J. Am. Soc. Mass Spectrom. (2018) 29:2360–2367
DOI: 10.1007/s13361-018-2051-6

RESEARCH ARTICLE

A Unique Collision-Induced Dissociation Reaction of Cholamine Derivatives of Certain Prostaglandins

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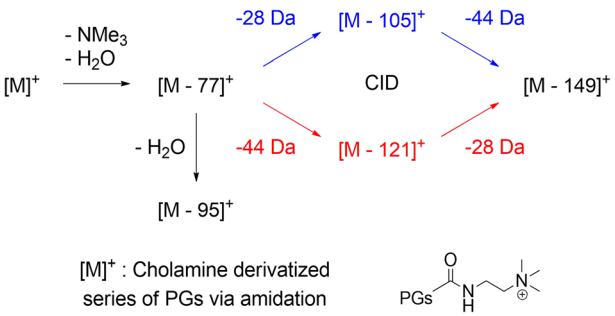

Abstract. Prostaglandins (PGs) are biologically active metabolites of arachidonic acid containing 20 carbon atoms, a cyclic moiety, and two side chains (A and B) in common. The bioassay of PGs requires high sensitivity because of their low concentration in tissues and blood and has usually been carried out by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in the negative ion mode. Chemical derivatization of PG carboxylic acid groups to introduce positive charge-carrying groups is an established strategy to improve the sensitivity and selectivity of such assays. In this study, we exploited this approach for structural identification of a series of PGs using cholamine derivatization through an amidation reaction. However, we observed that collision-induced dissociation of these derivatives gave rise to unexpected product ions that we postulated were formed by unique long-range intramolecular reactions resulting in dehydration of the B chain accompanied by fragmentation of the A chain through an unusual Hofmann rearrangement. Evidence for the proposed mechanism is presented based on ESI-MS/MS and high resolution mass spectrometry studies of cholamine derivatives of PGE_1, PGE_2, PGD_2, PGI_2, and C-17 methyl deuterium-labeled limaprost.

Keywords: Electrospray ionization tandem mass spectrometry, Prostaglandins, Cholamine derivatives, Macrocyclic intermediate, Hofmann rearrangement

Received: 27 April 2018/Revised: 18 July 2018/Accepted: 4 August 2018/Published Online: 27 August 2018

Introduction

Prostaglandins (PGs) are important participants in the regulation of physiological activity in various tissues of humans and animals. All PGs are synthesized in the cell from the essential fatty acid, arachidonic acid. Structurally, they contain 20 carbon atoms with at least one ring structure, one 7-carbon A chain with a terminal carboxylic acid group and one 8-carbon B chain incorporating an allylic alcohol moiety. Bioassay of PGs by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the negative ion mode usually requires an acidic mobile phase which tends to decrease the ionization efficiency of the carboxylic acid group. Therefore, derivatization to introduce positive charge-carrying groups has been applied to impart high-ionization efficiency for the lipid acid analytes in the positive ion mode [1–5].

Various quaternary ammonium derivatization reagents have been applied to introduce such moieties to PG molecules such as salts of the N-(4-aminomethylphenyl)pyridinium (AMPP) and (2-(4-aminophenoxy)ethyl) (4-bromophenethyl)dimethyl ammonium (4-APEBA) ions [6–11]. Among them, cholamine yields benefits not only with regard to the improved signal response of precursor ions but also to their greater stability due to the formation of a dihydrooxazolium ring [10]. Furthermore, these stabilized product ions are derived from the analyte residue rather than from the derivatization reagent which enables further structure-related fragmentation studies.

Prostaglandin E1 (PGE_1, also known as alprostadil) has been clinically available for many years as an injectable vasodilator

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and inhibitor of platelet aggregation. A synthetic analog of PGE_1, limaprost, is more stable than PGE_1 and can be taken orally. In the collision-induced dissociation (CID) of cholamine-derivatized limaprost (1), a series of intense signals arising from exclusive neutral mass loss was observed [10] together with a set of weak but highly specific product ions in the fragmentation pattern of 1. These additional product ions are also present in the MS^2 and MS^3 spectra of cholamine derivatives of other structurally similar prostaglandins viz PGE_1, PGE_2, and PGD_2. Such fragmentation behavior is reminiscent of the structurally specific McLafferty rearrangement which is observed in the MS of molecules containing a keto-group undergoing β-cleavage.

In the present study, we used electrospray ionization tandem mass spectrometry (ESI-MS/MS) and high-resolution time-of-flight mass spectrometry (HR-TOFMS) to investigate the mechanism of formation of these additional product ions in the CID fragmentation patterns of cholamine-derivatized prostaglandins. We maintain an understanding of this common fragmentation behavior will be helpful in future MS-based lipidomic studies.

