Prog. Lipid Res. Vol. 30, No. 4, pp. 301–322, 1991
Printed in Great Britain.

0163-7827/91/$0.00 + 0.50

FAB/MASS SPECTROMETRY OF LIPIDS

TOSHIKO MATSUBARA and AKIRA HAYASHI
Department of Chemistry, Faculty of Science and Technology, Kinki University, 3-4-1, Kowakae, Higashiosaka 577, Japan

CONTENTS

ABBREVIATIONS

GC-MS	mass spectrometry combined with gas chromatography
Cf-PDMS	Cf-plasma desorption mass spectrometry
SIMS	secondary ion mass spectrometry
FAB/MS	fast atom bombardment mass spectrometry
LDMS	laser desorption mass spectrometry
CAD	collisionary activated dissociation
TLC	thin layer chromatography
HPLC	high performance liquid chromatography
CF	continuous flow
PC	phosphatidylcholine
SM	sphingomyelin
PS	phosphatidylserine
PI	phosphatidylinositol
PE	phosphatidylethanolamine
PAF	platelet activating factor
SEA	sphingoethanolamine
PnSL	phosphonosphingolipids
CAEPn	ceramide aminoethylphosphonate
AEPn	aminoethylphosphonic acid
MAEPn	*N*-methyl derivative of AEPn
CMAEPn	*N*-methyl derivative of CAEPn
PnGSL	phosphonoglycosphingolipid

I. INTRODUCTION

Since the 1960s, when Beynon,[1] McLafferty[2] and Budzikiewicz *et al.*[3] established the fundamental theory of mass spectrometry for application to organic chemistry, mass spectrometry has become one of the important analytical tools for the structural determination of organic compounds. Especially useful is mass spectrometry combined with gas chromatography (GC–MS) using Ryhage's jet separator[4] for elucidating the structures of many lipids. In the GC–MS method, lipids are first hydrolyzed to their constituent fatty acids, long chain bases and water-soluble components, and then each component is derivatized to the volatile compound and separated by GC followed by analysis by MS. This method remains the most useful technique for analyzing component sugars and methylated sugars, which is essential for structure determination of the sugar chain and for determining the double bond position and branching point of long chain bases and fatty acids that are the ceramide portion of complex lipids. However, this method is restricted by the molecular size of the lipids analyzed and could not be used for intact lipids.

In the latter half of the 1970s, direct ionization of surface molecules with high energy particles, called particle-induced desorption ionization,[5] was developed. It is suitable for the analysis of high-molecular-weight, polar or thermally labile compounds and has been developed in many forms, ^{252}Cf-plasma desorption mass spectrometry (^{252}Cf-PDMS),[6] secondary ion mass spectrometry (SIMS),[7] fast atom bombardment mass spectrometry (FAB/MS)[8] and laser desorption mass spectrometry (LDMS).[9] These soft ionization methods are applied to lipid analysis.[10–14] FAB/MS and SIMS are used most often for the analysis of middle-molecular-weight compounds such as complex lipids.

An important characteristic of mass measurements for FAB and SIMS is the use of a liquid phase (e.g. glycerol), called the matrix. A "liquid drop", consisting of sample solution and the matrix, is bombarded with the fast beam, and the secondary ion produced during the sputtering process is measured. The difference between SIMS and FAB/MS is what is used as the fast beam, in SIMS, it is a cation beam, such as Ar^+ or Xe^+ (3–5 keV), while in FAB/MS, it is an electrically neutral atom beam, such as Ar^0 or Xe^0 (3–8 keV). However, the energy levels of the fast beam are about the same, and no significant difference can be recognized between the FAB/MS and SIMS spectra obtained. Therefore, in this review, SIMS and FAB/MS are dealt with in the same way.

As $[M+H]^+$, $[M-H]^-$ and fragment ions in FAB/MS can be observed for several minutes or more by using the matrix, a linked scan technique can be used for the specific ion to clarify the fragmentation pathway. The related FAB/MS/MS method is also used, in which the ions produced are separated by one mass spectrometer, the specific ion is selected and activated by collision, and then the daughter ions are analyzed by a second mass spectrometer. Some interfaces for the direct coupling of liquid chromatography (LC) and FAB/MS have been developed[15,16] and the convenient TLC/FAB/MS method was designed for the direct analysis of spots on a TLC plate without elution.[17]

At present, lipids are analyzed by mass spectrometry in their intact form without hydrolysis or derivatization. This review summarizes how FAB/MS is used in lipid research.

II. METHODS

A. *FAB/MS*

The main features of FAB/MS are shown schematically in Fig. 1. As an atom beam was used as the primary beam, an Ar^+, Xe^+ beam produced from the atom beam in the primary ionic chamber was introduced to the collision chamber which was filled with Ar and Xe gases, and Ar^+ and Xe^+ were changed to Ar^0 and Xe^0 by charge exchange, with the resulting neutral Ar^0 and Xe^0 being used as the bombarders. Both positive and negative ions are produced as secondary ions during the sputtering process.[8] A matrix is required for this analysis. The sample must be completely dissolved in the matrix to make a clear

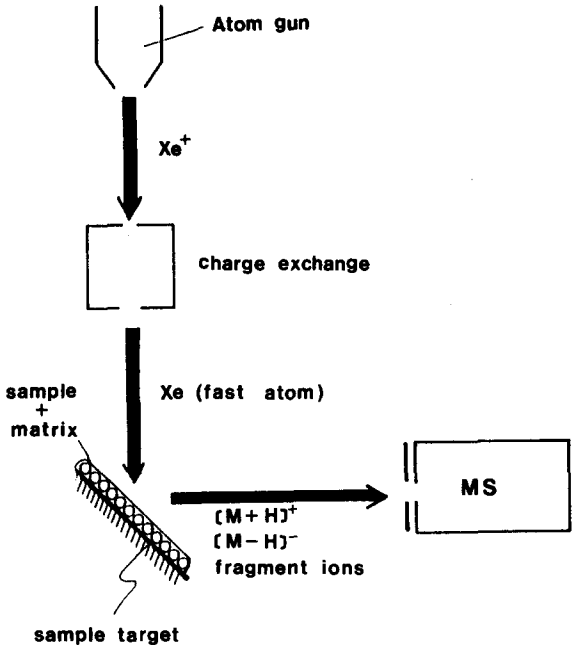


FIG. 1. Principle of FAB ionization.

solution, in order to obtain good spectra. For the lipid solvent, chloroform–methanol or methanol–glacial acetic acid mixtures are usually used and many kinds of matrices have been tried with different lipids (see Table 1).

B. *FAB/MS/MS*

Collisionary activated dissociation (CAD) mass spectrometry is a new field developed by McLafferty.[18] The ions which are associated with the molecular weight or fragment ion are selected in the first mass spectrometer MS-1. They collide with an inert gas such as helium in a collision cell located in the field-free region between MS-1 and the second mass spectrometer, MS-2. Finally, the product ions are analyzed in MS-2. The advantage of FAB/MS/MS is that collision spectra of the individual components from mixtures can be obtained, and it is possible to exclude most of the obstructive signals derived from the matrix in order to interpret the fragment ions. Therefore, this method can be used to characterize the important ions and examine the fragmentation pathway of pertinent ions.

C. *Linked Scan MS*

In a mass spectrometer with an E (electric field) B (magnetic field) or BE type arrangement, characteristic spectra by linked scan MS are obtained when an ion m_1^+ subjected to an accelerating voltage is decomposed to m_2^+ and m_3^+ before entering the electric field, and both magnetic and electric fields are scanned under fixed condition.[19] A B/E constant scan yields spectra of all m_2^+ (daughter ions) produced from m_1^+, whereas a B^2/E constant scan produces spectra of the m_1^+ (parent ion) produced from a specific m_2^+ ion. Combining this method and the FAB ionization method is effective for studying fragmentation.

D. *TLC/FAB/MS*

Efforts have been made to combine SIMS and TLC. SIMS is suitable for analyzing high-molecular-weight, non-volatile and thermolabile compounds, and TLC is a simple and powerful separation method. Complex mixtures of lipids are separated on a TLC plate

TABLE 1. Matrices used for FAB Mass Spectrometry of Lipids

Matrix	MW	b.p. (°C)	Proton affinity (kJ/mol)	Lipids (references)
Glycerol (G)	92.09	290	828	PC (21, 22, 24, 34, 37), PE (21, 22, 24, 37), LPC (21), PS (21), PG (21, 24), PA (24, 34), PI (34), PAF (39, 40, 42), lyso-PAF (43), ether lipid (44), CMAEP (45), CAEP (45), CMH (51, 58), CDH (51), CTH (51), Glob (51), CSE (79), GM1 (52, 58), GM2 (79), GM3 (50, 72, 79), asiloGM1 (50, 58), asialoGM2 (51), GD1a (58, 79), GD1b (58), psychosine (63), Cer (86)
Triethanolamine (TEA)	149.19	170	975	PC (23, 26, 28, 38), PE (36), LPC (26), PS (23), PG (36), PI (36), PAF (36, 41), SM (26, 28), CAEP (45), CMAEP (45), CMH (50, 70), CDH (50, 53), CTH (50, 53, 70), Glob (50), Forss (50), GM1 (50, 53, 63), GM2 (53, 70), GM3 (50, 53, 72), asialoGM1 (50, 53), asialoGM2 (50, 53, 63), 9-0-Ac-GD3 (73), Gal-6GL (57), sulfoGL (49, 82), phosphonoGL (57, 85)
Tetramethyl urea (TMU)	116.6	176.5		Gal-6GL (57)
TEA + TMU				CDH (53), CTH (53, 54), Glob (54), Forss (54), GM1 (53, 71, 80), GM1b (74), GM2 (53, 71), GM3 (50, 53, 54, 71, 72), asialoGM1 (53, 71), asialoGM2 (53, 71), GD1a (52, 54, 72), GD1b (54, 72), GT1a (54, 72), GT1b (54, 72), ether lipid (44)
Diethanolamine (DEA)	105.14	183	954	PC (25, 34), PE (25, 34, 35), PS (34), PI (34), SM (34), LPC (34), LPE (34), LPS (34), DPG (35)
3-Nitrobenzyl alcohol (NBA)	153.14	175–180 (3 mmHg)		PC (25, 26, 28), PE (25), LPC (26), SM (26, 28), CAEP (63), CMAEP (63), phosphonoGL (85), permethylated ganglioside (77), Cer (86)
Dithioerythritol + dithiothreitol	154.25 154.25	m.p. 82–84 125–130 (2 mmHg)		PE (36), CAEP (46), CMAEP (46)
Thioglycerol (TG)	108.16	118 (5 mmHg)		PC (22), PE (22), GM1 (75), FucGM1 (75), GM3 (75), GD1a (75), GD1b (75), FucGD1b (75), GT1b (75), GQ1b (75), permethylated sulfoGL (81)
TG + G-15-Crown-5	220.27	100–135 (0.2 mmHg)		Permethylated ganglioside (77)
TG + G				PE (33), permethylated ganglioside (78)

and then analyzed directly on the plate by SIMS without elution from the TLC plate. An aluminum- or plastic-backed silica gel TLC plate is used for good separation and easy attachment to the secondary ion mass spectrometer probe.[17,20] After separation of the sample on the TLC plate, the area of interest on the plate is cut out as a small piece, which is then attached to the ion target with a strip of double-faced masking tape and then mixed with a suitable solvent and matrix. For more convenience, the so-called scanning TLC/SIMS apparatus or scanning TLC/FAB/MS apparatus can used. This enables direct bombardment of each chromatogram with fast Xe^+ ions or Xe atoms without being cut from the TLC plate.

E. *HPLC/FAB/MS*

Combined use of FAB/MS and high performance liquid chromatography (HPLC) has also been expected to be useful. FAB/MS and SIMS are suitable for the analysis of

high-molecular-weight, polar or thermally labile compounds, while HPLC has superior chromatographic separation efficiency. Attempts have been made to develop a suitable interface for HPLC/FAB/MS. One is the moving belt method in which the test solute is ionized in a liquid matrix such as glycerol. However, the results have not been good and samples separated by HPLC are usually collected as a single peak for FAB/MS analysis. Some recent methods include micro HPLC/FAB/MS[15] and continuous flow (CF) FAB/MS[16] using fused-silica or an open tubular capillary tube and a stainless-steel frit. Glycerol, premixed in the mobile phase, serves as the matrix of FAB ionization. The mobile phase solvent is immediately vaporized on the surface of the frit, while the solute and matrix are left on the surface and subjected to bombardment by the Xe or Ar beam.

