Characterization of GM_1 ganglioside by direct inlet chemical ionization mass spectrometry

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Abstract Intact permethylated and permethylated-reduced ($LiAlH_4$) derivatives of GM_1 ganglioside were analyzed by direct inlet ammonia chemical ionization (CI) mass spectrometry. In addition, the trimethylsilylated derivative of the permethylated-reduced sample of this ganglioside was similarly analyzed. CI mass spectrometry proves to be a highly satisfactory method for structural studies of GM_1 ganglioside because of the simple fragmentation pattern and the presence of prominent molecular ions and fragment ions in the high mass region. Complete information on the carbohydrate sequence and lipophilic composition can be easily obtained.—**Ariga, T., R. K. Yu, M. Suzuki, S. Ando, and T. Miyatake.** Characterization of GM_1 ganglioside by direct inlet chemical ionization mass spectrometry. *J. Lipid Res.* 1982. **23:** 437–442.

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During the last decade, mass spectrometry has come to be a rapid and sensitive method for the identification and structural determination of glycosphingolipids. Several derivatives have been studied, such as the trimethylsilylated (1–4), peracetylated (5), and permethylated derivatives (6–11) of glycosphingolipids. The permethylated derivatives are preferred for mass spectrometric analysis because of the relatively small mass increase compared to other derivatives. Karlsson et al. (14–21) report that permethylation followed by reduction with $LiAlH_4$ of amide groups to the corresponding tertiary amines is of particular value in structural studies of complex glycosphingolipids.

Chemical ionization (CI) mass spectrometry, the so-called soft ionization method, generally enhances the intensities of the molecular ions and fragment ions in the high mass range and has been proved useful in structural studies on glycosphingolipids (22–27). We have recently reported that CI mass spectrometry can provide information about the molecular weights, the ceramide structures, and the sugar sequences of the oligosaccharides of a variety of neutral glycosphingolipids (25–27). However, the feasibility of employing this technique as an aid in the structural identification of gangliosides has not been elucidated. In the present study, we describe the characterization of GM_1 ganglioside from bovine brain by direct inlet mass spectrometry as its completely methylated, methylated and reduced ($LiAlH_4$), and methylated, reduced, and trimethylsilylated derivatives.

MATERIALS AND METHODS

Materials

GM_1 ganglioside from adult bovine brain was purified by DEAE-Sephadex A-25 column and Iatrobeads column chromatography according to the procedure of Momoi, Ando, and Nagai (28). Permethylation was done by the method of Ando et al. (24) with slight modifications. The sample containing 60 μg of sialic acid was dissolved in 50 μl of dimethyl sulfoxide, and 100 μl of dimethylformamide and 100 μl of sodium hydride solution (freshly prepared, 1 mg in 1 ml of dimethylformamide) was added. After the solution was allowed to stand for 30 min at 0°C, 150 μl of methyl iodide (freshly distilled) was added. The reaction mixture was incubated for 30 min at 0°C and then allowed to stand for 2 hr at room temperature. The reaction was terminated by the addition of 3 ml of chloroform. The chloroform layer was washed three times by 3 ml of water and evaporated to dryness under a stream of nitrogen. Reduction of permethylated GM_1 ganglioside was performed with $LiAlH_4$ in diethyl ether for 3 hr at room temperature according to the method of Karlsson (21). The purification of the methylated GM_1 ganglioside was achieved by thin-layer

Abbreviations: CI, chemical ionization; EI, electron impact ionization.

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chromatography with a solvent system of chloroform–methanol–n-hexane 40:10:20 (by volume). Trimethylsilylation of the methylated and reduced derivative was carried out with 0.1 ml of hexamethyldisilazane–trimethylchlorosilane–pyridine 2:1:5 (by volume) at 60°C for 20 min.

