Gas–liquid chromatography–mass spectrometry of synthetic ceramides

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ABSTRACT Two series of ceramides with either sphingosine (sphing-4-enine) or sphinganine as base and with one of the saturated fatty acids C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, or oleic acid were analyzed as the 1,3-di-*O*-trimethylsilyl ether derivatives by gas chromatography–mass spectrometry. The fragments formed on electron impact can be divided into three main groups, namely "molecular weight fragments," "long-chain base fragments," and "fatty acid fragments." The m/e values of these fragments can be used to determine unequivocally the structures of the long-chain base and fatty acid of a ceramide derived from a sphingolipid.

SUPPLEMENTARY KEY WORDS trimethylsilyl ethers · sphingosine · sphinganine

Sphingolipids have been subjected to extensive analysis with respect to the structures of the LCB (1–7) and of the fatty acids (7–11) obtained on hydrolysis. However, the structures of the individual molecular species of ceramides resulting from the combination of various fatty acids and LCB have not been determined.

Ceramides can be derived from sphingomyelins and glycosphingolipids by enzymatic and chemical methods (12–14), and studies in our laboratories have shown that ceramides with different LCB and fatty acid compositions can be analyzed by GLC and also by GLC–mass spectrometry (15). These experiments have been extended in the present study, which is concerned with analysis of synthetic ceramides as TMS ether derivatives by GLC–mass spectrometry. This method has been found to provide an efficient tool for determining the structures of ceramides.

Abbreviations: LCB, long-chain bases; GLC, gas–liquid chromatography; TMS, trimethylsilyl; TLC, thin-layer chromatography.

MATERIALS AND METHODS

The ceramides were prepared with either DL-sphinganine[1] or DL-sphingosine (DL-sphing-4-enine) obtained from Miles Laboratories, Inc., Elkhart, Ind. In both series ceramides with the fatty acids 16:0, 18:0, 18:1, 20:0, 22:0, 24:0, 26:0, and perdeuterated 18:0 (the last-named kindly supplied by Professor Einar Stenhagen) were prepared by direct coupling, using carbodiimide to activate the carboxylic acid. The procedure will be described elsewhere. The ceramides were purified by silicic acid chromatography and the purity was established by TLC and GLC.[2]

GLC–Mass Spectrometry

The ceramides were converted into TMS derivatives by treatment of 200 μg with 100 μl of pyridine, 20 μl of hexamethyl disilazane, and 10 μl of trimethyl chlorosilane at room temperature for 20 min (2).

An LKB gas chromatograph–mass spectrometer, model 9000, was used. The electron energy was 22.5 ev and the trap current 120 μamp. The column consisted of a 1.2 m coiled glass column (3 mm I.D.) with a packing of 1% OV-1 (nonpolar silicone phase) on 60–80 mesh Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa.). The column was conditioned at 350°C for 24 hr. The column temperature was 275°C, and the flash heater and separator temperatures were about 300°C. A gas–liquid chromatogram of TMS-*N*-oleoyl sphinganine is shown in Fig. 1.

[1] The nomenclature for sphingolipid bases is based on recommendations of the Commission on Biochemical Nomenclature of IUPAC and IUB (see 1967 J. Lipid Res. **8:** 523). Fatty acids are denoted by chain length: No. of double bonds.

[2] Samuelsson, B., and K. Samuelsson. To be published.

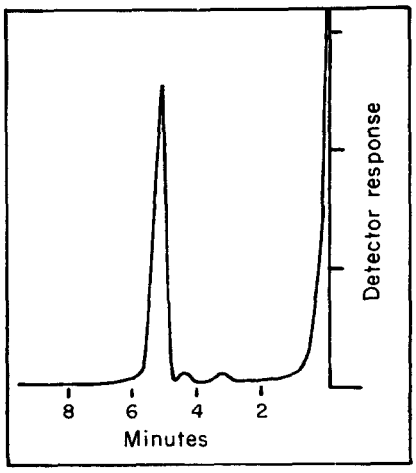

FIG. 1. Gas–liquid chromatography of 1,3-di-*O*-trimethylsilyl-*N*-oleoyl sphinganine. Column: 1% OV-1 on Gas-Chrom Q; column temperature: 275°C.

