FFI RAPPORT

CHARACTERISATION OF A MUNITION GRADE LEWISITE MIXTURE

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8) ABSTRACT

The three compounds *trans*-lewisite I, all-*trans*-lewisite II and all-*trans*-lewisite III have been identified in a munition grade lewisite mixture probably originating from World War II. all-*trans*-Lewisite II and all-*trans*-lewisite III were the main constituents in the mixture. NMR was used to confirm the identifications made by GC-MS and to identify the correct stereoisomers (all-*trans*).

The ESI-MS spectra turned out to be difficult to interpret. The two major compounds were tentatively identified as lewisite III oxide and lewisite II dioxidised dimer.

The 3,4-dimercaptotoluene (DMT) derivative of lewisite I or of 2-chlorovinylarsonous acid/lewisite oxide was identified by GC-MS. 2-chlorovinylarsonous acid (in equilibrium with lewisite oxide) and lewisite I give identical DMT-derivatives. The NMR result shows that this derivative originated from lewisite I and not from the acid/oxide.

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CHARACTERISATION OF A MUNITION GRADE LEWISITE MIXTURE

1 INTRODUCTION

The vesicant 2-chlorovinyldichloroarsine (lewisite I) was first produced by the USA towards the end of World War I (1). It is normally produced by reacting arsenic trichloride with acetylene in the presence of aluminium trichloride as a catalyst (2). In this process, the final product is a mixture of lewisite I, bis(2-chlorovinyl)chloroarsine (lewisite II) and tris(2-chlorovinyl)arsine (lewisite III) (Figure 1.1). Only two *cis/trans* isomers are possible for lewisite I, three isomers are possible for lewsite II and four isomers for lewisite III.
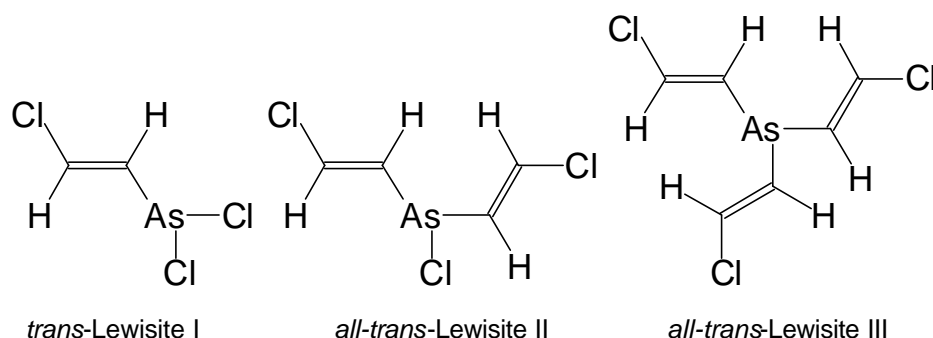

| *trans*-Lewisite I | *all-trans*-Lewisite II | *all-trans*-Lewisite III |

Figure 1.1   The all-trans isomers of lewisites I, II and III

The product ratioes of lewisite I isomers typically encountered in lewisite preparations reflects mainly the thermodynamic stability of the three isomers (*trans > cis > gem*, based on *ab initio* MP2/6-311+G(2d,p)//MP2/6-311+G(2d,p) calculations) (3). Using Boltzmann's law of distribution (4) and the published relative energies of the most stable conformers of lewisite I, the estimated thermodynamic distribution is: *trans* 66 %, *cis*: 24 % and *gem* 10 % (kinetics, solubility and preparation procedures not taken into account). The preparation of lewsite I by the method described above leads mainly to the *trans* isomer of lewisite (95 %), a lesser amount of the *cis* isomer and only a neglible amount of a geminal isomer 1-chlorovinyldichloroarsine, see Figure 1.2 (5)(6).
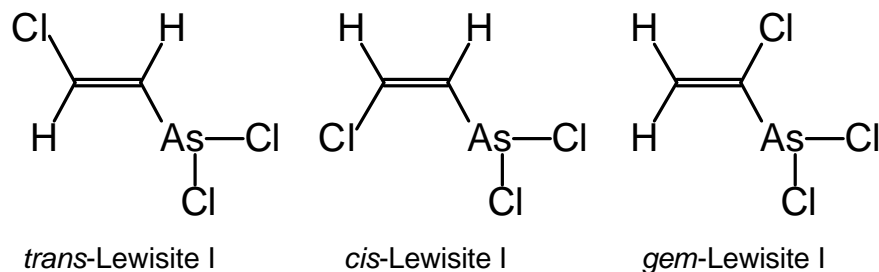

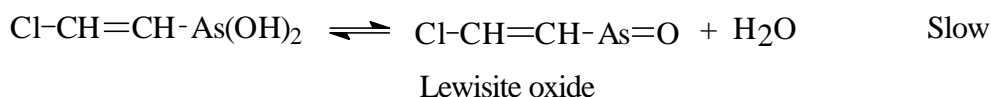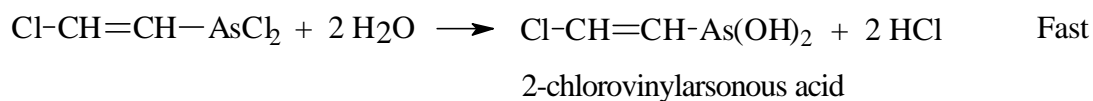| *trans*-Lewisite I | *cis*-Lewisite I | *gem*-Lewisite I |

Figure 1.2   Isomers of lewisite I

After World War I, both the former Soviet Union and the USA stockpiled lewisite. The more common vesicant mustard gas was also mixed with lewisite in a ratio 2:3 to lower its freezing point. A mustard gas/lewisite mixture (2:3) has a freezing point of −25.4 °C (2). After World

War II the production plants were destroyed and the stockpiles sunk at sea or deposited in dumping grounds. The deposits could still pose an environmental risk today.

Lewisite I is highly soluble in water and decompose rapidly according to the following scheme (7)(8)(9):

$$Cl-CH=CH-AsCl_2 + 2 H_2O \longrightarrow Cl-CH=CH-As(OH)_2 + 2 HCl \qquad \text{Fast}$$

2-chlorovinylarsonous acid

$$Cl-CH=CH-As(OH)_2 \rightleftharpoons Cl-CH=CH-As=O + H_2O \qquad \text{Slow}$$

Lewisite oxide

$$Cl-CH=CH-As=O \rightleftharpoons \text{Polymer} \qquad \text{Slow}$$

Lewisite oxide (2-chlorovinylarsenoxide) slowly oxidise further to 2-chlorovinylarsonic acid (CVAA) (8):

$$Cl-CH=CH-As=O \xrightarrow{[O]} Cl-CH=CH-\overset{\displaystyle O}{\underset{\displaystyle OH}{\overset{\|}{\underset{|}{As}}}}-OH$$
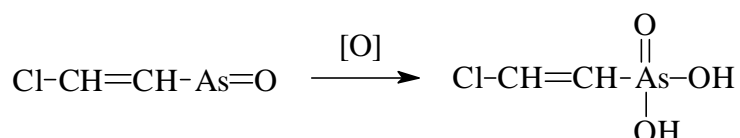
A sample of an old munition grade lewisite, probably from World War II, was found at Raufoss, Norway. The aim of this investigation was to characterise this sample and, if present, try to identify lewisite degradation products. Analytical methods for identification of lewisite and its decomposition products are important for assessing the environmental risk posed by old chemical munition dumped at sea or deposited on shore.

The most common technique for determination of chemical warfare (CW) agents is gas chromatography (GC), often combined with mass spectrometry (MS). Lewisite I is, however, reactive and rather difficult to analyse directly by GC. Furthermore, the decomposition products of lewisite I are polar or involatile and therefore impossible to determine directly by GC. Derivatization of the decomposition products by use of different thiols (5)(9)(10) (11)(12)(13)(14), thioglycolic acid methyl ester (15) or 3,4-dimercaptotoluene (16) followed by gas chromatographic analysis has been proposed. Both lewisite I and some of its decomposition products have been analysed by High Performance Liquid Chromatography (HPLC) with UV and electrochemical detectors (17), ion chromatography (18) and by HPLC-ICP-MS (19). HPLC-electrospray-MS (HPLC-ESI-MS) with postcolumn derivatisation using 2-mercaptopyridine has also been used (14).

