
Fast Atom Bombardment and Collision-Induced Dissociation of Prostaglandins and Thromboxanes: Some Examples of Charge Remote Fragmentation

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The mass spectra of products found by collisional activation of selected prostaglandins and thromboxanes were studied by tandem mass spectrometry as barium carboxylate salts and as carboxylate anions. Collision-induced dissociation (CID) of these closed shell ions generated by fast atom bombardment mass spectrometry reveals a wealth of structural information for these hydroxy acids. Decomposition reactions were found to be dependent upon the eicosanoid ring structure and the type of ion being studied, either positive or negative ion. The bariated carboxylate salts undergo reactions by processes that are similar to those previously characterized as charge remote mechanisms in which neutral species are lost as in thermal and photolytic decompositions. The most abundant ion is formed by loss of water from each of the hydroxyl groups present on the prostaglandin or thromboxane structure. For these multifunctionalized eicosanoids, typical patterns of decomposition emerge as characteristic of the oxygen substituents present along the carbon chain of the eicosanoid structure. The structural information obtained from the barium salts along with those from the carboxylate anions is substantially different, yet the structural information from each process is complementary. The CIDs of positive ions (metalated salts) provide structural information concerning the substituents between the carboxyl group and C₁₂ of the eicosanoid structure, whereas the decompositions of the carboxylate anions (negative ion mode) provide data concerning structure alterations of the eicosanoid structure between C₁₅ and C₂₀. (*J Am Soc Mass Spectrom* 1990, 1, 325-335)

Arachidonic acid can be enzymatically oxygenated into a host of structurally diverse molecules; in fact, more than 300 different structural species have been characterized as being derived from enzymatic oxygenation of arachidonic acid in various biological systems. One large class of oxygenated products is that of prostaglandins and thromboxanes, which are derived from the incorporation of two molecules of oxygen into the arachidonic acid structure to form a chemically reactive intermediate endoperoxide [1]. This cyclic endoperoxide can be enzymatically isomerized into numerous species called prostaglandins with a five-membered ring between C₈ and C₁₂ and different oxygen substituents at C₉ and C₁₁ (Figure 1). Thromboxanes result from a more complex rearrangement of the cyclic endoperoxide struc-

ture with formation of a cyclic oxarane ring followed ultimately by rearrangement to a six-membered cyclic hemiacetal. Interest in these molecules resides not only in the complex biosynthetic pathways involved in their production, but also in the profound biological activities they possess, which are thought to be an important aspect of cell-cell communication. Furthermore, prostaglandins are pharmacologically active substances that have been the subject of many investigations in the development of agents active in the human body. Since the discovery of the structure of the first prostaglandin [2], there has been continuing interest in these eicosanoids and in means to deduce their structures and the structures of their catabolic products [3].

Mass spectrometry has played a central role in studies of structural characterization of prostaglandins and has served as an analytical means to quantify such substances in biological fluids. Largely, the techniques that have been developed to analyze these unsaturated hy-

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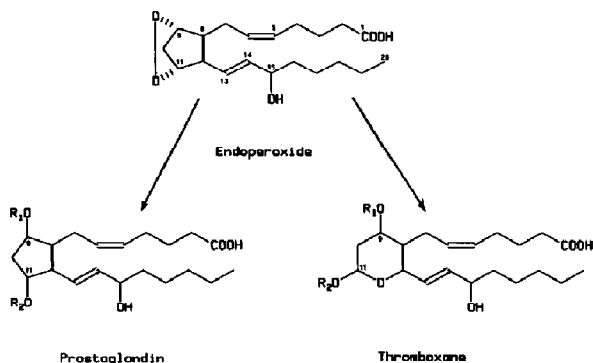


Figure 1. The cyclic endoperoxide (PGH_2) derived from arachidonic acid by the action of cyclooxygenase. This endoperoxide is isomerized enzymatically into several prostaglandins and thromboxanes that differ in oxygen functionalities (R_1 and R_2). The accepted carbon skeleton numbering is indicated.

droxy acids involve derivatization of the polar functionalities and analysis by combined gas chromatography/mass spectrometry (GC/MS) [4]. Typically, derivatives such as the methyl ester trimethylsilyl ethers have been employed for this purpose. Useful information can be obtained from the electron ionization (EI) fragmentation of such derivatives; major ions arise from α cleavages at the C_{15} hydroxyl position [5] and loss of derivatizing moieties such as trimethylsilyanol. Prostanoids do undergo fragmentation reactions that indicate positions of branching between the alkyl chain and the five-membered ring characteristic of the prostaglandins [6]; however, much of the fragment ion abundance of prostaglandin derivatives centers around the derivative itself. For example, the most abundant of ions of m/z 73, 129, 147, and 191 observed in the EI mass spectra of most prostanoid methyl ester trimethylsilyl ethers are in fact clearly related to the hydroxy acid nature of these molecules and are common to many derivatives, not only to the prostaglandins [7]. Unfortunately, structurally significant ions are typically much less abundant, the molecular ion often being absent.

In the past several years, new techniques for the analysis of underivatized, highly polar molecules have been developed, including fast atom bombardment mass spectrometry (FAB/MS). Such matrix-assisted desorption techniques have enabled investigators to analyze underivatized molecules lacking volatility. The FAB/MS spectra of underivatized prostaglandins characteristically show an abundance of $[\text{M}+\text{H}]^+$ ions and an even more abundant population of $[\text{M}-\text{H}]^-$ ions because of the stability of the closed shell carboxylate anion.

Recently, the study of the decompositions of such closed shell carboxylate anions or cationized species from simple carboxylic acids has revealed a wealth of structural information [8-10]. High-energy collision-induced dissociation (CID) of such ions gives rise

to a unique type of fragmentation termed "charge remote," a term suggesting that fragmentations can be induced in regions of the molecule remote from the localized charge. It has been suggested that the mechanism for some of these reactions is very similar to that observed for thermolytic and photochemical processes [11]. Of particular interest is the fact that charge remote fragmentations (CRFs) reveal substantial information concerning the sites of substitution along an alkyl chain, including the position of double bonds, alkyl branch points, cyclopropyl rings, and the position of hydroxyl substitution. Because EIMS of prostanoid derivatives provides limited information concerning these structural features, it was felt that a detailed study of the CID of closed shell ions from underivatized prostaglandins and thromboxanes might reveal unique and structurally specific ions. Furthermore, the prostaglandins are structurally complex and provide structural variation within a single class of molecules to probe our understanding of the CRF process itself, including fragmentations when multiple processes may compete for molecular ion decomposition.

