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Derivatization of 10-chloro-5,10-dihydrophenarsazine (Adamsite) for gas chromatographic analysis

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Abstract

Two methods are presented for the derivatization of Adamsite [578-94-9]: bromination to give 2,2',4,4',6,6'-hexabromodiphenylamine [64524-09-0] (method A) and pyrolytic ethylation with dimethylformamide diethylacetal [1188-33-6] to give 10-ethyl-5,10-dihydrophenarsazine [53573-13-0] (method B). For the gas chromatographic analysis, atomic emission and mass spectrometric detection were applied. With regard to practicability and reliability, method B proved to be superior to method A.

Keywords: Warfare agents: Derivatization, GC; 10-Chloro-5,10-dihydrophenarsazine; Adamsite

1. Introduction

The compound 10-chloro-5,10-dihydrophenarsazine was first synthesized in 1913 by Wieland et al. [1,2] and, independently, in 1918 by R. Adams, from whom the trivial name Adamsite originates [3]. One of the striking features of Adamsite is its thermal and hydrolytic stability: the compound withstands heating up to 410°C (i.e. about the boiling point) without major decomposition and its hydrolysis under environmental conditions is immeasurably slow [4]. Further information about the physical, chemical and physiological properties can be drawn from Jackson's comprehensive review [5] and two newer sources [3,4].

Because of its irritating properties, the substance was considered as a potential warfare agent obviously from the early beginning. During World War II, in Germany about 4000 t Adamsite has been produced, formulated and stockpiled for chemical warfare purposes [6]. After 1945, the plants were destroyed and the stockpiles deposited in dumping grounds or elsewhere. These deposits pose a serious risk still today. For mapping out contaminated areas, monitoring the ground water etc., appropriate analytical methods are required.

In a review from 1990, various methods are summarized for detecting Adamsite by thin-layer chromatography [7]. In 1986 Kuronen [8] published a procedure for analyzing Adamsite by high-performance liquid chromatography, using retention indices and photometric detection for its identification and quantitation.

Gas chromatography (GC) of Adamsite, however, was reported to yield irreproducible results

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[7] or to fail at all [9]. Accordingly, we found upon repeated GC-injections of Adamsite that the corresponding peak became successively smaller and, finally, stayed away at all. This behaviour is surprising in view of the thermal stability of the compound. On the other hand, it resembles our experience with other organoarsine chlorides, which likewise lead to irreversible damage of the GC column–irrespective of its type–upon repeated chromatography [10].

Some years ago, atomic emission spectrometry has been introduced as a gas chromatographic detection principle [11,12]. The atomic emission detector (AED) offers the possibility to determine the elemental composition of the GC peak, which (i) complements the usual mass spectrometric identification (GC–MS) in an ideal manner and (ii) allows at the same time to quantify the analyte. In our laboratory, the combined application of both GC–MS and GC–AED is established as a one of the standard operating procedures for analyzing environmental samples. Many compounds of relevance, which are not gas chromatographable as such, could be included into this analytical regime by converting them into GC-capable derivatives [10,13–15].

Thus, it seemed worthwhile to look for suitable methods for derivatizing Adamsite. After a series of preliminary experiments we ended up with two reactions (A and B), which both eventually served the envisaged purpose:

(A) Bromination in boiling glacial acetic acid, yielding 2,2′,4,4′,6,6′-hexabromodiphenylamine (HBDP) [16].
(B) Conversion into 10-ethyl-5,10-dihydrophenarsazine (EtPA) with N,N-dimethylformamide diethylacetal in the hot GC-injector.

Both reactions were optimized using (i) the pure substance and (ii) Adamsite-spiked extracts from soil samples. In the Experimental section, the final procedure as applied to real soil samples is described.

