Gas–liquid chromatography–mass spectrometry of synthetic ceramides containing 2-hydroxy acids

SVEN HAMMARSTRÖM, BENGT SAMUELSSON, and KARIN SAMUELSSON

Department of Medical Chemistry, Royal Veterinary College, Department of Neurology, Karolinska sjukhuset, Stockholm, Sweden

ABSTRACT Ceramides containing either sphingosine or sphinganine and one of the 2-hydroxy acids, 14h:0, 16h:0, 18h:0, 20h:0, 22h:0, 24h:0, and 26h:0 were prepared and separated by gas chromatography as the 1,3,2′-tri-*O*-trimethylsilyl derivatives. Mass spectrometric analyses of these derivatives showed that the ions formed on electron impact can be used to determine unequivocally the structures of the long-chain base and the fatty acid residue in the ceramide. Proposed structures of ions and the mechanisms of reaction of their formation are supported by mass spectra of homologous derivatives, by deuterium labeling experiments, and by high-resolution on mass spectrometry.

SUPPLEMENTARY KEY WORDS trimethylsilyl ethers · sphingosine · sphinganine · deuterium labeling · silicic acid chromatography

CERAMIDES ARE CONSTITUENTS of all sphingolipids. The structures of their fatty acids and LCB have been studied in detail (1–11). Recently a method for the gas chromatographic separation of ceramides as di-*O*-trimethylsilyl ethers was developed in our laboratory, and it was also shown that mass spectrometry can be used to determine the structures of individual molecular species (12, 13). Analyses of free ceramides in plasma (14) and of ceramides derived from sphingomyelin (15) and other sources (16) by combined GLC–mass spectrometry have also been reported.

The occurrence of ceramides containing 2-hydroxy acids as constituents of cerebrosides, especially in brain and kidney, prompted their investigation by combined GLC–mass spectrometry. The present report describes gas chromatographic separation and mass spectrometric analysis of tri-*O*-trimethylsilyl ethers of synthetically prepared ceramides containing 2-hydroxy acids.

MATERIALS AND METHODS

Chemicals

DL-Sphinganine (DL-*erythro*-1,3-dihydroxy-2-aminooctadecane) and DL-sphingosine (DL-*erythro-trans*-1,3-dihydroxy-2-aminooctadec-4-ene) were obtained from Miles Laboratories, Inc., Elkhart, Ind.; DL-2-hydroxytetradecanoic and DL-2-hydroxyhexadecanoic acids were from Fluka A.G., Buchs, Switzerland; DL-2-hydroxyhexacosanoic acid was from Mann Research Labs., Inc., New York; DL-2-hydroxyeicosanoic, DL-2-hydroxydocosanoic, and DL-2-hydroxytetracosanoic acids were obtained from Applied Science Laboratories, Inc., State College, Pa. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was purchased from the Ott Chemical Company, Muskegon, Mich. d_9-TMCS (>99 at.%) was synthesized and provided as a special order from Merck, Sharp & Dohme of Canada, Ltd., Montreal. DL-2-Hydroxyoctadecanoic acid (K & K Laboratories, Inc., Plainview, N.Y.) was purified by chromatography on a column of silicic acid, (Mallinckrodt Chemical Works, St. Louis, Mo.) 100 mesh, activated at 115°C. All other reagents were used without further purification.

Preparation of Ceramides

Ceramides were prepared according to a procedure which will be described in detail.[1] The 2-hydroxy acid was acetylated with acetic anhydride in pyridine at room temperature for 16 hr. The 2-acetoxy acid was coupled with the LCB using a carbodiimide to activate the car-

Abbreviations: LCB, long-chain base; GLC, gas–liquid chromatography; TMS, trimethylsilyl; TLC, thin-layer chromatography; TGCU, triglyceride carbon units; m.u., mass unit; HMDS, hexamethyldisilazane; TMCS, trimethylchlorosilane; d_9-TMCS, perdeuterated TMCS.

[1] Hammarström, S. To be published.

boxyl group (40°C, 16 hr). The 2′-acetoxy ceramide was isolated by extraction with ether and was purified by chromatography on silicic acid. The purified 2′-acetoxy ceramide was converted to the 2′-hydroxy ceramide by alkaline hydrolysis for 1 hr at 20°C, using 0.3 N NaOH in chloroform–methanol 1:1. Diastereoisomers were separated on TLC.[1]

Ceramide Diastereoisomer Used

Of the two diastereoisomeric pairs obtained in the preparation of ceramides using the DL-hydroxy acids and the DL-LCB listed under *Chemicals*, one enantiomer of the racemic diastereoisomer consisting of *N*-(2′-D-hydroxyacyl)-D-*erythro*-sphingosine and *N*-(2′-L-hydroxyacyl)-L-*erythro*-sphingosine should correspond to the naturally occurring isomer in sphingolipids (footnote 1, reference 17). This mixture of enantiomers was used to obtain mass spectra of ceramides containing either sphingosine or sphinganine.

Preparation of Trimethylsilyl Ether Derivatives for GLC and Mass Spectrometry

To 100 μg of a 2′-hydroxy ceramide, dissolved in 100 μl of dry pyridine, 20 μl of HMDS and 10 μl of TMCS were added. The mixture was left at room temperature for 30 min, and then was evaporated to dryness using an oil pump. The residue was dissolved in 100 μl of carbon disulfide. To prepare deuterated TMS ethers, 20 μl of d_9-TMCS (18) were added instead of HMDS and TMCS.