Experimental Procedures

Materials

Synthetic prostaglandins (PGA_1 , PGA_2 , PGA_3 , PGB_1 , PGB_2 , PGD_2 , PGE_2 , 13,14-dihydro-15-keto- PGA_2 , 13,14-dehydro-15-keto- PGE_2 , $\text{PGF}_{2\alpha}$, and 6-keto- $\text{PGF}_{1\alpha}$) as well as the thromboxanes TxB_2 , 11-dehydro- TxB_2 , and 2,3-dinor- TxB_2 were obtained from Cayman Chemical (Ann Arbor, MI). These prostaglandins were also analyzed by GC/MS (70 eV, EI) as the methyl ester trimethylsilyl ether derivative (and methoxime derivatives for those containing keto functions) to check identity and purity.

Fast Atom Bombardment Mass Spectrometry

Dynamic (continuous-flow) and static FAB/MS of the prostaglandin and thromboxane anions were carried out either by using a VG 7070E in normal mode or using the collision cell in the first field-free region (FFR1) and performing linked scans at constant B/E to study product ions, or linked scans at constant B^2/E to study precursor ions. Dynamic FAB was performed with a microsyringe pump (ISCO Inc., Model $\mu\text{LC-500}$) by using a mobile glycerol-based matrix (methanol-water-glycerol, 80/20/2) at 5 $\mu\text{L}/\text{min}$ through a 1 $\text{m} \times 50 \mu\text{m}$ untreated fused silica tube. The sample (200-500 ng) dissolved in 80% methanol-water was injected through a 2 μL loop injector. Static FAB experiments employed glycerol as matrix, and samples were applied in methanol (1-2 μL). No difference was observed in the mass spectra obtained by static FAB or dynamic FAB. This mass spectrometer was equipped with a saddle-field FAB

gun and a commercial FAB ion source. Secondary negative ions were produced with 8-keV Xe atoms with a gun current of 2 mA. Collision-induced dissociation was investigated by using He in the FFR1 collision cell with 10-50% suppression of the molecular ion beam. Linked scan B/E data from the VG 7070E were obtained by using a Teknivent data acquisition system and software. These scans were calibrated at each parent mass by linking the accelerating voltage with the B/E scan and then linear scanning all three parameters (B , E , V) simultaneously. This arrangement permitted transmission of all source-formed ions at their proper m/z ratio. The mass range was calibrated with glycerol cluster ions. Accelerating voltage was then unlinked, and the B/E scan was obtained for product ions.

Tandem mass spectrometry of CID of secondary positive ions (FAB/MS/CID/MS) was performed by using a Kratos MS 50 Triple Analyzer with EBE optics [8]. The instrument consists of a high-resolution mass spectrometer of Nier-Johnson geometry (MS-I) followed by an electrostatic analyzer used as MS-II. It was equipped with a Kratos FAB source, an Ion Tech saddle-field gun, and a Kratos DS-55 data system. Parent ions were produced in the FAB source by bombarding the sample with 7-8-keV Ar atoms, at an atom gun current of 2 mA. Secondary ions produced were accelerated to 8 keV translational energy. Desorption of ions by FAB was accomplished by mixing the solid prostaglandin or thromboxane (0.1-1 mg) on a copper probe tip with 3-nitrobenzyl alcohol (3-NBA) saturated with BaCl_2 and enriched with 5-10% glycerol to increase signal persistence. For the $[\text{M}-\text{H}+2\text{Li}]^+$ ion, the ions were desorbed from 3-NBA that was saturated with LiI. The CID experiments were performed with MS-I set at 3000 resolution (full peak width resolved), and decomposition spectra were acquired by scanning MS-II. The mass-selected ions were induced to decompose by collision with He (50% suppression of the ion beam) in a collision cell in the FFR3. The CID spectra were the average of 10-20 scans processed by software written at the Nebraska laboratory [12]. Although the presentation of spectra (Figures 2-6) allows comparison of the abundances of production with respect to precursor ions from compound to compound, comparisons should be interpreted with caution because important parameters such as collision gas pressure were not rigorously controlled for these experiments.

Synthesis of Stable-Isotope-Labeled Prostaglandins and Thromboxanes

Carboxylate ^{18}O -labeled isotopimers of PGB_2 ($[\text{1,1-}^{18}\text{O}_2]$ PGB_2 , PGB_1 , and TxB_2 ($[\text{1,1,11-}^{18}\text{O}_3]$ TxB_2) were made by using an enzymatically catalyzed oxygen atom exchange reaction with H_2^{18}O , as previously described [13]. The ring-opened form of TxB_2 permitted rapid incorporation of the third ^{18}O atom into carbon-11 aldehyde. Porcine liver esterase was obtained from Sigma Chemical Co. (St. Louis, MO) and H_2^{18}O from

Isotec Inc. 15-OD- PGA_2 and 15-OD- PGB_2 (Miami, OH) were obtained by exchange of the 15-hydroxy labile hydrogen atoms with deuterium by dissolving the respective eicosanoid in deuterated methanol (CH_3OD , Aldrich, Milwaukee, WI) and performing static FAB in d_3 -glycerol (Aldrich).

