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Fragmentation pathways of O-trimethylsilyl ethers of dihydroxy long-chain bases analysed by linked-scan mass spectrometry

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ABSTRACT

The fragmentation pathways of O-trimethylsilyl (TMS) ethers of long-chain bases were studied by the linked-scan technique and exact mass measurements of the characteristic ion of O-trimethylsilyl long-chain bases detected by gas chromatography combined with electron-impact mass spectrometry. The results indicate that there are three pathways: the $M^+ - 15$ (CH_3) $-$ 90 (TMSOH) $-$ 74 [$OSi(CH_3)_2$] series, the $M^+ - 103$ (CH_2OTMS) $-$ 90 (TMSOH) series and the $M^+ - 132$ ($CHNH_2CH_2OTMS$) series.

INTRODUCTION

Long-chain bases (LCBs), which are important constituents of sphingolipids, are usually analysed by gas chromatography (GC) as O-trimethylsilyl (TMS) [1] or N-acetyl-O-TMS [2] derivatives. However, each peak on the gas chromatogram must be checked by mass spectrometry (MS) to verify the structures of the individual LCBs. The fragmentation pattern of O-TMS-LCBs has been interpreted and widely used to determine the structure of LCBs [3,4]. The ion at m/z 132 was detected as the base peak of O-TMS-LCBs [4] and the fragment ions $[M - 15]^+$, $[M - 103]^+$, $[M - 105]^+$ and $[M - 132]^+$ were used to determine the molecular weight of O-TMS-LCBs. The mass spectrum of N-acetyl-O-TMS-LCBs also gave useful information for LCB analysis [5], but it has a long retention time in GC and separates into *erythro* and *threo* isomers [6]. Therefore, analysis of N-acetyl-O-TMS-LCBs was more complex than that of O-TMS-LCBs as it was a mixture of many types of LCBs. The fragmentation pathway of N-acetyl-O-TMS-LCBs was studied by Krisnangkura and Sweeley [7] using deuterium-labelled N-acetyl-O-TMS-octadecasphinganine. However, fragmentation pathways of O-TMS-LCBs have not been reported. In this study, fragment ions observed in GC–MS of O-TMS octadecasphinga-4-enine were analysed by the linked-scan technique, followed by exact mass measurement. These methods were extended to monoenoic LCBs having chain lengths of 16, 17, 19 and 20 and

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dienoic LCBs having chain lengths of 18, 20 and 22. Based on the results, the existence of three fragmentation pathways for O-TMS-LCB was confirmed.

EXPERIMENTAL

Preparation of O-TMS-long-chain bases

Octadecasphinga-4-enine was prepared from ox brain cerebroside by methanolysis with aqueous methanolic hydrochloric acid [2]. Hexa- and heptadecasphinga-4-enines were obtained from abalone (*Haliotis japonica*) phosphonosphingolipid [8], nonadeca- and dodecasphinga-4-enines from cerebroside of chiton (*Liolophura japonica*) (unpublished work) and octadecasphinga-4,8-, eicosasphinga-4,11- and docosasphinga-4,15-dienines were prepared from phosphonosphingolipids of oyster (*Ostrea gigas*), chiton (*L. japonica*) and top shell (*Turbo cornutus*), respectively [9].

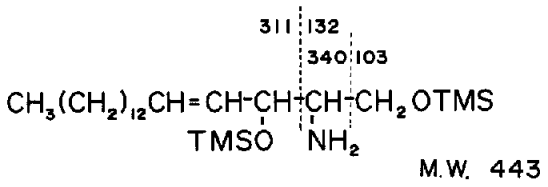
Each long-chain base was converted to the O-TMS ether with pyridine–hexamethyldisilazane–trimethylchlorosilane (10:2:1, v/v) [2].

Conditions of mass spectrometry

All mass spectra were recorded directly with a JEOL HX-100 mass spectrometer using the GC effluents. GC–MS of O-TMS-LCBs was carried out using a 2-m column packed with 3% SE-30 on 100–120 mesh Chromosorb W AW at 230°C. The ion source temperature was maintained at 230°C and the electron energy was 70 eV. The accelerating voltage was 5000 V and the ionization current 300 μA. Some characteristic ions of LCBs in GC–MS were analysed by linked scanning. Accurate mass measurements were performed by high-resolution mass spectrometry using perfluorokerosene as a mass calibrant. All mass errors were reported in millimass units (m.u.).