2. Experimental

2.1. Chemicals

The following chemicals were used: anhydrous sodium sulfate, granulated (Promochem, nanograde); sodium sulfite (Merck, P.A.); sodium carbonate, decahydrate (Merck, P.A.); bromine (Merck, P.A.); *tert.*-butylmethyl ether (Promochem, nanograde, redistilled over potassium hydroxide); ethanol (Merck, P.A.); pyridine (Aldrich, 99.8%); acetic acid (Merck, >99.8%); N,N-dimethylamine diethylacetal and -dimethylacetal (both from Aldrich, deriv. grade); 1-phenyldodecane (dodecylbenzene, 'DB', Aldrich 97%), used as an internal standard. Adamsite was made available by the Bundesministerium der Verteidigung, Bonn, via the Wehrwissenschaftliche Dienststelle der Bundeswehr, Munster (Germany); according to elemental analysis (Mikroanalytisches Laboratorium Göttingen), the purity of the sample was ≥98%.

2.2. Extraction of soil samples

A sample of 30 g of the soil to be analyzed was mixed with 30 g anhydrous sodium sulfate and extracted with *tert.*-butylmethyl ether (BME) for 24 h in a Soxhlet apparatus (ca. 5 flushings per hour).

2.3. Working up and analysis

Method A

Experimental procedure.

The extract was divided (by weight) in thirds. Part 1 was spiked with 1 μg dodecylbenzene (DB) and evaporated to 0.1 ml (or as far as possible, respectively) by blowing a gentle stream of nitrogen on the surface of the liquid, followed by GC–MSD and GC–AED analysis for diphenylamine and DB. After addition of 3 μg Adamsite to part 2, both parts 2 and 3 were evaporated to dryness, ending up in a 25-ml two-neck flask (due to the low vapour pressure

of 10^{-11} Pa at 20°C [4], losses of Adamsite have never been observed). The residue was re-dissolved in 1 ml glacial acetic acid under reflux (internal temperature 130°C), followed by the addition of bromine: 10 μl after 0, 5, 10, 25 min and, in case of decolouring too fast, a further portion of 40 μl. After the last addition, refluxing was continued for 60 min. The mixture was allowed to cool down to room temperature and, under cooling and magnetic stirring, 5 ml distilled water was added. Next, solid sodium sulfite was added up for decolouration of the bromine, followed by solid sodium carbonate (up to the end of CO_2 evolution, ca. pH 10). The mixture was then extracted with 4 ml BME (including 2 μg DB). The organic phase was evaporated to 200 μl and subjected to GC analysis. Relative retention time, RRT, of HBDP is 1.40 [$t_{R,DB}$(GC–AED) = 15.31 min].

Evaluation of results.

In the chromatograms obtained from parts 2 and 3, first the identity of DB and HBDP was verified on the basis of the retention times (t_R) and mass spectra. Next, both compounds were quantitated using the individual AED signals together with the respective calibration functions. From the amount of DB found, the "concentration factor" ($= DB_{found}/DB_{introduced}$) was calculated, to be used for correcting the amounts of HBDP correspondingly. From the difference between the amounts of HBDP found in parts 2 and 3, the derivatization yield was calculated ($= 100 \times HBDP_{found}/HBDP_{introduced}$), which, in turn, served for evaluating the final figure for the content of HBDP in part 3.

The chromatograms obtained from part 1 were treated accordingly to give the amount of diphenylamine–if any–in this fraction. Multiplying by 3.797 gave the corresponding amount of HBDP (assuming 100% conversion of the diphenylamine). Subtracting this from the HBDP content in part 3 yielded that portion of HBDP which in fact represented the original amount of Adamsite in 10 g soil sample (1 g HBDP corresponding to 0.432 g Adamsite).

Method B

Experimental procedure.

The Soxhlet extract from 30 g soil was divided (by weight) into halfs. After adding Adamsite (0.15–1.5 μg) to part 1, both parts 1 and 2 (or equally sized aliquots of them) were evaporated almost to dryness. The residue was redissolved with 0.25 ml ethanol (containing 1.5 μg DB), if necessary aided by ultrasonication. After centrifugation, the supernatant was evaporated to about 25 μl and mixed with 25 μl pyridine and 50 μl N,N-dimethylformamide diethylacetal (DMFDEA). From this mixture, 1 μl was injected into the 290°C hot injector, followed by GC of the reaction products. $RRT_{EtPA} = 1.16$ ($t_{R,DB} = 15.31$ min).