Results and Discussion

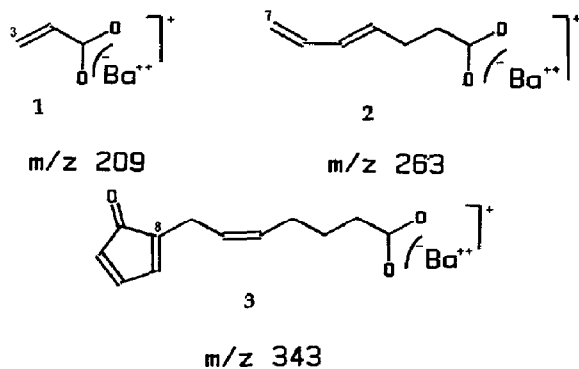
Prostaglandin A Series

Prostaglandins of the A series do not occur naturally, but rather arise from dehydration of PGE_2 during isolation procedures [14]. Nonetheless, the CID of the closed shell ions generated from either the bariated or lithiated (one example is presented) species or carboxylate anions were studied (Figures 2 and 3). The ions resulting from high-energy CID (FAB/MS/CID/MS) of the PGA_2 bariated molecular ion, $[\text{M}-\text{H}+\text{Ba}]^+$, yielded a characteristic pattern that was also seen for most of the other prostanoids. Peaks for low-mass ions from barium and the bariated carboxylate ion itself can be seen (m/z 138, $[\text{Ba}]^+$; m/z 155, $[\text{BaOH}]^+$; m/z 182, $[\text{BaCO}_2]^+$; m/z 196, $[\text{BaCO}_2\text{CH}_2]^+$). The ion at m/z 209 (1) is characteristically abundant for all prostanoids studied by MS/MS.

After the m/z 209 ion, going to higher masses, a gap in abundant ions is observed until the ion of m/z 263, which is formed by cleavage at the branching point of the alkyl chain with a cyclopentenone ring. This position of fragmentation is also allylic to the C_5 - C_6 double bond (2). Typically, a further gap of abundant ions is observed toward higher mass, until the ions of m/z 345, which arise by loss of C_2H_2 and 1-hexanal to give product 3. Formation of this ion involves a proton transfer to C_{12} and is suggested to result from a charge remote process; the most likely site for the rearranging proton is from the C_{15} hydroxyl moiety in a six-membered ring transition stage.

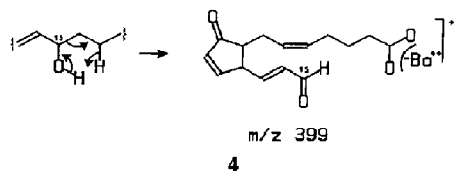
The next gap in masses of abundant ions is defined by an abundant ion at m/z 399. Cleavage of the C_{15} - C_{16} bond with migration of the hydroxyl proton (Scheme 1) and the cleaving alkyl chain accounts for formation of this ion (4). The mechanisms involved in these fragmentation reactions and the gaps in abundant ions were previously discussed in original references to CRF [6-9]. Finally, an ion formed by the loss of water (at m/z 453) from the bariated molecular ion was observed to be quite abundant. The collision-induced loss of water is characteristic for all eicosanoids containing hydroxyl substituents along the carbon skeleton.

The spectrum of lithiated PGA_2 , $[\text{M}-\text{H}+2\text{Li}]^+$, has been reported and is very similar to that of the barium salt except for the specific barium-containing ions (Figure 2b). The major ions are now at m/z 85 and 139; these originate from cleavage of the $\text{C}-\text{C}$ bonds of C_3 , C_4 , C_7 , and C_8 and correspond to $\text{C}_3\text{H}_3\text{O}_2\text{Li}_2$ and $\text{C}_7\text{H}_9\text{O}_2\text{Li}_2$, respectively. The intervening gap was



also observed for fatty acids. The other ions at m/z 219 and 275 are analogous to those described for the barium salt except that the m/z 219 ion is relatively less abundant than the corresponding barium-containing ion. A loss of water is the most facile CID process for this closed shell ion, and a peak corresponding to the loss of LiOH is observed at m/z 323. The reactions of the $[M-H+2Li]^+$ ion are often similar to those of the $[M-H+Ba]^+$ ion. A more detailed discussion of these species will be the subject of a future paper.

Collision-induced dissociation of the carboxylate anion from PGA₂ (Figure 2c) behaves in a completely different fashion than that described for the metalated cations. The loss of water is still the most abundant collision-induced process, as is the case for the metalated positive ions (see above). As seen in Figure 2c, relatively few decompositions were observed in the B/E studies of FFR1 decompositions compared to the FAB/MS/CID/MS experiments with the metalated PGA₂ (Figure 2a and b). The most abundant one is to give the ion of m/z 233, from loss of 100 u. This most likely results from fission of the bond between C₁₄ and C₁₅ with transfer of a proton from one of the carbons in the C₁₅-C₂₀ regions. Experiments carried out with *d*₃-glycerol reveal that this ion shifts by 1 u, indicating that the proton that rearranges is an exchangeable hydroxyl proton. Because the bond that apparently is cleaved is also vinylogous, two potential rearrangement processes could be postulated. The first would be facilitated by the carboxylate anion to involve removal of a proton from the bridgehead carbon, C₁₂, resulting in isomerization of the double bond and anion formation at C₁₄ (Scheme II, top). The proton on the C₁₅



