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ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Determination of Methylphosphonic Acid and Its Esters as Chemical Markers of Organophosphorus Chemical Warfare Agents

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Abstract—The feasibility of isolation and quantitative determination by gas chromatography of methylphosphonic acid and its mono- and dialkyl etsers, which originate from destruction of organophosphorus chemical warfare agents and have been detected in bitumen–salt compounds is examined.

Organophosphorus chemical warfare agents (OPCWAs), the most known of which are tabun, sarin, soman, and VX-group compounds, are extremely strong cholinergic antagonists.

By now, basic principles of OPCWA analysis have been developed and recommended for use in the practice of international examinations [1–3]. For practically all of the known OPCWAs, the physicochemical constants are known, and therefore their "confirming" identification involves no problems. However, the experience of chemical appraisals of the consequences of emergencies involving OPCWAs shows that, in most cases, the initial OPCWAs cannot be detected at sites of their application. Decomposition products of OPCWAs, formed in various media, cannot be predicted *a priori*, the more so as the type of the weapons used may be unknown. Therefore, it is very important to reveal long-lived "witness" compounds that would be always present among products of OPCWA decomposition and would retain information about the initial compound.

Such "witnesses," or markers, should also be revealed for setting up analytical and sanitation-chemical monitoring of processes used for destruction of chemical weapons. First, it is necessary to study the composition of degradation products of war gases and of intermediates released into the contact medium in the process. This stage involves identification of OPCWA conversion products and revealing of chemical markers. The next stage should involve examination of the emission of markers to various media and assessment of possible pathways of their further transformation. These results can serve as a basis for environmental monitoring of potentially hazardous sites.

At present, a two-stage flowsheet is being put into operation in Russia for destruction of OPCWAs, with chemical decomposition and subsequent bituminous grouting of the resulting solid wastes to obtain so-called bitumen–salt compounds (BSCs) [4, 5].

It has been shown previously that among the products of yperite decomposition, including its decomposition within BSCs, a versatile marker is 1,4-dithiane [6]. In this work, with the aim to reveal chemical markers, we studied by GC–MS the composition of BSCs containing the products of sarin, soman, and VX breakdown, and also that of gases released from, and aqueous extracts of BSCs. With respect to their origin, BSC components can be subdivided in three groups: products of OPCWA breakdown, deactivating agents, and bitumen components. The chemical markers should belong to the first group; in individual determinations, they should correspond to specific types of OPCWAs. They should also be stable in the matrix and selectively detectable against the background signals.

After disposal of BSCs, emergency seal failure of containers is possible, accompanied by washout of soluble BSC components by groundwater. To estimate the extent of marker emission into an aqueous-salt system simulating the chemical composition of groundwater at the site of a repository to be constructed, it was necessary to develop procedures for recovery of the markers from aqueous solutions and for their quantitative chemical analysis. For this purpose, we used gas chromatography (GC).

To reveal chemical markers, we initially identified components always present in gases released from,

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Table 1. Procedures of sample preparation for analysis

Procedure	Analysis object	Analysis procedure
A	BSCs	Static head-space analysis
B	Aqueous extracts of BSCs	Extraction with dichloromethane (pH 7)
C	Aqueous extract after procedure B	Evaporation to dryness, redissolution in dichloromethane–acetonitrile (1 : 1), silylation
D	″	Saturation with NaCl, extraction with acetonitrile (pH 2), drying, redissolution, silylation

and aqueous extracts of, BSCs. The sample preparation procedures are summarized in Table 1. Such a fourstep scheme allowed us to expand to the maximum possible extent the group of BSC components determinable by GC analysis: from the most volatile (head-space analysis) to non-volatile (derivatives of aqueous extracts).

In the systems BSC–sarin and BSC–soman, the main decomposition products of sarin and soman are, respectively, isopropyl and pinacolyl alcohols, diisopropyl and dipinacolyl methylphosphonates, isopropyl and pinacolyl hydrogen methylphosphonates, and also methylphosphonic acid itself. The system BSC–VX is considerably more complex and includes several tens of volatile organic compounds; the major components are mono- and disulfides containing diethylaminoethyl group but lacking the phosphorus atom. The mass spectra of these compounds are difficultly discernible and in most cases contain only a single strong peak corresponding to the $(Et_2N{=}CH_2)^+$ ion. The major phosphorus-containing products are methylphosphonic acid and its mono- and dibutyl esters [by VX we mean *O*-isobutyl *S*-(2-diethylaminoethyl) methylphosphonothioate, whereas in the United States and some other countries its isomer, *O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate, is more widespread].