RESULTS AND DISCUSSION

The mass spectrum of the TMS derivative of *N*-stearoyl sphingosine will first be considered in detail (Fig. 2, see also Table 1). The molecular weight is indicated by ions at m/e 694 (M–15), m/e 619 (M–90), and m/e 606 (M–103) formed by elimination of a methyl group, trimethyl silanol, and the terminal $-CH_2-O-Si(CH_3)_3$, respectively. Eliminations involving the fatty acid residue produce ions at m/e 426 [M–(*b* + 1)] and m/e 336 [M–(*b* + 1 + 90)] by loss of stearoyl amide and, in the latter case, also trimethyl silanol. Loss of stearoyl amide plus $CH_3(CH_2)_{12}$ [M–(*b* + 1 + *e*)] gives an ion at m/e 243; cleavage between C-2 and C-3 with charge retention on the main sphingosine fragment results in an ion at m/e 311. The same cleavage but with charge retention on the other part of the molecule gives rise to an ion appearing at m/e 398 (M–*a*). Another fragmentation involving loss of the main part of the sphingosine molecule gives an ion at m/e 471 [M–(*a*–73)], which is tentatively ascribed to cleavage between C-2 and C-3 and transfer of the TMS group to the remaining fragment. An ion appearing at m/e 247 is also tentatively interpreted to be formed by this reaction and, in addition, β-cleavage of the stearoyl residue with transfer of hydrogen from the γ-carbon atom, i.e. [M–(*a*–73 + 224)]; the additional elimination of trimethyl silanol gives rise to an ion at m/e 157.

In order to obtain experimental evidence for the fragmentations proposed above, we prepared the corresponding derivative of *N*-perdeuterostearoyl sphingosine and subjected it to GLC–mass spectrometry. The mass spectrum is shown in Fig. 3. The fragments (M–15), (M–90), and (M–103) now appeared at m/e values 35 units higher than those of the corresponding nondeuterated derivative. This is also seen for other fragments that retain the fatty acid residue, i.e. (M–*a*) and [M–(*a*–73)]. However, fragments formed by elimination of the fatty acid, [M–(*b* + 1)], [M–(*b* + 1 + 90)], and [M–(*b* + 1 + *e*)] have the same m/e values as in the nondeuterated derivative. Evidence for the β-cleavage of the stearoyl residue with transfer of hydrogen from the γ-carbon atom to the carbonyl oxygen in the formation of fragments [M–(*a*–73 + 224)] and [M–(*a*–73 + 224 + 90)] was also obtained. The reaction involves retention of three hydrogens originating in the fatty acid; it was accordingly found that the fragments mentioned above appear at m/e 250 and 160 instead of 247 and 157, respectively.

It is evident from the results described above that the molecular weight of a ceramide can be determined from the fragments M–15, M–90, and M–103, and the nature of the LCB from the fragments formed by elimination of the acylamide, with or without trimethyl silanol, and by cleavage between C-2 and C-3. In the example discussed above these fragments appear at m/e 426, 336, and 311, respectively. The structure of the fatty acid moiety can be deduced from the fragments formed by cleavage between C-2 and C-3 with or without transfer of a TMS group. The resulting ions appear at m/e 471 [M–(*a*–73)] and m/e 398 (M–*a*), respectively.