With the increasing popularity of electrospray-mass spectrometry (ESI-MS) as an analytical method for chemical warfare agents and their decomposition products, it was of interest to investigate this technique for the characterisation of the munition grade lewisite sample.

2   EXPERIMENTAL

2.1   Chemicals

Lewisite I, lewisite II and lewisite III were synthesised in-house using the procedure described in (20). Samples for gas chromatographic-mass spectrometric (GC-MS) analysis (1 µg/ml) were made up in Uvasol dichloromethane (Merck, Darmstadt, Germany). Samples for nuclear magnetic resonance (NMR) analyses (40 mg/ml) were made up in deuterated chloroform (99.8 %D, Merck). The samples were given the following numbers:

Lewisite I and lewisite II mixture	1-202
Lewisite II	1-181
Lewisite III	1-82
Munition grade lewisite	1-179

Derivatization of the munition grade lewisite was carried out by dissolving 1 mg in 10 ml of a solution of 1 mg/ml 3,4-dimercaptotoluene (3,4-DMT) (90 %, Aldrich Chem Co, Milwaukee, WI, USA) in n-hexane (Uvasol, Merck). The derivatization was carried out by sonication for 10 min at room temperature.

HPLC Ultra Gradient grade acetonitrile (J T Baker, Deventer, Holland) and deionised distilled (USF Elga, Lowell, MA, USA) water, both with 0.01% trifluoroacetic acid (TFA, Merck), was used to make up the mobile phase for HPLC. Lewisite samples for ESI-MS analysis (0.1 mg/ml) were made up in a mixture of distilled water and acetonitrile (1:1).

2.2   Instrumentation

The first approach to characterise the munition grade lewisite sample (sample no 1-179) was made by GC-MS. Samples of 1 µg/ml of the munition grade lewisite and analytical standards of lewisite I, lewisite II and lewisite III in dichloromethane were analysed by GC-MS. The instrumentation used was a Finnigan MAT 95Q hybrid mass spectrometer (Bremen, Germany), coupled to a HP 5890 Series II gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). A sample volume of 0.8 µl was injected splitless for 1 min. The column used was 30 m x 0.25 mm with 0.25 µm DB-5 stationary phase from J&W Inc (Folsom, CA, USA). Helium (grade 6.0) was used as carrier gas at a flow rate of 32 cm/sec. The injector was held at 250 °C, the transfer line at 280 °C and the oven programme was 40 °C (1 min) - 10 °C/min - 280 °C (5 min). The mass spectrometer was operated in electron ionisation (EI) or chemical ionisation (CI) mode. Other instrumental parameters used are shown in Table 2.1. Finnigan Instrument Control Language (ICL) version 10.6 was used to control the instrument and Finnigan ICIS II version 8.03HB software was used for data handling.

	MS method	
	EI	CI
Ion source temp (°C)	190	150
CI gas pressure (mbar)		6×10^{-5} (*)
Electron energy (eV)	70	150
Filament current (mA)	1.0	0.2
Scan range (u)	25-1000	100-400
Cycle time (s)	1	1
Resolution	1000	1000
Reaction gas		Ammonia

Table 2.1   Instrumental parameters used for GC-MS analysis
*   (*) Measured in the ion source outside the ion box*

The derivatized samples were analysed using a Fisons MD800/250 quadrupole mass spectrometer (ThermoQuest, Manchester, UK) in EI mode, coupled to a Fisons 8060 GC (ThermoQuest). 1 µl of the samples was injected splitless for 1 min by an autoinjector. The column used was 30 m x 0.25 mm with 0.25 µm DB-5MS stationary phase from J&W Inc. Helium was used as carrier gas at a flow rate of 1 ml/min (flow controlled). The injector was held at 250 °C, the transfer line at 260 °C and the oven program was 40 °C (1 min) - 10 °C/min - 280 °C (10 min). Other instrumental parameters were ion source temperature = 190 °C, electron energy = 70 eV, filament current = 0.15 mA, scan range = 35-600 u, scan cycle time = 0.5 sec and unit resolution. Finnigan MassLab version 1.4 software (ThermoQuest) was used for instrument control and data handling.

HPLC separations were performed using a Waters 600MS pump (Milford, MA, USA) connected to a 15 cm x 0.32 mm ID fused-silica capillary column packed with Kromasil ODS (5 µm particles) at G&T Septech (Oslo, Norway). Injections were made through a Rheodyne 8125 injector (Cotati, CA, USA) with a 5 µl sample loop. The separations were made with a gradient from 10 % to 90 % acetonitrile in water (both with 0.01 % TFA) over 20 minutes. The mobile phase was delivered from the pump at 250 µl/min and split prior to the injector such that the flow through the column was 5 µl/min.

ESI-MS spectra were acquired on the Finnigan MAT 95Q hybrid mass spectrometer with a Finnigan ESI-II ion source (Bremen, Germany) operated in positive mode. The ESI capillary was operated at 2.4 kV and the ESI source tuned with Gramicidin S (Sigma Chemical Co, St Louis, MO, USA) before use (approximate tune values were: capillary –10 V, tube lens 90 V and skimmer 2.0 V). Nitrogen grade 5.0 was used as sheath gas at a pressure of 5 bar. Scan range was 100-600 u with scan speed 2 sec/decade and resolution 1300.

In tandem (MS/MS) mode, the MS-1 was held at constant mass, while MS-2 was scanned linearly from m/z 50 to 25 u above the parent ion with scan speed 1 sec/100 u. In order to get reproducible regulation of the collision gas pressure, the high-vacuum in the quadrupole analyser outside the collision cell was monitored. The collision gas (argon grade 5.0) pressure was adjusted to give a high-vacuum reading outside the collision cell of 1×10^{-5} mbar. The collision energy was set to −80 eV. MS/MS spectra were acquired by continuous injection using a Harward Apparatus 22 syringe pump (South Natic, MA, USA).

The NMR spectra were obtained using a Bruker Avance DRX 500 NMR spectrometer (Rheinstetten, Germany) operating at 500 MHz equipped with a 2.5 mm BBI inverse (^{1}H,X) z-gradient probe. Chemical shifts are reported in ppm from TMS using internal standards: ^{1}H (7.24 ppm, $CHCl_3$), ^{13}C (77.0 ppm, $CDCl_3$). XWINNMR (Bruker) was used for instrument control. XWINNMR and WINNMR version 960901 was used for data handling. WINNMR and PeakFit were used for integration (SPSS Science, Chicago, IL, USA).

1D acquisition parameters
^{1}H: 32k data points, spectral width 4310 Hz, recycle delay 1.5 s, 64 scans and 90° pulse.
^{13}C: gated CPD {^{1}H} decoupling or CPD {^{1}H} decoupling, 45k data points, spectral width 23148 Hz, recycle delay 1.5 s, 4k scans and 30° pulse.

2D acquisition parameters
^{1}H-^{13}C HMBC, 2k data points in t_2 and 128 data points in t_1, 4 t_1 increments, spectral width: 4310 Hz in t_2 and 10869 Hz in t_1, a recycle delay of 1 s and 65 ms mixing time, 1.5 ms gradient pulse with 100 μs gradient recovery.