Evaluation of results.

Analogously to the procedure described for method A, the "concentration factor", the derivatization yield and, finally, the content of Adamsite were calculated.

2.4. Instrumentation

Two gas chromatographs (GC) were used, one of them coupled with a mass selective detector (GC–MSD), the second with an atomic emission detector (GC–AED). The correlation of the retention times obtained in both systems was based on GC runs with a standard solution of 19 *n*-alkanes plus dodecylbenzene (DB). The individual retention times, t_R, were related to that of DB to give the "relative retention times, RRT" ($RRT = t_{R,analyte}/t_{R,DB}$). A plot of RRT_{AED} versus RRT_{MSD} served for the mutual assignment of the analyte peaks in the chromatograms from both systems.

Gas chromatography–mass spectrometry (GC–MSD)

Method A.

GC: Hewlett-Packard HP 5890 II with autosampler HP 76763; split/splitless injector with glass wool, 270°C, 1 min splitless; column HP-1,

50 m × 0.32 mm I.D., 0.17 μm, 5 min 35°C, then at 10°C/min to 280°C (30 min); EPS (Electronic Pressure Control) constant flow 0.93 ml He/min; injection volume 1 μl.

MSD: Hewlett-Packard HP 5972 A mass selective detector (EI, 70 eV), DOS-ChemStation, Standard spectra autotune.

Method B.

As for method A, except: split/splitless injector with insert single-Taper liner HP 5062-3587, 290°C, 3 min splitless; column HP-5, 30 m × 0.25 mm I.D., 0.25 μm; 3 min 100°C, then at 10°C/min to 280°C (23 min), EPC constant flow 0.5 ml He/min.

Gas chromatography–atomic emission spectrometry (GC–AED)

Methods A and B

GC: The same as for GC–MSD, method A and B, respectively, except: EPC constant flow 0.9 ml He/min.

AED: Hewlett-Packard HP 5921 A with ChemStation HP 9144, ferrule purge 30.4, cavity vent 76.4 ml/min; nitrogen 2 l/min, oxygen 140, helium 200, hydrogen 220 kPa; injection volume 1 μl.

The GC–AED system was calibrated for the elements (in parentheses: wavelength in nm) carbon (193.031; 495.724), H (486.133), Br (478.578) and N (174.200) by use of a set of differently concentrated standard solutions containing mixtures of hexadecane, dodecylbenzene, nitrobenzene, acridine, chinoline, 1-bromohexane, 1-bromo-1,5-dichlorobenzene, triphenylarsine.

Linear calibration functions [pg atoms/μl injected = f(peak area)] were obtained in each case (1 pg atom = $1 \cdot 10^{-12}$ g atoms) with the following lower limits of detection and determination, respectively, given in pg atoms/μl injected: 2, 10 (C_{193}); 10, 50 (C_{496}); 50, 200 (H); 0.5, 1 (As); 2, 4 (Br); 15, 30 (N).

Each of the calibration compounds contained carbon; all the data obtained, e.g. for C_{193}, when put together, resulted in one and the same straight line, thus indicating both the quantitative transfer of the calibration substances from the injector up to the detector and their complete atomization in the AED. Further details in operating the GC–AED are given in previous papers [10,13–15].

3. Results

In the procedure described as method A, the treatment with bromine in boiling glacial acetic acid converted the Adamsite into 2,2′,4,4′6,6′-hexabromodiphenylamine (HBDP) [16] (Scheme 1).

Diphenylamine, if present in the sample, also would have reacted to give HBDP. Hence, the HBDP found had to be corrected for the portion originating from diphenylamine. The content of diphenylamine was determined in a separate analysis ("part 1").

Under the conditions applied in method B, the pyrolytic ethylation of Adamsite yielded 10-ethyl-5,10-dihydrophenarsazine (EtPA) as the main product (Scheme 2).

In both assays, one part of the soil extract (but not the soil itself) was spiked with a known amount of Adamsite; from the difference between the results from the spiked and the non-spiked parts the actual derivatization yield was calculated.

