Journal of Chromatography, 423 (1987) 1–14
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3919

MASS SPECTROMETRIC EVALUATION OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF MONOHYDROXYEICOSATETRAENOIC ACIDS AND LEUKOTRIENES

S. STEFFENRUD and P. BORGEAT

Research Unit Inflammation and Immunology-Rheumatology, Centre Hospitalier de l'Université Laval, Quebec G1V 4G2 (Canada)

and

M.J. EVANS and M.J. BERTRAND*

Department of Chemistry, University of Montreal, P.O. Box 6128, Stn A. Montreal, PQ H3C 3J7 (Canada)

(First received May 26th, 1987; revised manuscript received August 25th, 1987)

SUMMARY

The *tert.*-butyldimethylsilyl (t-BDMS) derivatives of the hydroxyl and the carboxyl groups on the monohydroxyeicosatetraenoic acids (HETEs) 5-, 12- and 15-hydroxyeicosatetraenoic acid (5-HETE, 12-HETE and 15-HETE, respectively) as well as leukotriene B_4 (LTB_4), 20-carboxy-LTB_4 (20-COOH-LTB_4) and 20-hydroxy-LTB_4 (20-OH-LTB_4) have been prepared. The derivatization reagents N-methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide (MTBSTFA) and *tert.*-butyldimethylsilylimidazole (t-BDMSIM) were utilized and the derivatization yields evaluated by gas chromatographic analysis. While the derivatization of 12-HETE and 15-HETE was achieved with good yield using MTBSTFA, the 5-hydroxylated compounds could only be derivatized satisfactorily with t-BDMSIM. The t-BDMS ester derivatives of monohydroxy fatty acids and 20-COOH-LTB_4 were found quite susceptible to hydrolysis upon attempted isolation. The mass spectrometric fragmentation patterns of the HETEs, LTB_4, 20-COOH-LTB_4 and 20-OH-LTB_4 and their reduced analogues as the t-BDMS ester and ether derivatives were examined. The fragmentation of the unsaturated compounds was mainly influenced by the double bonds while, on the contrary, the saturated compounds showed intense high-mass ions derived from elimination of a *tert.*-butyl radical. Furthermore, it was observed that the t-BDMS ester moiety of the unsaturated compounds exerted certain influence upon the fragmentation pattern. With regard to earlier results obtained with the methyl ester t-BDMS ether derivative, it was demonstrated that the use of the t-BDMS ester derivative resulted in decreased intensities of high-mass ions with a few exceptions. Taking into account the mass spectrometric fragmentation and the poor hydrolytic stability of the t-BDMS ester derivatives of some of the studied compounds, the use of these derivatives was not found to constitute a major advantage over the use of the methyl ester derivative. However, a favourable fragmentation pattern with a very intense high-mass ion was obtained for 15-HETE and the levels of this compound were determined in human lung tissue samples.

0378-4347/87/$03.50

INTRODUCTION

In recent years, several methods have been devised for the derivatization of monohydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LTs) prior to gas chromatography–mass spectrometry (GC–MS). With the carboxyl group derivatized as its methyl ester, derivatives such as trimethylsilyl, allyldimethylsilyl, *tert.*-butyldimethylsilyl (t-BDMS), isopropyldimethylsilyl and pentafluorophenyldimethylsilyl ethers were examined as hydroxyl-protecting groups [1–10]. Further attempts have been directed towards protection of the carboxyl group by preparation of esters other than methyl ester such as pentafluorobenzyl [11–13] or t-BDMS esters [8,14]. However, in most of these studies, it was found that the mass spectral fragmentation patterns in the electron ionization mode were not suitable for a quantitative assay due to lack of intense high-mass ions. Furthermore, it was soon concluded that the presence of conjugated double bonds in HETEs and LTs was a reason for thermal rearrangement and degradation on a GC column [8]. Removal of the double bonds by catalytic hydrogenation before the derivatization of carboxyl and hydroxyl groups resulted in an enhancement of the GC sensitivity as well as the intensities of high-mass ions [8,15]. In one of these studies, the fragmentation patterns of leukotriene B_4 (LTB_4) with the carboxyl group derivatized as the methyl ester as well as the t-BDMS ester were compared. Increased abundance of high-mass ions was obtained with the t-BDMS ester of LTB_4 as compared to the methyl ester. Unfortunately, no such comparison was made for the reduced analogue of LTB_4 [8].

With regard to the favourable results obtained with the t-BDMS esters of carboxylic acids other than HETEs and LTs [16–21], it was considered being of interest to compare the MS fragmentation patterns of the methyl ester and t-BDMS ester derivatives of HETEs and LTs as well as their reduced analogues. In this investigation such a comparative study is presented with the emphasis on eventual applicability for quantitative MS. The t-BDMS ester-ether derivative was found applicable for measurements of 15-hydroxyeicosatetraenoic acid (15-HETE) in human lung tissue.

EXPERIMENTAL

Materials

The monohydroxy fatty acids (5-, 12- and 15-hydroxyeicosatetraenoic acids (5-HETE, 12-HETE and 15-HETE, respectively) were obtained from human polymorphonuclear leukocytes (PMNLs), human platelets and soybean lipoxygenase, respectively, as described earlier [22–25].

LTB_4 was a gift from Dr. J. Rokach, Merck Frosst Labs. (Montreal, Canada). 20-Carboxy-LTB_4 (20-COOH-LTB_4) and 20-hydroxy-LTB_4 (20-OH-LTB_4) were prepared from human neutrophils [26].

N-Methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide (MTBSTFA) with 1% *tert.*-butyldimethylsilylchlorosilane was obtained from Regis (Morton Grove, IL, U.S.A.). *tert.*-Butyldimethylsilylimidazole (t-BDMSIM) was obtained from Pe-

trarch Systems (Bristol, PA, U.S.A.). *tert.*-Butyldimethylchlorosilane (t-BDMSCl) was obtained from PCR Chemicals (Gainsville, FL, U.S.A.).

