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Systematic analysis of naturally occurring linear and branched polyamines by gas chromatography and gas chromatography–mass spectrometry

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ABSTRACT

Using heptafluorobutyryl derivatives of a series of 27 linear di-, tri-, tetra-, penta- and hexaamines containing various sets of isomers, and a series of four tertiary tetraamines and five quaternary pentaamines, mostly with three or four methylene chain units, their gas chromatographic (GC) and gas chromatographic–mass spectrometric (GC–MS) properties were compared and examined in detail. Several results useful for their systematic analysis were found: assured baseline separation of one methylene difference in linear di- and polyamines and tertiary tetraamines by GC; distinct pyrolytic decomposition patterns of quaternary pentaamines by GC; distinct cleavage patterns of three or four methylene chain units by GC–MS; and distinct mass spectra of linear polyamines and tertiary tetraamines by GC–MS.

INTRODUCTION

Putrescine, spermidine and spermine are the most common di- and polyamines in living organisms, and are usually the target molecules in the field of polyamine research [1]. In the last decade, however, "unusual" aliphatic polyamines, including those possessing tertiary amine or quaternary ammonium groups, have been discovered successively from a variety of natural sources. Most of them are shown with their chemical structures in Tables I and II [2–13]. These findings necessitated an improved and systematic method for their identification, and convincing proof of the major polyamines, spermidine and spermine, in natural sources.

During the course of the discovery of some unusual polyamines having linear or branched structures with three or four methylene chain units, we have extensively applied HPLC, GC and GC–MS to identify them, and found GC and GC–MS to be very useful in polyamine analysis [10–15, and references cited therein on methods for polyamine analysis]. This paper

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TABLE I

LINEAR DIAMINES AND POLYAMINES

Diamines	$H_2N(CH_2)_aNH_2$	Triamines	$H_2N(CH_2)_aNH(CH_2)_bNH_2$
	a		*a–b*
Diaminopropane	3	Norspermidine	3–3
Putrescine	4	Spermidine	3–4
Cadaverine	5	Homospermidine	4–4
		Aminopropylcadaverine	3–5

Tetraamines	$H_2N(CH_2)_aNH(CH_2)_bNH(CH_2)_cNH_2$
	a–b–c
Norspermine	3–3–3
Thermospermine	3–3–4
Spermine	3–4–3
Aminopropylhomospermidine	3–4–4
Canavalmine	4–3–4
Homospermine	4–4–4
Aminopentylnorspermidine	3–3–5
N,N′-Bis(3-aminopropyl)cadaverine	3–5–3

Pentaamines	$H_2N(CH_2)_aNH(CH_2)_bNH(CH_2)_cNH(CH_2)_dNH_2$
	a–b–c–d
Caldopentamine	3–3–3–3
Homocaldopentamine	3–3–3–4
Thermopentamine	3–3–4–3
Aminopropylcanavalmine	3–4–3–4
Aminobutylcanavalmine	4–3–4–4
Homopentamine	4–4–4–4
	3–3–4–4[a]
	3–4–4–3[a]
	4–3–3–4[a]
	3–4–4–4[a]

Hexaamines	$H_2N(CH_2)_aNH(CH_2)_bNH(CH_2)_cNH(CH_2)_dNH(CH_2)_eNH_2$
	a–b–c–d–e
Caldohexamine	3–3–3–3–3
Homocaldohexamine	3–3–3–3–4

[a] So far not discovered from natural sources.

deals with the simultaneous GC analysis of various polyamines, including those not previously discovered from natural sources, the characteristic degradation behaviour of branched quaternary polyamines by GC and several characteristic mass fragment ions by GC–MS, all these being useful for differentiating and identifying structurally similar polyamines.

TABLE II

BRANCHED POLYAMINES

Tertiary tetraamines	$H_2N(CH_2)_aN\begin{matrix}(CH_2)_cNH_2\\(CH_2)_bNH_2\end{matrix}$
	a(*b*)*c*
N^4-Aminopropylnor-spermidine	3(3)3
N^4-Aminopropyl-spermidine	3(3)4
	3(4)4[a]
	4(4)4[a]
Quaternary pentaamines	$H_2N(CH_2)_aN^+\begin{matrix}(CH_2)_cNH_2\\(CH_2)_dNH_2\\(CH_2)_bNH_2\end{matrix}$
	a(*b*)(*c*)*d*
N^4-Bis(aminopropyl)-norspermidine	3(3)(3)3
N^4-Bis(aminopropyl)-spermidine	3(3)(3)4
	3(3)(4)4[a]
	3(4)(4)4[a]
	4(4)(4)4[a]

[a] So far not discovered from natural sources.

