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Mass spectral characterization of the CWC-related isomeric dialkyl alkylphosphonothiolates/alkylphosphonothionates under gas chromatography/mass spectrometry conditions

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RATIONALE: The isomeric dialkyl alkylphosphonothiolates and dialkyl alkylphosphonothionates are listed as scheduled chemicals of the Chemical Weapons Convention (CWC) implemented by the OPCW. The P–S and P–R bond connectivity has to be correctly identified for the verification of the CWC. The present study demonstrates successful identification of the target isomers by selective fragmentation under electron ionization (EI) or chemical ionization (CI) conditions.
METHODS: All the studied isomeric compounds (27 in total) were synthesized in our laboratory using established methods, then analyzed by EI and CI gas chromatography/mass spectrometry (GC/MS) using an Agilent 6890 gas chromatograph equipped with a HP-5MS capillary column and interfaced to a 5973 N mass-selective detector. The retention index (RI) values of all the compounds were calculated using Van den Dool's formula. GC/MS/MS and GC/HRMS experiments were also performed using a VG-Autospec (magnetic sector) and JEOL-AccuToF (time-of-flight) mass spectrometer, respectively.
RESULTS: The EI mass spectra of all the compounds had an abundant molecular ion at *m/z* 182, except in the case of a few selected butyl-substituted compounds, where this ion was of low abundance. The EI fragmentation pathways include α-cleavage, McLafferty rearrangement, McLafferty + 1 rearrangement, O/S-alkyl radical loss, and an alkene loss with a hydrogen shift. The characteristic fragment ions and their relative abundances are significant in elucidating the alkyl group attached to the P/S/O-atoms as well as the P–S/P = S bond connectivity. The EI and CI mass spectra together with RI values enable unambiguous identification of all the studied isomeric compounds.
CONCLUSIONS: The present study highlights the structural characterization of the isomeric phosphonothiolates and phosphonothionates based on their selective EI fragmentation. The assigned fragmentation pathway helps in the assignment of P–S and P–alkyl connectivity in phosphonothiolates and phosphonothionates, consequently the structure of the unknown compounds. The EI mass spectra (27 compounds) of isomeric compounds are immensely useful in the OPCW official proficiency tests and for off-site analysis. Copyright © 2013 John Wiley & Sons, Ltd.

Chemical warfare agents (CWAs) include nerve agents, vesicants, and choking compounds, which can lead to mass destruction of humans, animals, and plants in a short span of time and contaminate the environment.[1,2] Over the past two decades, a great deal of attention has been focused on the development of analytical techniques for the rapid identification and analysis of CWAs, their precursors, degradation products and reaction products. Most CWAs are volatile and relatively non-polar and, hence, gas chromatography (GC) coupled with various selective and sensitive detectors such as flame ionization detector (FID), nitrogen phosphorus detector (NPD), flame photometric detector (FPD), Fourier transform infrared (FTIR) spectroscopy, and mass spectrometry (MS) is widely used for the analysis of non-polar CWAs.[3–8] Of these, the MS detector has been extensively used because of its high sensitivity and versatility. Compounds of polar nature can be analyzed directly by liquid chromatography (LC)/MS or by GC/MS after derivatization.[9–15] GC/MS analysis is typically performed under electron ionization (EI) or chemical ionization (CI) conditions.[16–20] EI is more routinely used than CI, because it provides extensive details about a compound through its characteristic fragmentations. In addition, EI mass spectra of many CWAs and their degradation products are available in the OPCW Central Analytical Database[21] and in other commercial library databases, thus helping in the identification of target compounds.

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The Chemical Weapons Convention (CWC) is an international treaty implemented in 1997, banning the production, stockpiling, and use of chemical weapons.[22,23] A part of the CWC involves on-site analysis (by the OPCW inspectors) or off-site analysis (by OPCW-designated laboratories) of CWAs and their degradation products in suspected environmental matrices. Since the verification process is an internationally sensitive issue, the analytical performance of the participating laboratories (the designated laboratories and the laboratories seeking designation) is periodically evaluated by the OPCW by conducting proficiency tests (PTs) twice a year.[24] The tests involve unambiguous identification of spiked scheduled chemicals and/or their degradation/reaction products in different environmental matrices.