Dimethylformamide (DMF) (ACS grade, Fisher Scientific) was refluxed and distilled twice from calcium hydride.

Hydrogenation catalyst, 5% rhodium on alumina, was obtained from Aldrich (Milwaukee, WI, U.S.A.).

Sephadex LH-20 was obtained from Pharmacia (Uppsala, Sweden) and Amberlite XAD-4 resin, 50–100 μm, from Serva Feinbiochemica.

Extraction procedure

Human lung tissue was extracted on Amberlite XAD-4 as described earlier [5,6].

Catalytic hydrogenation

Catalytic hydrogenation of HETEs and LTs was performed in methanol at 0°C using 5% rhodium on alumina as the catalyst as described earlier [8].

Preparation of t-BDMS esters-ethers

The HETEs and LTB_4 were reacted with 40 μl of DMF–MTBSTFA (1:1) for 1 h at 100°C, essentially as described earlier [27]. The reaction mixture was injected directly into the gas chromatographic column or passed through a 3×0.5 cm column of Sephadex LH-20 equilibrated with hexane–ethyl acetate (3:1, v/v) as described earlier [28].

Alternatively, LTB_4 was reacted with 40 μl of DMF-t-BDMSIM containing 1% t-BDMSCl for 1 h at 100°C whilst 20-COOH-LTB_4 and 20-OH-LTB_4 were reacted for 2 h at 100°C. The reagents were removed by chromatography on Sephadex LH-20 as described above.

Gas chromatography

GC was performed on a Varian 3700 gas chromatograph equipped with a flame ionization detector and a 2.5 m×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. The column temperature was kept isothermal at temperatures between 250 and 280°C depending on the compound analyzed. The temperatures of the injector and detector were set at 260°C.

Carbon values were determined by comparison of the retention times with those of straight-chain fatty acid methyl esters.

Gas chromatography–mass spectrometry

MS was performed on a Kratos MS-50 TCTA mass spectrometer interfaced to a Carlo Erba 4160 gas chromatograph. The chromatograph was equipped with a DB-1 (J&W Scientific) fused-silica capillary column, 15 m×0.25 mm I.D., con-

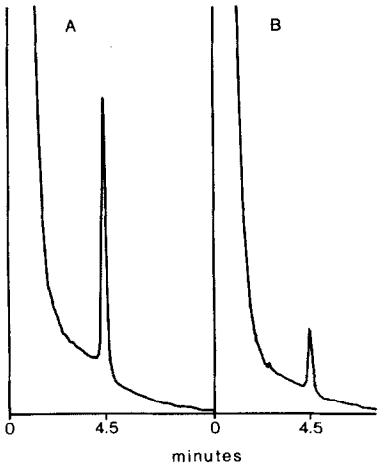

Fig. 1. (A) Gas chromatogram of 250 ng of LTB_4 as its t-BDMS ester-ether derivative prepared with t-BDMSIM. (B) Gas chromatogram of 250 ng of LTB_4 as its t-BDMS ester-ether derivative prepared with MTBSTFA. The column temperature was 270 °C.

nected to a Carlo-Erba on-column injector. The column oven temperature was programmed from 50 to 290 °C at a rate of 15 °C min^{-1}. The interface temperature was 260 °C and the temperature of the ion source was maintained at 200 °C.The accelerating voltage was 8 kV and the electron energy 70 eV. The spectra were acquired at a scan rate of 1 s/decade using the Kratos DS-55 data system.

Quantification by selected-ion monitoring

Quantitative analysis of 15-HETE was performed by measuring *m/z* 499 from the t-BDMS ester-ether derivative of the reduced compound. An external standard calibration curve was established by injections of aliquots of solutions of the same compound at different concentrations. Each point on the standard curve represents the mean value of three repetitive injections (see Fig. 8).

RESULTS AND DISCUSSION

Derivatization and gas chromatography

Despite the good results obtained earlier with MTBSTFA for the preparation of t-BDMS esters of various fatty acids and short-chain organic acids [18–20], good yields in this study were only obtained with the monohydroxy acids 12-HETE and 15-HETE. The yields were calculated by comparison of the GC response of the methyl ester t-BDMS ether derivative (data not shown). For all other compounds included in this study, t-BDMSIM had to be used in order to obtain satisfactory yields as exemplified in Fig. 1. The disadvantage with this reagent is the need for an extraction or a vacuum evaporation procedure to remove the reagents prior to GC. Such a procedure is, however, limited to those

TABLE I

CARBON VALUES (1% SE-30) OF 5-HETE, 12-HETE, 15-HETE, LTB_4, 20-COOH-LTB_4, 20-OH-LTB_4, AND THEIR REDUCED ANALOGUES 5-HEA, 12-HEA, 15-HEA, 5,12-DHEA, 5,12-DHEDA AND 5,12,20-THEA AS THEIR t-BDMS DERIVATIVES

The methylene unit values of 20-COOH-LTB_4, 20-OH-LTB_4 and their reduced analogues are approximative since fatty acid standards of equivalent chain length were not available.

Compound	Carbon value	Compound	Carbon value
6-HETE	26.9	LTB_4	31.0
5-HEA	27.6	5,12-DHEA	32.0
12-HETE	27.0	20-COOH-LTB_4	36
12-HEA	27.8	5,12-DHEDA	36
15-HETE	27.1	20-OH-LTB_4	36
15-HEA	28.0	5,12,20-THEA	36

compounds that are sufficiently stable to undergo an isolation procedure without decomposition. It was experienced in this study that all HETEs and 20-COOH-LTB_4 could not be isolated from the derivatization mixture. On the contrary, their reduced analogues were considerably more stable and it was possible to isolate and reconstitute their t-BDMS derivatives in heptane prior to GC. The t-BDMS ester-ether derivative of LTB_4 and its reduced analogue 5,12-dihydroxy-eicosanoic acid (5,12-DHEA) were found to be stable for about two days upon storage in heptane at $-20\,^{\circ}C$, while the saturated dicarboxylic acid hydrolyzed within a few hours.