Thus, among products of sarin, soman, and VX decomposition by the two-stage process, we can distinguish homologous series of alkyl hydrogen and dialkyl methylphosphonates. Specifically these compounds, together with their hydrolysis product, methylphosphonic acid, seem to be the most suitable chemical markers for assessing the possible environmental pollution. Additional advantages of these compounds are the possibility of their selective detection and low probability of false positive results, because compounds with a P–C bond are very rare in nature.

Table 2 gives mass spectra of phosphorus-containing compounds that originate from the breakdown of OPCWAs and are present in BSCs. Among the compounds given in Table 2, only methylphosphonic acid diesters $MeP(O)(OR)_2$ can be determined by direct gas-chromatographic analysis. As compared with methylphosphonic acid and alkyl hydrogen methylphosphonates, dialkyl methylphosphonates are considerably more seldom mentioned in the literature as OPCWA decomposition products, although they are included in the lists of compounds to be monitored according to the Convention on the Prohibition of the Development, Production, Stockpiling, and Use of Chemical Weapons and on Their Destruction [7]. Under conditions of the existing process for OPCWA destruction, they can form by reaction of the monoester MeP(O)(OH)(OR) with the alcohol ROH (R = *i*-Pr, pinacolyl, or *i*-Bu for sarin, soman, and VX, respectively). Also, diesters of methylphosphonic acid can be present as impurities in the initial OPCWAs, especially after their prolonged storage. A scheme for classification of these compounds according to their mass spectra was proposed in [8]. Comparison of the intensity of the peak at m/z = 47, corresponding to the $[P{=}O]^+$ fragment, with the preset threshold is the first step of the algorithm. If the threshold is not exceeded, this fact is regarded, according to [8], as the absence of the compounds under consideration in a sample. However, according to our experience, the intensity of this signal in the mass spectra of alkyl methylphosphonates is so low that at their low concentrations (especially in such matrices as soil) it can hardly be detected.

Under the experimental conditions, the content of diisopropyl and diisobutyl methylphosphonates in BSCs was determined with sufficient reproducibility by static head-space analysis. Dipinacolyl methylphosphonate present in BSCs can be quantitatively determined only in extracts. Table 2 shows that the strongest peak in the mass spectra of all these three diesters is that at m/z = 97. Group analysis of dialkyl methylphosphonates in groundwater and soil by GC–MS is possible in the selective ion monitoring mode. Samples were prepared for analysis by procedure B. The sensitivity of the analysis is high: the de-

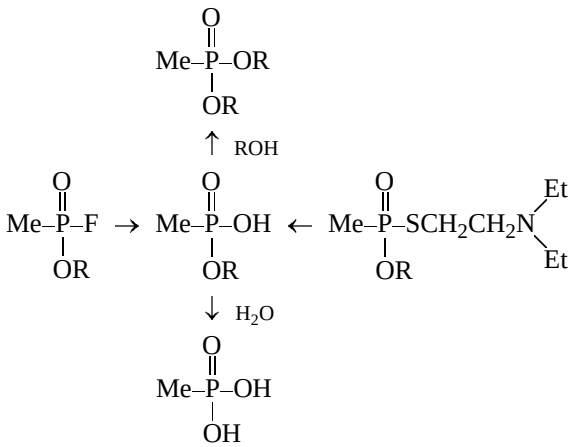
Table 2. Alkylphosphonates recovered from BSCs containing products of OPCWA destruction*

