

# Unusual collision-induced dissociation of fluorated and non-fluorated $\alpha$ -nitrotoluene analogs in a gas chromatograph triple-stage quadrupole mass spectrometer under electron-capturing negative-ion chemical ionization conditions

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## Abstract

Unusual collision-induced dissociation (CID) of perfluorated and non-perfluorated  $\alpha$ -nitrotoluene analogs in a gas chromatograph triple-stage quadrupole (TSQ) mass spectrometer (GC–QqQ–MS) under electron-capturing negative-ion chemical ionization conditions is reported. CID of  $[M - 1]^-$  of  $\alpha$ -nitro-2,3,4,5,6-pentafluorotoluene ( $C_6F_5CH_2-NO_2$ ) and  $\alpha$ -nitro-2,5-difluorotoluene ( $C_6H_3F_2CH_2-NO_2$ ) produced an intense ion with  $m/z$  66. By using  $^{15}N$ - or  $^{18}O$ -labelled  $C_6F_5CH_2-NO_2$  analogs, we found that this anion has the formula  $C_3NO$ . By contrast, CID of  $[M - 1]^-$  of  $\alpha$ -nitrotoluene ( $C_6H_5CH_2-NO_2$ ) and  $\alpha$ -nitro-3,5-difluorotoluene ( $C_6H_3F_2CH_2-NO_2$ ) produced an anion with  $m/z$  86 with the formula  $C_3H_4NO_2$ . The expected CID of the C–N-bond of all  $\alpha$ -nitrotoluene analogs to form the nitrite anion ( $NO_2^-$ ,  $m/z$  46) did not occur. We propose mechanisms for the formation of the anions  $C_3NO$  and  $C_3H_4NO_2$  in the collision chamber of the TSQ mass spectrometer. The most likely structures for the anion  $C_3NO$  are  $:C=C=C=N-O^-$  and  $N\equiv C-C\equiv C-O^-$ . The unique CID behavior of  $C_6F_5CH_2-NO_2$  can be utilized to unequivocally identify and accurately quantify nitrite in biological fluids by GC–tandem MS.

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**Keywords:** Nitrite; Pentafluorobenzyl; Stable isotopes; Tandem mass spectrometry

## 1. Introduction

As a rule, analysis by gas chromatography–mass spectrometry (GC–MS) of most compounds requires preceding chemical derivatization of one or more functionalities to increase volatility and thermal stability and also to improve the electron-capture negative-ion chemical ionization (ECNICI) behavior of the original analyte. Unlike electron ionization (EI), ECNICI in the ion source of GC–MS instruments leads to formation of a few anions. Usually, ECNICI is accompanied by loss of moieties introduced into the molecules by derivatization. For instance, ECNICI of pentafluorobenzyl (PFB) esters of carboxylic acids, which are obtained from the reaction of the carboxylic acid with PFB bromide (PFB–Br), leads to the formation of the carboxylate anion by loss of the

PFB radical [1]. Analogous, ECNICI of  $\alpha$ -nitro-2,3,4,5,6-pentafluorotoluene (PFB– $NO_2$ ), which is formed from the reaction of nitrite ( $NO_2^-$ ) with PFB–Br in aqueous acetone, acetonitrile or methanol (Fig. 1), produces  $NO_2^-$  by loss of the PFB radical (Fig. 2) [2–5]. The reduced fragmentation occurring under ECNICI conditions can be utilized to sensitively quantify by GC–MS in biological fluids various classes of compounds including carboxylic acids [1] and inorganic anions such as nitrite and nitrate [2–5].

Subjection of intense and/or specific parent anions to collision-induced dissociation (CID) with a target gas such as argon in the collision chamber of tandem mass spectrometers may generate characteristic product ions [1,6]. Generation of product ion mass spectra from only a few picogram amount of an analyte or monitoring of specific product ions can be used for structure elucidation as well as for highly specific quantification of various compounds in complex biological fluids such as urine and plasma [1,6,7].

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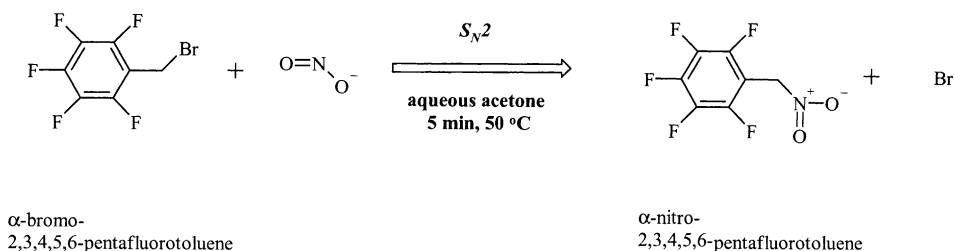


Fig. 1. Nucleophilic substitution reaction ( $S_N2$ ) between  $\alpha$ -bromo-2,3,4,5,6-pentafluorotoluene (PFB-Br) and nitrite ( $\text{NO}_2^-$ ) in aqueous acetone to form  $\alpha$ -nitro-2,3,4,5,6-pentafluorotoluene (PFB- $\text{NO}_2$ ).

Cleavage of C–C-bonds during CID of anions produced by ECN/CI of simply or multiply derivatized compounds rarely occurs [1,6]. For instance, CID of the carboxylate anions of PFB esterified, methoximated and trimethylsilylated eicosanoids is characterized by consecutive loss of trimethylsilylanol and the methoxy groups [1]. First after complete loss of all functionalities, cleavage of the C-1/C-2 bond occurs leading to loss of  $\text{CO}_2$ . An unusual C–C-bond cleavage at C-4/C-5 has been observed during CID of the carboxylate anion of the trimethylsilyl ether derivative of 5-hydroxyeicosanoic acid that apparently involves rearrangement of the trimethylsilyl group from the hydroxyl group at C-5 to the carboxyl group [6].

In previous work, we observed unexpectedly that CID of  $[\text{M} - 1]^-$  of  $\text{C}_6\text{F}_5\text{CH}_2\text{NO}_2$  in tandem mass spectrometry performed in a gas chromatograph triple-stage quadrupole (TSQ) mass spectrometer (GC–QqQ-MS) did not result in formation of  $\text{NO}_2^-$  ( $m/z$  46) [7], in contrast to nitroaromatics such as 1-nitro-2,4,6-trimethoxybenzene [8], but it yielded a product ion with  $m/z$  66. The corresponding product ion from  $\text{C}_6\text{F}_5\text{CH}_2\text{NO}_2$  was found to have an  $m/z$  value of 67. The utility of GC–QqQ-MS to quantify nitrite and nitrate in human urine and plasma as  $\text{C}_6\text{F}_5\text{CH}_2\text{NO}_2$  was demonstrated [7].