The results from derivatization of LTB_4 with the two different t-BDMS reagents are shown in Fig. 1. As can be seen, the peak representing the t-BDMS derivative of LTB_4 prepared with t-BDMSIM (Fig. 1A) is much higher than the one representing the t-BDMS derivative prepared with MTBSTFA (Fig. 1B). Neither the use of acetonitrile as the silylation solvent for MTBSTFA or the silylation mixture in acetonitrile, as described earlier [14], increased the effectiveness of MTBSTFA for derivatizing LTB_4. Since identical procedures were used for the derivatization with the two reagents and GC, the difference between the peak heights indicates different yields in the derivatization reactions. Assuming a steric hindrance to the simultaneous introduction of t-BDMS moieties on the carboxyl group and the hydroxyl group on C-5, the t-BDMSIM reagent would be more efficient according to the relative donor strengths of different leaving groups as reported earlier [29]. A steric hindrance to the simultaneous *tert.*-butyldimethylsilylation of the carboxyl group and the hydroxyl group on C-5 was confirmed by the preparation of the methyl ester t-BDMS derivative of LTB_4 employing the two t-BDMS reagents. Subsequent GC showed similar peak heights for the LTB_4 methyl ester t-BDMS ether prepared with either reagent (data not shown).

The retention times of HETEs and LTs as their t-BDMS derivatives, expressed as methylene units (carbon values), are summarized in Table I. It is notable that the carbon values are quite higher than those reported for the methyl ester t-BDMS derivative in earlier studies [5,6].

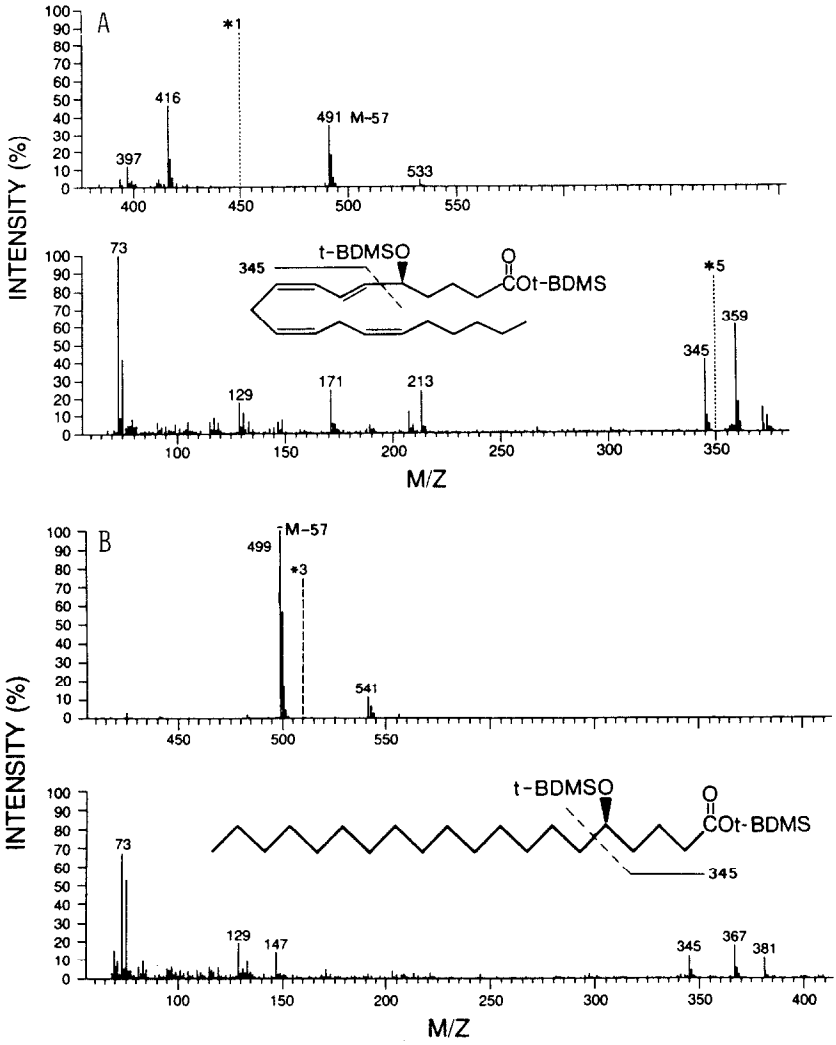

Fig. 2. (A) Mass spectrum of the t-BDMS ester-ether of 5-HETE. (B) Mass spectrum of the t-BDMS ester-ether of 5-HEA. * = Amplification factor.

Mass spectrometry

The mass spectrum of 5-HETE as its t-BDMS derivative is depicted in Fig. 2A. It can be seen that an α-cleavage at C-5 is still an important feature and results in a prominent ion at *m/z* 345. The portion of the total ion abundance, however, is not more than 5.6% (Table II), which is quite similar to the corresponding ion in the mass spectrum of the methyl ester t-BDMS derivative [5]. The ion at *m/z* 491, likely derived from the elimination of a *tert.*-butyl radical, is even weaker (Table II). Likewise, in the mass spectra of 12-HETE (Fig. 3A) and 15-HETE (Fig. 4A) prominent ions involving α-cleavage at C-12 and C-15,

TABLE II

PROMINENT HIGH-MASS IONS IN THE MASS SPECTRA OF THE t-BDMS DERIVATIVES OF 5-HETE, 12-HETE, 15-HETE, LTB_4, 20-COOH-LTB_4, 20-OH-LTB_4 AND THEIR REDUCED ANALOGUES 5-HEA, 12-HEA, 15-HEA, 5,12-DHEA, 5,12-DHEDA AND 5,12,20-THEA