3   RESULTS AND DISCUSSION

3.1   Gas chromatographic - mass spectrometric analysis

The chromatogram from GC-MS analysis of the munition grade lewisite sample using EI is shown in Figure 3.1 below. The compounds lewisite I, lewisite II and lewisite III were tentatively identified in the sample.
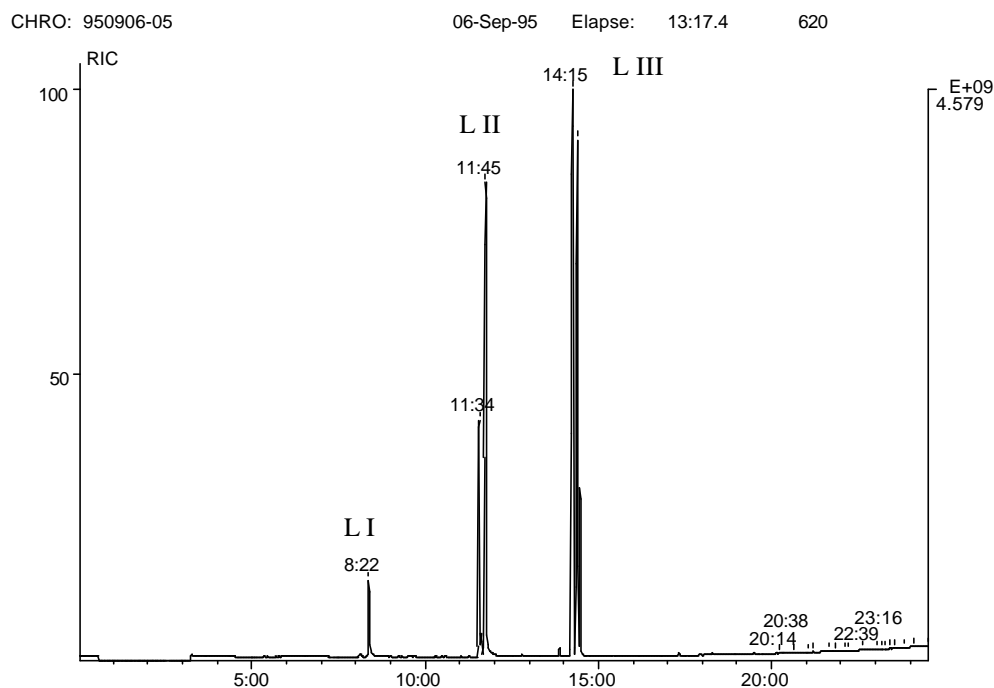
Figure 3.1   *Electron ionisation total ion chromatogram from 1 µg/ml munition grade lewisite. Lewisite I (L I), lewisite II (L II) and lewisite III (L III) were tentatively identified*

The retention times and spectra of the compounds were compared with authentic standards and were in good agreement with the proposed compounds. The chromatogram shows multiple peaks with identical mass spectra for both lewisite II and lewisite III (Figure 3.2). The multiplets are probably due to combined *cis/trans* isomers as explained by Smith et al (5) and Koehler et al (21) and might possibly be caused by heating in the GC-inlet. It has been reported that the *cis* isomer of lewisite I is obtained from the synthesised *trans* isomer as a consequence of energy changes such as heating (22).

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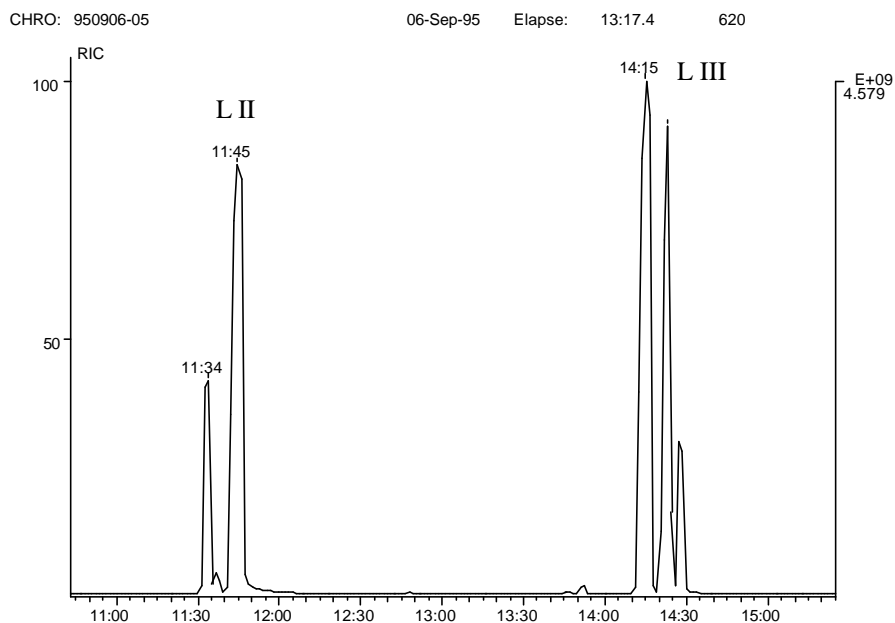CHRO: 950906-05   06-Sep-95   Elapse: 13:17.4   620

Figure 3.2   Electron ionisation total ion chromatogram from 1 µg/ml munition grade lewisite showing multiple peaks for lewisite II (L II) and lewisite III (L III)

Chemical ionisation spectra of the compounds using ammonia as reagent gas showed molecular or quasi molecular ions with relatively low intensities and abundant fragment ions as shown for lewisite III in Figure 3.3. The CI spectra confirmed the presence of lewisite I, lewisite II and lewisite III in the sample.

The analysis of the derivatized munition grade lewisite gave no indication of other compounds than the 3,4-DMT derivative of lewisite I. In addition, underivatized lewisite II and lewisite III was identified in the sample. The EI mass spectrum of the 3,4-DMT derivative of lewisite I is shown in Figure 3.4. This compound could result from derivatization of lewisite I itself or its decomposition product 2-chlorovinylarsonous acid in equilibrium with lewisite oxide. (9)(10).
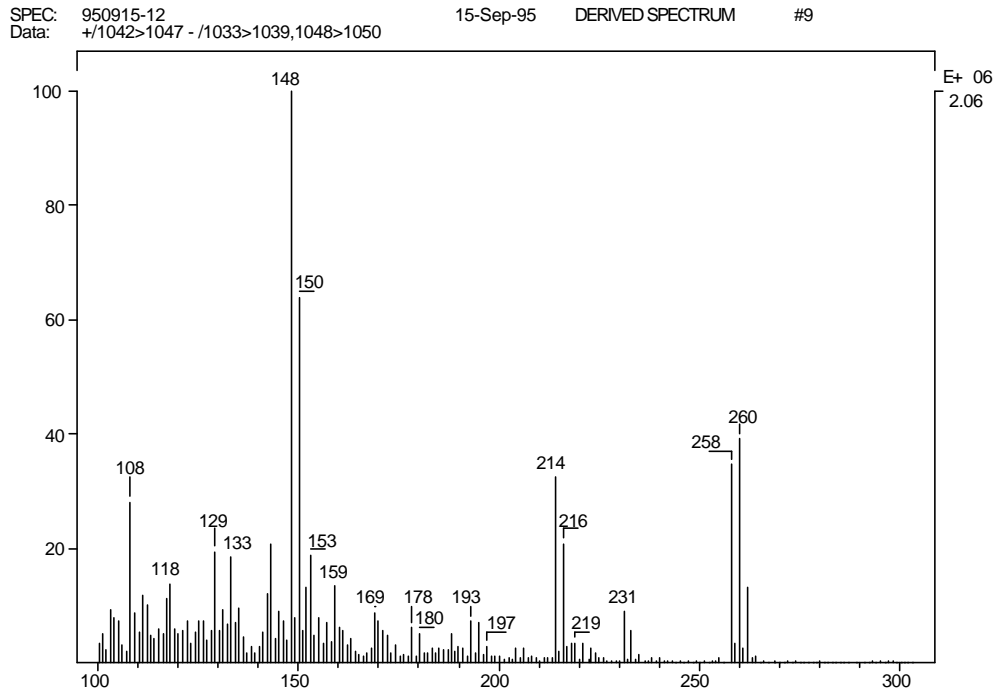
Figure 3.3   Ammonia CI mass spectrum of lewisite III (Molecular weight, MW=258)
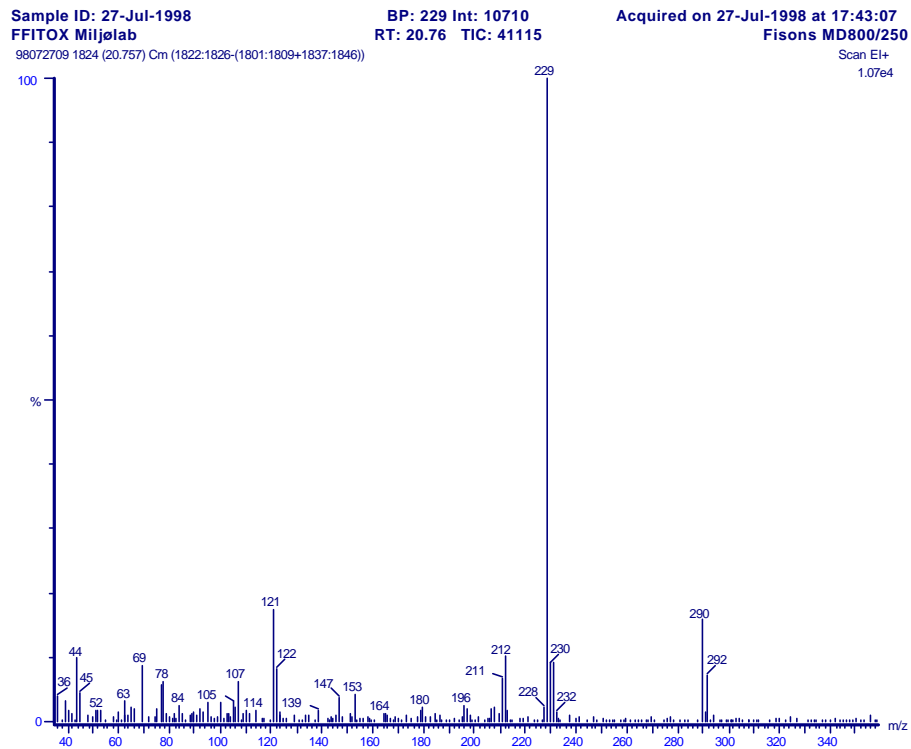