Scheme I

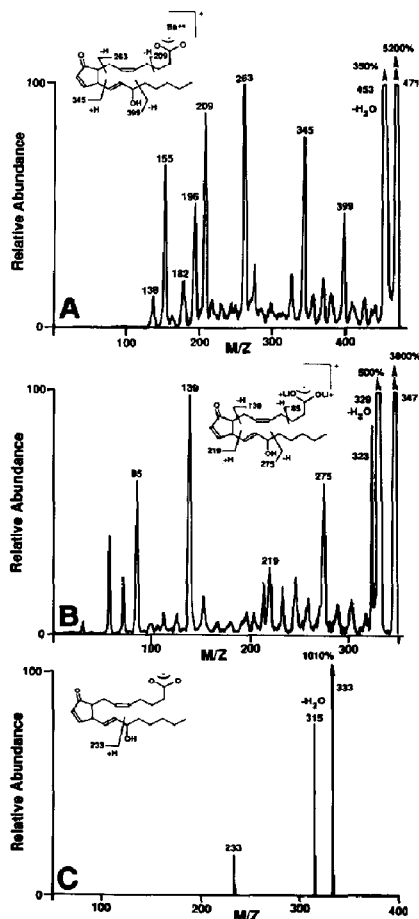
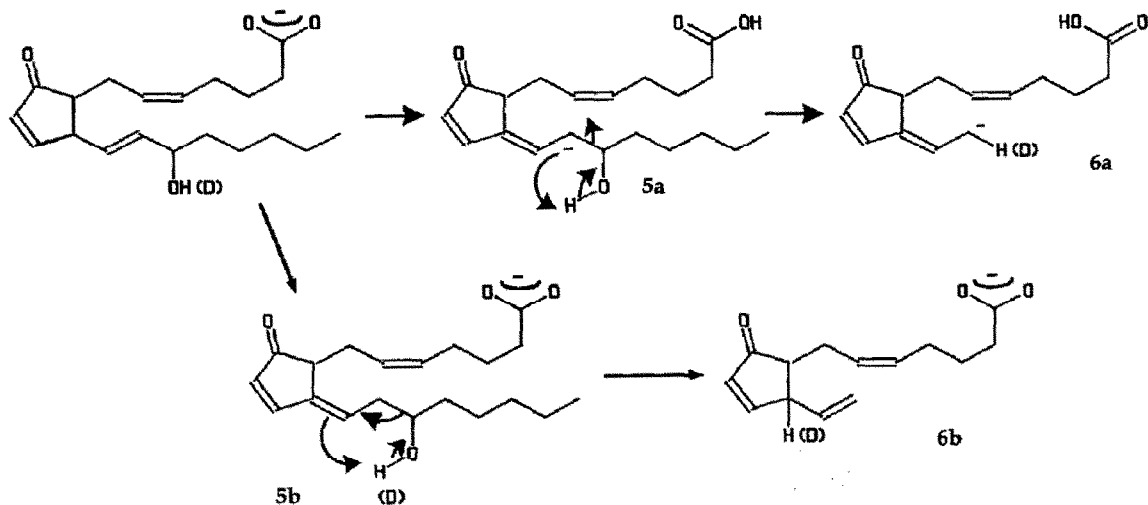


Figure 2. (a) FAB/MS/CID/MS spectrum of bariated PGA₂ (m/z 471). (b) FAB/MS/CID/MS spectrum of lithiated PGA₂ (m/z 347). (c) B/E spectrum of the carboxylate anion (m/z 333) of PGA₂. The indicated cleavage sites also involve hydrogen transfer reactions as described in the text.

hydroxyl group could then be transferred to this anionic site followed by cleavage of the highly stabilized conjugated dienone anion (6a). Of course, this process is not charge remote but rather charge driven. It is conceivable that the anionic site in 5a is produced in the matrix upon desorption rather than by a gas-phase process. A second mechanism would involve a charge remote 1,4-proton migration as suggested in Scheme II, bottom. The corresponding ions for the CID of the carboxylate anion for PGA₁, PGA₃, and 13,14-dihydro-15-keto-PGA₂ are presented in Table 1.

Prostaglandin B Series

Collisional activation of the bariated carboxylate anion of PGB₂ (Figure 3a) follows a decomposition pathway similar to that of PGA₂. For example, the ions



Scheme II

characteristic of barium salts of carboxylic acids at m/z 138, 155, 182, and 196 are observed. Furthermore, the ions at m/z 209 and 263 described above are also formed; however, it should be noted that the abundance of the m/z 263 ion is substantially reduced, probably because this fragmentation now involves formal cleavage of the vinylogous bond between C_7 and C_8 . PGB_2 shows a characteristic loss of a single water molecule as discussed above for PGA_2 . The same ion discussed above at m/z 399 (4) for bariatated PGA_2 is also observed in bariatated PGB_2 , but the ion at m/z 345 (3) is much less abundant. One interpretation of the change in ion abundances for m/z 345 and m/z 399 in PGB_2 is that the C—C bond being cleaved for m/z 345 is allylic/vinylic for PGA_2 whereas for PGB_2 it is doubly vinylic, and the abundance of the product ion is reduced relative to the ion abundance of m/z 399, which involves a fragmentation reaction at a site in these two molecules, which are chemically similar. A rather abundant ion of m/z 369 is observed in PGB_2 that formally results from loss of 102 u, conceivably by cleavage between C_{14} and

C_{15} with a hydrogen rearrangement. The m/z 369 ion may be formed more readily from PGB_2 because of the stability of the conjugation maintained in structure 7 (Scheme III). This is a rather curious feature that was also observed in the previous published spectra of lithiated PGB_2 [15] and may be related to the fact that the C_{12} — C_{13} bond is doubly vinylic because it is part of a conjugated dieneone. Elucidation of the mechanism requires additional study.

Decomposition of the carboxylate anion of PGB_2 (Figure 3b) is substantially different from that of PGA_2 . The loss of a single water molecule is the predominant process. The next most abundant ion, however, arises by the loss of 98 u to give an ion of m/z 235. Because PGB_2 is a unique unsaturated ketoprostaglandin, it is not likely that interruption of this highly conjugated system is involved. Formation of this ion is consistent with cleavage of the bond between C_{14} and C_{15} along with a rearrangement of three hydrogen atoms from C_{15} — C_{20} . PGB_2 labeled at the carboxyl oxygen, (1,1- $^{18}O_2$ - PGB_2) and 15-OD- PGB_2 , display this ion at m/z 239 and 236, respectively.

Table 1. CID mass spectra of FAB-generated closed shell anion $[M - H]^-$ of various prostaglandins

Structure	PGA_1	PGA_3	PGA_2	15-OD- PGA_2^*	13,14-DH- 15-keto- PGA_2
M^{**}	335	331	333	334	333
$[M - H_2O]^-$	317	313	315	316	315
6	235	233	233	234	
Other ions	209 (w) [†]	—	207	208	235 (8)
	221 (M - 114)	—	221 (w) [†]		207
					176

* Run using d_3 -glycerol as FAB matrix.

** $[M - H]^-$ for the free anion species.

† w = weak (less than 0.1% of the parent ion beam).