Compound	*m/z*	Percentage base peak	Percentage total ion abundance	Fragment
5-HETE	345	41.2	5.6	
	491	35.6	4.8	M−57
5-HEA	499	100	12.4	M−57
12-HETE	491	18.6	3.3	M−57
12-HEA	499	100	7.5	M−57
15-HETE	491	19.9	3.1	M−57
15-HEA	499	100	13.5	M−57
LTB_4	409	46.1	5.0	
	435	40.1	4.3	
	621	31.9	3.5	M−57
5,12-DHEA	629	100	13.9	M−57
20-COOH-LTB_4	765	23.4	3.9	M−57
	435	36.5	4.6	
5,12-DHEDA	773	100	9.9	M−57
20-OH-LTB_4	409	32.7	3.7	
	435	28.0	3.2	
5,12,20-THEA	759	100	15.6	M−57

respectively, are found at *m/z* 255 and 215, respectively. Accordingly, the ions at *m/z* 491 are also of minor importance.

On the contrary, the mass spectra of the t-BDMS derivatives of the reduced analogues 5-, 12- and 15-hydroxyeicosanoic acid (5-HEA, 12-HEA and 15-HEA, respectively) shown in Figs. 2B-4B all exhibit an intense ion at *m/z* 499 originating from elimination of a *tert.*-butyl radical, and carrying from 7.5 to 14% of the total ion abundance (Table II), while carbon–carbon cleavages are of minor importance.

In a similar manner, the mass spectrum of LTB_4 (Fig. 5A) can be interpreted. The two most intense high-mass ions at *m/z* 409 and 435 probably involve a cleavage of the carbon chain and the elimination of a *tert.*-butyldimethylsilanol. The ion at *m/z* 621 likely derived from elimination of a *tert.*-butyl radical represents only a few per cent of the total ion abundance (Table II).

Similarly, the mass spectra of 20-COOH-LTB_4 (Fig. 6A) and 20-OH-LTB_4 (Fig. 7A) have two prominent ions at *m/z* 409 and 435 but neither carry more than a few per cent of the total ion abundance (Table II).

Like the reduced analogues of HETEs, the mass spectra of the reduced analogues of LTs, 5,12-DHEA, 5,12-dihydroxyeicosadioic acid (5,12-DHEDA) and 5,12,20-trihydroxyeicosanoic acid (5,12,20-THEA), depicted in Fig. 5B–7B, show completely different fragmentation patterns. All spectra are dominated by an intense high-mass ion derived from elimination of a *tert.*-butyl radical and which constitutes 10 to 16% of the total ion abundance (Table II).

A clear advantage of using the t-BDMS ester-ether derivative was obtained

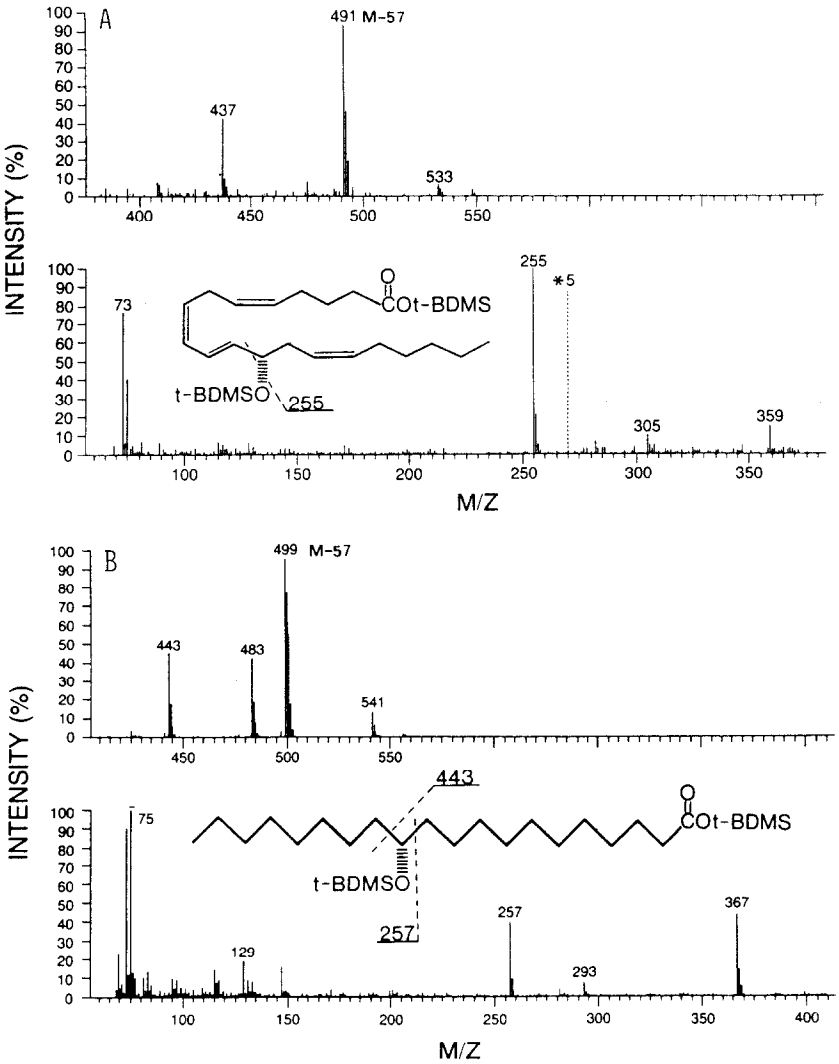

Fig. 3. (A) Mass spectrum of the t-BDMS ester-ether of 12-HETE. (B) Mass spectrum of the t-BDMS ester-ether of 12-HEA. * = Amplification factor.

only with 15-HETE, for which a quantitative assay was developed. Following extraction of lung tissue on XAD-4 resin, catalytic hydrogenation and derivatization, an aliquot was injected into a capillary column interfaced to a mass spectrometer. As can be seen, the total ion chromatogram displayed in Fig. 8 shows few impurities. The two last eluting peaks at retention times about 7 min contained *m/z* 499; however, only the last peak had a retention time corresponding to the reduced analogue of 15-HETE (15-HEA). The sensitivity was sufficient to determine by external standard calibration the levels of 15-HETE in cancerous human lung tissue (parenchyma) to 5.5 ± 0.75 ng (mean $\pm$S.D., $n=3$) per gram wet weight of lung tissue.

