ANALYSIS OF PERFLUOROALKYL ANION FRAGMENTATION PATHWAYS FOR BRANCHED PERFLUOROOCTANOIC ACIDS DURING LC/ESI-MS/MS.

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1. Introduction

LC-electrospray ionization-MS (LC/ESI-MS) and LC/ESI-MS/MS can be considered the current standards for analysis of perfluorinated compounds.[1] The majority of reports found in the literature have employed LC/ESI-MS/MS as the preferred analytical method with multiple transitions of the molecular ion used for qualitative and quantitative purposes.

It is known[1] that under LC/ESI-MS/MS conditions, the perfluoroalkyl carboxylate anion ($R_FCO_2^-$) first loses CO_2 to give a perfluoroalkyl anion R_F^-, $[(M-H)-CO_2]^-$, which subsequently fragments to give (*inter alia*) lower mass carbanions. It has been suggested in a previous study[2] that such secondary fragmentation involves cleavage of C_nF_{2n} segments. However, the results of a recent study[3] of the LC/ESI-MS/MS of a series of linear ^{13}C-labelled perfluoroalkylcarboxylic acids (PFCAs) indicate that fragmentation of the R_F^- anion does not entail simple "unzipping" of a primary perfluoroalkyl anion of the type $F_3C(CF_2)_xCF_2^-$. A fragmentation mechanism was proposed[3] that involves rapid fluorine shifts, after the initial decarboxylation, which generate a series of new anions prior to secondary and tertiary fragmentation. This work has now been extended to branched PFOA isomers. These give predictable mass spectra lending support to the newly proposed fragmentation pathway.[3]

2. Materials & Methods

2.1 Chemicals

The native perfluoroalkyl carboxylic acids (R_f-CO_2H, see Scheme 1) were synthesized at Wellington Laboratories using proprietary methods. HPLC grade methanol (MeOH) and water were purchased from Caledon.

2.2 LC/ESI-MS/MS

LC/ESI-MS/MS experiments were conducted on a Waters Acquity Ultra Performance LC interfaced to a Micromass Quattro micro atmospheric pressure ionization (API) mass spectrometer. The isomer stock solutions were diluted to a concentration of approximately 2 ppm with 75:25 MeOH:water and infused into the MS at a rate of 10 µL/min. The LC conditions to the MS source were set at 80:20 MeOH/water at a flow rate of 0.15 mL/minute. The Micromass Quattro *micro* API MS was set up in the negative-ion electrospray mode with the following conditions: capillary voltage (kV) = 0.50; source temperature (°C) = 110; cone gas flow (L/Hr) = 60; desolvation gas flow (L/Hr) = 600; desolvation gas temperature (°C) = 325; gas cell pressure ~ $3.5e^{-3}$ mbar. In-source fragmentation of the isomers was utilized to obtain the $[R_f–CO_2]^-$ anion and subsequent collision-induced dissociation provided the desired product ions. The cone voltage (V) and collision energy (eV) for the product ion experiments involving the transition $[R_f–CO_2]^-$ to $[R_f]^-$ were optimized for each individual isomer.

3. Results & Discussion

For the PFOA isomers studied, the LC/ESI-MS/MS conditions were optimized for the production of secondary product ions. The mass spectra are summarized in Figure 1.

As expected from previous results obtained for perfluoro-1,2,3,4-$^{13}C_4$-octanoic acid[3], LC/ESI-MS/MS of linear PFOA (**1**), when optimized to produce secondary daughter ions, generates fragments m/z 119, m/z 169 and m/z 219 (Fig. 1a). These anions arise after primary fragmentation involving the loss of CO_2 from **1** to produce a perfluoroalkyl fragment $[C_7F_{15}]^-$ which then undergoes rapid fluorine migration to generate more stable secondary anions before secondary fragmentation (see Scheme 1 in reference 3).

