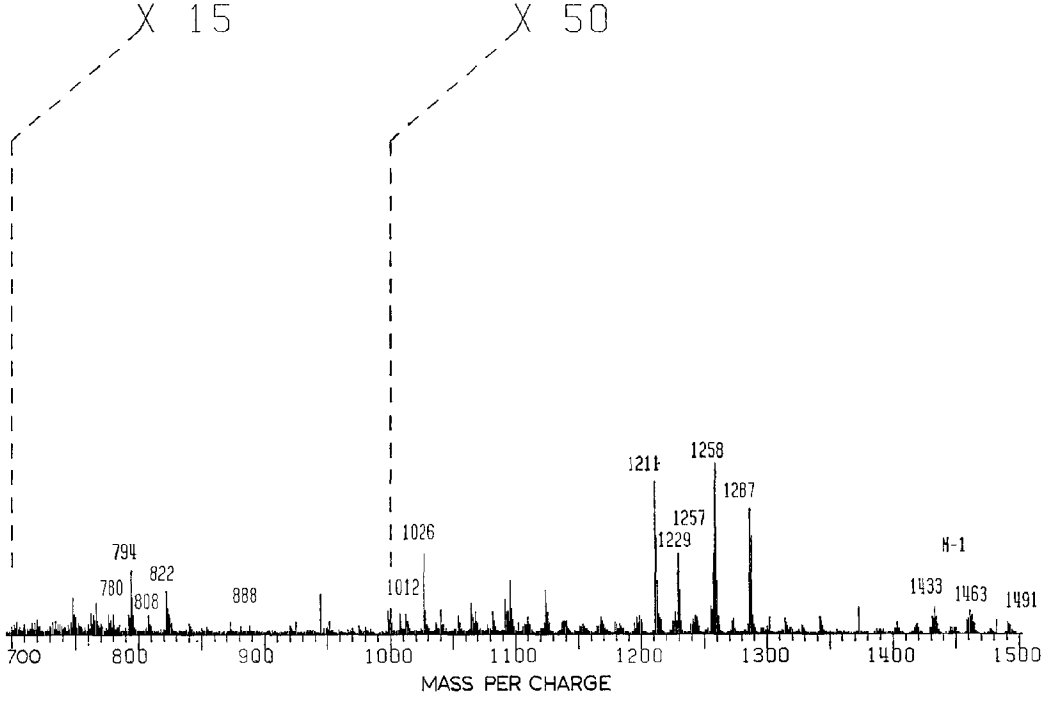
1400
1500
MASS PER CHARGE


X 15
X 50
794
780
822
808
888
1026
1012
1211
1258
1287
1257
1229
M-1
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1463
1491
700
800
900
1000
1100
1200
1300
1400
1500
MASS PER CHARGE

m/z 464 and *m/z* 913 clearly preponderate (Scheme 1). The intensity of the ion at *m/z* 668 corresponding to a Gal→GlcNAc→Gal$^+$ fragment is only twice that of the background level produced by glycerol. The ion at *m/z* 1362 indicates that, in addition to a tetrabranched molecule, the sample also contains a tribranched isomer having one $(Gal\rightarrow GlcNAc)_3$ chain. Thus, the facile cleavage and the ion-stabilized, aminoglycoside linkages may be used to monitor heterogeneity of the oligosaccharide.

The glycolipid **2** (see Scheme 2) was selected to illustrate the behavior of permethylated glycosphingolipids under f.a.b. conditions. For comparison, both e.i. and f.a.b. spectra of this compound are given in Fig. 3. E.i. fragmentation pathways are shown on Scheme 2 and are consistent with those previously described for this type of molecule[15]. Fragmentation of the molecule is extensive despite the use of low-energy electrons (20 eV) for ionisation, and the molecular-ion region is very weak (note the ×50 multiplication factor >*m/z* 1000). In contrast, abundant molecular-ion species are present in the f.a.b. spectrum at *m/z* 1493 $(M + H)^+$, 1465 $(M + H)^+$, 1461 (*m/z* 1493 − methanol), and 1433 (*m/z* 1465 − methanol). The major fragment ions observed in the f.a.b. spectra may be attributed to the ceramide residue (*m/z* 576 and 604) and to cleavage at the amino sugar (*m/z* 464 and 228).