Preliminary investigations had showed that the product ion with  $m/z$  66 may contain up to three C atoms, thus suggesting that during CID of PFB- $\text{NO}_2$  the aromatic ring must have been cleaved. This unusual fragmentation prompted us to investigate the CID of  $\text{C}_6\text{F}_5\text{CH}_2\text{NO}_2$  in more detail, and, if possible to identify the structure of the unusual product ion with  $m/z$  66 and to elucidate the underlying mechanism. For this purpose, we used  $^{15}\text{N}$ - and  $^{18}\text{O}$ -labelled  $\text{C}_6\text{F}_5\text{CH}_2\text{NO}_2$  analogs and  $\alpha$ -nitrotoluene analogs with distinctly positioned fluorine atoms (F) in the benzene ring.

## 2. Experimental

### 2.1. Materials and chemicals

Sodium [ $^{15}\text{N}$ ]nitrite (98 at.% at  $^{15}\text{N}$ ) was bought from Cambridge Isotope Laboratories (Andover, MA, USA). Sodium nitrite, acetone, acetonitrile and toluene were purchased from Merck (Darmstadt, Germany). 2,3,4,5,6-Pentafluorobenzyl (PFB) bromide, 2,5-difluorobenzyl (DFB)

bromide, 3,5-difluorobenzyl bromide and benzyl bromide were obtained from Aldrich (Steinheim, Germany).  $\text{H}_2^{18}\text{O}$  (99 at.% at  $^{18}\text{O}$ ) was obtained from CAMPRO Scientific (Emmerich, Germany).  $^{18}\text{O}$ -Labelled nitrite was prepared by dissolving solid sodium nitrite (6.9 mg) in  $\text{H}_2^{18}\text{O}$  (100  $\mu\text{l}$ ) and acidifying with 1 M HCl followed by neutralization with 1 M NaOH. GC–Q-MS analysis of the PFB derivatives of the reaction product revealed a mixture consisting of  $\text{O}=\text{N}-\text{O}^-$  ( $m/z$  46; 24%),  $\text{O}=\text{N}-^{18}\text{O}^-$  ( $m/z$  48; 48%), and  $^{18}\text{O}=\text{N}-^{18}\text{O}^-$  ( $m/z$  50; 28%).

### 2.2. Derivatization procedure and product isolation

Derivatization of nitrite in aqueous acetone with PFB bromide and other benzyl bromide agents was performed as described elsewhere [5]. Briefly, aliquots (100  $\mu\text{l}$ ) of aqueous solutions of unlabelled and labelled nitrite (10 mM) were diluted with acetone (400  $\mu\text{l}$ ), the bromide agent (10  $\mu\text{l}$ ) was added, and the reaction mixtures were allowed to stand at  $50^\circ\text{C}$  for 5–60 min [5]. After cooling to room temperature, acetone was removed under nitrogen and reaction products were extracted by vortex-mixing with toluene (1 ml) for 1 min.

### 2.3. GC–Q-MS and GC–QqQ-MS conditions

Single-stage quadrupole GC–MS (GC–Q-MS) analyses were performed on a Hewlett-Packard MS engine 5890A connected directly to a gas chromatograph 5890 series II equipped with an autosampler Hewlett-Packard model 7673 (Waldbronn, Germany). GC–Q-MS and triple-stage quadrupole GC–MS (GC–QqQ-MS) were carried out on a Thermoquest TSQ 7000 apparatus (San Jose, CA, USA) connected directly to a Thermoquest Carlo Erba Instruments gas chromatograph Trace 2000 equipped with an autosampler model AS 2000. Optima 17 (15 m  $\times$  0.25 mm i.d., 0.25- $\mu\text{m}$  film thickness) fused capillary columns from Macherey-Nagel (Düren, Germany) were used. The following temperature program was used in GC–Q-MS and GC–QqQ-MS analyses: the column was held at  $70^\circ\text{C}$  for 1 min then increased to  $280^\circ\text{C}$  at a rate of  $30^\circ\text{C}/\text{min}$ . In GC–QqQ-MS analyses, helium (at a constant pressure of 70 kPa) and methane (530 Pa) were used as carrier and reactant gases, respectively. For CID argon (usually at 0.130 Pa) was used at collision energies of