Figure 3.4   EI mass spectrum of 3,4-DMT derivative of lewisite I (MW=290)

3.2   Liquid chromatographic – mass spectrometric analysis

The chromatogram from HPLC-ESI-MS analysis of a 0.1 mg/ml solution of the munition grade lewisite sample in a mixture of acetonitrile and water (1:1) is shown in Figure 3.5.
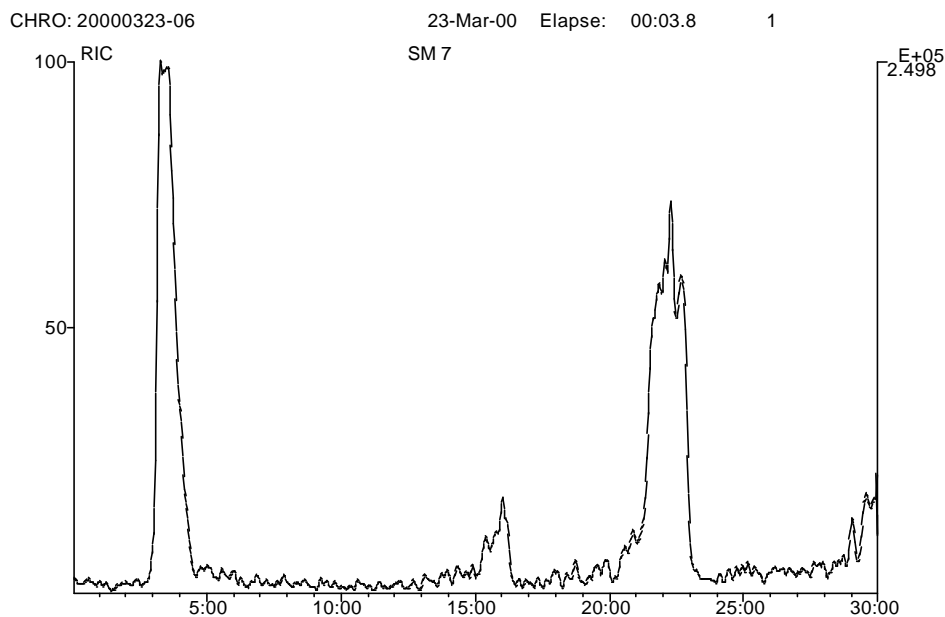
Figure 3.5   HPLC-ESI-MS total ion chromatogram from 0.1 mg/ml munition grade lewisite

Two large peaks are observed at retention times approximately 3 and 22 minutes and a smaller peak at 16 minutes. The ESI-MS spectra from the two larger peaks are shown in Figure 3.6 and Figure 3.7 respectively. The smaller peak at 16 min has identical ESI mass spectrum with the peak at 3 minutes.
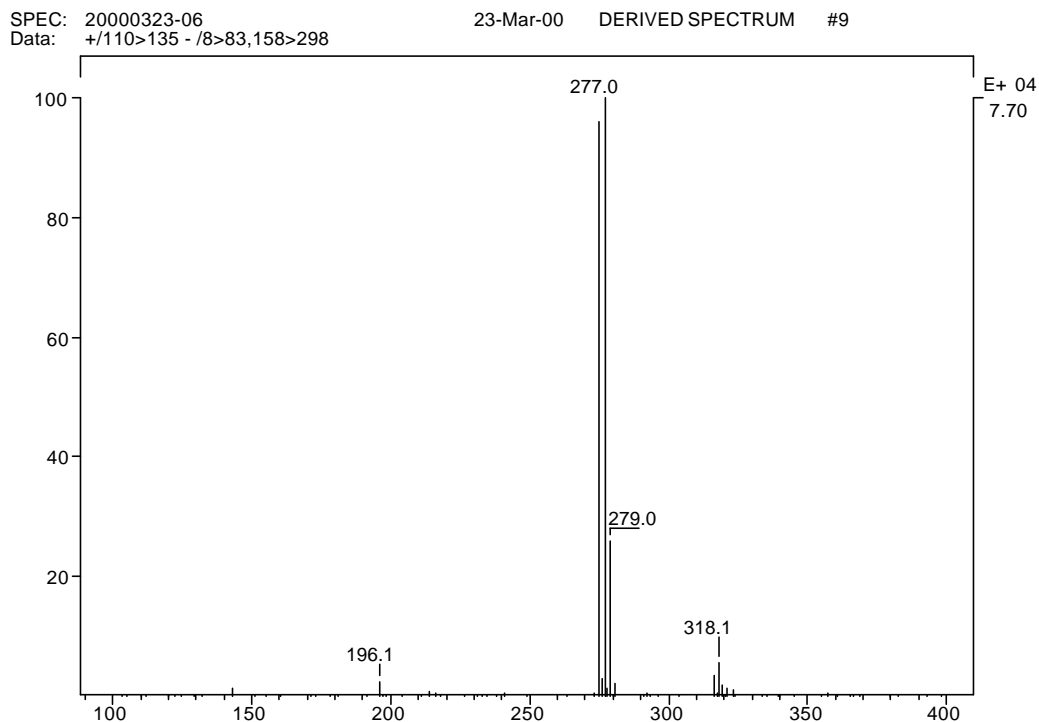

Figure 3.6   ESI-MS spectrum of the compound with retention time approximately 3 minutes
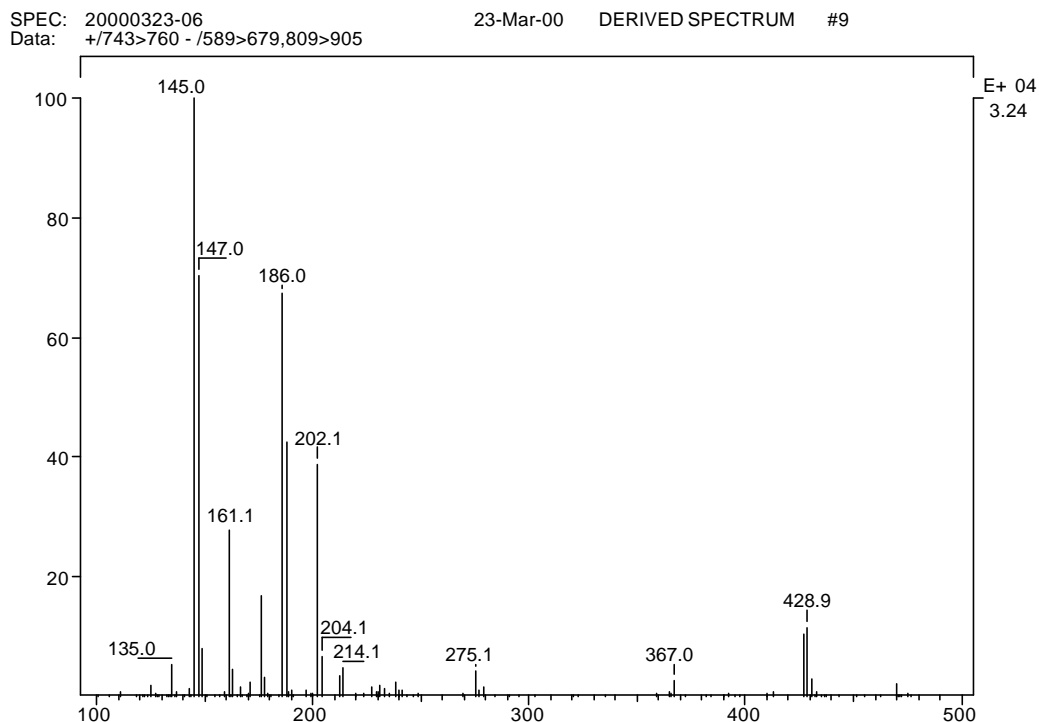
Figure 3.7   ESI-MS spectrum of the compound with retention time approximately 22 minutes

The ESI mass spectra could not easily be interpreted as lewisite I, II or III. Standards of lewisite II and III and a mixture of lewisite I and lewisite II were therefore made up separately in a mixture of acetonitrile and water (1:1) and analysed under identical conditions. It became evident that the compounds observed in the HPLC-ESI-MS analysis of the standards and the munition grade sample were not lewisite I, lewisite II or lewisite III themselves. By comparing the retention times and spectra with the standards, it was observed that the peak at 3 min was related to lewisite III and the peak at 22 min was related to lewisite II. The small peak at 16 min was also observed in the lewisite III reference sample. No peak related to lewisite I was observed in the HPLC-ESI-MS chromatogram of the munition grade sample.

