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GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF MONOHYDROXYEICOSATETRAENOIC ACIDS AS THEIR METHYL ESTERS TRIMETHYLSILYL, ALLYLDIMETHYLSILYL AND *tert.*-BUTYLDIMETHYLSILYL ETHERS

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SUMMARY

The gas chromatographic and mass spectrometric properties of the monohydroxy acids 5-hydroxyeicosatetraenoic acid (5-HETE), 12-hydroxyeicosatetraenoic acid (12-HETE) and 15-hydroxyeicosatetraenoic acid (15-HETE) as their methyl ester trimethylsilyl, methyl ester allyldimethylsilyl and methyl ester *tert.*-butyldimethylsilyl ethers were investigated. The gas chromatographic properties of the trimethylsilyl and *tert.*-butyldimethylsilyl derivatives were found to be excellent while the allyldimethylsilyl derivative required a well deactivated column. The mass spectra of these silyl derivatives with the exception for 12-HETE did not exhibit particulary intense ions in the upper mass region. A quantitative analysis by selected-ion monitoring of the most intense ion in the upper mass region of respective mass spectrum demonstrated that a detection limit in the low picogram range could only be obtained for 12-HETE. Since the mass spectra indicated that the double bonds exerted a strong influence on the fragmentation pattern, the trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl ethers of the methyl esters of the reduced analogues of the monohydroxy acids were prepared. The saturation of the double bonds completely altered the fragmentation patterns and

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very intense ions carrying a high percentage of the total ion abundance were found in all of the mass spectra. The developed technique was utilized for measurements of 5-HETE in lung tissue samples from patients with lung cancer.

INTRODUCTION

Early studies on the conversion of arachidonic acid in human platelets led to the discovery of 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) [1]. A few years later, investigations of the transformation of arachidonic acid in polymorphonuclear leukocytes (PMNLs) resulted in the characterization of other similar monohydroxy fatty acids such as 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) and 15-hydroxy-5,8,10,13-eicosatetraenoic acid (15-HETE) [2–4].

Besides the chemotactic and chemokinetic properties of 5-HETE towards PMNLs [5], the putative role of the monohydroxy fatty acids in certain inflammatory disorders [6, 7] makes them priority targets for a sensitive and specific assay method such as gas chromatography–mass spectrometry (GC–MS). At present, a few GC–MS assay methods have been developed, employing methyl ester trimethylsilyl ethers [8, 9], methyl ester pentafluorophenyldimethylsilyl ethers [10], pentafluorobenzyl ester trimethylsilyl ethers [11, 12] and methyl ester *tert.*-butyldimethylsilyl ethers [13]. With derivatives carrying strong electron withdrawing substituents such as pentafluorophenyldimethylsilyl or pentafluorobenzyl, subpicomole sensitivity is often obtained when MS is performed in the negative-ion chemical-ionization (NICI) mode. However, such derivatives of polyunsaturated compounds often result in poor gas chromatographic properties [14]. In this latter study, it was concluded that the *tert.*-butyldimethylsilyl derivative was superior with respect to thermal stability and it showed a favourable fragmentation upon electron-impact (EI) ionization. In view of the recent introduction of the novel reactive N,O-bis(allyldimethylsilyl)trifluoroacetamide (BASTFA) for the preparation of allyldimethylsilyl ethers of leukotrienes, prostaglandins and steroids [15, 16], this report presents a comparative study of the MS properties of allyldimethylsilyl, *tert.*-butyldimethylsilyl and trimethylsilyl ethers with emphasis on their eventual applicability for quantitative MS of monohydroxy fatty acids.

One of the examined derivatization techniques was found very suitable for measurement of 5-HETE levels in human lung tissue.

EXPERIMENTAL

Materials

The monohydroxy fatty acids 5-HETE, 12-HETE and 15-HETE were prepared from human PMNLs, human platelets and soybean lipoxygenase, respectively, as described earlier [1–4]. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from PCR Research Chemicals (Gainsville, FL, U.S.A.). N-Methyl-N-*tert.*-butyldimethylsilyl trifluoroacetamide (MTBSTFA) with 1% *tert.*-butyldimethylsilylchlorosilane was obtained from Regis (Morton Grove, IL,

U.S.A.). BASTFA was prepared as described [15]. ACS-grade pyridine (Fisher Scientific) was refluxed and distilled twice from potassium hydroxide and a third time from calcium hydride. ACS-grade dimethylformamide (DMF) (Fisher Scientific) was refluxed and distilled twice from calcium hydride. Hydrogenation catalyst, 5% rhodium on alumina, was purchased from Aldrich (Milwaukee, WI, U.S.A.). Amberlite XAD-4 resin (50–100 μm) was obtained from Serva Feinbiochemica (Canadian supplier: Terochem Labs., Edmonton, Canada).

Extraction procedure

The lung tissue was homogenized in one volume of methanol (v/w) and precipitated proteins were removed by centrifugation as described [17]. The supernatant was concentrated in vacuo, acidified to pH 4 with 1 *M* hydrochloric acid and extracted on an XAD-4 column using pyridine–water–tetrahydrofuran (50:45:5, v/v/v) for elution of leukotrienes as reported [18].

Catalytic hydrogenation

Catalytic reduction of double bonds in monohydroxy acids was performed in methanol using 5% rhodium on alumina as the catalyst as described [14].

Methyl ester trimethylsilyl ether formation

The methyl ester obtained by reaction with etheral diazomethane in methanol was treated with 40 μl of pyridine–BSTFA (1:1) for 30 min at room temperature.

Methyl ester tert.-butyldimethylsilyl ether formation

The methyl ester was treated with 40 μl of DMF–MTBSTFA (1:1) at 60°C for 1 h as described [19]. The solvent and reagent were subsequently evaporated under a stream of nitrogen.

Methyl ester allyldimethylsilyl ether formation

The methyl ester was treated with 40 μl of pyridine–BASTFA (1:1) for 1 h at room temperature. Finally, the reagent and solvent were removed under a stream of nitrogen.

Gas chromatography

Gas chromatography was carried out with a Varian 3700 gas chromatograph equipped with a flame-ionization detector and a 2.5 m$\times$2 mm I.D. silanized glass column packed with 1% SE-30 on Chromosorb W HP 80–100 mesh. Argon at a flow-rate of 20 ml min^{-1} was used as the carrier gas.