Chemical ionization mass spectrometry

A Shimadzu-LKB gas chromatography–mass spectrometer 9000A equipped with a CI source was used. Ammonia was used as the reagent gas. The temperature of the ion source was kept at 220°C. The mass spectra were obtained at an electron energy of 500 eV, an emission current of 400 μA, and an accelerating voltage of 1.75 kV. Samples (50–100 μg) were introduced through a direct probe. The temperature of the direct probe inlet was programmed from 100°C to 400°C at the rate of about 50°C per minute. The total ion collector and mass spectra were monitored continuously during the heating. The mass marker was calibrated by measuring tri(perfluorononyl)-s-triazine (mol wt = 1485, PCR Research Chemicals, USA) and Ultramark (mol wt = 1621, PCR Research Chemicals, USA).

RESULTS AND DISCUSSION

GM_1 ganglioside in adult mammalian brain characteristically contains about equal amounts of C18 and C20 sphingosine, and less than 5% of other bases. The predominant fatty acid is stearic acid (28, 29). Therefore the ions originating from GM_1 ganglioside with C18 sphingosine as well as those with the C20 homologue should be predominantly detected. The ammonia CI mass spectra of the three derivatives of GM_1 ganglioside, namely the completely methylated, the methylated and reduced, and the methylated, reduced, and trimethylsilylated, are presented.

Fig. 1 shows the ammonia CI mass spectrum of the completely methylated GM_1 ganglioside. The fragmentation diagram of GM_1 ganglioside with C20 sphingosine is shown in **Fig. 2.** The mass spectrum shows six recognizable ion groups including the ceramide structure that are of diagnostic value in the structural elucidation for GM_1 ganglioside. The CI mass spectrum of the completely methylated derivative is characterized by the presence of prominent molecular ions. The protonated molecular ions $[MH]^+$ at m/z 1826 and 1854, and the fragment ions due to a loss of methanol [MH-32; MH-$CH_3OH]^+$ at m/z 1794 and 1822 are clearly detected and these ions provide information on the molecular weights of GM_1 ganglioside with stearic acid and C18 and C20 sphingosines, respectively. The CI mass spectrometry of permethylated glycosphingolipids and its merits for the molecular weight information have been previously described (27). In the case of the completely methylated GM_1 ganglioside, the sialic acid may be split from the molecule and the ions containing lipophilic parts are produced (Fig. 1). The ions due to a loss of methanol from asialo GM_1 structures are detected at m/z 1418 and 1446. The fragment ions corresponding to the loss of terminal galactose moiety are of low intensity. These ions are recorded at m/z 1199 and 1227. However, the ions due to the loss of the disaccharide moiety (m/z 464) are prominent at m/z 954 and 982, providing information on the sequence of sugars. The ceramide and

