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Gas chromatography-mass spectrometric studies of *O*-alkyl *O*-2-(*N,N*-dialkylamino) ethyl alkylphosphonites(phosphonates) for Chemical Weapons Convention verification

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The electron ionization (EI) mass spectra of a series of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites(phosphonates), which are precursors of nerve agents, were studied for Chemical Weapons Convention (CWC) verification. General EI fragmentation pathways were constructed and discussed. Proposed fragment structures were confirmed through analyzing fragment ions of deuterated analogs and density functional theory (DFT) calculations. The observed fragment ions are due to different fragmentation pathways such as hydrogen and McLafferty+1 rearrangements, alkene, amine and alkoxy elimination by α- or β-cleavage process. Fragment ions distinctly allow unequivocal identification of the interested compounds including those of isomeric compounds. The presence and abundance of fragment ions were found to depend on the size and structure of the alkyl group attached to nitrogen, phosphorus and oxygen atoms.

Keywords: mass spectrometry, Chemical Weapons Convention, alkylphosphonites(phosphonates), DFT calculations, McLafferty+1 rearrangement

Introduction

An ongoing area of research in our laboratory in recent years has been the utilization of microsynthesis and mass spectral studies of organophosphorus compounds for verification purposes under the Chemical Weapons Convention (CWC).[1–3] This convention, which prohibits development, production, stockpiling and use of chemical weapons, was available for signature on January 13, 1993 and came into force on April 29, 1997. For CWC implementation, toxic chemicals, precursors and most related chemicals are listed in three distinct CWC schedules contained in the annex on chemicals. Schedule 1 includes chemicals which were used in a chemical weapon such as mustard gas or nerve agents (Part A) and four main precursors for the preparation of nerve agents (Part B). Schedule 2 lists three toxic chemicals not included in Schedule 1 and the degradation products and precursors of these toxic chemicals as well as of those of Schedule 1. Schedule 3 lists four toxic chemicals and some precursors not listed in the other schedules. The state parties established the Organization for the Prohibition of Chemical Weapons (OPCW) to achieve the object and purpose of CWC.[4] In order to detect and identify CWC-related chemicals in different matrices, OPCW maintains a network of designated laboratories. Only designated laboratories are authorized to undertake the task of off-site analysis in verification activities. Analytical

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performance of designated laboratories and laboratories seeking designation is regularly evaluated by OPCW through official proficiency tests.[5] For unequivocal identification of the CWC-related chemicals in real samples or proficiency tests, the availability of mass spectra and interpretation skills are essential requirements. The OPCW Central Analytical Database (OCAD) is the major source for CWC-related reference mass spectra.[6] This database has been developed by laboratory contributions of the member states. It is impossible to have a complete collection of mass spectroscopic data for all CWC-related chemicals, in part due to the extreme toxicity of these materials, which restrict research on such chemicals. In recent years, some research results have been reported on microsynthesis and interpretation of mass spectra of CWC-related chemicals.[7–12]

Among the scheduled chemicals, nerve agents constitute the greatest worry, due to their highly toxic effects on humans and facile and inexpensive synthesis. Well known chemicals are tabun, sarin, soman and VX. Nerve agents inhibit the enzyme acetylcholinesterase (AChE) which hydrolyzes and terminates the action of the neurotransmitter acetylcholine. Inhibition of AChE results in overstimulation of cholinergic nerves leading to respiratory paralysis.[13,14] Therefore, fast analysis and identification of these agents, their precursors and degradation/reaction products (CWC-related chemicals) are essential in selecting the most effective countermeasure against them. *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites with general structure (**I**) are placed in CWC as Schedule 1.B.10 (Figure 1). These chemicals are precursors of highly toxic nerve agents known as V-agents. For example, 2-ethyl *O*-2-(*N,N*-diisopropylamino)ethyl methylphosphonite (QL) is used as a starting material for the synthesis of VX. The OPCW Central Analytical Database (OCAD) has very poor information on the data of alkylphosphonites. At this time, there is only one spectrum for alkylphosphonite compound (QL). On the other hand, oxidation of *O*-alkyl *O*-2-(*N,N*-dialkylamino) ethyl alkylphosphonites gives *O*-alkyl *O*-2-(*N,N*-dialkylamino) ethyl alkylphosphonates (**II**), which are also included in the CWC Schedule 2.B.4. Although mass data of some derivatives of **II** were entered in the OCAD, no detailed investigation on the mass spectral fragmentation process of these chemicals (**I** and **II**) and their isotopically-labeled derivatives are found in the literature.

Since verification activities, in general, rely on gas chromatography-mass spectrometry (GC/MS), this paper is devoted to electron ionization (EI) mass spectra of **I** and **II** with possible fragmentation routes. Structures of fragments were confirmed using analysis of deuterated analogs. Density functional theory (DFT) was used to reveal preferred fragmentation pathways. This paper will make a substantial contribution to OCAD.

Experimental

Reagents and chemicals

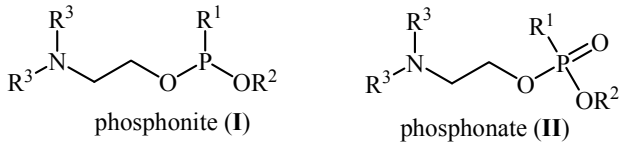
All chemicals required for microsynthesis of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites(phosphonates) were purchased from Sigma–Aldrich (St Louis, MO, USA), Fluka (Neu-Ulm, Germany) and Merck (Darmstadt, Germany) and were used as received. Alkyl dichlorophosphine, alkylphosphonic dichloride were synthesized using the existing method.[15,16] Isopropanol-d_6 was prepared through reduction of acetone-d_6 by sodium borohydride.[17]

GC/MS analyses

The GC/MS analyses were performed using an Agilent 6890N gas chromatograph equipped with a 5973 mass selective detector, a HP-5MS capillary column (30 m, 320 µm i.d. and 0.25 µm film thickness) and helium as the carrier gas at a constant flow of 1.8 mL min^{-1}. The oven temperature was set at 40°C for 3 min and was then increased to 280°C with a ramp of 10°C min^{-1} and held for 6 min. The samples were injected in splitless mode at an injection temperature of 250°C. The temperatures of the EI source and analyzer were kept at 230°C and 150°C, respectively. The ionization energy was set at 70 eV. The scan range was *m/z* 40–500. Automated mass spectral deconvolution and identification system (AMDIS) software was used to calculate the retention indices (RI) of the synthesized compounds. An alkane mixture [octane (C_8) to tetracosane (C_{24})] was used for retention indices calculations.

Computation details

All geometry optimizations and frequency calculations for all species were performed by using the Gaussian 03 program.[18] Density functional theory (DFT) calculations with the Becke three parameters hybrid functional (DFT-B3LYP) were carried out, with a 6-311 ++G (d, p) basis set for all atoms. Vibrational frequencies were calculated at the same level to ensure that each stationary point is a true minimum. The harmonic-oscillator approximation was also used for the thermodynamic



$R^1 = CH_3, C_2H_5, R^2 = CH_3, C_2H_5, CD_3, C_2D_5, i\text{-}C_3HD_6, R^3 = CH_3, C_2H_5, i\text{-}C_3H_7$

Figure 1. General structure of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites(phosphonates).

partition functions. After geometry optimization and frequency calculations, zero-point energies (*ZPE*s) and thermal corrections were obtained at 298 K.

General procedure for microsynthesis of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites I

A solution of alcohol (R^2OH, 0.2 mmol), 2-(*N,N*-dialkylamino) ethanol (0.2 mmol) and triethylamine (0.4 mmol) in CH_2Cl_2 (200 µL) was added to a solution of alkyl dichlorophosphine (0.2 mmol) in dry dichloromethane (300 µL), under nitrogen atmosphere dropwise, while stirring. The reaction mixture was stirred at ambient temperature for an hour. Any precipitate was filtered off and the resulting solution of *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites **I** was analyzed using GC/MS as required.

General procedure for microsynthesis of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonates II

Alcohol (R^2OH, 0.2 mmol), 2-(*N,N*-dialkylamino)ethanol (0.2 mmol) and triethylamine (0.4 mmol) in CH_2Cl_2 (200 µL) was added to a solution of alkylphosphonic dichloride (0.2 mmol) in dichloromethane (300 µL) dropwise, while stirring. The reaction mixture was stirred at ambient temperature for an hour. Any precipitate was filtered off and the solution was analyzed using GC/MS as required.

CAUTION: These compounds are extremely toxic and, therefore, should be handled only by a trained professional in an efficient fume cupboard equipped with an active charcoal filtration system.