3.1. Identification of the derivatives

The derivatives HBDP and EtPA were identified by means of their mass spectra, obtained by GC–MSD-runs, and their molecular formulae as evaluated from the respective GC–AED results (see Table 1).

In Fig. 1 the mass spectrum of HBDP is given. The isotopic distribution in the molecular-ion cluster around mass 643 corresponds to the six bromine atoms in the molecule. The mass spectrum of EtPA (Fig. 2) shows, in addition to the molecular ion 271, only two prominent peaks, representing the hydrophenarsazine (242) and carbazole (167) species, respectively [17]. These ions served later, together with the appropriate retention window, for the routine GC–MS analysis by single-ion monitoring (SIM mode).

Table 1
Molecular formulae as determined by GC–AED analysis

Compound		C	H	As	Br	N
Hexabromodiphenylamine (HBDP)	calc.	12	5		6	1
	found	11.9	4.5		6.0	1.0
10-Ethyl-5,10-dihydrophenarsazine (EtPA)	calc.	14	14	1		1
	found	13.8	14.4	1.0		0.9

3.2. Derivatization yield, lower limits of determination

To evaluate the derivatization yields, the GC–AED results were used. Consequently, the respective values represent overall recoveries, counting from the amount introduced into the reaction up to the amount reaching the detector.

Method A

Subjecting pure Adamsite or diphenylamine to the bromination procedure, the derivatization yielded up to 90% HBDP. With soil extracts spiked with Adamsite, the derivatization yields ranged between 20 and 80%, depending on the type of soil and the presence and quantity of other contaminants.

From the bromination of matrix-free solutions and based upon the Br signal of the AED, the lower determination limit was 0.4 ng HBDP per μl injected. This would correspond to 4 μg Adamsite per kg soil sample, assuming 100% yield for both soil-extraction and derivatization. The lower determination limit for diphenylamine, based upon the C_{193} signal, was 0.14 ng/μl, which, after 100% conversion into HBDP, would correspond to 2 μg Adamsite per kg soil sample. In the chromatograms from soil extracts, however, considerable background levels of organic carbon often occurred around the di-

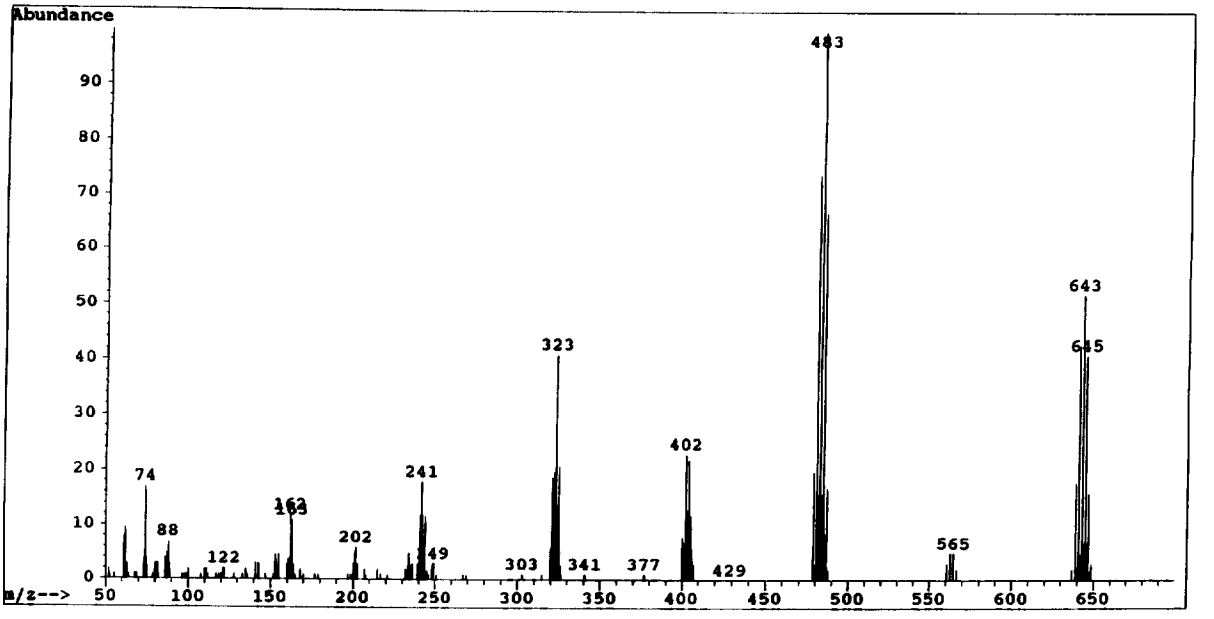

Fig. 1. EI-mass spectrum of 2,2',4,4',6,6-hexabromodiphenylamine (HBDP).