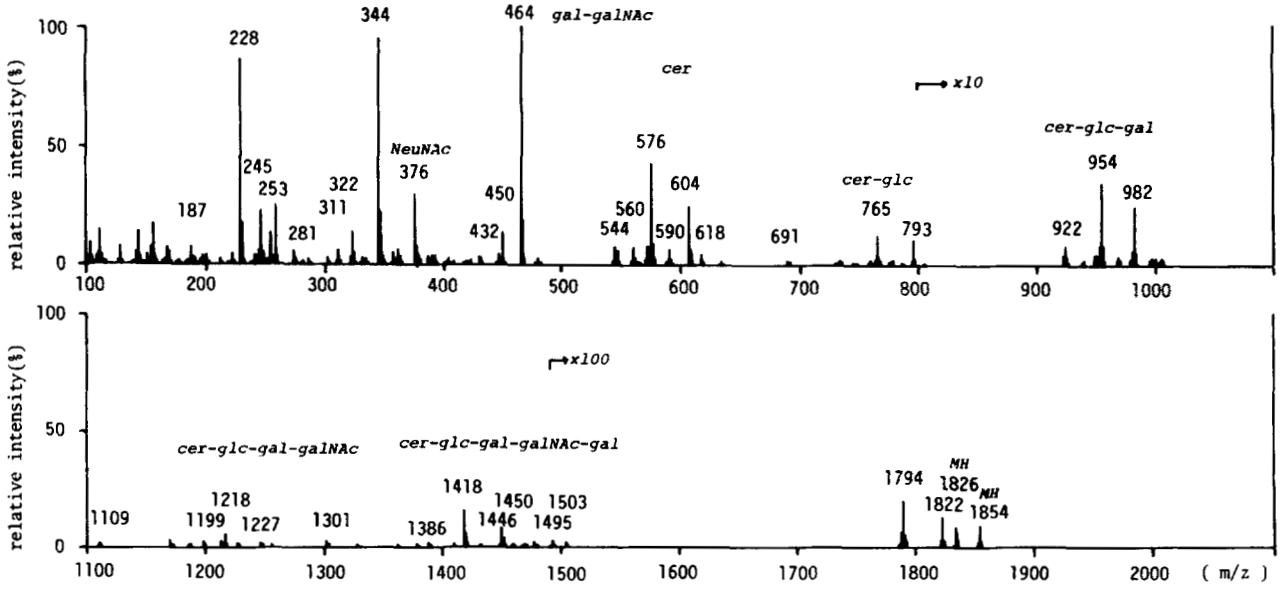

Fig. 1. Chemical ionization mass spectrum of the completely methylated GM_1 ganglioside.

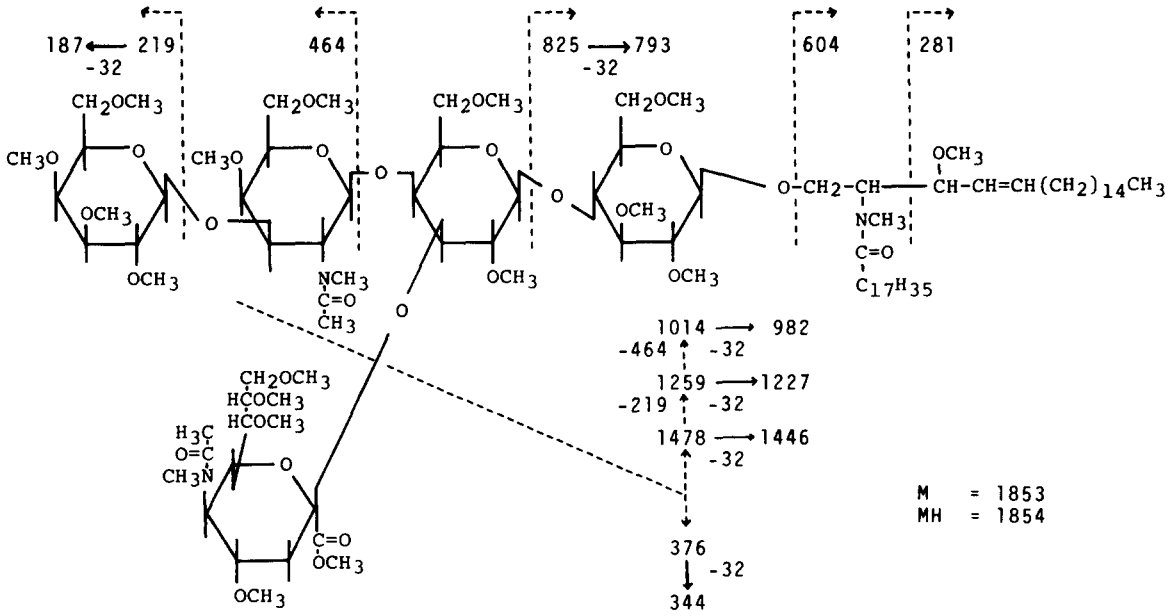

Fig. 2. Fragmentation diagram of the completely methylated GM_1 ganglioside with C20 sphingosine.