GLC

The 1,3,2′-tri-*O*-TMS derivatives of ceramides were analyzed in an F & M Biomedical gas chromatograph, Model 400, (F & M Scientific Corp., Avondale, Pa.) with a hydrogen flame ionization detector. The column contained 1% OV-1, a nonpolar silicone phase on 60–80 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.), in a U-shaped glass column 1.2 m in length (3.5 mm I.D.) and was conditioned at 350°C for 24 hr. The column temperature was varied between 260° and 290°C depending on the particular ceramide to be analyzed. The detector and flash heater temperatures were kept 25°C above the column temperature. Helium was used as carrier gas with an inlet pressure of 3.0 kg/cm².

Mass Spectrometry

An LKB gas chromatograph–mass spectrometer, model 9000, was used. The electron energy was 22.5 ev, the trap current was 60 μA, the accelerator voltage was 3.5 kv, and the multiplier voltage was 2.9 kv. The separator temperature was 280°C, the ion source temperature was 290°C, the scan speed was 6, and the scan limits were m/e 4-1000. All conditions for GLC were identical with those described above, except that a 1.2 m coiled glass column (3 mm I.D.) was used. High resolution mass spectra were obtained with an Atlas SM-1 instrument by direct probe analysis of the 1,3,2′-tri-*O*-trimethylsilyl derivatives. The comparator which was used (Bell & Howell/Projectina A.G., Heerbrugg, Switzerland) had an accuracy of ±15 p.p.m.

RESULTS AND DISCUSSION

Fig. 1a shows the mass spectrum of the 1,3,2′-tri-*O*-TMS derivative of *N*-(2′-hydroxystearoyl)sphingosine.[2] The designation of fragments is given in the structural formula shown in this figure. Many ions are analogous to those seen for ceramides with normal fatty acids (12, Table 4). The molecular ion, m/e 797, is of low intensity, and the molecular weight is more readily determined from the ions m/e 782 (M-15), m/e 707 (M-90), m/e 694 (M-103), m/e 617 (M–2 × 90), and m/e 604 (M–103-90). These are called "molecular weight fragments" and are presumably formed by elimination of $\cdot CH_3$ from one of the TMS groups, one molecule of trimethylsilanol, the terminal $CH_2{=}\dot{O}{-}Si(CH_3)_3$ radical, two molecules of trimethylsilanol, and $CH_2{=}\dot{O}{-}Si(CH_3)_3$ plus one molecule of trimethylsilanol, respectively. The spectrum has a prominent base peak at m/e 486. This can be visualized to be formed by homolytic cleavage of the C—C bond between C-2 and C-3 after initial charge localization on the nitrogen at C-2. Cleavage of the same bond with charge localization on the oxygen at C-3 is presumably involved in the formation of the ion appearing at m/e 311. The ion appearing at m/e 599 [M–(a-73)][3] is probably also formed by cleavage between C-2 and C-3, but in this case the odd electron on the nitrogen is paired by transfer of a trimethylsilyl radical as shown in Fig. 2.

Transfer of a hydrogen atom to the nitrogen in [M–(a-73)] followed by cleavage of the bond between C-2 and the nitrogen could yield the ion at m/e 444 (b + 1 + 73).[3] An ion at m/e 426 [M–(b + 1)] is assumed to arise by elimination of acylamide. Additional elimination of one molecule of trimethylsilanol yields m/e 336 [M–(b + 1)-90] which is also seen regularly. The ion at m/e 372 (b + 2)[3] is presumably formed by transfer of two hydrogens to fragment b. Cleavage between C-1′ and C-2′ with charge retention on the oxygen at C-2′ is probably involved in the formation of m/e 327, f,[3] which is specific for 2′-hydroxy ceramides. The ion

[2] Below m/e 100 the following major ions are present (relative abundance is in parentheses): m/e 55 (4%), m/e 57 (5%), m/e 75 (27%), m/e 83 (5%), m/e 85 (7%), m/e 97 (5%).

[3] The observed mass of the corresponding ion in the high resolution mass spectrum of 1,3,2′-tri-*O*-trimethylsilyl-*N*-(2′-hydroxytetradecanoyl) sphinganine ceramide differed less than 15 p.p.m. from the calculated m/e value for the ion.