The ESI spectrum at 3 min (Figure 3.6) shows large ions at m/z 275/277/279 with an isotope pattern corresponding to a quasi molecular ion [MH]$^+$ with three chlorine atoms. A small cluster of peaks is observed at m/z 316/318/320 with the same isotope pattern. It was assumed that the smaller cluster was an acetonitrile adduct ([MH+CH$_3$CN]$^+$). This was confirmed by substituting acetonitrile with acetone as solvent. The isotope cluster then shifted to m/z 333/335/337 ([MH+(CH$_3$)$_2$C(O)]$^+$).

The spectrum at 22 min (Figure 3.7) shows a distinct ion cluster at m/z 427/429/431/433 with an isotope pattern corresponding to a quasi molecular ion with four chlorine atoms. The base peak (m/z 145/147/149) with two chlorine atoms coming from [AsCl$_2$]$^+$ is common in lewisite spectra. The ion cluster m/z 186/188/190 is again assumed to be an acetonitrile adduct of [AsCl$_2$]$^+$.

To assist in the interpretation of the ESI spectra, some MS/MS experiments were carried out on the munition grade lewisite sample and on a lewisite II standard, both diluted in water : acetonitrile (1:1). Continuous injections with a Harward Apparatus syringe pump were carried out. Firstly, the daughter spectrum of m/z 275 observed in the ESI spectrum from the

peak with retention time 3 min was recorded. The MS/MS spectrum in Figure 3.8 shows fragmentation similar to what can be found in the EI spectrum of lewisite III (with the exception of an isotopic pattern). By combining molecular weight information with structural information obtained by MS/MS, the chromatographic peak at 3 min was tentatively identified as the oxidised form of lewisite III ($[As(O)(CH=CH-Cl)_3H]^+$, MW=275).

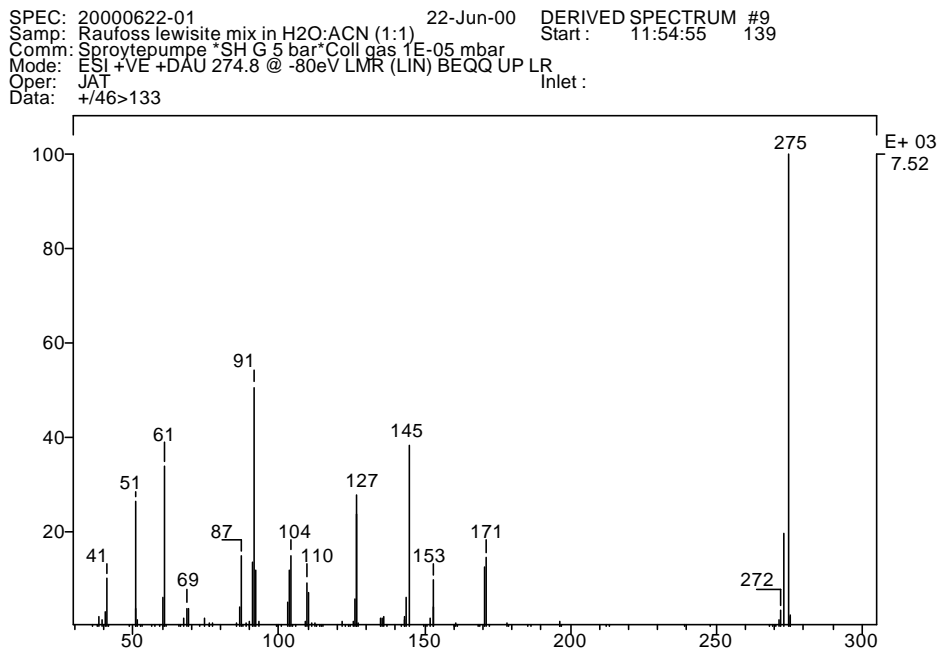*Figure 3.8   MS/MS daughter spectrum of m/z 275 from the munition grade lewisite sample*

The intensity of m/z 427 from the munition grade lewisite sample (peak at retention time 22 min) was not high enough to obtain any daughter spectrum. The daughter spectrum of m/z 427 was therefore recorded by a MS/MS experiment carried out on a lewisite II standard solution (Figure 3.9).
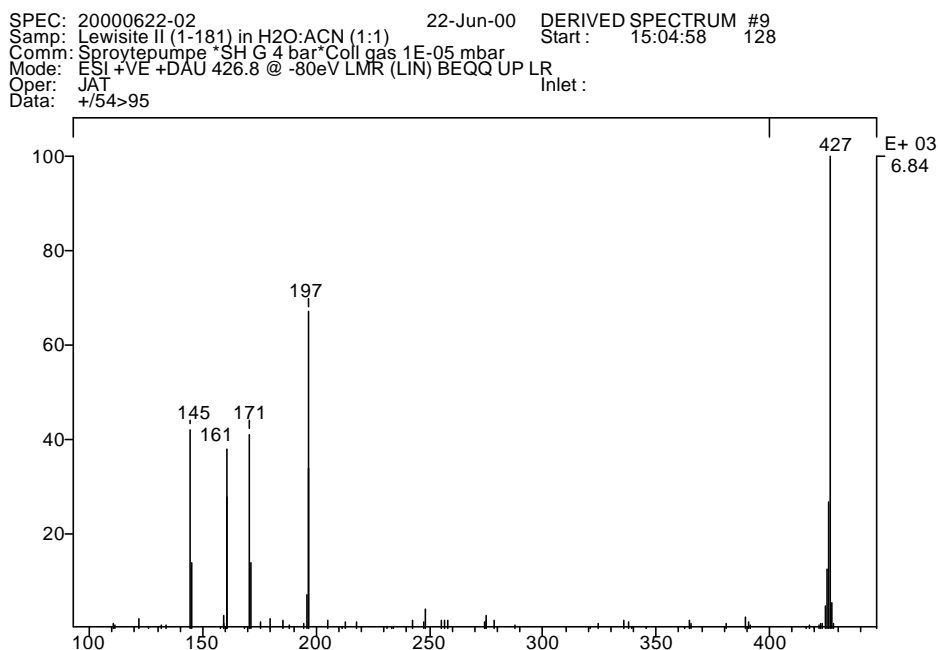
*Figure 3.9   MS/MS daughter spectrum of m/z 427 from a (0.5 mg/ml) lewisite II standard
solution (the masses between m/z 100 and m/z 400 are enlarged four times)*

Based on molecular weight information and structural information obtained by MS/MS, the
compound with retention time 22 min was tentatively identified as an oxidised dimer of
lewisite II (Figure 3.10).
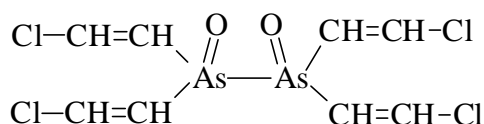
Figure 3.10   Proposed structure (MW 426) of the peak with retention time 22 min

The proposed structures of the daughters of m/z 427 are shown in Table 3.1.