Results and discussion

EI-MS of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites I

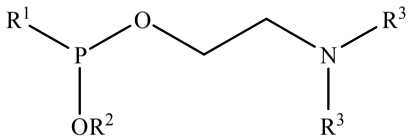
GC retention indices (RI) for contribution to the OCAD were calculated using the Van Den Dool method.[19] It is interesting to note the effect of heavy isotopes on retention indices of isotopically-labeled compounds. Deuterosubstitutions can have an influence on the vaporization, diffusion, partition and lipophilicity of the analyte which are influencing its interactions with the GC stationary phase.[20] Retention index depends on vapor pressure.[21] For most unlabeled and labeled compounds, inverse vapor pressure isotope effects have generally been seen, $p_D > p_H$.[22] In this study, the RI values of labeled compounds are less than unlabeled compounds (Table 1).

EI mass spectra of **I** showed that their fragmentation follows a general pathway involving α- and β-cleavage and amine, alkene and aldehydes elimination etc. Prominent EI fragment ions of **I** are listed in Table 1 and their corresponding structures are depicted in Figure 2.

The most important fragment ion [A] is formed through α-cleavage of P–C bond. The intensity of this special ion is influenced by the alkyl group on nitrogen. When alkyl on nitrogen (R^3) becomes larger, the intensity of [A] decreases or vanishes. In $R^3 = CH_3$ analogs, this ion abundance is the third largest peak in the mass spectra. Ion [A] was clearly distinguishable in the spectra of deuterated analogs. In addition, formation of the ion [D] from [A] through a 1,3-hydrogen shift is an evidence for α-cleavage of a P–C bond, which can be seen in the spectra of deuterated analogs. It is interesting to note that ion [A] could be related to three possible structures (Figure 3), in which [A_1] and [A_2] are resonance forms. The free energy of structure [A_1], in the case of $R^2 = R^3 = CH_3$ was calculated to be 112 kJ mol^{-1} and 23 kJ mol^{-1} less than that of structure [A_2] and [A_3], respectively. This indicates a preference for the formation of structure [A_1]. The C–N or C–H bonding electrons in [A_1] can delocalize to stabilize the electron deficient oxygen atom. In other words, the effect of bonding electrons of C–N or C–H on stabilization of [A_1] can be represented by hyperconjugation ($\sigma \rightarrow \sigma^*$, Figure 4).[23] The implication of this resonance structure is that some electron density is transferred from a C–N or C–H bond (σ, as HOMO) to the empty σ^* orbital (as LUMO) of a C–O bond. The C–O bond with the electron deficient oxygen atom is better able to accept electron density.

Elimination of the 2-(*N,N*-dialkylamino)ethanol radical from molecular ion ($M^{+\bullet}$) by α-cleavage caused formation of ion [B]. Fragmentation of deuterated analogs also showed this ion. The intense ions [E], [F] and [G] were produced from the 2-(*N,N*-dialkylamino)ethanol group. Presence of these fragments in the mass spectra is a good indication of *2-(N,N*-dialkylamino)ethanol in **I**. Fragment ion [C] could be formed through the loss of an alkoxy radical from the $M^{+\bullet}$. Ion [C] further fragmented to ion [H] by the migration of one hydrogen to the P=O group. Elimination of alkoxy as a radical and one alkyl group on nitrogen as the neutral alkene gave rise to ion [I]. Fragment ion [J] is attributed to the loss of *N,N*-dialkylethylamine as a radical via β-cleavage. Its corresponding deuterated ion in the mass spectra of deuterated analogs could support such a fragmentation pathway. Leaving a neutral aldehyde and alkene from [J] gave rise to ions [M] and [N], respectively. Direct elimination of the *N,N*-dialkylamine moiety from $M^{+\bullet}$ yielded ion [K]. The ions [L]/[L′] can be postulated to arise via hydrogen shift involving both oxygen atoms and a phosphorus atom (Figure 5). The radical cation can be produced by knocking out one non-bonding electron either from phosphorus (route L), or from one of the two oxygen atoms (route L′). The proposed fragmentation pathway (L) involves an initial hydrogen migration (1,4 C-P H shift) to the phosphorus-bearing radical cation site, leading to a distonic species. The second hydrogen is abstracted as a hydride in a concerted manner, with simultaneous hydride migration (1,3 C–O H^- shift) and single bond cleavage involving a four-membered cyclic state. The proposed fragmentation pathway (L′) also involves an initial hydrogen migration (1,5 C–O H shift) to the oxygen-bearing radical cation site, leading to a distonic species followed by a 1,3 C–O H^- shift to the other oxygen atom through a four-membered transition state with expulsion of a *N,N*-dialkylethenamine radical.

Table 1. GC-MS data of *O*-alkyl *O*-2-(*N*,*N*-dialkylamino)ethyl alkylphosphonites I.



Entry	R1	R2	R3	Retention Index (RI)	Fragment ions (% relative abundances)													
					[A]	[B]	[C]	[D]	[E]	[F]	[G]	[H]	[I]	[J]	[K]	[L]	[M]	[N]
1	C_2H_5	C_2H_5	C_2H_5	1307	192 (26)	—	176 (2)	93 (2)	86 (100)	100 (59)	99 (21)	77 (3)	148 (1)	121 (<1)	148 (<1)	123 (<1)	77 (3)	93 (2)
2	C_2H_5	C_2D_5	C_2H_5	1303	197 (27)	110 (1)	176 (1)	98 (3)	86 (100)	100 (60)	99 (22)	77 (<1)	148 (1)	126 (<1)	—	128 (<1)	78 (3)	94 (2)
3	C_2H_5	CH_3	C_2H_5	1257	178 (31)	91 (1)	176 (1)	79 (4)	86 (100)	100 (45)	99 (15)	77 (<1)	148 (1)	107 (1)	134 (1)	109 (1)	77 (<1)	—
4	C_2H_5	CD_3	C_2H_5	1256	181 (32)	94 (2)	176 (1)	82 (5)	86 (100)	100 (46)	99 (17)	—	148 (1)	110 (1)	137 (<1)	—	—	94 (2)
5	C_2H_5	C_2H_5	CH_3	1164	164 (63)	105 (1)	148 (3)	93 (3)	58 (76)	72 (100)	71 (35)	77 (4)	—	121 (<1)	148 (3)	123 (<1)	77 (4)	93 (3)
6	C_2H_5	C_2D_5	CH_3	1160	169 (54)	110 (1)	148 (2)	98 (1)	58 (77)	72 (100)	71 (36)	77 (1)	—	126 (<1)	—	128 (<1)	78 (3)	94 (2)
7	C_2H_5	CD_3	CH_3	1109	153 (74)	94 (2)	148 (1)	82 (8)	58 (100)	72 (97)	71 (31)	77 (1)	—	110 (1)	—	112 (2)	—	94 (2)
8	CH_3	C_2H_5	C_2H_5	1162	192 (3)	91 (2)	162 (2)	93 (<1)	86 (100)	100 (28)	99 (30)	63 (4)	134 (<1)	107 (<1)	—	109 (<1)	63 (4)	79 (3)
9	CH_3	C_2D_5	C_2H_5	1160	197 (3)	96 (1)	162 (1)	98 (2)	86 (100)	100 (28)	99 (30)	63 (1)	134 (<1)	—	—	—	64 (4)	80 (3)
10	CH_3	CH_3	C_2H_5	1171	178 (4)	77 (3)	162 (1)	79 (1)	86 (100)	100 (22)	99 (27)	63 (2)	—	93 (3)	120 (<1)	95 (<1)	63 (2)	79 (1)
11	CH_3	CD_3	C_2H_5	1169	181 (4)	80 (3)	162 (1)	82 (2)	86 (100)	100 (22)	99 (28)	63 (<1)	—	96 (3)	—	98 (2)	64 (1)	80 (3)
12	CH_3	C_2H_5	*i*— C_3H_7	1353	220 (<1)	91 (2)	190 (1)	93 (<1)	114 (100)	128 (18)	127 (23)	63 (4)	148 (2)	107 (1)	134 (1)	109 (<1)	63 (4)	79 (3)
13	CH_3	C_2D_5	*i*— C_3H_7	1349	225 (<1)	96 (1)	190 (1)	98 (4)	114 (100)	128 (19)	127 (23)	63 (1)	148 (1)	112 (11)	—	114 (100)	64 (4)	80 (3)