III. APPLICATION TO STRUCTURAL ASSIGNMENT

A. *Phospholipids*

1. *Glycerophospholipids*

Naturally occurring lipids are usually mixtures of multiple molecular species and their mass spectra are complex. Among the glycerophospholipids, phosphatidylcholine (PC) has been a popular choice for study,[21–27] because PC with a known structure can be prepared easily from egg yolk and soy beans and a synthetic one having simple molecular species with component fatty acids of known kind and position, is commercially available. FAB mass spectra of dipalmitoylglycerylphosphocholine and 1-palmitoyl-2-stearoylglycerophosphocholine are shown in Fig. 2.

The molecular weight of glycerophospholipids is usually determined from the m/z value of both $[M + H]^+$ ions and $[M + Na]^+$ ions, which appear in the positive ion mode and $[M - H]^-$ ions which appear in the negative ion mode. In the case of PC, $[M + H]^+$ ions are detected in the positive ion mode, but $[M - H]^-$ ions are not obtained in the negative ion mode.[23,24,26] Instead, triplet ions of $[M - 15]^-$, $[M - 60]^-$ and $[M - 86]^-$ appear as ions indicating molecular weight. They have been assigned structures of $[M - CH_3]^-$, $[M - NH(CH_3)_3]^-$ and $[M - CH_2 = CHN(CH_3)_3]^-$ by measurement of their exact mass numbers and the MS/MS of each triplet ion. The origin of the triplet ion was found to be the matrix-ion adduct to the target molecule, using the B^2/E linked scan technique.[26,28] The occurrence of triplet ions is characteristic for choline containing lipids and is seen also in lyso PC and sphingomyelin (SM).[26]

Information as to the kind of acyl group and its position in the glycerol backbone can be obtained as follows. Collisional activation of carboxylate anions causes fragmentation that can be used to characterize the fatty acid substituent. When the $[RCOO]^-$ ion in the negative ion mode is subjected to measurement of CAD spectra, fragmentation takes place from the alkyl end as shown in Fig. 3. Collisional activation of the $[RCOO]^-$ ion yields a series of fragments arising from loss of the elements of $C_n H_{2n+2}$ from the alkyl terminus remote from the charge site. Identification of the carboxylic acid and determination of the length of its carbon chain is possible because a series of fragment ions is formed starting with the loss of CH_4 and ending with its loss which yields an ion of m/z 58. The presence of unsaturation on the acid chain can cause perturbation of this fragmentation pattern. Therefore, the total carbon number and degree of unsaturation of the acyl group can be deduced from the spectra.[23,26,29,30] If the acyl group is unsaturated fatty acid, the positions of up to three double bonds can be determined.[23,26,29,30] The branching point can also be determined.[30]

The position of the acyl group is judged from the relative intensities of deacylated fragment ions. In the two pairs of ions produced by deacylation from glycerophospholipids, i.e. the deacyl doublet, $[MH - RCH = C = O]^+$ and $[MH - RCH = C = O - 2H]^+$, and the deacyloxy doublet, $[MH - RCH_2 \cdot COOH + 2H]^+$ and $[MH - RCH_2COOH]^+$, the intensities of these ions produced from release of the acyl and acyloxy groups at the 2-position are usually stronger than those from the 1-position. This makes possible

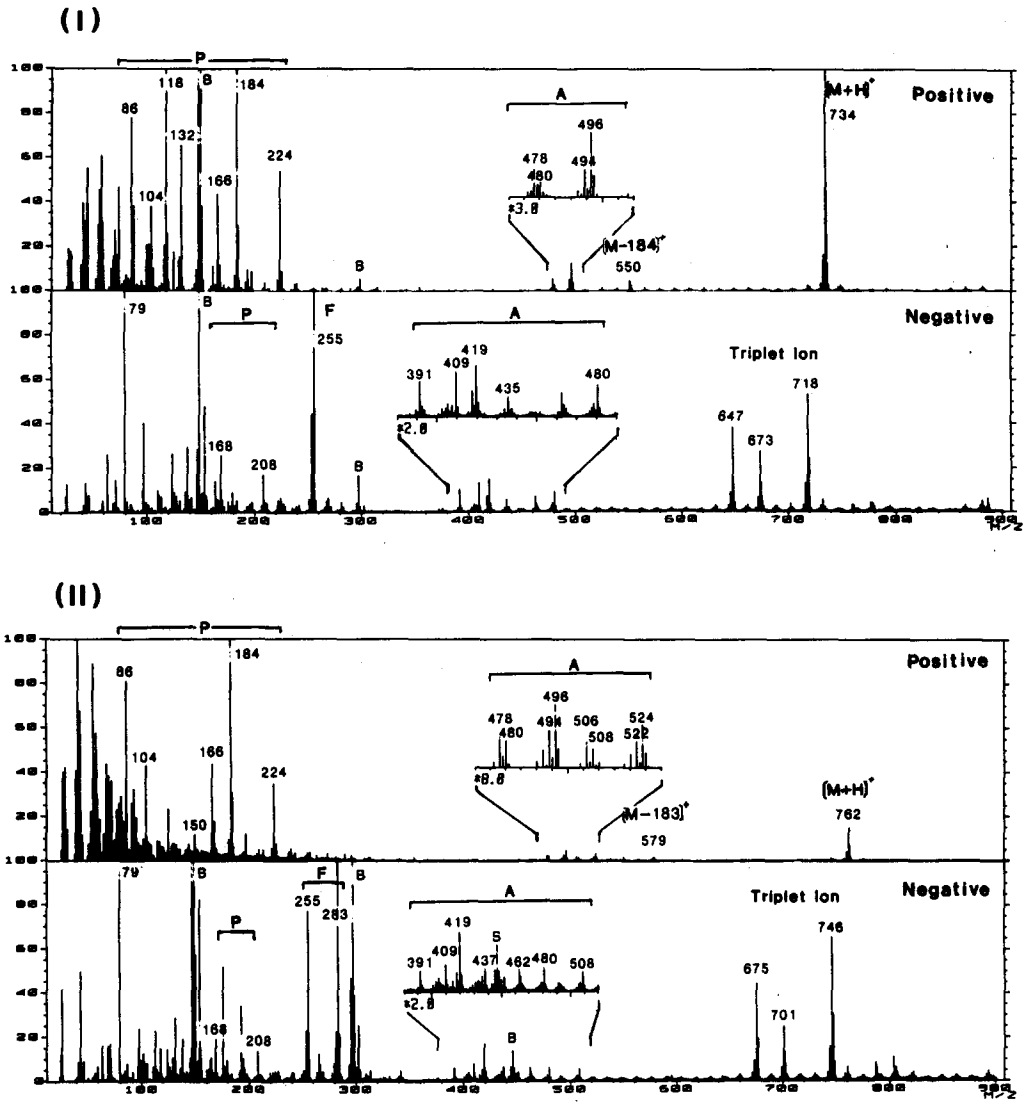


FIG. 2. FAB mass spectra of phosphatidyl choline. (I) Dipalmitoylglycerylphosphocholine, (II) 1-palmitoyl-2-stearoylglycerylphosphocholine. Matrix: glycerol (positive), triethanolamine (negative), P: polar group ion region, B: ions arising due to the matrix, A: deacylated ion region, M: molecular ion region.

clarification of the acyl groups combined with 1- and 2-positions of glycerol.[22,23,26] Another method compares the intensity of the $[RCOO]^-$ ion produced in the negative ion mode from the molecular ion by B/E constant linked scanning or CAD spectra. $[M-15]^-$ and $[M-60]^-$ ions among the triplet ions in PC produce the $[RCOO]^-$ ion by MS/MS, and the intensity of the $[RCOO]^-$ ion released from the 2-position of PC is always stronger than that from the 1-position. Kayganich and Murphy[27] reported that the ratio of $[RCOO]^-$ ions for the 1-position and the 2-position is 1:3. Moreover, $[RCOO]^-$ ions appearing in the negative ion FAB mass spectrum show that the ions from the 2-position are also more intense than those from the 1-position.

The polar head group has a characteristic m/z value which reveals the kind of water-soluble compound it is. In PC, the ions at m/z 184 and m/z 224 in the poistive ion mode[21,26] and m/z 168 in the negative ion mode[32] indicate the presence of phosphorylcholine. These ions have been confirmed to arise from phosphorylcholine by the CAD spectrum of the $[M+H]^+$ ion. The ion at m/z 184 in the positive ion mode shows the presence of phosphorylserine in phosphatidylserine (PS),[30] and the ion at m/z 142 in the positive ion mode and m/z 140 in the negative ion mode[30] show the presence of phosphorylethanolamine.

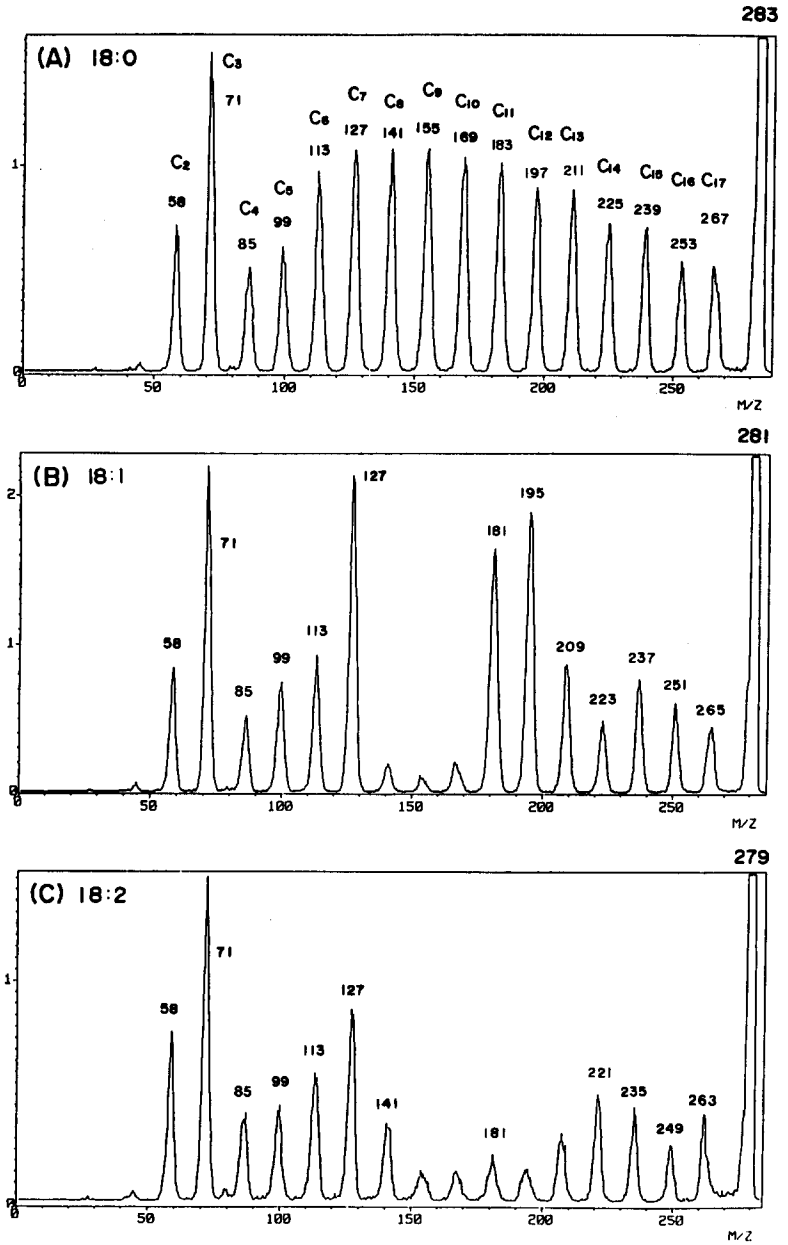
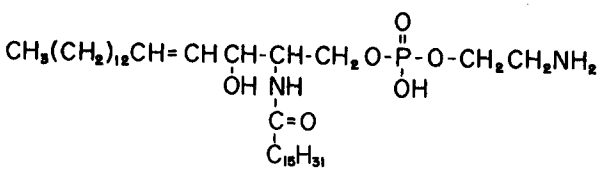


FIG. 3. CAD spectra of $[RCOO]^-$ ions at m/z 283, 281 and 279 obtained in the negative ion mode FAB/MS of phosphatidylcholine.