Mass (u)	Proposed structure
197	$[As(CH=CHCl)_2]^+$
171	$[ClAsCH=CHCl]^+$
161	$[As(O)Cl_2]^+$
145	$[AsCl_2]^+$

Table 3.1   Proposed structures of the daughters of m/z 427

Note that the same two tentatively identified compounds were also observed by HPLC-ESI-
MS of authentic lewisite II and lewisite III standards.

3.3 ^{1}H and ^{13}C nuclear magnetic resonance analysis

Proton and carbon NMR spectral parameters for lewisites were measured with the aim of identifying the main components in the munition grade lewisite sample. Figure 3.11 shows the notations used.

Figure 3.11   NMR notations used for lewisites I, II and III, as depicted by the all-trans lewisites

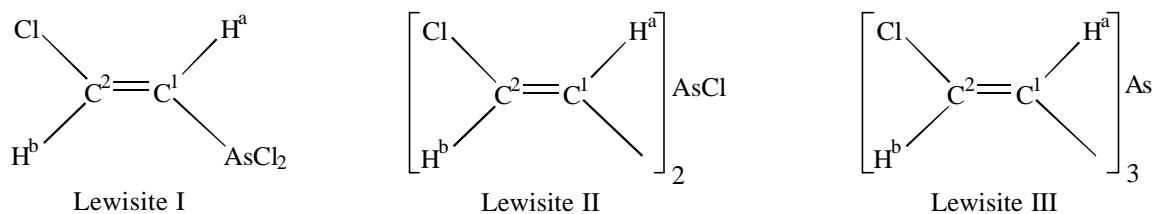The experimental spectral values for the lewisite standards (in CDCl$_3$) are tabulated in Table 3.2. Proton-carbon connectivities were established from inverse proton-carbon correlated spectroscopy (HMBC). The ^{1}H NMR and ^{13}C NMR chemical shifts and coupling constants correspond well with the literature data also given in Table 3.2 for *trans*-lewisite I, all-*trans*-lewisite II and all-*trans*-lewisite III (acquired in CDCl$_3$).

The $^3J_{Ha\text{-}Hb}$ coupling constants (15 Hz) clearly shows that all the three lewisites have an all-*trans* configuration (15 Hz (5)(21)) and not a single *cis* (7 Hz (5)(21)) or *gem* (3 Hz (5)) vinylic fragment. In olefinic systems, J_{trans} is always larger than J_{cis} (23). Vinylic protons are expected to have *trans* J_{HH} coupling constants between 12 and 18 Hz and *cis* J_{HH} coupling constants between 8-12 Hz (5). Using the empirical equations of Abraham for vinylic vicinal H-H couplings (23), assuming additivity and using the electronegativities estimated by Pauling (24), J_{trans} = 16 Hz and J_{cis} = 8 Hz for the lewisite systems, in good agreement with the experimental literature values. However, the calculated J_{gem} = 6 Hz is somewhat high.

Note that the differences in chemical shifts due to solvent effects (25) for literature spectra acquired in other solvents (Table 3.2) would have made comparisons with experimental shifts difficult. The exception from this is the ^{13}C NMR chemical shifts that even so are comparable due to the large range of the ^{13}C NMR chemical shift scale.

Also included in Table 3.2 are the experimental spectral values for the munition grade lewisite sample, for which the ^{1}H NMR spectrum is shown in Figure 3.12. The ^{13}C {^{1}H} NMR spectrum is shown in Figure 3.13 and the ^{13}C gated {^{1}H} NMR spectrum in Figure 3.14. Proton-carbon connectivities were established from inverse proton-carbon correlated spectroscopy (HMBC). A comparison between the spectral parameters for the lewisite standards (all-*trans*) and the munition grade lewisite sample shows that the munition grade sample contains all three all-*trans* lewisites.

Sample	Lewisite[a]	Solvent	Chemical shifts (ppm)				Coupling constants (Hz)						References
			$\delta_H a$	$\delta_H b$	$\delta_C 1$	$\delta_C 2$	$^1J_{C1\text{-}H}a$	$^1J_{C2\text{-}H}b$	$^2J_{C1\text{-}H}b$	$^2J_{C2\text{-}H}a$	$^3J_{C1\text{-}As\text{-}C1'\text{-}H}a'$	$^3J_{H}a\text{-}_{H}b$	
1-202	L I	CDCl₃	7.13	6.92	139.2	133.1	171.3	190.9	7.8	1.9	n/a	14.8	This work
1-181	L II	CDCl₃	6.85	6.70	134.2	131.7	175.4	198.9	2.8	9.4	m	14.7	This work
1-82	L III	CDCl₃	6.45	6.37	129.6	128.2	167.8	198.2	7.3	8.7	m	14.6	This work
1-179	L I[b]	CDCl₃	7.13	6.92	139.2	133.1	-	-	-	-	n/a	14.8	This work
	L II		6.85	6.69	134.1	131.6	175.6	199.0	2.8	9.6	m	14.7	
	L III		6.45	6.36	129.6	128.1	167.6	198.3	7.3	8.7	m	14.6	
	L I	CDCl3	7.146	6.936	139.29	133.17	n m	n m	n m	n m	n/a	14.81	(26)
	L I	CDCl₃	7.15	6.94	139.2	133.1	n m	n m	n m	n m	n/a	14.8	(5)
	L I	CDCl3	7.146	6.936	139.27	133.17	n m	n m	n m	n m	n/a	14.8	(27)
	L I	CDCl3	7.15	6.93	n m	n m	n m	n m	n m	n m	n/a	14.8	(27)
	L I	CDCl3	7.14	6.93	139.09	133.07	n m	n m	n m	n m	n/a	14.8	(27)
	L I	CDCl₃	7.145	6.935	n m	n m	n m	n m	n m	n m	n/a	14.8	(28)
	L I	C₆D₆	6.34	5.78	139.43	132.58	172.3	196.6	6.2	7.3	n/a	15.0	(21)
	L I	CCl₄	7.07	6.87	138.5	132.5	174.6	197.2	4.2	8.2	n/a	14.4	(6)
	L II	CDCl3	6.863	6.713	134.23	131.65	n m	n m	n m	n m	n m	14.73	(26)
	L II	CDCl3	6.864	6.713	134.22	131.66	n m	n m	n m	n m	n m	14.7	(27)
	L II	CDCl3	6.87	6.71	134.12	131.56	n m	n m	n m	n m	n m	14.6	(27)
	L II	C₆D₆	6.20	5.83	134.34	131.19	171.1	198.5	7.3	8.2	2.9	14.7	(21)
	L III	CDCl3	6.470	6.386	129.68	128.26	n m	n m	n m	n m	n m	14.6	(27)
	L III	CDCl3	6.46	6.38	129.67	128.19	n m	n m	n m	n m	n m	14.6	(27)
	L III	C₆D₆	5.84	5.72	129.90	127.97	167.8	199.1	5.0	8.7	2.5	14.7	(21)

