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Daughter Ion Mass Spectra of 11-Pentafluorobenzyl Ester Derivatives of 11-Dehydrothromboxane B_2 and B_3

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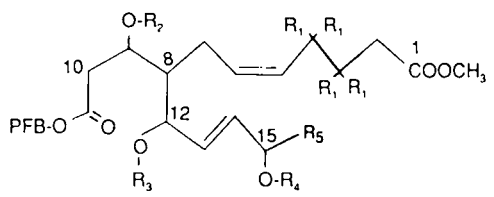
Collisionally activated decomposition (CAD) mass spectra of the $[M - C_6F_5CH_2^{\cdot}]^-$ ions of 1-methyl ester-11-pentafluorobenzyl ester-9,12,15-tris(trimethylsilyl) and 9,12,15-tris(ethyldimethylsilyl) ether derivatives of 11-dehydrothromboxane B_2 are presented and discussed. The spectra are interpreted with the aid of those of a corresponding 3,3,4,4-tetradeutero compound and of an analogous derivative of 11-dehydrothromboxane B_3. Proposed fragmentation pathways are based on internal consistency of data from all four compounds. The migration of a trimethylsilyl or an ethyldimethylsilyl group is the salient feature of all the CAD spectra.

INTRODUCTION

Thromboxane A_2 (TXA_2), one of the most powerful vasoconstrictors and platelet agonists, is the major eicosanoid synthesized by platelets from arachidonic acid. Because of its remarkable biological activities, the synthetic level of TXA_2 *in vivo* is of interest to clinicians and physiologists and also to nutritional biochemists investigating the effect of diets on indices of cardiovascular function and health.

Currently, one of the methods of choice used to assess TXA_2 endogenous synthesis is to measure the urinary excretion of 11-dehydrothromboxane B_2 (11-$DTXB_2$), a major and stable metabolite of TXA_2, in 24 h urine.[1,2] The use of tetradeuterated analogs as internal standards has become routine in the quantification of eicosanoids based on either gas chromatography/electron-capture negative-ion mass spectrometry (GC/EC-NIMS) or GC/tandem mass spectrometry (GC/MS/MS).[3] It is of practical significance to know the fragmentation pathways of the derivatized analytes under the chosen set of conditions in order to be able to select both the site of the (stable-isotope) label and the specific fragment ion for either selected-ion monitoring or MS/MS.

We have recently developed a GC/MS/MS method for the quantification of urinary 11-dehydro- thromboxane B_2 where, contrary to existing methods, the pentafluorobenzyl (PFB) moiety was introduced at C(11).[4] This type of derivative has not been described previously. In this paper we report on a study of collisionally activated decompositions (CAD) of the $[M - PFB]^-$ ions of the four closely related 11-dehydrothromboxane derivatives shown in Fig. 1. The study of compounds **3** and **4** was carried out with a view toward validating the tentative fragmentation pathways we first observed in compounds **1** and **2**.



1 $R_1 = H$; $R_2 = R_3 = R_4 = Si(CH_3)_3$; $R_5 = H_2C\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$

2 $R_1 = {}^2H$; $R_2 = R_3 = R_4 = Si(CH_3)_3$; $R_5 = H_2C\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$

3 $R_1 = H$; $R_2 = R_3 = R_4 = Si(CH_3)_2(C_2H_5)$; $R_5 = H_2C\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$

4 $R_1 = H$; $R_2 = R_3 = R_4 = Si(CH_3)_3$; $R_5 = H_2C\text{-}CH{=}CH\text{-}CH_2\text{-}CH_3$

Figure 1. Structures of the 11-dehydrothromboxane derivatives studied.

EXPERIMENTAL

Materials

Pentafluorobenzyl bromide (PFBBr) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Pierce Chemical (Rockford, IL), ethyldimethylsilylimidazole from TCI American (Atlanta, GA) and Sep-Pak C_{18} cartridges from Waters Associates (Milford, MA). Diazomethane was prepared from Diazald (Aldrich Chemical, Milwaukee, WI) and silica gel G TLC plates (5 cm × 20 cm), 500 μm layer thickness, were purchased from Analtech (Newark, DE). 11-Dehydrothromboxane B_2 (11-$DTXB_2$) and 3,3,4,4-[$^2H_4$]-11-$DTXB_2$ were obtained from Cayman Chemical (Ann Arbor, MI). Solvents were of analytical grade and distilled in glass.