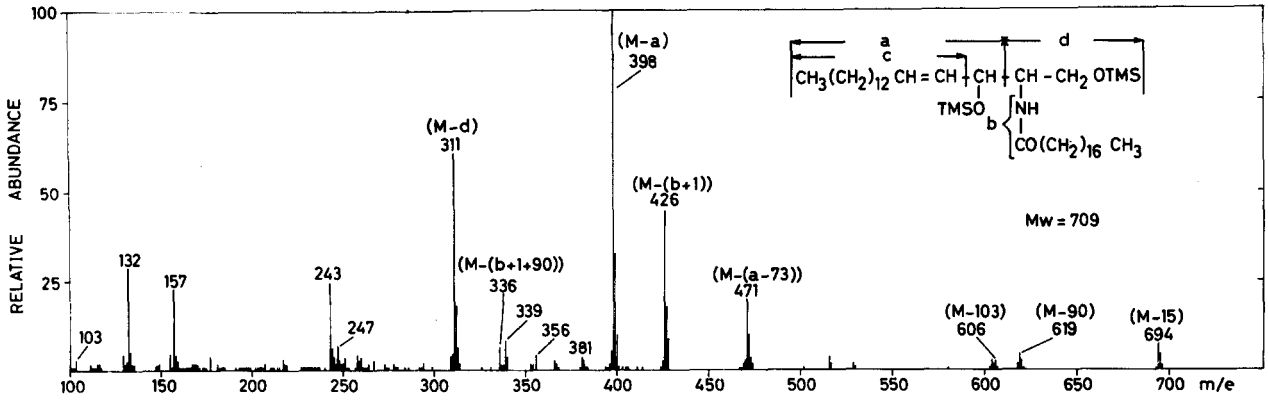

FIG. 2. Mass spectrum of 1,3-di-*O*-trimethylsilyl-*N*-stearoyl sphingosine.

Support for the proposed fragmentations can also be found in the mass spectrum of the TMS derivative of *N*-oleoyl sphingosine, which is shown in Fig. 4. Compared with the stearoyl analogue, the "molecular weight fragments" have m/e values which are two units lower, i.e. m/e 692, 617, and 604, whereas the "LCB fragments" are the same, i.e. m/e 426, 336, and 311. In accord with these data, the "fatty acid fragments" have m/e values (396 and 469) that are two units lower than corresponding fragments of the stearoyl analogue.

Some of the fragmentations described above have also been observed by Gaver and Sweeley, who characterized *N*-acetyl derivatives of sphingosine and sphinganine (16).

The mass spectrum of the TMS derivative of *N*-stearoyl sphinganine is shown in Fig. 5. The molecular weight is again obtained from the eliminations M–15,

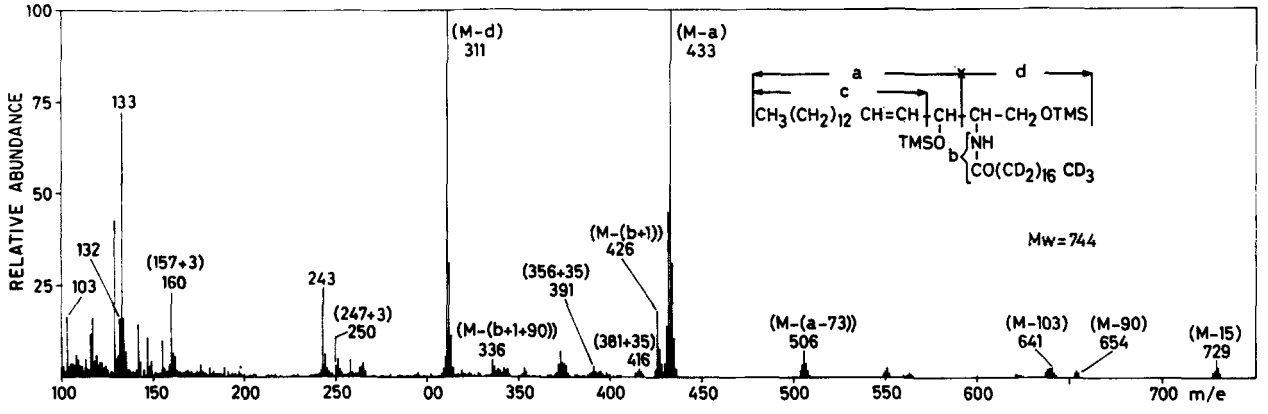

FIG. 3. Mass spectrum of 1,3-di-*O*-trimethylsilyl-*N*-perdeuterostearoyl sphingosine.