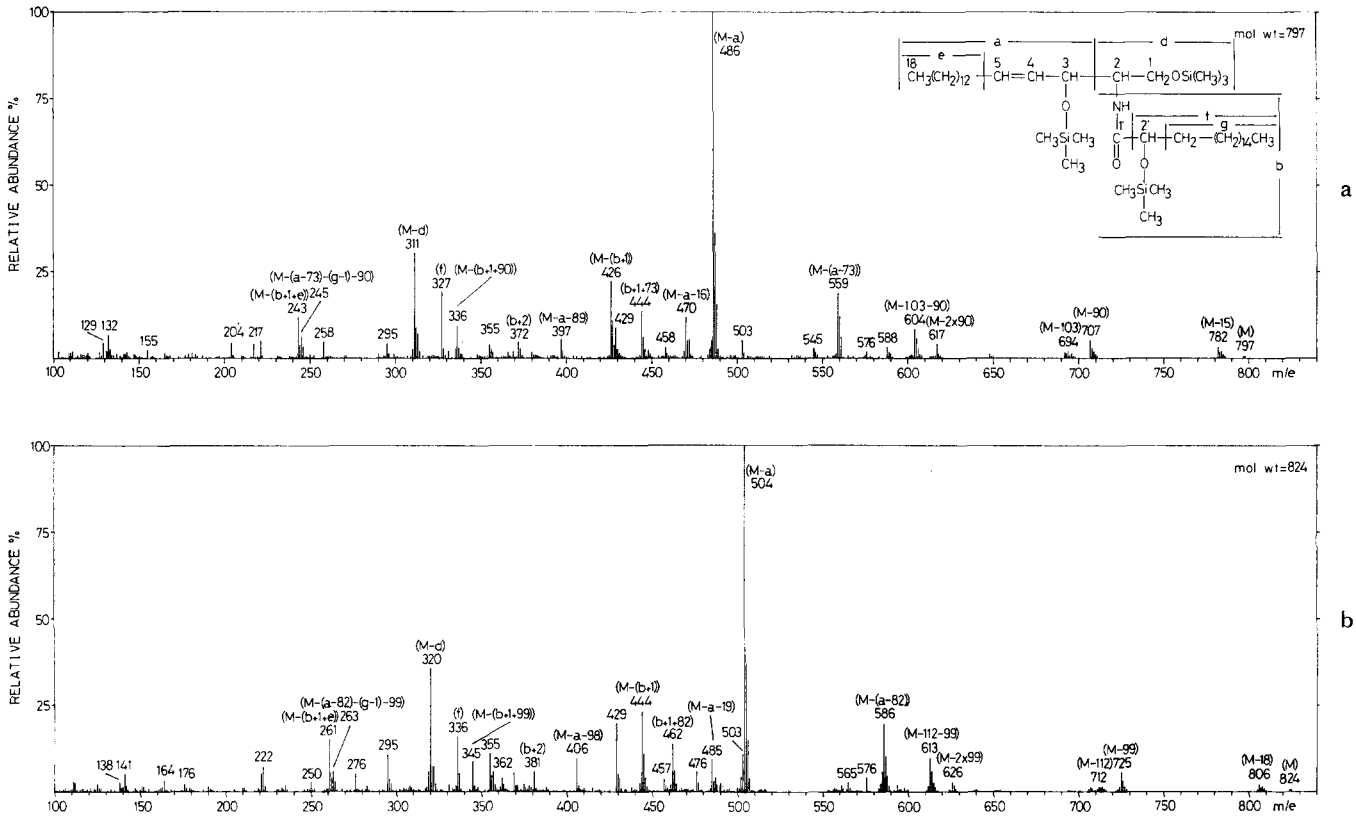

FIG. 1. Mass spectrum of (*a*) 1,3,2′-tri-*O*-methylsilyl-*N*-(2′-hydroxystearoyl) sphingosine[2] and (*b*) 1,3,2′-d_{27}-tri-*O*-trimethylsilyl-*N*-(2′-hydroxystearoyl) sphingosine.

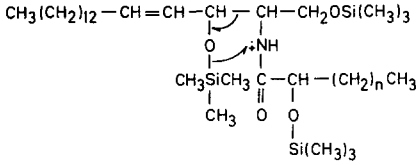

FIG. 2. Proposed formation of the ion m/e 559 [M–(a-73)].

[M–(b + 1 + e)] at m/e 243, resulting from elimination of acylamide and $CH_3(CH_2)_{12}$, is present in sphingosine ceramides containing normal fatty acids (12) or 2-hydroxy acids but is absent in sphinganine ceramides.

In order to obtain mass spectrometric data and to aid us in the interpretation of fragmentations, two series of homologous ceramides containing 2-hydroxy acids ranging from 14h:0 to 26h:0 were prepared. The mass spectrometric data for sphingosine ceramides is shown in Fig. 3, and for sphinganine ceramides in Fig. 4. Two types of ions can be seen, i.e. ions that appear at the same m/e value in all homologs, and ions that shift 28 m.u. between adjacent homologs. The latter ions retain the fatty acyl part of the ceramide. Furthermore, the ions can be divided into the following three classes: "molecular weight fragments," "fatty acid fragments," and "LCB fragments" (Table 1). The molecular weight fragments and the fatty acid fragments retain the fatty acyl part of the molecule. The five molecular weight fragments have been considered above. There are seven fatty acid fragments in sphingosine ceramides (Fig. 3): [M–(a-73)], (M–a), (M–a-16), (M–a-89), (b + 1 + 73), (b + 2), and f which are formed by elimination of the main part or the whole of the sphingosine carbon skeleton. The LCB fragments consist of the following three ions: m/e 426 [M–(b + 1)], m/e 336 [M–(b + 1 + 90)], and m/e 311 (M–d). These are formed by elimination of the fatty acid part of the ceramide. In addition, there is an ion at m/e 243 [M–(b + 1 + e)] which appears to be specific for sphingosine ceramides, as it is not present in sphinganine ceramides.

Fig. 4 shows the mass spectra of the homologous series of sphinganine ceramides containing 2-hydroxy acids, and in Fig. 5a there is shown the mass spectrum of 1,3,2′-tri-*O* - trimethylsilyl - *N* - (2′ - hydroxystearoyl) sphinganine.[4] Inspection of the latter mass spectrum shows that the molecular weight can be determined from the following ions: m/e 799 (M), m/e 784 (M–15), m/e 709

[4] Below m/e 100 the following major ions are present (relative abundance is in parentheses): m/e 57 (4%), m/e 73 (4%), m/e 75 (13%), m/e 77 (8%), m/e 82 (4%), m/e 83 (4%), m/e 97 (5%).

