Mass spectra of tert-butyldimethylsilyl ether derivatives of the major metabolite of prostaglandin F

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Abstract

The EI mass spectra of four tert-butyldimethylsilyl ether derivatives of the major metabolite of prostaglandins \( F_1 \) and \( F_2 \) (PGF-M) are presented and discussed. Proposed ion assignments and fragmentation pathways are based on substituent shifts, on data from a deuterium-labeled methoxime analog, and on the analysis of collision-induced dissociation spectra of selected ions. Fragment ions suitable for identification and quantification work are proposed.

Keywords: Prostaglandin F; Metabolite; PGF-M; Derivatives; Electron ionization; Mass spectrometry

1. Introduction

Prostaglandins (PG) belong to a broad group of naturally occurring compounds called eicosanoids which are synthesized in all mammalian systems from \( C_{20} \) polyunsaturated fatty acids via the cyclooxygenase pathway. Prostaglandins \( F_1 \) and \( F_2 \) are two of the so-called ‘primary’ PG because they were among the first to be discovered. The need to quantify changes of their endogenous synthesis prompted the development of assays based on the non-invasive measurement of urinary metabolites by gas chromatography/mass spectrometry (GC/MS) [1–3]. This is our third report [4,5] on the mass spectrometry of derivatives of \( 9\alpha,11\alpha\)-dihydroxy-15-oxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid, the major human metabolite of prostaglandin F (PGF-M). It should be noted that minor contributions to PGF-M excretion may originate from prostaglandin \( E_2 \) and, possibly, other oxo-prostaglandins. The derivatives under investigation are shown in Fig. 1. The data herein reported will be helpful in the identification of PGF-M in complex mixtures, in setting up quantitative as-
says based on selected ion monitoring, and in site selection for stable isotope labeling.

2. Experimental

2.1. Materials

Synthetic PGF-M was a gift from Drs. J. Pike and U. Axen of Upjohn (Kalamazoo, MI, USA). Diazomethane and diazoethane were prepared as described previously [6]. Methoxylamine hydrochloride was purchased from Baker Chemical (Phillipsburg, NJ, USA), tert-butyldimethylchlorosilane/imidazole (1:1) from Alltech Associates (Deerfield, IL, USA), and [3H3]methoxylamine hydrochloride from Regis Chemical (Morton Grove, IL, USA). All analytical operations were carried out in silanized glassware.

2.2. Synthetic procedures

Derivatives for mass spectral analysis were prepared on a 20-μg scale.

2.3. Preparation of compounds 1 and 2

Because PGF-M exists in the C(1)/C(9) lactone form in neutral and acidic media, esterification at C(1) must be preceded by a delactonization step which consists of heating the substrate with water/pyridine/triethylamine (10:10:1, v/v) at 40°C for 30 min [4]. Immediately after removal of the solvent under a stream of dry nitrogen, the residue was dissolved in one drop of methanol and treated with excess ethereal diazomethane (for 1) or diazoethane (for 2). After evaporation of the ether, the residue was treated with methoxylamine hydrochloride in pyridine as described previously [4]. Finally, the hydroxyl groups at C(9) and C(11) were derivatized with 50 μl of tert-butyldimethylchlorosilane/imidazole (1:1) at 60°C for 30 min. The reaction mixture was then diluted with excess water and extracted with hexane. Portions of the residue after evaporation were injected into the GC/MS system.

2.4. Preparation of compounds 3 and 4

PGF-M was first directly esterified at C(20) with either diazomethane (for 4) or diazoethane (for 3). This was followed by delactonization and esterification at C(1) (with the proper reagent). Methoxymation and tert-butyldimethylsilylation were then carried out as described above.