Preparation of 11-dehydrothromboxane derivatives

Compounds 1 and 2. A 10 μg amount of 11-$DTXB_2$ or 3,3,4,4-[$^2H_4$]-11-$DTXB_2$ was treated with excess of ether-

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eal diazomethane and the sample was evaporated to dryness (under nitrogen) after 15 min. The residue was treated with 10 µl of pyridine and 50 µl of 0.06 M NH_4HCO_3 to open the lactone ring. After being allowed to stand for 3 h at room temperature, the sample was evaporated to dryness after addition of three drops of absolute ethanol. The residue was then treated immediately with 20 µl of diisopropylethylamine (DIPEA) and 20 µl of 35% PFBBr in acetonitrile and heated at 40 °C for 30 min. After thorough evaporation, 20 µl of BSTFA in pyridine (1 : 1, v/v) were added to the residue. The sample was then heated at 40 °C for 15 min and, after evaporation, the residue was treated with 40 µl of 2,2,4-trimethylpentane, vortex mixed and centrifuged. The supernatant was then transferred into a clean vial and, after solvent evaporation, the residue was dissolved in 20 µl of 5% BSTFA–pyridine (1 : 1, v/v) in 2,2,4-trimethylpentane. A volume of 1 µl containing 2.5 ng of derivative was used for GC/MS.

Compound 3. This was prepared in an identical fashion to **1** and **2**, except that ethyldimethylsilylimidazole in pyridine (1 : 1, v/v) was used for derivatization of the hydroxyl groups.

Compound 4. This was a natural product obtained by processing at 10 cm^3 portion of a 24 h urine pool from a human subject who had participated in a fish oil supplementation study.[5] Briefly, urine was acidified [water–HCl (1 : 1, v/v)] to pH 2.7 and, after 1 h at room temperature, was passed through a pre-washed Sep-Pak C_{18} cartridge. The cartridge was sequentially rinsed with 10 cm^3 of acidified (pH 2.7, formic acid) water and 10 cm^3 of methyl formate–light petroleum (5 : 95, v/v). The thromboxanes (both 11-$DTXB_2$ and 11-$DTXB_3$) were then eluted with 10 cm^3 of methyl formate–light petroleum (1 : 1, v/v). The residue from solvent evaporation was methylated with diazomethane, then delactonized with aqueous NH_4HCO_3 and pyridine as usual. After 3 h at room temperature followed by addition of 1 cm^3 of water, the sample was extracted three times with ethyl acetate. The aqueous phase was re-acidified to pH 2.7 (dilute HCl) and, after 1 h, was extracted (× 3) with ethyl acetate. The organic phase was evaporated to dryness and the residue was placed on a pre-washed [$CHCl_3$–MeOH (2 : 1, v/v)] silica gel plate (2.5 cm channel). The plate was developed with ethyl acetate–2,2,4-trimethylpentane (4 : 1, v/v) saturated with 0.1% acetic acid. (A reference plate spotted with 11-$DTXB_2$ methyl ester was developed alongide.) A 2 cm band centered at R_F 0.6 was scraped off the plate and the absorbed material was eluted with 3 cm^3 of 10% methanol in ethyl acetate. After solvent evaporation, preparation of the PFB ester at C(11) and of the trimethylsilyl ether derivative at C(9), C(12) and C(15) were carried out as described for compounds **1** and **2**.