Per-O-acetylated oligosaccharides. — After reduction with sodium borohydride and peracetylation, the neutral, L-fucose-containing fraction of human-milk oligosaccharides was separated into more than 50 components. The composition of each component was readily established from its molecular weight, as each residue (Gal, Glcol, GlcNAc, or Fuc) contributes a specific mass increment: for example, reduced and acetylated lacto-*N*-tetraose has a M_r of 1297, and the mass increments are 230, 287, and 288 m.u. for Fuc, HexNAc, and Hex, respectively. For characterizing these substances, procedures using f.d.m.s. have been developed to establish the M_r values of the alditol acetates, and subsequent e.i.m.s. of the deacetylated, permethylated samples to ascertain the sequence[13]. Because of the potential of f.a.b.m.s. for providing both M_r and sequence data in a single experiment, it appeared to be an attractive alternative to combined f.d.–e.i.m.s. analysis. Therefore, the analysis of alditol

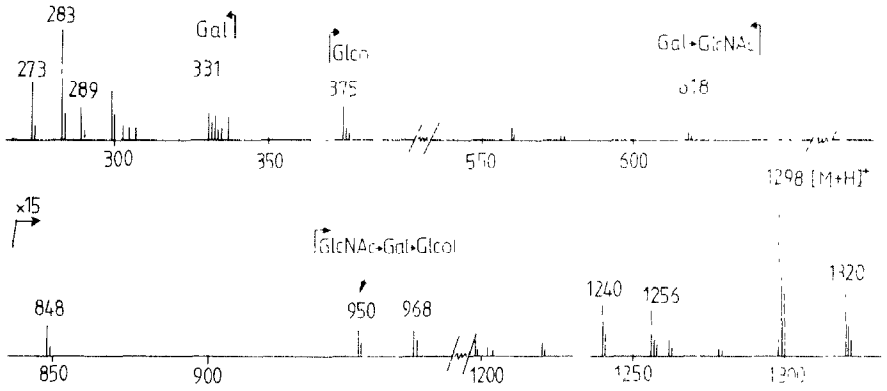
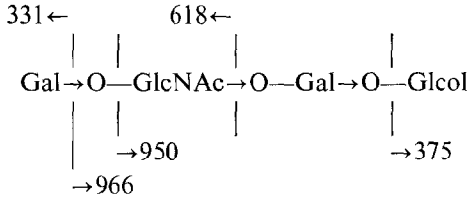


Fig. 4. F.a.b. spectrum of reduced, peracetylated lacto-*N*-tetraose (**3**).

acetates by f.a.b.m.s. was attempted. In contrast to the successful studies of unmodified and permethylated oligosaccharides, all but the smallest member of the family, namely lacto-*N*-tetraose (**3**) were difficult to study. This compound gave an excellent f.a.b. spectrum (Fig. 4). Abundant molecular-ions were present at *m/z* 1298 $(M + H)^+$ and 1320 $(M + Na)^+$, these were accompanied by signals at 42 and 58 m.u. lower, a pattern that was also observed for all sequence-ions from the nonreducing end. The fragmentation of the molecule is described in Scheme 3. The ions at *m/z* 273, 289, and 848 (Fig. 4) are derived from an L-fucose-containing impurity.



Scheme 3. Structure of lacto-*N*-tetraose (**3**) and fragmentation pattern of per-*O*-acetylated derivative.

The next higher homolog, fucopentaose I (M_r 1527) did not give an f.a.b. spectrum when examined at ambient temperature. In contrast, difucohexaose I, which was shown by f.d.m.s. to consist of a mixture of fully acetylated and monodeacetylated derivatives (see Fig. 5), gave an excellent f.a.b. spectrum corresponding to the partially acetylated species (see Fig. 6) with abundant molecular-ions at *m/z* 1716 $[(M - Ac) + H]^+$. No signal arising from the fully *O*-acetylated carbohydrate residue was obtained from the sample bombarded at ambient temperature. After heat had been applied to the target with the heating unit, supplied for direct c.i.m.s. studies, of the ZAB mass spectrometer, the successful ionization of the carbohydrate residue was finally achieved. The results obtained with a 2-A current supplied to the target are shown in Fig. 6. The protonated molecular-ion at *m/z* 1758 was obtained immediately after the sample had been heated, and subsequent scans contained the cationized species at *m/z* 1780 $(M + Na)^+$. The spectrum lasted until glycerol had

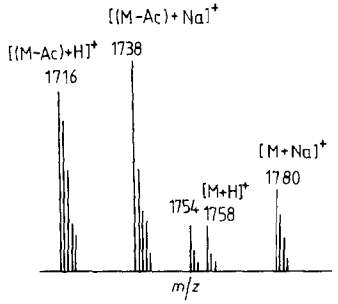


Fig. 5. F.d. spectrum of reduced, acetylated difucohexaose I showing the presence of a monodeacetylated derivative.