EXPERIMENTAL

Chemicals

All the polyamines listed in Tables I and II were obtained as hydrochloride salts. The following commercially available amines were used after recrystallization: diaminopropane and putrescine from Tokyo Kasei Kogyo (Tokyo, Japan), cadaverine from Wako (Osaka, Japan) and spermidine and spermine from Sigma (St. Louis, MO, USA). The other polyamines were synthesized in our laboratory [16,17]. Heptafluorobutyric anhydride (HFBA) was purchased from Wako.

Preparation of HFB derivatives of polyamines

In the presence of 0.2 ml each of acetonitrile and HFBA, 200–300 nmol of dry residue of each polyamine hydrochloride were heated in a sealed tube at 100°C for 30 min. After cooling, the reaction mixture was evaporated with a stream of nitrogen and the residue was dissolved in 0.5 ml of diethyl ether. The ether solution was washed with 0.5 ml of 0.5 *M* Na_2CO_3 and centrifuged. The ether phase was displaced into a sample tube and an aliquot of the solution was injected into the GC or GC–MS system.

Instruments

A GC-9A gas chromatograph (Shimazu, Kyoto, Japan) equipped with a flame ionization detector was employed. A Pyrex glass column (2.1 m × 3 mm I.D.) was packed with 3% SE-30 on 100–120 mesh Chromosorb W HP (Gasukuro Kogyo, Tokyo, Japan). Helium was used as the carrier gas at a flow-rate of 40 ml/min. The temperatures of the injector and the detector were 300°C and the column oven temperature was programmed from 180 to 280°C at 8°C/min or from 100 to 280°C at 16°C/min.

A JMS-DX 300 mass spectrometer (JEOL, Tokyo, Japan) was employed for GC–MS and operated in the electron impact mode at an ionization energy of 70 eV. The GC conditions were almost the same as above.

RESULTS AND DISCUSSION

In the GC analysis of polyamines, their pentafluoropropionyl (PFP) derivatives have often been used [3,9,18–20]. We also tried PFP derivatives in the initial stage of this work, but often encountered unknown decomposition peaks appearing behind each PFP polyamine peak on the gas chromatograms when PFP derivatives stored at −20°C were injected one day after derivatization. As instability seemed to be inevitable for PFP polyamines and made it difficult to analyse simultaneously as many polyamines as possible, we tested heptafluorobutyryl (HFB) derivatives of polyamines, and confirmed them to be very stable, *e.g.*, for at least one month at −20°C in diethyl ether extracts, as has also been reported by Fujihara *et al.* [21]. Subsequent studies were carried out with HFB derivatives of polyamines.

Separation of HFB derivatives of diamines, linear polyamines and tertiary tetraamines by GC

A typical gas chromatogram of HFB derivatives of diamines and linear polyamines with

three, four, or five methylene chain units is shown in Fig. 1, in which the compounds listed in Table I are shown with their abbreviated forms expressing the numbers of methylene chain units. As can be seen, their retention times increased with the increasing number of amino groups, except for 4–4–4–4 and 3–3–3–3–3. Moreover, one methylene difference in a series of di-, tri-, tetra-, penta- or hexaamines was sufficient to obtain a baseline separation under the conditions described. Among the five pairs of tetraamines with identical molecular mass in each pair, *i.e.*, 3–3–4 and 3–4–3, 4–3–4 and 3–4–4, 3–3–5 and 3–5–3, 4–3–4 and 3–3–5, and 3–4–4 and 3–5–3, each isomer of the first three pairs was recognized as a separated peak, whereas those of the last two pairs were not separated. A difficulty in separation was also observed with a triamine pair, 4–4 and 3–5. With pentaamines, there are three sets of isomers, *i.e.*, 3–3–3–4 and 3–3–4–3; 4–3–4–4 and 3–4–4–4; and 4–3–3–4, 3–3–4–4, 3–4–3–4 and 3–4–4–3. Each isomer of the first two pairs was recognized as a separated peak, and the last four isomers gave three adjoining peaks with an overlapped centre peak of 3–3–4–4 and 3–4–3–4. These results showed that an isomer having an aminobutyl group at its terminal portion eluted faster than the other isomer having an aminopropyl group.