LC/ESI-MS/MS of the isopropyl branched PFOA (**7**) produces, after secondary fragmentation, one major fragment at m/z 169 and a very weak fragment at m/z 219 (Fig. 1b). It is well known[4] that the order of stabilities of perfluoro carbanions is 3° > 2° > 1°. Therefore, after primary fragmentation, the equilibrium would be expected to favor carbanion **7e** which, after secondary fragmentation, results in formation of the ion at m/z 169 (see Fig. 2, pathway G). We can exclude the possibility that this ion (m/z 169) comes from **7c** by investigating the fragmentation of a PFNA analog. LC/ESI-MS/MS of perfluoro-6-methyloctanoic acid (**2**) gives one major fragment at m/z 219 and a very weak fragment at m/z 169 (Fig. 1j). It can be seen from figure 3 that formation of the tertiary carbanion **2f** leads to the major fragment m/z 219 (Fig. 3, pathway I). The carbanion **2d**, similar to **7c,** produces a very minor secondary fragment. Therefore, it can be concluded that secondary fragmentation results from formation of the more stable tertiary carbanion which then yields the secondary fragments.

Extending this investigation to the other mono-methyl branched PFOAs, isomers **4**-**6** produce major fragments m/z 169, 119 and 119, respectively (Fig. 1c, 1d and 1e). These results are expected when the primary daughter anion rearranges to the tertiary carbanion before secondary fragmentation. Isomer **3** is different from the other mono-methyl branched PFOA isomers in that the carboxyl group is situated on a secondary carbon. Therefore, after decarboxylation, the m/z 369 anion produced during fluorine migration is identical to the one produced from linear PFOA and, as a result, the same major secondary fragment is seen at m/z 119 and m/z 169 (Fig. 1f).

The geminal dibranched PFOA isomers (**8**-**9**) are unique in that 1,2-fluorine shifts after decarboxylation would be halted at the quaternary carbon atom. Isomer **8** fragments to give the very stable tertiary-butyl carbanion m/z 219 (Fig.1g). Isomer **9** after decarboxylation can only give two anions **9a** and **9b** through fluoride migration before secondary fragmentation to give either m/z 119 or 219 (see Fig. 4). We only observe m/z 119 for isomer **9** (Fig. 1h), again indicating that formation of the secondary anion after decarboxylation is favored over the primary anion before secondary fragmentation. It should be noted that isomer **9** contains another PFOA isomer as an impurity which is most likely responsible for the observation of the fragment at m/z 169.

The dibranched PFOA isomers **10** and **11** exist as a 5:3 mixture. It would be expected that these isomers would give predominantly fragments m/z 119 and 169, respectively, via the proposed mechanism of decarboxylation, fluorine migration and then secondary fragmentation through the more stable 3° anion. Indeed, these two fragments are observed in the mass spectrum (see Fig. 1i).

The relative response factors for the branched PFOA isomers were determined using MPFOA (M+4) as an internal standard under SIM conditions optimized for linear PFOA (**1**). The results are summarized in Table 1. Isomer **3** showed a very weak relative response factor. This is not surprising knowing that the carboxyl group is attached to a secondary carbon which would be expected to more readily decarboxylate to give directly a secondary perfluoroalkyl anion. Isomers **4** and **11** are similar in structure in that they both have trifluoromethyl groups beta to the carboxyl functional group and it is interesting to note that both have similar low response factor of about 40%. Isomers **9** and **10** also have lower response factors. However, we remain unsure as to why branching affects the ease of fragmentation of these four isomers which results in lower relative response factors under SIM conditions.

Analysis of the LC/ESI-MS/MS spectra of a series of branched PFOA isomers confirms that fragmentation of the R_F^- anions, after initial loss of CO_2 from the $[M\text{-}H]^-$ ions, proceeds via rapid fluorine shifts thus generating a series of new anions prior to secondary fragmentation. The favored pathway is dictated by the order of stabilities of perfluoro carbanions which is 3° > 2° > 1°. Branching also has an impact on relative response factors under SIM conditions.

