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Hydrolysis of Sesquimustards

Timothy D. St Quintin,[A] *D. Ralph Leslie*[B] *and J. Grant Collins*[A,C]

[A] School of Chemistry, University College, University of New South Wales, Australian Defence Force Academy, Canberra 2600, Australia.

[B] Aeronautical and Maritime Research Laboratory, Defence Science and Technology Organisation, P.O. Box 4331, Melbourne 3001, Australia.

[C] Author to whom correspondence should be addressed (email: g-collins@adfa.edu.au).

The hydrolysis of the sesquimustards 1,2-bis(2-chloroethylthio)ethane (QN2) and 1,3-bis(2-chloroethylthio) propane (QN3) has been studied by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. For both sesquimustards, stable cyclic sulfonium ions were observed in hydrolysis experiments in a 1 : 1 mixture of [D_6]acetone and D_2O. The cyclic sulfonium ions, which persist in the aqueous solution for up to a week, are likely to retain some toxicity and could possibly be used as markers for sesquimustards in the analysis of mustard-contaminated soil for chemical weapons treaty verification. The formation of a macrocyclic oxadithiaether was also demonstrated for QN2 but not QN3.

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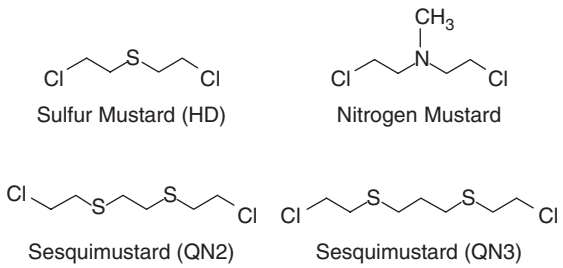
Introduction

Due to its use as a chemical warfare agent, the reactions of sulfur mustard (2,2′-dichlorodiethylsulfide; HD) have been extensively studied.[1–8] As a highly lipid-soluble molecule, sulfur mustard can rapidly penetrate the skin and alkylate a range of biomolecules. Vesication (blistering of the skin) results from the cleavage of the basal cell membrane from the basement membrane (the two lower layers of the epidermis).[9] However, the mechanism of vesication is not well understood. One theory proposes that, via a sequence of enzymatic processes, DNA alkylation leads to the reduction in cellular levels of nicotinamide adenine dinucleotide (NAD^+), an important component of glycolysis. The disruption of glycolysis leads, ultimately, to the activation of cellular proteases that are thought to cause disruption to the dermal–epidermal attachments, resulting in blister formation.[10,11] Alternatively, it has been proposed that DNA interstrand cross-linking prevents the unwinding of the DNA helix required for protein, RNA, and DNA synthesis.[11] Consequential cell death results in separation of the epidermis from the dermis, forming blisters.

Sesquimustards, Scheme 1, were first described in the literature in 1921.[12] Of the sesquimustards, 1,2-bis(2-chloroethylthio)ethane (QN2) and 1,3-bis(2-chloroethylthio) propane (QN3) have the greatest vesicant power.[13] Although QN2 and QN3 have significantly greater vesicant properties than HD, sesquimustards have to date not been stockpiled or used as chemical warfare agents, primarily due to their very low vapour pressures. However, dissemination of these agents will produce a highly persistent liquid contact hazard.



Scheme 1. Structure of sulfur mustard (HD), nitrogen mustard, and the sesquimustards (QN2 and QN3).

Consequently, as has been the case for HD, it is now important to develop an understanding of the reactions of sesquimustards with water and various biomolecules in order to develop antidotes and/or pretreatments.