As these ions sometimes overlap with the cluster ions derived from the matrix, careful attention must be paid when identifying them. However, this problem can be resolved by measuring negative ion FAB/MS using a surface precipitation technique.[32] The polar groups of the phosphoester type can be identified from the daughter ions produced by B/E constant scanning of $[M + H]^+$ ions and four kinds of deacylated ions mentioned above in positive ion FAB/MS.

In addition to PC, FAB/MS spectra of PS,[21,23,32] phosphatidylethanolamine (PE),[24,30,32,33,36] phosphatidylinositol(PI),[32,34–36] phosphatidylglycerol(PG),[24,30,36] phosphatidic acid[24,36] and the lyso-homolog[32] of PC, PE and PS were measured. Also FAB/MS/MS of PC,[23] PS,[23] PE,[36] PI,[36] PG[36] and cardiolipin[36] were measured.

Plasmalogen type phospholipids have also been analyzed by SIMS[35] and FAB/MS.[37,38] As they differ from conventional diacyl type phospholipids by the lack of an oxygen atom



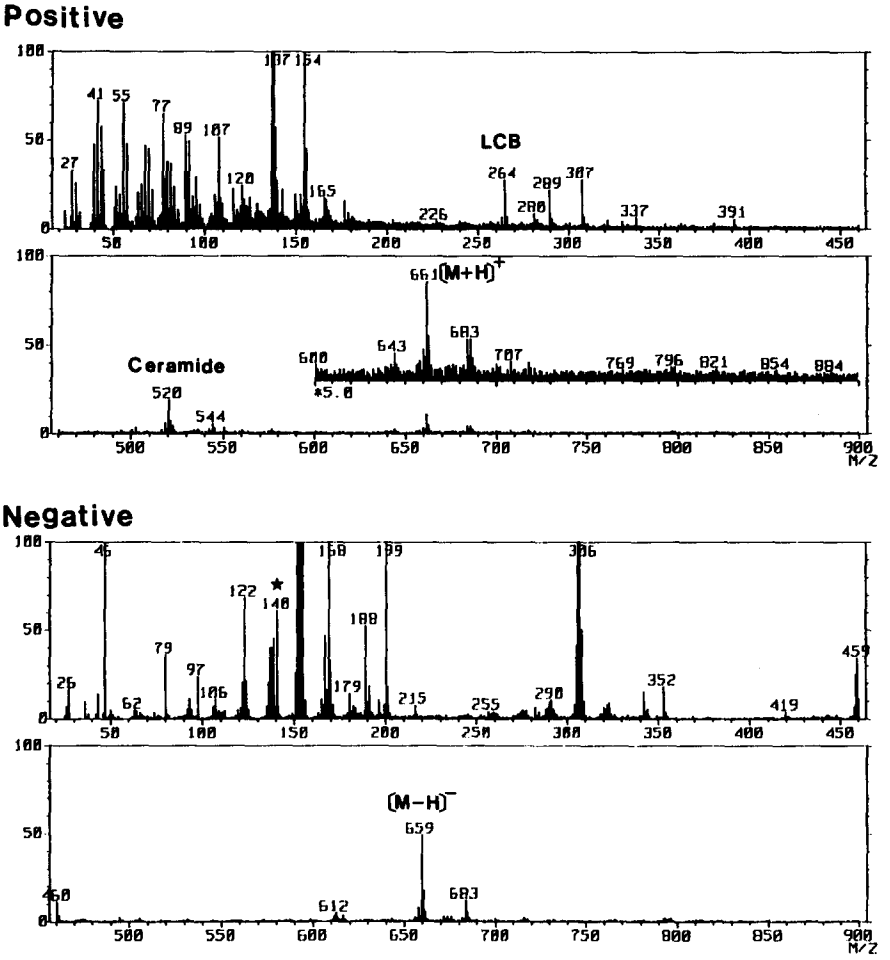


FIG. 4. FAB mass spectra of sphingoethanolamine. Matrix: 3-nitrobenzyl alcohol.

at the 1-position, the $[M + H]^+$ ions are detected at 16 mass units less than that of the diacyl type phospholipid.

The structure of the platelet activating factor (PAF) (1-*O*'-alkyl-2-*O*-acetyl-*sn*-glycerophosphocholine) is similar to the plasmalogen type phospholipid, because the long aliphatic chain at the 1-position is attached by an ether linkage. As PAF is of biological interest, it has been subjected not only to qualitative analysis,[40,42] but also to quantitative analysis[39,41] using stable isotope dilution and the FAB ionization technique. The ratio of $[M + H]^+$ ions produced from native and trideuterio derivatives used as internal standards is measured and quantities as low as 10 ng can be determined.[39] Moreover, analysis in the picogram range is possible by the stable isotope method and FAB/MS/MS.[41] Lyso-PAF, considered to be a precursor of PAF, has also been analyzed by the FAB/MS method; its detection limit is 5 ng.[43] FAB/MS/MS analysis of archaeobacterial lipids was reported.[44] These lipids consist of a glycerol backbone with a polar head group, but differ from other glycerophospholipids by the fact that phytanyl chains are ether-linked to glycerol.

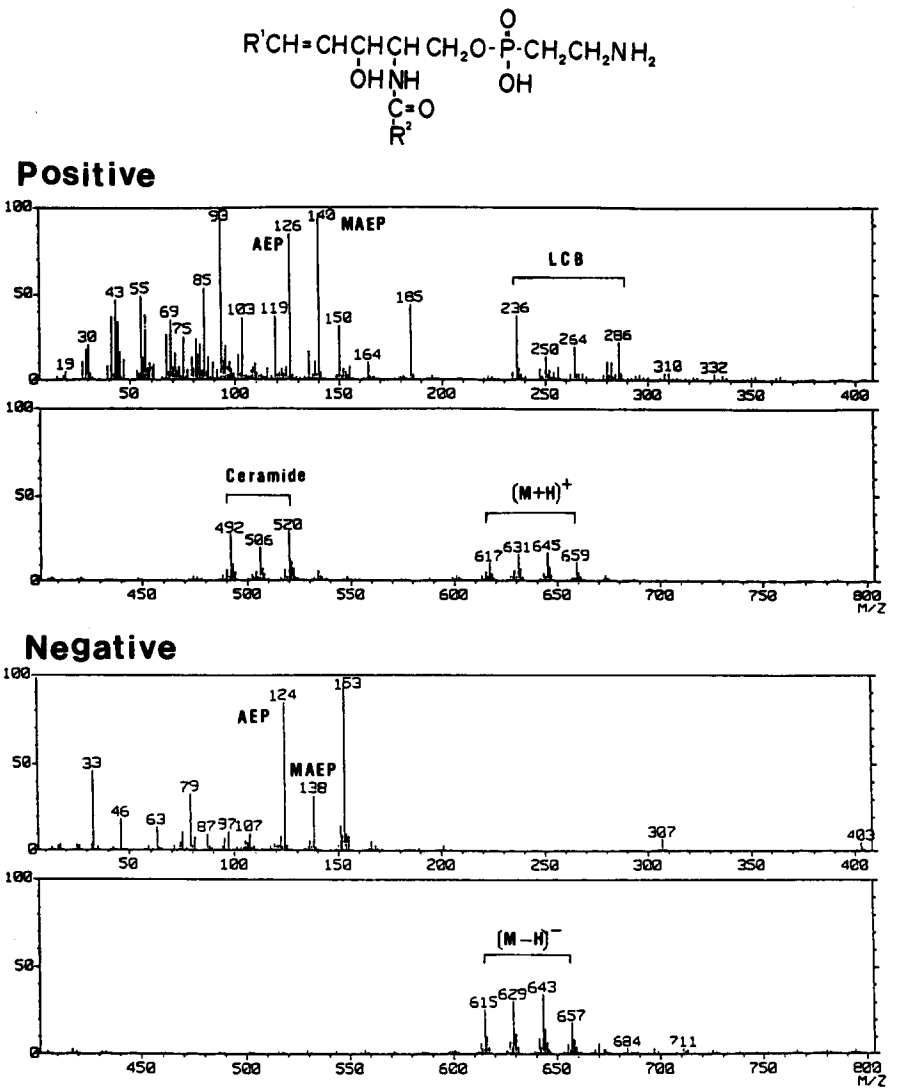


FIG. 5. FAB mass spectra of sphingophosphonolipids (mixtures of ceramide aminoethylphosphonate and ceramide *N*-methylaminoethylphosphonate). Matrix: glycerol.

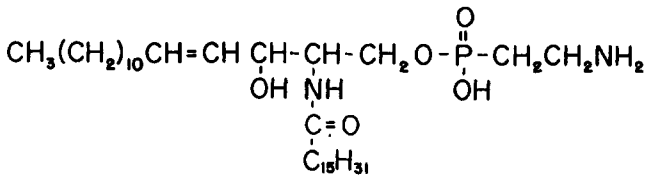
2. *Phosphosphingolipids*

Sphingomyelin (SM)[26,37] and sphingoethanoamine (SEA) were analyzed by FAB/MS. As SM contains choline as its constituent, triplet ions occur instead of the $[M-H]^-$ ion in the negative ion mode, as for PC. In the positive ion mode, ions which indicate the presence of ceramide and a long chain base appear in addition to the $[M+H]^+$ ion. The ion at m/z 184 shows the presence of phosphorylcholine.

Positive and negative ion FAB mass spectra of SEA, *N*-palmitoyl-octadecasphingenyl phosphorylethanolamine, are shown in Fig. 4. The $[M+H]^+$ ion at m/z 661, the ceramide ion at m/z 520 and the LCB ion at m/z 264 appear in the positive ion mode. In addition to the $[M-H]^-$ ion at m/z 659, the ion at m/z 140 is detected in the negative ion mode. This ion shows the presence of phosphorylethanolamine.

3. *Phosphonosphingolipids*

Two phosphonosphingolipids (PnSL) have been shown to be naturally occurring sphingolipids containing aminoalkylphosphonic acid. One is a ceramide aminoethylphosphonate (CAEPn) which has aminoethylphosphonic acid (AEPn) as a water-soluble component,



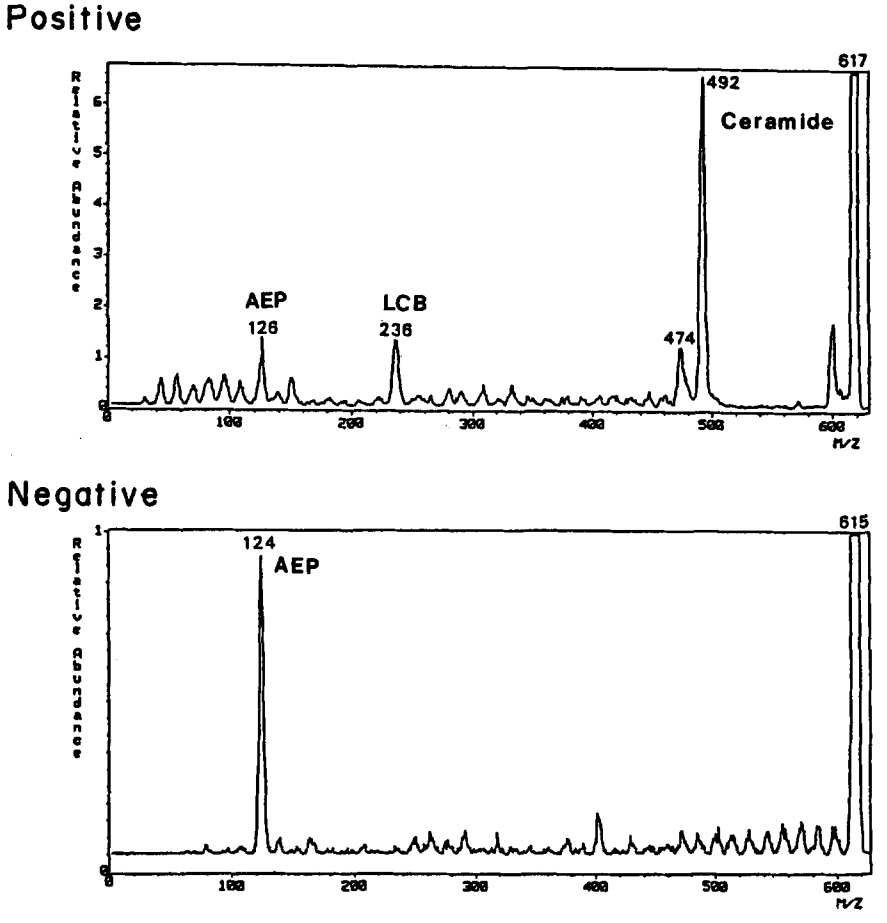


FIG. 6. CAD spectra of $[M + H]^+$ and $[M - H]^-$ ions at m/z 617 and 615 obtained in the FAB/MS of ceramide aminoethylphosphonate.

and another, PnSL, has an *N*-methyl derivative (MAEPn) of AEPn and is ceramide *N*-methylaminoethylphosphonate (CMAEPn). FAB mass spectra of a mixture of CAEPn and CMAEPn are shown in Fig. 5. In addition to the $[M + H]^+$ ions, those indicating ceramide and a long chain base were detected in the positive ion mode and the $[M - H]^-$ ion was detected in the negative ion mode. There are also two pairs of ions, at m/z 126 and 140 in the positive ion mode and at m/z 124 and 138 in the negative ion mode. These ions were assigned to be ions indicating AEPn and MAEPn from the results of exact mass measurement of the CAD spectral data of each ion.[45,46] Therefore, these ions can be used to distinguish AEPn and MAEPn in CAEPn and CMAEPn.