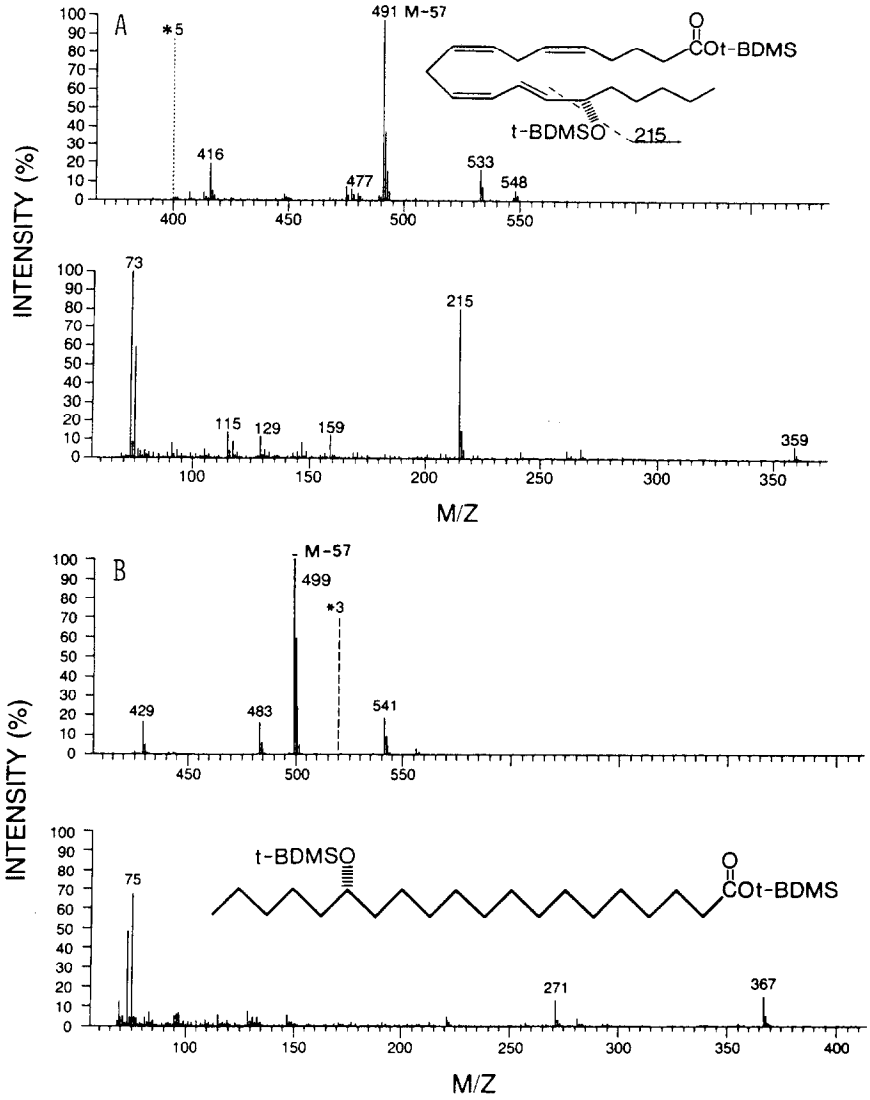

Fig. 4. (A) Mass spectrum of the t-BDMS ester-ether of 15-HETE. (B) Mass spectrum of the t-BDMS ester-ether of 15-HEA. * = Amplification factor.

CONCLUSION

As demonstrated in this study, the use of t-BDMS esters for MS of polyunsaturated compounds did not generally increase the intensities of high-mass ions sufficiently for a quantitative assay as compared to earlier results with the methyl ester derivative [5,6]. This is also in line with investigations of fatty acids where it was observed that with an increasing degree of unsaturation, the t-BDMS ester moiety had a decreasing influence on the fragmentation pattern [16,21,30]. However, as found in earlier investigations [5–8], removal of the double bonds