Gas chromatography–mass spectrometry

Mass spectra were acquired on a Kratos MS 50 magnetic sector instrument interfaced to a Carlo Erba 4160 gas chromatograph. GC was performed on a DB-1 (J&W Scientific) fused-silica capillary column (30 m$\times$0.25 mm I.D.) connected to a Carlo Erba on-column injector. The column oven temperature was linearly programmed from 50 to 280°C at a rate of 15°C min^{-1}. The interface temperature was 250°C and the temperature of the ion source was maintained at

TABLE I

CARBON VALUES (1% SE-30) OF THE MONOHYDROXY FATTY ACIDS 5-HETE, 12-HETE AND 15-HETE AND THE CORRESPONDING SATURATED FATTY ACIDS 5-HAA, 12-HAA AND 15-HAA AS THEIR METHYL ESTER TRIMETHYLSILYL (Me TMS), METHYL ESTER ALLYLDIMETHYLSILYL (Me ADMS) AND METHYL ESTER *tert.*-BUTYLDIMETHYLSILYL (Me t-BDMS) DERIVATIVES

Compound	Me TMS	Me ADMS	Me t-BDMS
5-HETE	21.3	23.1	23.6
5-HAA	22.1	23.9	24.3
12-HETE	21.3	23.0	23.5
12-HAA	22.1	23.8	24.2
15-HETE	21.3	23.0	23.5
15-HAA	22.2	24.0	24.5

200 °C. Mass spectra were acquired at an accelerating voltage of 8 kV and electron energy of 70 eV. The mass spectra were recorded by a Kratos DS-55 data system and the scan-rate was 1 s per decade.

Quantification by selected-ion monitoring (SIM)

Quantitative analysis was performed in the SIM mode of *m/z* 399 from reduced 5-HETE as its methyl ester *tert.*-butyldimethylsilyl ether derivative. A calibration curve was established by injections of aliquots of standard solutions containing different concentrations of the same compound. Each point on the standard curve represents the mean value of five repetetive injections (see Fig. 8).

RESULTS AND DISCUSSION

Gas chromatography

The trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl ethers of methyl esters of the monohydroxy fatty acids 5-HETE, 12-HETE and 15-HETE were prepared and subjected to GC using a 1% SE-30 packed column. The retention times, expressed as carbon values calculated from comparison with methyl esters of saturated fatty acids [20], are summarized in Table I. As demonstrated in Fig. 1A, the GC sensitivity of the methyl ester allyldimethylsilyl derivative of the monohydroxy acids represented by 12-HETE was below those of the methyl ester trimethylsilyl and methyl ester *tert.*-butyldimethylsilyl derivatives. This finding is similar to earlier results obtained with the polyunsaturated fatty acid leukotriene B_4 (LTB_4) as its allyldimethylsilyl derivative [16]. Furthermore, it is shown in Fig. 1A that the 6-*trans* isomer of 12-HETE (an impurity) elutes later as a very broad peak indicating a considerable decomposition on the column. Since it has been demonstrated earlier that saturation of the double bonds in LTB_4 results in a considerable improvement of its GC properties [14], a catalytic hydrogenation was performed and subsequently the trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl ethers were prepared from the methyl esters of the hydroxyarachidic acids (HAA). As demonstrated in Fig. 1B, the response

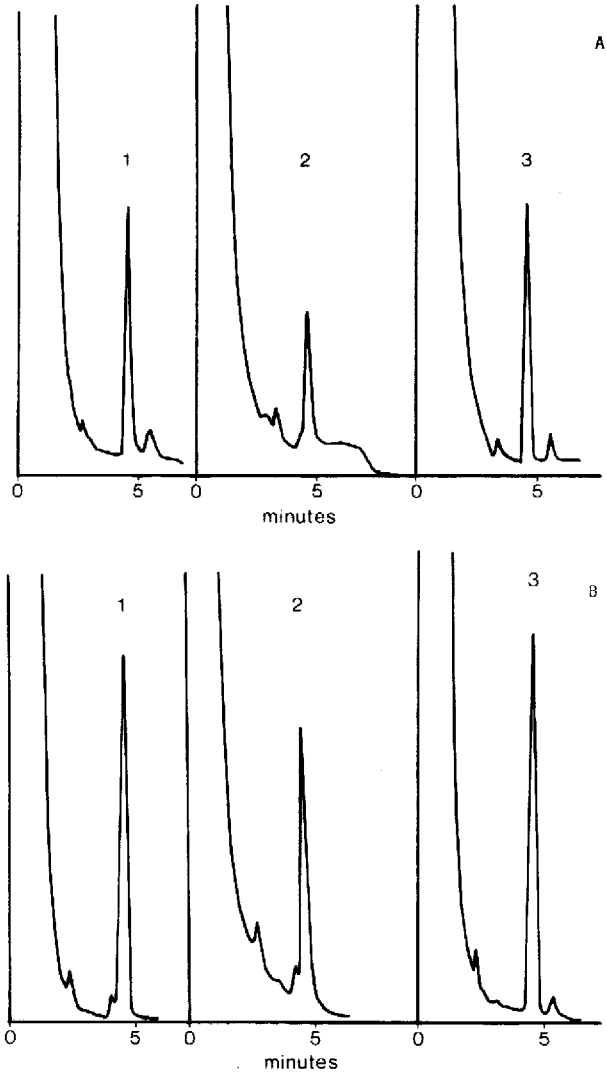

Fig. 1. (A) Gas chromatogram of 12-HETE as its methyl ester trimethylsilyl (1), methyl ester allyldimethylsilyl (2) and methyl ester *tert.*-butyldimethylsilyl ether (3). (B) Gas chromatogram of 12-HAA as its methyl ester trimethylsilyl (1), methyl ester allyldimethylsilyl (2) and methyl ester *tert.*-butyldimethylsilyl ether (3). The oven temperature was adjusted in order to obtain identical retention times for all derivatives. The injected quantity was 200 ng.

increased for all derivatives but most dramatically for the allyldimethylsilyl derivative.

The retention times of 5-hydroxyarachidic acid (5-HAA), 12-hydroxyarachidic acid (12-HAA) and 15-hydroxyarachidic acid (15-HAA) as their methyl ester trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl ethers expressed as carbon values have been included in Table I.