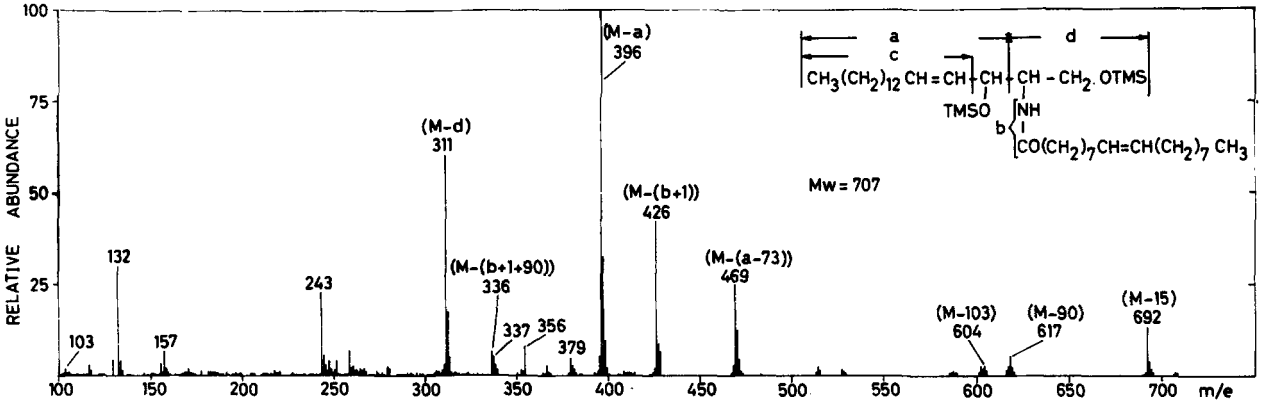

FIG. 4. Mass spectrum of 1,3-di-*O*-trimethylsilyl-*N*-oleoyl sphingosine.

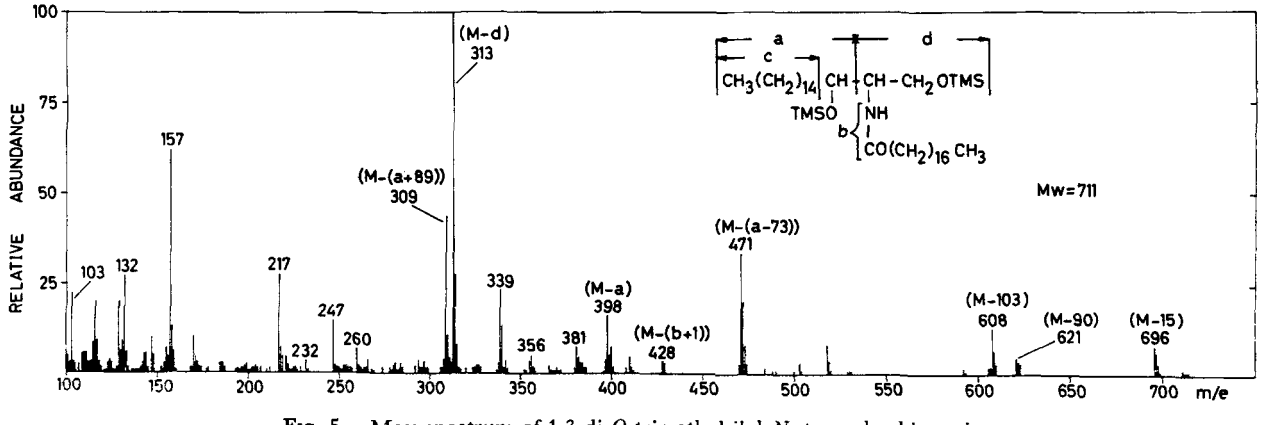

FIG. 5. Mass spectrum of 1,3-di-*O*-trimethylsilyl-*N*-stearoyl sphinganine.