Table 3.2 ^1H and ^{13}C NMR data for all-trans lewisite standards and the munition grade lewisite mixture (1-179) compared with reference data . For notations, see Figure 3.11. m: multiplet, -: not observed, n m: not measured, n/a: not applicable.
[a]L I denotes trans-lewisite, L II denotes all-trans-lewisite II and L III denotes all-trans-lewisite III.
[b]Coupling constants from ^{13}C NMR are not available due to a low concentration of this isomer
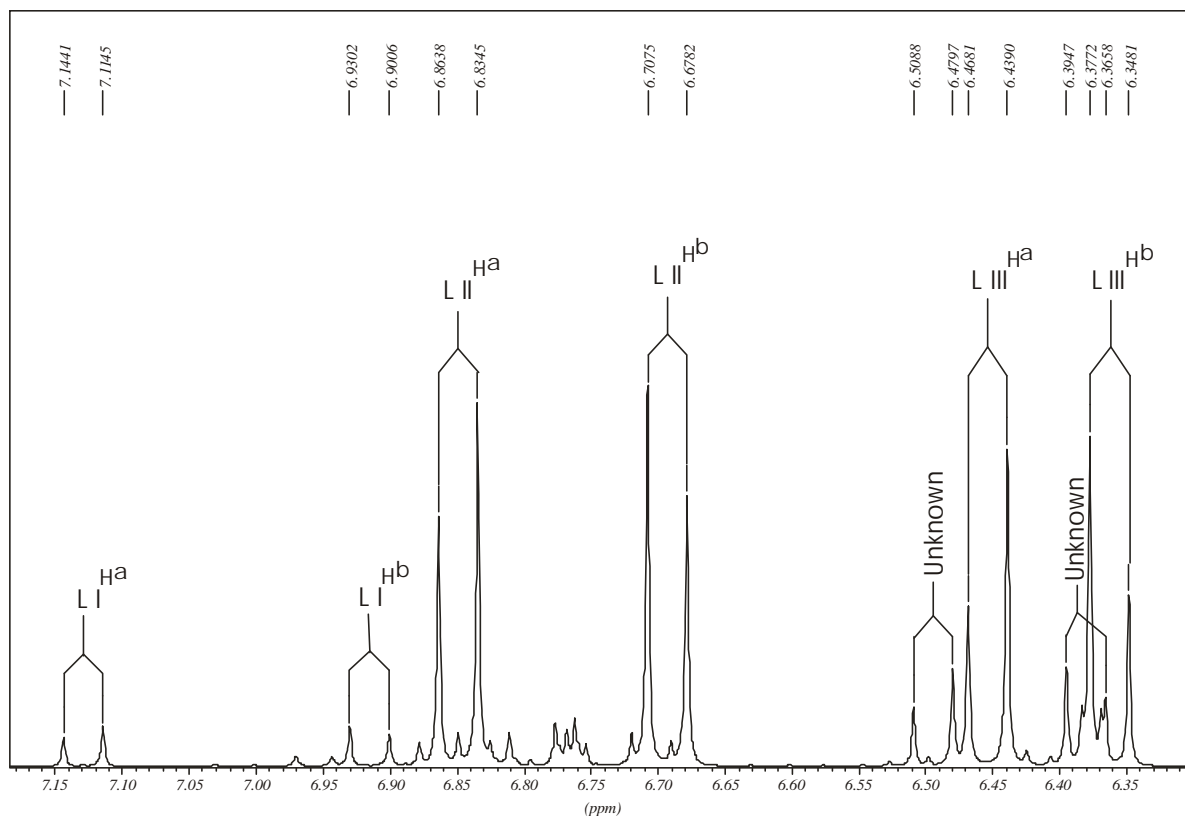
Figure 3.12   *^{1}H NMR spectrum of a munition grade lewisite mixture (1-179). trans-*
Lewisite I (L I), all-trans-lewisite II (L II) and all-trans-lewisite III (L III)
were identified. For notations, see Figure 3.11

The ^{1}H NMR spectrum also shows that the munition grade lewisite sample contains an additional compound (marked "Unknown" in Figure 3.12). This compound has two non-identical protons, resonating at 6.49 (d) ppm and 6.38 (d) ppm (CDCl$_3$), which couples with each other with a coupling constant $J = 14.5$ Hz. The size of the coupling constant and the fact that the chemical shifts are similar to the chemical shifts for H^a and H^b in all-*trans*-lewisite III indicate that the signals are caused by a lewisite related compound with a *trans* configuration. The signals for this unknown compound have a higher intensity than the signals for *trans*-lewisite I, which has the lowest concentration of the all-*trans* lewisites in the munition grade lewisite sample. Note that this unknown was also found in the lewisite II and lewisite III standards.

The ratio between the different resonances has been determined by integrating the experimental ^{1}H NMR spectrum of the munition grade lewisite sample both with WinNMR and PeakFit. Both integration routines gave integrals of equal magnitude. The ratios between the identified compounds are shown in Table 3.3 and show that the munition grade lewisite sample is composed mostly of all-*trans*-lewisite II and all-*trans*-lewisite III. No *cis*-isomers were encountered.

Sample	Area ratio
trans-Lewisite I	1
all-*trans*-Lewisite II	8
all-*trans*-Lewisite III	6
Unknown	2

Table 3.3   Relative areas of the compounds in the munition grade lewisite sample (1-179) determined from integration of the different signals (Random Mean Squared = 0.93)
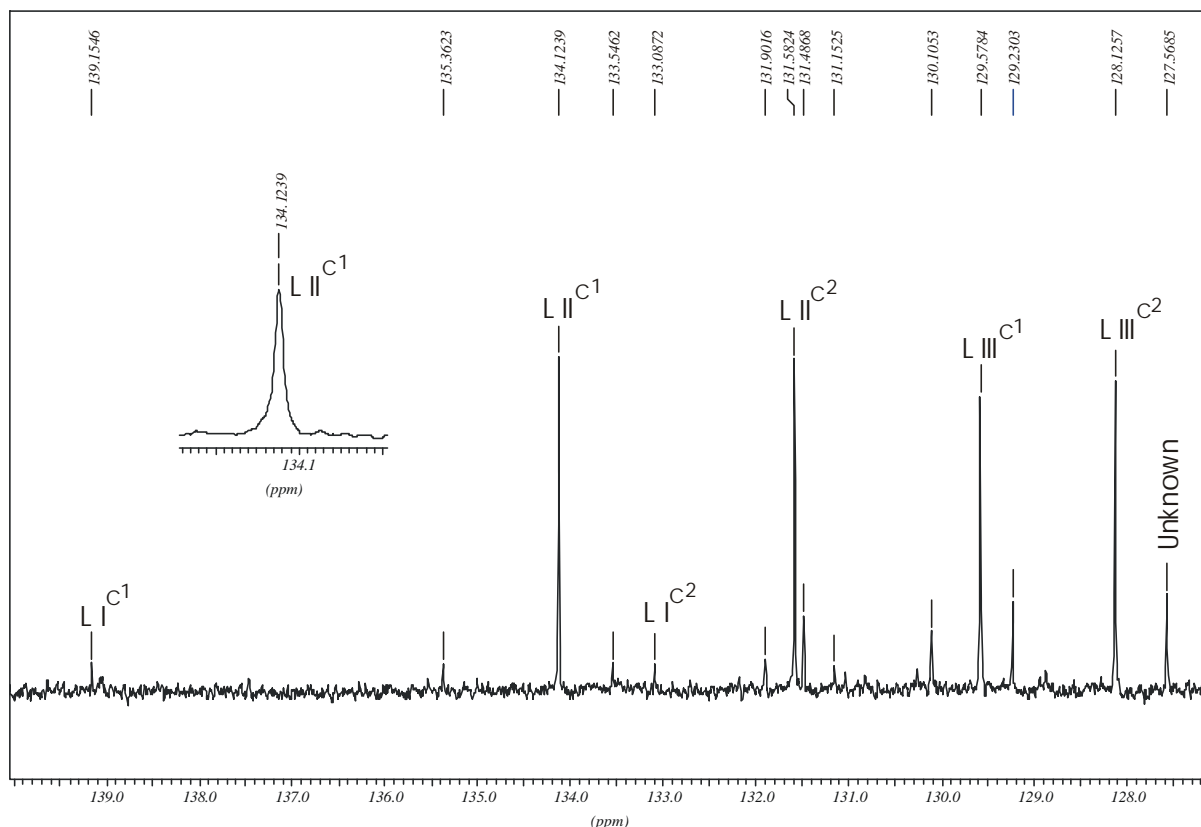

Figure 3.13 ^{13}C {^{1}H} NMR spectrum of a munition grade lewisite mixture (1-179). trans-Lewisite I (L I), all-trans-lewisite II (L II) and all-trans-lewisite III (L III) were identified. For notations, see Figure 3.11

Two additional unknown signals were found in the ^{13}C {^{1}H} NMR and ^{13}C gated {^{1}H} NMR spectra of the munition grade lewisite sample. The first resonates at 127.6 ppm (dd) (CDCl$_3$) with $J = 198.4$ Hz and $J = 8.9$ Hz. As seen from Table 3.2, this is the same set of coupling constants as for C^2 in all-*trans*-lewisite III. The signal also appears at a frequency only slightly lower than for all-*trans*-lewisite III C^2, strongly indicating its familiarity with C^2 in lewisite III. Note that this unknown signal was also found in the lewisite III standard, but only slightly above noise threshold.

The second signal is weaker and appears at 134.1 ppm (dd) (CDCl$_3$) with $J = 143$ Hz and $J = 2$-3 Hz. Note that this signal co-resonates with the signal for L II^{C1} in Figure 3.13 as shown in Figure 3.14. However, both coupling constants are very different from the coupling constants for C^1 and C^2 published for *cis* and *trans* isomers of lewisites, which are $^1J_{C-H} = 164$-172 Hz (C^1), $^1J_{C-H} = 194$-199 Hz (C^2) and $^2J_{C-CH} = 4$-9 Hz (21). The unknown signal was also found in the lewisite II standard, but only slightly above noise threshold.