hexosylceramide structure are characterized by the presence of the ions at m/z 576 and 604, and at m/z 765 and 793, respectively. Therefore the sialic acid must be bound as a branch to the second hexose from ceramide. These ions including the ceramide structure have been characterized for the CI mass spectra of the completely methylated derivatives of neutral glycosphingolipids (27). The most important fragment ions containing only the carbohydrate part are as follows. The N-acetylneuraminic acid gives characteristic ions at m/z 376 and 344 [376-32; 376-CH_3OH]$^+$. The ion at m/z 344 is very prominent for GM_1 ganglioside. The ions at m/z 219 and 187 [219-32; 219-CH_3OH]$^+$ for terminal hexose are of relatively low intensity, while the fragment ion which is characteristic of terminal disaccharide units (gal-galNAc) is abundant. The prominent ion at m/z 228 is probably arising from N-acetylgalactosamine (6). The ions at m/z 253 and 281 give structural information about the long chain base, which is 1,3-dihydroxy-2-amino-4-octadecene and 1,3-dihydroxy-2-amino-4-eicosene, respectively (27).

In order to increase the volatility and stabilize the

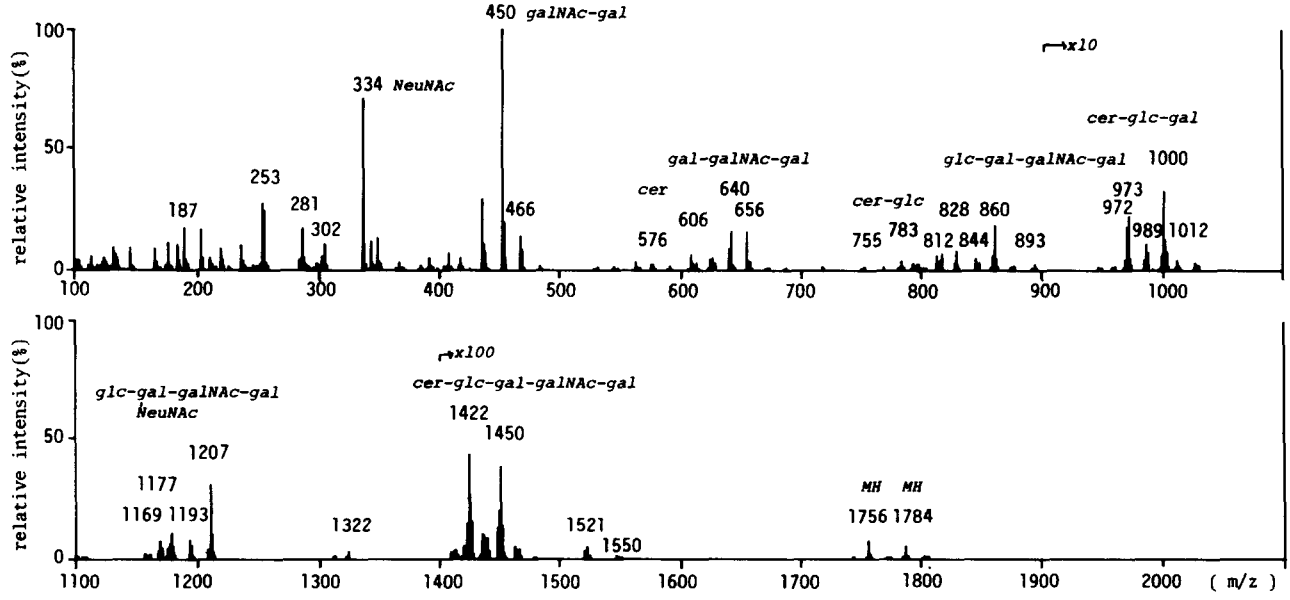

Fig. 3 Chemical ionization mass spectrum of the methylated and reduced GM_1 ganglioside.