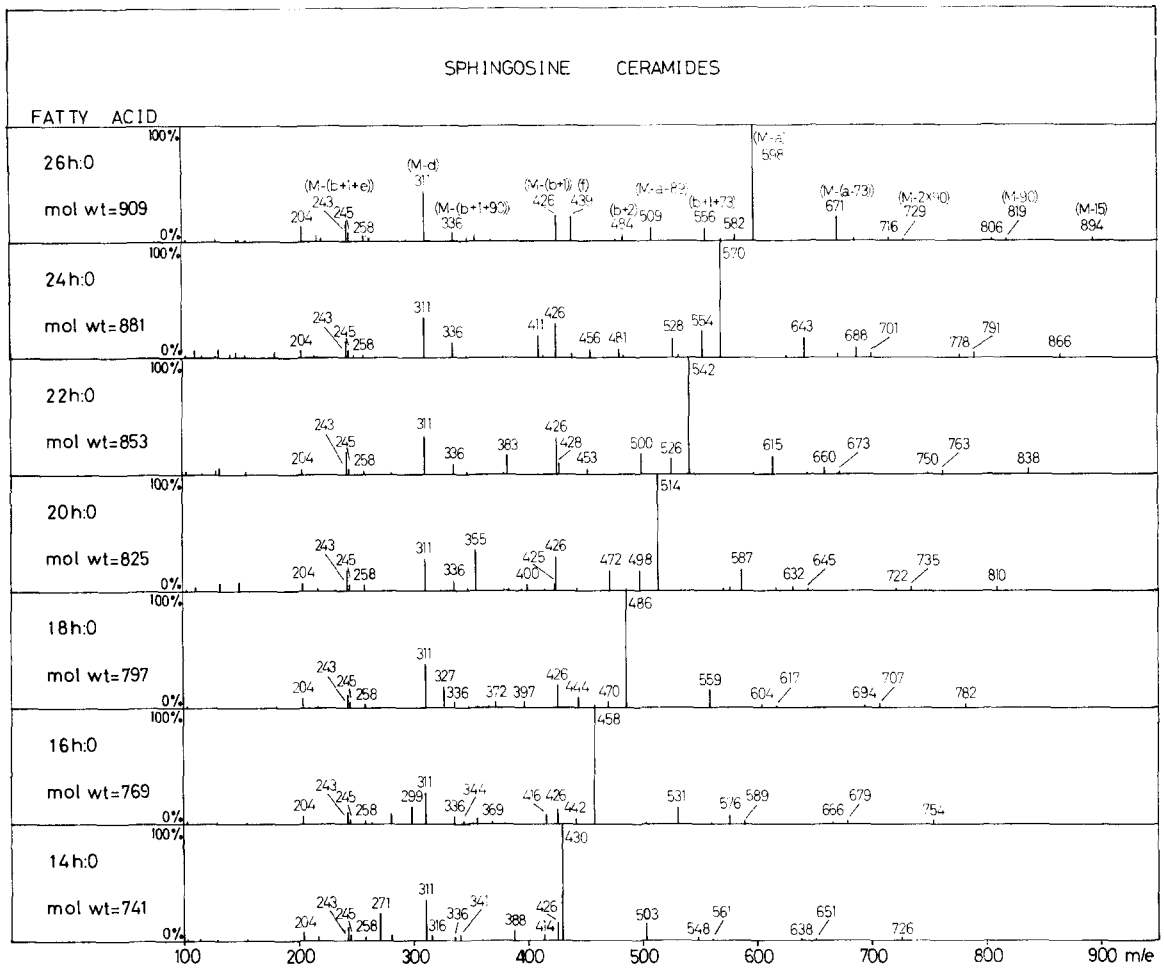

FIG. 3. Mass spectrometric data for TMS derivatives of 2′-hydroxy sphingosine ceramides.

(M–90), m/e 696 (M–103), m/e 619 (M–2 × 90), and m/e 606 (M–103-90). These fragmentations are analogous to those described above for sphingosine ceramides. Some of the fatty acid fragments are also analogous to those described above, i.e. m/e 559 [M–(a-73)], m/e 486 (M–a), m/e 470 (M–a-16), m/e 372 (b + 2), m/e 327 (f), and m/e 444 (b + 1 + 73). A prominent ion appearing at m/e 397 is considered to be due to (M-a-89)[3] (Fig. 6). This ion is probably formed by cleavage between C-2 and C-3 and elimination of the trimethylsilyloxy radical at C-1.

Ions at m/e 543 and m/e 381 are considered to be formed from [M–(a-73)] and (M–a-89), respectively, by elimination of one molecule of methane (18, 19). Elimination of one molecule of trimethylsilanol from [M–(a-73)] would give an ion at m/e 469.