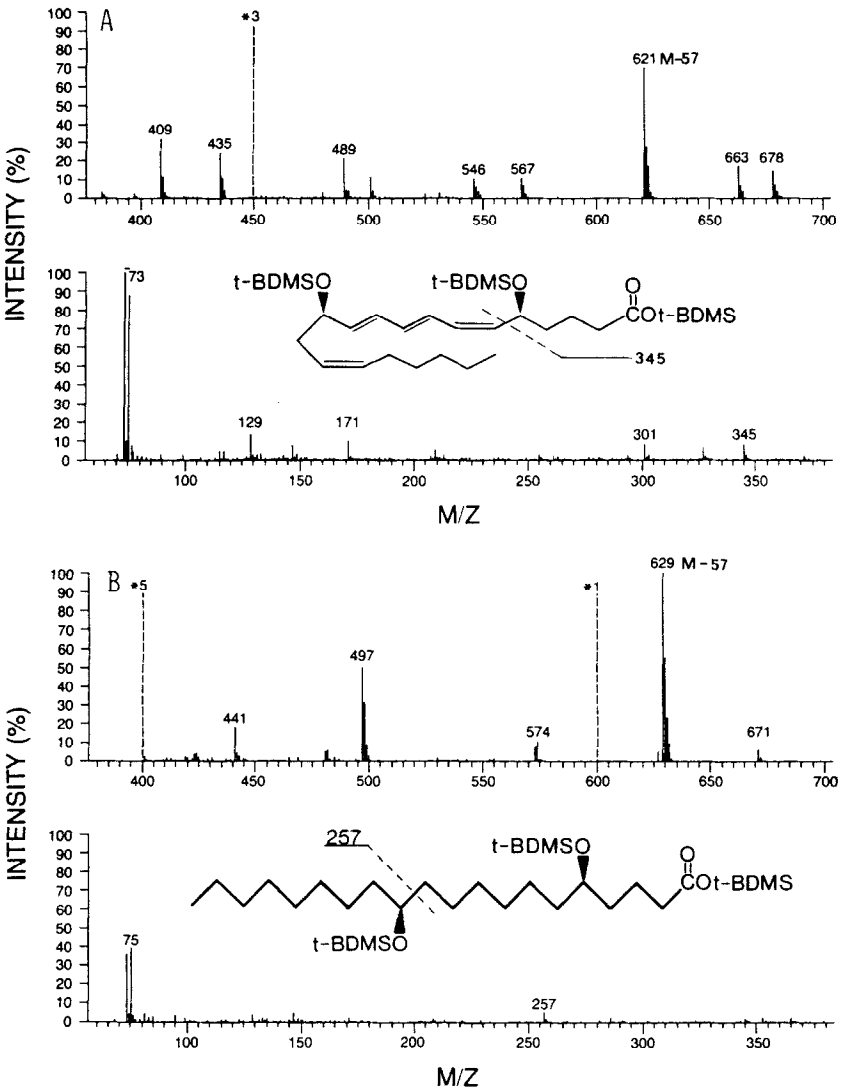

Fig. 5. (A) Mass spectrum of the t-BDMS ester-ether of LTB_4. (B) Mass spectrum of the t-BDMS ester-ether of 5,12-DHEA. * = Amplification factor.

in polyunsaturated fatty acids considerably decreased the MS fragmentation. Furthermore, in one of these studies it was indicated that replacement of the methyl ester for the t-BDMS ester derivative in a saturated compound might even further decrease the fragmentation upon electron impact [8]. The conclusion from the present investigation is, however, that the t-BDMS ester derivative is advantageous only for certain compounds.

In the mass spectra of the saturated HETEs, 5-HEA and 12-HEA (Fig. 2B and 3B, respectively), the portion of the total ion abundance carried by the most intense high-mass ion at *m/z* 499 is actually lower (Table II) than found earlier

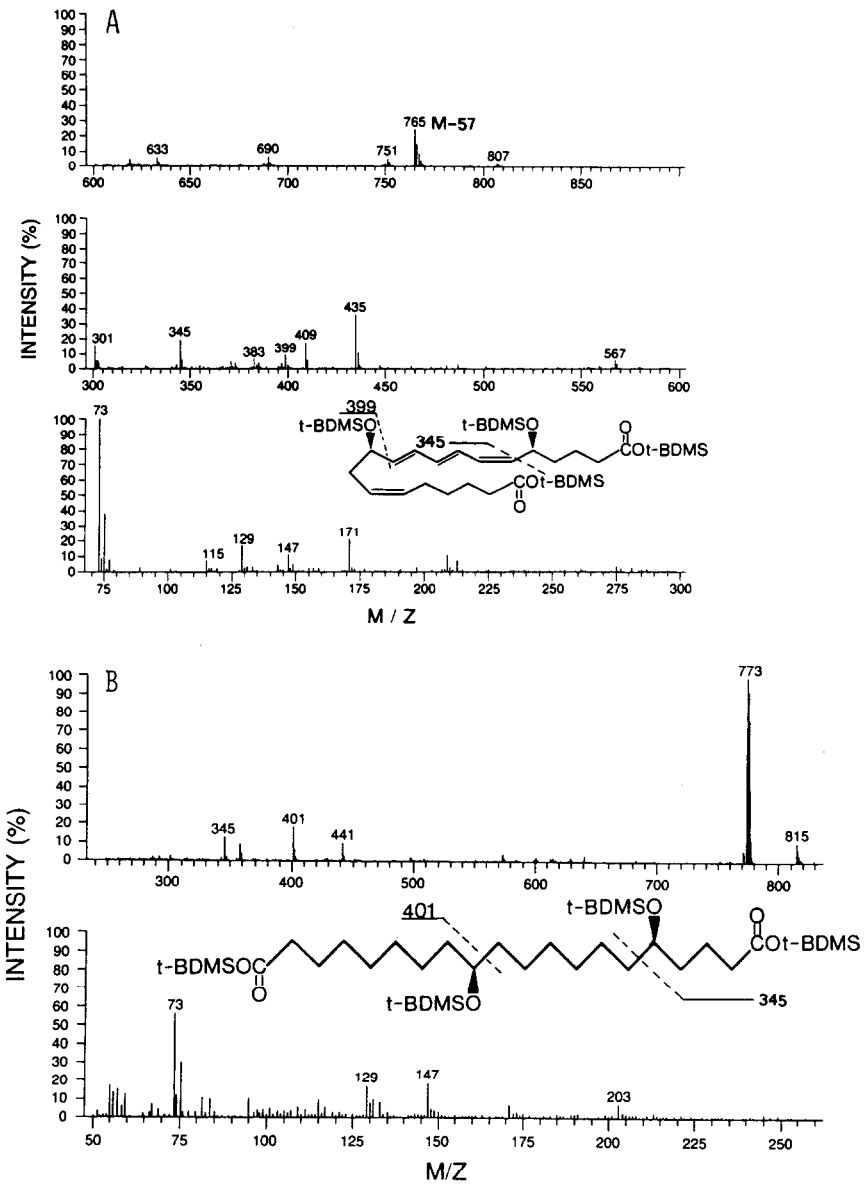

Fig. 6. (A) Mass spectrum of the t-BDMS ester-ether of 20-COOH-LTB$_4$. (B) Mass spectrum of the t-BDMS ester-ether of 5,12-DHEDA. * = Amplification factor.