Materials and Methods

Derivatization Protocol

All chemicals were of analytical grade and purchased from Sigma (St. Louis, MO, USA). The derivatization protocol was as follows: To an aliquot (1 mL) of a 3 μM prostaglandin solution in acetonitrile, 100 μL aliquots of 1 mM N,N-diisopropylethylamine (DIPEA) and 1 mM 1-[bis(dimethylamino)methylene] -1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) in acetonitrile were added and the mixture incubated for 15 min at ambient temperature. Then a 100 μL aliquot of an aqueous solution of 1 mM cholamine or ethanolamine was added and the mixture incubated for 1 h at ambient temperature. Previous research showed that an incubation of 1 h duration is sufficient for the reactions to go to completion.

Mass Spectrometry

MS was performed on a triple TOF 5600 mass spectrometer (SCIEX, Concord, Canada) and a QTRAP 5500 mass spectrometer (SCIEX, Toronto, Canada), each equipped with a TurboIonSpray™ ESI source. Data acquisition was achieved using Analyst software 1.6.1 and 1.5.2. In fragmentation experiments, analyte standard solutions were infused into the MS through a syringe pump at 10 μL/min. Optimized MS parameters were as follows: Source temperature 0 °C; ion spray voltage 5500 V; nebulizer, heater, and curtain gas (N_2) 20, 0, 20 psi respectively. DP, CE, and AF2 values were tuned manually in each experiment.

Results and Discussion

Fragmentation of Cholamine-Derivatized Limaprost

In our previous research, to identify product ions of cholamine-derivatized limaprost (1), a set of stabilized positive CID

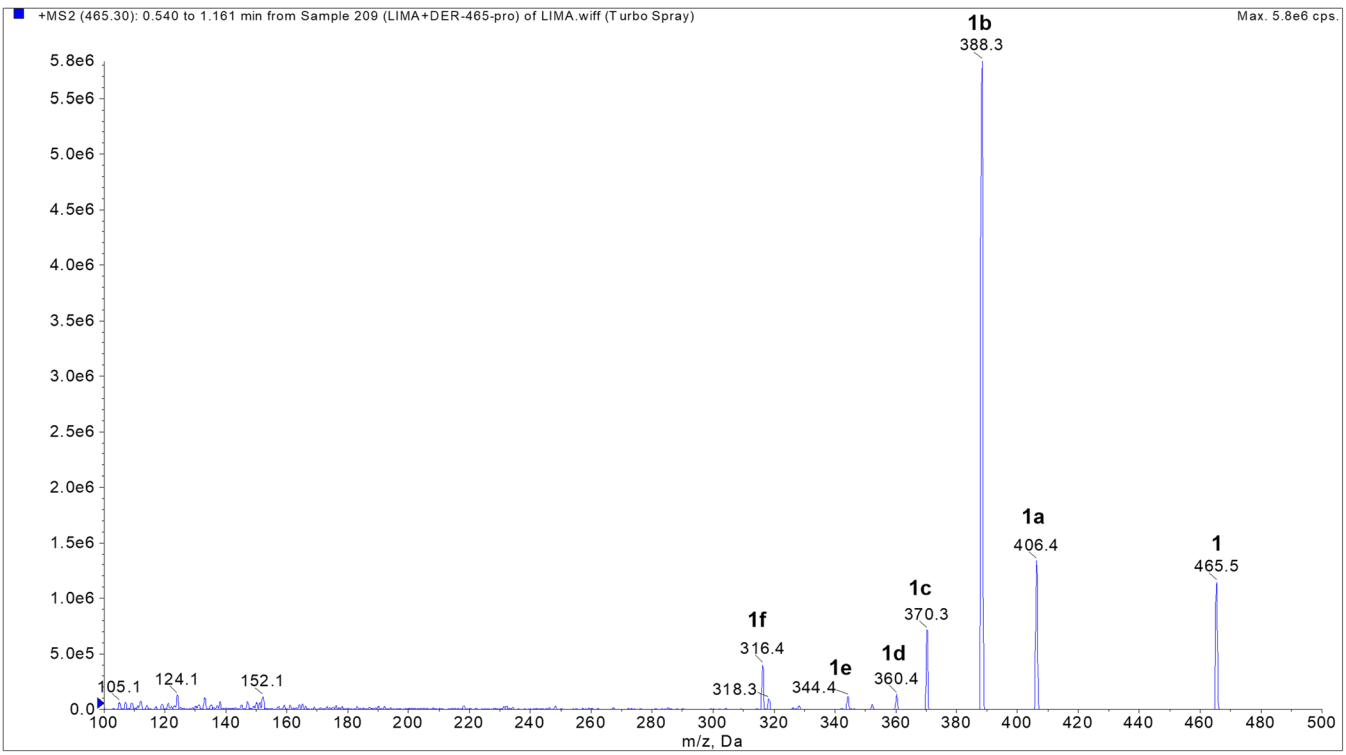


Figure 1. Product ion scan of cholamine-derivatized limaprost (1) from *m/z* 100 to 500 collected by QTRAP 5500 MS with a TurboIonSpray™ ESI