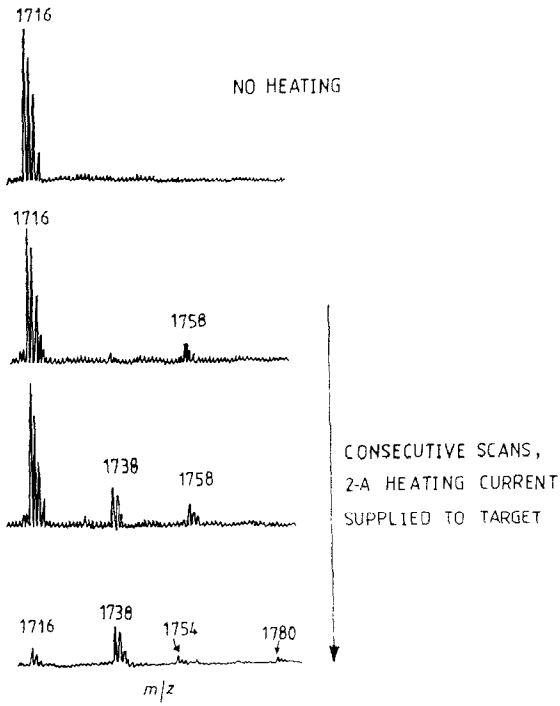


Fig. 6. F.a.b. spectrum of the molecular-ion region of reduced, peracetylated difucohexaose I from human milk showing the influence of target heating; *m/z* 1716 is derived from the mono-deacetylated derivative.

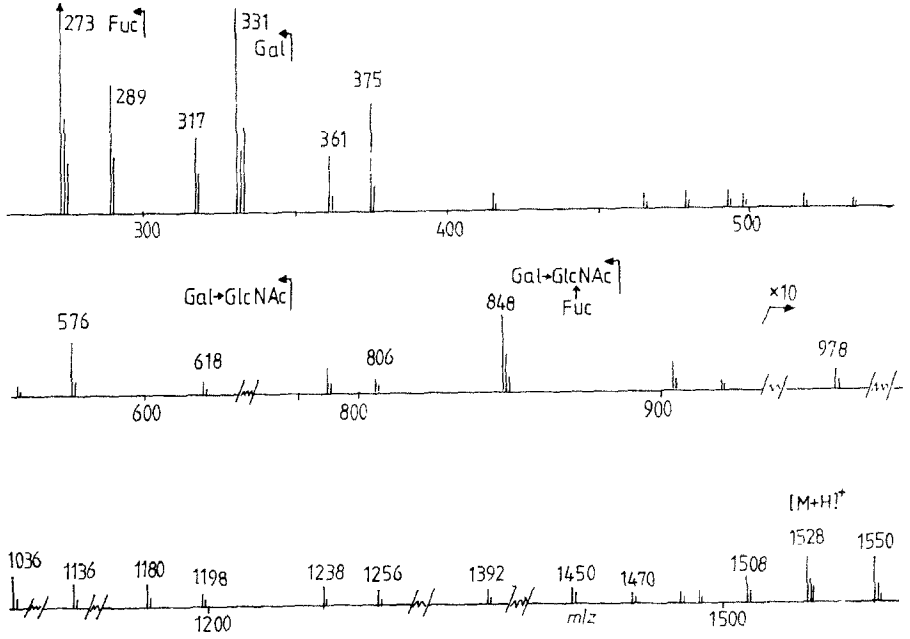


Fig. 7. F.a.b. spectrum of reduced, peracetylated fucopentaose (**4**).

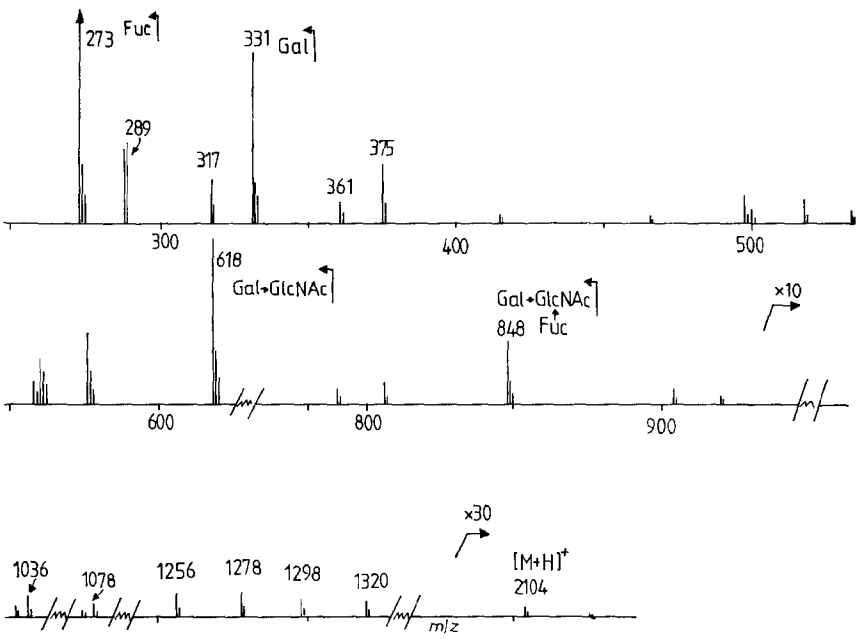
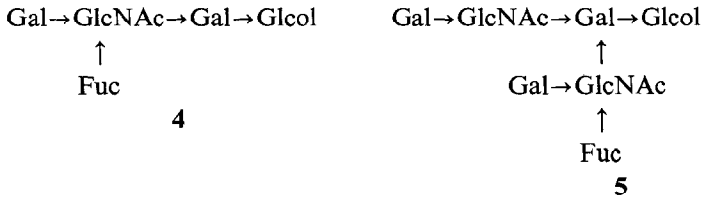