Compound	Mass spectrum, *m*/*z* (I_{rel}, %)	OPCWA being destroyed	Sample preparation procedure (Table 1)
Methylphosphonates:			
diisopropyl	165(4.1), 139(7.2), 137(3.1), 123(65.1), 121(9.0), 97(100.0), 80(7.2), 79(20.9), 65(3.6), 47(4.5), 45(7.3), 43(9.1)	Sarin	A, B**
diisobutyl	165(1.6), 153(4.5), 137(8.6), 135(3.1), 111(4.7), 110(8.7), 97(100.0), 80(7.8), 79(10.6), 57(11.1), 43(3.5)	VX	A, B**
dipinacolyl	208(4.0), 207(38.1), 181(1.7), 180(1.4), 165(11.5), 124(60.1), 123(99.5), 111(2.6), 97(100.0), 85(35.9), 80(12.4), 69(11.1), 57(15.6)	Soman	B
isopropyl hydrogen (converted to trimethylsilyl ester)	195(5.0), 169(14.9), 155(3.6), 154(8.3), 153(100.0), 152(5.1), 151(15.0), 137(4.4), 123(2.3), 121(4.4), 77(7.2), 75(22.7), 73(7.9), 45(8.6)	Sarin	C, D**
isobutyl hydrogen (converted to trimethylsilyl ester)	209(2.0), 170(2.5), 169(24.3), 167(3.9), 154(7.8), 75(20.2), 73(10.3), 153(100.0), 151(14.5), 137(3.0), 123(2.0), 77(6.7), 45(7.8)	VX	C, D**
pinacolyl hydrogen (converted to trimethylsilyl ester)	237(1.2), 196(14.7), 195(27.1), 179(3.7), 169(32.5), 153(100.0), 152(9.8), 151(25.5), 137(6.4), 121(10.5), 77(6.3), 75(19.8), 73(13.9), 45(6.7)	Soman	C, D**
Methylphosphonic acid [converted to bis(trimethylsilyl) ester]	240(7.6), 227(7.6), 226(17.8), 225(100.0), 209(3.8), 195(3.4), 153(7.2), 147(10.3), 135(7.3), 133(9.3), 105(7.7), 75(11.4), 73(18.5), 45(11.6)	Sarin, Soman, VX	C,** D

* Underlined are the *m*/*z* values for the strongest peaks.

** The best procedure for sample preparation.

tection limit in water is as low as 0.1 μg l^{-1}. At the same time, esters of dibasic carboxylic acids, alkenes, alkylthiophenes, and some other compounds often present in soils also give in mass spectra a strong peak at *m*/*z* = 97. Therefore, if the concentration level does not allow analysis of the total mass spectrum, identification of dialkyl methylphosphonates, especially in soil, requires additional confirmation. Dialkyl methylphosphonates fully meet the requirements to BSC chemical markers. Their main advantages are simple recovery from matrices and the possibility of direct selective gas-chromatographic determination. At the same time, it is known [9] that, in the presence of water, dialkyl methylphosphonates are hydrolyzed to give alkyl hydrogen methylphosphonates and then methylphosphonic acid. Thus, just alkyl hydrogen methylphosphonates and methylphosphonic acid, initially present in BSCs and formed by hydrolysis of dialkyl methylphosphonates, can be considered "long-lived" BSC markers. Alkyl hydrogen methylphosphonates in which the alkyl group is specific to a particular OPCWA can be regarded as individual chemical markers, and methylphosphonic acid, as group marker (Table 2):



where R = *i*-Pr (sarin), pinacolyl (soman), or *i*-Bu (VX).

Methylphosphonic acid and alkyl hydrogen methylphosphonates are readily soluble in water; therefore,

Table 3. Comparative estimation of the efficiency and reproducibility of recovery of acidic methylphosphonates from aqueous solutions

Compound	Method of sample preparation	S_{av}	S_x	R
		%		
Hydrogen phosphonate:				
isopropyl	Calibration	0.920	–	–
	Evaporation	0.031	48.9	3.3
	Extraction*	0.074	17.1	80.0
isobutyl	Calibration	0.357	–	–
	Evaporation*	0.068	49.7	18.9
	Extraction	0.303	23.8	85.0
pinacolyl	Calibration	0.590	–	–
	Evaporation	0.094	67.0	15.8
	Extraction*	0.581	21.6	98.6
Methylphosphonic acid	Calibration	0.805	–	–
	Evaporation	0.610	23.6	75.8
	Extraction*	0.413	28.8	51.0