Gas chromatography/tandem mass spectrometry

Gas chromatography was carried out with a Varian 3400 instrument operated in the splitless mode with a 30 m × 0.25 mm i.d. DB-1 (J. & W. Scientific, Rancho Cordova, CA) capillary column, phase thickness 0.25 µm. The injector temperature was 250 °C. The oven was kept at 100 °C for 0.5 min after injection, then heated to 300 °C at 27 °C min^{-1} and held at 300 °C for 10 min. The chromatograph was interfaced with a Finnigan-MAT TSQ-70B triple-stage mass spectrometer operated in the negative-ion detection mode with methane as ionization gas. The interface temperature was 300 °C and the ion-source temperature 150 °C. Methane was supplied at a pressure of 7 Torr (1 Torr = 133.3 Pa), the argon collision cell pressure was 1 mTorr, collision energy 19 eV, electron energy 70 eV and emission current 0.2 mA and the electron multiplier was operated at 1400 V.

RESULTS AND DISCUSSION

The parent ions P^-, $[M - PFB]^-$, from compounds **1–4** that underwent CAD are at *m/z* 615, 619, 657 and 613, respectively (Figs 2–5). The P^- of **1**, **2** and **4** lose

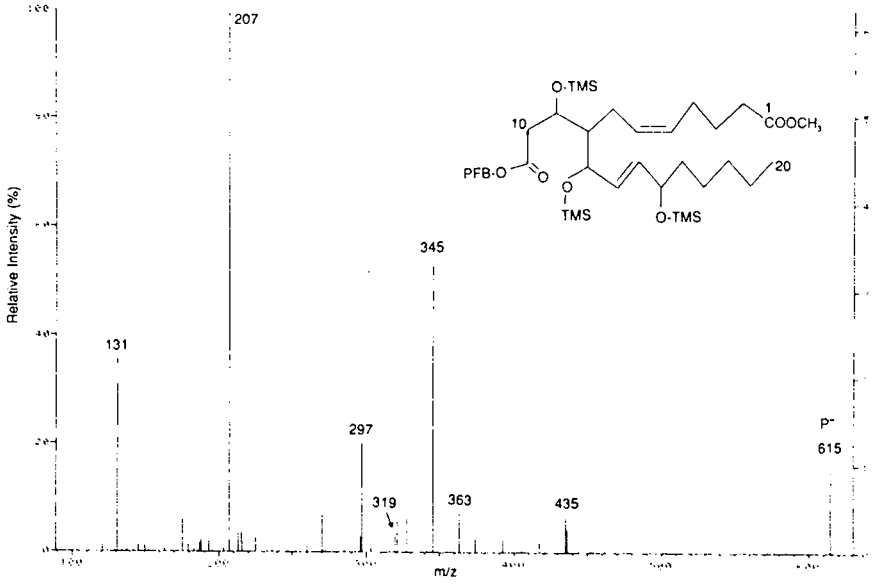

Figure 2. CAD mass spectrum of the $[M - PFB]^-$ ion (*m/z* 615) of the 1-methyl ester-11-pentafluorobenzyl ester-9,12,15-tris-(trimethylsilyl) ether derivative of 11-dehydrothromboxane B_2 (**1**).