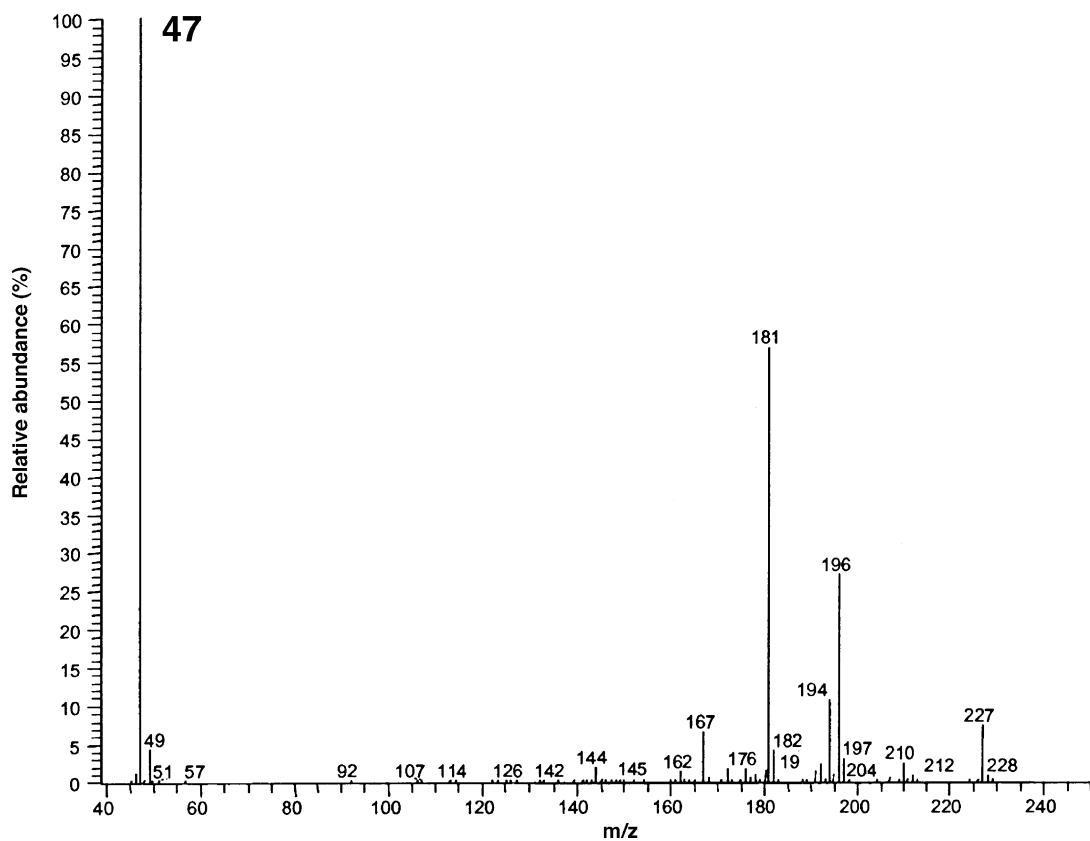
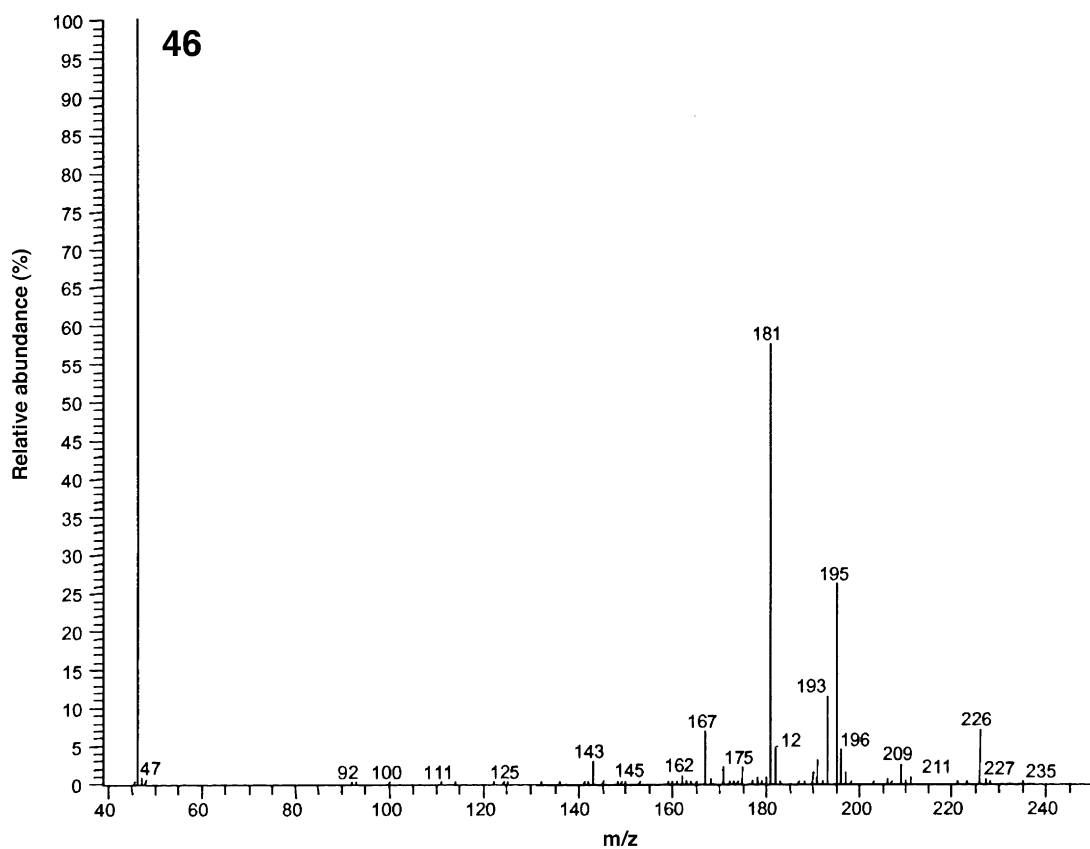


Fig. 2. ECNICI mass spectra of (upper panel)  $^{14}\text{N}$ - and (lower panel)  $^{15}\text{N}$ -labelled  $\alpha$ -nitro-2,3,4,5,6-pentafluorotoluene. Instrument, TSQ 7000.

5–25 eV. Electron energy and electron current were 230 eV and 300  $\mu$ A, respectively. Injector, interface and ion source were held constant at 200, 280 and 180 °C, respectively. Aliquots (1  $\mu$ l) were injected in the splitless mode in both instruments. PFB derivatives of unlabelled and labelled nitrite had practically identical retention times. Mass spectra were generated by a scanning rate of 1 s per scan. The dwell time in the selected-ion monitoring (SIM) as well as in the selected-reaction monitoring (SRM) was 50 ms for all ions.

### 3. Results and discussion

#### 3.1. Mass spectra and mechanisms of fragmentation

The ECNICI mass spectra of  $^{14}$ N- and  $^{15}$ N-labelled  $\alpha$ -nitro-2,3,4,5,6-pentafluorotoluene (PFB- $^*NO_2$ ,  $^*N$  means  $^{14}N$  or  $^{15}N$ ) are shown in Fig. 2. The most significant ions in the ECNICI mass spectra of all compounds investigated in this study are summarized in Table 1. Fig. 2 and Table 1 show that ECNICI of  $\alpha$ -nitrotoluene analogs results in the cleavage of the C–N-bond to produce the nitrite anion, i.e.  $[NO_2]^-$ , as the most intense anion, and less intense anions due to  $[M - 1]^-$ . The latter anions still contain the nitro group and are most likely formed by loss of H from the methylene group.

CID of the parent ion at  $m/z$  226 ( $[M - 1]^-$ ) of PFB- $NO_2$  resulted in the generation of an intense product ion with  $m/z$  66 (Fig. 3, upper panel). CID of  $m/z$  227 ( $[M - 1]^-$ ) of PFB- $^{15}NO_2$  resulted in the generation of an intense product ion with  $m/z$  61 indicating that this product ion carries one  $^{15}N$  atom (Fig. 3, lower panel). The ions with  $m/z$  66 and  $m/z$  67 were present in the ECNICI mass spectra of PFB- $NO_2$  and PFB- $^{15}NO_2$  at an intensity of 0.06% with respect to  $m/z$  46 and  $m/z$  47 (100%). This indicates that the ions with  $m/z$  66 and  $m/z$  67 are characteristic CID product ions. The product ion mass spectra of all compounds investigated in this study are summarized in Table 2. CID of all of these compounds did not result in formation of the nitrite anion ( $m/z$  46,  $[NO_2]^-$ ).

