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Electron ionization mass spectral studies of phenoxide derivatives of mustards: Implications for analysis to support chemical weapons convention

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ABSTRACT

Mass spectrometric (MS) analytical features of phenoxide derivatives of sulfur and nitrogen mustards were described. Electron ionization (EI) mass spectra of title chemicals with possible fragmentation routes were investigated via analysis of fragment ions of deuterated analogs, MS–MS experiments and density functional theory calculations. EI-MS and EI-MS/MS analysis revealed phenoxide, ethane, CO, CS and H_2S exclusions, α-cleavages, retro Diels–Alder cycloaddition, hydrogen rearrangements and a previously unknown intramolecular Claisen-type rearrangement. The results would be valuable during toxic chemical destruction monitoring in support of chemical weapons convention (CWC) and for the verification of state-parties activities, based on CWC context.

1. Introduction

Sulfur and nitrogen mustards are potent vesicants, blisters and cytotoxic agents. Sulfur mustard, H, was used during World War I and subsequently the Iran-Iraq war. Sesquimustard, Q, and oxygen mustard, T, are more vesicant and persistent than sulfur mustard [1]. On the other hand nitrogen mustards, HN1, HN2 and HN3, analogs of sulfur mustard, are highly reactive alkylating agents. Interestingly various nitrogen mustard derivatives have been widely used as anti-tumor drugs in cancer chemotherapy [2–4]. Monitoring and analysis of mustards, their precursors and degradation/reaction products in environmental and biological samples is an important part of verification activities in support of the chemical weapons convention (CWC), which came into force on 29 April 1997. Sulfur and nitrogen mustards are placed in CWC as schedule 1A.04 and 1A.06, respectively. The Organization for the Prohibition of Chemical Weapons (OPCW) is responsible for CWC implementation through executing its strict verification protocols. Always verification activities requires chemical analysis which involves two steps: (1) collection of samples from production, storage and suspected sites such as waste containers during inspections and (2) on- or off-site analysis of collected samples by appropriated methods and techniques. Mass spectrometer coupled with gas chromatograph (GC/MS) is an indispensable analytical instrument for the detection and identification of mustards during on- and/or off-site analyses [5]. In case of off-site analysis the samples are sent to at least two designated laboratories which are certified by OPCW Director-General after participation in proficiency tests (PTs) [6]. For unequivocal identification of CWC-related chemicals in real samples or PTs, the availability of mass spectra and interpretation skills are essential requirements. Despite global efforts, due to the extreme toxicity of the CWC-related chemicals, there are a limited number of research reports available for such chemicals [7–13]. Toxic chemical destruction can be defined as a method involving the conversion of toxic chemicals into harmless and non-toxic products by suitable chemical reaction [14,15]. Aqueous solution of sodium phenoxide has been used as decontamination solution [16]. One of the great advantages of this solution is that it converts the toxic chemicals completely into non-toxic compounds during short time. Detection and identification reaction products of mustards with sodium phenoxide solution by gas chromatography–mass spectrometry would be valuable during chemical weapons destruction monitoring and during verification activities. As shown in Scheme 1, reaction products of sodium phenoxide with mustards can be phenoxide derivatives. They are considered as existing markers of blistering agents in suspected

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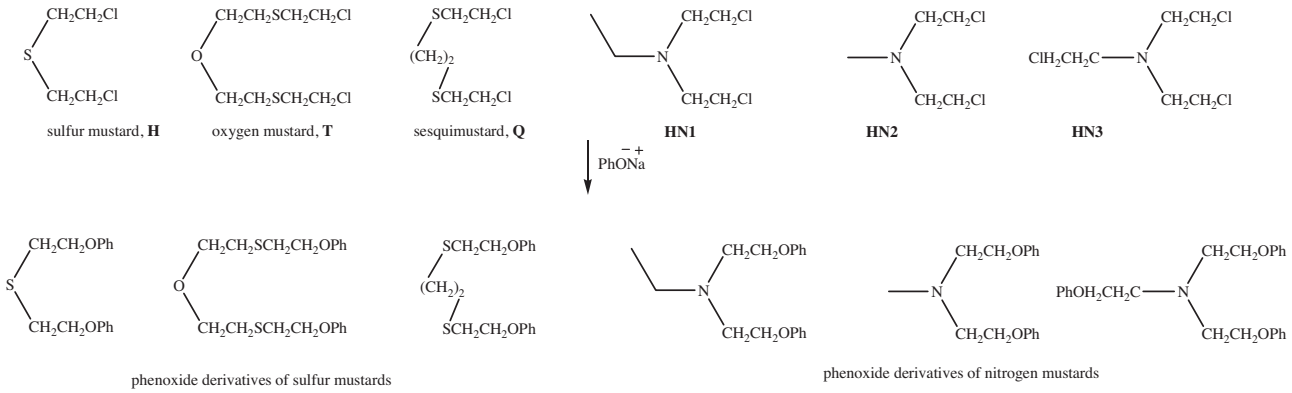

Scheme 1. General structures and pathway for the reaction of mustards with sodium phenoxide.