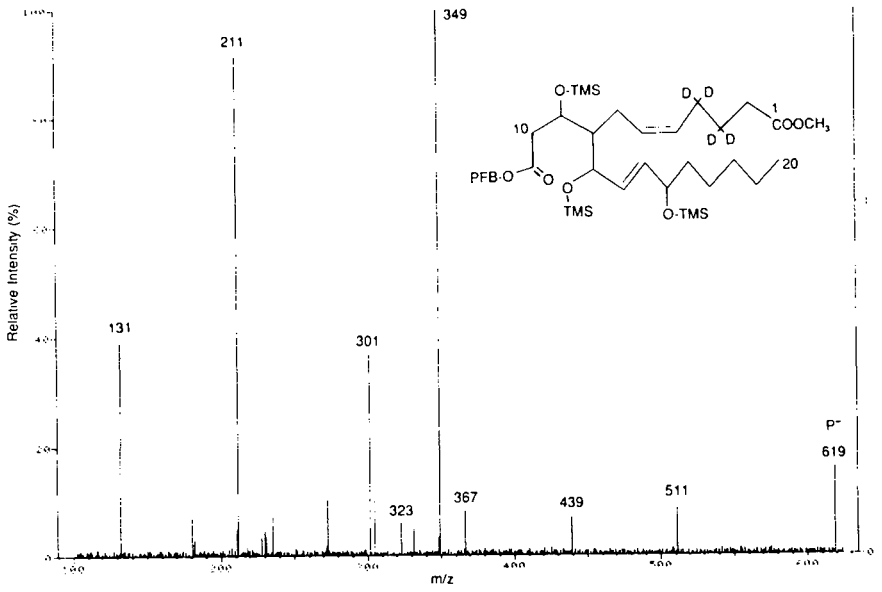

Figure 3. CAD mass spectrum of the $[M - PFB]^-$ ion (*m/z* 619) of the 3,3,4,4-$[^2H_4]$-1-methyl ester-11-pentafluorobenzyl ester-9,12,15-tris(trimethylsilyl) ether derivative of 11-dehydrothromboxane B_2 (**2**).

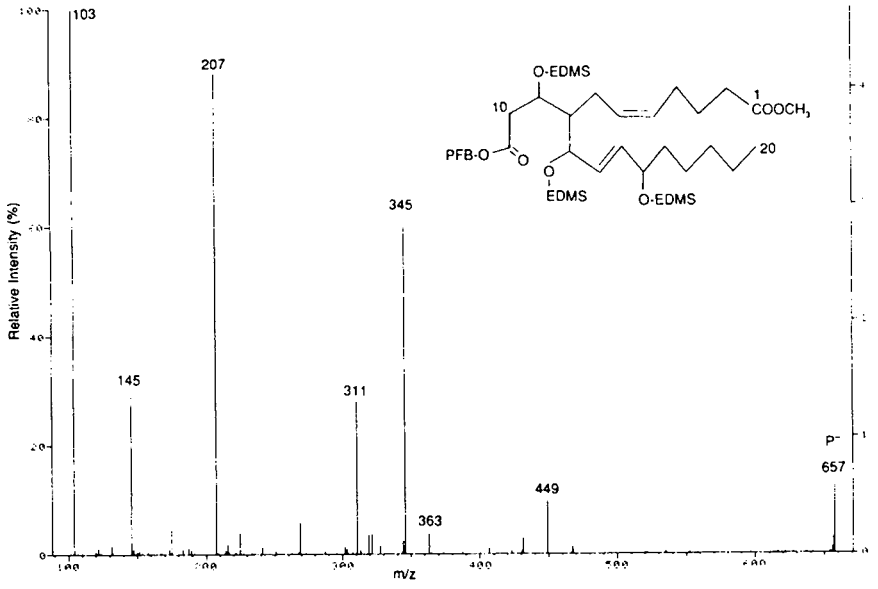

Figure 4. CAD mass spectrum of the $[M - PFB]^-$ ion (*m/z* 657) of the 1-methyl ester-11-pentafluorobenzyl ester-9,12,15-tris(ethyldimethylsilyl) ether derivative of 11-dehydrothromboxane B_2 (**3**).

two molecules of trimethylsilanol (180 u) whereas that of **3** loses two ethyldimethylsilanol molecules (208 u) at C(9) and C(15) to give fragment ions at *m/z* 435, 439, 433 and 449, respectively. These charge-remote site fragmentations are followed by migration of the trimethylsilyl group (or of the ethyldimethylsilyl group in the case of **3**) at C(12) to the C(11) carboxylate group with concomitant loss of the neutral fragment C(12)–C(20). In the case of **1**, **2** and **3**, the neutral molecule is nonadienal ($C_9H_{14}O$, 138 u) (Fig. 6); in the case of **4** it is a molecule of nonatrienal ($C_9H_{12}O$, 136 u). Similar migrations of TMS groups in negative-ion mass spectrometry have been observed previously.[6] The resulting rearrangement ions at *m/z* 297, 301 and 311 all eliminate another trimethylsilanol molecule (ethyldimethylsilanol in the case of **3**) as indicated in Fig. 6 for **1**. The structures of the resulting fragments at *m/z* 207 and 211 are uncertain. We believe that the set of shifts in the ion masses observed in the daughter ion spectra of P^- from **1**–**4** as described provides strong support for the proposed decompositions.