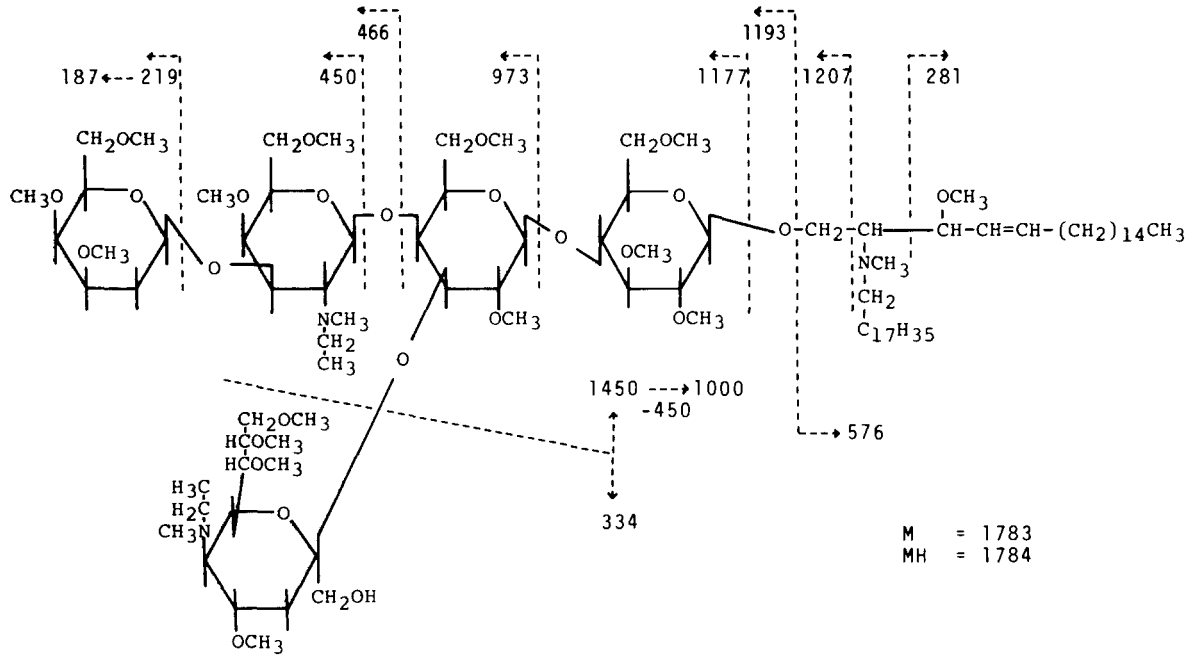

Fig. 4. Fragmentation diagram of the methylated and reduced GM_1 ganglioside with C20 sphingosine.

molecular ions, the permethylated and reduced derivatives of a variety of complex glycosphingolipids have been successfully applied to the electron impact ionization (EI) mass spectrometry for structural studies (12–15). Gangliosides are characterized by the presence of sialic acid. By reduction with $LiAlH_4$, the sialic acid of the methylated gangliosides was converted to the corresponding alcohol form. Karlsson et al. (15–21) have reported that conversion of the remaining alcohol group to a trimethylsilyl ether group of the reduced ganglioside derivatives is more useful for providing information on the carbohydrate sequences and molecular weights than the permethylated derivatives and the methylated and reduced derivatives, especially for complex gangliosides. We have also applied this method to GM_1 ganglioside for ammonia CI mass spectrometric analysis.

Fig. 3 shows the ammonia CI mass spectrum of the methylated and reduced derivative of GM_1 ganglioside. **Fig. 4** shows the fragmentation diagram of GM_1 ganglioside with C20 sphingosine. The mass spectrum is