Theoretically, the ions with  $m/z$  66 and  $m/z$  67 could be adducts of unlabelled and labelled nitrite (molecular mass 46) with a molecule of HF (molecular mass 20), which is also

Table 1  
ECNICI mass spectra<sup>a</sup> of  $\alpha$ -nitrotoluene analogs investigated in this study

$\alpha$ -Nitrotoluene	[Nitrite] <sup>-</sup> , $m/z$	$[M - 1]^-$ , $m/z$
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	46	226
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	47	227
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> N <sup>18</sup> O <sub>2</sub>	50	230
C <sub>6</sub> H <sub>3</sub> [2,5]F <sub>2</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	46	172
C <sub>6</sub> H <sub>3</sub> [2,5]F <sub>2</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	47	173
C <sub>6</sub> H <sub>3</sub> [3,5]F <sub>2</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	46	172
C <sub>6</sub> H <sub>3</sub> [3,5]F <sub>2</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	47	173
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	46	136
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	47	137

<sup>a</sup> For simplicity only the nitrite ion and  $[M - 1]^-$  are summarized. Instrument, MS engine 5890A.

Table 2

C<sub>3</sub>-product ions in ECNICI mass spectra generated by CID of the parent ions  $[M - 1]^-$  of various  $\alpha$ -nitrotoluene analogs<sup>a</sup>

$\alpha$ -Nitrotoluene analog	$[M - 1]^-$ $m/z$	C <sub>3</sub> -ion $m/z$	Ion assignment
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	226	66	C <sub>3</sub> <sup>14</sup> NO
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	227	67	C <sub>3</sub> <sup>15</sup> NO
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> N <sup>18</sup> O <sup>16</sup> O	228	66, 68	C <sub>3</sub> <sup>14</sup> N <sup>16</sup> O, C <sub>3</sub> <sup>14</sup> N <sup>18</sup> O
C <sub>6</sub> F <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> N <sup>18</sup> O <sub>2</sub>	230	68	C <sub>3</sub> <sup>14</sup> N <sup>18</sup> O
C <sub>6</sub> H <sub>3</sub> [2,5]F <sub>2</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	172	66	C <sub>3</sub> <sup>14</sup> NO
C <sub>6</sub> H <sub>3</sub> [2,5]F <sub>2</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	173	67	C <sub>3</sub> <sup>15</sup> NO
C <sub>6</sub> H <sub>3</sub> [3,5]F <sub>2</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	172	86	C <sub>3</sub> H <sub>4</sub> <sup>14</sup> NO <sub>2</sub>
C <sub>6</sub> H <sub>3</sub> [3,5]F <sub>2</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	173	87	C <sub>3</sub> H <sub>4</sub> <sup>15</sup> NO <sub>2</sub>
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> - <sup>14</sup> NO <sub>2</sub>	136	86	C <sub>3</sub> H <sub>4</sub> <sup>14</sup> NO <sub>2</sub>
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> - <sup>15</sup> NO <sub>2</sub>	137	87	C <sub>3</sub> H <sub>4</sub> <sup>15</sup> NO <sub>2</sub>

<sup>a</sup> Instrument, TSQ 7000.

produced in the collision chamber during CID. However, the following line of evidence eliminates the possibility of such an adduct formation. CID of  $[M - 1]^-$  from PFB-N<sup>18</sup>O<sub>2</sub> did not result in formation of a product ion with  $m/z$  70 but only of  $m/z$  68. This observation clearly indicates that the product ion with  $m/z$  68 carries only one <sup>18</sup>O atom. Other findings (Table 2) strongly suggest that these product ions also contain a single N atom. Therefore, we may assume that the product ion at  $m/z$  66 has the formula RNO, whereas R is a residue with a molecular mass of 36.

Another possible explanation for the product ion with  $m/z$  66 could be adduct formation of NO<sup>-</sup> with one molecule of the reactant gas CH<sub>4</sub> (molecular mass, 16) and one molecule of HF (molecular mass, 20), i.e. a species with the formula CH<sub>5</sub>FNO. However, this possibility has to be rejected for several reasons.

First, the peak area ratio of  $m/z$  66 to  $m/z$  68 produced by CID of  $m/z$  226 of  $[PFB-NO_2 - 1]^-$  and  $m/z$  228 of  $[PFB-N^{18}O_2 - 1]^-$ , respectively, did not depend on CH<sub>4</sub> pressure (130–530 Pa) in the ion source or argon pressure (0.026–0.26 Pa) in the collision chamber (i.e.  $1.040 \pm 0.021$ , mean  $\pm$  SD; RSD, 1.98%). However, a CH<sub>4</sub> pressure of 260 Pa was necessary to obtain abundant formation of the parent ions  $[M - 1]^-$ . An argon pressure of 0.13 Pa was found to be optimum. The ion  $m/z$  66 was observed at 0.026 Pa argon with an abundance one-seventh of that at 0.13 Pa. The product ion was observed already at a collision energy of 5 eV, but maximum formation required a collision energy of 25 eV.

Second, SRM of  $m/z$  67 and  $m/z$  66 from PFB- $NO_2$  resulted in a peak area ratio of  $0.039 \pm 0.004$  (mean  $\pm$  SD,  $n = 5$ ). By means of Eq. (1), the abundance ratio of  $m/z$  67 ( $P_{M+1}$ ) to  $m/z$  66 ( $P_M$ ) for a compound containing C, N and O atoms was calculated according to Biemann [9]. It resulted in the ratios of 0.015 for one C atom, 0.0262 for two C atoms, 0.037 for three C atoms, and 0.0484 for four C atoms. Thus, the measured ratio of 0.039 is very close to the theoretical value of 0.037 for three C atoms, suggesting that the residue R in the formula RNO of the product ion with  $m/z$  66 is a C<sub>3</sub>