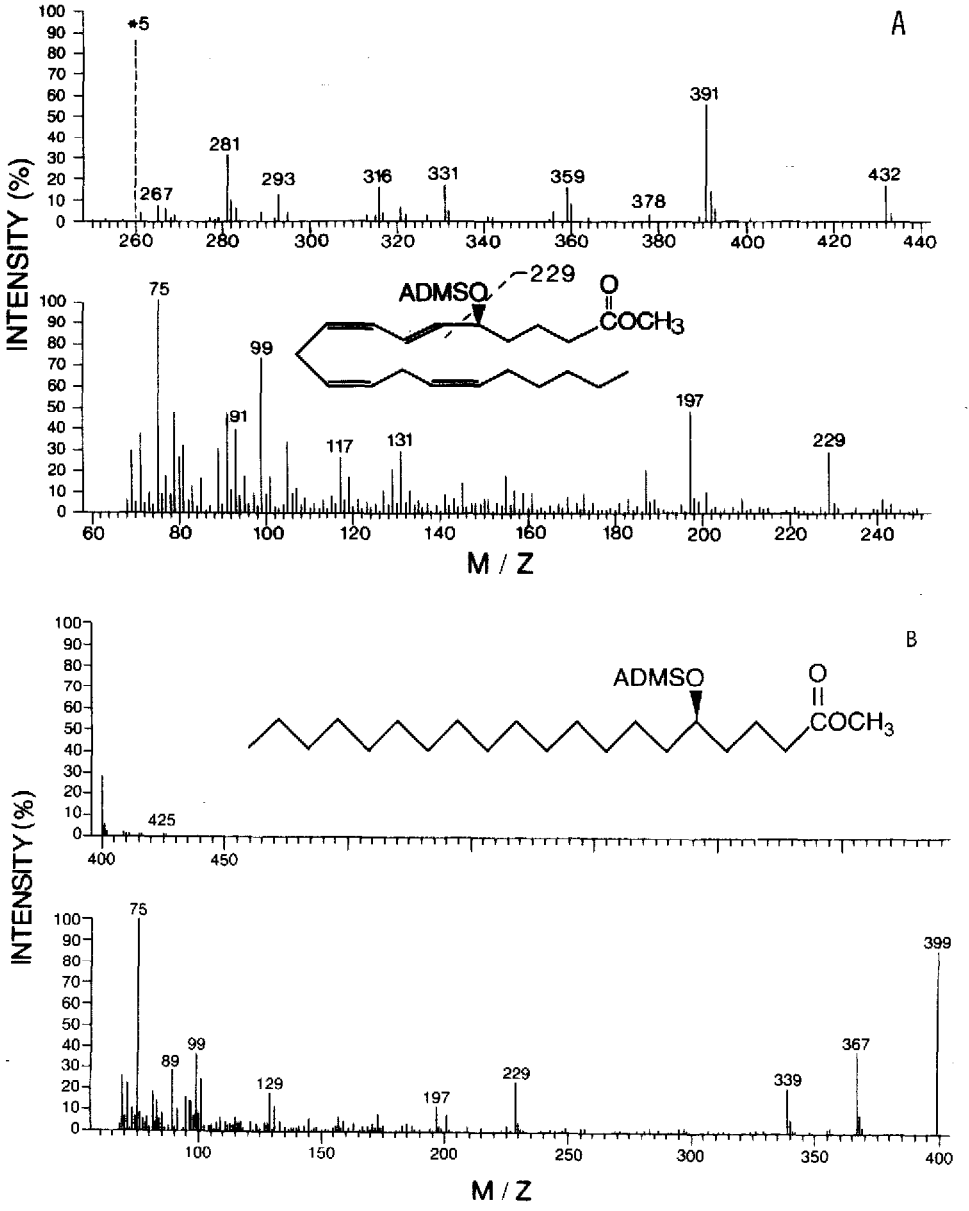

Fig. 2. (A) Mass spectrum of the methyl ester allyldimethylsilyl ether of 5-HETE. (B) Mass spectrum of the methyl ester allyldimethylsilyl ether of 5-HAA.

Mass spectrometry

The mass spectra of the methyl ester allyldimethylsilyl ethers and methyl ester *tert.*-butyldimethylsilyl ethers of the monohydroxy fatty acids 5-HETE (Figs. 2A and 3A, respectively), 12-HETE (Figs. 4A and 5A, respectively) and 15-HETE (Figs. 6A and 7A, respectively) were compared with the mass spectra of their methyl ester trimethylsilyl ethers. Important ions from the trimethylsilyl ether spectra are listed in Table II. Since three different derivatives were employed for

