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Unusual collision-induced dissociation of fluorated and non-fluorated α-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 α-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 α-nitro-2,3,4,5,6-pentafluorotoluene ($C_6F_5CH_2{-}NO_2$) and α-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 α-nitrotoluene ($C_6H_5CH_2{-}NO_2$) and α-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 α-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.

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 α-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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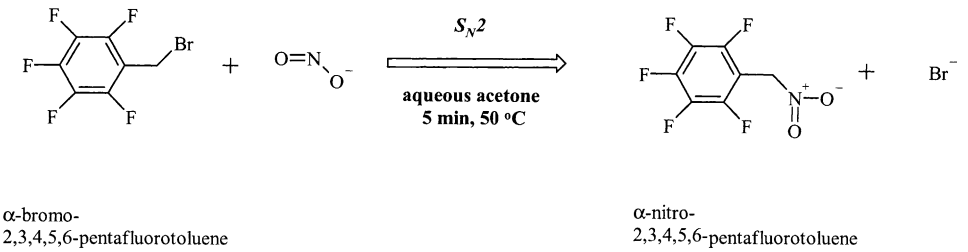

Fig. 1. Nucleophilic substitution reaction (S_N2) between α-bromo-2,3,4,5,6-pentafluorotoluene (PFB-Br) and nitrite (NO_2^-) in aqueous acetone to form α-nitro-2,3,4,5,6-pentafluorotoluene (PFB-NO_2).

Cleavage of C–C-bonds during CID of anions produced by ECNICI 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 trimethylsilanol 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 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 carboxylic group [6].

In previous work, we observed unexpectedly that CID of $[M-1]^-$ of $C_6F_5CH_2{-}NO_2$ in tandem mass spectrometry performed in a gas chromatograph triple-stage quadrupole (TSQ) mass spectrometer (GC–QqQ-MS) did not result in formation of 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 $C_6F_5CH_2{-}^{15}NO_2$ 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 $C_6F_5CH_2{-}NO_2$ 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-NO_2 the aromatic ring must have been cleaved. This unusual fragmentation prompted us to investigate the CID of $C_6F_5CH_2{-}NO_2$ 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}N- and ^{18}O-labelled $C_6F_5CH_2{-}NO_2$ analogs and α-nitrotoluene analogs with distinctly positioned fluorine atoms (F) in the benzene ring.

2. Experimental

2.1. Materials and chemicals

Sodium [^{15}N]nitrite (98 at.% at ^{15}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). $H_2{}^{18}O$ (99 at.% at ^{18}O) was obtained from CAMPRO Scientific (Emmerich, Germany). ^{18}O-Labelled nitrite was prepared by dissolving solid sodium nitrite (6.9 mg) in $H_2{}^{18}O$ (100 μl) 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 $O{=}N{-}O^-$ (*m/z* 46; 24%), $O{=}N{-}^{18}O^-$ (*m/z* 48; 48%), and $^{18}O{=}N{-}^{18}O^-$ (*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 μl) of aqueous solutions of unlabelled and labelled nitrite (10 mM) were diluted with acetone (400 μl), the bromide agent (10 μl) was added, and the reaction mixtures were allowed to stand at 50 °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 × 0.25 mm i.d., 0.25-μm 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 °C for 1 min then increased to 280 °C at a rate of 30 °C/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