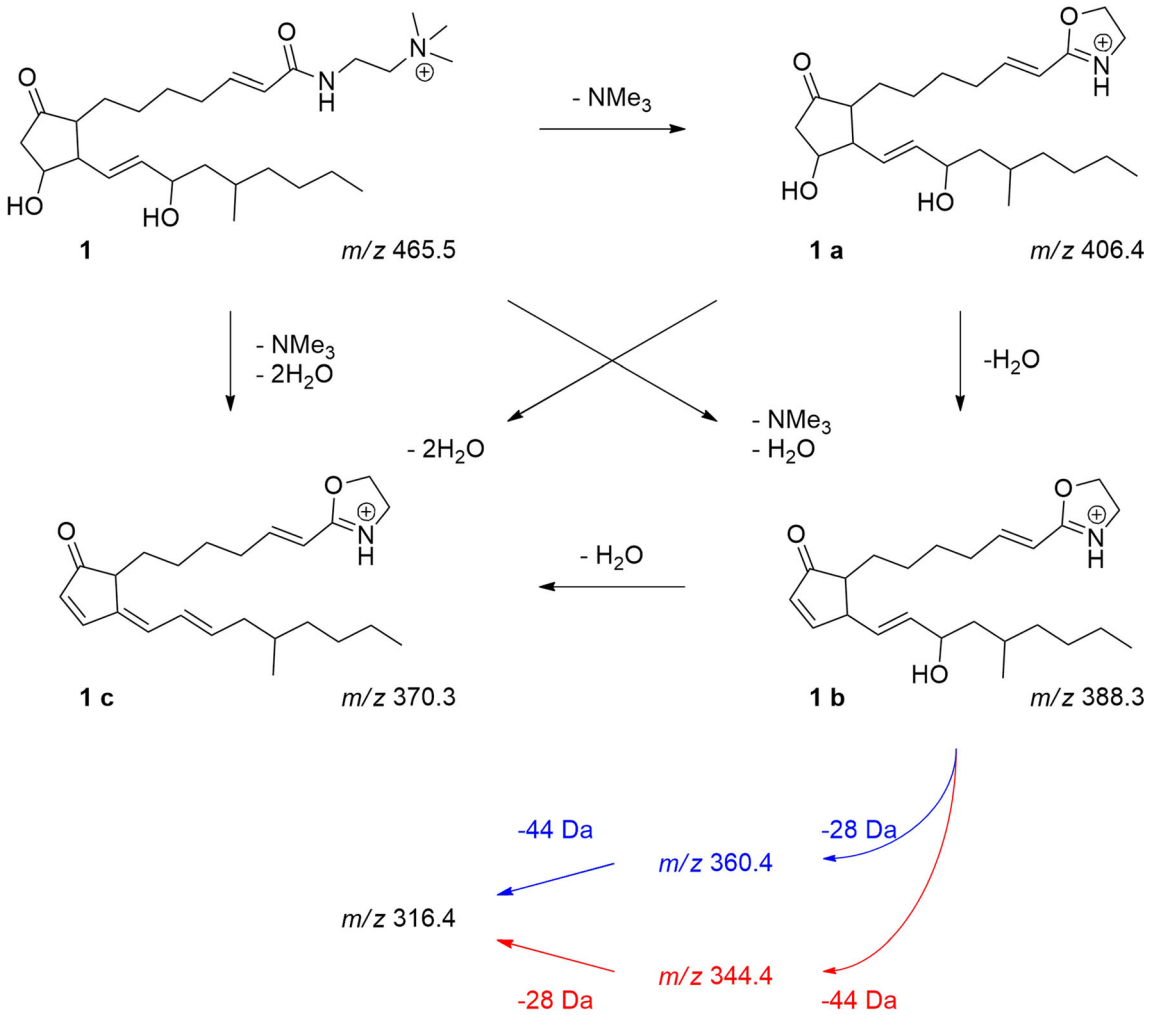
product ions of the parent ion at *m/z* 465.5 were observed (Figure 1) [10]. The set included ions at *m/z* 406.4 (1a, 1-NMe_3), 388.3 (1b, 1-NMe_3-H_2O), and 370.3 (1c, 1-NMe_3-$2H_2O$) formed by stepwise neutral mass loss. In addition to the doubly dehydrated product ion 1c, the MS^2 spectrum of 1b also contained a set of specific product ions with weaker intensity at *m/z* 360.4 (1d), 344.4 (1e), and 316.4 (1f) (Scheme 1). These arise from two independent fragmentation pathways via 1d in one case (shown in blue) and 1e in the other (shown in red) (Scheme 2). These unique fragmentation pathways culminating in 1f therefore involve sequential mass loss of 28 and 44 Da in one case and 44 and 28 Da in the other. There was no interchange between the two intermediate fragments and no connection with the dehydration pathway leading to 1c.