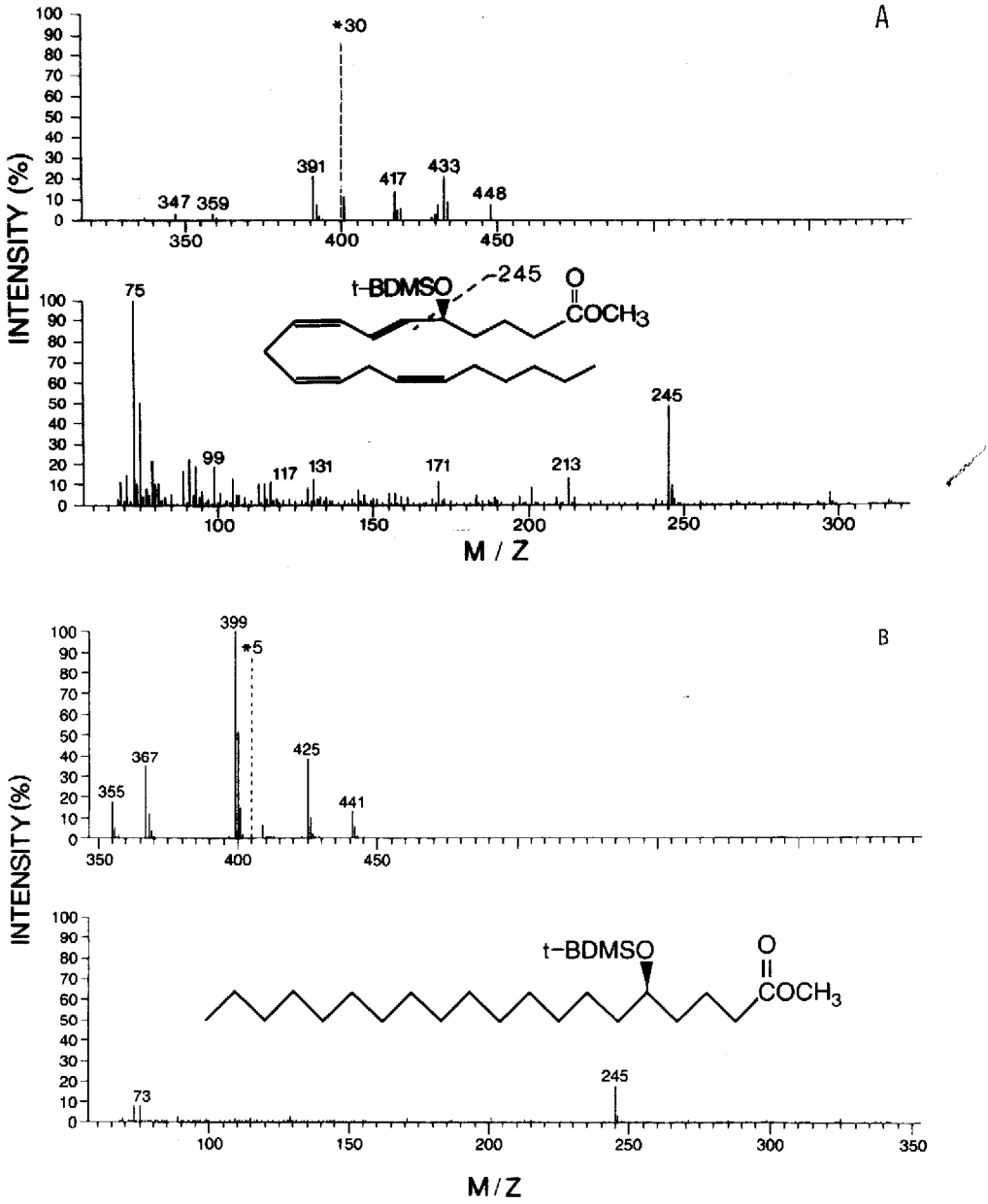

Fig. 3. (A) Mass spectrum of the methyl ester *tert.*-butyldimethylsilyl ether of 5-HETE. (B) Mass spectrum of the methyl ester *tert.*-butyldimethylsilyl ether of 5-HAA.

each of the compounds in this study, there was an opportunity to examine more in detail the fragmentation pathways reported earlier for the methyl ester trimethylsilyl ether derivative of 5-HETE where an unusual cleavage was proposed [2]. In the mass spectrum of the methyl ester trimethylsilyl ether of 5-HETE (Table II), one of the intense ions, which constitutes 68% of the base peak, is

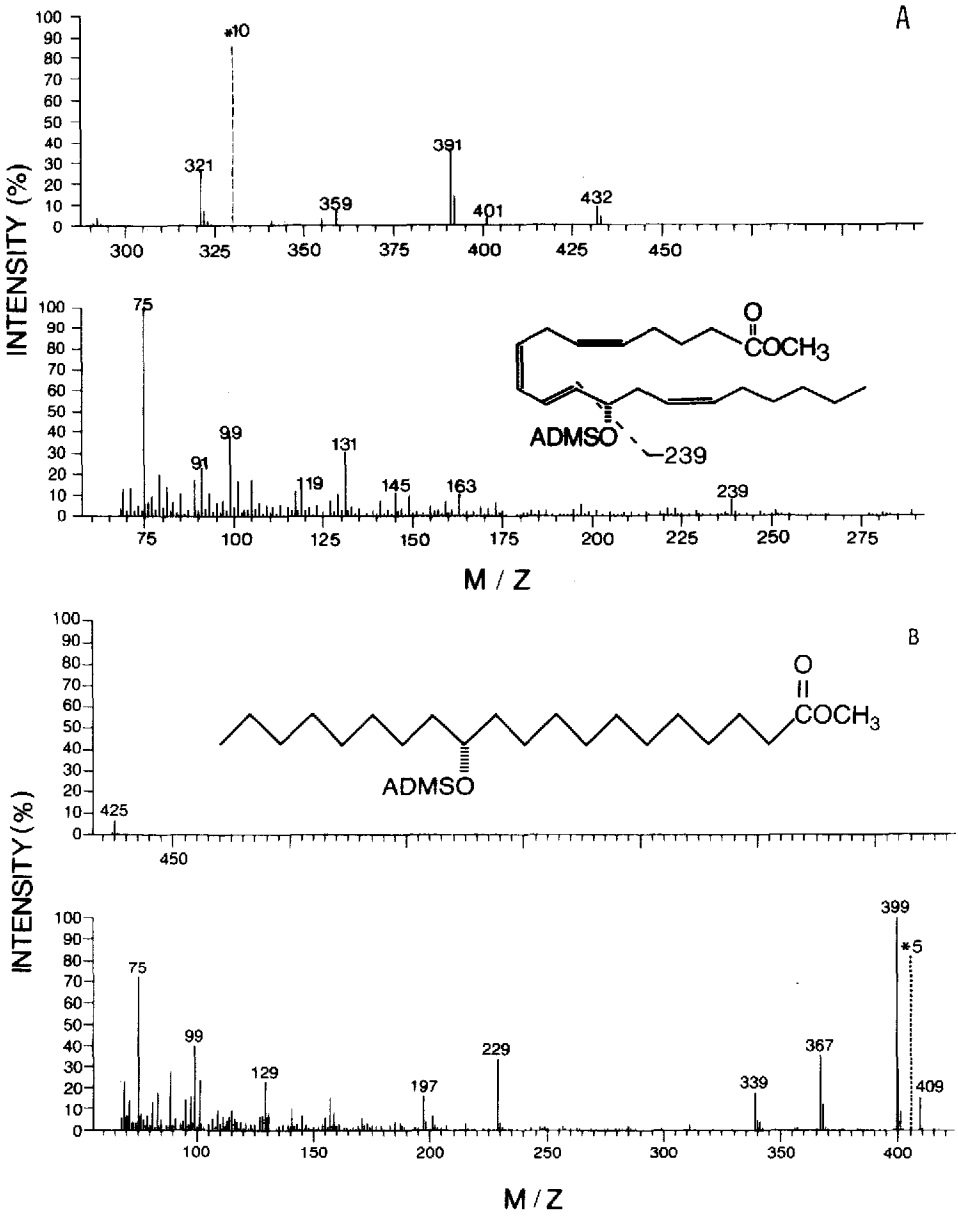

Fig. 4. (A) Mass spectrum of the methyl ester allyldimethylsilyl ether of 12-HETE. (B) Mass spectrum of the methyl ester allyldimethylsilyl ether of 12-HAA.

found at *m/z* 203. However, the mechanism for its formation is in competition with the cleavage at C-4–C-5 and fragmentations of the alkyl chain and the ester group. Based on the fragmentation patterns of several alkyldimethylsilyl ethers, a rationalisation for the formation of the major ions in the mass spectra of methyl esters alkyldimethylsilyl ethers is given in Fig. 8. It can be seen that there are four fragmentation pathways which can be related to different ionization sites.