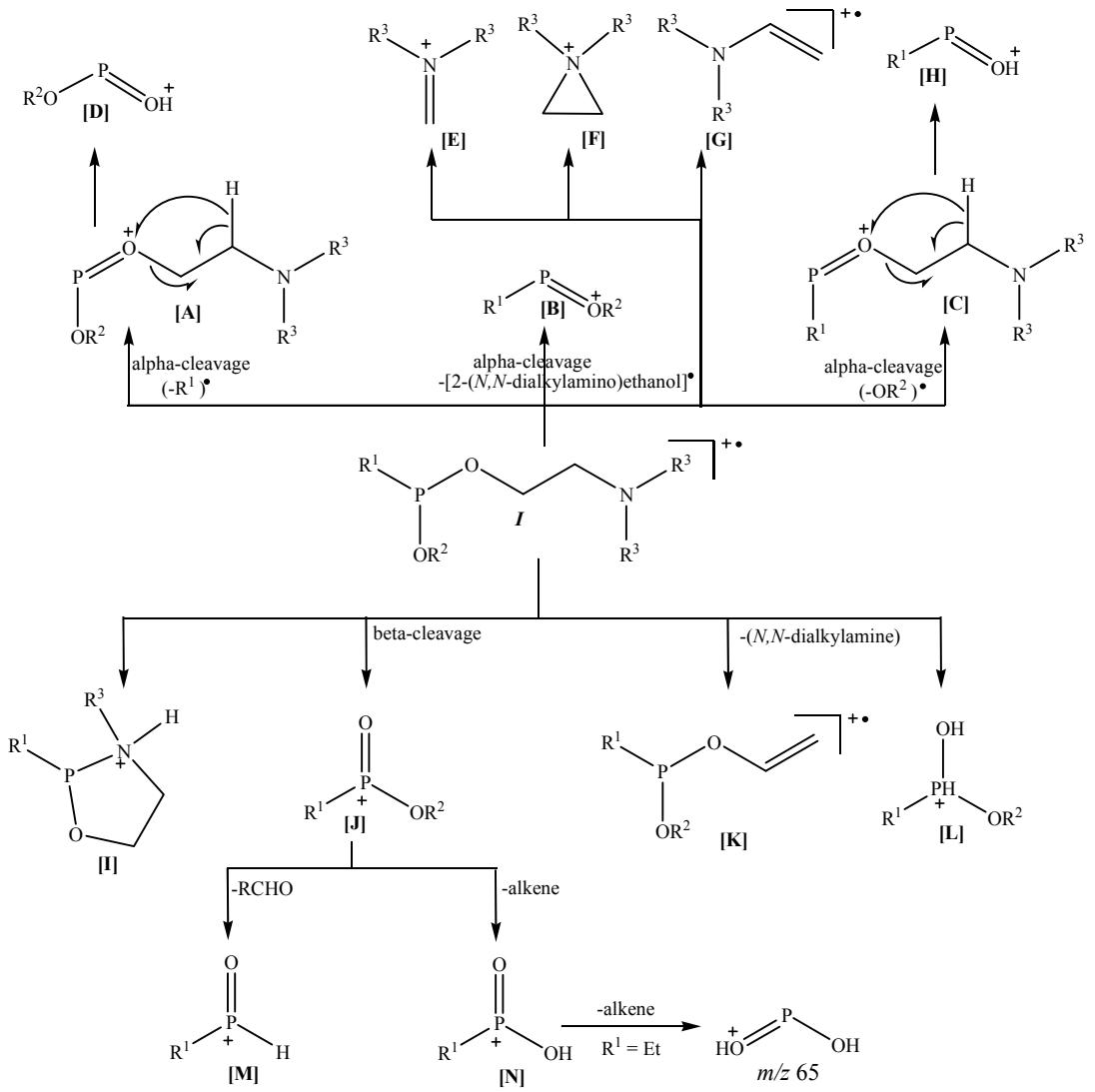


Figure 2. General fragmentation pattern of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites **I** in EI-MS.

Ions [L] and [L′] with different structures have the same molecular formulas and could give rise from different radical cations. Calculations revealed that isomer [L] is 21 kJ mol^{-1} more stable than [L′] in the case of $R^1 = R^2 = CH_3$. This could be attributed to delocalization of positive charge over two oxygen atoms.

With these encouraging results, attention was focused on the spectra of *O*-2-(*N,N*-diisopropylamino)ethyl methylphosphonites (QL) and its deuterated analog (QL-d_5, entries 12 and 13 Table 1). As mentioned above, the QL is a precursor for the synthesis of VX and its oxygen analogs. Therefore, unambiguous identification of QL and its related

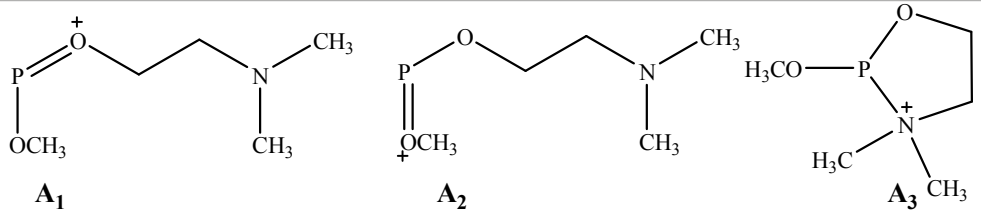


Figure 3. Three possible structures of [A].

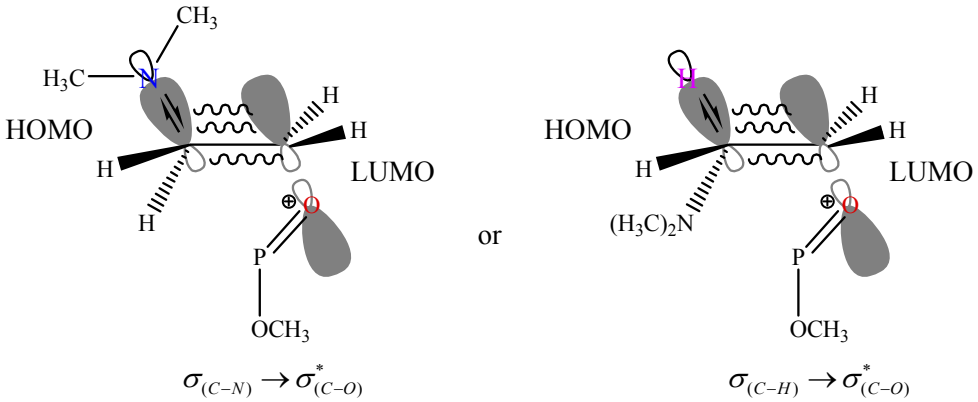


Figure 4. Hyperconjugation ($\sigma \rightarrow \sigma^*$) in fragment ion [A].

chemicals in environmental samples is of vital interest. The EI-MS spectra of QL and QL-d_5 are shown in Figure 6. The intensity of fragment ion [A] in both spectra, unlike the others, is very low. Despite the other methylphosphonite derivatives, elimination of an isopropyl radical on nitrogen from $M^{+\bullet}$ was observed with low intensity in the spectra of the QL (*m/z* 192) and QL-d_5 (*m/z* 197), respectively. These differences may be considered as a hint for unambiguous identification of QL in a real sample analysis.

EI-MS of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonates II

As mentioned above, the *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonates **II** are listed in Schedule 2.B.4 of CWC. They