TABLE 1 SOME IMPORTANT FRAGMENTS FOR STRUCTURE DETERMINATION OF CERAMIDES BY MASS SPECTROMETRY

"Molecular Weight Fragments"	"LCB Fragments"	"Fatty Acid Fragments"	Sphinganine Derivatives	Sphingosine Derivatives
M–15	M–(b + 1)	M–a	M–(b + 1 + c)	M–(b + 1 + e)
M–90	M–(b + 1 + 90)	M–(a + 89)		
M–103	M–d	M–(a–73)		

For explanation of portions a–d of the molecule, see upper right-hand corner of Fig. 2; e is $CH_3(CH_2)_{12}$ from sphinganine.

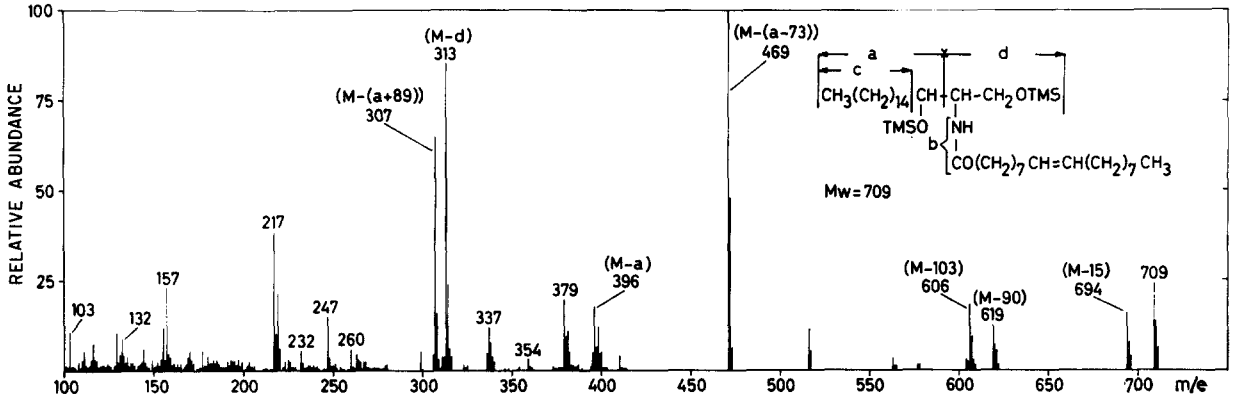

FIG. 6. Mass spectrum of 1,3-di-*O*-trimethylsilyl-*N*-oleoyl sphinganine.