TABLE 1 CLASSES OF MASS SPECTRAL IONS IN CERAMIDES

Molecular Weight Fragments	LCB Fragments	Fatty Acid Fragments	Sphinganine Fragment	Sphingosine Fragment
M–15	M–(b + 1)	M–a	M–(b + 1 + c)	M–(b + 1 + e)
M–90	M–(b + 1 + 90)	M–a-16		
M–103	M–d	M–(a-73)		
M–2 × 90	M–(g-1)	M–(a-73)-16)		
M–103-90		M–(a-73)-90		
		M–a-89		
		M–a-89-16		
		b + 2		
		f		
		b + 1 + 73		

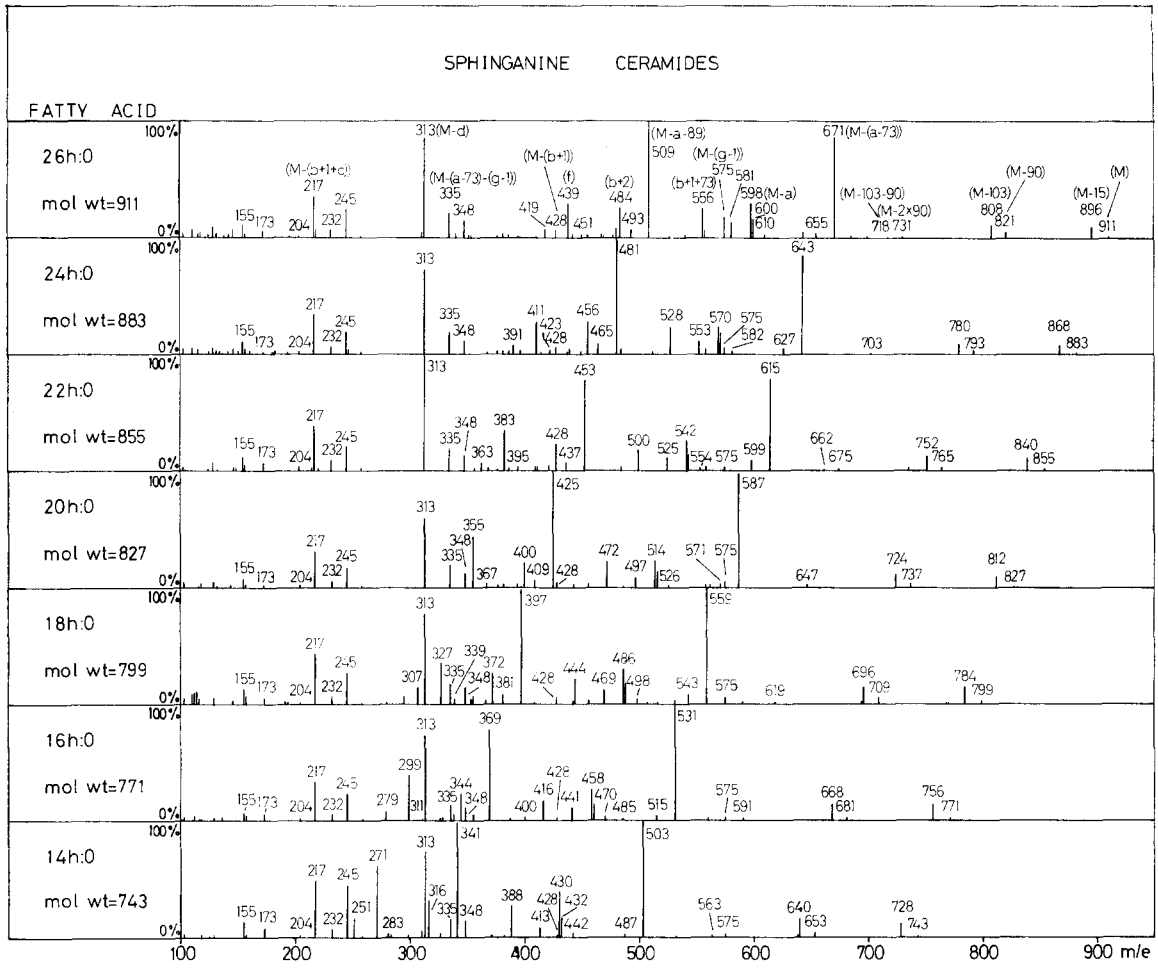

FIG. 4. Mass spectrometric data for TMS derivatives of 2′-hydroxy sphinganine ceramides.

Reactions leading to the LCB-fragments m/e 428 [M–(b + 1)] and m/e 313 (M-d) have been discussed above for the sphingosine derivatives where similar fragmentations are observed. However, an ion of the same category appearing at m/e 575 [M–(g-1)][3] has been attributed to rearrangement of a γ-hydrogen atom and cleavage of the bond β to the carbonyl group to give the ion shown in Fig. 7.

A similar fragmentation was observed earlier for sphinganine ceramides with normal fatty acids in forming the ion [M–(a-73)-(g-1)]. Its structure was supported by deuterium labeling experiments (12).

The β-cleavage described above, and the cleavage between C-2 and C-3 with transfer of a trimethylsilyl radical are presumed to be responsible for the formation of two ions. One of these ions appears at m/e 335 and is assumed to be due to [M–(a-73)-(g-1)].[3] The presence of a metastable peak at the calculated m/e 200.76 indicates that the initial reaction is a cleavage of the C—C bond between C-2 and C-3 with transfer of the trimethylsilyl radical to the nitrogen. The ion formed in this way is subsequently subjected to β-cleavage (Fig. 8).