References

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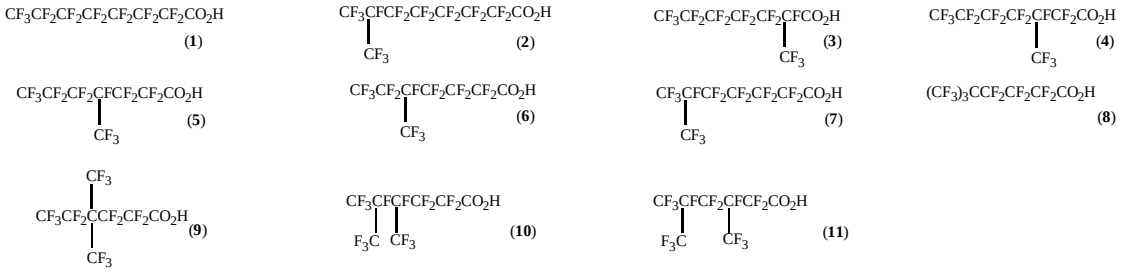
2. Lyon P.A., Tomer K.B., Gross M.L. *Anal Chem.* 1985; **57**: 2984-2989.
3. Arsenault G., McAlees A., McCrindle R., Riddell N. *Rapid Commun. Mass Spectrom.* 2007; **21**: 3803-3814.
4. Kirsch, P. 2004. Modern Fluoroorganic Chemistry. Wiley-VCH, 103.

Scheme 1: Structures of the perfluoroalkylcarboxylic isomers.



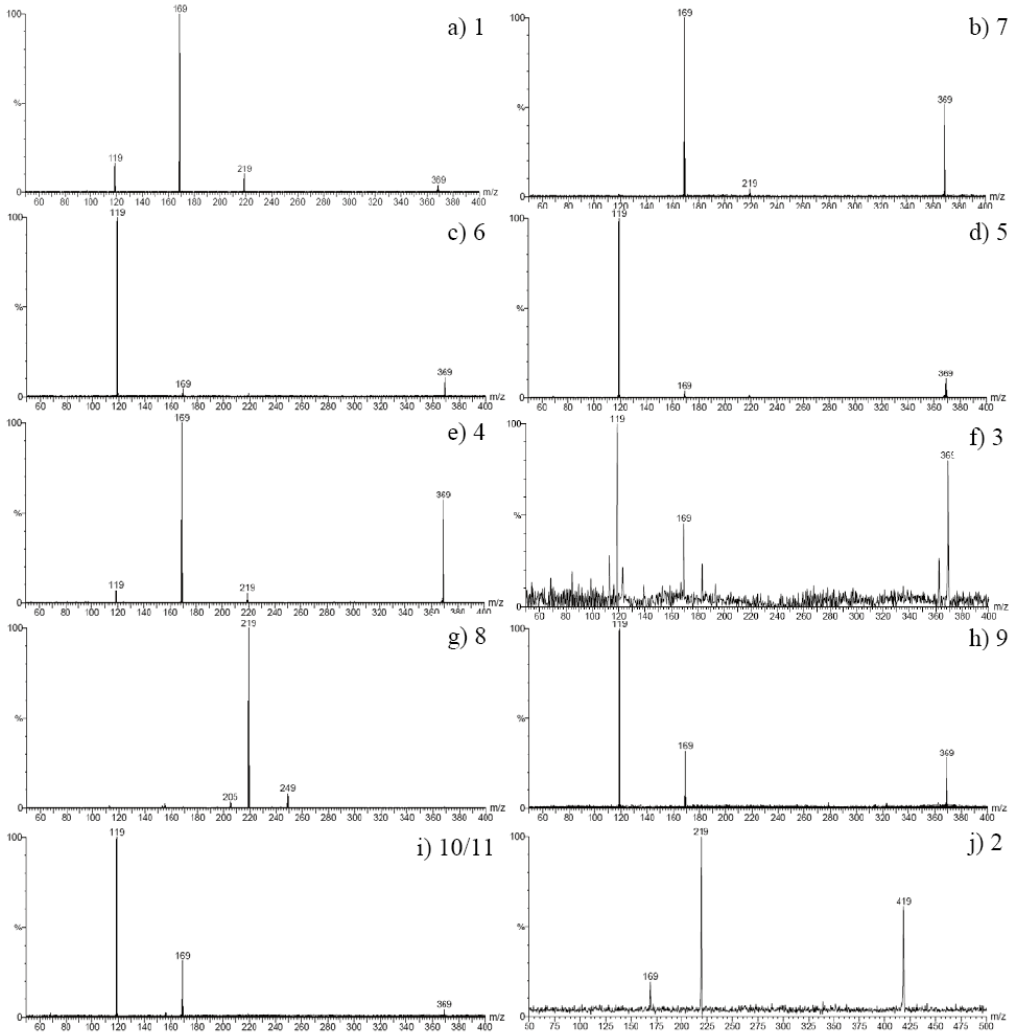


Figure 1. Product ion spectrum of the [(M-H)-CO_2] ion of a) **1**; b) **7**; c) **6**; d) **5**; e) **4**; f) **3**; g) **8**; h) **9**; i) **10/11** and j) **2**.