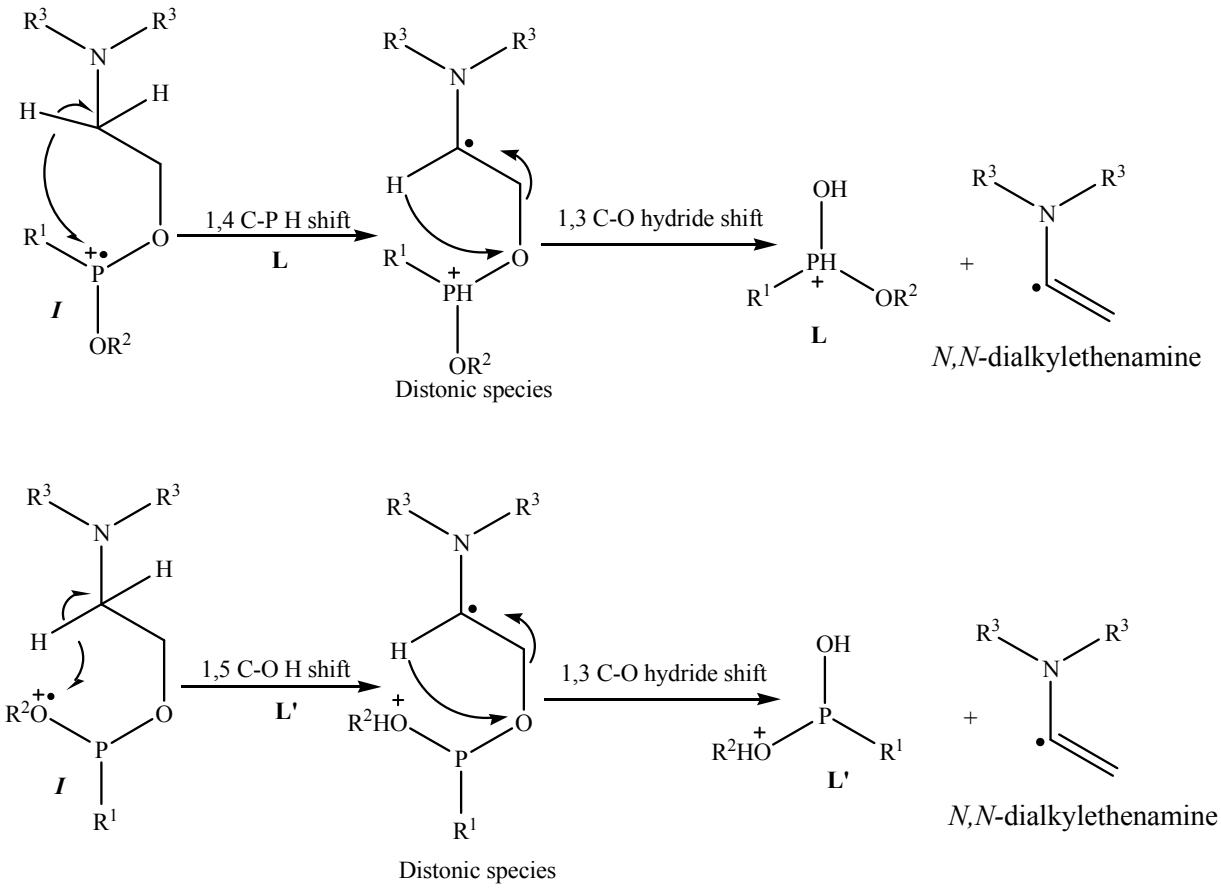


Figure 5. Proposed mechanisms for formation of ion [L].

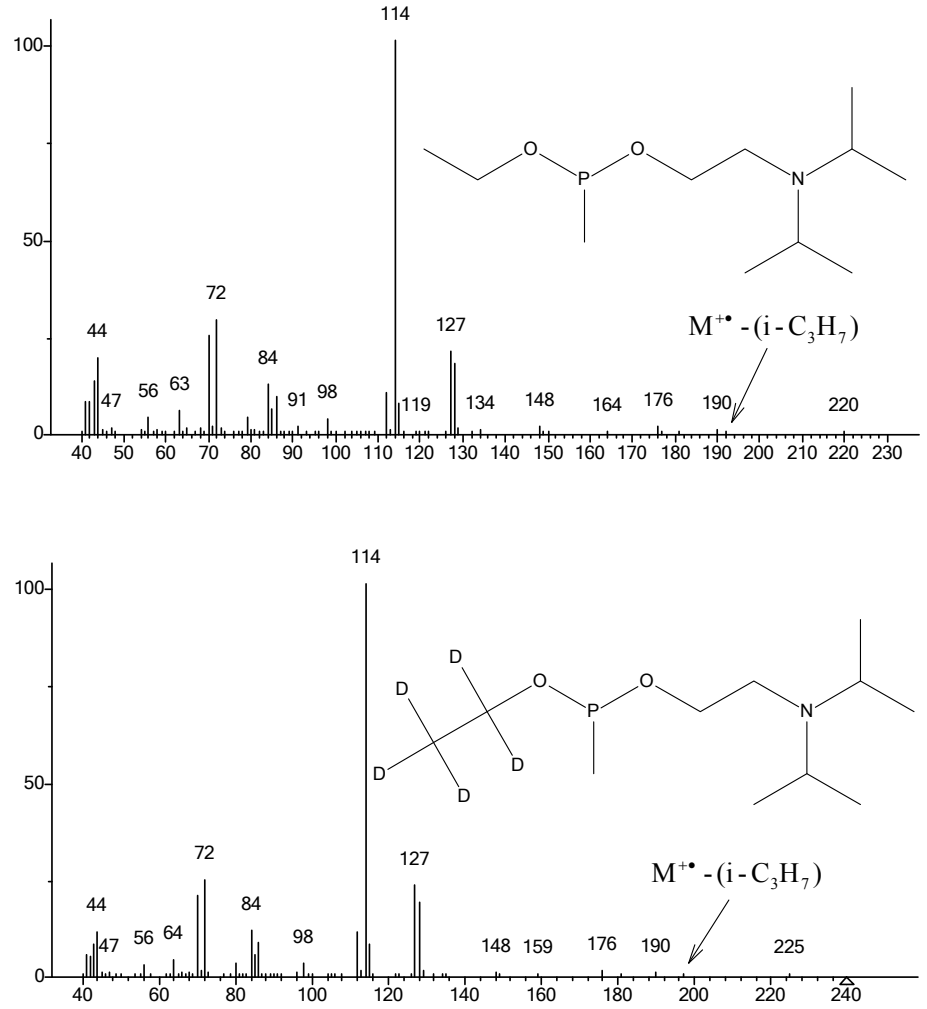


Figure 6. EI-MS mass spectra of the QL and QL-d_5.

are by-products in the synthesis of VX. Their presence in the environmental sample indicates the alleged preparation of restricted chemicals.

Therefore, the knowledge on their fragmentation mechanisms in EI-MS spectra becomes important from the verifications point of view for on/off site analysis. We have studied 32 *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonate MS spectra. The EI fragment ions of **II** are given in Table 2 and their routes of fragmentation are shown in Figure 7. The $M^{+\bullet}$ of **II** were not present in the mass spectra. Chemical ionization (CI)-MS of most of the studied chemicals were recorded to support their structure elucidation (see supporting information).

All compounds **II** show fragment *N*-methylideneaminium [L] as the base peak which resulted from $M^{+\bullet}$ by cleavage of the C–C bond of 2-(*N,N*-dialkylamino)ethanol. This ion could be used for *N,N*-dialkylamino group distinction in such chemicals. Analysis of mass spectra revealed that the type of alkyl groups present on nitrogen influenced the mass spectrum. Based on the alkyl group, the chemicals can be classified in three different classes: *O*-alkyl *O*-2-(*N,N*-diethylamino)ethyl alkylphosphonates (Series-1, entries 1–11, Table 2), *O*-alkyl *O*-2-(*N,N*-diisopropylamino)ethyl alkylphosphonates (Series-2, entries 12–22, Table 2), and *O*-alkyl *O*-2-(*N,N*-dimethylamino) ethyl alkylphosphonates (Series-3, entries 22–32, Table 2). As typical examples, the spectra of three chemicals, along with their deuterated analogs, are given in Figure 8. Fragment ion [A] was formed by direct elimination of the alkoxy group as a radical from $M^{+\bullet}$ with moderate relative abundance. This ion is not found in the mass spectra of series-2, with the exception of the *O*-isopropyl analogs (entries 16 and 17, Table 2). However, when the *O*-alkyl chain on series-1 and 3 become smaller, the intensity of the [A] signal decreases or vanishes. Therefore, existence and intensity of [A] in the mass spectra of **II** depends on the alkyl group size on the nitrogen and oxygen atoms. Fragment [B] can be formed from $M^{+\bullet}$ with double hydrogen transfer (McLafferty+1 rearrangement).[2] Formation of ion [B] through McLafferty+1 rearrangement was proposed by observing the corresponding fragment ions in EI-MS of deuterated analogs of **II**. This process involves an initial hydrogen migration (1,5 C–O H shift) to the oxygen-bearing