A gas chromatogram of tris-HFB derivatives of a series of four tertiary tetraamines listed in Table II is shown in Fig. 2, with the indication of the elution positions of tetrakis-HFB derivatives of the corresponding linear tetraamines. Again, one methylene group difference among the tertiary tetraamines resulted in a baseline separation. Each tertiary tetraamine eluted faster than the corresponding linear tetraamine with the same molecular mass, and the retention times of 3(3)4 and 3(4)4 were almost the same as those of 3–3–3 and 3–3–4, respectively. This tendency might be simply explained by the difference in molecular mass of corresponding HFB derivatives, *e.g.*, tris-HFB 3(3)3 and tetrakis-HFB 3–3–3, but it should be noted that tertiary tetraamines modified by HFB still retain basic properties.

Gas chromatography of HFB derivatives of quaternary pentaamines

GC analysis of quaternary ammonium compounds has usually been performed with the

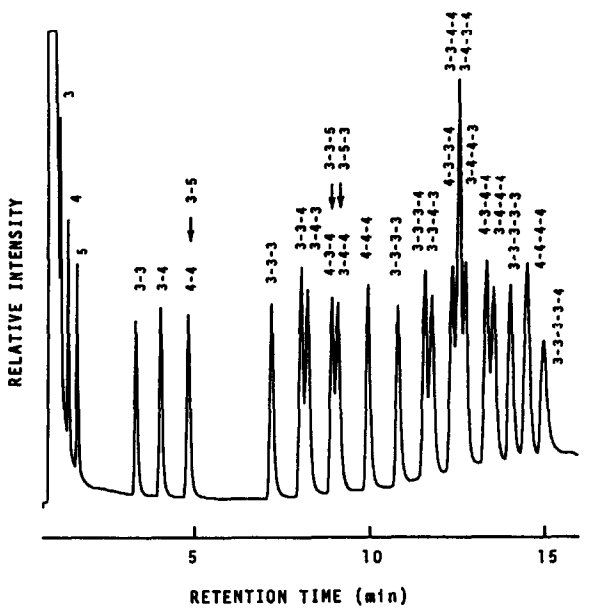

Fig. 1. Gas chromatogram of HFB derivatives of diamines and linear polyamines. The amount of each amine injected was *ca.* 1 nmol. For peak abbreviations, see Table I. GC column temperature increased from 180 to 280°C at 8°C/min.

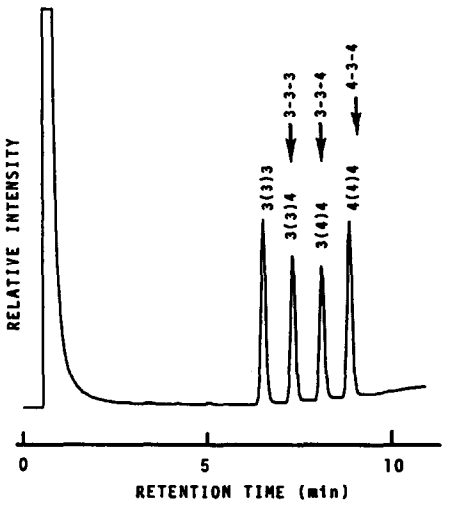

Fig. 2. Gas chromatogram of HFB derivatives of tertiary tetraamines. For peak abbreviations, see Table II. GC column temperature increased from 180 to 280°C at 8°C/min.