Fig. 8. F.a.b. spectrum of reduced, peracetylated fucosyllacto-*N*-hexaose (**5**).

completely evaporated (30 s–1 min) and could be regenerated by adding glycerol to the dried sample, on the target, and reapplying the heating current.

By use of this heating procedure, excellent spectra for a variety of fully acetylated, neutral milk sugars were obtained, and typical results are shown in Figs. 7 and 8 for a fucopentaose [$(M + H)^+$, m/z 1528] and fucosyl lacto-*N*-hexaose [$(M + H)^+$, m/z 2104], respectively. The latter sample was contaminated with lacto-*N*-tetraose [$(M + H)^+$, m/z 1298]. All samples fragmented at the glycoside linkages to yield sequence ions from both the reducing and nonreducing ends.

The spectra shown in Figs. 7 and 8 are in agreement with the simplified structures **4** and **5**, respectively.



In conclusion, the results described herein demonstrate that the f.a.b. technique is applicable to unmodified oligosaccharides and their derivatives, and to glycosphingolipids having an M_r of at least 3000. In all cases, the M_r data were readily

obtained on samples weighing 1–5 μg. A limited number of fragment ions were observed in the f.a.b. spectra of unmodified oligosaccharides, and examination of permethylated derivatives is recommended in order to obtain fragment ions of high diagnostic-value.

REFERENCES

1 H. RAUVALA, J. FINNE, T. KRUSIUS, J. KÄRKKÄINEN, AND J. JARNEFELT, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 389–416.
2 P. HANFLAND, H. EGGE, U. DABROWSKI, S. KUHN, D. ROELCKE, AND J. DABROWSKI, *Biochemistry*, 20 (1981) 5310–5319.
3 M. E. BREIMER, G. C. HANSSON, K.-A. KARLSSON, H. LEFFLER, W. PIMLOTT, AND B. E. SAMUELSSON, *FEBS Lett.*, 124 (1981) 299–303.
4 P. FREDMAN, J.-E. MANSSON, L. SVENNERHOLM, B. E. SAMUELSSON, I. PASCHER, W. PIMLOTT, K.-A. KARLSSON, AND G. W. KLINGHARDT, *Eur. J. Biochem.*, 116 (1981) 553–564.
5 M. LINSCHLID, J. D'ANGONA, A. L. BURLINGAME, A. DELL, AND C. E. BALLOU, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 1471–1475.
6 H. R. MORRIS, A. DELL, AND R. A. MCDOWELL, *Biomed. Mass Spectrom.*, 8 (1981) 463–473.
7 M. BARBER, R. S. BORDOLI, R. D. SEDGWICK, AND A. N. TYLER, *Nature (London)*, 293 (1981) 270–273.
8 L. S. FORSBERG, A. DELL, D. J. WALTON, AND C. E. BALLOU, *J. Biol. Chem.*, 257 (1982) 3355–3363
9 R. KUHN AND H. EGGE, *Chem. Ber.*, 96 (1963) 3338–3348.
10 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
11 G. STRECKER AND J. MONTREUIL, *Biochimie*, 61 (1979) 1199–1246.
12 H. VON NICOLAI, H. E. MULLER, AND F. ZILLIKEN, *Hoppe Seyler's Z. Physiol. Chem.*, 359 (1978) 393–398.
13 H. EGGE, J. DABROWSKI, P. HANFLAND, H. VON NICOLAI, U. DABROWSKI, AND A. DELL, *Proc. Int. Symp. Glycoconjugates, 6th*, (1981) 318–319.
14 P. HANFLAND AND H. EGGE, *Chem. Phys. Lipids*, 16 (1976) 201–214.
15 H. EGGE, *Chem. Phys. Lipids*, 21 (1978) 349–360.