RESULTS AND DISCUSSION

The mass spectrum of the O-TMS ether of octadecasphinga-4-enine (C_{18}-sphingosine) is shown in Fig. 1. The molecular ions of the O-TMS ethers of LCBs are usually not detected. Octadecasphinga-4-enine showed an $[M - 15]^+$ ion at m/z 428, representing the loss of the methyl radical assumed to be derived from one of the trimethylsilyl groups. Two structures can be proposed for this ion, as shown in Fig. 2a, but we could not distinguish whether the methyl group was lost from C-1 or C-3. The elemental composition of this ion was found to correspond to $C_{23}H_{50}O_2NSi_2$ (found, 428.3367; -1.5 m.u.). Linked scanning of the ion at m/z 428 produced three daughter ions at m/z 399, 338 and 264 as shown in Fig. 2a. The ion at m/z 338 corresponds to loss of the TMSOH from the precursor ion at m/z 428. The elemental composition of this ion was $C_{20}H_{40}ONSi$ (found, 338.2879; 0.0 m.u.). This ion is usually represented as the $[M - 105]^+$ ion in the GC–MS of TMS-LCBs. The ion at m/z 264 seems to be produced by the loss of



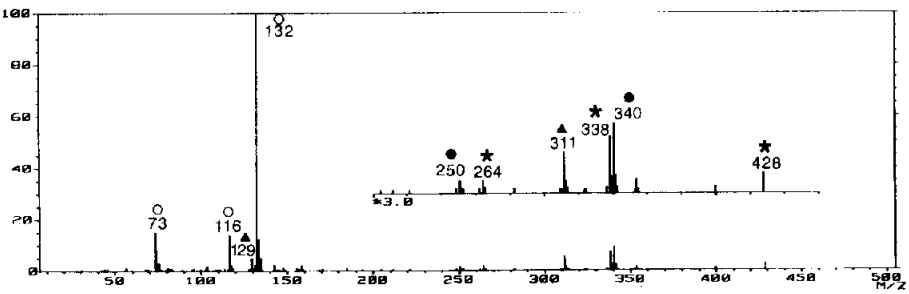


Fig. 1. Mass spectrum of O-trimethylsilyloctadecasphinga-4-enine. The symbols on the peaks (★, ●, ▲, ○) are explained in the text. ★, M − 15 series; ●, M − 103 series; ▲ and ○, M − 132 series.

OSi $(CH_3)_2$ from *m/z* 338. This ion corresponds to the so-called Z^+ ion $[CH_2C(NH_2)=CHR]^+$ which appears in positive-ion mode fast atom bombardment MS or positive secondary ion MS [10]. Ohashi *et al.* [10] confirmed by means of the linked-scan technique that the ion at *m/z* 264 was derived from sphingolipids containing octadecasphinga-4-enine as an LCB constituent. The elemental composition of the ion at *m/z* 264 corresponds to $C_{18}H_{34}N$ (found, 264.2701; +1.0 m.u.). The structure of the ion at *m/z* 399 is uncertain and this ion may be an artifact peak. The above results indicate that one fragmentation pathway of O-TMS-octadecasphinga-4-enine was the ion at *m/z* 428 $[M - 15]^+ \rightarrow$ *m/z* 338 $[M - 15 - 90]^+ \rightarrow$ *m/z* 264 $[M - 15 - 90 - 74]^+$. Linked scanning of the ion at *m/z* 264 did not produce any significant ions. This series was designated as the M − 15 series and each ion is marked with asterisk in Fig. 1. C_{16}-, C_{17}-, C_{19}- and C_{20}-sphingosine and C_{18}-, C_{20}- and C_{22}-sphingadienine showed $[M - 15]^+$ ions as shown in Table I. Linked scanning showed that each $[M - 15]^+$ ion gave two daughter ions as shown in Table I.

The fragment ion at *m/z* 340 in Fig. 1 was formed by loss of the terminal trimethylsilyloxymethylene moiety from the original octadecasphinga-4-enine and designated as the $[M - 103]^+$ ion [4]. The elemental composition of the *m/z* 340 ion corresponds to $C_{20}H_{42}ONSi$ (found, 340.3049; +1.4 m.u.). A daughter ion was observed at *m/z* 250 by linked scanning as shown in Fig. 2b. Therefore, the ion at *m/z* 250 is considered to be formed by loss of TMSOH from the ion at *m/z* 340. The elemental composition of the *m/z* 250 ion corresponds to $C_{17}H_{32}N$ (found, 250.2493; −4.2 m.u.). The mechanism of the formation of the ion at *m/z*