The daughter ions resulting from fragmentation of three trialkylsilanol molecules all give very prominent peaks in the CAD spectra of $[M - PFB]^-$ from **1**, **2**, **4** and **3** at *m/z* 345, 349, 343 ($[P - 3 \times 90]^-$) and 345 ($[P - 3 \times 104^-$), respectively. The fragment ions at *m/z* 363, 367 and 361 in the daughter-ion spectra of $[M - PFB]^-$ from **1**, **2** and **4**, respectively, correspond to the elimination of two TMSOH molecules plus 72 u, presumably the neutral fragment $Me_2Si{=}CH_2$. Finally,

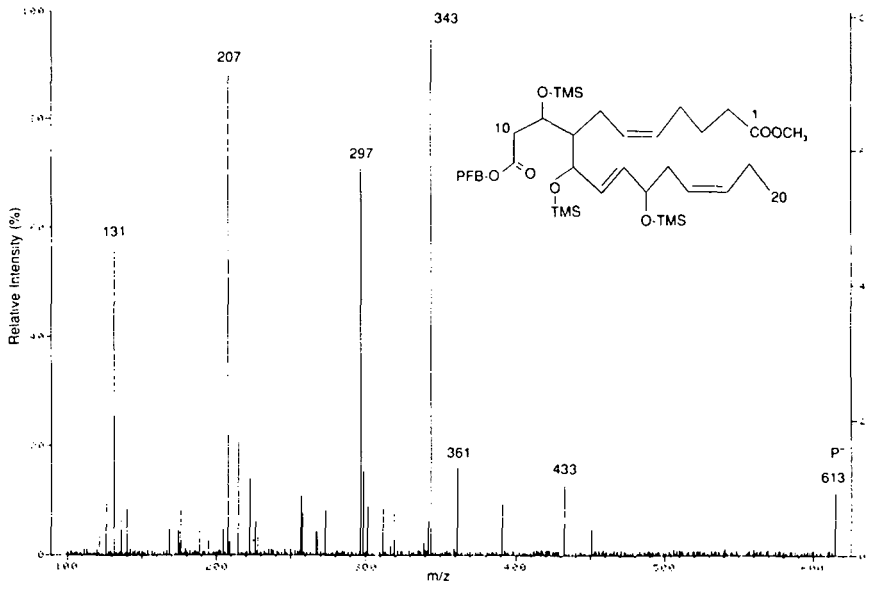

Figure 5. CAD mass spectrum of the $[M - PFB]^-$ ion (*m/z* 613) of the 1-methyl ester-11-pentafluorobenzyl ester-9,12,15-tris-(trimethylsilyl) ether derivative of 11-dehydrothromboxane B_3 (**4**).

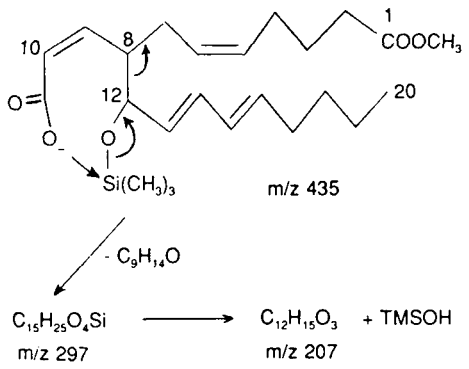

Figure 6. Migration of the trimethylsilyl group at C(12) and simultaneous elimination of the elements of nonadienal, followed by loss of the remaining TMSOH.

the CAD spectra of $[M - PFB]^-$ of **1** and **2** show an ion $[P - (2 \times TMSOH) - (Me_2Si{=}CH_2) - CO_2]^-$ at *m/z* 319 and 323, respectively.

In our GC/MS/MS method for quantification of urinary 11-DTXB_2 we proposed the use of the ion pair *m/z* 345/349 ($[P - 3 \times 90]^-$).[4] Based on the results presented here, it is clear that the *m/z* 297/301 and the 207/211 pairs are at least equally satisfactory because the deuterium label is still present in the corresponding fragment ions.

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