detection of pyrolytic decomposition products derived from the ammonium compound [22–25]. Gas chromatograms of tetrakis-HFB derivatives of a series of five quaternary pentaamines listed in Table II are shown in Fig. 3. Each HFB derivative was decomposed under the conditions described to reveal corresponding tertiary tetraamine(s) and HFB monoamine, *i.e.*, HFB allylamine derived from an eliminated aminopropyl group or HFB pyrrolidine derived from an eliminated aminobutyl group, which was identified by GC–MS, the latter being further confirmed using an authentic pyrrolidine. Three quaternary pentaamines, 3(3)(3)4, 3(3)(4)4 and 3(4)(4)4, were expected to show two kinds of tertiary tetraamines, 3(3)3 and 3(3)4, 3(3)4 and 3(4)4, and 3(4)4 and 4(4)4, respectively. However, 3(4)(4)4 showed a single tertiary tetraamine corresponding to 3(4)4. Moreover, the major tertiary amine from 3(3)(3)4 was 3(3)3, its peak area being about five times greater than that of 3(3)4, and the major one from 3(3)(4)4 was 3(3)4, its peak area being about 20 times greater than that of 3(4)4. These results clearly

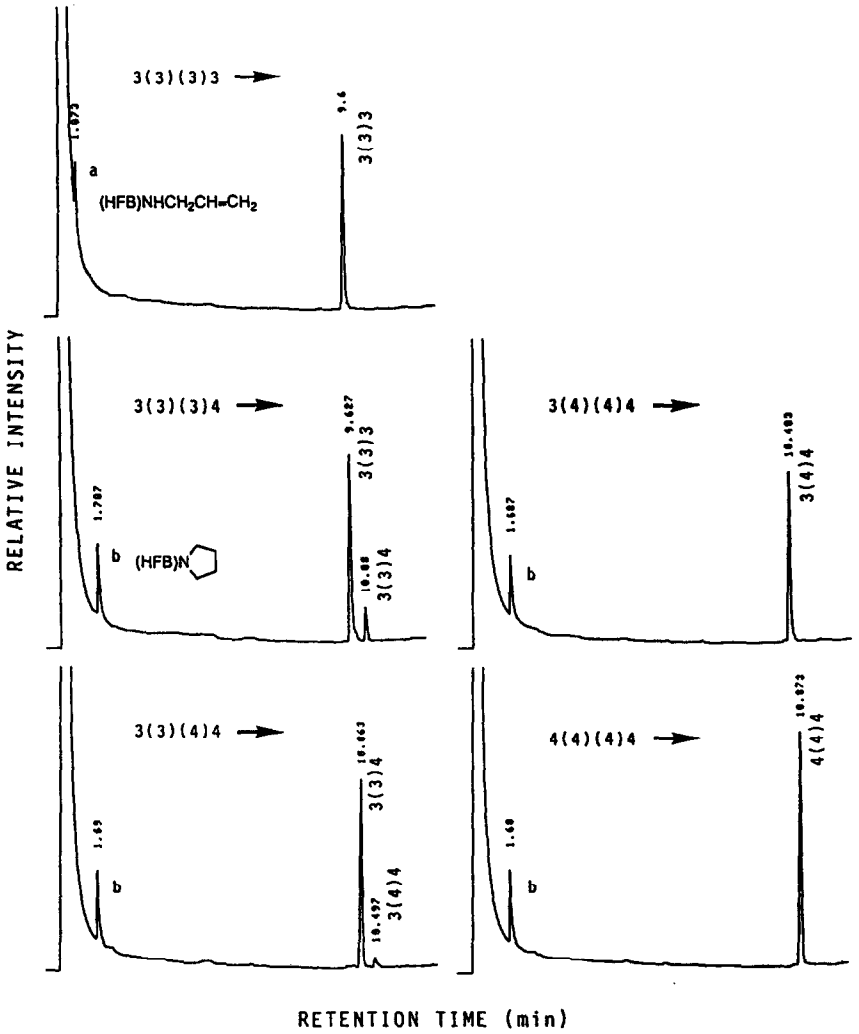

Fig. 3. Gas chromatograms of HFB derivatives of quaternary pentaamines. For peak abbreviations, see Table II. GC column temperature increased from 100 to 280°C at 16°C/min.