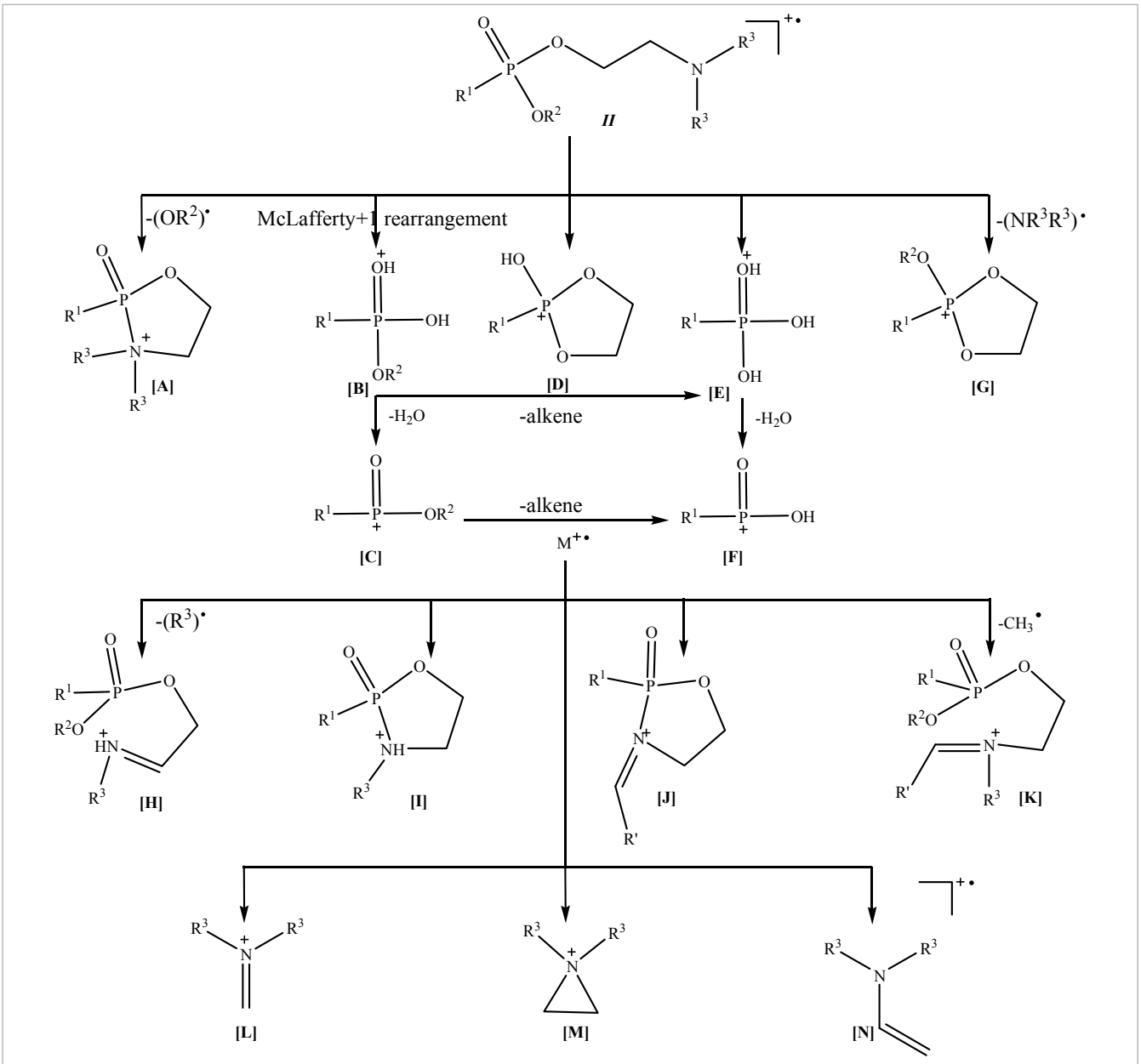


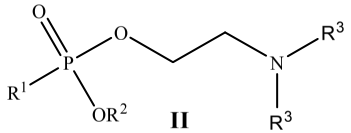
Figure 7. Proposed fragmentation pathways of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonates II in EI-MS.

radical cation site, leading to a distonic species. A radical cation and a neutral enamine (radical cation–neutral complex) are produced through single bond cleavage. Then, a hydrogen atom transfers from an enamine to a radical cation, yielding a cation and an enamine radical fragment (Figure 9).[24]

It should be mentioned that the corresponding ion [B] for compounds bearing *an O*-isopropyl group is not observed. On the other hand, the β-cleavage process is observed in compounds bearing an isopropyl group on nitrogen which can form fragment ion [K]. These facts could be used for identification of an isopropyl group on oxygen and nitrogen in such chemicals. Loss of H_2O from ion [B] is found in most cases which produces the fragment ion [C]. Its corresponding deuterated ion in the mass spectra of deuterated analogs is an evidence of the occurrence of such a mechanism. Stepwise expulsion of the *N,N*-dialkylamine moiety, which took place to yield ion [G] and elimination of an alkene from $M^{+\bullet}$, was another important mode of fragmentation that gave rise to ion [D]. It is important to note that ion [D] was not produced in all **II** bearing methyl groups on oxygen atoms. It is of interest that ion [D] can assume two structures, $[D_{1}]$ and $[D_2]$, as shown Figure 10. Energy calculation shows that the former ion is 137 kJ mol^{-1} more stable than the latter.

Formation of fragment ions [E] and [F] was attributed to stepwise loss of a neutral alkene and water molecule from ion [B]. Generally, these fragment ions were present in the

Table 2. MS data of *O*-alkyl *O*-2-(*N*,*N*-dialkylamino)ethyl alkylphosphonates II.



II

Entry	R^1	R^2	R^3	$M^{+\bullet}$	Fragment ions (% relative abundances)													
					[A]	[B]	[C]	[D]	[E]	[F]	[G]	[H]	[I]	[J]	[K]	[L]	[M]	[N]
1	CH_3	CH_3	C_2H_5	209	—	111 (2)	93 (9)	—	—	79 (2)	137 (3)	180 (1)	—	134 (1)	—	86 (100)	100 (5)	99 (29)
2	CH_3	CD_3	C_2H_5	213	—	114 (2)	96 (10)	—	98 (2)	80 (1)	140 (3)	183 (1)	—	134 (1)	—	86 (100)	100 (5)	99 (27)
3	CH_3	C_2H_5	C_2H_5	223	178 (2)	125 (1)	—	123 (3)	97 (3)	79 (7)	151 (1)	194 (1)	150 (2)	134 (1)	—	86 (100)	100 (9)	99 (33)
4	CH_3	C_2D_5	C_2H_5	—	178 (2)	130 (1)	—	124 (4)	98 (6)	80 (10)	156 (2)	199 (1)	150 (1)	134 (1)	—	86 (100)	100 (8)	99 (33)
5	CH_3	i—C_3H_7	C_2H_5	—	178 (11)	—	—	123 (4)	97 (4)	79 (4)	—	—	150 (6)	134 (1)	—	86 (100)	100 (13)	99 (50)
6	CH_3	i—C_3HD_6	C_2H_5	—	178 (9)	—	—	124 (4)	98 (5)	80 (3)	—	—	150 (4)	134 (1)	—	86 (100)	100 (11)	99 (43)
7	C_2H_5	CH_3	C_2H_5	223	—	125 (2)	107 (5)	—	—	93 (1)	151 (3)	194 (1)	164 (1)	148 (1)	—	86 (100)	100 (7)	99 (33)
8	C_2H_5	CD_3	C_2H_5	226	—	128 (1)	110 (5)	—	—	—	154 (3)	197 (1)	—	—	—	86 (100)	100 (6)	99 (33)
9	C_2H_5	C_2H_5	C_2H_5	237	192 (2)	—	—	137 (3)	111 (2)	93 (4)	165 (2)	208 (1)	164 (2)	—	—	86 (100)	100 (10)	99 (41)
10	C_2H_5	C_2D_5	C_2H_5	242	—	—	—	138 (3)	112 (2)	94 (3)	170 (1)	213 (1)	—	148 (1)	—	86 (100)	100 (9)	99 (42)
11	C_2H_5	i—C_3H_7	C_2H_5	—	192 (20)	—	—	137 (6)	111 (4)	93 (4)	179 (1)	—	164 (8)	148 (2)	—	86 (100)	100 (17)	99 (57)
12	CH_3	CH_3	i—C_3H_7	237	—	111 (4)	93 (12)	—	97 (8)	79 (3)	137 (6)	194 (5)	—	148 (1)	222 (6)	114 (100)	128 (9)	127 (27)
13	CH_3	CD_3	i—C_3H_7	240	—	114 (100)	96 (13)	—	98 (5)	—	140 (6)	197 (4)	—	—	225 (5)	114 (100)	128 (8)	127 (23)
14	CH_3	C_2H_5	i—C_3H_7	251	—	125 (2)	—	123 (5)	97 (5)	79 (7)	151 (3)	208 (4)	164 (2)	148 (1)	236 (4)	114 (100)	128 (12)	127 (22)
15	CH_3	C_2H_5	i—C_3H_7	256	—	130 (1)	—	124 (3)	98 (8)	80 (8)	156 (3)	213 (5)	164 (1)	148 (1)	241 (4)	114 (100)	128 (9)	127 (23)

Table 2 (continued). MS data of *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonates II.