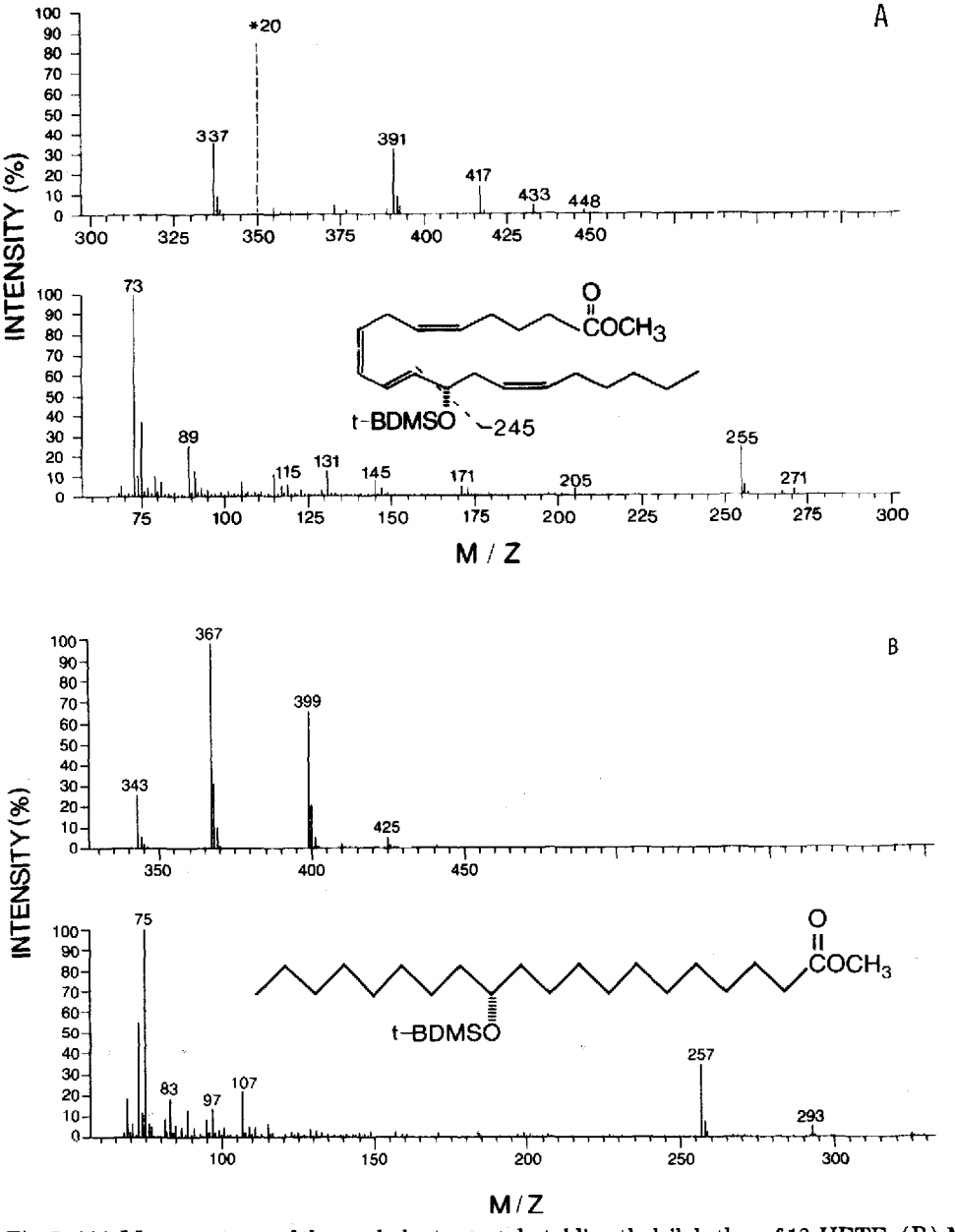

Fig. 5. (A) Mass spectrum of the methyl ester *tert.*-butyldimethylsilyl ether of 12-HETE. (B) Mass spectrum of the methyl ester *tert.*-butyldimethylsilyl ether of 12-HAA.

Fragmentation of the ester group results in the elimination of a methoxy radical followed by elimination of a ketene which can further loose C_2H_4 to form an oxonium-type structure which is present at *m/z* 305, 331 and 347 in the spectra of the trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl derivatives, respectively. A second pathway consists of the elimination of an alkyl radical on the silicon atom followed by the elimination of dimethylsilanol to yield a polyene

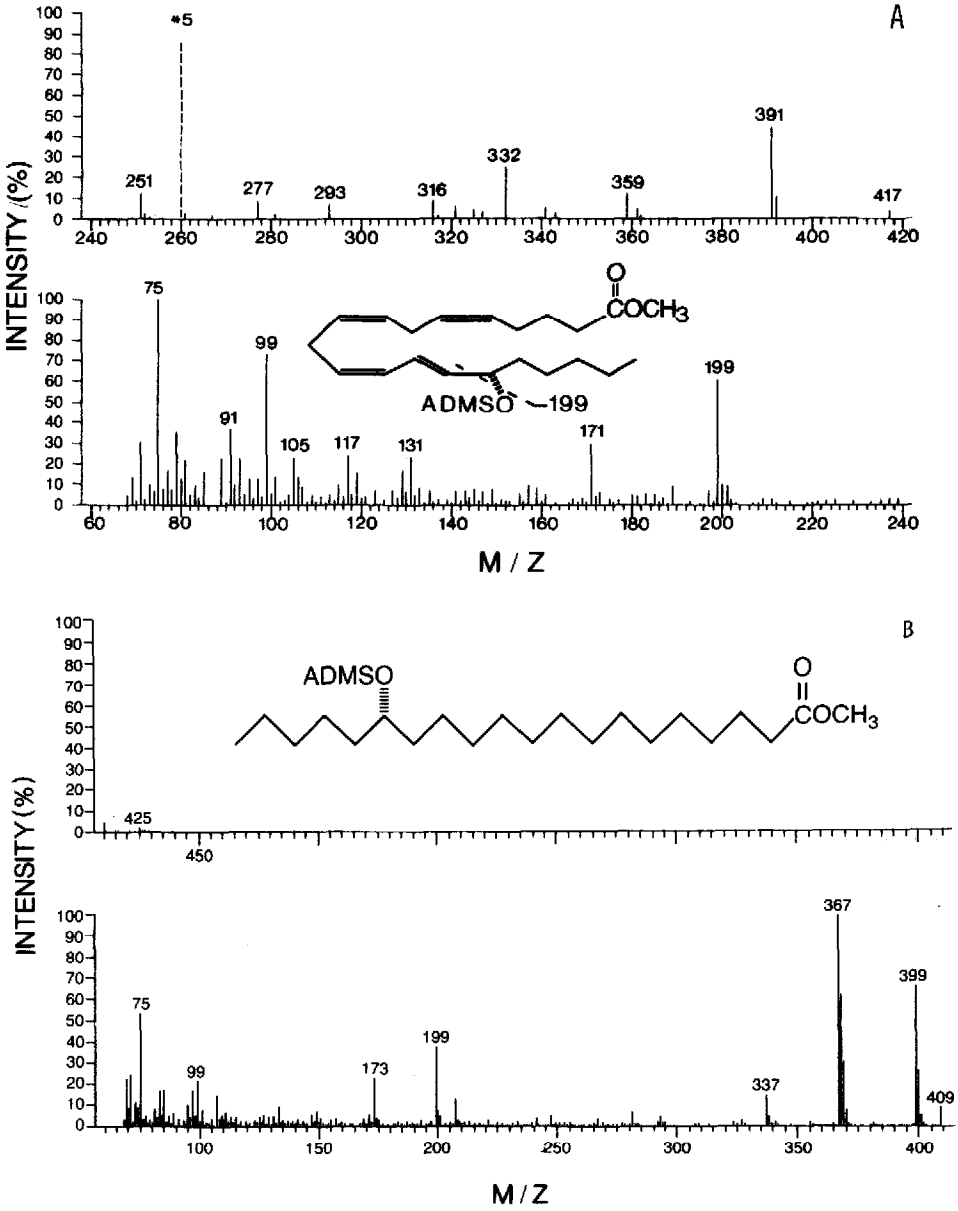

Fig. 6. (A) Mass spectrum of the methyl ester allyldimethylsilyl ether of 15-HETE. (B) Mass spectrum of the methyl ester allyldimethylsilyl ether of 15-HAA.