The other ion, [M–(a-73)-(g-1)-90][3] which is formed by additional elimination of one molecule of trimethylsilanol appears at m/e 245. An ion appearing at m/e 217 was earlier observed in the mass spectra of sphinganine ceramides with normal fatty acids. This ion, which was interpreted to be [M–(b + 1 + c)], is also present in sphinganine ceramides containing 2-hydroxy acids.

In order to provide additional support for the proposed fragmentations, 1,3,2′-d_{27}-tri-*O*-trimethylsilyl-*N*-(2′-hydroxystearoyl) sphingosine was prepared using d_9-TMCS (18). The mass spectra of corresponding nondeuterated and deuterated ceramide derivatives are shown in Fig. 1a[2] and 1b.[5] The presence of three intact TMS groups is shown by the shift of the molecular ion by 27 m.u., from m/e 797 in Fig. 1a to m/e 824 in Fig. 1b. Loss of a methyl

[5] Below m/e 100 the following major ions are present (relative abundance is in parentheses): m/e 57 (3%), m/e 76 (9%), m/e 81 (30%), m/e 85 (4%), m/e 97 (5%).

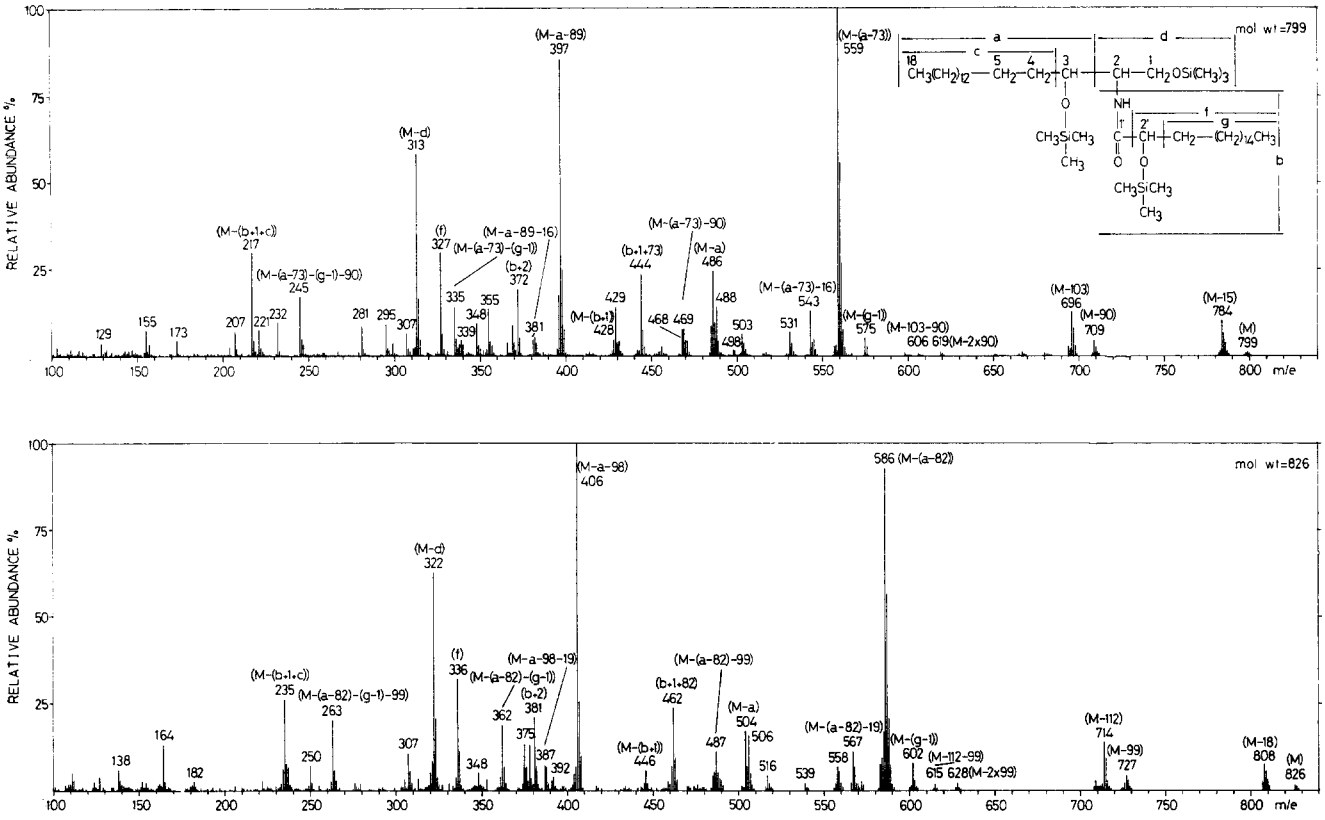

FIG. 5. Mass spectrum of (*a*) 1,3,2′-tri-*O*-trimethylsilyl-*N*-(2′-hydroxystearoyl) sphinganine[4] and (*b*) 1,3,2′-d_{27}-tri-*O*-trimethylsilyl-*N*-(2′-hydroxystearoyl) sphinganine.[6]

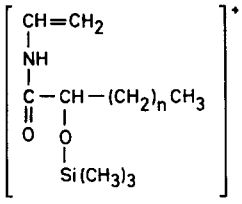

FIG 6. Proposed structure of the ion m/e 397 (M–a-89).