The mechanism of hydrolysis of HD has been extensively studied,[1–5] as its substitution by biological nucleophiles shares a common reaction mechanism and also because water is a major component of biological systems. Kinetic and mechanistic studies have shown that HD is hydrolyzed by the initial formation of a transient cyclic sulfonium ion with the loss of a chloride ion.[3] The sulfonium ion then rapidly reacts with water, forming 2-chloroethyl-2-hydroxyethylsulfide and a hydrogen ion. The second chloroethyl group is hydrolyzed by the same mechanism to give thiodiglycol. However, for the sesquimustards, there is the possibility for the formation of larger-ringed cyclic sulfonium ions through the nucleophilic attack of the sulfur atom distal to the carbon bearing the chloro group. These larger ringed cyclic sulfonium ions

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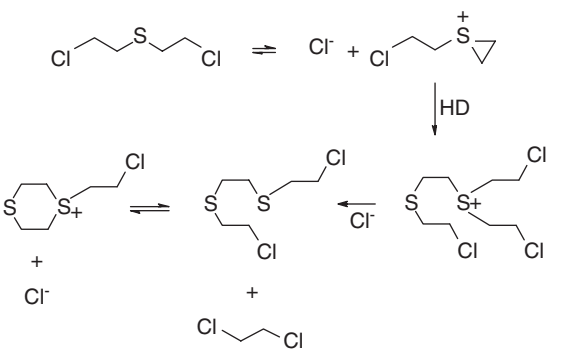
should be considerably more stable than the 3-membered ring formed by HD. Indeed, 2-chloroethyl-1,4-dithianium has been recently identified, and a pathway for its formation proposed (Scheme 2), as the major decomposition product of HD in storage containers.[14] It is important to confirm the formation and stability of the cyclic sulfonium ions formed by sesquimustards, and subsequently, their physiological effects and toxicities. In this paper we present the results of an NMR study of the hydrolysis of QN2 and QN3.

Results and Discussion

Hydrolysis of QN3

Figure 1 shows the ^{1}H NMR spectra of QN3 at various times after it was dissolved in a 1 : 1 solution of D_2O and [D_6]acetone. At 10 minutes the major peaks are assigned, using the nomenclature shown in Figure 2, to the H1 (2.75), H2 (1.90), Hα (2.93), and Hβ (3.77 ppm) of QN3. After 30 days, the reaction has proceeded to one dominant product, QN3–OH: H1′ (2.70), H2′ (1.88), Hα′ (2.72), and Hβ′ (3.73 ppm). The structure of QN3–OD was confirmed by gas chromatography–mass spectroscopy (GCMS). During the course of the reaction, small but significant additional peaks are also observed in the ^{1}H NMR spectra, e.g. 4.16, 3.18, and 2.27 ppm. As the purity of the QN3 sample was determined to be >99% by GC analysis, these peaks must represent intermediates in the hydrolysis reaction (see Scheme 3). These peaks are not consistent with QN3–III, the expected product of the first step of the hydrolysis.

In the ^{13}C NMR spectrum of the mixture after 7 days (see Fig. 3), resonances from QN3 and the QN3–OH product are observed along with one apparent set of six resonances of



Scheme 2. Proposed pathway by which 2-chloroethyl-1,4-dithianium is formed from HD.[14]

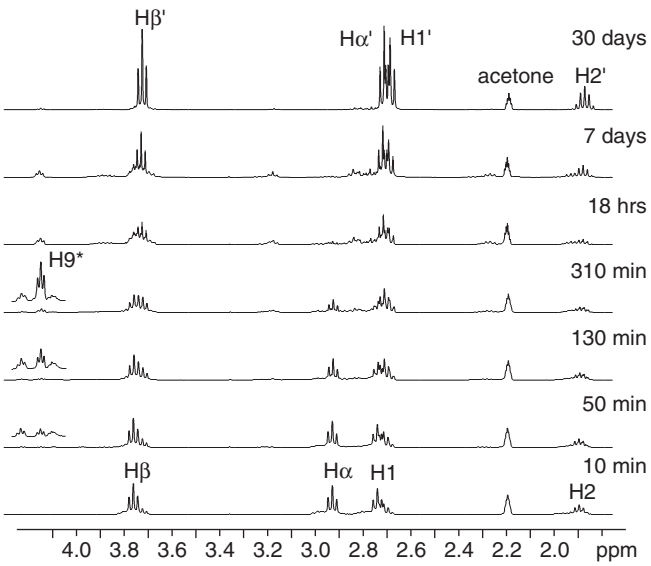


Fig. 1. ^{1}H NMR spectra of QN3 in 1 : 1 [D_6]acetone/D_2O at 25°C as a function of time after the addition of QN3 to the [D_6]acetone/D_2O mixture. H9* indicates the H9 resonance assigned to QN3–VI at 4.16 ppm.