* The best procedure is marked with an asterisk.

they will migrate into groundwater in the case of a seal failure of BSC containers. At the same time, being polar and nonvolatile, these compounds are difficult objects for chemical analysis. However, they can be readily determined by HPLC. In this case, it is necessary to ensure efficient detection, which can be provided by introducing groups imparting UV absorption to a substance [10] or enhancing its electrical conductivity [11]. The feasibility of direct analysis of alkyl hydrogen alkylphosphonates by liquid chromatography, combined with mass spectrometry in the electrospray mode, was demonstrated in [12]. The main limitations of the procedures involving HPLC separation are problems with obtaining narrow peaks [10, 11] and the high cost of the equipment [12].

The majority of the methods developed for analysis of alkyl hydrogen alkylphosphonates are based on GC with various modification procedures for increasing the volatility of the compounds being analyzed. To prepare a sample for GC analysis, it is necessary to quantitatively recover it from aqueous solution and convert to a volatile form. These tasks are solved successively, since extractive alkylation procedures are inapplicable to these compounds. To recover alkyl hydrogen alkylphosphonates, Soderstrom *et al*. [9] evaporated aqueous solutions and biological fluids to dryness; Minami *et al*. proposed an alternative approach: extraction with acetonitrile [13].

In this study, we used both the procedures. To find the best conditions for recovery and modification of methylphosphonic acid and isopropyl, isobutyl, and pinacolyl hydrogen methylphosphonates, we used GC with a flame-ionization detector. The areas of the chromatographic peaks of the trimethylsilyl esters were measured, and their ratios to the area of the peak of tetradecane, used as internal reference, (relative peak areas) were determined.

The average relative peak areas S_{av} for each component in calibration solutions and in samples after evaporation and extraction are given in Table 3. The reproducibility of the analysis was characterized by the root-mean-square deviation S_x, and the efficiency of sample preparation, by the degree of recovery R. Data in Table 3 were obtained for the analyte concentration range 1–100 mg l^{-1}.

Table 3 shows that, in analysis of alkyl hydrogen methylphosphonates, satisfactory results are obtained only in extraction with acetonitrile (method D). We found that methylene chloride (method B) does not recover methylphosphonic acid and its acidic esters from both neutral and acidic (pH 1–6) aqueous solutions to a noticeable extent (R < 1%). Thus, there is practically no loss of methylphosphonic acid and its acidic esters in stage B of sample preparation (Table 1).

Methylphosphonic acid is recovered from aqueous solutions most difficultly. At the same time, with methylphosphonic acid, satisfactory analytical parameters are achieved by evaporation of the aqueous sample. Thus, the best procedures for analysis of methylphosphonic acid and its acidic esters are C and D, respectively (Table 1). However, in analysis of large

volumes of natural waters expected to contain methylphosphonic acid in low concentrations, procedure C is very power-consuming. In this case, procedure D becomes more appropriate, with correction made for the actual degree of recovery.

To choose a procedure for conversion of methylphosphonic acid and its acidic esters to volatile derivatives, we compared, using solutions of the corresponding reference samples in dichloromethane, the efficiencies of methylation (with diazomethane and methyl iodide) and silylation [with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA)]. The highest relative yields, at a minimum of false positive results, were achieved with silylation (irrespective of the silylating agent used). The effect of trimethylchlorosilane (TMS) addition on the yield of silylation with BSTFA was studied in [13]; the highest yield was achieved at the ratio BSTFA : TMS = 1 : 9. Under our conditions, addition of TMS had no significant influence on the silylation efficiency and resulted only in sample contamination. As already noted, when choosing the optimal conditions for recovery from aqueous solutions and modification of alkyl hydrogen methylphosphonates, we used as recording device a gas chromatograph equipped with a flame-ionization detector, which was very convenient as applied to model, relatively concentrated (0.1–100 mg l^{-1}) solutions. However, for actual water samples, the sensitivity and selectivity of this procedure were insufficient. In this case, we used in GC analysis a thermoionic detector, with the same recovery and modification procedures. The detection limit of methylphosphonic acid and its esters was as low as 0.1–1 μg l^{-1}, approaching the sensitivity level of GC–MS with selective ion monitoring (m/z = 153 for alkyl hydrogen methylphosphonates and 225 for methylphosphonic acid).