CAD spectra of the $[M + H]^+$ ion at m/z 617 and the $[M - H]^-$ ion at m/z 615 of PnSL are shown in Fig. 6. The $[M + H]^+$ ion produces a ceramide ion at m/z 492, long chain base ions at m/e 236 (C_{16}-sphingosine; d16:1), and a polar group ion at m/z 126 (AEPn). On the other hand, the $[M - H]^-$ ion gave only strong polar group ions at m/z 124 (AEPn).

Separation of the PnSL mixture into CAEPn and CMAEPn at the molecular species level was carried out by reversed phase HPLC. Each peak was collected separately and analyzed by FAB/MS. As shown in Fig. 7, one peak consists of almost a single molecular species.

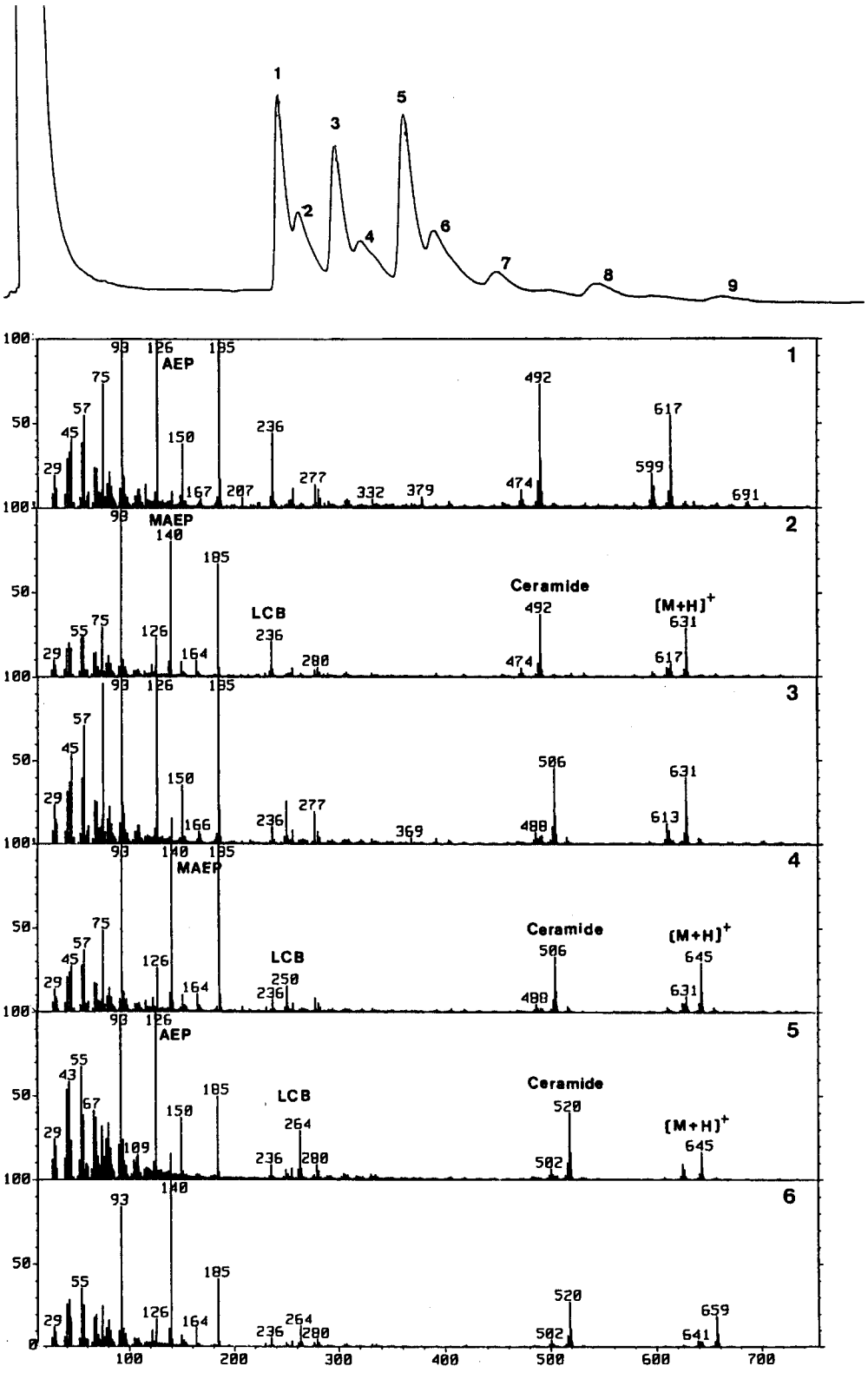
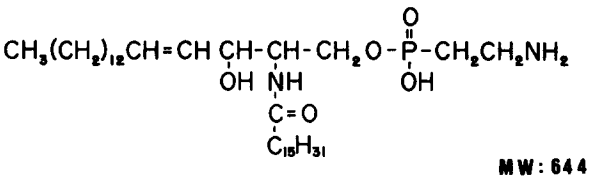


FIG. 7. Reversed phase HPLC of sphingophosphonolipids and positive ion FAB/MS of each peak. AEP: aminoethylphosphonic acid, MAEP: *N*-methyl-aminoethylphosphonic acid, LCB: long chain base.



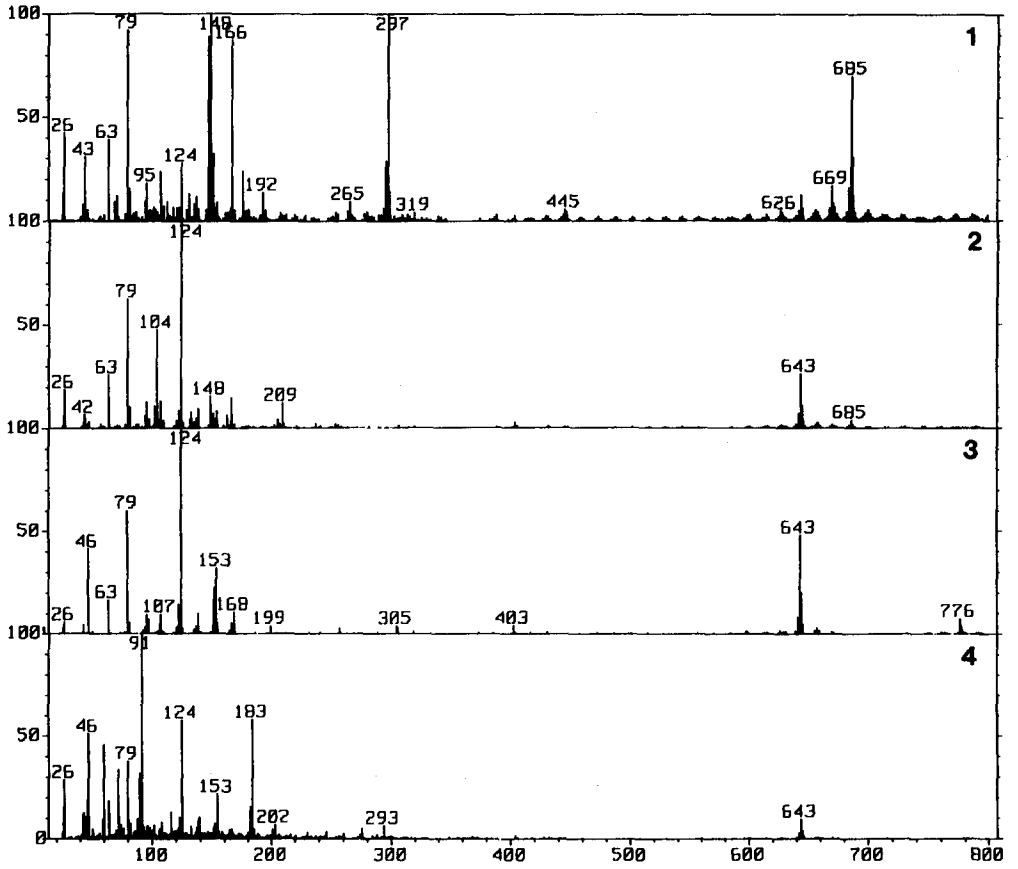


FIG. 8. Influence of matrix on the measurement of FAB/MS. 1: Triethanolamine, 2: diethanolamine, 3: 3-nitrobenzyl alcohol, 4: glycerol.

Triethanolamine (TEA) has been reported to be better than glycerol as a matrix for phospholipids. However, when TEA was used as a matrix in the negative ion mode, FAB/MS of phospholipids containing a free amino group, such as PE, PS, CAEPn and SEA, $[M - H + 26]^-$ and $[M - H + 42]^-$ ions appeared in addition to the $[M - H]^-$ ion.[47] As the intensities of these adduct ions between the matrix and lipid are usually stronger than that of $[M - H]^-$ ion, the molecular weight should be determined with caution. These phenomena did not occur in the diethanolamine (DEA) matrix. This problem is also described by Jensen.[48]

The sensitivity of detection of the $[M - H]^-$ ion is good in the order of DEA, 3-nitrobenzyl alcohol (3-NBA) and glycerol, with slight differences being noted with the type of phospholipid. Intensities of the $[M - H]^-$ ion of CAEPn using four kinds of matrices are shown in Fig. 8.

B. *Glycolipids*

1. *Glycoglycerolipids*

Sulfates of monogalactosylalkylacylglycerol and its lyso-homologue were analyzed by SIMS.[49] In the positive SIMS, cationized molecular species were very intense. Desulfo and

desulfo-degalacto ions derived from the molecular ions were also prominent. In the negative SIMS, $[M-H]^-$ ions were detected with strong intensities in the high mass region, and the fragment ions corresponding to the terminal sulfated galactose residue at m/z 241 $[HSO_3\text{-Gal}]^-$ and m/z 257 $[HSO_3\text{-Gal-O}]^-$ were identified.

Glycolipids containing dialkylglycerol can also be analyzed by negative ion FAB/MS.[50] The $[M-H]^-$ ion and ions indicating the sugar sequence were detected clearly, but an ion corresponding to the dialkylglycerol was not observed. This differs greatly from the spectra of glycosphingolipids which produce ceramide ions as described below. It is very interesting that the spectra reflected a difference in the linkage position of the glycolipid. When dimannosyl-di-*O*-alkyl glycerols with different linkages, such as 1–6, 1–2 and 1–4 linkages, were analzyed under identical conditions, clearly different relative intensities of $[M-H]^-$ and [mannosyl-di-*O*-alkyl glycerol $-$ H]$^-$ ions were observed.

2. *Glycosphingolipids*

FAB/MS spectra of a large number of glycosphingolipids as well as gangliosides have been studied, because they yielded information about molecular weight and sugar sequence.[51–58] TEA alone or a mixture of TEA and tetramethylurea (TMU), and recently 3-NBA, are used as matrices for glycosphingolipids. Glycerol is not suitable for analyzing glycosphingolipids and gangliosides, because little structural information can be obtained from both positive and negative ion FAB mass spectra. Molecular weight was determined from $[M+H]^+$, $[M+Na]^+$ and $[M+H-H_2O]^+$ ions in the positive ion mode and from $[M-H]^-$ ions in the negative ion mode. These quasi molecular ions were observed usually as multiple peaks with differences of 10, 12, 14, and 16 mass units. These phenomena reflect the heterogeneity of the ceramide moiety, i.e. a mixture of some molecular species. The difference of 10 mass units shows the presence of 2 double bonds, 12 mass units, 1 double bond and 16 mass units a hydroxyl group in the ceramide moiety. Negative ion FAB was used to define the sugar sequence. As shown in Fig. 9a, a series of ions with a specific m/z value which was produced by cleavage of glycosidic linkages were detected clearly from the start at the ceramide containing an oxygen atom in the glycolipid with a linear sugar chain.[59,60] From these m/z values, the composition of sugar [hexose (162), pentose (132), deoxyhexose (146), *N*-acetylhexosamine (203), methylated hexose (176) and so on] and sugar sequence can be determined. However, isomeric monosaccharides (glucose, galactose, mannose etc.) cannot be distinguished from each other. If the sugar chain has a branching structure, the spectra shows a complex pattern from the branching point (Fig. 9b).