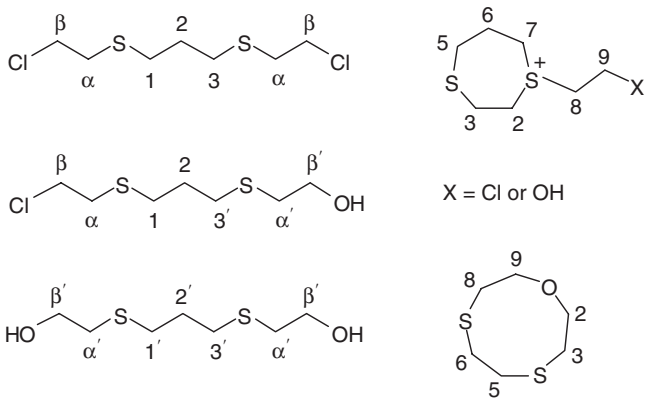
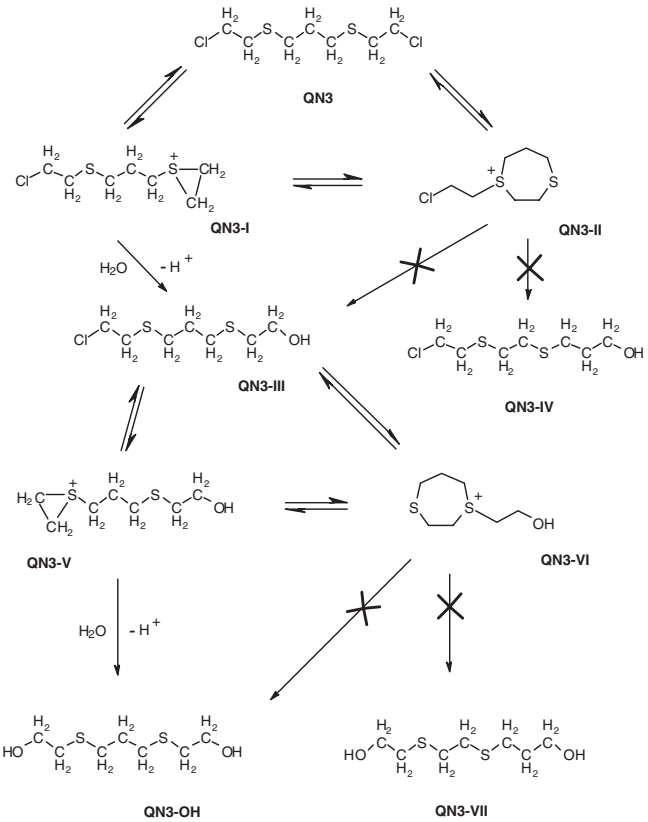


Fig. 2. Structure and atom numbering of QN3, QN3–III, and QN3–OH (top to bottom on left), the cyclic sulfonium ions QN3–II (X = Cl) and QN3–VI (X = OH), and 1-oxa-4,7-dithiacyclononane.



Scheme 3. Proposed reaction pathway for the hydrolysis of QN3.

similar significant intensity that do not belong to the QN3. The three resonances in the 39–45 ppm region are consistent with carbon atoms adjacent to either chlorine atoms or a positively charged sulfur atom.[4] The resonances from carbon atoms next to an oxygen atom occur significantly further downfield (50–65 ppm). QN3–VI is the only possible intermediate that would be expected to give three, and only three, resonances in the 39–45 ppm region. The assignment of the six ^{13}C resonances to QN3–VI (see Table 1) was confirmed by the ability to assign corresponding ^{1}H resonances to the ^{13}C resonances in a GHSQC (pulsed field gradient, heteronuclear single-quantum correlation) spectrum, and the subsequent confirmation of the H2–H3, H5–H6–H7, and H8–H9 *J*-coupled spin systems in a ^{1}H–^{1}H DQFCOSY (double-quantum filtered correlated spectroscopy) spectrum. The seventh carbon resonance (C5) of QN3–VI was identified under the acetone peaks.