Mechanism of 1b → 1f Pathways

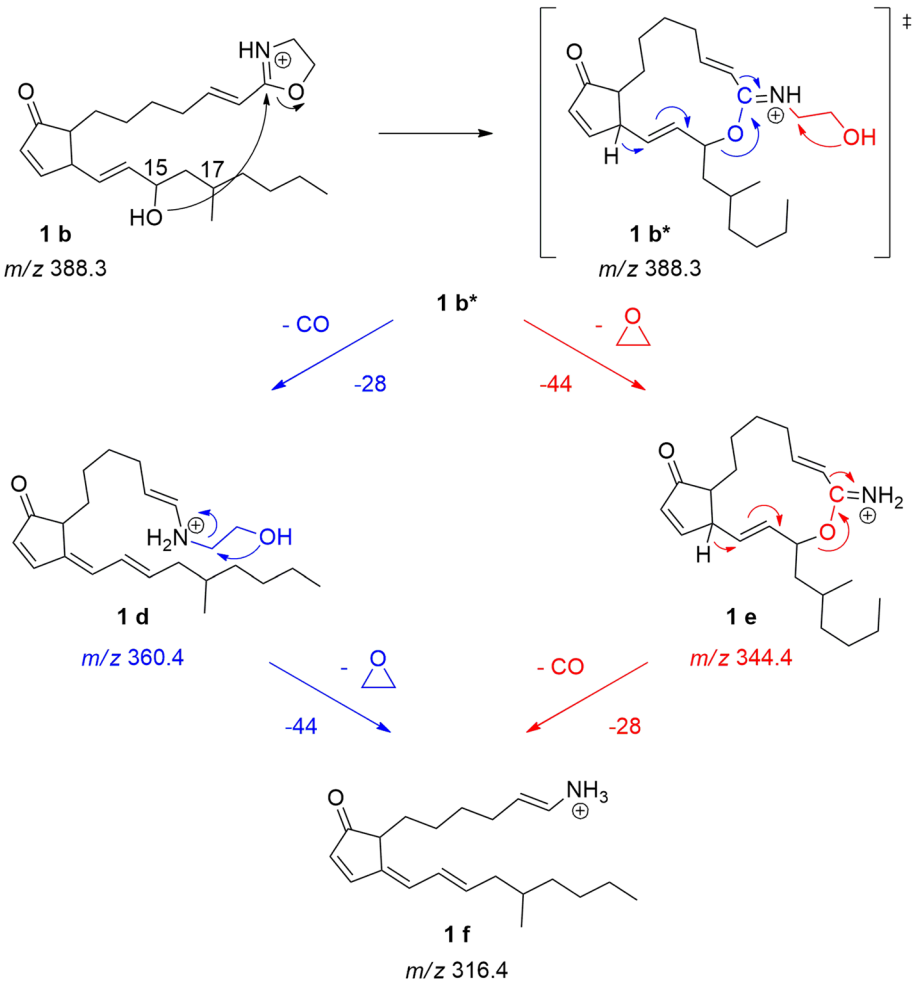
The elimination of the C-15 hydroxyl group of PGs is known to involve a [1,3]-sigmatropic rearrangement with a high-energy barrier to double-bond migration [12]. Therefore, the active intermediate 1b will tend to accumulate leading to the possibility of other fragmentation pathways as found here. A mechanistic explanation of these alternative pathways based on the rules of gas phase reactions is shown in Scheme 2.

The first step involves an intramolecular cyclization through nucleophilic attack of the C-15 hydroxyl group on the iminoester carbon. Such a long range intramolecular interaction between the C-15 hydroxyl group of the B chain and the carboxylate anion of the A chain has been postulated to explain the specific CID of PGE_2 using ESI-MS in the negative ion mode [12]. Furthermore, formation of a macrocyclic intermediate may not be favorable in solution but is possible in the gas phase or under vacuum as has been found to occur in a CID reaction of the $[MH]^+$ ions of Leu-enkephalin amide and a variety of heptapeptide amides [13]. The fact that 1c has the highest signal intensity among the product ions derived from 1b is due to the high-energy barrier of ester exchange reactions.

In the blue route in Scheme 2, a [1, 3] sigmatropic double-bond shift initiates a Hofmann rearrangement leading to an unusual isocyanate that decomposes spontaneously with neutral mass loss of CO (28 Da). Subsequently, an ethylene oxide (or possibly an acetaldehyde, 44 Da) moiety departs from 1d leaving the amine product ion 1f.