Positive ion FAB/MS gave less information about the structure of the sugar chain than the negative ion mode type, but much information was obtained about the constitution of ceramide and long chain base, especially when 3-NBA was used as the matrix.[61,62] The ion at m/z 264 in the positive ion FAB/MS was found to be derived from C_{18}-sphingosine (d18:1) which is one of the most abundant naturally occurring long chain bases.[63] The chain length of fatty acid can be calculated by subtracting the m/z value of the long chain base ion from that of the ceramide ion.

Recently, a more detailed study on the structure of glycosphingolipids and the fragmentation pathway of some ions have been carried out[58] using FAB ionization combined with high performance tandem mass spectrometry.

In the positive ion FAB mass spectra of permethylated glycolipid, the sugar ion series produced by cleavage of the glycosidic linkage from the nonreducing terminal was detected[64–66] in addition to ceramide ions. Elimination of MeOH (32 mass units) from the sugar ion is a very typical feature similar to the ion found by EI mass spectra of permethylated glycolipid. The terminal hexose residue gives rise to ions at m/z 219 and 187 (219 $-$ MeOH) and deoxyhexose is represented by ions at m/z 189 and 157 (189 $-$ MeOH). The ion at m/z 260 and 228 (260 $-$ MeOH) is characteristic of terminal *N*-acetylhexosamine. The glycosidic bonds of *N*-acetylhexosamine residues are most favored points of cleavage. Thus, glycolipid with repeated lactosamine units (Gal1–4GalNAc) gave the ion at m/z 464 and 432 (464 $-$ MeOH) and m/z 913 and 881 (913 $-$ MeOH). If the

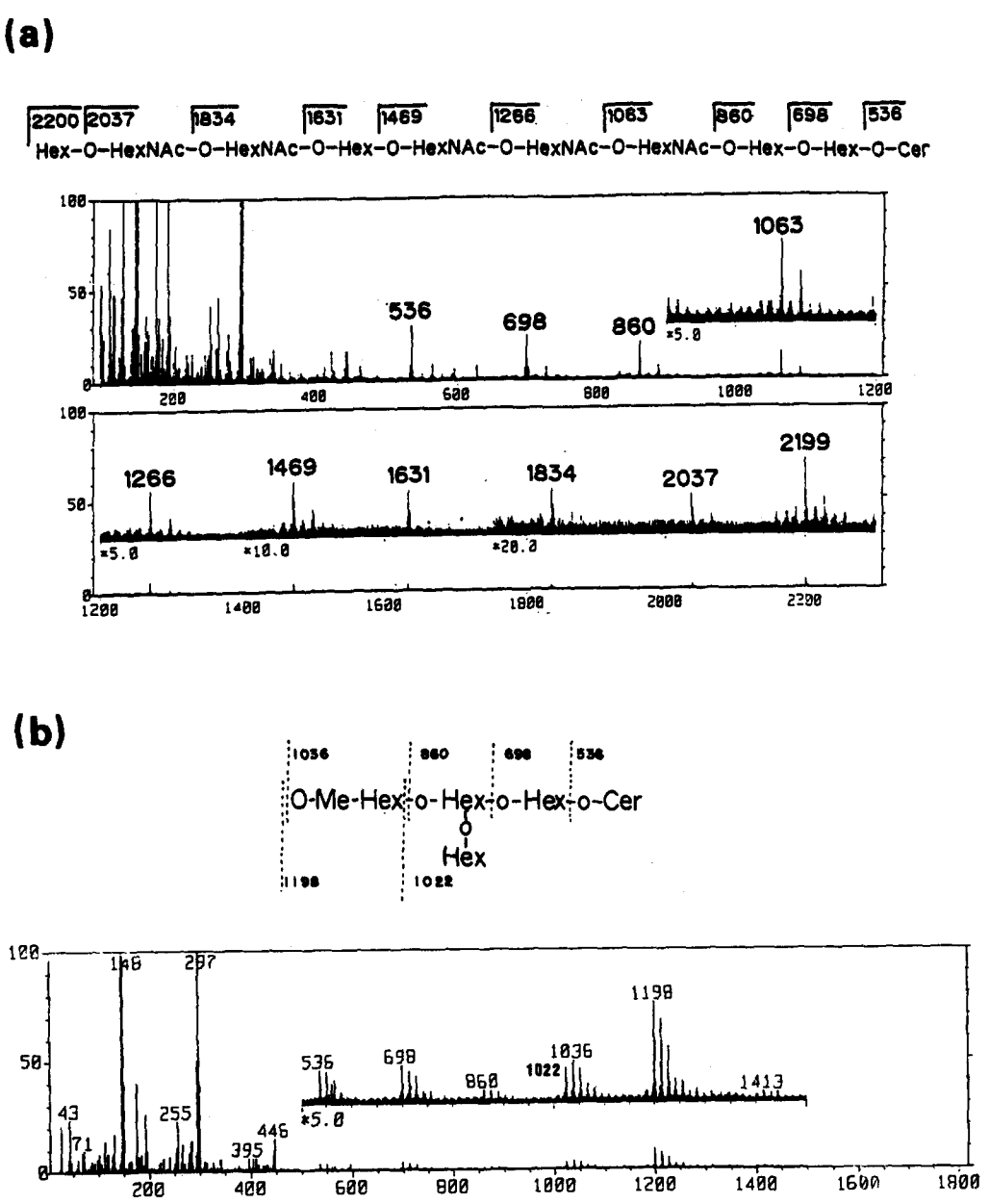


FIG. 9. Negative ion FAB mass spectra of glycosphingolipids with linear sugar chain (a) and branched sugar chain (b). Matrix: triethanolamine.

Gal1–3GalNAc structure is present at the terminal end of the glycolipid, the ion at m/z 464 is accompanied by the ion at m/z 228 instead of m/z 432. Egge *et al.* analyzed oligoglycosylceramide containing up to 25 sugars by this method.[64,66]

Many applications of LC/MS for the analysis of glycolipid have been reported, e.g. separation of glycolipid classes using the moving belt LC/MS interface with CI mass spectrometry,[67] separation of molecular species of cerebrosides by reversed phase LC and atmospheric pressure ionization.[68] Recently, a new system of HPLC/FAB/MS which involves a frit interface between micro column HPLC and MS was developed and applied to the separation and characterization of molecular species of glycosphingolipids with one and five sugars.[69]

Analysis of glycolipid accumulated in some lipidoses has been carried out using TLC/SIMS.[70]

3. *Gangliosides*

A large number of FAB mass spectra of intact gangliosides[71–75] and permethylated gangliosides[65,76–79] have been measured. For determination of the sugar sequence, the negative ion mode is useful, similar to the case of the neutral glycolipid. Cleavage of glycosidic bonds takes place only from the side of the ceramide including the oxygen atom. As sialic acid is usually attached to the sugar chain at the non-reducing end and this ketoside linkage is cleaved easily, the ion at m/z 308 and its dehydration ion at m/z 290 were observed in the case of *N*-acetylneuraminic acid containing ganglioside (Fig. 10).

The isomeric gangliosides (GM1a and GM1b,[74] GD1a and GD1b,[54] GT1a and GT1b[54]) can be clearly distinguished on the basis of fragment ions. As polysialogangliosides have many negative charges, they have a tendency to bind cations like Ca^+, K^+ or Na^+, which leads to a very complex cluster of cationized molecules. This problem can be overcome partly by the addition of acid, such as hydrochloric, oxalic or citric acids, to the matrix, although this can lead to a pronounced tendency for lactone formation. Glycerol is not a good matrix for analyzing ganglioside, but when hexamethylphosphoric triamide or triethylene glycol was added to glycerol, the $[M - H]^-$ ion and prominent fragment ions caused by sequential cleavage of glycoside linkage were clearly observed.[80]

4. *Sulfoglycolipids*

As sulfoglycolipids have a negative charge coming from the sulfate group, the negative ion mode is expected to be better than the positive ion mode for analyzing them, as in the case of gangliosides. Indeed, negative ion FAB[81] and negative SIMS[82,83] of sulfoglycolipids proved the presence and location of the sulfate group and also revealed the sugar sequence and ceramide composition, as shown in Fig. 11.

The positive ion mode is superior to the negative ion mode for analyses of permethylated neutral glycolipids and gangliosides, but the negative ion mode is better for analyses of permethylated sulfatides.

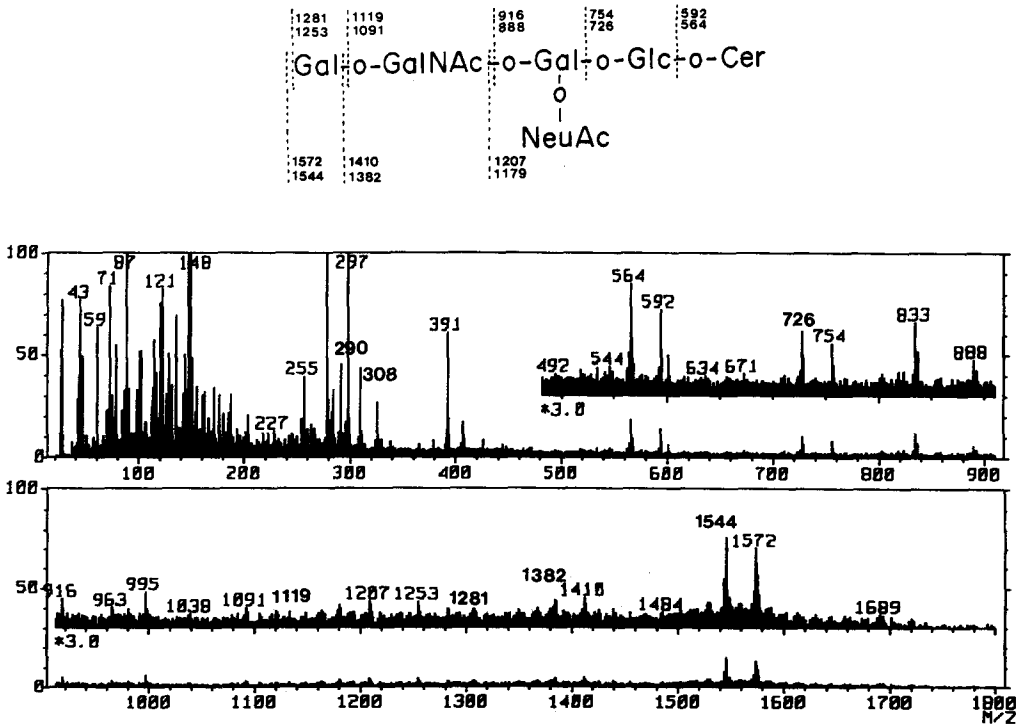


FIG. 10. Negative ion FAB mass spectrum of ganglioside GM_1. Matrix: triethanolamine.