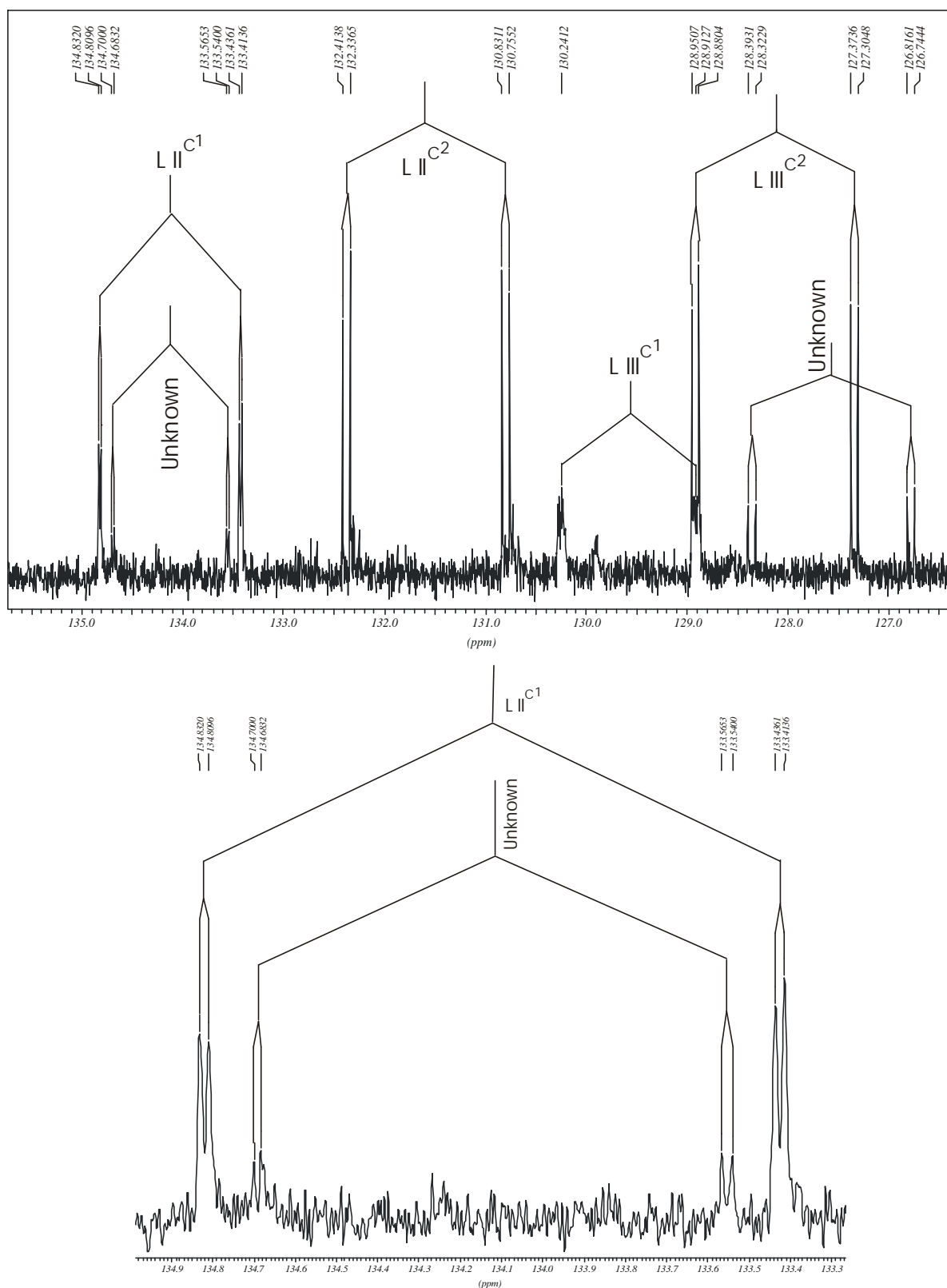*Figure 3.14* ^{13}C *gated {^1H} NMR spectrum of a munition grade lewisite mixture (1-179). all-trans-Lewisite II (L II) and all-trans-lewisite III (L III) were identified. The concentration of trans-lewisite (L I) was too weak to be detected in the coupled spectrum. For notations, see Figure 3.11*

Both ^{13}C NMR signals are stronger than the signals for *trans*-lewisite I, possibly indicating that both signals might be caused by the same compound as the unknown compound in Figure 3.12 (^{1}H NMR). This compound seems to be *trans*-lewisite related, however, the environment sensed by C^1 must be different from the normal lewisite structures as opposed to the environment sensed by C^2.

As described in Chapter 1, lewisite oxide in equilibrium with 2-chlorovinylarsonous acid is a decomposition product of lewisite I. Thus we wanted to check our unknown signals against this compound. A ^{13}C NMR spectrum of lewisite oxide in MeOH-d_4 has been published by O'Connor et al (29) where the carbon atoms resonate at 132 and 140-141 ppm. This is significantly different from the resonances of the unknown compound, even when taking different solvents into account.

It has recently been published by Haas et al (30)(31) that lewisite I reacts quickly with alcohols, giving an equilibrium with the corresponding ether. Similarly, lewisite II quickly gives the corresponding mono- and diethers at room temperature. The mono- and diethers of lewisite I with methanol was found to be stable.

The published ^{13}C NMR chemical shifts for lewisite oxide (29) may thus be in error (MeOH-d_4 solvent). However, O'Connor et al detected no ^{13}C NMR signal indicating the presence of a methyl-d_3 ether group in the spectrum for lewisite oxide. But such a signal would, due to deuterium coupling, appear as a heptet, and with no enhancement from Nuclear Overhauser Effect (NOE) by irradiation (due to deuterium substitution/spin rotation relaxation of methyl groups), such a signal would have been weak and could have gone undetected. Thus, it is uncertain whether O'Connor acquired the spectrum of lewisite oxide or of its corresponding methyl-d_3 ethers. ^{13}C NMR reference spectra of the methyl ethers of lewisite I were not available for comparison during this study.

It is therefore not possible to conclude whether or not the unknown signals in the muniton grade lewisite mixture are caused by lewisite oxide.

Another possibility is that the unknown NMR signals are caused by lewisite III oxide or lewisite II dioxidised dimer, which were tentatively identified by ESI-MS. We have not sufficient data to verify this hypothesis.

In addition, several signals of similar magnitude as compared to the weak signals for *trans*-lewsite I, were found in the decoupled ^{13}C NMR spectrum (Figure 3.13). These signals appear in the region occupied by the signals from the lewsites I, II and III, indicating that they too might be related to lewisite.

4   CONCLUSIONS

The three compounds *trans*-lewisite I, all-*trans*-lewisite II and all-*trans*-lewisite III have been identified in a munition grade lewisite mixture probably originating from World War II. all-*trans*-Lewisite II and all-*trans*-lewisite III were the main constituents in the mixture. NMR was used to confirm the identifications made by GC-MS and to identify the correct stereoisomers (all-*trans*).

The gas chromatograms showed multiple peaks with identical mass spectra from lewisite II and lewisite III, probably due to mixed *cis/trans* isomers. In the NMR spectra, however, only all-*trans* isomers were observed. The reason for this is not fully understood, but it could be related to isomerisation of the more stable *trans* isomers due to heating in the GC-MS.

An unknown *trans*-lewisite related compound was found by NMR. This compound was also observed in the NMR spectra of authentic lewisite II and lewisite III standards.

The ESI-MS spectra turned out to be difficult to interpret. The two major compounds were tentatively identified as lewisite III oxide and lewisite II dioxidised dimer. The same two compounds were also observed by HPLC-ESI-MS of authentic lewisite II and lewisite III standards.

We can thus not exclude the possibility that the unknown NMR signals are caused by lewisite III oxide or lewisite II dioxidised dimer.

The 3,4-dimercaptotoluene derivative of lewisite I or of 2-chlorovinylarsonous acid/lewisite oxide was identified by GC-MS. 2-chlorovinylarsonous acid (in equilibrium with lewisite oxide) and lewisite I give identical DMT-derivatives. The NMR result shows that this derivative originated from lewisite I and not from the acid/oxide.

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