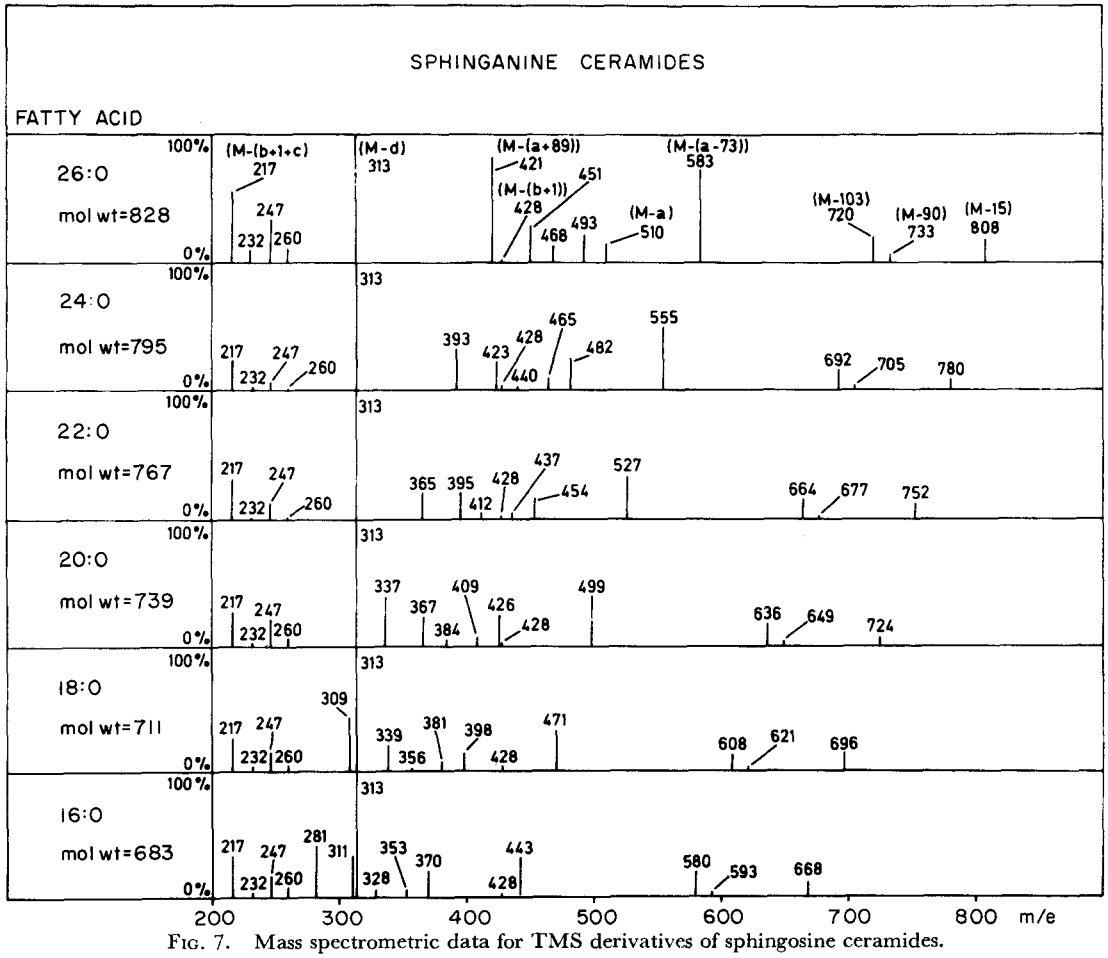

FIG. 7. Mass spectrometric data for TMS derivatives of sphingosine ceramides.