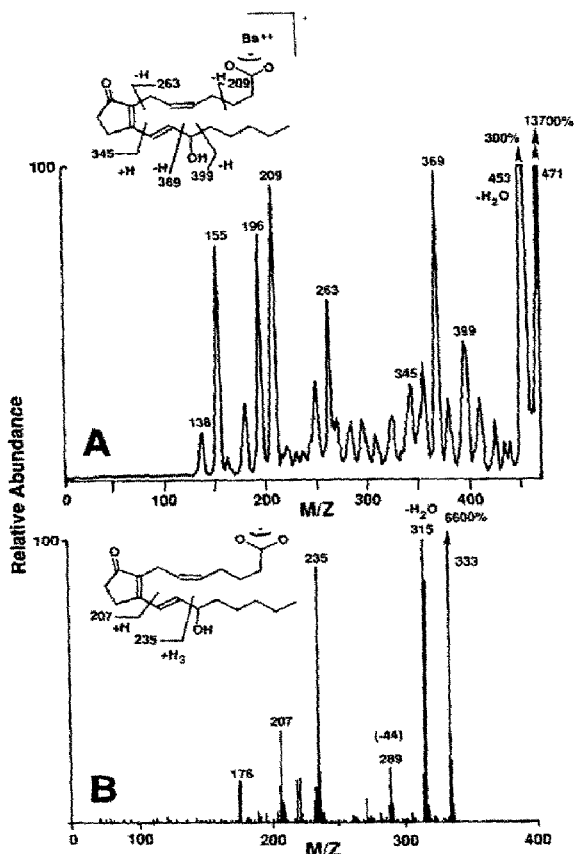
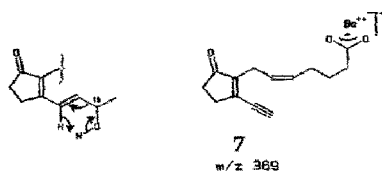


Figure 3. (a) EAB/MS/CID/MS spectrum of bariated PGB₂ (m/z 471). (b) B/E spectrum of the carboxylate anion of PGB₂ (m/z 333).

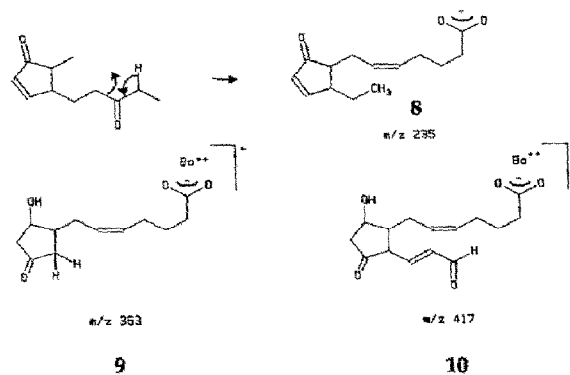
Another prostanoid that undergoes a loss of 98 u in the CID spectrum of the carboxylate anion is 13,14-dihydro-15-keto-PGA₂. In this case, a CRF mechanism involving proton migration from C₁₆ to C₁₄ with loss of an alkyl ketene (8) (Scheme IV) accommodates the result.

Prostaglandin D Series

Prostaglandin D₂ is a naturally occurring prostanoid produced in large quantities by the mast cell [16]. It has



Scheme III



Scheme IV

profound biological activities and thus is of substantial interest. Decomposition of the bariated PGD₂ (Figure 4a) gives unique ions for this metabolite in addition to the common ions discussed above. The ions at m/z 399 and 345 were also observed for bariated PGA₂, suggesting the loss of water from PGD₂ and formation of an ion structure similar to that derived from PGA₂ in the collision process. The ions at m/z 363 (9) and m/z 417 (10) are isomeric to the nondehydrated ions 3 and 4 previously described.

The carboxylate anion of PGD₂ (Figure 4b) behaves in a similar fashion to that of PGA₂ except that it undergoes the loss of two molecules of water to give ions at m/z 333 ($M-18$) and m/z 315 ($M-2(18)$). Like PGA₂, this carboxylate anion decomposes by the loss of hexanal or the loss of hexanal with an additional 18 u to give ions of m/z 251 and m/z 233, respectively. Because the proton at C₁₂ in PGD₂ is allylic to as well as adjacent to the ketone moiety, this enhanced acidity would probably facilitate the rearrangement process described in the formation of ion 6 for PGA₂ and the corresponding loss of hexanal.

Prostaglandin E Series

These biologically active, naturally occurring prostaglandins are isomeric to the PGD series in having a ketone at C₉ and a hydroxyl at C₁₁. The $[M-H+Ba]^+$ from PGE₂ (m/z 489) decomposes upon collisional activation similarly to the corresponding ions of PGD₂ and PGA₂ (Figure 5a). Abundant ions result from the losses of one and two molecules of water as well as the low-mass fragment ions that are characteristic of a carboxylic acid. The most abundant ion results from cleavage between C₇ and C₈ to give an ion at m/z 263 (2). The masses of the remaining ions in this spectrum are very similar to those of PGA₂, suggesting that substantial dehydration of PGE₂ occurred during the collisional activation process leading to the subsequent fragment ions. FAB/MS/CID/MS experiments of the $[M-H-H_2O+Ba]^+$ ion (m/z 471) substantiated

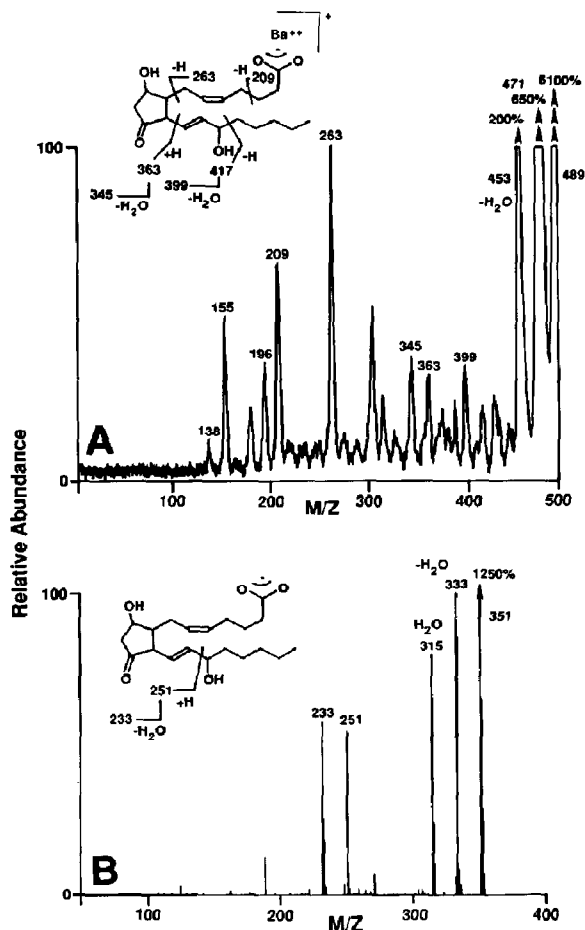
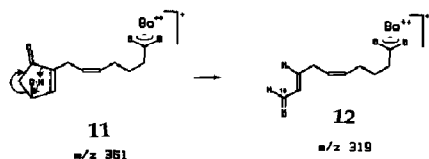