Nerve agents constitute one of the most important groups of the CWAs and they are categorized as some of the most toxic species; the alleged use of these nerve agents in the Iran-Iraq conflict and the Tokyo subway incident has been well documented.[25,26] Most of the precursor/degradation products of nerve agents such as phosphonofluoridates, phosphonic acids, cyclic and acylic phosphonates have been studied extensively for their unambiguous identification.[27–29] Dialkyl alkylphosphonothiolates and the corresponding phosphonothionates (schedule 2.B.4), which are precursors of the nerve agents and/or are by-products during the synthesis of the nerve agents,[30] are less toxic and are stable in normal environmental samples. Thus, they are often used as the spiking chemicals in the samples of the official PTs conducted by the OPCW. During the PT, the participating laboratories must identify the P-alkyl groups and establish the correct connectivity between P and S (phosphonothiolates and phosphonothionates). The O/S-alkyl groups may be reported using the generic name of the alkyl, without identification of the exact structure. The scheduled list shows that thousands of phosphonothiolates and phosphonothionates are possible under schedule 2.B.4. A few reports have been published on the isomeric differentiation of dialkylphosphonothiolates, but not of phosphonothionates.[31] Moreover, to the best of our knowledge, there is no detailed study emphasizing the differentiation of phosphonothiolates and phosphonothionates based on their EI fragmentation patterns. This prompted us to synthesize a series of isomeric *O,S*-dialkyl alkylphosphonothiolates and O,O′-dialkyl alkylphosphonothionates and investigate their EI/CI mass spectra. As a first approach, we synthesized 27 isomeric compounds fixing the molecular formula as $C_6H_{15}O_2PS$ (molecular weight 182), where the alkyl group attached to P is C_1–C_3, and that attached to O or S is C_1–C_4; and the alkyl groups, i.e., methyl, ethyl and propyl, are interchanged among the three heteroatoms (O, P and S) whereas the butyl group is interchanged between the oxygen and sulfur atoms. A few phosphonothionates are known to undergo thiono-thiolo rearrangement under EI and electrospray ionization (ESI) conditions[32] and this may pose a difficulty in differentiation of phosphonothionates from their rearranged products, phosphonothiolates. Thus, we have also focused on the impact of this rearrangement in the selected set of compounds. Herein, we report the characterization of the isomeric phosphonothiolates and phosphonothionates using GC/EIMS and GC/CIMS.

EXPERIMENTAL

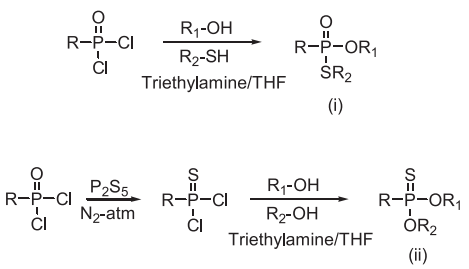
Materials

All the compounds (**1–27**) were synthesized in-house according to a reported procedure (Scheme 1).[33,34] Briefly, the alkylphosphonic dichloride (0.02 mmol) was treated with the corresponding alkyl alcohol/thiol (0.02 mmol) in the presence of triethylamine (0.025 mmol in dry tetrahydrofuran) at room temperature (25 °C) for 30 min. The residue was extracted with ethyl acetate and the extracts were directly analyzed by GC/MS. The O,O′-diethyl methylphosphonothionate was procured from Sigma-Aldrich (Steinheim, Germany) and other chemicals and solvents used for the synthesis were purchased from Sd-Fine Chemicals (Mumbai, India). Analytical grade solvents for chromatographic and mass spectrometric analysis were obtained from E-Merck (Mumbai, India).

Mass spectral analysis

The GC/EIMS analyses were performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped a HP-5MS capillary column (length 30 m, 250 μm i.d. and 0.25 μm film thickness) and interfaced to a 5973 N mass-selective detector (a single quadrupole instrument). The column oven was programmed initially from 50 °C with a 2 min hold time to the final temperature of 280 °C with 10 °C/min ramp. The final temperature hold time was 5 min (total run: 30 min). Helium was used as the carrier gas in constant flow mode at a flow rate of 1.0 mL/min. The inlet and GC/MS interface temperatures were 250 °C and 280 °C, respectively. The samples were introduced in split injection mode at a split ratio of 10:1. The temperatures of the EI source and quadrupole analyzer were 230 °C and 150 °C, respectively. Similar experimental conditions were used for the GC/CIMS experiments using methane as the reagent gas. The mass spectrometer was scanned from *m/z* 29 to 600 and from *m/z* 60 to 600 for EI and CI, respectively. All the spectra were recorded under identical conditions. The RI values were calculated for all the isomeric compounds by using Van den Dool's formula[5] with respect to standard n-alkane hydrocarbons (Supplementary Table S1, Supporting Information).