for the corresponding ions in the mass spectra of the methyl ester t-BDMS derivative [5]. Ions derived from α-cleavage at C-5 and C-12, respectively, can be seen in these spectra. However, when the t-BDMS ether moiety is positioned at C-15 (Fig. 4B) there seems to be less tendency for α-cleavage. Accordingly, the ion at *m/z* 499 in the mass spectrum of 15-HEA (Fig. 4B) carries about 5% more of the total ion abundance (Table II) than the methyl ester t-BDMS derivative [5]. Thus for this reduced analogue of 15-HETE, the t-BDMS derivative was

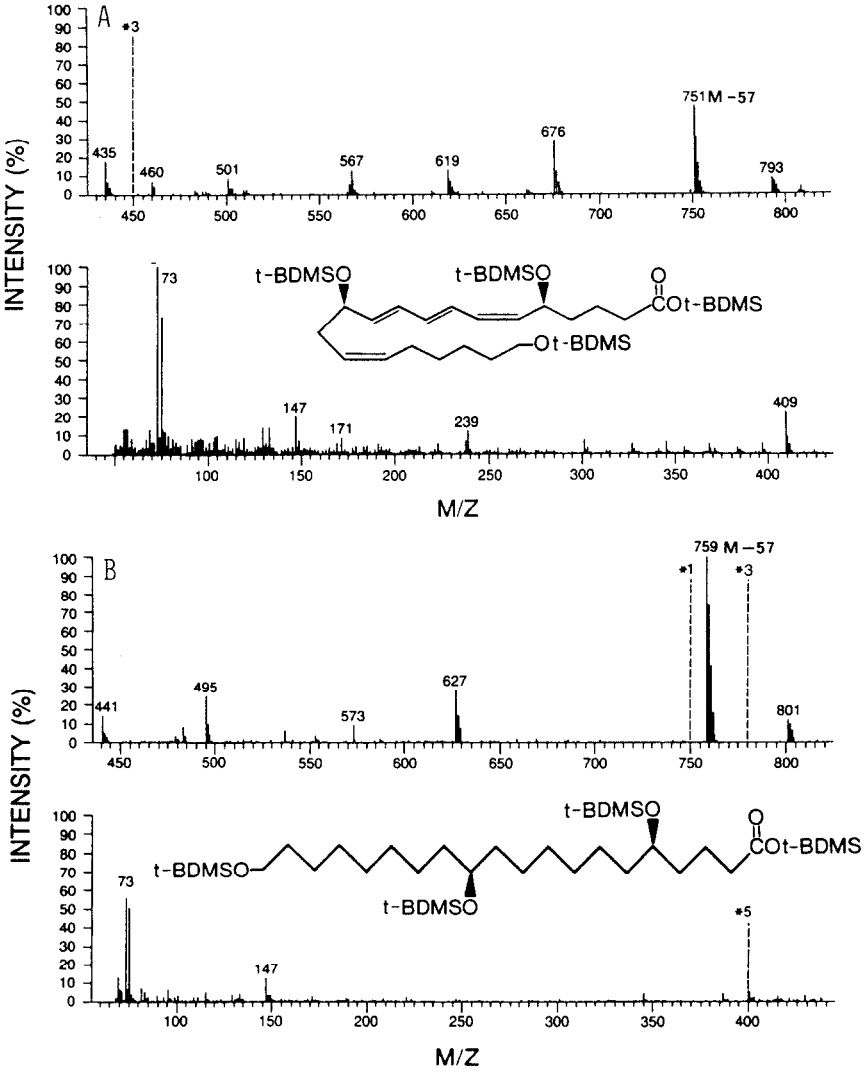

Fig. 7. (A) Mass spectrum of the t-BDMS ester-ether of 20-OH-LTB_4. (B) Mass spectrum of the t-BDMS ester-ether of 5,12,20-THEA. * = Amplification factor.

used with advantage for quantitative assay. A further important factor is the possibility to utilize MTBSTFA for the derivatization with no time-consuming extraction afterwards.

Likewise, for the saturated analogues of LTs, 5,12-DHEA (Fig. 5B), 5,12-DHEDA (Fig. 6B) and 5,12,20-THEA (Fig. 7B), the ions originating from elimination of a *tert.*-butyl radical carry a decreased portion of the total ion abundance as compared to the methyl ester t-BDMS ether derivative with the exception of 5,12-DHEDA [6]. In the spectrum of this latter compound, the ion at *m/z* 773 showed a slightly increased intensity (Table II), about 1–2% as compared to ear-

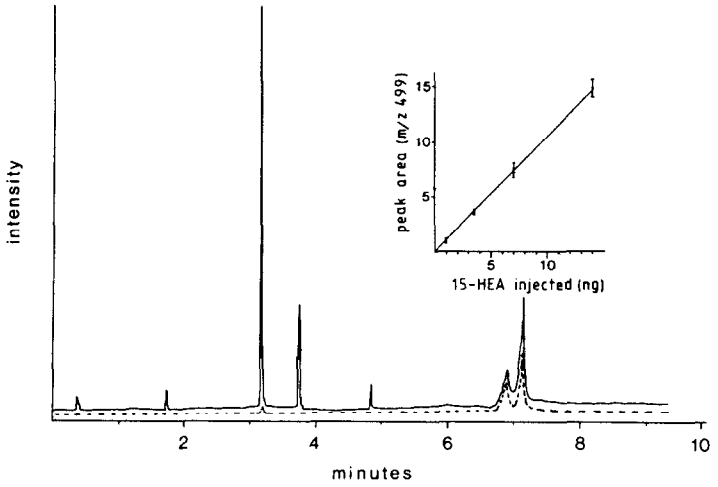

Fig. 8. Total ion chromatogram (—) and *m/z* 499 (---) following injection of an aliquot of one lung tissue sample after XAD-4 extraction, hydrogenation and preparation of the t-BDMS ester-ether derivative. The inserted curve shows the external standard calibration for levels between 1 and 14 ng. The peak areas for *m/z* 499 are given in arbitrary units.

lier results with the methyl ester [6]. However, for dicarboxylic acids the susceptibility towards hydrolysis should also be taken into consideration [17].