Figure 4. (a) FAB/MS/CID/MS spectrum of bariated PGD₂ (m/z 489). (b) B/E spectrum of the carboxylate anion of PGD₂ (m/z 351).

that the ions at m/z 209, 263, 345, and 399 arise from the ion of m/z 471. A new ion appeared at m/z 319 for the bariated PGE₂ that was 42 u below m/z 361 (11). This ion may form (Scheme V) from the loss of ketene and formation of structure 12.

As seen in Figure 5, the B/E linked spectrum of the PGE₂ anion is characterized by the losses of one and two molecules of water as well as by an ion at m/z 233 (6), which forms by the loss of water and the loss of hexanal. The mechanism for this latter process is likely to be similar to that for the formation of ion 6 from PGA₂ after the dehydration of PGE₂ to PGA₂



Scheme V

during the CID process. There is also an ion of very low abundance at m/z 251, arising by the direct loss of 100 u (hexanal) without prior loss of water from the cyclopentene ring. The high abundance of one ion of m/z 233 with respect to that of m/z 251 may be accounted for by a facile water loss to introduce into the cyclopentenyl ring a double bond that is conjugated with the double bond at position 13. This conjugation is not achieved for a 1,2-H₂O loss from the anion of PGD₂. The only other ion present in the B/E linked scan spectra of PGE₂ anion is at m/z 271 (M-80) corresponding to the loss of carbon dioxide as well as two molecules of water. The behavior of PGE₁ is nearly identical to that of PGE₂.

Prostaglandin F Series

Two important prostanoids appear in the F series; one is PGF_{2 α} , a naturally occurring biologically active prostaglandin, and the other is 6-keto-PGF_{1 α} , the metabolite of prostacyclin (PGI₂), which is typically measured as an index of the formation of PGI₂ in vivo. Both of these molecules have the F ring, a cyclopentane ring with two hydroxyls, at C₉ and C₁₁. They differ essen-

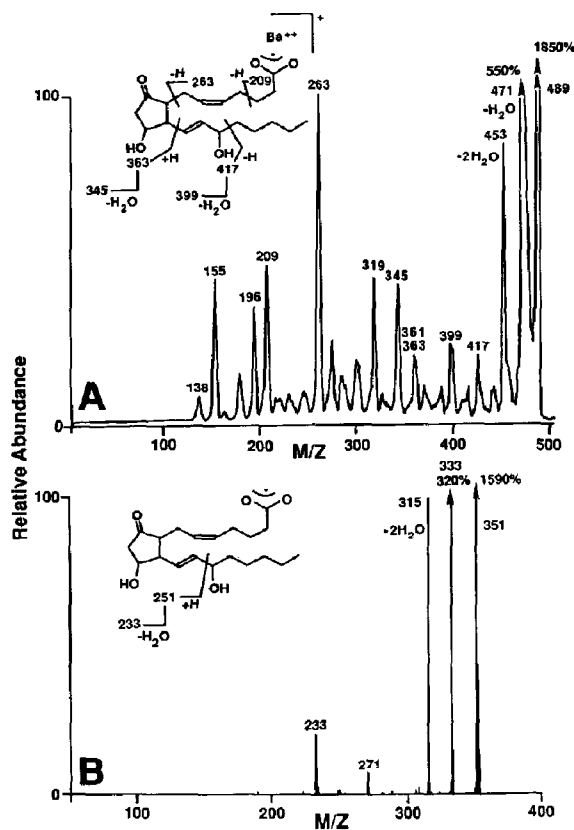


Figure 5. (a) FAB/MS/CID/MS spectrum of bariated PGE₂ (m/z 489). (b) B/E spectrum of the carboxylate anion of PGE₂ (m/z 351).

Table 2. CID mass spectra of FAB-generated closed shell ions from metalated and free anion of selected F-ring prostanooids*

Major ion	PGF _{2α}		6-Keto-PGF _{1α}	
	Ba†	Anion‡	Ba†	Anion‡
M [‡]	491	353	507	369
M - H ₂ O	473	335	489	351
M - 2 H ₂ O	455	317 (w)	471	333
M - 3 H ₂ O	437	299 (w)	453	315 (w)
M - 44	447 (w)	309 (s)	463 (w)	325 (w)
M - (44 + 18)	429 (w)	291	445 (w)	307
Additional ions	209 (1)		209 (1)	
	263 (2)		220	
	302		280	
	399 (4)		318	

*w = weak (less than 0.01% of the parent ion beam); s = strong (greater than 1% of the parent ion beam). Boldface numerals refer to specific structures (see text).

† FAB/MS/CID/MS, tandem mass spectrometry.

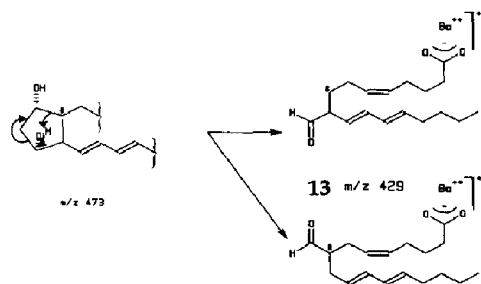
‡ B/E, FFR1 CID.

§ [M - H + Ba]⁺ for bariated species; [M - H]⁻ for free anion species.

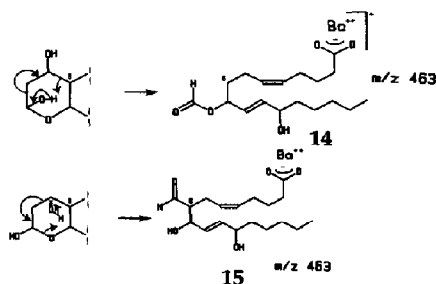
tially in C₅ and C₆, where PGF_{2α} has a double bond but 6-keto-PGF_{1α} has a methylene group at C₅ and a keto moiety at C₆.