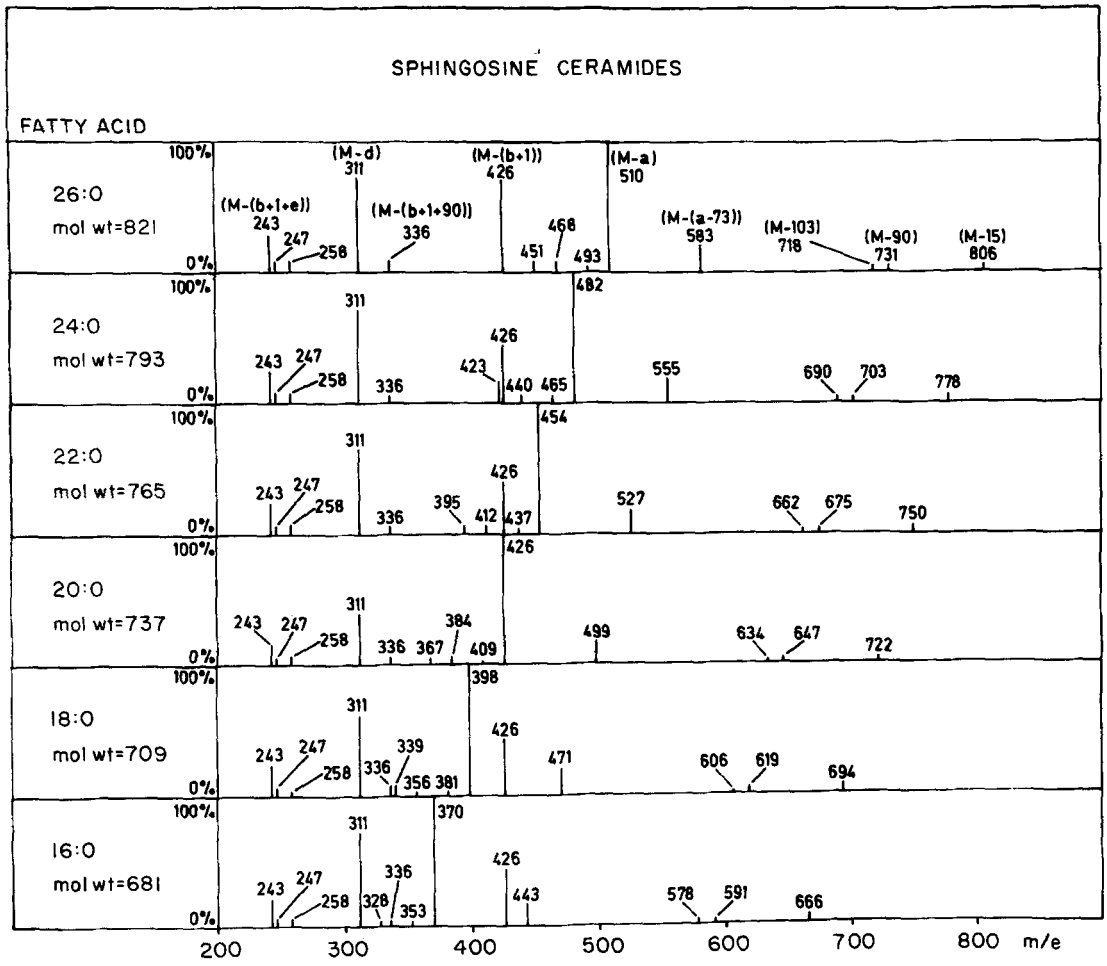

FIG. 8. Mass spectrometric data for TMS derivatives of sphinganine ceramides.

M–90, and M–103, which give rise to ions appearing at m/e 696, 621, and 608, respectively. Information on the structure of the LCB is provided by M–*d* (cleavage between C-2 and C-3) appearing at m/e 313, whereas charge retention on the other part of the molecule gives a fragment (m/e 398) which can be used to determine the structure of the fatty acid residue. Other "fatty acid fragments" are formed by additional elimination of a trimethyl silyloxy group [M–(*a* + 89)] or capture of the TMS group [M–(*a*–73)] and appear at m/e 309 and 471, respectively.

An ion that is absent from the mass spectrum of the sphingosine ceramide derivatives but that is relatively prominent in the sphinganine series appears at m/e 217. This ion most probably consists of C–1 to C–3 of the LCB from which acyl amide has been eliminated.

The mass spectrum of the oleoyl analogue of the sphinganine ceramide derivative is shown in Fig. 6. The molecular ion at m/e 709 is relatively prominent and the molecular weight of the compound can also be found from the fragments M–15, M–90, M–103, and M–(90 + 103). The structure of the LCB is obtained from M–*d* appearing at m/e 313 and the sphinganine nature of the LCB from fragments appearing at m/e 217. The "fatty acid fragments," i.e. M–*a*, M–(*a*–73), and M–(*a* + 89), appear at m/e 396, 469, and 307 and show conclusively that a monounsaturated C_{18} acid is present in the ceramide.