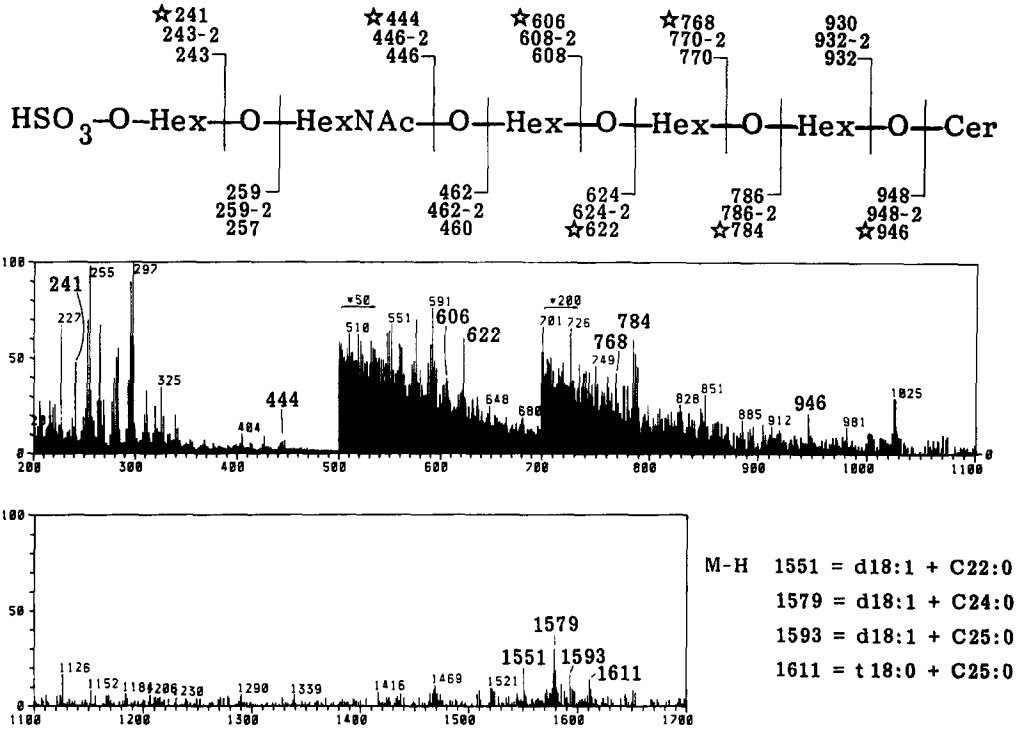


FIG. 11. Negative secondary ion mass spectrum of sulfoglycolipid.

Lyso-sulfatide can be analyzed by both positive and negative ion modes.[84] Weak $[M + H]^+$ ions, and strong ions corresponding to galactosylpsychosine and the dehydrated ion of the protonated long chain base were detected in the positive ion mode. Besides a large amount of $[M - H]^-$ ions, the ions derived from sulfated galactose at m/z 257 and 241 and deprotonated sulfate at m/z 97 were observed in the negative ion mode.

5. *Phosphonoglycosphingolipids*

Information on the structure of the sugar chain and ceramide composition were obtained from negative ion FAB mass spectra as well as neutral glycolipids. Aminoalkylphosphonic acid was identified using the ion at m/z 124 and 138 in the negative ion mode and the ion at m/z 126 and 140 in the positive ion mode as described in Section III.A.3. The position of aminoalkylphosphonic acid on the sugar chain was revealed by comparing each negative ion mode FAB mass spectra of phosphonoglycosphingolipid (PnGSL) before and after treatment with HF (Fig. 12).[81] The molecular species of the long chain base and aminoalkylphosphonic acid also can be determined by linked scanning of $[M + H]^+$ ions and ceramide ions of intact PnGSL, respectively.

C. *Non-Polar Moiety*

1. *Ceramides*

The positive ion FAB/MS of intact sphingolipid mainly yields fragments related to the non-polar portion (ceramide) whereas negative ion FAB/MS yields more informative ions for the water-soluble part. FAB mass spectra of free ceramide gave $[M + H]^+$ and $[M + H - H_2O]^+$ ions in the positive ion mode and $[M - H]^-$ ions in the negative ion mode. Therefore, the molecular weight could be estimated from these ions. In our observations, LCB ions and a series of ions for hydrocarbons were detected clearly when 3-NBA was used as the matrix (Fig. 13).

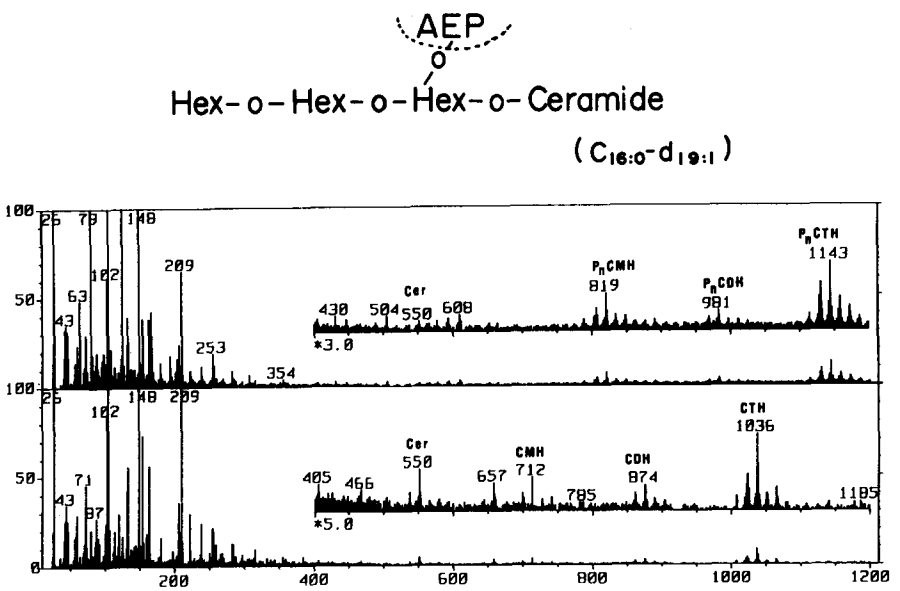


FIG. 12. Negative ion FAB mass spectra of phosphonoglycosphingolipids. Upper: intact lipid, lower: lipid treated with hydrogen fluoride. Matrix: diethanolamine.

However, Domon *et al.*[86] reported that the response of the characteristic ceramide ion is very weak in positive ion FAB/MS, and the MS/MS experiment is difficult to apply to microscale analysis. Hence, ceramide was chemically modified and analyzed by MS/MS, i.e. reduction with borane (conversion of the amide to amine) followed by oxidation (conversion of allylic alcohol to a vicinal diol). Highly sensitive results with signal enhancement 10 times that of intact ceramide can be obtained.[86]

2. *Fatty Acids*

Fatty acids, one of the important components of conjugated lipid, have been analyzed by GC/MS as methyl esters. The double bond position of unsaturated fatty acid can be determined as trimethylsilyl, pirrolidide and picolinyl derivatives also using GC/MS. Recently, free fatty acid can be analyzed by FAB/MS/MS.[87-94] Free acids dissolved in the TEA matrix are readily desorbed in the negative ion mode by FAB to yield $[M-H]^-$ ions. When the $[M-H]^-$ ions are selected in a tandem mass spectrometer and collisionally activated, they undergo parallel loss of the elements CH_4, C_2H_6, C_3H_8 etc. These C_nH_{2n+2} losses begin at the alkyl terminus and progress along the entire alkyl chain. The ion fragmentations are called "charge-remote". Analytical applications and fundamental studies of charge-remote fragmentation are reviewed by Adams.[95] The patterns are well defined for saturated fatty acid,[88] e.g. for palmitic acid, the ions at m/z 58, 71, 85, 99, 113, 127, 141, 155, 169, 183, 197, 211, 225 and 239 are formed as daughter ions from the $[M-H]^-$ ion at m/z 255 by the loss of C_nH_{2n+2}. If unsaturation or substituents are present in the alkyl chain, this pattern is interrupted at that point, enabling determination of the double bond position. Polyunsaturated fatty acids are reduced with deuterium-labelled diimide (N_2D_2) and the resulting saturated acid with deuterium labelling at the original unsaturated side is analyzed by this method. The location of double bonds in the original acid can be determined by the mass shifts caused by incorporation of deuterium into C_nH_{2n+2} fragments.[89,92] The FAB/MS/MS method has been extended to the structural determination of fatty acids containing branching (iso- and anteiso-[90]), epoxy rings, cyclopropane rings, cyclopropene rings and hydroxy groups.[93]

In the case of the derivatized fatty acid, the $[M+H]^-$ ion is used. Deterding and Gross[93] reported that picolinyl ester derivatives can also be readily desorbed by FAB as positive ions and then collisionally activated. CAD spectra of the $[M+H]^+$ ions of the derivatized

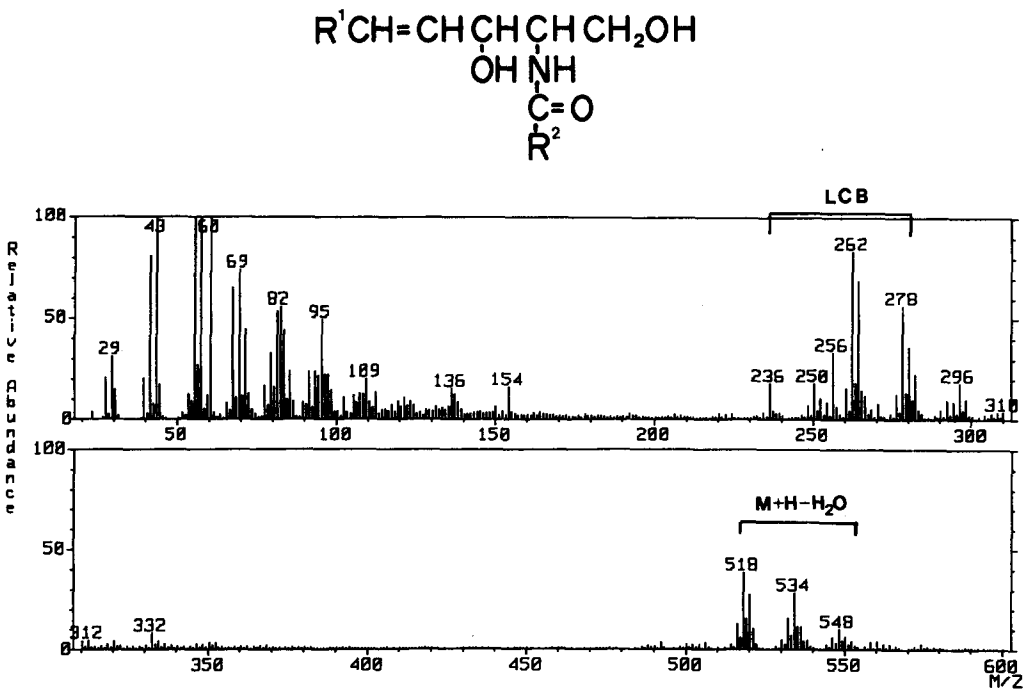


FIG. 13. Positive ion FAB mass spectra of ceramide. Matrix: 3-nitrobenzyl alcohol.

fatty acids revealed that structurally informative charge-remote fragmentations occur just as they do for fatty acid carboxylates. About 50 ng of material is needed to obtain a CAD spectrum for the piconilyl derivative of stearic acid, and about 10 ng for the oleic acid for the $[M-H]^-$ ion. Thus, the detection limits are similar. In addition to the picolinyl derivative, methyl-, ethyl- and propyl-esters, amide, and pirrolidide derivatives have also been analyzed using $[M+H]^+$ ions in the positive ion mode.[94]

3. *Long Chain Bases*

The ion at m/z 264 which appeared in positive ion FAB/MS of sphingolipids has already been proved to be the LCB ion (d18:1) by B/E constant linked scanning of the ceramide ion.[63] Long chain bases in lysosphingolipids (psychosine) were analyzed by positive ion FAB/MS.[96] Sphingenine containing psychosine produced $[M+H-Gal]^+$, $[M+H-H_2O]^+$ and $[M+H-Gal-2H_2O]^+$ ions in addition to $[M+H]^+$ and $[M+H-H_2O]^+$ ions, whereas sphinganine containing psychosine gave $[M+H-Gal-H_2O]^+$ and $[M+H-Gal-2H_2O]^+$ ions with weak intensity. Therefore, sphingenine and sphinganine were distinguished by comparing the intensity of these ions. There are two reports concerning the analysis of free long chain base by FAB/MS.[96,97] Only $[M+H]^+$ and $[M+H-H_2O]^+$ ions are detected in the positive ion mode and the ion at m/z 264 corresponding to $[M+H-2H_2O]^+$ was not found.