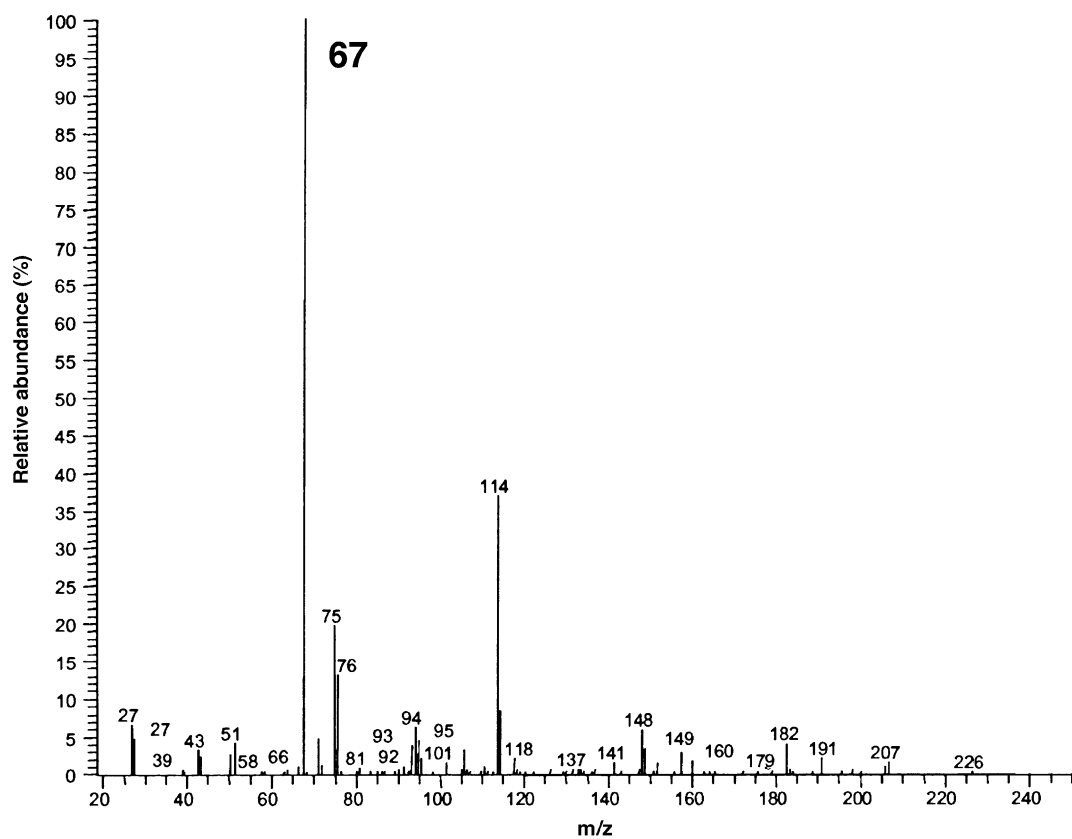
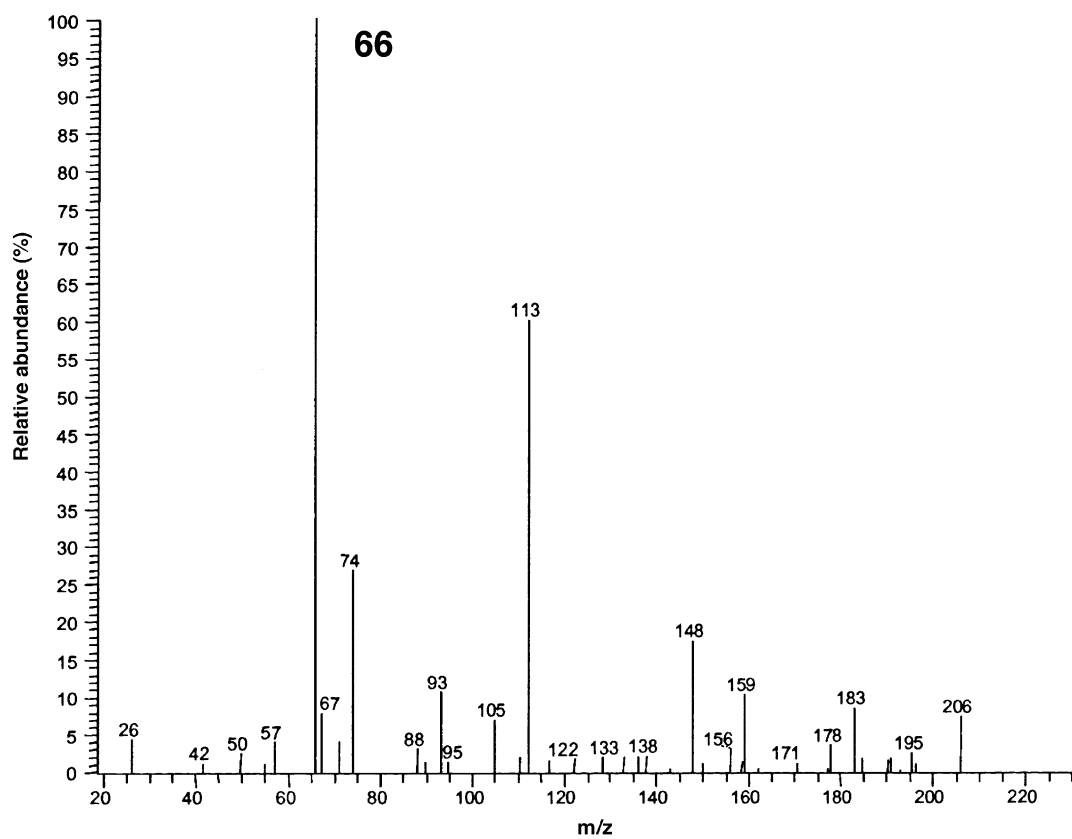


Fig. 3. Product ion mass spectra generated by CID of  $[M - 1]^-$  from (upper panel)  $^{14}\text{N}$ - and (lower panel)  $^{15}\text{N}$ -labelled  $\alpha$ -nitro-2,3,4,5,6-pentafluorotoluene. Instrument, TSQ 7000.

moiety.

$$\frac{P_{M+1}}{P_M} = N_C \left[ \frac{^{13}\text{C}}{100 - ^{13}\text{C}} \right] + N_N \left[ \frac{^{15}\text{N}}{100 - ^{15}\text{N}} \right] + N_O \left[ \frac{^{17}\text{O}}{100 - ^{17}\text{O} - ^{18}\text{O}} \right] \quad (1)$$

where  $P_M$  is the abundance of molecules containing no heavy isotopes;  $P_{M+1}$  is the abundance of molecules containing  $^{13}\text{C}$  (i.e. 1.1%),  $^{15}\text{N}$  (i.e. 0.37%), or  $^{17}\text{O}$  (i.e. 0.037%); the natural abundance of  $^{18}\text{O}$  is 0.2%;  $N_C$ ,  $N_N$  and  $N_O$  is the number of C, N and O atoms present in the molecule, respectively.