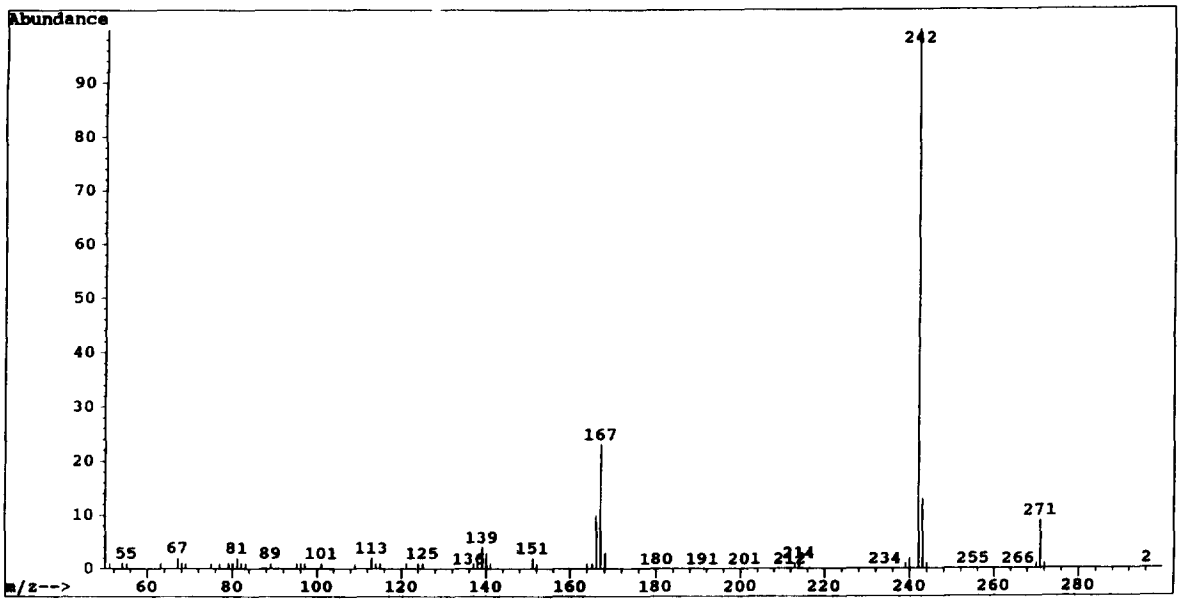

Fig. 2. EI-mass spectrum of 10-ethyl-5,10-dihydrophenarsazine (EtPA).

phenylamine peak, so that, instead of C_{193}, the N signal of the AED must be used for the quantitation of diphenylamine. On account of the lower sensitivity and the presence of only one nitrogen atom per molecule, the determination limit in such cases increased up to 5 ng/μl, corresponding to 80 μg Adamsite/kg.

Method B

With ten matrix-free solutions of Adamsite in the reaction mixture (1–20 ng Adamsite/μl injected), the GC–AED runs showed derivatization yields of 51 ± 11%. With spiked extracts (1 ng/μl injected) from ten different soil samples derivatization yields of 53 ± 14% were obtained, indicating the reaction to be rather insensitive to matrix effects.

The lower determination limit, based upon the As signal of the AED, was 0.5 ng Adamsite per μl injected. Assuming 100% extraction yield, this would correspond to about 3 μg Adamsite per kg soil sample. From "dirty" extracts, however, in order to save the GC inlet, only portions down to 1/10 of parts 1 and 2, respectively, were used for making up the reaction mixture, entailing correspondingly higher determination limits.