Scheme 1. Product ions of cholamine-derivatized limaprost (1)



Scheme 2. Mechanistic explanation of the CID of cholamine-derivatized limaprost

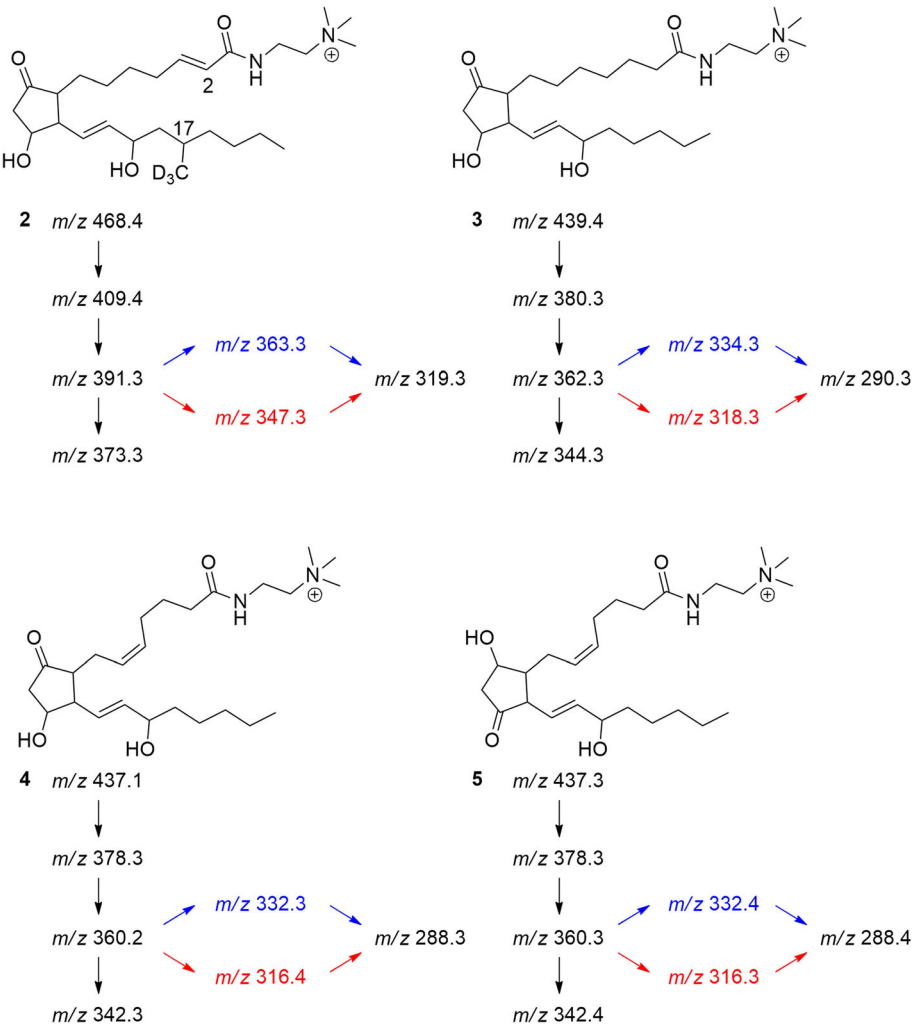
In contrast, in the red route in Scheme 2, the elimination of an ethylene oxide moiety precedes the Hofmann rearrangement leading to the same amine product ion 1f. Since 1f is the only product ion resulting from both pathways and since an amine group has excellent ionization efficiency, the signal intensity of 1f is expected to be higher than the signals of its precursor ions 1d and 1e. This is actually what is observed (Table 1).

Evidence for the Mechanism

HR-TOFMS and Deuterium Labeling MS^2 and MS^3 relationships alone are not adequate to validate a fragmentation mechanism. Accordingly, the exact mass of all product ions derived from 1 were measured by HR-TOFMS and found to match their corresponding theoretical values

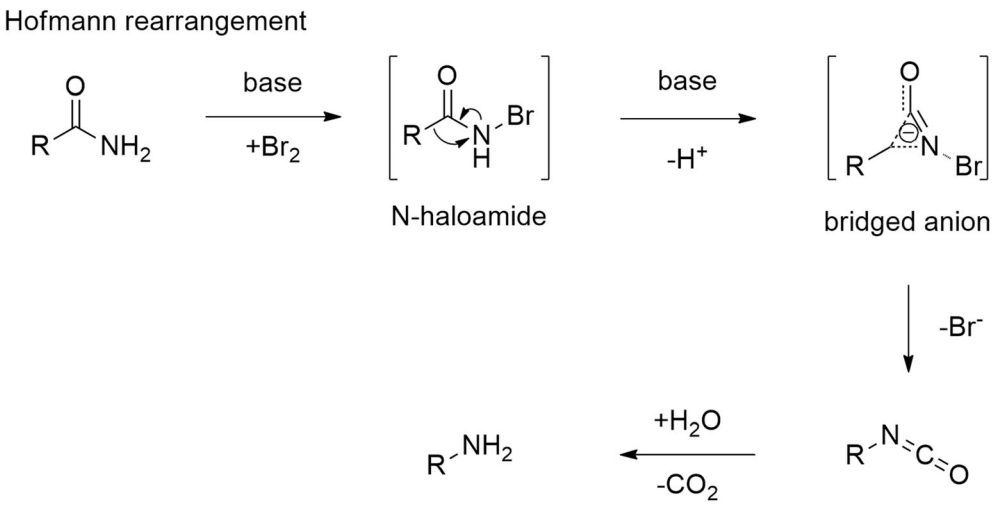
Table 1. HR-TOFMS Data of Cholamine-Derivatized Limaprost (1), C-17 Methyl Deuterium-Labeled Limaprost (2) and PGE_1 (3)