Although the formation of QN3–VI was established, there was no evidence of QN3–VII in the 43rd day ^{13}C NMR spectrum. Therefore, QN3–VI must only lead to QN3–OH, and without the formation of another stable intermediate, since attack on the carbons positioned α to the S^+ should result in the observation of a significant amount of QN3–VII. This suggests that the mechanism of hydrolysis for the stable sulfonium ion QN3–VI, and hence QN3–II (should it form), through QN3–V and QN3–I, respectively, is strongly favoured over direct hydrolysis of QN3–VI and QN3–II. While QN3–VI builds up to a significant extent in the 7th day ^{13}C NMR spectrum, QN3–II does not accumulate to any extent throughout the hydrolysis experiment. This could be due to the ability of QN3–II to undergo an S_N2-type reaction with water to form QN3–VI, which in turn can only undergo hydrolysis. However, this seems unlikely given that the half-life for the hydrolysis of the first 2-chloroethylsulfide group in HD is approximately 12 min,[5] and the rate of hydrolysis of 2-chloroethyl methyl sulfide is 10^6 times that of *n*-butyl chloride.[15] A more likely explanation is that while the formation of QN3–I is greatly favoured over the formation of QN3–II, QN3–VI can build up because there is no competing reaction for the sulfur atom on the β-carbon to the hydroxyl group in QN3–III.

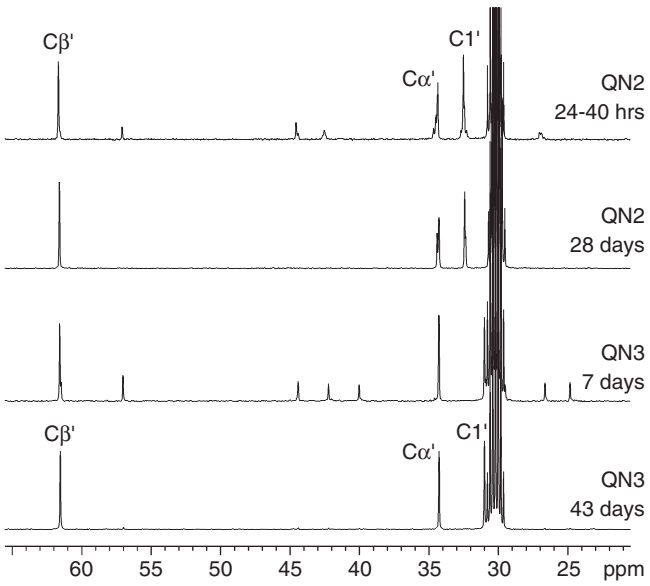


Figure 3. ^{13}C NMR spectra of QN3 and QN2 in 1 : 1 [D_6]acetone/D_2O at 25°C at several time points after the addition of QN3 or QN2 to the [D_6]acetone/D_2O mixture.

Table 1. ^{1}H and ^{13}C NMR assignments (ppm) of the resonances observed in the hydrolysis of QN3 at 25°C in 1 : 1 [D_6]acetone/D_2O[A]

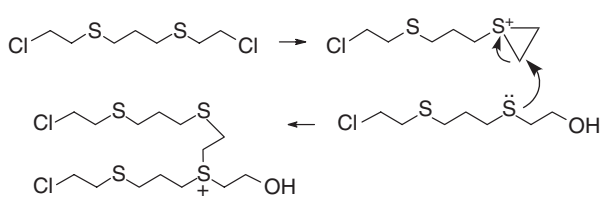
QN3	^{1}H	QN3–OH	^{1}H	^{13}C	QN3–VI	^{1}H	^{13}C
1/3	2.75	1′/3′	2.70	31.0	2	3.89	42.2
2	1.90	2′	1.88	30.5	3	3.18	26.6
α	2.93	α′	2.72	34.3	5	2.84	30.9
β	3.77	β′	3.73	61.5	6	2.27	24.8
					7	3.70	40.0
					8	3.76	44.4
					9	4.16	57.0

[A] Positional numbering refers to the numbering scheme given in Figure 2.