Entry	R^1	R^2	R^3	$M^{+\bullet}$	Fragment ions (% relative abundances)													
					[A]	[B]	[C]	[D]	[E]	[F]	[G]	[H]	[I]	[J]	[K]	[L]	[M]	[N]
16	CH_3	i—C_3H_7	i—C_3H_7	—	206 (2)	—	121 (2)	123 (6)	97 (4)	79 (8)	165 (1)	222 (4)	164 (13)	148 (3)	250 (3)	114 (100)	128 (21)	127 (23)
17	CH_3	i—C_3HD_6	i—C_3H_7	—	206 (2)	—	127 (30)	124 (6)	98 (12)	80 (4)	—	228 (4)	164 (11)	148 (3)	256 (3)	114 (100)	128 (21)	127 (30)
18	C_2H_5	CH_3	i—C_3H_7	251	—	125 (4)	107 (6)	—	—	93 (1)	151 (5)	208 (4)	—	—	236 (4)	114 (100)	128 (9)	127 (23)
19	C_2H_5	CD_3	i—C_3H_7	254	—	128 (12)	110 (6)	—	112 (31)	—	154 (5)	211 (4)	178 (2)	—	239 (4)	114 (100)	128 (11)	127 (22)
20	C_2H_5	C_2H_5	i—C_3H_7	265	—	139 (1)	121 (1)	137 (1)	111 (6)	93 (5)	165 (3)	222 (5)	178 (1)	—	250 (3)	114 (100)	128 (10)	127 (27)
21	C_2H_5	C_2D_5	i—C_3H_7	270	—	144 (1)	126 (2)	138 (3)	112 (36)	94 (5)	170 (3)	227 (6)		—	255 (3)	114 (100)	128 (9)	127 (24)
22	CH_3	CH_3	CH_3	181	150 (1)	111 (3)	93 (8)	—	—	79 (2)	137 (2)	—	—	—	—	58 (100)	72 (6)	71 (5)
23	CH_3	CD_3	CH_3	184	150 (1)	114 (2)	96 (9)	—	—	80 (2)	140 (1)	—	—	—	—	58 (100)	72 (3)	71 (44)
24	CH_3	C_2H_5	CH_3	195	150 (5)	125 (1)	—	123 (2)	97 (5)	79 (7)	151 (1)	—	—	—	—	58 (100)	72 (11)	71 (74)
25	CH_3	i—C_3H_7	CH_3	—	150 (26)	—	121 (1)	123 (4)	97 (7)	79 (8)	—	—	—	—	—	58 (100)	72 (22)	71 (91)
26	CH_3	i—C_3HD_6	CH_3	—	150 (19)	—	127 (1)	124 (3)	98 (5)	80 (4)	—	—	—	—	—	58 (100)	72 (20)	71 (99)
27	C_2H_5	CH_3	CH_3	195	164 (1)	125 (4)	107 (5)	—	—	93 (1)	151 (2)	—	—	—	—	58 (100)	72 (10)	71 (71)
28	C_2H_5	CD_3	CH_3	198	—	128 (4)	110 (5)	—	—	—	154 (2)	—	—	—	—	58 (100)	72 (9)	71 (77)
29	C_2H_5	C_2H_5	CH_3	209	164 (4)	139 (1)	121 (1)	137 (2)	111 (4)	93 (5)	165 (1)	—	—	—	—	58 (100)	72 (16)	71 (96)
30	C_2H_5	C_2D_5	CH_3	214	164 (2)	—	—	138 (2)	112 (5)	94 (5)	—	—	—	—	—	58 (100)	72 (13)	71 (98)
31	C_2H_5	i—C_3H_7	CH_3	—	164 (20)	—	135 (1)	137 (3)	111 (6)	93 (4)	—	—	—	—	—	58 (87)	72 (21)	71 (100)
32	C_2H_5	i—C_3HD_6	CH_3	—	164 (15)	—	—	138 (3)	112 (5)	94 (3)	—	—	—	—	—	58 (79)	72 (18)	71 (100)

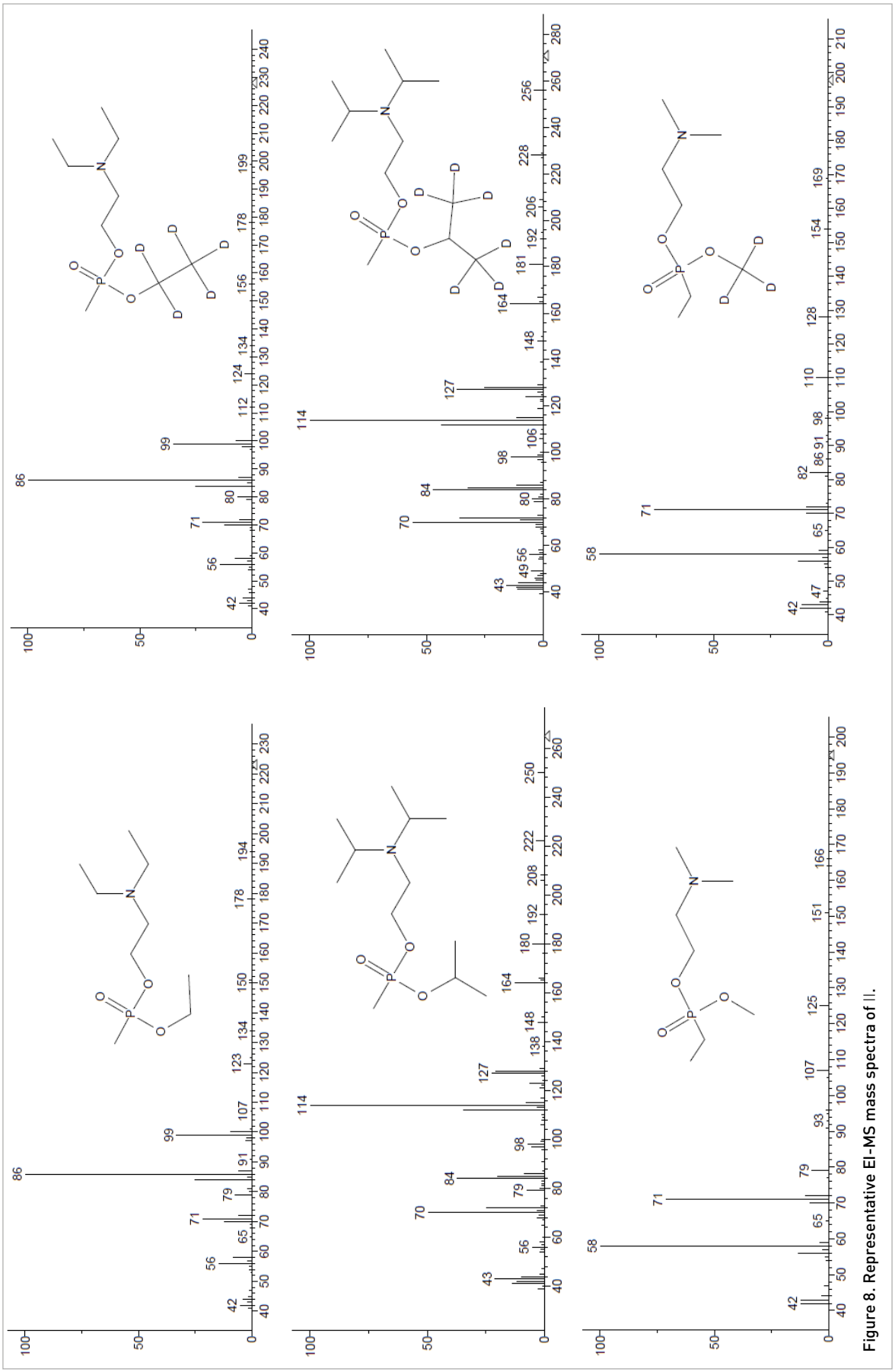


Figure 8. Representative EI-MS mass spectra of II.