Furthermore, with the exception of 15-HEA (Fig. 4B) an increased tendency was observed for carbon–carbon cleavage of the t-BDMS esters-ethers of the saturated compounds as compared to the methyl ester t-BDMS ethers [5,6]. This might indicate that the t-BDMS ester group exerts a certain influence on the fragmentation.

The simple purification and derivatization procedure used in the present study also leads to the simultaneous detection of 5-HETE, 12-HETE and 15-HETE. However, as demonstrated in Table I, the reduced analogues of HETEs have different retention times (carbon values) as their t-BDMS ester-ether derivatives and are easily separated on a capillary column.

Another advantage of the t-BDMS ester-ether derivative is the avoidance of preparation and handling of toxic and explosive diazomethane, which is the most commonly used reagent for preparation of methyl esters of fatty acids.

ACKNOWLEDGEMENTS

The financial supports from the Medical Research Council and the Natural Sciences and Engineering Research Council of Canada (NSERC) are acknowledged. The skillful assistance of Mr. Serge Picard in the preparation of monohydroxy fatty acids is also acknowledged.

REFERENCES

1 J. Larrue, M. Rigaud, G. Razaka, D. Daret, J. Demond-Henri and H. Bricaud, Biochem. Biophys. Res. Commun., 112 (1983) 242.

2 J.M. Boeynaems, A.R. Brash, J.A. Oates and W.C. Hubbard, Anal. Biochem., 104 (1980) 259.
3 S. Steffenrud and P. Borgeat, Prostaglandins, 28 (1984) 593.
4 S. Steffenrud, P. Borgeat, M.J. Evans and M.J. Bertrand, Biomed. Mass Spectrom., 13 (1986) 657.
5 S. Steffenrud, P. Borgeat, H. Salari, M.J. Evans and M.J. Bertrand, J. Chromatogr., 416 (1987) 219.
6 S. Steffenrud, P. Borgeat, M.J. Evans and M.J. Bertrand, Biomed. Environ. Mass Spectrom., 14 (1987) 313.
7 P.M. Woollard and A.I. Mallet, J. Chromatogr., 306 (1984) 1.
8 R.C. Murphy, Prostaglandins, 28 (1984) 597.
9 I. Izumi, T. Shimizu, T. Kasama, Y. Seyama, H. Sumimoto, K. Takeshige, S. Minakami, A. Wetterholm and O. Radmark, Biochem. Biophys, Res. Commun., 134 (1986) 512.
10 H. Rabinovitch-Chable, J. Durand, J.C. Aldigier, P. Chebroux, N. Gualde, J.L. Beneytout and M. Rigaud, Prostaglandins Leukotrienes Med., 13 (1984) 9.
11 R.J. Strife and R.C. Murphy, J. Chromatogr., 305 (1984) 3.
12 R.J. Strife and R.C. Murphy, Prostaglandins Leukotrienes Med., 13 (1984) 1.
13 J. MacDermot, C.R. Kelsey, K.A. Wadell, R. Richmond, R.K. Knight, P.J. Cole, C.T. Dollery, D.N. Landon and I.A. Blair, Prostaglandins, 27 (1984) 163.
14 A.I. Mallet, R.M. Barr and J.A. Newton, J. Chromatogr., 378 (1986) 194.
15 H. Gleispach, R. Moser, B. Mayer, H. Esterbauer, U. Skriletz, L. Zierman and H.J. Leis, J. Chromatogr., 344 (1985) 11.
16 G. Phillipou, D.A. Bigham and R.F. Seamark, Lipids, 10 (1975) 714.
17 A.P.J.M. de Jong, J. Elema and B.J.T. van de Berg, Biomed. Mass Spectrom., 7 (1980) 359.
18 W.F. Schenk, P.J. Berg, B. Beaufrere, J.M. Miles and M.W. Haymond, Anal. Biochem., 141 (1984) 101.
19 D.L. Schooley, F.M. Kubiak and J.V. Evans, J. Chromatogr. Sci., 23 (1985) 385.
20 F.S. Abbott, J. Kassam, A. Acheampong, S. Ferguson, S. Panesar, R. Burton, K. Farrell and J. Orr, J. Chromatogr., 375 (1986) 285.
21 H. Parsons, E.M. Emken, L. Marai and A. Kuksis, Lipids, 21 (1986) 247.
22 M. Hamberg and B. Samuelsson, Proc. Natl. Acad. Sci., 7 (1974) 3400.
23 P. Borgeat, M. Hamberg and B. Samuelsson, J. Biol. Chem., 251 (1976) 7816.
24 P. Borgeat and B. Samuelsson, Proc. Natl. Acad. Sci., 76 (1979) 2148.
25 E. Goetzl and F. Sun, J. Exp. Med., 150 (1979) 406.
26 M. Nadeau, B. Fruteau de Laclos, S. Picard, P. Braquet, E.J. Corey and P. Borgeat, Can. J. Biochem. Cell Biol., 62 (1984) 1321.
27 A.C. Bazan and D.R. Knapp, J. Chromatogr., 236 (1982) 201.
28 R.W. Kelly and P.L. Taylor, Anal. Chem., 48 (1976) 465.
29 C.F. Poole and A. Zlatkis, J. Chromatogr. Sci., 17 (1979) 115.
30 P.M. Woollard, Biomed. Mass Spectrom., 10 (1983) 143.