In the ^{1}H NMR spectra of the QN3 hydrolysis, several additional sets of resonances are also observed near the resonances from QN3–VI. These resonances, which appear after about 10 min but then decrease in relative proportion to those of QN3–VI, are most likely due to acyclic sulfonium ions, such as that formed from the reaction of QN3–I with QN3–III (see Scheme 4). The chemical shift for most of the resonances in such ions would be similar to those from QN3–VI or QN3. For example, as shown in Figure 1, either of the triplets (4.25 and 4.11 ppm) near the triplet assigned to H9 of QN3–VI (4.16 ppm) could be assigned to the $S^+CH_2CH_2$–OH of the ion in Scheme 4. The formation of other acyclic sulfonium ions cannot be excluded. Hydrolysis of these potential dimeric sulfonium ions would be through the reverse reaction, to give the episulfonium ion, analogously to the proposed hydrolysis pathway for QN3–VI.

Hydrolysis of QN2

Figure 4 shows the ^{1}H NMR spectra of QN2 at various times after it was dissolved in a 1 : 1 solution of D_2O and [D_6]acetone. After 10 min the major peaks observed are assigned to QN2, while after 7 h the major peaks are assigned to the product QN2–OH (see Scheme 5). Similar to the hydrolysis of QN3, the ^{1}H and ^{13}C NMR (see Fig. 3) spectra showed evidence of long-lived intermediates consistent with cyclic sulfonium ions. The assignment of the ^{1}H resonances and the ^{13}C resonances in a GHSQC spectrum, and the subsequent confirmation of the ^{1}H–^{1}H *J*-coupled spin systems



Scheme 4. Potential acyclic sulfonium ion formed between QN3–I and QN3–III.

in a DQFCOSY spectrum, confirmed the assignment of these resonances to QN2–V. In addition, spin–lattice relaxation time (T_1) experiments recorded after 3 d showed that the resonances assigned to QN2–V have significantly shorter T_1 values (H2 1.1, H3 0.7, H7 1.9, and H8 1.1 s) than those resonances from QN2–OH (H1′ 1.5, Hα′ 2.0, Hβ′ 2.3 s). This is consistent with QN–V being a less flexible cyclic structure. The ^{1}H and ^{13}C NMR chemical shifts for QN2, QN2–OH, and QN2–V are given in Table 2. Similar to the QN3 hydrolysis, a significant build up of QN2–V was observed compared with QN2–II, which did not accumulate to any extent throughout the experiment.

Interestingly, an additional set of peaks is also clearly observed after 6 d that have very similar chemical shifts to the major product QN2–OH (see Fig. 4 and Table 2). In addition, a white precipitate was also observed in the NMR tube after 6 d, which after isolation was shown to account for the additional product peaks in the spectra of the hydrolysis mixture. From the combined NMR data, this additional product was determined to be 1-oxa-4,7-dithiacyclononane (see Figure 2). A satisfactory elemental analysis was obtained for the isolated 1-oxa-4,7-dithiacyclononane. The formation of this macrocycle could result from the intramolecular ring-opening attack by the oxygen of QN2–V, or more likely (given that no evidence of nucleophilic attack at the carbon adjacent to a S^+ has been observed) of QN2–IV.