Molecule		$[M\text{-}NMe_3\text{-}H_2O]^+$	$[M\text{-}NMe_3\text{-}2H_2O]^+$	$[M\text{-}NMe_3\text{-}H_2O\text{-}28]^+$	$[M\text{-}NMe_3\text{-}H_2O\text{-}44]^+$	$[M\text{-}NMe_3\text{-}H_2O\text{-}72]^+$
1	Theor. value (/Da)	388.2788 (1b)	370.2741 (1c)	360.2897 (1d)	344.2584 (1e)	316.2635 (1f)
	HRMS (m/z^{-1})	388.2846	370.2696	360.2855	344.2533	316.2596
	RE δ (/ppm)	−15	−12	−12	−15	−12
	Relative intensity	18.2	5.2	1.3	1.0	3.4
	Peak CE(/eV)	34	41	43	42	43
2	Theor. value (/Da)	391.3035	373.2929	363.3085	347.2772	319.2823
	HRMS (m/z^{-1})	391.3028	373.2942	363.3091	347.2774	319.2819
	RE δ (/ppm)	−2	+3	+2	+1	−1
3	Theor. value (/Da)	362.2690	344.2584	334.2741	318.2428	290.2478
	HRMS (m/z^{-1})	362.2658	344.2539	334.2695	318.2410	290.2447
	RE δ (/ppm)	−9	−13	−14	−6	−11
	Relative intensity	30.5	7.0	1.0	2.7	8.5
	Peak CE(/eV)	32	39	43	42	45

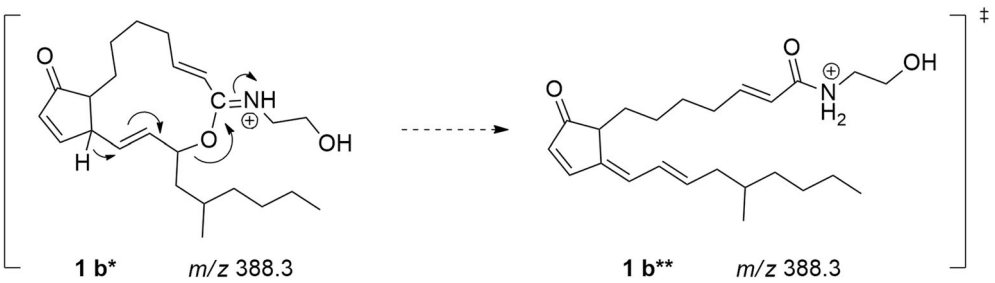


Scheme 3. CID patterns of cholamine-derivatized limaprost and other structurally similar PGs

with relative errors (RE) of −15 to −12 ppm (Table 1). Subsequently, the cholamine derivative of C-17 methyl deuterium-labeled limaprost (2) was studied to eliminate the possibility of coincidental matches. In this case, theoretical values were matched with relative errors of only −2 to +3 ppm (Table 1). HR-TOFMS of PGE_1 (3) with relative errors of −6 to −14 ppm (Table 1) also supports the proposed mechanism.



Scheme 4. Mechanism of the Hofmann rearrangement



Scheme 5. Alternative mechanism for the fate of the macrocyclic intermediate (1b*)

Other possible explanations for neutral mass loss of 28 Da such as ethylene or of 44 Da such as carbon dioxide were ruled out by HR-TOFMS. On the other hand, a study of the fragmentation of benzyl isocyanate showed neutral loss of CO and formation of benzylamine in support of the proposed mechanism.

Effect of the C-2 Double Bond In the proposed mechanism, the double bond in the A chain of limaprost does not play a role. To verify that this is the case, experiments with cholamine-derivatized PGE_1 (3), PGE_2 (4), and PGD_2 (5) were carried out. These prostaglandins have the same carbon skeleton as limaprost but without the C-2 double bond in the A chain. The MS^2 spectrum of 3 resulting from *m/z* 439.4 includes three very intense product ions at *m/z* 380.3 ($-NMe_3$), 362.3 ($-NMe_3$, $-H_2O$), and 344.4 ($-NMe_3$, $-2H_2O$). The MS^3 spectrum of *m/z* 362.3 then includes three independent pathways viz the second dehydration to give *m/z* 344.4, sequential loss of 28 then 44 Da to give *m/z* 334.3 and 290.3, and sequential loss of 44 then 28 Da to give *m/z* 318.3 and 290.3. The MS^2 and MS^3 spectra of 4 and 5 also show the same fragmentation pattern as 3 (Scheme 3). On this basis, it is clear that the C-2 double bond of limaprost does not participate in or influence the CID fragmentation.