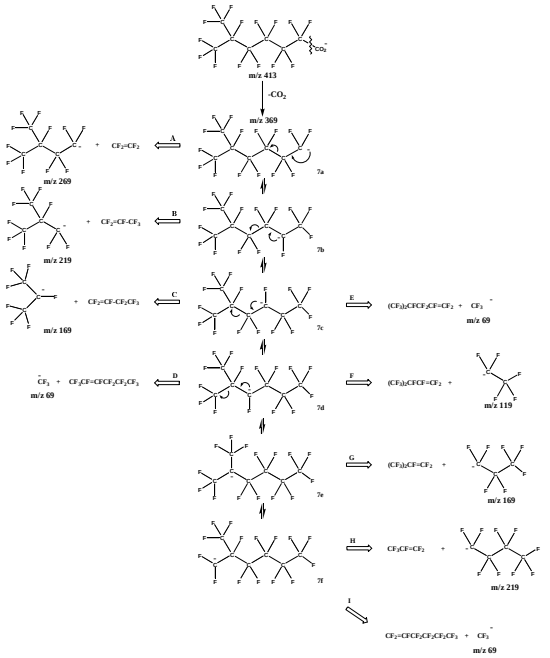


Figure 2. Fragmentation pathways for the M-H ion of **7**. (Arrows show the fragmentation mechanism for the secondary pathway of A to D only).

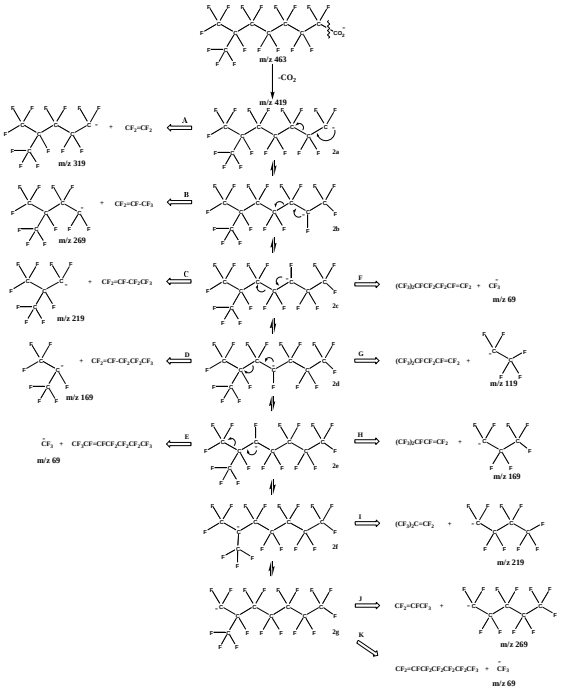


Figure 3. Fragmentation pathways for the M-H ion of **2**. (Arrows show the fragmentation mechanism for the secondary pathway of A to E only).

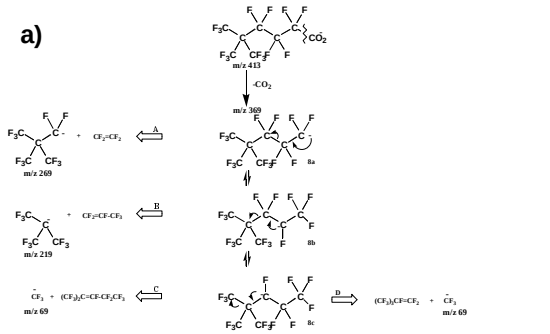


Figure 4. Fragmentation pathways for the M-H ion of a) **8** and b) **9**. (Arrows show the fragmentation mechanism for the secondary pathway)

Table 1. LCMS relative response factors for the various branched PFOA isomers against linear PFOA (**1**)

Isomer	3	4	5	6	7	8	9	10	11
%Relative response factor[a]	1	40	90	110	100	90	50	75	36

a The relative response factors for the branched PFOA isomers were determined using MPFOA (M+4) as an internal standard under SIM conditions for linear PFOA (**1**). The uncertainty measurement for these experiments is estimated at ±16%.