In order to provide data for the identification of ceramides of natural origin and to facilitate the interpretation of fragmentation processes, two series of ceramides have been prepared, one with sphingosine as base and the other with sphinganine as base. In both cases the fatty acid residues are saturated normal acids ranging from C_{16} to C_{26}. The results have been summarized in Figs. 7 and 8 and Table 1, where the fragments that are important for structure determination of ceramides are summarized.

It is evident from Fig. 7 that in the sphinganine derivatives the "LCB fragments" M–(*b* + 1) and M–*d* appear at the same m/e value in all members of the series, whereas the "fatty acid fragments" M–*a*, M–(*a*–73), and M–(*a* + 89) are shifted 28 units between neighboring members of the series. This is also true for

fragments appearing at m/e values 451, 468, and 493 in the C_{26} homologue and the molecular weight fragments M–15, M–90, and M–103. Three ions that appear at m/e 217 [M–(b + 1 + c)], 232, and 260 are the same for all of the homologues of the sphinganine series. They are absent from the sphingosine series and might be useful for the identification of sphinganine derivatives, although additional studies with LCB of different chain lengths are required before definitive conclusions can be drawn.

The sphingosine derivatives (Fig. 8) show similar groups of fragments. Thus the "LCB fragments" M–(b + 1), M–(b + 1 + 90), and M–d appear at the same m/e value in all of the homologues. The "fatty acid fragments" M–a and M–(a–73) and "the molecular weight" fragments appear at m/e values that differ by 28 units between homologues. This is also seen for the fragments appearing at m/e 451, 468, and 493 in the C_{26} homologue, and since the same ions are present in the sphingosine and sphinganine series they can probably be referred to as "fatty acid fragments" although no structures have as yet been assigned. Two ions appearing at m/e 243 and 258 in all of the sphingosine homologues are absent from the sphinganine series and might be important for the identification of the former series. An ion at m/e 247 is present in all of the homologues of both the sphingosine and sphinganine series.

It is evident from the data presented that mass spectrometry can be used to determine the structure of the fatty acid residue and the LCB in ceramides. In combination with gas chromatography it provides an efficient method for identification of molecular species of sphingolipids.

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References

1. Sweeley, C. C., and E. A. Moscatelli. 1959. *J. Lipid Res.* **1:** 40.
2. Gaver, R. C., and C. C. Sweeley. 1965. *J. Amer. Oil Chem. Soc.* **42:** 294.
3. Karlsson, K. A., and G. A. L. Holm. 1965. *Acta Chem. Scand.* **19:** 2423.
4. Karlsson, K. A. 1967. *Acta Chem. Scand.* **21:** 2577.
5. Carter, H. E., and R. C. Gaver. 9167. *Biochem. Biophys. Res. Commun.* **29:** 886.
6. Polito, A. J., T. Akita, and C. C. Sweeley. 1968. *Biochemistry.* **7:** 2609.
7. Carter, H. E., P. Johnson, and E. J. Weber. 1965. *Annu. Rev. Biochem.* **34:** 109.
8. Sweeley, C. C. 1963. *J. Lipid Res.* **4:** 402.
9. Kishimoto, Y., and N. S. Radin. 1963. *J. Lipid Res.* **4:** 437.
10. O'Brien, J. S., and G. Rouser. 1964. *J. Lipid Res.* **5:** 339.
11. Ställberg-Stenhagen, S., and L. Svennerholm. 1965. *J. Lipid Res.* **6:** 146.
12. Sribney, M. 1966. *Biochim. Biophys. Acta.* **125:** 542.
13. Renkonen, O. 1965. *J. Amer. Oil Chem. Soc.* **42:** 298.
14. Carter, H. E., J. A. Rothfus, and R. Gigg. 1961. *J. Lipid Res.* **2:** 228.
15. Samuelsson, B., and K. Samuelsson. 1968. *Biochim. Biophys. Acta.* **164:** 121.
16. Gaver, R. C., and C. C. Sweeley. 1966. *J. Amer. Chem. Soc.* **88:** 3643.