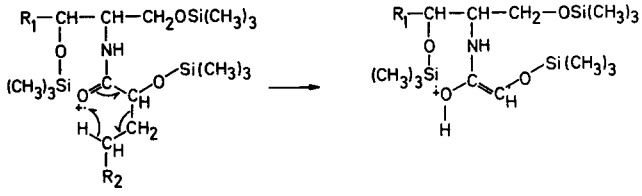

FIG. 7. Proposed formation of the ion m/e 575 [M–(g-1)].

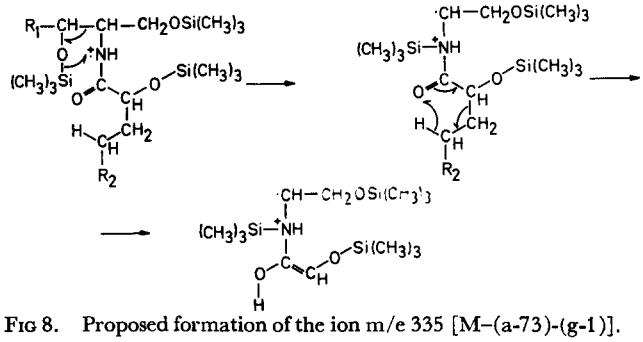

FIG 8. Proposed formation of the ion m/e 335 [M–(a-73)-(g-1)].

radical from a TMS group is confirmed by the shift of (M-15) from m/e 782 to m/e 806.

The ions formed during the reactions discussed above for sphingosine ceramides can be divided into three groups, i.e. those containing one, two, or three TMS groups per molecule. The results, which have been summarized in Table 2, show that there is complete agreement between the proposed number of TMS groups in the ions and the Δ m/e between the deuterated and non-deuterated derivatives. Of specific interest is the intramolecular transfer reaction of a trimethylsilyl group occurring during the formation of [M–(a-73)] and (b + 1 + 73). This reaction is strongly supported by the observed shifts of 27 m.u. for the former ion and 18 m.u. for (b + 1 + 73), demonstrating the presence of three and two TMS groups, respectively. The ion m/e 470 (M-a-16) shifts 15 m.u. and is considered to be formed from (M-a) by elimination of one TMS methyl group as methane after rearrangement of a hydrogen atom (18, 19).

TABLE 2 MASS SHIFTS OF IONS IN TRI-*O*-d_9-TMS-18h:0-SPHINGOSINE CERAMIDE

Ion	Number of Methyl Groups Originating in TMS Groups	Mass Shift Observed (m.u.)
M	9	27
M–(a-73)	9	27
M–15	8	24
M–90	6	18
M–103	6	18
M–a	6	18
b + 1 + 73	6	18
M–(b + 1)	6	18
M–(a-73)-(g-1)-90	6	18
M–(b + 1 + e)	6	18
M–a-16	5	15
M–2 × 90	3	9
b + 2	3	9
M–(b + 1 + 90)	3	9
f	3	9
M–d	3	9
M–103-90	3	9
M–a-89	3	9

TABLE 3 MASS SHIFTS OF IONS IN TRI-*O*-d_9-TMS-18h:0 SPHINGANINE CERAMIDE

Ion	Number of Methyl Groups Originating in TMS Groups	Mass Shift Observed (m.u.)
M	9	27
M–(g-1)	9	27
M–(a-73)	9	27
M–(a-73)-(g-1)	9	27
M–15	8	24
M–(a-73)-16	8	24
M–90	6	18
M–103	6	18
M–a	6	18
M–(a-73)-90	6	18
b + 1 + 73	6	18
M–(b + 1)	6	18
M–(a-73)-(g-1)-90	6	18
M–(b + 1 + c)	6	18
M–a-89	3	9
b + 2	3	9
f	3	9
M–d	3	9
M–2 × 90	3	9
M–103-90	3	9
M–a-89-16	2	6

Table 3 summarizes the corresponding results for sphinganine ceramides. Mass spectra of 1,3,2′-d_{27}-tri-*O*-trimethylsilyl-*N*-(2′-hydroxystearoyl) sphinganine[6] and 1,3,2′ - tri - *O* - trimethylsilyl - *N* - (2′ - hydroxystearoyl) sphinganine[4] are shown in Fig. 5. Conclusions similar to those described above can be drawn from these data. Additional support for the intramolecular transfer of a TMS group is derived from shifts of 27 and 18 m.u., respectively, for [M–(a-73)-(g-1)] and [M–(a-73)-(g-1)-90]. The ions m/e 543 [M–(a-73)-16] and m/e 381 (M–a-89-16) show shifts of 24 m.u. and 6 m.u., respectively, and are probably formed in the same way as (M–a-16) (cf. above).

Table 4 shows the occurrence of mass spectral ions in sphingosine and sphinganine ceramides containing normal and 2-hydroxy acids. There are several ions that occur in all the ceramides listed, namely the molecular weight fragments (M), (M-15), (M-90), (M-2 × 90), (M-103), (M–103-90), the fatty acid fragments (M-a), [M–(a-73)], (b + 1 + 73), [M–(a-73)–(g-1)], [M–(a-73)-(g-1)-90], and the LCB fragments (M-d), [M–(b + 1)]. The ion [M–(b + 1 + e)] at m/e 243 seems to be specific for ceramides containing sphingosine as LCB. With sphinganine or 4-hydroxy-sphinganine (phytosphingosine) (cf. reference 20) as LCB, [M–(b + 1 + c)] appears instead of [M–(b + 1 + e)]. The presence of a 2′-hydroxyl group in the ceramides causes cleavage of the C—C bond between C-1′ and C-2′ with formation of the ion f. This ion is fairly abundant and is specific for 2′-hydroxy ceramides. Furthermore, [M–(g-1)] is found only in 2′-hydroxy sphinganine ceramides. The molecular weight fragments and the fatty acid fragments appear at m/e values which are 88 m.u. higher in ceramides containing 2′-hydroxy