Third, we measured the  $^{13}\text{C}$  to  $^{12}\text{C}$  ratio in the product ion derived by CID of PFB- $^{15}\text{NO}_2$ . SRM of  $m/z$  68 ( $[\text{C}_3^{13}\text{C}_1^{12}\text{C}_2^{15}\text{NO}]^-$ ) and  $m/z$  67 ( $[\text{C}_3^{12}\text{C}_3^{15}\text{NO}]^-$ ) that were produced by CID of  $m/z$  228 ( $[\text{C}_6\text{F}_5\text{CH}_2^{15}\text{NO}_2 - 1]^-$ ) resulted in the ratio of  $0.72 \pm 0.04$  (mean  $\pm$  SD,  $n = 5$ ). This ratio is very close to the ratio of the probability for  $^{13}\text{C}$  in PFB- $^{15}\text{NO}_2$  ( $\text{C}_7\text{F}_5\text{H}_2^{15}\text{NO}_2$ ) which is calculated as  $3/7$  (in  $\text{CFCCH}_2^{15}\text{NO}_2$ ) to  $4/7$  (in  $\text{C}_4\text{F}_4$ ) = 0.75 (Fig. 4). Thus, all observations strongly suggest that the formula of the product anion with  $m/z$  66 is  $\text{C}_3\text{NO}$ .

Formation of  $[\text{C}_3\text{NO}]^-$  implies cleavage of C–C-bonds of the aromatic ring of PFB- $\text{NO}_2$ . Product ions with this formula were obtained from PFB- $\text{NO}_2$  and 2,5-DFB- $\text{NO}_2$  but not from 3,5-DFB- $\text{NO}_2$  and other  $\alpha$ -nitrotoluene analogs (Table 2). This observation strongly indicates that F in *ortho*-position is required for the formation of  $[\text{C}_3\text{NO}]^-$ , and is further supported by the observation that CID of  $m/z$  206 ( $[\text{M} - \text{HF} - 1]^-$ ) from PFB- $\text{NO}_2$  and of  $m/z$  207 ( $[\text{M} - \text{HF} - 1]^-$ ) from PFB- $^{15}\text{NO}_2$  did not produce  $m/z$  66 and  $m/z$  67 (data not shown), respectively. The most abundant product ions generated by CID of  $[\text{M} - 1]^-$  from 3,5-

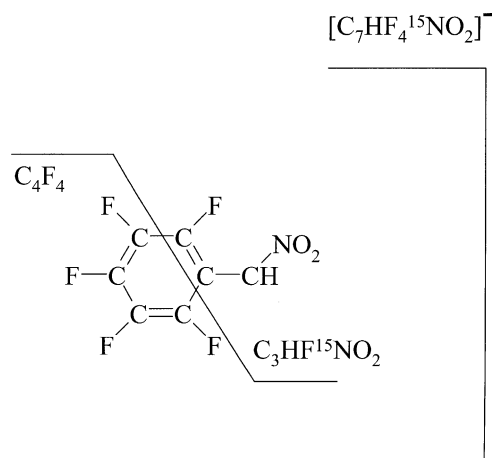


Fig. 4. Structure of PFB- $^{15}\text{NO}_2$  ( $\text{C}_7\text{F}_5\text{H}_2-^{15}\text{NO}_2$ ) and cleavage between C-2/C-3 and C-1/C-6. The probability for  $^{13}\text{C}$  in PFB- $^{15}\text{NO}_2$  ( $\text{C}_7\text{F}_5\text{H}_2-^{15}\text{NO}_2$ ) is calculated as  $3/7$  in  $\text{CFCCH}_2^{15}\text{NO}_2$  and  $4/7$  in  $\text{C}_4\text{F}_4$ . The calculated ratio of the probabilities, i.e.  $3/7$  to  $4/7 = 0.75$ , agrees well with the measured ratio of 0.72.

DFB- $\text{NO}_2$  and 3,5-DFB- $^{15}\text{NO}_2$  were  $m/z$  86 and  $m/z$  87, respectively, with the most likely formulas  $\text{C}_3\text{H}_4\text{NO}_2$  and  $\text{C}_3\text{H}_4^{15}\text{NO}_2$ , respectively (Table 2). In the case of 2,5-DFB- $\text{NO}_2$  and 2,5-DFB- $^{15}\text{NO}_2$ , the corresponding ions were, however,  $m/z$  66 and  $m/z$  67 due to  $\text{C}_3\text{NO}$  and  $\text{C}_3^{15}\text{NO}$ , respectively.

The existence of each three C atoms in all of these product ions may imply a common mechanism of fragmentation, i.e. intramolecular nucleophilic aromatic substitution. In the case of  $\alpha$ -nitrotoluenes with a F atom in *ortho* position, such as PFB- $\text{NO}_2$ , this F atom serves as a leaving group. Nucleophilic attack on C-2 may occur both by the carbanion (Fig. 5A) and by the mesomeric nitro-anion (Fig. 5B). Nucleophilic aromatic substitution of F in *ortho* position would produce  $\text{F}^-$ -molecule complexes to finally form the anions due to  $[\text{M} - 1 - \text{HF}]^-$ , which were indeed present in the product ion mass spectra of PFB- $\text{NO}_2$  (i.e.  $m/z$  206) and PFB- $^{15}\text{NO}_2$  (i.e.  $m/z$  207) (Fig. 3). Taking into consideration the results presented here and the gas-phase anionic chemistry presently known [10–12], the mechanisms shown in Fig. 5 are likely to take place in the collision chamber of the TSQ mass spectrometer. For PFB- $\text{NO}_2$  both mechanisms provide anions with the same formula  $\text{C}_3\text{NO}$  but distinctly different structures, i.e. the heterocumulene  $:\text{C}=\text{C}=\text{C}=\text{N}-\text{O}^-$  (Fig. 5A) via the carbanion attack and  $\text{N}=\text{C}-\text{C}=\text{C}-\text{O}^-$  via the nitro-anion attack (Fig. 5B). It is worth mentioning that anions of cumulenes such as  $:\text{C}=\text{C}=\text{C}=\text{C}=\text{C}=\text{O}$  and carbene cumulenes such as  $:\text{C}=\text{C}=\text{C}=\text{C}=\text{CH}_2$  and  $:\text{C}=\text{C}=\text{C}=\text{CH}_2$  have been repeatedly produced in the gas phase in mass spectrometers and detected in remote galactic regions [13–18]. Interestingly, the anion  $\text{N}=\text{C}-\text{C}=\text{C}-\text{O}^-$  with  $m/z$  66 has been detected in the gas phase of an ion cyclotron resonance mass spectrometer from the nucleophilic attack of  $\text{O}^-$  on C-2 of 1,2-dicyanoethene ( $\text{N}=\text{C}-\text{CH}=\text{CH}-\text{C}=\text{N}$ ) which is accompanied by loss of one HCN molecule [10].