Collision-induced dissociation (CID) spectra were recorded using a VG Autospec M triple-sector (EBE geometry) mass spectrometer (Manchester, UK) equipped with a VG Masslynx data system, using a dedicated EI source. The source conditions for electron ionization were: accelerating



Scheme 1. Synthetic routes of (i) dialkyl alkylphosphonothiolates and (ii) dialkyl alkylphosphonothionates.

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voltage 7 kV, electron energy 70 eV, trap current 200 A and source temperature 250 °C. Spectra were acquired using a scan time of 1 s/decade and 0.3 s inter-scan delay. All the samples (**1–27**) were introduced through a HP 5890 Series II gas chromatograph using similar conditions to those described above. The *O,O′*-diethyl methylphosphonothionate was introduced into the EI source through a direct insertion probe. Tandem mass spectrometry (MS/MS) experiments were performed using linked scan techniques, where the first electric sector (E) and magnet sector (B) were scanned simultaneously so as to preserve a predetermined relationship between scan parameters to produce product ion and precursor ion spectra. CID product ion spectra were recorded at constant B/E under computer control, with air as the collision gas admitted into the first field-free region so that the main beam transmission was reduced to 20–30% of its original intensity. Precursor ion spectra were obtained at constant B^2/E.

High-resolution (HR) mass spectra were recorded using a JEOL GC-AccuTOF (Tokyo, Japan), a time-of-flight mass spectrometer, equipped with Agilent 7890A gas chromatograph. The GC and MS conditions were similar to those described above. The instrument mass resolution was 8000 (full width at half maxima (FWHM)) and perfluorokerosene was used as the internal reference.

RESULTS AND DISCUSSION

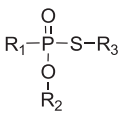
The structures of the studied isomeric compounds of *O*-alkyl, *S*-alkyl alkylphosphonothiolates (**1–18**) and *O,O′*-dialkyl alkylphosphonothionates (**19–27**) are shown in Scheme 2. All the studied compounds are isomeric and, hence, distinction of one isomer from another is a challenging task. With a view to differentiating the studied set of isomers, we analyzed their GC/EIMS, linked scan, GC/CIMS and GC-RI data. The impurities and/or by-products present in the reaction mixture were well resolved in the GC method; hence, the EI or CI mass spectra of target compounds thus obtained are pure without any interfering peaks from other products.

GC/EIMS analysis

The EI mass spectra of the studied phosphonothiolates (**1–18**) and phosphonothionates (**19–27**) are summarized in Supplementary Tables S2 and S3 (see Supporting Information), respectively. Some typical spectra are shown in Figs. 1, 2 and 5. All the compounds show an abundant molecular ion ($M^{+\bullet}$, **a**) at *m/z* 182 (13–78%), except for dialkyl alkylphosphonothionates bearing a butyl group (**21–23**, 0.3–2%). The isotopic patterns of $M^{+\bullet}$ ions matched well with the simulated isotopic pattern for the formula, $C_6H_{15}O_2PS$. EI mass spectra of nine of the 27 compounds were recently added in the OPCW Central Analytical Database (OCAD) (v14); however, there was no investigation of their mass spectral fragmentation.