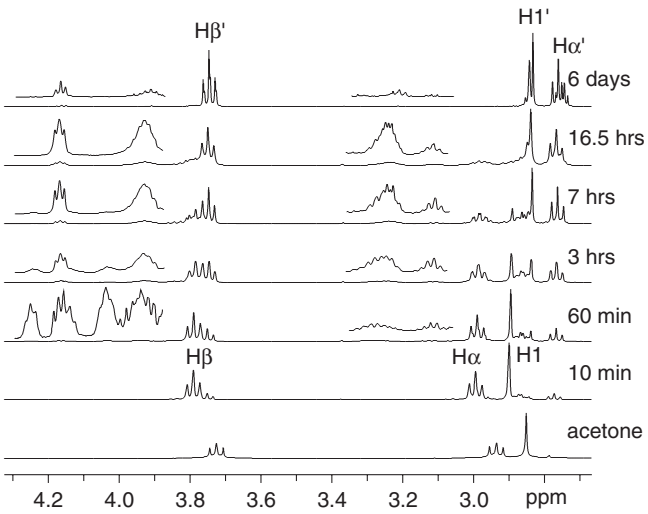
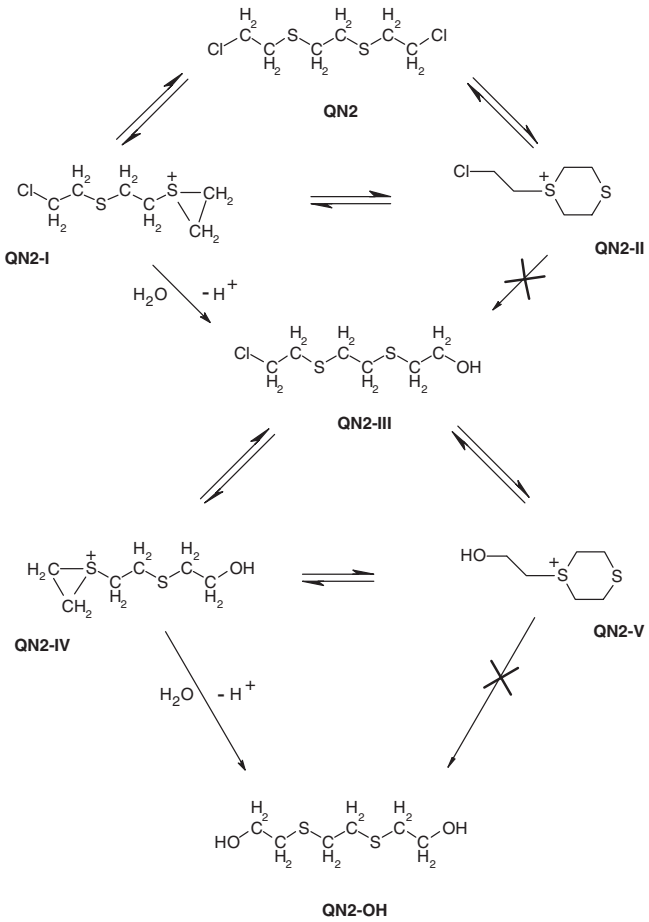


Figure 4. ^{1}H NMR spectra of QN2 in 1 : 1 [D_6]acetone/D_2O at 25°C as a function of time after the addition of QN2 to the [D_6]acetone/D_2O mixture.

Relevance

This paper constitutes the first detailed study of sesquimustard hydrolysis since the Second World War. Such studies are valuable, not only because they yield information about the sesquimustards themselves but, by contrasting the results with those of sulfur and nitrogen mustards, they may also provide insights into the differences in vesicatory action, and hence lead to a better understanding of mustard vesicant properties in general. In this study the formation of long-lived cyclic sulfonium ions has been demonstrated. The sulfonium ions are likely to retain some toxicity, via the reformation of the powerful alkylating agents QN3–V and QN2–IV, and could possibly be used as markers for sesquimustard in the analysis of mustard-contaminated soil for chemical weapons



Scheme 5. Proposed reaction pathway for the hydrolysis of QN2.

Table 2. ^{1}H and ^{13}C NMR assignments (ppm) of the resonances observed in the hydrolysis of QN2 at 25°C in 1 : 1 [D_6]acetone/D_2O[A]

QN2	^{1}H	QN2–OH	^{1}H	^{13}C	QN2–V	^{1}H	^{13}C	QN2-mc[B]	^{1}H	^{13}C
1/2	2.89	1′/2′	2.83	32.5	2/6	3.91	42.5	2/9	3.74	61.6
α	2.98	α′	2.76	34.3	3/5	3.23	27.1/26.9	3/8	2.75	34.5
β	3.78	β′	3.74	61.6	7	3.82	44.5	5/6	2.84	32.4
					8	4.16	57.0			

[A] Positional numbering is analogous to the numbering scheme given in Figure 2.
[B] QN2-mc = 1-oxa-4,7-dithiacyclononane.

treaty verification. The formation of a macrocyclic oxadithiaether was demonstrated for QN2, but not QN3. Whilst the acetone/water solvent used in these studies does not accurately reflect physiological conditions, it remains likely that this macrocycle could still form in vivo.