CID of Ethanolamine Derivatized PGs The classic Hofmann rearrangement (Scheme 4) occurs during reaction of a primary amide with bromine under basic conditions when loss of CO from an isocyanate intermediate produces a primary amine [14]. Formation of 1d from the iminoester 1b* can be considered a Hofmann rearrangement because it proceeds through an isocyanate intermediate and involves loss of CO but the reaction is unusual in that it occurs under acidic conditions and the product is a secondary rather than primary amine. Although loss of CO from 1e produces a primary amine 1f, it can also be considered an unusual Hofmann rearrangement because it occurs under acidic conditions.

Instead of a Hofmann rearrangement, a noncyclic amide ($1b^{**}$) is also a plausible contributor to the signal at *m/z* 388.3 and a potential intermediate in the subsequent neutral mass loss reactions (Scheme 5). To examine this possibility, ethanolamine derivatized limaprost (6), C-17 methyl deuterium labeled limaprost (7), PGE_1 (8), and PGE_2 (9) were studied (Figure 2).

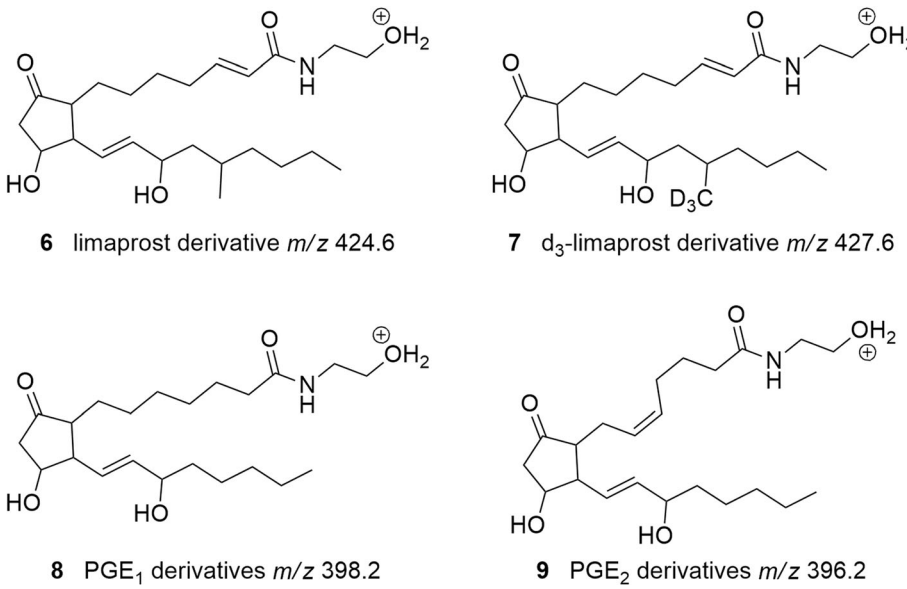


Figure 2. Ethanolamine derivatives of limaprost and other structurally similar PGs

The intermediate 1b** could be one of the CID dehydration products of 6. In MS^2 and MS^3 experiments on 6, 7, 8, and 9, the following common CID behavior was observed: (a) single dehydration; (b) double dehydration; (c) mass loss of 28 then 44 Da from b; (d) mass loss of 44 then 28 Da from b (Table 2).

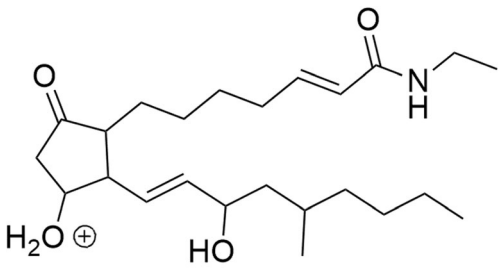
When compared with cholamine derivatives, the signals of product ions arising from ethanolamine derivatives are roughly one to two orders of magnitude weaker. These results illustrate that the amide 1b** may not be a popular fragmentation intermediate in this process. The weak intensities are likely to result from the poorer leaving group ability of a water molecule in the ethanolamide groups of 6, 7, 8, and 9. By losing one, two, or three water molecules, the CID pathways of ethanolamine derivatives may include formation of the stabilized cationic intermediate 1a, 1b, and 1c respectively.

Leaving Group Effects In order to identify the role of the leaving group in producing the parallel CID fragmentation patterns, we prepared an ethylamine derivative of limaprost (10, Figure 3). No trace of sequenced mass loss of 28/44 and 44/28 Da was observed in the MS^2 of 10 consistent with the absence of a leaving group.