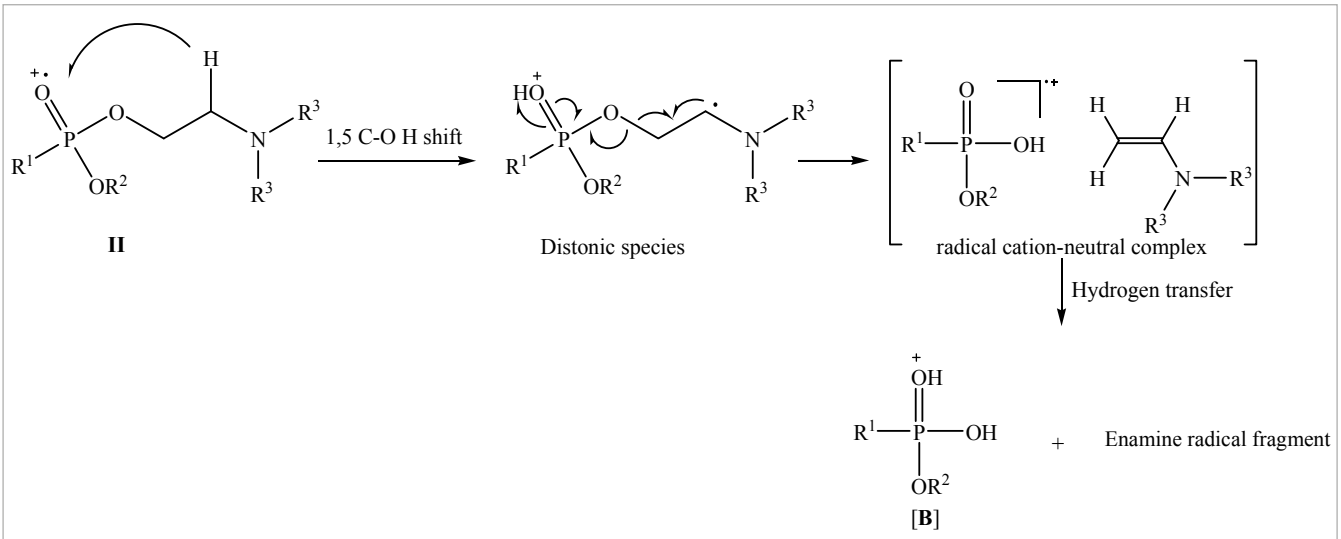


Figure 9. Proposed mechanism for formation of ion [B] in EI-MS of II.

mass spectra of alkylphosphonates. The [$M^{+\bullet} - R^3$] ions, where the alkyl group is other than methyl, show ion [H] corresponding to the loss of an alkyl via β-fission. Ion [H] was clearly distinguishable in the EI-MS of deuterated analogs. As expected, [$M^{+\bullet}$-R^3] is prominently observed in Series-2, in which two isopropyl groups are attached to nitrogen. Stepwise loss of alkoxy (OR^2) and an alkyl radical on nitrogen gave rise to the ion [I]. The cation [I] could further disintegrate by elimination of a methyl radical and produce ion [J]. The ions [H], [I], [J] and [K] are not present in the mass spectra of series-3. Therefore, spectra for these chemicals are distinct. The lower part of the spectra of all **II** was dominated by the ions [L], [M], [N], [O], [P] and [Q] which were produced from *2-(N,N*-dialkylamino)ethanol with hydrogen rearrangements and alkyl and alkene elimination.

It is noteworthy that among the studied alkylphosphonates **II**, six isomeric pairs (Table 3) are present for **II**. The isomerism is exclusively due to the size of the alkyl group attached to the nitrogen, phosphorous and oxygen atoms. These isomeric compounds could easily be differentiated based on differences in their EI-MS fragmentation pathways. Among these, three isomeric pairs have different alkyl groups on nitrogen (entries 1, 2 and 3, Table 3) and, hence, can easily be differentiated from one another by considering the lower part of the mass spectra; especially ions [L], [M] and [N], which reveal the size of the alkyl group on nitrogen in the 2-(*N,N*-dialkylamino) ethanol moiety. The [L] is the base peak for such chemicals which, in the case of R^3 = Et, *i*-Pr and Me are *m/z* 86, *m/z* 114 and *m/z* 58, respectively. Three other isomeric pairs (entries 4, 5 and 6, Table 3) have different alkyl groups on phosphorous and oxygen atoms. The isomeric pairs show a resemblance in their EI-MS spectra. The mass spectra for the isomeric pair consisting of isopropyl on nitrogen (entry 4, Table 1), which exhibit very similar appearance, are shown in Figure 11 as example.

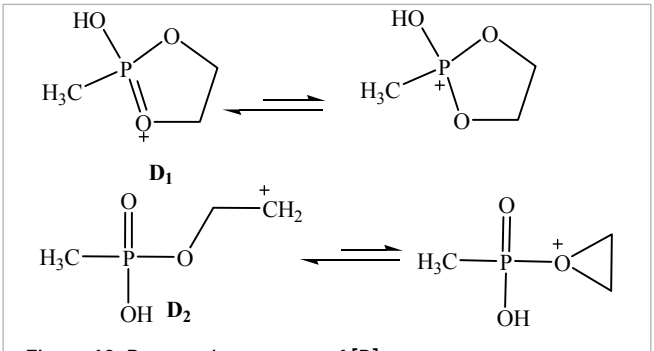


Figure 10. Proposed structures of [D].

Table 3. Some isomeric compounds for chemicals II.

Entry	$M^{+\bullet}$	Isomeric pairs		
		R^1	R^2	R^3
1	209	CH_3 C_2H_5	CH_3 C_2H_5	C_2H_5 CH_3
2	237	C_2H_5 CH_3	C_2H_5 CH_3	C_2H_5 *i*-C_3H_7
3	223	C_2H_5 C_2H_5	CH_3 *i*-C_3H_7	C_2H_5 CH_3
4	223	CH_3 C_2H_5	C_2H_5 CH_3	C_2H_5 C_2H_5
5	251	CH_3 C_2H_5	C_2H_5 CH_3	*i*-C_3H_7 *i*-C_3H_7
6	195	CH_3 C_2H_5	C_2H_5 CH_3	CH_3 CH_3

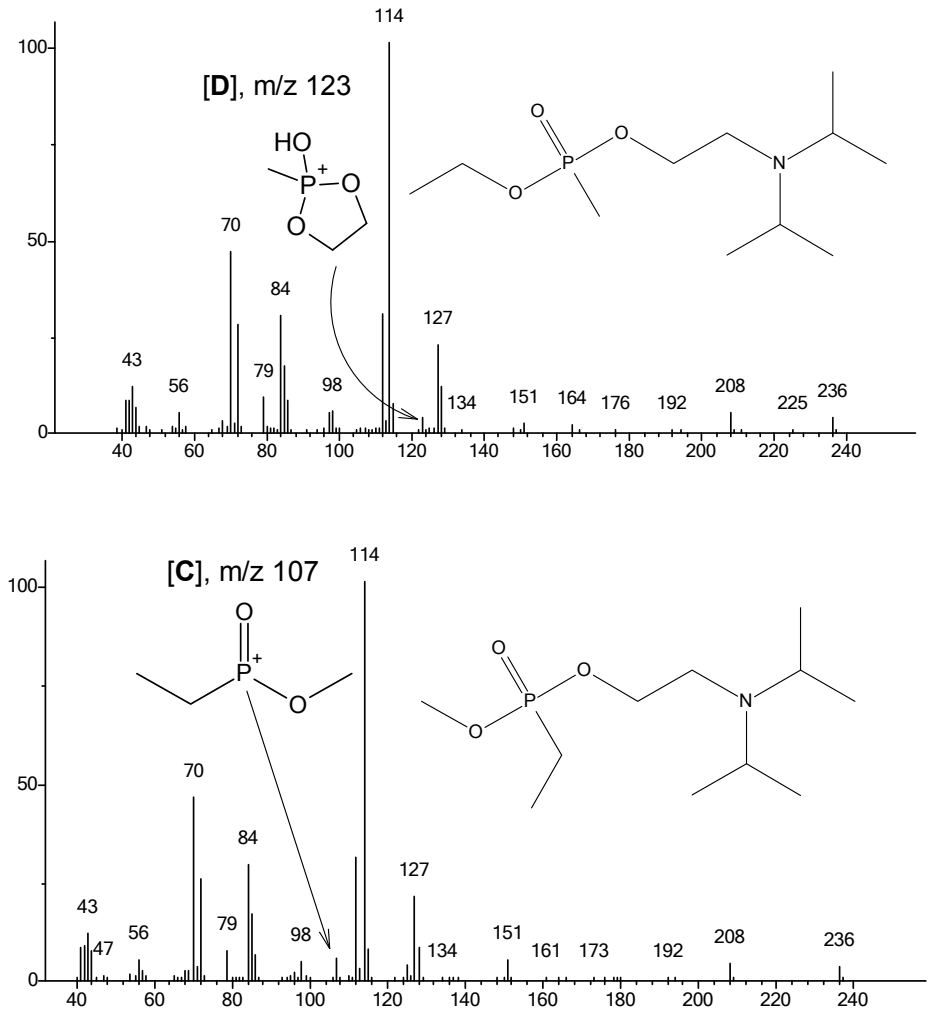


Figure 11. EI mass spectra for isomeric pair consisting isopropyl on nitrogen (entry 5, Table 3).