2.5. Gas chromatography/mass spectrometry (GC/MS)

Gas chromatography was performed on a Varian 3400 instrument operated in the splitless mode with a 23 m × 0.25 mm DB-1 (J&W Scientific Inc., Rancho Cordova, CA) capillary column. The He flow was 40 cm/s and the injector temperature was 250°C. The oven was kept at 100°C for 1 min after injection, then it was heated to 300°C at the rate of 27°C/min. The gas chromatograph was interfaced to a Finnigan-Mat TSQ-70B triple stage quadruple mass spectrometer operated in the EI mode (70 eV, 200 μA). The transfer line temperature was 300°C and the ion source temperature was 150°C. Low-energy (E₂ = 5–25 eV) spectra were recorded.

![Molecular Weight of Compounds](image)

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<tr>
<td>4</td>
<td>C₂H₅</td>
<td>CH₃</td>
<td>629</td>
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</table>

Fig. 1. Structure of the four compounds studied.
3. Results and discussion

3.1. Mass spectra of compounds 1–4

The EI mass spectrum of 1 is shown in Fig. 2. Proposed ion assignments and fragmentation pathways are based on substituent shifts and on data from the $[2H_3]$methoxime analog of 1 ($1-2H_3$). They were supported by the analysis of collision-induced dissociation (CID) spectra of selected ions (Fig. 3).

In all four spectra, the fragments at the highest m/z correspond to the loss of a methyl radical while the molecular ion does not emerge from the background noise. The presence of fragment ions at m/z 584 (M−34) and m/z 587 (M−31) in an approximately 2:1 ratio in the spectrum of $1-2H_3$ indicates that the [M−31]$^+$ ion in the spectrum of 1, and presumably of compounds 3 and 4, arises predominantly from the loss of the methoxime CH$_3$O. Analysis of the CID spectrum of the $[2H_3]$methoxime analog of 1 further indicates that the loss of the ester CH$_3$O involves preferentially the ester moiety at C-1 rather than at C-20. Elimination of a tert-butyl radical (57 amu) produces relatively prominent peaks at m/z 558 and 586 in the spectra of 1 and 2, respectively, and at m/z 572 in those of 3 and 4, while the corresponding ion is shifted to m/z 561 in the spectrum of $1-2H_3$. The sequential elimination of a tert-butyl radical plus the elements of methanol (32 amu) produces a low-intensity peak at m/z 526 in the spectrum of 1, and analogous peaks in the spectra of 2–4. Because no shift is observed in the
spectrum of the deuterium-labeled analog of 1, the \( m/z \) 526 ion must originate from a rearrangement-elimination involving the methoxime OCH₃ moiety. The spectra of 2, 3 and 4 all exhibit peaks of relative intensity < 5%, corresponding to the loss of an \(^{1}OC_{2}H_{5}\) radical (45 amu).
The mid-mass range is characterized by the presence of several relatively abundant ions resulting from the elimination of a tert-butylidemethylsilyloxide radical (131 amu), of tert-butylidemethylsilanol (132 amu), or from combination losses of the above plus fragments of the C-13/20 chain. Thus, the fragment of m/z 484 ([M-(131)]+) in the spectrum of 1 has its counterparts at m/z 512, 498 and 498 in the spectra of 2, 3 and 4, respectively. Similarly, the abundant ion at m/z 452 ([M-(131+32)]+ or [M-(132+31)]+) has its analogs at m/z 480, 466 and 466, respectively. In addition to the fragment of m/z 452 ([M-(132+CD3)]+), the spectrum of the \(^{2}D_{3}\)-labeled methoxime of 1 displays a related fragment of comparable intensity at m/z 455 originating from loss of (132+CH\(_3\)OH) or (131+CH\(_{2}\)O) from the M\(^{+}\). The combined elimination of tert-butylidemethylsilanol and of 131 amu (57+74 or a tert-butylidemethylsilyloxy radical) produces the most intense peaks in the mid-mass range at m/z 352, 380, 366 and 366 in the spectra of 1, 2, 3 and 4, respectively. The anticipated shift to m/z 355 was observed in the spectrum of 1-\(^{2}D_{3}\). Further loss of 32 amu generates fragments of comparable abundance at m/z 320 (1), 348 (2), and 334 (3 and 4). The fact that no shift is observed in the spectrum of 1-\(^{2}D_{3}\) indicates that the elements of methanol in this transition come almost exclusively from the methoxime moiety.