D. Water-Soluble Components

1. *Sugars*

FAB/MS of sugars has been studied for glycolipids, and also for oligosaccharides derived from glycoprotein[98–101] and naturally occurring oligosaccharides.[102–105] Egge *et al.*[65,102,106] analyzed a large number of sugar chains obtained from oligosaccharides, glycolipids and glycoproteins as intact, permethylated and peracetylated forms by FAB/MS. They concluded that negative ion FAB/MS could be used for analysis of non-derivatized neutral and acidic oligosaccharides, while positive ion FAB/MS was better for methylated and

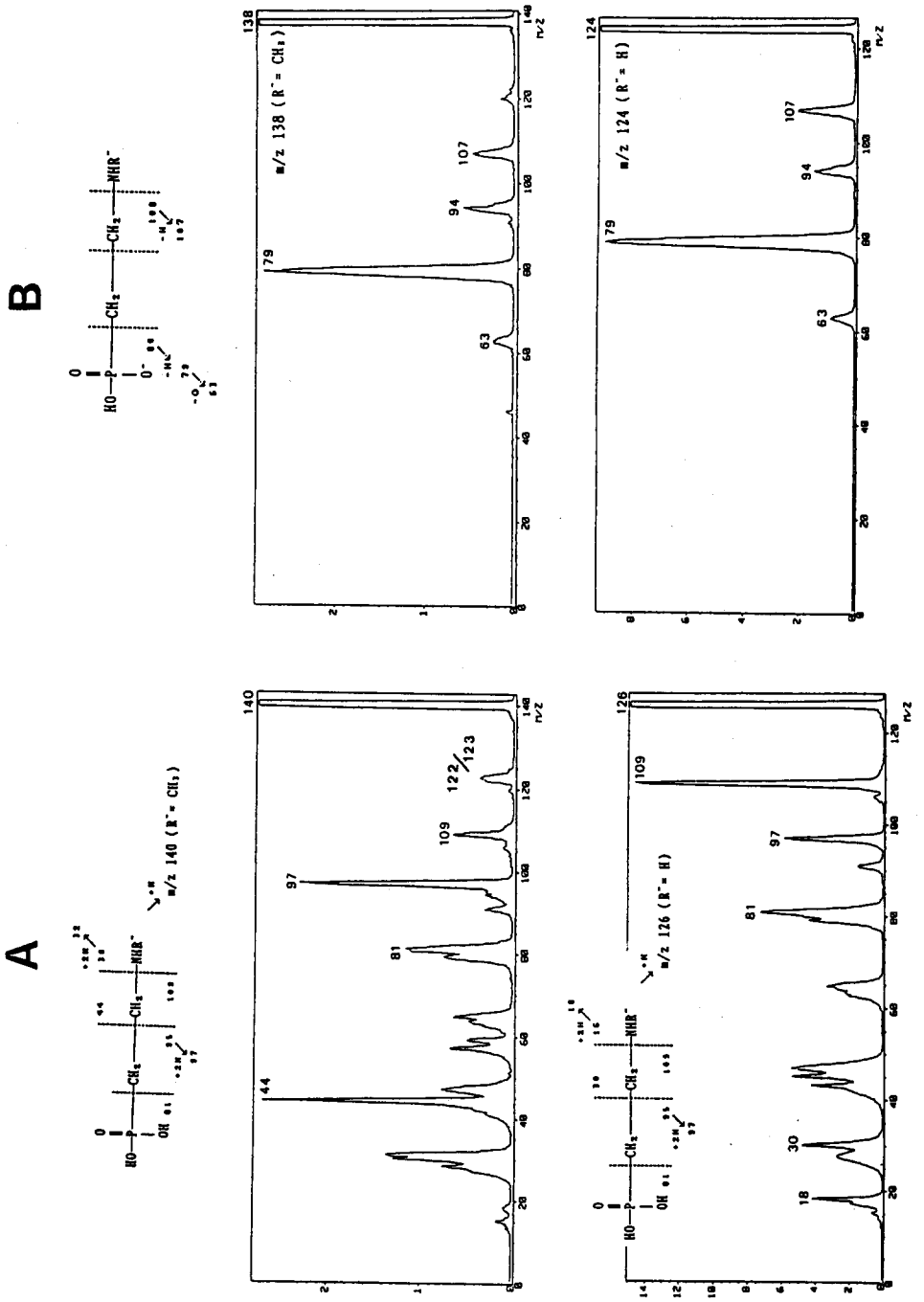


FIG. 14. CAD spectra of aminoalkylphosphonic acids. A: positive ion mode, B: negative ion mode.

acetylated derivatives, because very clear cut fragmentation could be obtained from the non-reducing terminal of the sugar chain. A detailed FAB/MS study of sugar derived from glycoconjugates has been reviewed by Dell.[107] For a more fundamental study, fragmentations of free sugar,[108] sugar phosphate[109] and sugar sulfate[109] have been analyzed systematically. Analyses of anomeric configuration are being tried by FAB/MS coupled with chromic oxidation.[110] Identification of interglycosidic linkages of oligosaccharides are also being tried by high performance tandem mass spectrometry.[111,112]

2. *Aminoalkylphosphonic Acids*

CAD spectra of AEPn and MAEPn were measured.[45] From the ion at m/z 138 and 124, daughter ions such as m/z 107, 94, 79, and 63 were detected and also daughter ions at m/z 109, 97, 81 and 44 or 30 from both ions at m/z 140 and 126, as shown in Fig. 14. A large amount of ion at m/z 79 in the negative ion mode is strong evidence for phosphonic acid. The existence of a daughter ion at m/z 44 in the positive ion mode produced from the ion at m/z 140 shows that the hydrogen of the amino group of AEPn is substituted by a methyl group. Therefore, the ion at m/z 138 and 124 in the negative mode and the ion at m/z 140 and 126 in the positive mode represent MAEPn and AEPn, respectively.

IV. CONCLUSION

The introduction of FAB/MS to the study of lipids released many workers from the tedious work of chemical analyses by trial-and-error methods. FAB/MS made it possible to obtain valuable information on the structure of complex lipids, such as homogeneity, molecular weight, molecular species, composition of fatty acid, LCB and sugar, fatty acid position in the glycerol backbone, and the sequence of sugars, although it cannot be used for linkage analysis of the sugar chain. These highly reproducible data can be obtained with comparatively small amounts of material in a very short time. Therefore, FAB/MS measurement prior to chemical analysis is essential for lipid analysis today.

Mass spectrometry, unfortunately, cannot give stereochemical information, e.g. *cis* and *trans* configuration of the double bond in fatty acids and long chain bases, anomeric configuration and the site of substitution in the sugar chain. These can be determined using one- or two-dimensional ^{1}H-NMR, although NMR studies require larger amounts of sample. If NMR sensitivity can be increased, the structure of native lipids including the conformation can be determined by a combination of FAB/MS and NMR. This would make it possible to clarify the relationships between structures and many biological functions.

For pure samples, FAB ionization coupled with high performance tandem mass spectrometry is now being used to obtain more detailed structure information including linkage analysis of the sugar chain. This would make it possible to determine the conformation of intact lipids using only mass spectrometry in the near future. For heterogeneous samples, high performance liquid chromatography combined with FAB mass spectrometry is the most powerful analytical tool. However, although many research groups have tried using it, they have not been successful due to the lack of a good interface, like the jet separator used in GC/MS. Two promising systems have been developed for HPLC/FAB/MS. One is a FRIT-FAB LC/MS, which has already been applied to the analysis of glycolipids, and the other is a CF-FAB LC/MS, which has attracted much interest, but has not yet been applied widely to the analysis of lipids.

(*Received 4 April 1991*)

REFERENCES

1. BEYNON, J. H. *Mass Spectrometry and Its Applications to Organic Chemistry*. Elsevier, Amsterdam, 1960.
2. MCLAFFERTY, F. D. (ed.) *Mass Spectrometry of Organic Ions*. Academic Press, New York, 1963.
3. BUDZIKIEWICZ, H., DJERASSI, C. and WILLIAMS, D. H. *Mass Spectrometry of Organic Compounds*, Holden-Day, London, 1967.

4. RYHAGE, R. *Anal. Chem.* **36,** 759–764 (1964).
5. MCNEAL, C. J. *Anal. Chem.* **54,** 43A–50A (1982).
6. SUNDQUVIST, B. and MACFARLANE, R. D. *Mass Spectrom. Rev.* **4,** 421–460 (1985).
7. BENNINGHOVEN, A. and SICHTERMAN, W. *Org. Mass Spectrom.* **12,** 595–597 (1977).
8. BARBER, M., BORDOLI, R. S., ELLIOT, G. J., SEDGWICK, R. D. and TYLER, A. N. *Anal. Chem.* **54,** 645A–657A (1982).
9. COTTER, R. J. and TABET, J. C. *Int. Mass Spectrom. Ion Phys.* **53,** 151–166 (1983).
10. HELLER, D. N., FENSELAU, C., COTTER, R. J., DEMIREV, P., OLTHOFF, J. K., HONOVICH, J., UY, M., TANAKA, T. and KISHIMOTO, Y. *Biochem. Biophys. Res. Commun.* **142,** 194–199 (1987).
11. FENSELAU, C., HELLER, D. N., OLTHOFF, J. K., COTTER, R. J., KISHIMOTO, Y. and UY, O. M. *Biomed. Environ. Mass Spectrom.* **18,** 1037–1045 (1989).
12. YANG, Y. M., SOKOLOSKI, E. A., FALES, H. M. and PANNELL, L. K. *Biomed. Environ. Mass Spectrom.* **13,** 489–492 (1986).
13. DEMIREV, P. A. *Biomed. Environ. Mass Spectrom.* **14,** 241–246 (1987).
14. COTTER, R. J., HONOVICH, J., QURESHI, N. and TAKAYAMA, K. *Biomed. Environ. Mass Spectrom.* **14,** 591–598 (1987).
15. ITO, Y., TAKEUCHI, T., ISHII, D. and GOTO, M. *J. Chromatogr.* **358,** 201–207 (1986).
16. MOSELEY, M. A., DETERDING, L. J., DE WIT, J. S. M., TOMER, K. B., KENNEDY, R. T., BRAGG, N. and JORGENSON, J. W. *Anal. Chem.* **61,** 1577–1584 (1989).
17. KUSHI, Y. and HANDA, S. *J. Biochem. (Tokyo)* **98,** 265–268 (1985).
18. MCLAFFERTY, F. W. and SAKAI, I. *Org. Mass Spectrom.* **7,** 971–983 (1973).
19. BOYD, R. K. and BEYNON, J. H. *Org. Mass Spectrom.* **12,** 163–165 (1977).
20. CHANG, T. T., LAY, T. O., JR and FRANCEL, R. J. *Anal. Chem.* **56,** 109–111 (1984).
21. FENWICK, G. R., EAGLES, J. and SELF, R. *Biomed. Mass Spectrom.* **10,** 382–386 (1983).
22. OHASHI, Y. *Biomed. Mass Spectrom.* **11,** 383–385 (1984).
23. JENSEN, N. J., TOMER, K. B. and GROSS, M. L. *Lipids* **21,** 580–588 (1986).
24. MÜNSTER, H., STEIN, J. and BUDZIKIEWICZ, H. *Biomed. Environ. Mass Spectrom.* **13,** 423–427 (1986).
25. MÜNSTER, H. and BUDZIKIEWICZ, H. *Biol. Chem. Hoppe-Seyler* **369,** 303–308 (1988).
26. HAYASHI, A., MATSUBARA, T., MORITA, M., KINOSHITA, T. and NAKAMURA, T. *J. Biochem. (Tokyo)* **106,** 264–269 (1989).
27. KAYGANICH, K. and MURPHY, R. C. *J. Am. Soc. Mass Spectrom.* **2,** 45–54 (1991).
28. HAYASHI, A., MATSUBARA, T., MORITA, M., KINOSHITA, T. and NAKAMURA, T. *Proc. Jap. Soc. Med. Mass Spectrom.* **12,** 203–206 (1987).
29. DASGUPTA, A., AYANOGLU, E., WEGMANN-SZENTE, A., TOMER, K. B. and DJERASSI, C. *Chem. Phys. Lipids* **41,** 335–347 (1986).
30. DASGUPTA, A., AYANOGLU, E., TOMER, K. B. and DJERASSI, C. *Chem. Phys. Lipids* **43,** 101–111 (1987).
31. ZHANG, M.-Y., LIANG, X.-Y., CHEN, Y.-Y. and LIANG, X.-G. *Anal. Chem.* **56,** 2288–2290 (1984).
32. CHEN, S., BENFENATI, E., FANELLI, R., KIRCHNER, G. and PREGNOLATO, F. *Biomed. Environ. Mass Spectrom.* **18,** 1051–1056 (1989).
33. PRAMANIK, B. N., ZECHMAN, J. M., DAS, P. R. and BARTNER, P. L. *Biomed. Environ. Mass Spectrom.* **19,** 164–170 (1990).
34. SHERMAN, W. R., ACKERMANN, K. E., BATEMANN, R. H., GREEN, B. N. and LEWIS, I. *Biomed. Mass Spectrom.* **12,** 409–413 (1985).
35. BENFENATI, E. and REGINATO, R. *Biomed. Mass Spectrom.* **12,** 643–651 (1985).
36. JENSEN, N. J., TOMER, K. B. and GROSS, M. L. *Lipids* **22,** 480–489 (1986).
37. GROSS, R. W. *Biochemistry* **23,** 158–165 (1984).
38. CHILTON III, F. H. and MURPHY, R. C. *Biomed. Mass Spectrom.* **13,** 71–76 (1986).
39. CLAY, K. L., STENE, D. O. and MURPHY, R. C. *Biomed Mass Spectrom.* **11,** 47–49 (1984).
40. VARENNE, P., DAS, B. C., POLONSKY, J. and TENCE, M. *Biomed. Mass Spectrom.* **12,** 6–10 (1985).
41. HAROLDSEN, P. E. and GASKELL, S. J. *Biomed. Environ. Mass Spectrom.* **18,** 439–444 (1989).
42. PLATTNER, R. D., STACK, R. J., AL-HASSAN, J. M., SUMMERS, B. and CRIDDLE, R. S. *Org. Mass Spectrom.* **23,** 834–840 (1988).
43. HAROLDSEN, P. E., CLAY, K. L. and MURPHY, R. C. *J. Lipid Res.* **28,** 42–49 (1987).
44. FREDRICKSON, H. L., DE LEEUW, J. W., TAS, A. C., VON DER GREEF, J., LAVOS, G. F. and BOON, J. J. *Biomed. Environ. Mass Spectrom.* **18,** 96–105 (1989).
45. MATSUBARA, T., MORITA, M. and HAYASHI, A. *Biochim. Biophys. Acta* **1042,** 280–286 (1990).
46. HAYASHI, A., MATSUBARA, T., NAKAMURA, T. and KINOSHITA, T. *Chem. Phys. Lipids* **52,** 57–67 (1990).
47. MORITA, M., MATSUBARA, T. and HAYASHI, A. *Proc. Jap. Soc. Med. Mass Spectrom.* **15,** 103–106 (1990).
48. JENSEN, N. J. and GROSS, M. L. *Mass Spectrom. Rev.* **7,** 41–69 (1988).
49. KUSHI, Y., HANDA, S. and ISHIZUKA, I. *J. Biochem. (Tokyo)* **97,** 419–428 (1985).
50. IWAMORI, M., OHASHI, Y. and NAGAI, Y. In *Mass Spectrometry in Health and Life Sciences*, pp. 379–399 (BURLINGAME, A. L. and CASTAGNILI, JR, eds) Elsevier, Amsterdam, 1985.
51. HANDA, S., KUSHI, Y., KAMBARA, H. and SHIZUKUISHI, K. *J. Biochem. (Tokyo)* **93,** 315–318 (1983).
52. ARITA, M., IWAMORI, M., HIGUCHI, T. and NAGAI, Y. *J. Biochem. (Tokyo)* **93,** 319–322 (1983).
53. ARITA, M., IWAMORI, M., HIGUCHI, T. and NAGAI, Y. *J. Biochem. (Tokyo)* **94,** 249–256 (1983).
54. ARITA, M., IWAMORI, M., HIGUCHI, T. and NAGAI, Y. *J. Biochem. (Tokyo)* **95,** 971–981 (1984).
55. REINHOLD, V. N. and CARR, S. A. *Mass Spectrom. Rev.* **2,** 153–221 (1983).
56. HEMLING, M. E., YU, R. K., SEDWICK, R. D. and RINEHART, K. L. *Biochemistry* **23,** 5706–5718 (1983).
57. MATSUBARA, T. and HAYASHI, A. *J. Biochem. (Tokyo)* **99,** 1401–1408 (1986).
58. DOMON, B. and COSTELLO, C. E. *Biochemistry* **27,** 1534–1543 (1988).
59. SEKINE, M., SUZUKI, M., INAGAKI, F., SUZUKI, A. and YAMAKAWA, T. *J. Biochem. (Tokyo)* **101,** 553–562 (1987).
60. SUGITA, M., INAGAKI, F., NAITO, H. and HORI, T. *J. Biochem. (Tokyo)* **107,** 899–903 (1990).

