Journal of Chromatography A, 1227 (2012) 163–173

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Characterization and study of piperazinium salts, degradation products of nitrogen mustards by nuclear magnetic resonance spectroscopy and liquid chromatography–mass spectrometry

Jin Young Lee*, Yong Han Lee, Yong Gwan Byun

Agency for Defense Development (ADD), PO Box 35-5, Yuseong-gu, Daejeon, 305-600, South Korea

ARTICLE INFO

Article history:
Received 19 September 2011
Received in revised form
26 December 2011
Accepted 30 December 2011
Available online 11 January 2012

Keywords:
Chemical warfare agent
Nitrogen mustard
Piperazinium salt
NMR
LC–MS

ABSTRACT

We synthesized and analyzed the degradation products, piperazinium salts from bis(2-chloroethyl)methylamine (HN2) and bis(2-chloroethyl)ethylamine (HN1) using ^{1}H nuclear magnetic resonance (NMR) and liquid chromatography–mass spectrometry (LC–MS). Piperazinium salt is the major degradation product of HN2, not *N*-methyldiethanolamine above a concentration of 0.01 M in water and is a non-scheduled chemical that may be generally assumed relevant to the Chemical Weapons Convention (CWC) within the context of the Organization for the Prohibition of Chemical Weapons (OPCW) proficiency test. In verification analysis, ^{1}H NMR offers real-time information about degradation pathway of nitrogen mustards and LC–MS is expected to play an increasing role in the analysis of environmental samples for the degradation products of chemical warfare agents.

1. Introduction

Nitrogen mustards, bis(2-chloroethyl)ethylamine (HN1), bis(2-chloroethyl)methylamine (HN2) and tris(2-chloroethyl)amine (HN3) are blistering agents, and belong to the same class of chemical agents with vesicant properties as lewisites and sulfur mustards [1,2]. Nitrogen mustards were developed in the 1930s and stockpiled during World War II for military use. However, the use of such chemical warfare agents (CWA) is now prohibited by Chemical Weapons Convention (CWC). According to this treaty, the development, production, stockpiling and use of chemical weapons are prohibited [3–5]. In 1995, AUM Shinrikyo terrorists released the nerve agent sarin in the Tokyo subway system [6]. This real incident points to the great threat of terrorism by chemical warfare. In addition, the disarmament of stockpiled chemical weapons is now an urgent issue. In both chemical warfare terrorism and disarmament, the detection and identification of CWA is important for verifying their presence and possible exposure to casualties.

The transformations undergone by the nitrogen mustards in water were chosen as the first point of attack since a detailed knowledge of these reactions seemed essential to an understanding of the general chemistry of the nitrogen mustards. Furthermore, the transformations of the nitrogen mustards in water are of interest from the biochemical point of view, since water is a major constituent of all biological systems. Finally, the possibility that the nitrogen mustards might be employed in chemical warfare as water contaminants made it imperative to study the chemical reactions exhaustively undergone by these agents in water.

Nitrogen mustards usually undergo hydrolysis in the presence of water to form relevant ethanolamines. *N*-ethyldiethanolamine (EDEA), *N*-methyldiethanolamine (MDEA) and triethanolamine (TEA), are produced from bis(2-chloroethyl)ethylamine (HN1), bis(2-chloroethyl)methylamine (HN2) and tris(2-chloroethyl)amine (HN3), respectively. Piperazinium salts are also degradation products of the nitrogen mustards and non-scheduled chemicals that may be generally assumed relevant to the CWC within the context of the OPCW proficiency test [7–9].

Surprisingly few attempts have been made to characterize and study piperazinium salts in solution. Golding and Kebbell reported the study of HN2 nitrogen mustard by NMR [10]. In this paper as an extension of this work, we have investigated piperazinium salts, degradation products of the nitrogen mustards (HN1, HN2) via ^{1}H nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography–mass spectrometry (LC–MS). NMR spectroscopy offers real-time information of degradation products of nitrogen mustards and LC–MS is being increasingly applied to the analysis

---

* Corresponding author. Tel.: +82 42 821 0483; fax: +82 42 823 3400.
E-mail address: marlintime@add.re.kr (J.Y. Lee).

0021-9673/$ – see front matter. Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.12.107

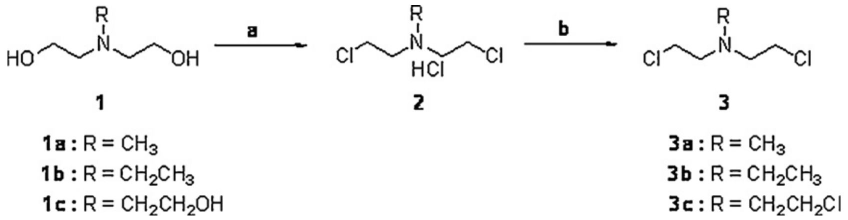

Fig. 1. The preparation of compound **3**: (a) $SOCl_2$/benzene; (b) Na_2CO_3/diethylether.