place, for example in a waste container. At the time being there is no mass spectrum for such chemicals in commercial library databases and OPCW Central Analytical Database (OCAD) [17]. In the 35th OPCW proficiency test conducted in spring 2014, one of the spiking chemicals was tris (2-phenoxyethyl)amine which is the reaction product of HN3 with phenoxide anion. This spiking chemical was not identified by eleven out of sixteen regular participating laboratories. This chemical elutes quite late, typically at retention times slightly over 30 min with the normal columns in the typical GC condition used in PTs. On the other hand, no availability of mass spectral data and quite different EI-MS fragmentation pathway for this compound may be formed some reasons for such high percentage (68%) of false negative identification [18]. As continuation of our research on analysis of CWC-related chemicals in support of CWC, we have carried out a general microsynthesis protocol to prepare phenoxide derivatives of mustards (Scheme 1). Subsequently, electron ionization (EI) mass spectra of these chemicals, with possible fragmentation routes, were investigated via analysis of fragment ions of deuterated analogs, MS–MS experiments and density functional theory (DFT) calculations.

2. Experimental

2.1. Reagents and chemicals

All chemicals required for the microsynthesis of phenoxide derivatives of sulfur and nitrogen mustards were purchased from Sigma–Aldrich (St. Louis, MO, USA), Fluka (Neu-Ulm, Germany), and Merck (Darmstadt, Germany), and were used as received. Deuterium-labeled phenols were synthesized by use of deuteronation of phenol using D_2SO_4 at room temperature [19]. As shown in Scheme 2, proton exchange on oxygen and *ortho*/*para* positions with deuterium yields deuterium-labeled phenol derivatives. EI-MS spectra of deuterium-labeled phenol derivatives and their corresponding phenyl acetate derivatives show that major products of the reaction are d_3- and d_4-phenol (electronic supplementary material, Figs. 1S and 2S).

2.2. GC/MS and GC/MS–MS analysis

GC/MS analyses were performed using an Agilent 6890N gas chromatograph equipped with a 5973 quadrupole Mass Selective Detector (Agilent Technologies, Inc., Santa Clara, CA, USA), a HP-5MS (5% phenyl, 95% dimethylpolysiloxane, J&W Scientific) capillary column (30 m, 320 μm i.d. and 0.25 μm film thickness), and helium as carrier gas at constant flow of 1.8 mL min^{-1}. The oven temperature was set at 40 °C for 3 min and then was increased to 280 °C with ramp of 10 °C/min and held at 280 °C for 6 min. The samples were injected in splitless mode at an injection temperature of 250 °C. The temperatures of the EI source and analyzer were kept at 230 and 150 °C, respectively. The scan range was *m*/*z* 35–500. GC/MS–MS analyses were performed using an Agilent 7890N gas chromatograph interfaced to a 7000A triple quadruple mass spectrometer (Agilent Technologies, Inc., Wilmington, DE, USA). GC conditions were as noted above. The ionization energy was set at 70 eV in both MS instruments. MS–MS analyses were carried out using nitrogen as collision gas, at collision energy of 10 eV and source temperature of 230 °C.

2.3. ESI-MS and ESI-MS–MS analysis

LC/MS analysis was performed on an Agilent 1200 LC system (Agilent Technologies, Inc., Waldbronn, Germany) equipped with Agilent 6410 triple quadrupole tandem mass spectrometer and managed by a Mass Hunter workstation (Agilent Technologies Inc., CA, USA). The column used for separation was an Agilent rapid resolution HT zorbax SB-C18 (3 mm × 150 mm, 3.5 μm) (Agilent Technologies Inc., Santa Clara, CA, USA). The column temperature was set at 25 °C. A gradient mobile phase of (A) water plus 20 mM formic acid and (B) acetonitrile plus 20 mM formic acid was used. The initial condition was set at 5% of B. The following solvent gradient was applied: from 95% A and 5% B to 5% A and 95% B within 20 min, hold for 10 min. Flow rate was set at 0.25 mL min^{-1} and 4 μL of samples were injected using autosampler. The electrospray ionization (ESI) and fragmentor voltages were set at 4000 and 60 V, respectively. The ultra-high pure nitrogen was used as the nebulizer and collision gas. The heated capillary temperature was maintained at 300 °C. The drying gas flow rate and nebulizer gas pressure were 10 L min^{-1} and 40 psi, respectively. Mass spectra were obtained by scanning from *m*/*z* 80–1000 with 0.5 s scan time.

2.4. NMR analysis

A Bruker (Avance DRX-250 MHz, Germany) NMR instrument was employed for ^1H and ^{13}C NMR experiments. All spectra were recorded at ambient temperature using $CDCl_3$ as a solvent.

2.5. Computational details

All geometry optimizations and frequency calculations of all species were carried out using the Gaussian 03 program [20]. Density Functional Theory (DFT) with the Becke three parameters hybrid functional (DFT-B3LYP) calculations were performed with a 6-31+G (d, p) basis set for all atoms. Vibrational frequencies were calculated at the same level to ensure that each stationary point is a real minimum. Harmonic-oscillator approximation was also used for the thermodynamic partition functions. After