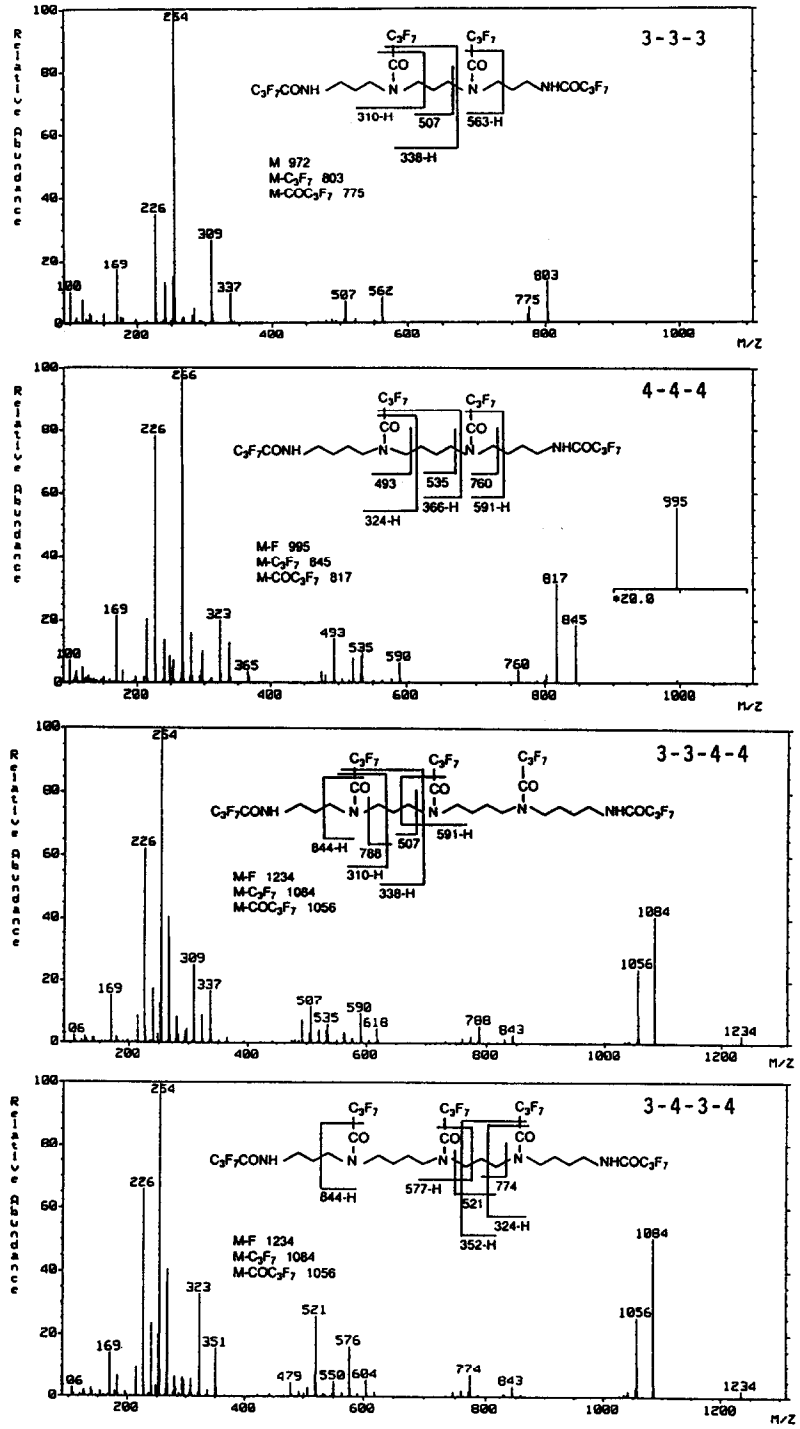

Fig. 4. Mass spectra of HFB derivatives of two comparable tetraamines and pentaamines.

show that elimination of the aminobutyl group occurred much more rapidly than elimination of the aminopropyl group in the quaternary pentaamines. The significant difference in the elimination rates of the two groups might be referred to two distinct mechanisms of elimination as were indicated by the two elimination products, HFB allylamine probably formed by Hofmann elimination [26] and HFB pyrrolidine by a cyclization reaction. These GC properties indicate that the presence of quaternary pentaamines must be tested by HPLC whenever a tertiary tetraamine is detected from unknown samples.