TABLE 4 MAJOR IONS IN MASS SPECTRA OF CERAMIDES

LCB Fatty Acid	Sphingosine		Sphinganine	
	Normal*	2-Hydroxy	Normal*	2-Hydroxy
	%		%	
M	1	1	1	2
M–15	7	4	8	11
M–90	5	6	5	5
M–103	3	3	13	13
M–2 × 90	2	4	1	2
M–103-90	4	9	8	1
M–a	100	100	16	25
M–a-16	—	13	—	8
M–(a-73)	20	19	33	100
M–(a-73)-16	—	3	—	8
M–(a-73)-90	—	3	—	8
M–a-89	—	6	44	86
M–a-89-16	—	1	—	5
M–(a.73)-(g-1)	7	3	15	14
M–(a-73)-(g-1)-90	23	6	62	17
M–(b + 1)	45	22	4	5
M–(b + 1 + 90)	6	10	—	—
M–(b + 1 + e)	24	12	—	—
M–(b + 1 + c)	—	4	28	30
b + 1 + 73	4	14	5	24
b + 2	—	5	—	20
M–d	60	31	100	58
f	—	20	—	30
M–(g-1)	—	—	—	5

Relative abundancies given are for the C_{18}-fatty acid ceramides.
* These data were obtained from reference 12.

[6] Below m/e 100 the following major ions are present (relative abundance is in parentheses): m/e 44 (2%), m/e 55 (3%), m/e 56 (5%), m/e 69 (4%), m/e 70 (4%), m/e 71 (5%), m/e 76 (9%), m/e 81 (13%), m/e 97 (7%).

TABLE 5 RETENTION TIMES FOR TMS DERIVATIVES OF CERAMIDES ON 1% OV-1 AT 280°C EXPRESSED AS TGCU

LCB Fatty Acid	Triglyceride Carbon Units			
	Sphingosine		Sphinganine	
	Normal*	2-Hydroxy	Normal*	2-Hydroxy
14:0		36.1 ± 0.1†		36.0 ± 0.1†
16:0	37.5 ± 0.1	38.1 ± 0.1	37.5 ± 0.1	38.0 ± 0.1
18:0	39.4 ± 0.2	40.0 ± 0.1	39.7 ± 0.1	40.0 ± 0.1
20:0	41.4 ± 0.2	42.0 ± 0.1	41.6 ± 0.2	41.9 ± 0.2
22:0	43.5 ± 0.1	43.9 ± 0.1	43.7 ± 0.1	43.9 ± 0.1
24:0	45.5 ± 0.1	45.8 ± 0.2	45.8 ± 0	45.8 ± 0.2
26:0		47.7 ± 0.1		47.7 ± 0.2

* These data were obtained from reference 13.
† Standard deviation, five determinations.

acids than in the normal fatty acid analogs, because of the extra trimethylsilyloxy group. The LCB fragments, with the exception of [M–(g-1)], do not show this shift. The latter ion retains C-2′.

The retention times for ceramides have been expressed as TGCU by making the linear plot of the logarithm of the retention times for trilaurin, trimyristin, and tripalmitin against their total number of carbon atoms (39, 45, and 51, respectively) and interpolating the logarithm of the retention time for the ceramide. The data for ceramides of sphingosine and sphinganine with normal and 2-hydroxy fatty acids are given in Table 5. It is evident that the retention times for the various types of ceramides containing the same number of carbon atoms are rather similar. Thus analyses of mixtures of ceramides of natural origin, require group separation before GLC–mass spectrometry (cf. reference 15).

The authors are indebted to Dr. R. Ryhage and Dr. J. Sjövall for providing facilities for mass spectrometry, and to Mrs. Saga Elwe for capable technical assistance. This work was supported by grants from the Swedish Natural Science Research Council (project No. 2931 1 ans. 7952K) and from Reservationsanslaget till främjande av ograduerade forskares vetenskapliga verksamhet, Royal Veterinary College.

Manuscript received 16 May 1969; accepted 5 November 1969.

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. 1967. *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. Samuelsson, B., and K. Samuelsson. 1969. *J. Lipid Res.* **10:** 41.
13. Samuelsson, B., and K. Samuelsson. 1968. *Biochim. Biophys. Acta.* **164:** 421.
14. Samuelsson, K. 1969. *Biochim. Biophys. Acta.* **176:** 211.
15. Samuelsson, B., and K. Samuelsson. 1969. *J. Lipid Res.* **10:** 47.
16. Casparrini, G., E. C. Horning, and M. G. Horning. 1969. *Chem. Phys. Lipid.* **3:** 1.
17. Hammarström, S. 1969. *FEBS Letters.* **5:** 192.
18. McCloskey, J. A., R. N. Stillwell, and A. M. Lawson. 1968. *Anal. Chem.* **40:** 233.
19. Draffan, G. H., R. N. Stillwell, and J. A. McCloskey. 1968. *Org. Mass Spectrom.* **1:** 669.
20. Hammarström, S. 1970. *J. Lipid Res.* In press.