system which is present at *m/z* 316 in the mass spectra of the trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl derivatives of 5-HETE. Cleavage of the C-5–C-6 bond leads to ions at *m/z* 203, 229 and 245 for trimethylsilyl, allyldimethylsilyl and *tert.*-butyldimethylsilyl derivatives, respectively. The ions formed can further decompose loosing methanol and a ketene or through a McLafferty-type rearrangement to yield the stable conjugated forms seen at *m/z* 129, 155 and 171 in the spectra of the trimethylsilyl, allyldimethylsilyl and *tert.*-

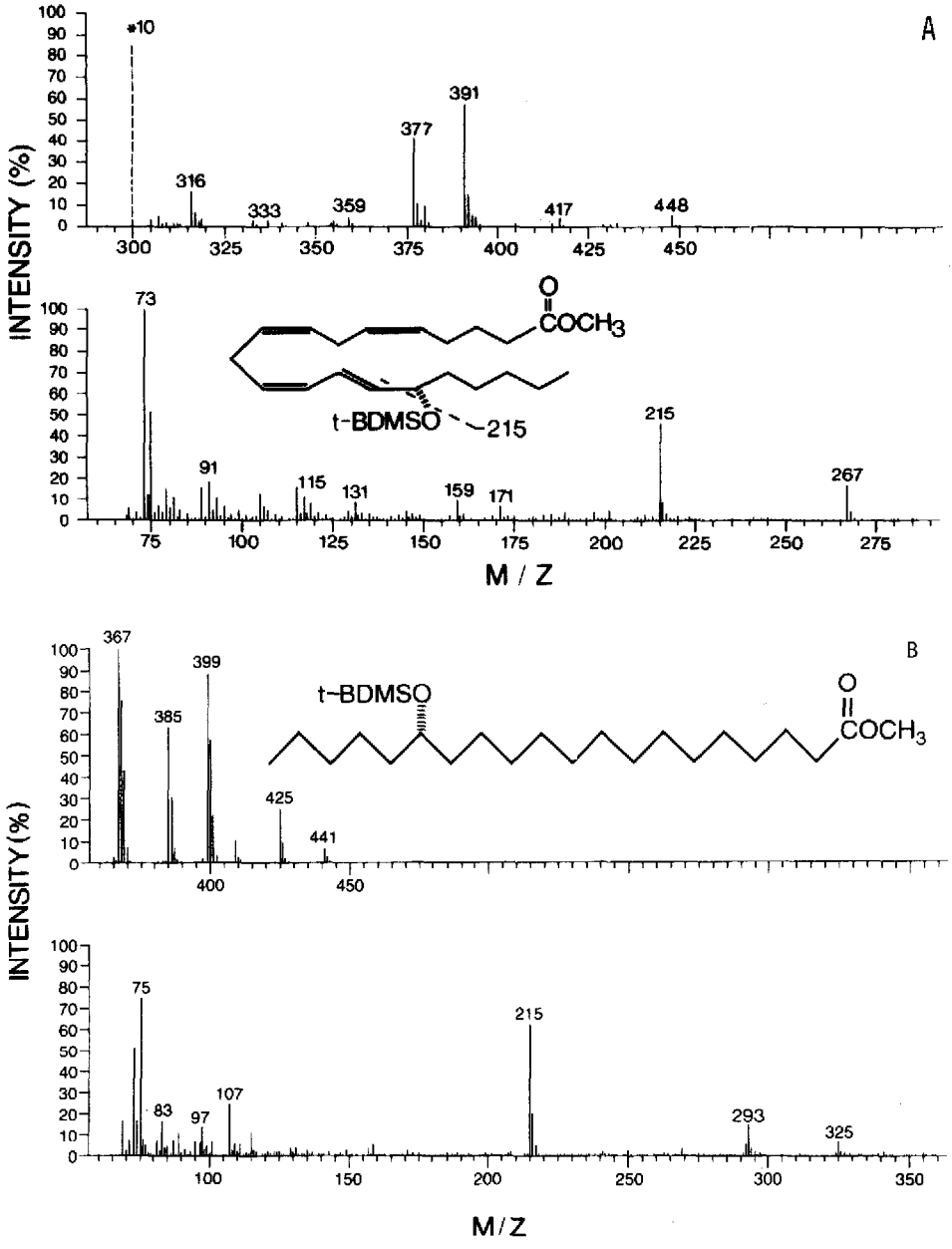

Fig. 7. (A) Mass spectrum of the methyl ester *tert.*-butyl dimethylsilyl ether of 15-HETE. (B) Mass spectrum of the methyl ester *tert.*-butyldimethylsilyl ether of 15-HAA.

butyldimethylsilyl derivatives, respectively. In all cases ions at *m/z* 129, 155 and 171 are characteristic of 5-hydroxy fatty acids and are also observed for leukotrienes hydroxylated in the C-5 position [2, 21, 22]. In the mass spectra of 12-HETE and 15-HETE, a corresponding cleavage of the C-11–C-12 and C-14–C-15 bonds, respectively, leads to ions at *m/z* 213 and 173 (trimethylsilyl, respec-

TABLE II

IMPORTANT IONS IN THE MASS SPECTRA OF THE METHYL ESTER TRIMETHYLSILYL DERIVATIVE OF THE MONOHYDROXY FATTY ACIDS 5-HETE, 12-HETE AND 15-HETE

5-HETE	12-HETE	15-HETE
m/z 406 M	*m/z* 406 M	*m/z* 406 M
391 M−15	391 M−15	391 M−15
375 M−31	375 M−31	335 M−71
316 M−90	316 M−90	316 M−90
305 M−101	295 M−111	245 M−(71+90)
255 M−151	213 M−193	225 M−181
203 M−203	205 M−(111+90)	173 M−233
171	173 M−(111+90+32)	103
143	131	73
129	103	
117	73	
73		

tively), 239 and 199 (allyldimethylsilyl, respectively) and 255 and 215 (*tert.*-butyldimethylsilyl, respectively).