Decompositions of [M-H+Ba]⁺ from the PGF series (Table 2) are characterized by abundant ions formed by losses of one, two, and three molecules of water from the closed shell cation. Furthermore, ions observed for the other prostanooids at low mass, in particular at *m/z* 209 (1) and *m/z* 263 (2) are also observed for these molecules. The most characteristic ion for the F series occurs with the loss of 44 u from the monodehydrated metalated molecular ion (*m/z* 429), which was previously studied [15, 17] and found to involve a facile rearrangement of the proton on the hydroxyl at C₉ or C₁₁ to C₁₂ or C₈, respectively, and loss of C₂H₄O from the five-membered ring (13, Scheme VI).

The decompositions of the carboxylate anion of PGF_{1α}, PGF_{2α}, and 6-keto-PGF_{1α} are characterized by the loss of water as well as the loss of 44 u (C₂H₄O) as observed for the metalated closed shell cations. The similarity between these two decomposition pathways from both the cations and anions supports the suggestion that these ions arise from a CRF mechanism.



Scheme VI



Scheme VII

Thromboxane Series

Thromboxane B₂ is the chemically stable metabolite of the biologically active TxA₂, which is substantially different from the prostaglandins because it contains a six-membered ring hemiacetal and hydroxyl substituents at C₉ and C₁₁. Nevertheless, low-mass ions 1 and 2 are observed in the FAB/MS/CID/MS of bariated TxB₂ (Figure 6a) at *m/z* 209 and *m/z* 263 as for the prostaglandins, since the C₁-C₈ chain is common to both structural types. The most abundant ions are of *m/z* 463 and *m/z* 307. The ion of *m/z* 463 (Scheme VII; 14 and 15) is the loss of 44 u, and considering the 1,3-diol nature of hemiacetal ring, this loss is postulated to be identical to that observed for PGF_{2α} (13). The production of one ion of *m/z* 307 (16) probably involves rearrangement in the hemiacetal ring with the loss of C₂H₄O and neutral aldehyde from the methyl terminus (Scheme VIII).

Some of the decompositions of the [M-H]⁻ of TxB₂ (Figure 6b) are substantially different from those of prostaglandins because abundant ions in the range below *m/z* 200 are produced. Yet, as for the anions of the prostaglandin F series, abundant ions are formed by the loss of 44 u, undoubtedly due to the 1,3-dihydroxy structure in the six-membered ring. The mechanism for loss of 44 u is expected to be similar to that described for formation of ions 12 and 13. There are also abundant ions arising by loss of water and by loss of water plus 44 u (C₂H₄O). The ion of *m/z* 169 can form from a concerted rearrangement of the cyclic acetal (Scheme IX), leading to formation of the same conjugated and neutral aldehyde and loss of C₂H₄O as a neutral fragment to give the ion of *m/z* 169 (17), carrying the charged carboxylate anion. Rather than loss of C₂H₄O, the ion at *m/z* 195 (18) may be derived from the [M-H₂O] ion by a cleavage of the acetal structure and loss of a neutral conjugated aldehyde. The ¹⁸O-labeled thromboxane mass spectrum is presented in Table 3 to support the postulated rearrangements shown in Scheme X. Peaks for these ions are also seen in the mass spectrum of 2,3-dinorththromboxane B₂ except that they are shifted by 28 u owing to the chain shortening of this thromboxane B₂ metabolite (Table 3).

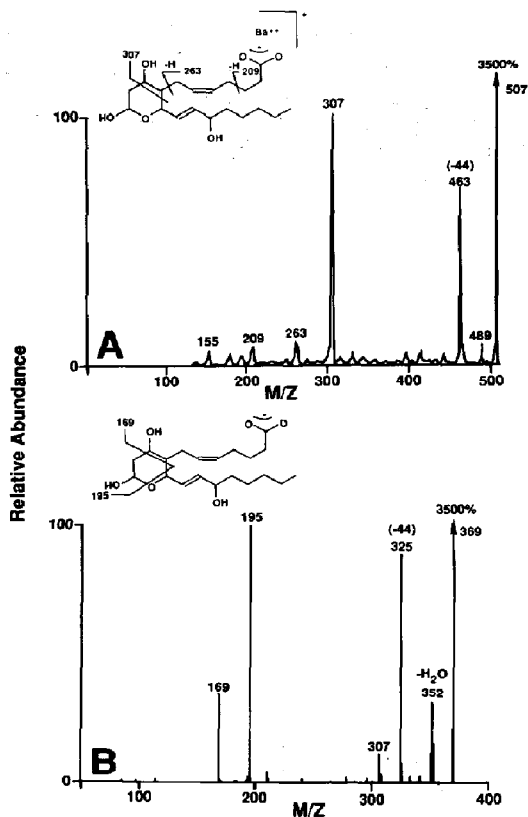
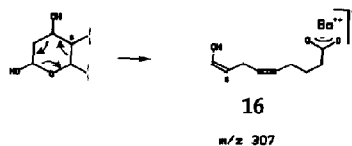
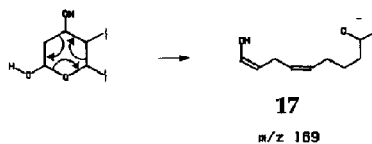


Figure 6. (a) FAB/MS/CID/MS spectrum of bariated thromboxane B_2 (m/z 507). (b) B/E spectrum of the carboxylate anion of TxB_2 (m/z 369).

The mass spectrum of 11-dehydrothromboxane B_2 (Table 3), however, is substantially different because these ions do not appear, no doubt because of the alteration of the hemiacetal into the lactone. For the 11-dehydro- TxB_2 anion, the most abundant product ion is of m/z 241, and it arises by cleavage of the C_7 - C_8 bond facilitated through attack of the carboxylate group at



Scheme VIII.



Scheme IX

C_5 as seen in structure 19 (Scheme XI). This was only a minor ion in thromboxane B_2 itself, suggesting the importance of the rearrangements of the lactone ring in 11-dehydrothromboxane B_2 during the CID process and the stability of the resultant carboxylate anion.