In order to compensate for this, we later changed to the more sensitive single-ion monitoring method (SIM), using GC–MS alone for both (tentative) identification and quantitation. With the SIM analysis (ions 271, 242 and 167) applied on the reaction mixture out of 1/10 of parts 1 and 2, respectively, again a determination limit of 3 μg Adamsite/kg could be established.

4. Discussion

The bromination method A was adopted from a synthetic procedure described by Elson et al. [17] and modified for the actual analytical purpose. The recoveries turned out to be very sensitive to variations in the reaction conditions such as temperature, heating time, the mode of adding the bromine and its amount. Occasionally isomeric tetrabromo benzenes and tetrabromo anilines were found as by-products, indicating the breakdown of the diphenylamine molecule. Disadvantageous is the fact that diphenylamine, if present in the sample, would also react to give HBDP. As a consequence, the sensitivity of the method depends on the determination limit of

diphenylamine, because this is, in general practice, higher than that for HBDP. In the evaluation of results, we assume a 100% conversion of diphenylamine into HBDP, which is, strictly spoken, incorrect. We decided to accept this inaccuracy rather than to add a further control assay for determining the derivatization yield with respect to diphenylamine.

Regarding the pyrolytic alkylation method B, initial experiments were carried out with dimethylformamide dimethylacetal. They yielded a product which, on the basis of its mass spectrum (Fig. 3), was identified by the internal Wiley MS-library to be 10-methyl-5,10-dihydrophenarsazine (MePA). In our chromatograms, however, the MePA peak was accompanied by a shoulder containing a by-product with a very similar mass spectrum. After various ineffectual attempts to separate the peaks or to suppress the by-product, we changed to dimethylformamide diethylacetal (DMFDEA) as the derivatizing reagent.

The application of DMFDEA resulted in a prominent and uniform product peak (Fig. 4). The molecular formula of the derivative, as

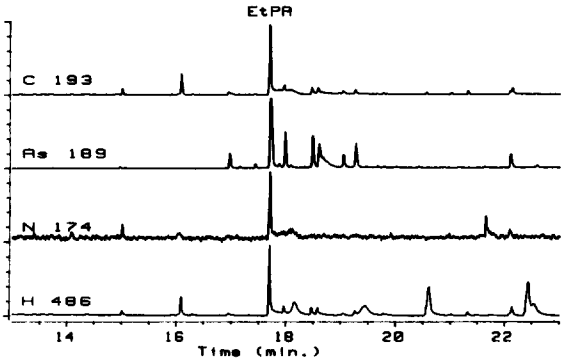

Fig. 4. GC–AED chromatograms from a matrix-free solution, containing 100 ng Adamsite/μl, subjected to pyrolytic ethylation according to method B; injection volume 1 μl; 17.72 min: EtPA, 55 ng/μl injected, corresponding to 56% derivatization yield; 18.40 min: the by-product supposed to be EtOPA.

calculated from the AED data, together with the mass spectrum (Fig. 2) closely resembling that of MePA, evidenced its identity to be 10-ethyl-5,10-dihydrophenarsazine (EtPA).

Irrespective of the injector temperature applied, substituting the pyridine in the reaction