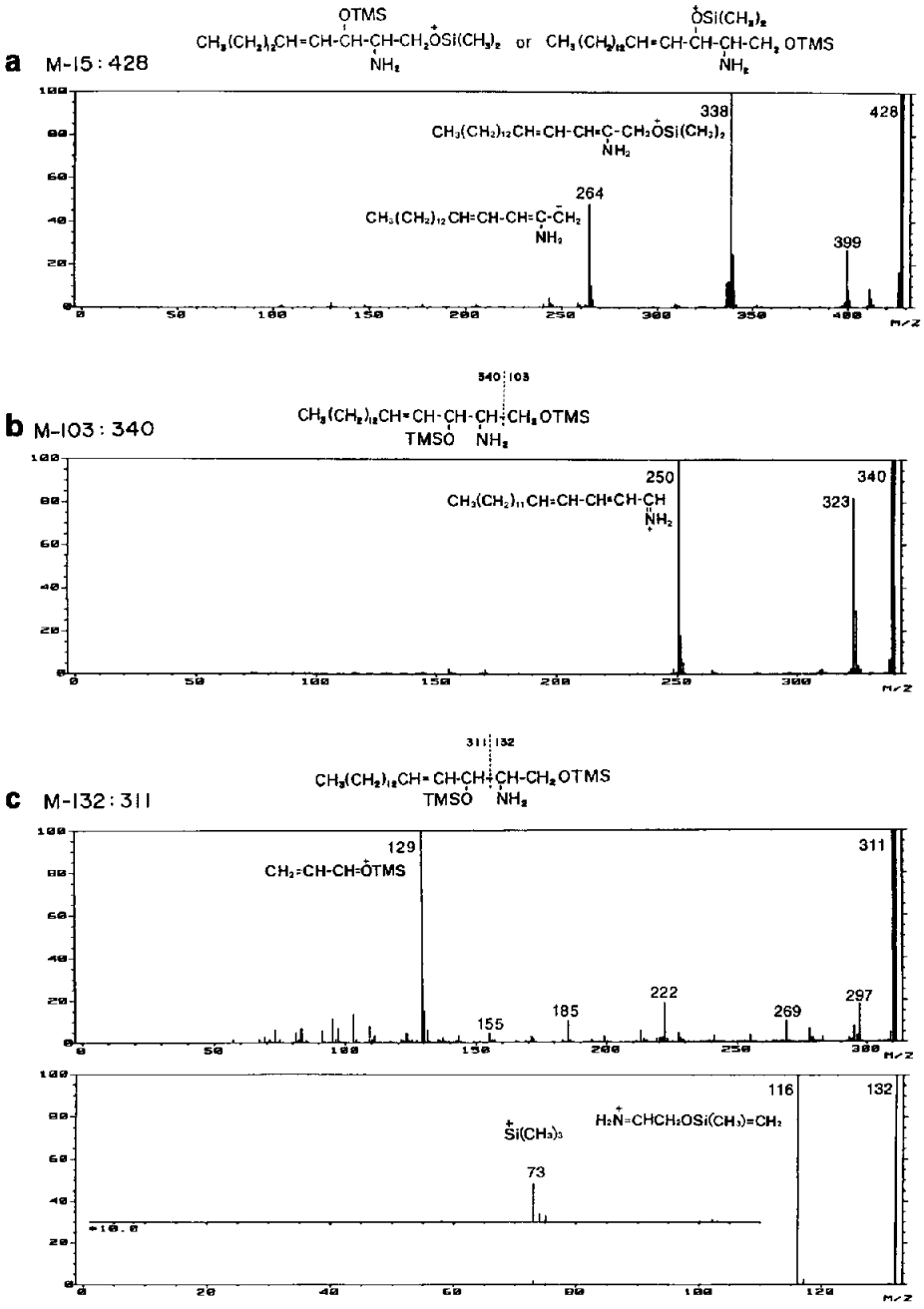


Fig. 2. Linked-scan mass spectra of O-trimethylsilyloctadecasphinga-4-enine. Precursor ion: (a) *m/z* 428; (b) *m/z* 340; (c) *m/z* 311 (upper) and *m/z* 132 (lower).

TABLE I

CHARACTERISTIC IONS OF THE THREE FRAGMENTATION PATHWAYS FOR O-TRIMETHYLSILYL DIHYDROXY LONG-CHAIN BASES.