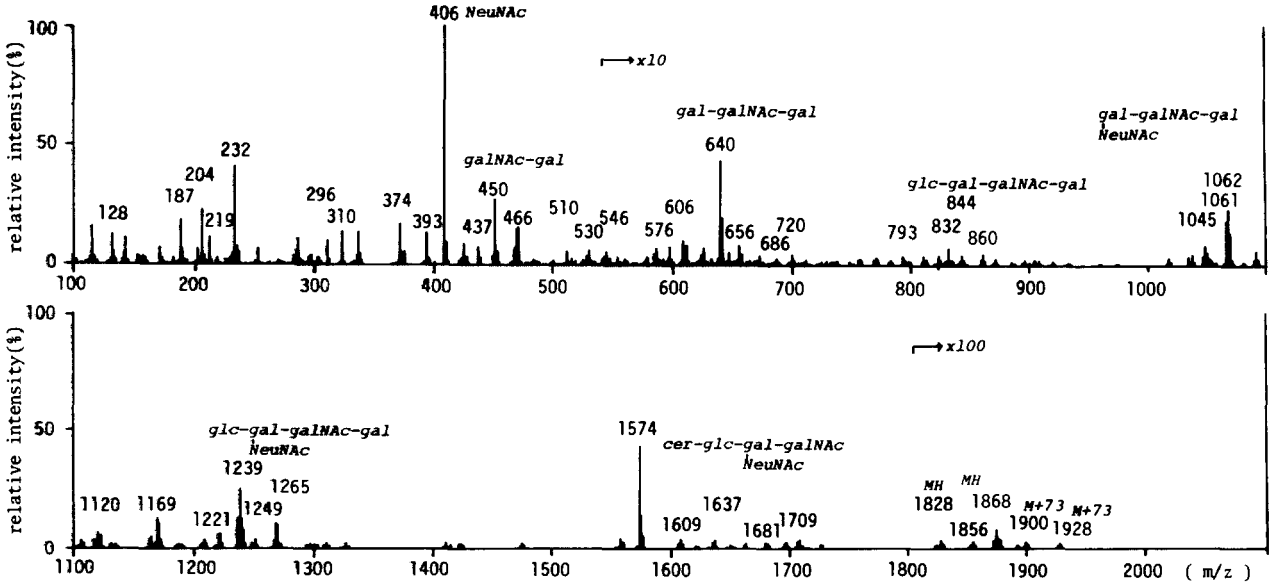

Fig. 5. Chemical ionization mass spectrum of the methylated, reduced, and trimethylsilylated GM_1 ganglioside.

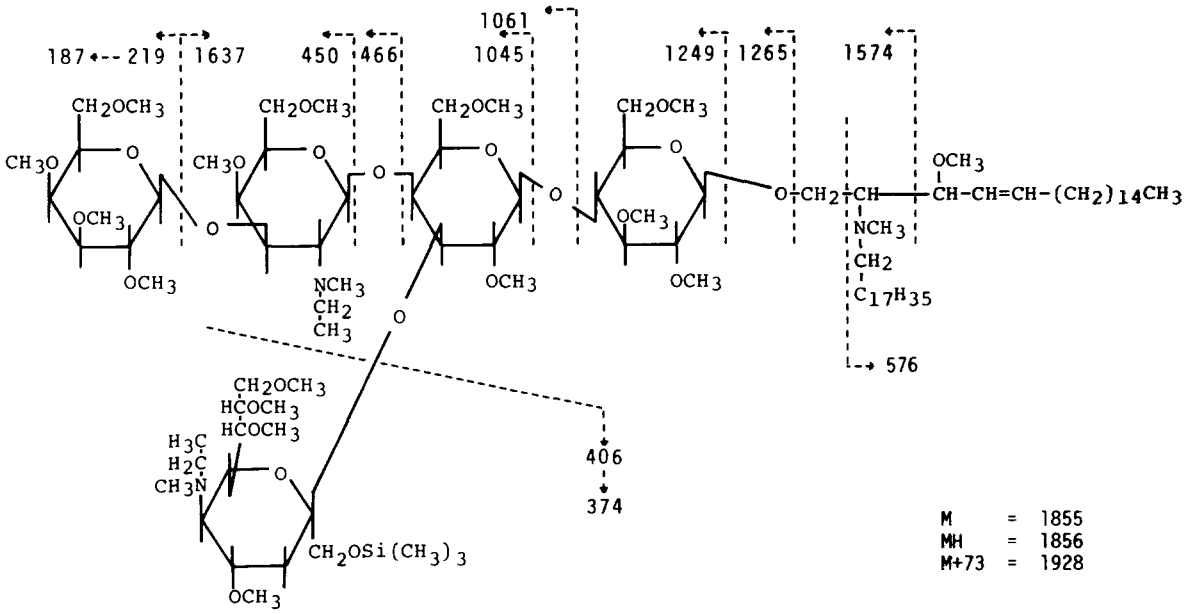

Fig. 6. Fragmentation diagram of the methylated, reduced, and trimethylsilylated GM_1 ganglioside with C20 sphingosine.