The mass spectra of the allyldimethylsilyl and *tert.*-butyldimethylsilyl ethers were also examined for the presence of intense ions in the upper mass region suitable for a quantification by SIM. However, ions derived from the elimination of an allyl or a *tert.*-butyl radical at *m/z* 391 carried only a few percent of the total ion abundance (Table III). Furthermore, the mass spectra of 12-HETE as its methyl ester allyldimethylsilyl ether (Fig. 4A) and methyl ester *tert.*-butyldi-

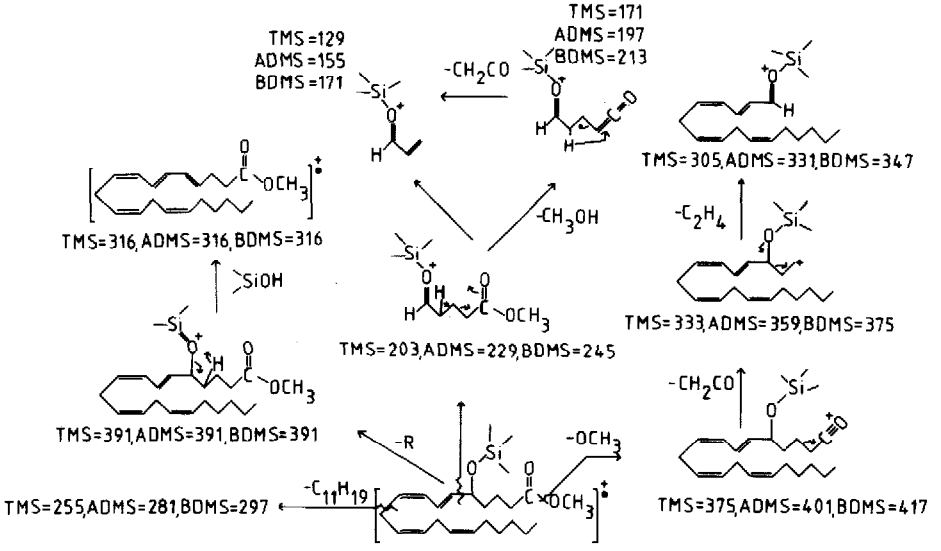

Fig. 8. Fragmentation patterns of trimethylsilyl (TMS), allyldimethylsilyl (ADMS) and *tert.*-butyldimethylsilyl (BDMS) ethers of 5-HETE.

TABLE III

MOST PROMINENT IONS IN THE UPPER MASS REGION OF THE MASS SPECTRA OF THE TRIMETHYLSILYL (TMS), ALLYLDIMETHYLSILYL (ADMS) AND *tert.*-BUTYLDIMETHYLSILYL (t-BDMS) DERIVATIVES OF METHYL ESTERS (Me) OF THE MONOHYDROXY FATTY ACIDS 5-HETE, 12-HETE AND 15-HETE

The percentage of the total ion abundance and the detection limits in SIM are shown. The detection limit was defined as the quantity injected that resulted in a signal-to-noise ratio of 5:1.

Compound	*m/z*	Percentage base peak	Percentage total ion abundance	Detection limit
5-HETE Me TMS	203	68.3	7.2	250 pg
5-HETE Me ADMS	391	11.0	0.8	4 ng
5-HETE Me t-BDMS	391	21.4	2.6	1 ng
	245	48.5	5.9	500 pg
12-HETE Me TMS	295	76.9	11.4	170 pg
12-HETE Me ADMS	321	28.4	3.6	2.5 ng
12-HETE Me t-BDMS	337	35.0	7.1	40 pg
15-HETE Me TMS	225	34.0	4.9	350 pg
15-HETE Me ADMS	391	8.7	0.8	4 ng
15-HETE Me t-BDMS	267	16.9	2.6	1 ng

methylsilyl ether (Fig. 5A) showed more prominent ions derived from an α-cleavage at C-12 resulting in ions at *m/z* 321 and 337, respectively. The methyl ester *tert.*-butyldimethylsilyl ether derivative of 15-HETE exhibits an important cleavage between C-10 and C-11 resulting in a fragment at *m/z* 267. These fragmentation patterns, strongly influenced by the presence of several double bonds as found earlier [13], result in decreased intensities of the ions in the upper mass region. The most prominent ions from the upper mass regions of the mass spectra of the allyldimethylsilyl and *tert.*-butyldimethylsilyl derivatives are found in Table III together with those of the trimethylsilyl ether derivatives. Except ions from the mass spectra of the trimethylsilyl ethers and the methyl ester *tert.*-butyldimethylsilyl ether derivative of 12-HETE, the ions listed do not carry a high percentage of the total ion abundance which is important for a sensitive assay method. However, since an earlier study of the allyldimethylsilyl ether derivative of prostaglandins and steroids demonstrated sensitivities in the low picogram range upon SIM in spite of the measured ions carried only a few percent of the total ion abundance [16], SIM was performed on the ions listed in Table III. As demonstrated the detection limits obtained are not especially low and were approximately proportional to the intensities of the analyzed ions. Only for the methyl ester *tert.*-butyldimethylsilyl ether derivative, an acceptable limit of detection of 40 pg was obtained.

Thus, because of the strong influence of the double bonds upon the mass spectral fragmentation patterns irrespective of the derivative employed, it was concluded that the double bonds should be saturated in order to reduce the number of fragmentation pathways and thus obtain mass spectra with a few intense ions,

TABLE IV

PROMINENT IONS FROM THE MASS SPECTRA OF THE TRIMETHYLSILYL (TMS), ALLYLDIMETHYLSILYL (ADMS) AND *tert.*-BUTYLDIMETHYLSILYL (t-BDMS) DERIVATIVES OF THE METHYL ESTER (Me) OF 5-HAA, 12-HAA AND 15-HAA