LCB[a]	M^+	M − 15 series			M − 103 series		M − 132
		M − 15	M − 15 − 90	M − 15 − 90 − 74	M − 103	M − 103 − 90	
d16:1	415	400	310	236	312	222	283
d17:1	429	414	324	250	326	236	297
d18:1	443	428	338	264	340	250	311
d19:1	457	442	352	278	354	264	325
d20:1	471	456	366	292	368	278	339
d18:2	441	426	336	262	338	248	309
d20:2	469	454	364	290	366	276	337
d22:2	497	482	392	318	394	304	365

[a] d = dihydroxy long-chain bases.

323 remains unexplained. The second pathway was the ion at *m/z* 340 $[M - 103]^+ \rightarrow$ *m/z* 250 $[M - 103 - 90]^+$. The $[M - 103]^+$ ions and their daughter ions of C_{16}-, C_{17}-, C_{19}- and C_{20}-sphingosine and C_{18}-, C_{20}- and C_{22}-sphingadienine were detected as shown in Table I. These ions, which belong to the M − 103 series, are marked by black circles in Fig. 1.

Two ions at *m/z* 311 and 132, representing cleavage between C-2 and C-3, were detected as shown in Fig. 1. The ion at *m/z* 311 was designated as the $[M - 132]^+$ ion. The elemental composition of this ion corresponds to $C_{19}H_{39}OSi$ (found, 311.2792; +2.2 m.u.). A daughter ion was detected at *m/z* 129 by linked scanning of the ion at *m/z* 311 as shown in Fig. 2c. The ion at *m/z* 129 seems to be formed by cleavage of the C-5–C-6 bond of the original LCB with transfer of one hydrogen atom. The elemental composition of this ion corresponds to $C_6H_{13}OSi$ (found, 129.0737; +0.2 m.u.). In addition to the ion at *m/z* 129, thirteen weak ions with fourteen mass intervals were found between *m/z* 311 and 129 as shown in Fig. 2c. The medium-intensity ion at *m/z* 222 should be at *m/z* 221 and may have been formed by loss of TMSOH from the precursor ion at *m/z* 311. The ions at *m/z* 311 and 129 were classified as the M − 132 series and are marked with triangles in Fig. 1. $[M - 132]^+$ ions of C_{16}-, C_{17}-, C_{19}- and C_{20}-sphingosine and C_{18}-, C_{20}- and C_{22}-sphingadienine were detected as shown in Table I. $[M - 132 - TMSOH]^+$ ions appeared as intense ions in dienoic bases.

The ion at *m/z* 132 is usually detected as the base peak in the mass spectra of all the O-TMS-LCBs [4]. Linked scanning of this ion produced two ions at *m/z* 116 and 73. The structure of the ion at *m/z* 116 may be $H_2N^+ = CHCH_2OSi(CH_3) = CH_2$ and that at *m/z* 73 TMS. The M − 132 series was the third pathway and these ions are marked with open circles in Fig. 1.

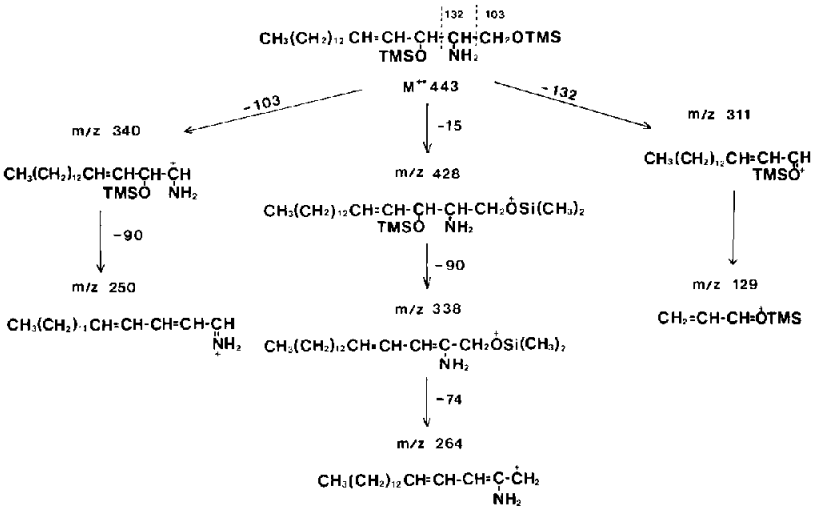


Fig. 3. Fragmentation pathways of O-trimethylsilyl long-chain bases. The scheme shows the pathways for O-trimethylsilyloctadecasphinga-4-enine.

CONCLUSION

The present results suggest that there are three fragmentation pathways for O-TMS-LCBs, as shown in Fig. 3, and almost all fragment ions which appeared in the GC–MS of O-TMS-LCBs could be characterized.

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