Ions of moderate abundance at m/z 295 in the spectrum of 1 and m/z 309 in the spectrum of 4 arise by elimination of the fragments of mass 132 and 31 discussed above plus a third fragment (with rearrangement) consistent with the elemental composition C\(_{8}\)H\(_{15}\)NO\(_{3}\) (157 amu) involving the C-13/20 chain (see Fig. 3). This assignment is confirmed by the fact that in the spectra of 2 and 3 (ethyl esters at C-20), the C\(_{8}\) fragment becomes C\(_{8}\)H\(_{17}\)NO\(_{3}\) (171 amu), and the corresponding ions in their spectra appear at m/z 309 and m/z 295, respectively. An analogous situation presents itself in regard to the m/z 253 ion in the spectrum of 1 and its m/z 267 analog in the spectrum of 4 arising from the sequential loss of 57, 132 and 173 amu. For the fragment of mass 173 we propose the empirical formula C\(_{8}\)H\(_{15}\)NO\(_{3}\) and the structure indicated in Fig. 3. Again, the 173 fragment becomes C\(_{9}\)H\(_{17}\)NO\(_{3}\) (187 amu) in the case of the C-20 ethyl esters 2 and 3 whose spectra display corresponding ions at m/z 267 and m/z 253. Finally, the m/z 179 ion ([M-(132+131+173)]+) in the spectra of 1 and 3 is shifted to m/z 193 in the spectra of 2 and 4.

We attribute the relatively abundant ion at m/z 371 to initial loss of a tert-butyl radical (57 amu) followed by elimination of the neutral molecule C\(_{9}\)H\(_{17}\)NO\(_{3}\) (187 amu) via a McLafferty rearrangement (Fig. 3). Corresponding ions, somewhat less abundant, are observed at m/z 385 in the spectrum of 4 and at m/z 385 and 371 in the spectra of 2 and 3 ([M-(57+201)]+), respectively. Cleavage at C-15/16 affords the base peaks at m/z 115 (compounds 1 and 4) and m/z 129 (2 and 3). In the low mass range, four peaks of nearly identical relative intensity at m/z 105, 119, 133 and 147 appear in the spectra of all four compounds. The genesis of the associated ions (except that at m/z 133) is proposed in Fig. 3. Finally, in keeping with the assigned structure, the low-abundance m/z 426 ion is shifted to m/z 429 in the spectrum of the 1-\(^{2}D_{3}\) analog, to m/z 454 in the spectrum of 2, and to m/z 440 in the spectra of 3 and 4.

4. Conclusion and summary

In the mass spectra of all four derivatives, the most abundant ions originate from direct loss, or combination losses, of 132, 131, 57, 32 and 31 amu. In sharp contrast to the mass spectrometric behavior of the 9,11-n-butylboronate ester derivatives [4], a single McLafferty-type rearrangement is observed in the spectra of 1–4: this involves the C-14/20 chain and the \(^{\gamma}\)-hydrogen at C-12.

Based on the data presented here, we conclude that, in conjunction with the molecular ion, the following fragment ions could be used to help identify PGF-M in biomatrices: m/z 558, m/z 452, m/z 371 and m/z 352. With proper deuterium labeling, all of these ions, with the exception of the one at m/z 371, are recommended for quantification by selected ion monitoring. It is noteworthy that, among the ions of m/z below 352, only those at m/z 269 and m/z 285 are shifted by 3 amu in the spectrum of the \(^{2}D_{3}\)methoxime.
analog of 1. Brash and coworkers [7,8] pioneered the use of tert-butyldimethylsilyl derivatives of PGF-M for analytical purposes. However, those authors dealt with analytes in which the carbonyl at C-15 was left underivatized. Their mass spectra were adequately discussed [7,8]. Because of the structural similarity between PGF-M and the metabolites of F-isoprostanes [9], some of the characteristic fragmentation modes described in this paper might also be exhibited by the F-isoprostane metabolites. Thus, our observations could conceivably aid in their identification.

References