The ion at *m/z* 107 corresponding to the fragment of [C] proves to be characteristic for the methyl moiety on the oxygen atom in such chemicals and the presence of ion [D] in the mass spectra of chemicals shows methyl attached to the phosphorous atom. Analysis of mass spectra of deuterated analogs also supported these points.

Conclusions

EI-MS of the *O*-alkyl *O*-2-(*N,N*-dialkylamino)ethyl alkylphosphonites(phosphonates) were collected and investigated with the aim of enriching OCAD, which may be used in OPCW verification activities, on/off site analysis and to improve MS interpretation knowledge. The presented EI-MS data of the title chemicals indicated that their fragmentations were governed by the size and structure of the alkyl group attached to nitrogen, phosphorus and oxygen atoms. Fragmentation processes were mostly dominated by hydrogen rearrangement, alkene, amine and alkoxy elimination by α- and β-cleavage process and McLafferty+1 rearrangement. The most important fragment ion in the EI-MS of phosphonites was formed through α-cleavage a P–C bond, which was clearly distinguishable in the spectra of the deuterated analogs. This fragment ion was not observed in the mass spectra of phosphonates. Presence and abundance of fragment ions formed through direct elimination of alkoxy groups in the mass spectra of phosphonates depends on the alkyl group size on nitrogen and oxygen atoms. The characteristic fragmentation processes and subtle differences in EI-MS spectra of phosphonites and phosphonates may create pitfalls in structural assignments of unknown CWC-related chemicals and isomeric compounds.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1255/ejms.1234.

References

1. H. Saeidian, D. Ashrafi M. Sarabadani, M.T. Naseri and M. Babri, "Mass spectrometric study on *O*(*S*)-alkyl *N,N*-dimethylamino alkylphosphonates (alkylphosphonothiolates) for Chemical Weapons Convention verification purposes", *Int. J. Mass Spectrom.* 319–320, 9 (2012). doi: 10.1016/j.ijms.2012.03.006
2. H. Saeidian, M. Babri, M. Abdoli, M. Sarabadani, D. Ashrafi and M.T. Naseri, "Microsynthesis and electron ionization mass spectral studies of *O*(*S*)-alkyl *N,N*-dimethyl alkylphosphono(thiolo)thionoamidates for Chemical Weapons Convention verification", *Rapid Commun. Mass Spectrom.* **26(23),** 2805(2012). doi: 10.1002/rcm.6407
3. H. Saeidian, M. Babri, D. Ashrafi, M. Sarabadani and M.T. Naseri, "Fragmentation pathways of *O*-alkyl methylphosphonothionocyanidates in the gas phase: toward unambiguous structural characterization of chemicals in the Chemical Weapons Convention framework", *Anal. Bioanal. Chem* **405(21),** 6749(2013). doi: 10.1007/s00216-013-7094-8
4. M. Mesilaakso, "Introduction", in *Chemical Weapons Convention Analysis, Sample Collection, Preparation and Analytical Methods*, Ed by M. Mesilaakso. John Wiley & Sons Ltd, Chichester, UK, p. 1 (2005).
5. J. Hendrikse, "Comprehensive review of the Official OPCW Proficiency Test", in *Chemical Weapons Convention Analysis, Sample Collection, Preparation and Analytical Methods*, Ed by M. Mesilaakso. John Wiley & Sons Ltd, Chichester, UK, p. 89 (2005).
6. C. Nyanyira, "The OPCW Central Analytical Database", in *Chemical Weapons Convention Analysis, Sample Collection, Preparation and Analytical Methods*, Ed by M. Mesilaakso. John Wiley & Sons Ltd, Chichester, UK, p. 133 (2005).
7. B. Papoušková, P. Bednář, J. Stýskala, J. Hlaváč, P. Barták and K. Lemr, "Mass spectrometry as a tool for characterization of *N,N*-dialkylaminoethane- 2-thiols-precursors and degradation products of chemical warfare agents", *J. Mass Spectrom.* **44(11),** 1604 (2009). doi: 10.1002/jms.1674
8. D. Pardasani, V. Tak, A.K. Purohit, P.K. Kanaujia and D.K. Dubey, "Gas chromatography-electron ionization mass spectrometry analysis of *O,O′*-dialkyl methylphosphonites for verification of Chemical Weapons Convention", *Eur. J. Mass Spectrom.* **17(1),** 57 (2011). doi: 10.1255/ejms.1109
9. M. Palit, D. Pardasani, A.K. Gupta, P. Shakya and D.K. Dubey, "Microsynthesis and electron ionization mass spectrometric analysis of chemical weapons convention (CWC)-related *O,O*-dialkylphosphoramidates", *Anal. Bioanal. Chem.* **381(2),** 477 (2005). doi:10.1007/s00216-004-2873-x
10. J. Stýskala, P. Cankař, M. Soural, P. Bednář and K. Lemr, "Preparation and characterization of some unsymmetrical 2-(dialkylamino)ethanthiols", *Arkivoc* **2007(15),** 171 (2007). doi: 10.3998/ark.5550190.0008.f17
11. D. Pardasani, P.K. Kanaujia, V. Tak, P. Garg, A. Mazumder and D.K. Dubey, "Gas chromatography electron ionization mass spectrometric analysis of *O*-alkyl methylphosphinates for verification of Chemical Weapons Convention", *Eur. J. Mass Spectrom.***15(5),** 579(2009). doi: 10.1255/ejms.999
12. S.E. Steinborner, A. Ramachandran and S.J. Blanksby, "The fragmentation pathways of protonated Amiton in the gas phase: towards the structural characterization of organophosphorus chemical warfare agents by electrospray ionization tandem mass spectrometry", *Rapid Commun. Mass Spectrom.* **20(12),** 1939 (2006). doi: 10.1002/rcm.2535
13. T.C. Marrs, R.L. Maynard and F.R. Sidell (Eds), in *Chemical Warfare Agents—Toxicology and Treatment*, 2nd Edn. John Wiley & Sons Inc., Chichester, UK (2007).
14. F. Worek, H. Thiermann, L. Szinicz and P. Eyer, "Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes", *Biochem. Pharmacol.* **68(11),** 2237(2004). doi: 10.1016/j.bcp.2004.07.038
15. J.B. Ledgard, "Preparation of methylphosphonic dichloride", in *The Preparatory Manual of Chemical Warfare Agents*. The Paranoid Publishing Company (2003).
16. J.A. Wojtowicz, "Process for making methylphosphonic dichloride", US Patent 4871486, (1989).
17. R.O. Hutchins and M.K. Hutchins, "Sodium borohydride", in *Handbook of Reagents for Organic Synthesis, Oxidizing and Reducing Agents*, Ed by S.D. Burke and R.L. Danheiser. John Wiley & Sons Ltd, Chichester, UK, p. 394 (1999).
18. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, Jr, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez and J.A. Pople, *Gaussian 03*, Revision E.01. Gaussian, Inc., Wallingford CT, USA (2004).
19. H. Van Den Dool and P.D. Kratz, "A generalization of the retention index system including linear temperature

programmed gas-liquid partition chromatography", *J. Chromatogr. A* **11,** 463(1963). doi: 10.1016/S0021-9673(01)80947-x

20. Y. Benchekroun, S. Dautraix, M. Désage and J.L. Brazier, "Isotopic effects on retention times of caffeine and its metabolites 1,3,7- trimethyluric acid, theophylline, theobromine and paraxanthine", *J. Chromatogr. B* **688,** 245(1997). doi: 10.1016/S0378-4347(96)00323-4

21. J. Kurz and K. Ballschmiter, "Vapour pressures, aqueous solubilities, Henry's law constants, partition coefficients between gas/water (K_{gw}), *N*-octanol/water (K_{ow}) and gas/Noctanol (K_{go}) of 106 polychlorinated diphenyl ethers (PCDE)," *Chemosphere* **38(3),** 573(1999). doi: 10.1016/S0045-6535(98)00212-4

22. H. Zhao, P. Unhannanant, W. Hanshaw and J.S. Chickos, "Enthalpies of vaporization and vapor pressures of some deuterated hydrocarbons. liquid–vapor pressure isotope effects", *J. Chem. Eng. Data*. **53(7),** 1545 (2008). doi: 10.1021/je800091s

23. F.A. Carey and R.J. Sundberg, "Chemical bonding and molecular structure" in *Advanced Organic Chemistry, Part A: Structure and Mechanisms*. Springer Science Business Media, LLC, New Yor, USA (2007).

24. D.G.I. Kingston, J.T. Bursey and M.M. Bursey, "Intramolecular hydrogen transfer in mass spectra. II. The McLafferty rearrangement and related reactions", *Chem. Rev.* **74(2),** 215 (1974). doi: 10.1021/cr60288a004