In  $\alpha$ -nitrotoluenes without F atoms in *ortho* position, such as 3,5-DFB- $\text{NO}_2$ , F does not serve as a leaving group for the nucleophilic attack of the carbanion (Fig. 5A, right panel). As a consequence the anion  $\text{C}_3\text{H}_4\text{NO}_2$  with  $m/z$  86 and the likely structure  $[\text{HCH}=\text{HC}-\text{CH}-\text{NO}_2]^-$  is formed. Theoretically, the ion  $m/z$  86 from  $[\text{M} - 1]^-$  of 3,5-DFB- $\text{NO}_2$  could be the ion  $m/z$  66, i.e.  $\text{N}=\text{C}-\text{C}=\text{C}-\text{O}^-$ , clustered with HF via hydrogen bonding.

The reasons for these unusual fragmentations could be the inability of the  $[\text{M} - 1]^-$  parent ions of  $\alpha$ -nitrotoluene analogs to form the nitrite ion and the corresponding stable benzyl radical under CID conditions because of the preceding loss of H from the methylene group during the ECNICI, and the formation of  $\text{F}^-$ -molecule complexes [11]. Remarkably, nitroaromates may show usual tandem mass spectrometric fragmentation such as 1-nitro-2,4,6-trimethoxybenzene, which yields abundantly nitrite [8]. But they may also show unusual fragmentation such as 1,3,5-trinitrobenzene in which the *ortho* effect leads to the loss of a OH radical from the nitro group and the aromatic ring [19].

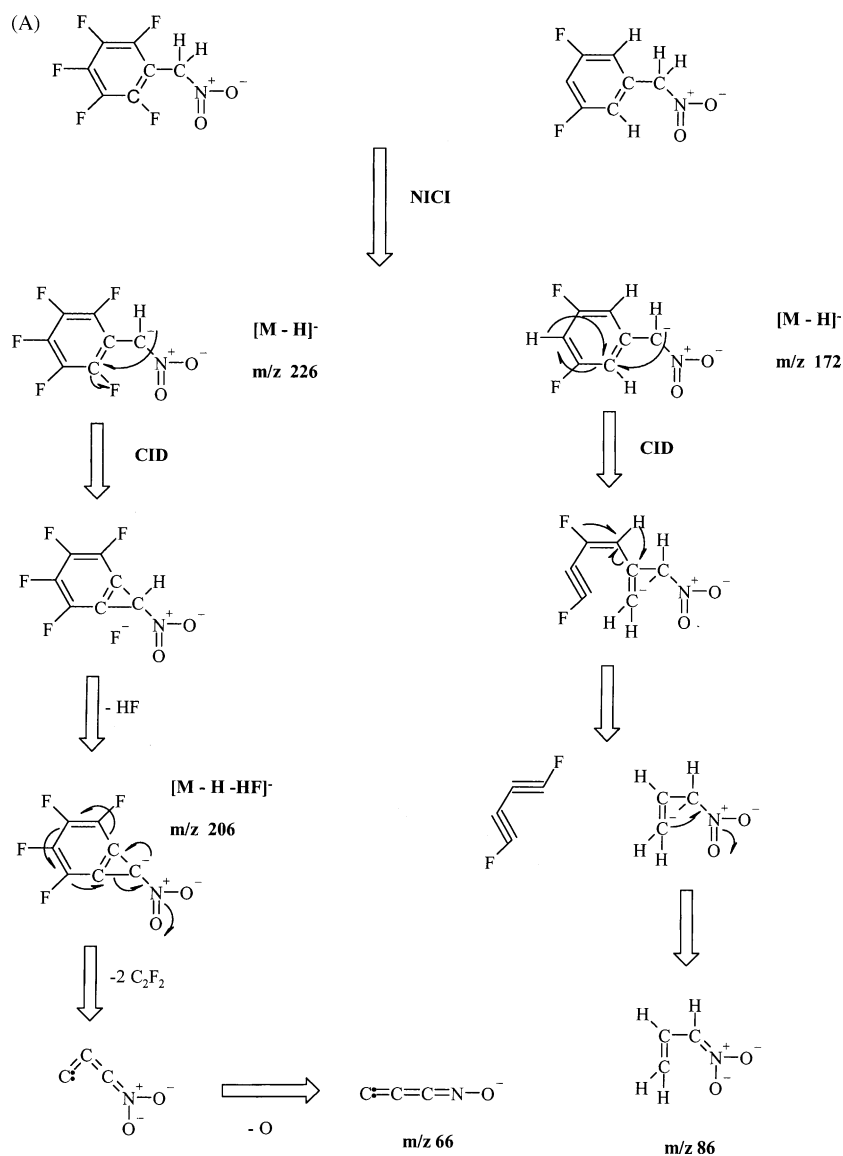


Fig. 5. Proposed mechanisms for CID occurring in  $\alpha$ -nitrotoluene analogs exemplified for  $\alpha$ -nitro-1,2,3,4,5-pentafluorotoluene and  $\alpha$ -nitro-3,5-difluorotoluene. Intramolecular nucleophilic aromatic substitution starting by the attack (A) of the carbanion and (B) of the nitro-anion.

### 3.2. Considerations from the quantitative point of view

Nitrite concentrations  $[\text{NO}_2^-]$  can be accurately calculated by multiplying the concentration of the internal standard  $[\text{}^{15}\text{NO}_2^-]$  with the peak area ratio of  $m/z$  46 to  $m/z$  47 ( $R_{46/47}$ ) measured by GC-Q-MS by using Eq. (2). Fig. 6 demonstrates the validity of Eq. (2). On the other hand, Fig. 6 shows that  $[\text{NO}_2^-]$  cannot be accurately calculated by simply multiplying  $[\text{}^{15}\text{NO}_2^-]$  with the peak area ratio of  $m/z$  66 to  $m/z$  67 ( $R_{66/67}$ ) measured by GC-QqQ-MS by using Eq. (3) for  $R_{66/67}$  values above 1.0, because the curve of  $[\text{NO}_2^-]$  becomes hyperbolic [20]. This phenomenon is mainly attributed to the  $^{13}\text{C}$  isotope of the anion  $\text{C}_3\text{NO}$  originating from  $\text{NO}_2^-$ , which considerably contributes to the anion  $\text{C}_3^{15}\text{NO}$  that originates from the internal standard, i.e.  $^{15}\text{NO}_2^-$ . Knowl-

edge of the composition of the anions with  $m/z$  66 and  $m/z$  67 now enables to develop an equation, which considers the contribution of the  $^{13}\text{C}$  isotope and allows calculation of true  $[\text{NO}_2^-]$  from the  $R_{66/67}$  measured in GC-QqQ-MS analyses.