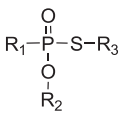
General EI fragmentation

The general EI fragmentation patterns of the phosphonothiolates and phosphonothionates, based on HRMS and linked scan (MS/MS) data, are given in Schemes 3 and 4, respectively. The HRMS data for the significant ions of the compound **2** is presented in Supplementary Table S4 (see Supporting Information). The alkyl (methyl, ethyl, propyl/isopropyl, and butyl/isobutyl/tert-butyl) groups attached to O, S and P influenced the overall fragmentation. The alkyl groups are lost either as radicals or as neutrals after proton migration. The alkene losses through McLafferty rearrangement are dominant in higher alkyl group-containing compounds (e.g., ions **c**, **e** and **h**). The fragment ions due to [Mclafferty + 1] rearrangement (ions **d** and **g**) are also observed in the spectra. Further loss of an alkene from the McLafferty rearrangement fragment ion is also found when that ion contains a higher alkyl group (other than a methyl group). Other ions include those due to homolytic fission of the P–R, P–OR or P–SR bond (α-cleavage) from the $M^{+\bullet}$. The $[M–OR]^+$ and $[M–(OR-H)]^{+\bullet}$ ions are diagnostic of the alkyl group attached to the oxygen, while the $[M–SR]^+$ and $[M–(SR-H)]^{+\bullet}$ ions indicate the alkyl group attached to the sulfur. Fragment ions formed by thiono/thiolo rearrangements are also observed in both alkylphosphonothiolates and alkylphosphonothionates



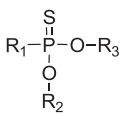
(**1-10**)

C. no	R_1	R_2	R_3
1	CH_3	n-C_3H_7	C_2H_5
2	CH_3	i-C_3H_7	C_2H_5
3	CH_3	C_2H_5	n-C_3H_7
4	CH_3	C_2H_5	i-C_3H_7
5	CH_3	n-C_4H_9	CH_3
6	CH_3	i-C_4H_9	CH_3
7	CH_3	t-C_4H_9	CH_3
8	CH_3	CH_3	n-C_4H_9
9	CH_3	CH_3	i-C_4H_9
10	CH_3	CH_3	t-C_4H_9



(**11-18**)

C. no	R_1	R_2	R_3
11	C_2H_5	n-C_3H_7	CH_3
12	C_2H_5	i-C_3H_7	CH_3
13	C_2H_5	CH_3	n-C_3H_7
14	C_2H_5	CH_3	i-C_3H_7
15	n-C_3H_7	CH_3	C_2H_5
16	n-C_3H_7	C_2H_5	CH_3
17	i-C_3H_7	CH_3	C_2H_5
18	i-C_3H_7	C_2H_5	CH_3



(**19-27**)

C. no	R_1	R_2	R_3
19	CH_3	C_2H_5	n-C_3H_7
20	CH_3	C_2H_5	i-C_3H_7
21	CH_3	CH_3	n-C_4H_9
22	CH_3	CH_3	i-C_4H_9
23	CH_3	CH_3	t-C_4H_9
24	C_2H_5	CH_3	n-C_3H_7
25	C_2H_5	CH_3	i-C_3H_7
26	n-C_3H_7	C_2H_5	CH_3
27	i-C_3H_7	C_2H_5	CH_3

Scheme 2. Structures of the studied dialkyl alkylphosphonothiolates (**1–18**) and dialkyl alkylphosphonothionates (**19–27**).