GC–MS of HFB derivatives of polyamines

GC–MS was very useful in identifying HFB derivatives of various polyamines, particularly when two compounds gave overlapping GC peaks, such as 3–3–4–4 and 3–4–3–4. Some useful information is presented below.

In a series of HFB derivatives of the diamines and the linear polyamines, molecular ions (M^+) were detected at a relative intensity of 10–20% for the diamines and less than 2% for the triamines. Detection of M^+ was difficult for the tetraamines, pentaamines and hexaamines. In these higher polyamines, instead, a pair of $[M - C_3F_7]^+$ and $[M - COC_3F_7]^+$ ions with relatively high intensity were useful for the determination of M^+. $[M - F]^+$ ions observed with most of the polyamines were also useful, although their intensities were low in general.

Mass spectra of two simple tetraamines, 3–3–3 and 4–4–4, which were suitable for assigning fragment ions, are shown in Fig. 4 together with cleavage sites on their chemical structures. Cleavage sites of the C–C bond or N–C bond of the polyamine skeleton were similar in the two tetraamines, but there were some ions in the 4–4–4 spectrum that had no counterpart in the 3–3–3 spectrum (*e.g.*, *m/z* 493 and 760). The mass spectra of the two pentaamines, 3–3–4–4 and 3–4–3–4, are also shown in Fig. 4. Fragment ions were assigned with reference to the information obtained from the 3–3–3 and 4–4–4 spectra. The two pentaamines were well distinguished by the comparison of fragment ions observed in the following four ranges of *m/z* values: 309–351, 479–521, 576–618 and 774–788. The fragment ions observed in these ranges were found to be derived as the results of preferred cleavages of a three methylene chain unit to a four methylene chain unit. That is, the ions at *m/z* 309 and 337 in the mass spectrum of 3–3–4–4 are assigned to $C_3F_7CONH(CH_2)_3N(CO)CH_2 - H$ and $C_3F_7CONH(CH_2)_3N(CO)(CH_2)_3 - H$, respectively, and those at *m/z* 323 and 351 in that of 3–4–3–4 to $C_3F_7CONH(CH_2)_4N(CO)CH_2 - H$ and $C_3F_7CONH(CH_2)_4N(CO)(CH_2)_3 - H$, respectively, showing a difference of 14 mass unit between their corresponding mass fragment ions derived from the cleavage at similar sites on the inside three methylene chain unit.

The characteristic cleavage sites on the three or four methylene chain unit of the linear polyamines are summarized in Fig. 5 after confirmation for all polyamines listed in Table I. Cleavages of methylene chains between primary and secondary amines occurred at the C–C bond situated α,β to the primary amine and at the C–N bond connected to the secondary amine. The mass fragment ion at *m/z* 254 corresponding to the terminal aminopropyl moiety usually appeared as a base peak in the mass spectra of such

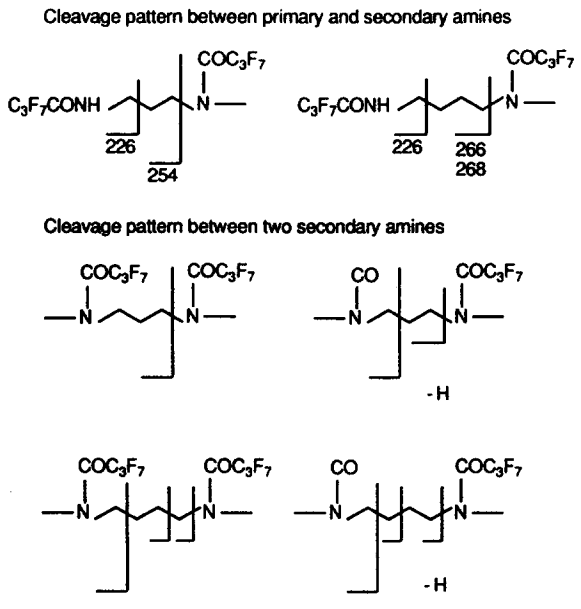

Fig. 5. Characteristic cleavage sites of the three and four methylene chain unit of the linear polyamines.