characterized by the presence of fragment ions containing terminal oligosaccharide units as well as those containing the ceramide part. Molecular weight information on GM_1 ganglioside is obtained from the protonated molecular ions at m/z 1756 and 1784, which correspond to different structures containing C18 and C20 sphingosine, respectively. The fragment ions at m/z 1422 and 1450 represent the asialo GM_1 structure with C18 and C20 sphingosine, respectively. The ions resulting from the loss of terminal disaccharide units from the asialo GM_1 structure are detected at m/z 972 and 1000, while the ions representing the ceramide and hexosylceramide are less prominent but still clearly discernible (Fig. 3). On the other hand, the ions resulting from terminal oligosaccharide units are found at m/z 450, 640, and 844, which are clearly derived from di-, tri-, and tetrasaccharides, respectively. These ions are sometimes detected as the corresponding ions containing the glycosidic oxygen also. Therefore, these ions provide information on the oligosaccharide sequences of GM_1 ganglioside (Fig. 3). The intact oligosaccharide units are also found at m/z 1177, 1193, and 1207, corresponding to cleavage of three different sites near the ceramide portion (Fig. 4). The N-acetylneuraminic acid gives characteristic ions at m/z 334 and 302 $[334\text{-}32;\ 334\text{-}CH_3OH]^+$.

Fig. 5 shows the ammonia CI mass spectrum of the methylated, reduced, and trimethylsilylated GM_1 ganglioside. The fragmentation diagram of GM_1 ganglioside with C20 sphingosine is shown in **Fig. 6.** In the case of this derivative, the fragment ions originating from the oligosaccharide units are prominent, while the ions including the ceramide structure are barely detectable. The protonated molecular ions are recorded at m/z 1828 and 1856. In the case of the trimethylsilylated derivative, the adduct ions due to $[M + 73;\ M + TMSi]^+$ are found at m/z 1900 and 1928. These ions provide information on the molecular weights of GM_1 ganglioside. The ion at m/z 1574 corresponding to the complete oligosaccharide plus the fatty acid is detected in the high mass range (19). Ions with N-acetylneuraminic acid-containing oligosaccharides are found at m/z 1045, 1061 and m/z 1249 and 1265, which correspond to the tetra- and pentasaccharides. The fragmentation of the oligosaccharide unit arising from the asialo GM_1 structure is almost identical to that of the reduced derivative (Fig. 5). The characteristic ions of terminal N-acetylneuraminic acid are detected at m/z 406 and 374 $[406\text{-}32;\ 406\text{-}CH_3OH]^+$.

Karlsson et al. (19, 20) have previously described the EI mass spectra of GM_1 ganglioside derivatives from bovine brain. Although the EI mass spectrum of the trimethylsilylated derivative of the methylated and reduced GM_1 ganglioside gives the molecular ions, suggesting a stabilizing effect, the spectra of the completely methylated and the methylated and reduced derivatives fail to show the molecular ions, and the fragment ions in the high mass range are not sufficiently intense to be useful for structural analysis. The CI method is based on the ion-molecular reaction between sample molecules and reactant ions and has been successfully used for the analysis of thermally labile compounds. CI mass spectrometry is therefore well suited for the structural studies of GM_1 ganglioside derivatives, because the molecular ions and the fragment ions in the high mass range, even the reduced derivatives, are clearly revealed, as shown in this

investigation. Extension of the CI mass spectrometry to other gangliosides is currently in progress.

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