EXPERIMENTAL

Head-space analysis (method A) was performed in the static mode: 1 g of a BSC sample was kept for 40 min in temperature-controlled 10-ml vessels with a Teflon membrane at 70°C, and 1-ml samples of the equilibrium vapor were taken for analysis.

Aqueous BSC samples were prepared as follows.[1] A bitumen–salt compound was poured into molds made of a chemically inert material and kept for 24 h, after which an aqueous-salt solution simulating the composition of natural waters at the site of intended BSC disposal was added, and the system was kept for 4 days at 25°C.

Neutral extracts from BSC aqueous extracts (method B) were prepared by treatment of 10 ml of an aqueous sample with methylene chloride (3 × 2 ml) at pH 7. The combined extract was dried, concentrated, and analyzed.

The aqueous residue was divided in two portions. An aliquot of the first portion was evaporated to dryness and dissolved in 10 μl of methylene chloride–acetonitrile (1 : 1), 20 μl of a methanolic solution of BSA or BSTFA was added, and the mixture was kept for 20 min at 60°C; after that it was diluted (if necessary) and analyzed (method C). The second portion was used for preparing acidic extracts from aqueous extracts. It was acidified to pH 1 with dilute sulfuric acid, saturated with sodium chloride, and extracted with acetonitrile (3 × 2 ml). The combined extract was dried, concentrated, again dried, and redissolved in 10 μl of methylene chloride–acetonitrile (1 : 1); 20 μl of a methanolic solution of BSA or BSTFA was added, and the mixture was kept for 20 min at 60°C (method D).

The solvents (methylene chloride and acetonitrile) were purified by standard procedures and distilled in a column; pure-grade anhydrous sodium sulfate was calcined in a muffle furnace at 400°C before use. BSA, BSTFA, and methyl iodide were purchased from Merck (Germany); diazomethane was prepared by decomposition of N-nitrosomethylurea.

The GC–MS analysis was performed on a Shimadzu device comprising a GC-17A gas chromatograph, a QP 5000 mass spectrometer equipped with a quadrupole analyzer, and a data processing system. The ionization was performed by electron impact at an ionizing electron energy of 70 eV. The ion source temperature was 280°C. The device was calibrated in the automated mode against perfluorotributylamine reference. A DB-5 25000 × 0.2-mm capillary column of fused quartz was used; the thickness of the stationary phase film was 0.33 μm. The flow rate of the carrier gas (He) was 1 ml min^{-1}. Liquid samples (0.5–1 μl) were injected in the splitless mode (0.3 min), and gas samples (1 ml), in the split mode. The injector temperature was 250°C. The column was heated from 40 to 270°C at a rate of 5 deg min^{-1} and kept at the final temperature for 15 min.

Compounds were separated on a Kristall-2000 gas chromatograph equipped with a flame-ionization de-

[1] The procedure was developed at the State Research Institute of Organic Chemistry and Technology (GosNIIOKhT); reference samples of methylphosphonic acid and its isopropyl, isobutyl, and pinacolyl esters were prepared and submitted by GosNIIOKhT staff members.

tector or a thermionic detector and a quartz capillary column (25 000 × 0.2 mm). The thickness of the stationary phase film (SE-54) was 0.2 μm. The flow rate of the carrier gas (nitrogen) was 1 ml min^{-1}; hydrogen and air were fed into the detector at rates of 15 and 250 ml min^{-1}, respectively. The temperature schedule was the same as in the GC–MS analysis.

Quantitative GLC analysis was performed with external reference.

CONCLUSIONS

(1) Methylphosphonic acid and its mono- and dialkyl esters present in bitumen–salt compounds as decomposition products of organophosphorus chemical warfare agents can be considered chemical markers.

(2) Dialkyl methylphosphonates can be quantitatively determined by gas chromatography. For determination of methylphosphonic acid and its acidic esters, they should be preliminarily extracted with acetonitrile from acidic solutions and silylated.

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