polyamines. Similarly, the mass fragment ions at m/z 266 and 268 corresponding to the terminal aminobutyl moiety appeared with high relative abundance, *e.g.*, 100 and 70%, respectively, for 4–4–4, 75 and 70% for 4–3–4, 75 and 80% for 4–3–3–4 and 100 and 75% for 4–4–4–4. Cleavages of methylene chains between two secondary amines, on the other hand, were more complicated, as shown in Fig. 5. They were classified into two groups: those which retain two entire HFB groups and those which lose C_3F_7 from one of the HFB groups. In the former, cleavages occurred at C–N bonds for the three methylene unit and at C–C bonds situated α,β to secondary amines in addition to C–N bonds for the four methylene unit. In the latter, with all fragment ions showing m/z values reduced by one mass unit, cleavages occurred at the C–N bond (nitrogen keeping HFB) and at the C–C bond situated α,β to another nitrogen losing C_3F_7 of HFB for the three methylene unit, and at the C–N bond (nitrogen keeping HFB) and the C–C bond situated α,β or β,γ to another nitrogen losing C_3F_7 of HFB for the four methylene unit. The decrease of one mass unit in these fragment ions can be explained by the loss of a hydrogen in the process of unstable dicationic ions generated on cleavage of two sites turning into stable monocationic ions. A mechanism specifying the hydrogen, however, is still open to question.

The mass spectra of two tertiary tetraamines, 3(3)4 and 3(4)4, are shown in Fig. 6. Significant fragment ions were observed at m/z 536 and 550 for 3(3)4 and at m/z 550 and 564 for 3(4)4. As the result of cleavage at the C–C bond situated α,β to the tertiary amine, the former ions for 3(3)4 and 3(4)4 were assigned as $M^+ - C_3F_7CONH(CH_2)_3$ and the latter as $M^+ - C_3F_7CONH(CH_2)_2$. It is a matter of course that a single significant ion was observed at m/z 536 for 3(3)3 and at m/z 564 for 4(4)4. These characteristic fragment ions often appeared as the base peak. It was easy from these results to distinguish linear tetraamines and tertiary tetraamines if they were overlapped, as in the case of 3–3–3 and 3(3)4 or 3–3–4 and 3(4)4 in Fig. 2.

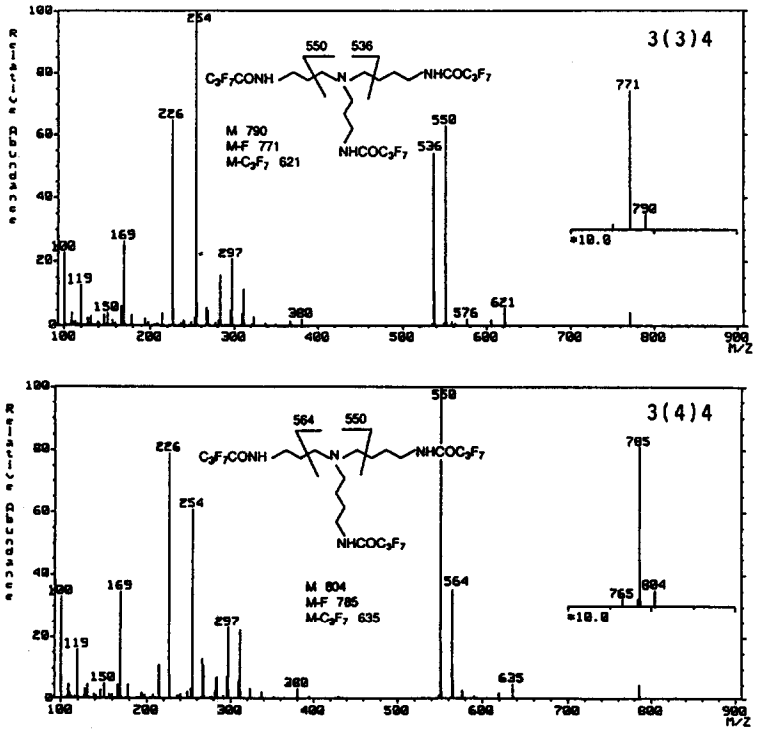

Fig. 6. Mass spectra of HFB derivatives of tertiary tetraamines containing both aminopropyl and aminobutyl groups.

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