Compound	*m/z*	Percentage base peak	Percentage total ion abundance	Fragment
5-HAA Me TMS	414	20.9	3.0	M
	399	59.6	8.4	M−15
	383	27.3	3.8	M−31
	313	100.0	14.1	M−101
	203	37.0	5.2	M−211
5-HAA Me ADMS	399	86.3	9.2	M−41
	367	38.4	4.1	M−(41+32)
5-HAA Me t-BDMS	399	100.0	26.5	M−57
	367	34.8	9.2	M−(57+32)
12-HAA Me TMS	301	84.6	18.6	M−113
	215	100.0	22.0	M−199
12-HAA Me ADMS	399	100.0	10.8	M−41
	367	36.5	3.9	M−(41+32)
12-HAA Me t-BDMS	399	65.9	8.7	M−57
	367	98.0	13.0	M−(57+32)
15-HAA Me TMS	343	49.8	10.7	M−71
	173	100.0	21.6	M−241
15-HAA Me ADMS	399	65.0	5.9	M−41
	367	100.0	9.1	M−(41+32)
15-HAA Me t-BDMS	399	87.9	7.5	M−57
	385	63.3	5.4	M−71
	367	100.0	8.5	M−(57+32)
	215	61.4	5.2	M−241

preferably in the upper mass region. The mass spectra of the hydrogenated products, e.g. 5-HAA, 12-HAA and 15-HAA as their methyl ester allyldimethylsilyl ether and methyl ester *tert.*-butyldimethylsilyl ether derivatives, are depicted in Figs. 2B–7B. As shown, the mass spectra are completely different and now a few intense ions are present in all of the spectra. The mass spectra of the allyldimethylsilyl ethers (Figs. 2B, 4B and 6B) are now dominated by intense ions derived from elimination of an allyl radical [M−41] at *m/z* 399 while the mass spectra of the *tert.*-butyldimethylsilyl ethers (Figs. 3B, 5B and 7B) show intense ions derived from elimination of a *tert.*-butyl radical [M−57] at *m/z* 399. The next, most intense ion in these mass spectra are due to the elimination of methanol as described [23], yielding intense ions [M−(41+32)] or [M−(57+32)] at *m/z* 367. This simplified fragmentation results in a pair of ions in the upper mass region carrying a large portion of the total ion abundance (Table IV). The most prominent ions from the mass spectra of the trimethylsilyl ethers are also included in Table IV, their full spectra have been described elsewhere [2, 9]. As indicated,

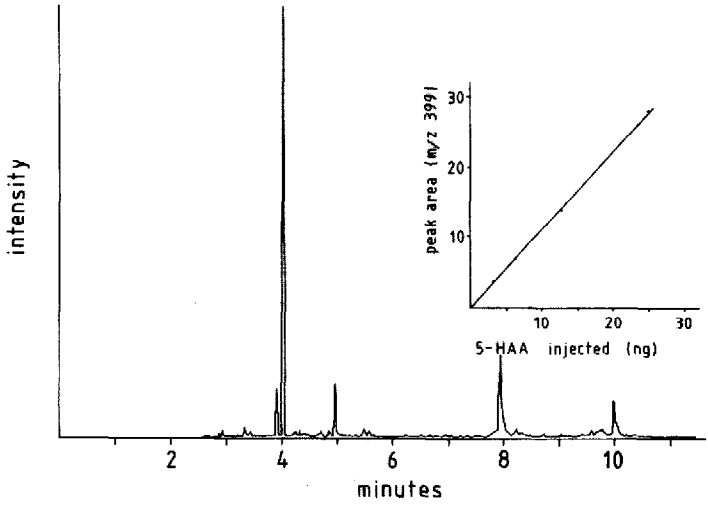

Fig. 9. Reconstructed total-ion chromatogram following injection of an aliquot (1/20) of the lung tissue sample after XAD-4 extraction and derivatization to the methyl ester *tert.*-butyldimethylsilyl ether derivative. The inserted curve displays the external standard calibration for levels between 3 and 25 ng. The peak areas for *m/z* 399 are given in arbitrary units.

the spectra of the trimethylsilyl ethers are dominated by fragments derived from α-cleavage on either side of the hydroxyl group and which carry a high percentage of the total ion abundance.

Finally, one of the derivatization techniques was applied for measurements of 5-HETE in human lung tissue (parenchyma) using the methyl ester *tert.*-butyldimethylsilyl ether derivative of the hydrogenated compound (5-HAA) after XAD-4 resin extraction. As can be seen in Fig. 9, the total-ion chromatogram (TIC) is relatively free from interfering compounds at the levels measured (15–25 ng). The ion *m/z* 399 was found to correspond with the peak eluting close to 8 min. The total levels in three samples were determined to 536 ± 129 ng (mean $\pm$ S.D., $n=3$) per gram wet weight of lung tissue. The levels of 5-HETE found were in accordance with those measured earlier by reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 280 nm [17].

CONCLUSION

The mass spectra of three alkyldimethylsilyl derivatives of the monohydroxy acids 5-HETE, 12-HETE and 15-HETE were compared to evaluate the ion abundances of high-mass ions. For the three types of derivatives, a common fragmentation pattern leading to several ions is found. The presence of multiple fragmentation pathways diminished the total ion current in high-mass ions, thus giving fairly high limits of detection in SIM. None of the derivatives employed for the quantification of unsaturated monohydroxy fatty acids were satisfactory, except the methyl ester *tert.*-butyldimethylsilyl ether derivative of 12-HETE which gave a detection limit of 40 pg.

Saturation of the double bonds improved the GC properties as well as the MS

fragmentation with regard to quantification by SIM. The portion of the total ion abundance was increased several-fold for the ions in the upper mass region, which is important for a sensitive assay method. In addition, it was found that once the double bonds were saturated, no striking differences were found between the different alkyldimethylsilyl derivatives employed with respect to intensities of ions in the upper mass region. Exception was the mass spectrum of the methyl ester *tert.*-butyldimethylsilyl ether of 5-HAA where the fragment *m/z* 399 carried as much as 26.5% of the total ion abundance. The GC–MS analysis of the saturated forms, because of the presence of intense ions in the upper mass region, is very likely to have detection limits in the low picogram range as described for 12-HAA [13].

Indeed, as demonstrated by determination of the levels of 5-HETE in human lung tissue (parenchyma), this can simply be performed by external standard calibration in the lower nanogram range with no interfering impurities. The resin extraction technique used, which gives better recoveries (>90%) and cleaner extracts than octadecyl silica extraction columns [18], made it possible to hydrogenate and derivatize the extract with no further purification. Although this simple purification and derivatization procedure would also lead to detection of *m/z* 399 from 12-HETE as well as 15-HETE, the production of these compounds in lung parenchyma is negligible as compared to 5-HETE [17]. Furthermore, in biological specimen where several HETEs are present, a reversed-phase chromatographic separation can be included after the preliminary extraction [17]. For measurements in the picogram range, an internal standard such as an isotope-labelled analogue must be added to the sample to avoid poor recoveries upon isolation and purification. Since deuterated analogues cannot be used as internal standards due to deuterium–hydrogen exchange during the catalytic reduction, the use of ^{18}O-labeled monohydroxy acids as internal standards seems presently to be an attractive alternative. The facile preparation of such standards by exchange of the carboxyl oxygen and their use as internal standards for quantification in the picomole range has been described previously for HETEs and leukotrienes [11, 14, 24, 25].

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