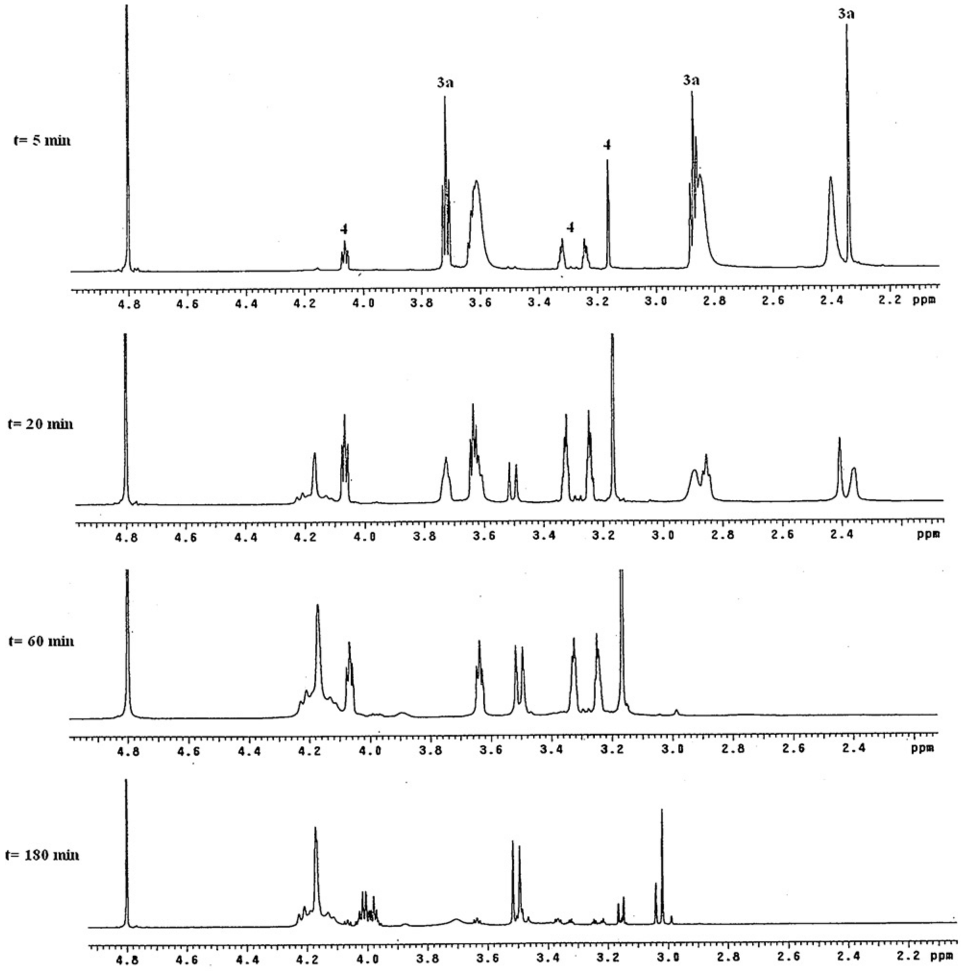

Fig. 2. The change of ^{1}H NMR spectra of bis(2-chloroethyl)methylamine (HN2) **3a** in D_2O. (a) $t = 5$ min, (b) $t = 20$ min, (c) $t = 60$ min, and (d) $t = 180$ min.

of CW agent residues using atmospheric pressure ionization techniques [11–14]. In addition to advantages with regard to sample preparation, LC–MS may identify polar degradation products that are not seen using GC–MS analysis [11,13].

2. Experimental

2.1. Reagents and materials

N-ethyldiethanolamine (EDEA) was obtained from the Tokyo Chemical Industry (Tokyo, Japan). *N*-methyldiethanolamine (MDEA) and triethanolamine (TEA) were obtained from Wako Pure Chemical (Osaka, Japan). Reagents for synthesis were purchased from Sigma–Aldrich (St. Louis, MO, USA). Gradient-grade solvents (benzene, diethylether) were purchased from Merck (Darmstadt, Germany).

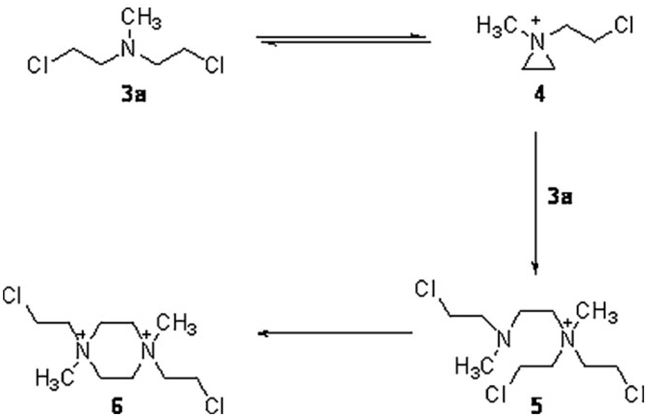

Fig. 3. The plausible reaction mechanism of bis(2-chloroethyl)methylamine (HN2) **3a**.