Comparison with Decompositions of Closed Shell Species

Previous MS/MS studies of prostaglandins and thromboxanes centered on the CID of positive ions generated either by EI of the methyl ester trimethylsilyl (and methoxime derivatives of keto groups) derivatives [18-22] or of the pentafluorobenzyl ester, trimethylsilyl ether derivatives [23]. In the latter investigations, negative ion electron capture chemical ionization mass spectrometry (CIMS) was employed. In these studies, the derivatives were made to enhance volatility to facilitate GC/MS, and the CID experiments were carried out in triple-quadrupole mass spectrometers with relatively low collision energies (15-20 eV). Although substantial decompositions were observed for these molecules, the most abundant ions arise from loss of the derivatizing groups, such as loss of trimethylsilyl or methanol from the methoxime derivative. Only a few ions of low abundance can be observed

Table 3. Abundant ions in the CID mass spectra (B/E of FAB-generated carboxylate anions from selected thromboxanes

Ion	[1,1,11- $^{18}O_3$]			
	TxB_2	TxB_2	2,3-Dinor- TxB_2	11-Dehydro- TxB_2
M*	369	375	341	367
M-OH	352	355, 357**	324	349†
M-44	235	329††	297	n.d.
M-(44+18)	307	311	279	305
18	195	201	167	n.d.
17	169	173	141	n.d.
19	n.d.	n.d.	n.d.	241

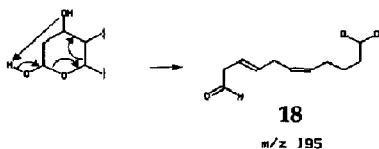
n.d. = not detected.

* [M - H]⁻ for free anion species.

** Loss of H_2O and loss of H_2 ^{18}O were equally evident.

† M - H_2O .

†† M - 46 (loss of C_2H_4 ^{18}O from the C_{11} - ^{18}O atom).



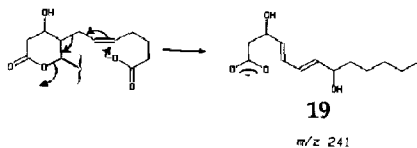
Scheme X

that arise from fragmentation of the carbon skeleton of the prostanoids [23]. In spite of the lack of useful structural information for this type of MS/MS, the MS/MS approach was shown to improve analytical utility by enhancing sensitivity for quantitative assays when compared to direct selected ion monitoring and single-stage mass spectrometric analysis.

In contrast to this, there have been several reports of high-energy collisional activation of carboxylate anions produced by either negative ion CIMS of fatty acid methyl esters [24] or electron capture negative ion CI of pentafluorobenzyl esters of carboxylic acids [25]. In these studies, substantial structural information was obtained for relatively simple fatty acids such as mycolic acids or monounsaturated fatty acids [25]. These studies were also carried out with GC/MS, and in the study involving the pentafluorobenzyl ester derivatives, as little as 10 ng was necessary to obtain complete collisional spectral data [25].

In the present study CID of closed shell ions either as bariated species or as free carboxylate anions generated by FAB/MS reveals a wealth of structural information. The particular CID reactions observed are often dependent on whether positive or negative ions are studied. This situation is different from that seen for simpler fatty acids for which CRF is seen for both positive and negative closed shell ions. The negative ions likely show these differences because the charge is *not* localized exclusively on the carboxylate.

The bariated carboxylate salts undergo reactions via processes that were previously characterized as charge remote mechanisms in which neutral species were lost in processes that are similar to thermal and photolytic reactions. The most abundant product ions arise by the loss of water involving each of the hydroxyl groups present in the prostaglandin or thromboxane structure. Water loss for the carboxylate anions is followed by the appearance of ions arising from cleavage (along with H rearrangement) of the carbon atoms from C₈ to C₁. For those eicosanoids that contain keto groups, hydroxy groups, and multiple unsaturations, some typical patterns emerge. Molecules that can dehydrate to a PGA₂-like structure (these include PGE₂ and PGD₂ as well as their various metabolite variants with one less and one more double bond at C₇, or C₁₇, respectively) show



Scheme XI

abundant ions in their CID spectra from cleavage at major branching points at the five-membered ring and from cleavage adjacent to the hydroxy group at C₁₅. These cleavages typically involve hydrogen migrations and loss of neutral aldehydes. The presence of these characteristic ions in combination with the number of water losses is consistent with the basic structure of PGA.

The ions of the PGF ring that are characterized by the presence of a 1,3-diol structure within the cyclopentyl ring display a characteristic loss of either C₂H₄O (44 u) or a greater loss of water and C₂H₄O (62 u). The CID of PGB₂ and members of this family is surprisingly different from that of other prostaglandins. No doubt this is due to the extended conjugated system present in this prostaglandin. The loss of 98 u (apparently as C₆H₁₀O) replaces the more common loss of heptanal (100 u) and appears to be a more complex process initiated by the negative charge in the form of a carbanion. Thromboxanes are characterized by rearrangements of the cyclic hemiacetal structure, loss of a neutral conjugated aldehyde, and formation of abundant ions retaining carbons 1-8 and 11. Thromboxanes also have a 1,3-diol structure, and these molecules also lose C₂H₄O (44 u).

One important feature of carboxylate anion decompositions is that these ions can be obtained with substantially less material than is needed to generate the positive ion (bariated or lithiated carboxylate ions). Typically, 10-50 ng of material was used for the anion B/E studies, but less material would suffice if pentafluorobenzyl derivatives were chosen as a source for the carboxylate anion. Studies of the decomposition of closed shell ions of prostaglandins and thromboxanes have revealed that multiple processes do occur in the high-energy CID of molecular ion species. Although abundant ions are obtained with the loss of water, and for certain prostaglandins with the loss of C₂H₄O, most ions arise following different formation processes. The structural information obtained from the various processes is complementary; CID of the positive ions provides information concerning the arrangement of atoms from C₁ through C₁₂, whereas the negative ion decomposition products provide information concerning alterations in the prostanoic structure between carbons in the C₁₅-C₂₀ region. Future studies will be focused on details of this mechanism.

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