61. MEILI, J. and SEIBL, J. *Org. Mass Spectrom.* **19,** 581–584 (1984).
62. SWEETMAN, B. J. and BLAIR, I. A. *Biomed. Environ. Mass Spectrom.* **17,** 337–340 (1988).
63. OHASHI, Y., IWAMORI, M., OGAWA, T. and NAGAI, Y. *Biochemistry* **26,** 3990–3995 (1987).
64. EGGE, H., DABROWSKI, J. and HANFLAND, P. *Pure Appl. Chem.* **56,** 807–818 (1984).
65. EGGE, H. and KATALINIC, J. P. *Mass Spectrom. Rev.* **6,** 331–393 (1987).
66. HANFLAND, P., KORDOWICZ, M., KATALINIC, J., EGGE, H., DABROSKI, J. and DABROSKI, U. *Carbohydr. Res.* **178,** 1–21 (1988).
67. EVANS, J. E. and MCCLUER, R. H. *Biomed. Environ. Mass Spectrom.* **14,** 149–153 (1987).
68. KATO, Y., TAKAHASHI, D., HIROSE, H., SAKAIRI, M. and KAMBARA, H. *Biomed. Environ. Mass Spectrom.* **16,** 331–334 (1988).
69. SUZUKI, M., SEKINE, M., YAMAKAWA, T. and SUZUKI, A. *J. Biochem. (Tokyo)* **105,** 829–833 (1989).
70. KUSHI, Y., ROKUKAWA, C. and HANDA, S. *Anal. Chem.* **175,** 167–176 (1988).
71. ARITA, M., IWAMORI, M., HIGUCHI, T. and NAGAI, T. *JEOL News* **19A,** 2–6 (1983).
72. IWAMORI, M., ARITA, M., HIGUCHI, T. and NAGAI, Y. *JEOL News* **20A,** 2–9 (1984).
73. THURIN, J., HERLYN, M., HINDSGAUL, O., STROMBERG, N., KARLSSON, K.-A., ELDER, D., STEPLEWSKI, Z. and KOPROWSKI, H. *J. Biol. Chem.* **260,** 14556–14563 (1985).
74. ARIGA, T. and YU, R. K. *J. Lipid Res.* **28,** 285–291 (1987).
75. EGGE, H., KATALINIC, J. P., REUTER, G., SCAUER, R., GHIDONI, R., SONNINO, S. and TETTAMANTI, G. *Chem. Phys. Lipids* **37,** 127–141 (1985).
76. FREDMAN, P., MANSSON, J.-E., WIKSTRAND, C. J., VRIONIS, F. D., RYNMARK, B.-M., BIGNER, D. D. and SVENNERHOLM, L. *J. Biol. Chem.* **264,** 12122–12125 (1989).
77. LEVERY, S. B., NUDELMAN, E. D., SALYAN, M. E. K. and HAKOMORI, S. *Biochemistry* **28,** 7772–7781 (1989).
78. NUDELMAN, E. D., MANDEL, U., LEVERY, S. B., KAIZU, T. and HAKOMORI, S. *J. Biol. Chem.* **264,** 18719–18725 (1989).
79. KUSHI, Y., HANDA, S., KAMBARA, H. and SHIZUKUISHI, K. *J. Biochem. (Tokyo)* **94,** 1841–1850 (1983).
80. ISOBE, R., KAWANO, Y., HIGUCHI, R. and KOMORI, T. *Anal. Biochem.* **177,** 296–299 (1989).
81. LEFFLER, H., HANSSON, G. and STOMBERG, N. *J. Biol. Chem.* **261,** 1440–1444 (1986).
82. IIDA, N., TOIDA, T., KUSHI, Y., HANDA, S., FREDMAN, P., SVENNERHOLM, L. and ISHIZUKA, I. *J. Biol. Chem.* **264,** 5974–5980 (1989).
83. NAGAI, K., ROBERTS, D. D., TOIDA, T., MATSUMOTO, H., KUSHI, Y., HANDA, S. and ISHIZUKA, I. *J. Biol. Chem.* **264,** 16229–16237 (1989).
84. TAKETOMI, T., HARA, A., KUTSUKAKE, Y. and SUGIYAMA, E. *J. Biochem. (Tokyo)* **107,** 680–684 (1990).
85. HAYASHI, A., MATSUBARA, T. and MORITA, M. *Proc. Jap. Soc. Med. Mass Spectrom.* **13,** 65–72 (1988).
86. DOMON, B., VATH, J. E. and COSTELLO, C. E. *Anal. Biochem.* **184,** 151–164 (1990).
87. TOMER, K. B., CROW, F. W. and GROSS, M. L. *J. Am. Chem. Soc.* **105,** 5487–5488 (1983).
88. JENNSEN, N. J., TOMER, K. B. and GROSS, M. L. *J. Am. Chem. Soc.* **107,** 1863–1868 (1985).
89. JENSEN, N. J., TOMER, K. B. and GROSS, M. L. *Anal. Chem.* **57,** 2018–2021 (1985).
90. JENSEN, N. J. and GROSS, M. L. *Lipids* **21,** 362–365 (1986).
91. TOMER, K. B., JENSEN, N. J. and GROSS, M. L. *Anal. Chem.* **58,** 2429–2433 (1986).
92. JENNSEN, N. J. and GROSS, M. L. *Mass Spectrom. Rev.* **6,** 497–536 (1987).
93. DETERDING, L. J. and GROSS, M. L. *Anal. Chim. Acta* **200,** 431–445 (1987).
94. DETERDING, L. J. and GROSS, M. L. *Org. Mass Spectrom.* **23,** 169–177 (1988).
95. ADAMS, J. *Mass Spectrom. Rev.* **9,** 141–186 (1990).
96. HARA, A. and TAKETOMI, T. *J. Biochem. (Tokyo)* **100,** 415–423 (1986).
97. KOBAYSHI, T., MITSUO, K. and GOTO, I. *Eur. J. Biochem.* **172,** 747–752 (1988).
98. FUKUDA, M., SPOONCER, E., OATES, J. E., DELL, A. and KLOCK, J. C. *J. Biol. Chem.* **259,** 10925–10935 (1984).
99. FUKUDA, M., BOTHNER, B., RAMSAMOOJ, P., DELL, A., TILLER, P. R., VARKI, A. and KLOCK, J. C. *J. Biol. Chem.* **260,** 12957–12967 (1985).
100. WANG, T. H., CHEN, T. F. and BAROFSKY, D. F. *Biomed. Environ. Mass Spectrom.* **16,** 335–338 (1988).
101. MIZUOCHI, T., LOVELESS, R. W., LAWSON, A. M., CHAI, W., LACHMANN, P. J., CHILDS, R. A., THIEL, S. and FEIZI, T. *J. Biol. Chem.* **264,** 13834–13839 (1989).
102. BRUNTZ, R., DABROWSKI, U., DABROWSKI, J., EBERSOLD, A., KATALINIC, J. P. and EGGE, H. *Biol. Chem. Hoppe-Seyler* **369,** 257–273 (1988).
103. BOULENGUER, P., LEROY, Y., ALONSO, J. M., MONTREUIL, J., RICART, G., COLBERT, C., DUQUET, D., DEWAELE, C. and FOURNET, B. *Anal. Biochem.* **168,** 164–170 (1988).
104. ORLAND, R., BUSH, C. A. and FENSELAU, C. *Biomed. Environ. Mass Spectrom.* **19,** 747–754 (1990).
105. ISHII, K., IWASAKI, M., INOUE, S., KENNY, P. T. M., KOMURA, H. and INOUE, Y. *J. Biol. Chem.* **264,** 1623–1630 (1989).
106. EGGE, H. and KATALINIC, P. In *Mass Spectrometry in the Health and Life Sciences*, pp. 401–424. (BURLINGAME, A. L. and CASTAGNOLI, N., JR, eds) Elsevier, Amsterdam, 1985.
107. DELL, A. *Adv. Carbohydr. Chem. Biochem.* **45,** 19–72 (1987).
108. DALLINGA, J. W. and HEERMA, W. *Biomed. Environ. Mass Spectrom.* **18,** 363–372 (1989).
109. DALLINGA, J. W., RINKEMA, F. D. and HEERMA, W. *Biomed. Environ. Mass Spectrom.* **18,** 241–246 (1989).
110. KHOO, K. H. and DELL, A. *Glycobiology* **1,** 83–91 (1990).
111. DOMON, B., MULLER, D. R. and RICHTER, W. J. *Org. Mass Spectrom.* **24,** 357–359 (1989).
112. DOMON, B., MULLER, D. R. and RICHTER, W. J. *Biomed. Environ. Mass Spectrom.* **19,** 390–392 (1990).