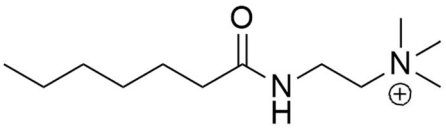
We next examined cholamine-derivatized heptanoic acid (11) in order to determine whether a stabilized dihydrooxazolium cation structurally similar to the A chain of cholamine-derivatized PGE_1 (3) would undergo the neutral mass loss sequence. In the CID pattern of 11, neutral mass loss of trimethylamine (−59 Da) is clearly evident but there was no signal indicative of sequential mass loss of 28/44 and 44/28 Da. Therefore, a stabilized cationic intermediate is not a sufficient condition for triggering the unique type of Hofmann rearrangement observed here.

*The Role of the Macrocyclic Intermediate 1b** Formation of the intermediate 1b* is crucial in giving rise to the parallel fragmentation pathways of our postulated mechanism. Evidence for the formation of 1b* is not only provided by structurally similar PGs, but also by cholamine-derivatized PGI_2 (12, Figure 4). PGI_2 differs from other PGs in incorporating an annealed bicyclic structure. Due to restriction imposed by its molecular skeleton, particularly the *Z*-double bond in the A chain, it is unable to form an intermediate of the form of 1b*. The MS^2 spectrum of 12 derived from *m/z* 437.4 includes three intense product ions at *m/z* 378.3 (-NMe_3), 360.4 (-NMe_3, -H_2O), and 342.4 (-NMe_3, -$2H_2O$) but there was no sequenced mass loss of 28/44 or 44/28 Da.

In summary, the results in toto support the view that the unusual fragmentation pattern of cholamine-derivatized PGs is specifically derived from the macrocyclic intermediate 1b*. The imidoester group in 1b* benefits from the [1, 3] sigmatropic rearrangement in the B chain and rearranges to the energetically more stable amine. This cascade rearrangement occurs in parallel with the elimination of ethylene oxide and gives rise to the unique CID fragmentation pattern.

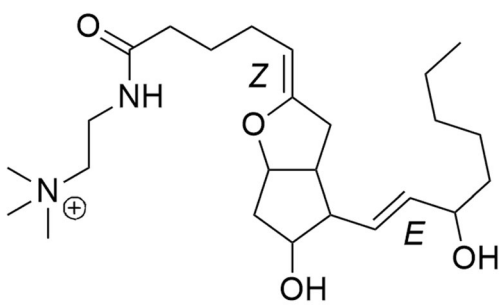


10 ethylamine derivatized limaprost *m/z* 408.5



11 Cholamine derivatized heptanoic acid *m/z* 215.5

Figure 3. Compounds designed to evaluate the leaving group effect



12 PGI_2 derivative *m/z* 437.4

Figure 4. The cholamine derivative of PGI_2 (12)

Table 2. Fragmentation Patterns of Ethanolamine-Derivatized Limaprost (6), C-17 Methyl Deuterium-Labeled Limaprost (7), PGE_1 (8), and PGE_2 (9) (Confirmed via MS^3)

Precursor ion (m/z^{-1})		-H_2O (m/z^{-1})	-$2H_2O$ (m/z^{-1})	-$2H_2O$-28/44 (m/z^{-1})	-$2H_2O$-72 (m/z^{-1})
6	424.6	406.5	388.5	360.2 (−28, w) 344.2 (−44, w)	316.3 (t)
7	427.6	409.5	391.5	363.5 (−28, w) 347.5 (−44, w)	319.3 (t)
8	398.2	380.5	362.5	334.2 (−28, w) 318.4 (−44, w)	290.3 (t)
9	396.2	378.2	360.3	332.2 (−28, w) 316.2 (−44, w)	288.3 (t)

w weak, *t* trace

Conclusion

Specific parallel neutral mass loss pathways of 28/44 and 44/28 Da occur in the CID of cholamine-derivatized limaprost, PGE_1, PGE_2, and PGD_2 under ESI-positive ion conditions. Such sequenced mass loss relates specifically to the molecular structure of cholamine-derivatized PGs and their ability to form a stabilized dihydrooxazolium cationic intermediate. This stabilized intermediate facilitates the formation of a unique macrocyclic product ion when a [1, 3] sigmatropic rearrangement in the B chain is coupled to and initiates an unusual Hofmann rearrangement in the A chain. At the same time, the elimination of an ethylene oxide occurs and gives rise to the specific CID pattern. Such specific fragmentation behavior in the cholamine derivatives of structurally similar PGs provides useful information in the lipidomic profiling of prostaglandins and their analogs.

Acknowledgments

The authors gratefully acknowledge financial support from the National Natural Science Foundation of China (Grant Nos. 81430087 and 81673396) and the Science and Technology Major Specialized Projects for "significant new drugs creation" of the 13th five-year plan (2017ZX09101001).

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