$$[\text{NO}_2^-] = [\text{}^{15}\text{NO}_2^-]R_{46/47} \quad (2)$$

$$[\text{NO}_2^-] = [\text{}^{15}\text{NO}_2^-]R_{66/67} \quad (3)$$

In a sample containing  $\text{NO}_2^-$  at a concentration of  $[\text{NO}_2^-]$  and  $^{15}\text{NO}_2^-$  (with an isotopic purity of 98 at.% at  $^{15}\text{N}$  in the present study) at a concentration of  $[\text{}^{15}\text{NO}_2^-]$ , the ratio  $R_{66/67}$  can be expressed by Eq. (4), when the contribution of  $^{17}\text{O}$  from the anion  $\text{C}_3\text{NO}$  ( $m/z$  66) to the anion  $\text{C}_3^{15}\text{NO}$

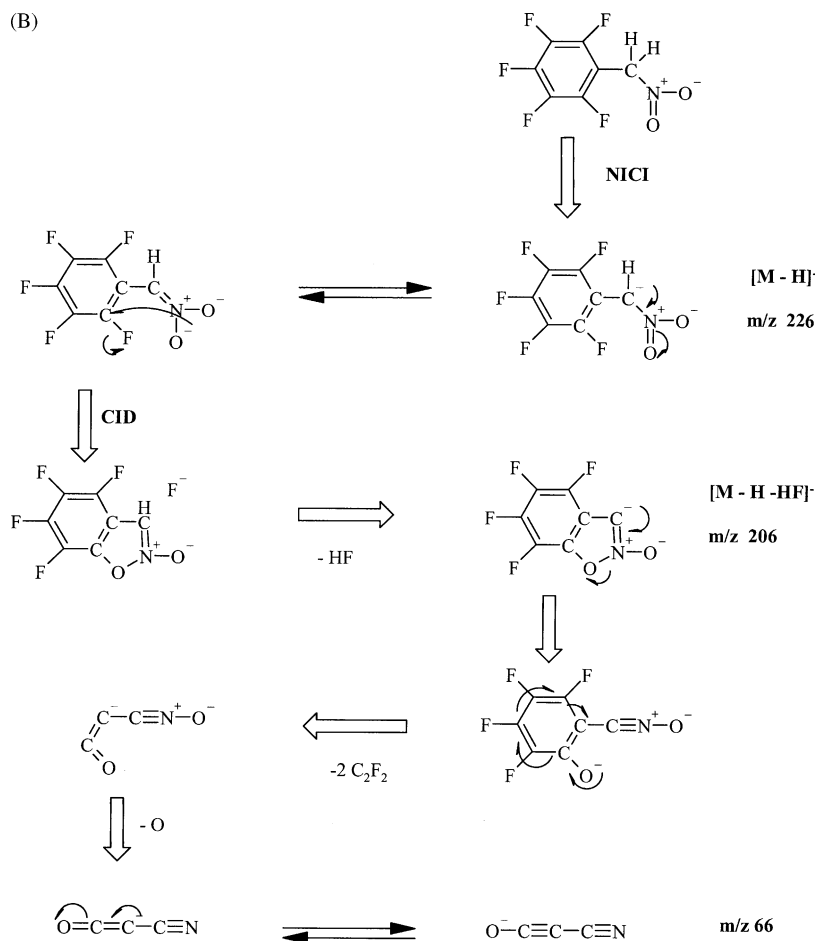


Fig. 5. (Continued).

( $m/z \ 67$ ) is neglected.

R66/67

$$= \frac{0.9963 \times [\text{NO}_2^-] + 0.02 \times [^{15}\text{NO}_2^-]}{0.0037 \times [\text{NO}_2^-] + 0.98 \times [^{15}\text{NO}_2^-] + 3 \times 0.011 \times [\text{NO}_2^-]} \quad (4)$$

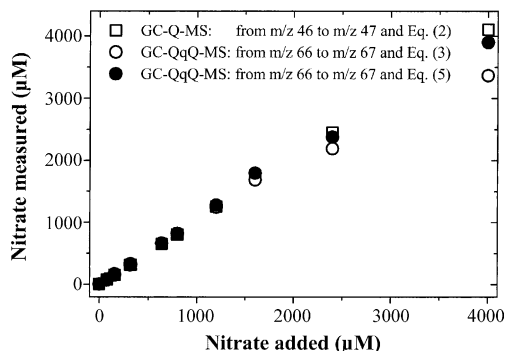


Fig. 6. Relationship between measured and added nitrate. Nitrite from reduced nitrate [7] was measured by GC-Q-MS and GC-QqQ-MS as pentafluorobenzyl derivative. Nitrite concentrations were calculated by means of three different Equations. The concentration of the internal standard  $^{15}\text{NO}_3^-$  was  $800 \mu\text{M}$ . For more details see the text. Instrument, TSQ 7000.

Rearrangement of Eq. (4) leads to Eq. (5):

$$[\text{NO}_2^-] = [^{15}\text{NO}_2^-] \times \frac{0.98 \times \text{R66/67} - 0.02}{0.9963 - 0.0367 \times \text{R66/67}} \quad (5)$$

where the term  $3 \times 0.011[\text{NO}_2^-]$  represents the contribution of the  $^{13}\text{C}$  isotope.

Fig. 6 shows that calculation of  $[\text{NO}_2^-]$  by means of Eq. (5) and the measured ratios R66/67 from GC-QqQ-MS analyses yields almost the same results as the measurement of the R46/47 by GC-Q-MS analysis and use of Eq. (2).

#### 4. Conclusions

Collision of argon atoms with  $[\text{M}-1]^-$  of  $\alpha$ -nitrotoluene analogs in the collision chamber of a GC-QqQ-MS instrument induces an unusual fragmentation that causes cleavages of C-C-bonds at C-2/C-3 and C-1/C-6 of the aromatic ring and produces unusual anions. Although this CID occurs in all  $\alpha$ -nitrotoluene analogs, the further CID process depends upon the presence and position of F atoms in the benzene ring.  $\alpha$ -Nitrotoluene analogs carrying F in *ortho* position form the



product anion  $C_3NO$  with  $m/z$  66.  $\alpha$ -Nitrotoluene analogs not carrying F at all or carrying F in other ring positions form the product anion with  $m/z$  86. This unusual CID can be utilized to unequivocally identify and accurately quantify nitrite in biological fluids by GC–QqQ-MS after its conversion by PFB bromide to PFB- $NO_2$ . Tandem mass spectrometry carried out in a triple-stage quadrupole mass spectrometer may be useful in the quest for interstellar cumulenes and heterocumulenes in the gas phase in the laboratory.

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