The biological consequences of the long-lived cyclic sulfonium ions are not known, however, it is possible that they could lead to a greater degree of DNA alkylation for the sesquimustards. As they are cationic they will be electrostatically attracted to polyanionic DNA, thereby leading to an increased sesquimustard concentration within the grooves of DNA. In contrast, the highly reactive three-membered cyclic sulfonium ion formed from sulfur mustard will not be able to effectively concentrate near DNA, as it will rapidly react with the numerous nucleophiles available within human cells. As DNA alkylation is thought to be responsible for the vesicant action of mustards,[10,11] any increase in the relative amount of DNA alkylation may result in greater vesicant properties.

Conclusion

^{1}H and ^{13}C NMR experiments have confirmed that stable cyclic sulfonium ions are formed in the hydrolysis reactions of the sesquimustards 1,2-bis(2-chloroethylthio)ethane and 1,3-bis(2-chloroethylthio)propane. The cyclic sulfonium ions could possibly be used as markers for sesquimustard in the analysis of mustard-contaminated soil for chemical weapons treaty verification.

Experimental

Materials

The 1,*n*-bis(2-hydroxyethylthio) *n*-alkanes ($n = 2$, 3) were provided by Ms S. Pantleidis (Defence Science and Technology Organisation, Melbourne). The deuterated solvents were obtained from Cambridge Isotope Laboratories (Cambridge, MA).

Synthesis of Sesquimustards

Due to the powerful vesicant properties of the sesquimustards extreme caution must be taken in their synthesis and then subsequent handling. Nitrile gloves were worn in addition to the usual protective clothing.

Approximately 120 mg of the corresponding dithioalkanediol was dissolved in a small amount of CH_2Cl_2 (3–4 mL). An excess of thionyl chloride (>6 equivalents) was dissolved in CH_2Cl_2 such that the combined volume was 10 mL, was added dropwise with stirring to the dithioalkanediol. The mixture was refluxed for 4 h. After cooling, the mixture was washed with three 15 mL portions of water, followed by three 10 mL portions of water and three 10 mL portions of 5% $NaHCO_3$ and then once with 15 mL of water. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 overnight, then filtered through glass wool. The CH_2Cl_2 was then removed by evaporation under a stream of nitrogen, and then purified by bulb-to-bulb distillation under reduced pressure. Yields: QN2 68%; QN3 70%. The products were characterized by ^{1}H NMR spectroscopy and the purity of each sesquimustard was determined to be >99% by GCMS. δ_H ($CDCl_3$) QN2: 3.66 (t, *J* 7.7), 2.89 (t, *J* 7.7), 2.81 (s); QN3: 3.65 (t, *J* 7.8), 2.87 (t, *J* 7.8), 2.69 (t, *J* 7.1), 1.89 (m, *J* 7.1).

Hydrolysis Experiments

The sesquimustard hydrolysis experiments were carried out in a NMR tube at 25°C in a solvent of 1 : 1 D_2O/[D_6]acetone. For QN3, a liquid at room temperature, approximately 10 μL of the sesquimustard was added to the NMR tube containing the mixed solvent, just prior to the commencent of the NMR spectra being recorded. For QN2, a solid at room temperature, a small quantity was dissolved in acetone prior to being added to the D_2O in the NMR tube. The final concentration of the sesquimustard in the NMR tube was 40–45 mM.

Instrumentation

The purity of the sesquimustards was determined using a HP6890 GC coupled to a HP5973 mass-selective detector with a 30 m HP-5MS column. NMR spectra were recorded on a Varian UnityPlus-400 spectrometer, operating at 400 MHz for the ^{1}H nucleus, and analyzed using the supplied Varian software VNMR. All NMR experiments were conducted at 25°C. One-dimensional proton spectra were accumulated using a spectral width of 5000 Hz with 16 000 data points and a pulse repetition delay of 2 s. Carbon spectra were accumulated using a spectral width of 25 000 Hz with 32 000 data points and a 1.7 s pulse repetition delay. DQFCOSY experiments were accumulated using 2048 data points in t_2 for 256 t_1 values with a pulse repetition delay of 1.7 s with 64 scans per FID. GHSQC experiments were accumulated with spectral widths of 5000 Hz in the proton dimension and 6500 Hz in the carbon dimension, using 2048 data points in t_2 for 128 t_1 values with a pulse repetition delay of 2.8 s.

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