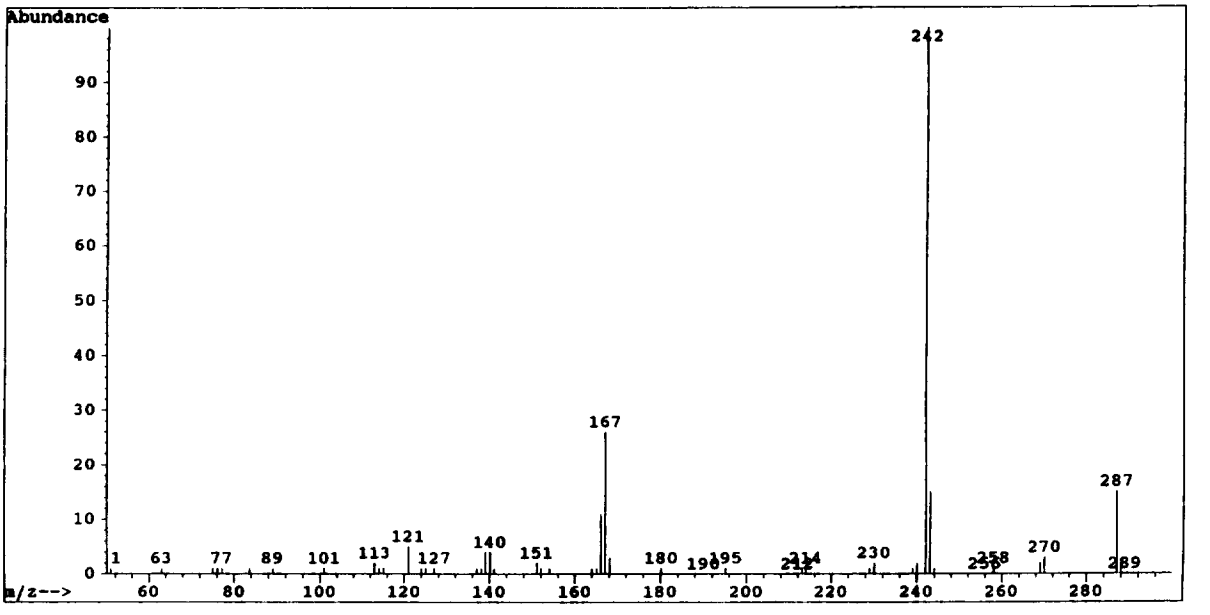

Fig. 3. EI-mass spectrum of a by-product formed out of Adamsite and DMFDEA, supposed to be 10-ethoxy-5,10-dihydrophenarsazine (EtOPA).

mixture by dimethylformamide resulted in lower yields, and without any catalyst even less EtPA was formed. With increasing injector temperature (190–350°C) the yields in EtPA ascended up to 290°C, followed by a slight decrease at higher temperatures. Concurrently, the amount of a by-product at RRT = 1.20 (mass spectrum given in Fig. 3) went down to reach, at 290°C, about 10% of that of EtPA; we consider this by-product to be 10-ethoxy-5,10-dihydrophenarsazine (EtOPA).

In another experiment, the reaction mixture (16 μg Adamsite in 25 μl ethanol + 25 μl pyridine + 50 μl DMFDEA) was stored for three days in a closed vial at 90°C; on column injection of 1 μl from this solution, no ethyl derivative could be detected, whereas splitless injection at 290°C injector temperature yielded EtPA in the expected amount.

From this finding we conclude that the reactive species is not Adamsite itself but a consecutive product formed in the hot injector. According to Vermeer et al. [16], at higher temperature Adamsite will split off chlorine and undergo dimerization to 10,10′-bis(5,10-dihydrophenarsazinyl). We suppose this dimer to react with DMFDEA to give both EtOPA (predominating below 230°C) and EtPA (prevailing above 230°C).

Compared to method A, the pyrolytic ethylation offers decisive advantages regarding the practicability, the insensitivity to matrix effects and the conclusiveness of results. Hence we consider method B as the method of choice for determining Adamsite. In turn, the more drastic bromination method A might prove to be useful in generally detecting the phenarsazine system as such, e.g. in the presumptive breakdown products 10,10′-bis(5,10-dihydrophenarsazinyl)oxide and 10-hydroxy-10-oxo-5,10-dihydrophenarsazine, which unfortunately were not available for being included in the present studies.

The soil-extraction procedure applied was based on the results from a series of optimization experiments with spiked soil samples (sterile "standard soils" of known composition and defined humidity), stored for 4 weeks after spiking [15]. The extraction yields in Adamsite were 60–90%, depending on the type of soil. It is well known, however, that for many soil contaminants the extractable percentage decreases with increasing time of storage [18]. For the real soil samples to be analyzed in our laboratory, the "storage" time was 50 years and more, so that the aforementioned recoveries here cannot be taken as to be representative. Hence, the actual extraction yields remain unknown, and, consequently, the analytical results obtained from these extracts merely present the lower limit of the true values.

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