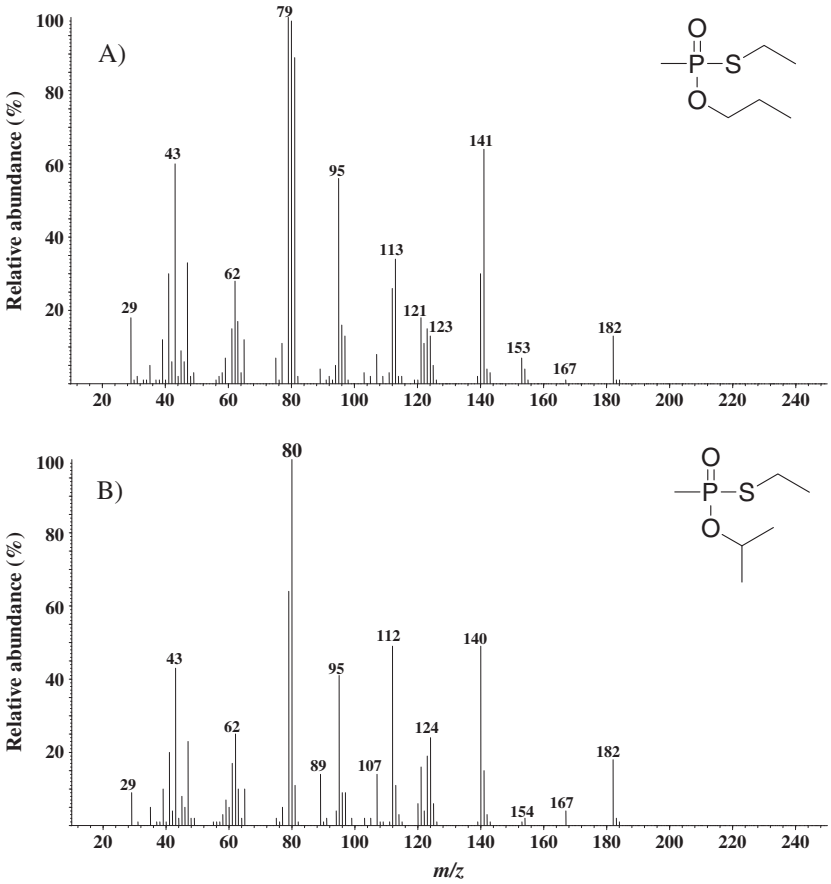


Figure 1. EI mass spectra of (A) compound **1** and (B) compound **2**.

(discussed in a separate section at the end), but they are low abundance and do not affect the structure indicative fragmentation pattern (discussed below). Apart from the ions discussed above, the spectra include a set of low-mass fragment ions at *m/z* 65 $[P(OH)_2]^+$ (**r**), 63 $(P\equiv S^+)$ (**s**), 47 $(P\equiv O^+)$ (**t**), 43 ($^{\cdot}C_3H_7$) (**u**), and 29 ($^{\cdot}C_2H_5$) (**v**) that reflect the connectivity and nature of the alkyl group attached to the heteroatoms.

Phosphonothiolates

The phosphonothiolates contain alkyl groups on three heteroatoms (P, O and S) and it is possible to identify the alkyl group attached to each heteroatom based on the observed selective fragmentation (Scheme 3).

O-Alkyl differentiation

The $[M–R_2/R_3]^+$ and $[M–(R_2/R_3\text{-}H)]^{+\cdot}$ ions provide information about the size of the alkyl group; however, the $[M–OR_2/R_3]^+$ and $[M–(OR_2/R_3\text{-}H)]^{+\cdot}$ ions confirm the attachment of the alkyl group (other than a methyl) to the oxygen atom. The *O*-methyl-group-containing compounds did not show the $[M–OCH_3]^+$ ion. As expected, the O-ethyl-, O-C_3H_7-alkyl-, and O-C_4H_9-alkyl-group-containing compounds yielded $[M–C_2H_4]^{+\cdot}$, $[M–C_3H_6]^{+\cdot}$, and $[M–C_4H_8]^{+\cdot}$ ions, respectively (McLafferty rearrangement ion). In addition, the compounds possessing O-C_3H_7-alkyl and O-C_4H_9-alkyl groups yielded McLafferty + 1 rearrangement ions. The McLafferty + 1 rearrangement ions are always higher in O-propyl group-containing compounds than in those with an O-isopropyl group, and the reverse trend is observed for McLafferty rearrangement products [Supplementary Table S2 (Supporting Information), compounds 1 and 2 (Fig. 1)]. This is in good agreement with the previous report, where the McLafferty + 1 ion was found to be more prominent in the n-alkyl-group-containing compounds than in those with branched alkyl groups.[34] Similarly, O-propyl-group-containing compounds showed a relatively more dominant $[M–OC_3H_7]^+$ ion than the O-isopropyl-group-containing compounds, while this trend is reversed in the relative abundances of the $[M–OC_3H_6]^{+\cdot}$ ions. Among the O-butyl-group-containing compounds, the McLafferty + 1 rearrangement ions are dominant over McLafferty rearrangement ions in all O-C_4H_9-alkyl-group-containing compounds, irrespective of the butyl structure. In a similar manner, the $[M–OC_4H_9]^+$ ion is always dominant over the $[M–OC_4H_8]^{+\cdot}$ ion and there are no significant differences among the isomeric butyl groups.

S-Alkyl differentiation

It is easy to identify the alkyl group attached to the sulfur by observing the presence of the $[M–SR]^+$ and/or $[M–(SR\text{-}H)]^{+\cdot}$ ions. All the S-methyl-containing compounds (**5**, **6**, **11**, **12**, **16**, and **18**) specifically show the $[M–SCH_3]^+$ ion (*m/z* 135). The S-ethyl-containing compounds (**1**, **2**, **15**, and **17**) consistently show a distinct $[M–SC_2H_5]^+$ ion (*m/z* 121) in

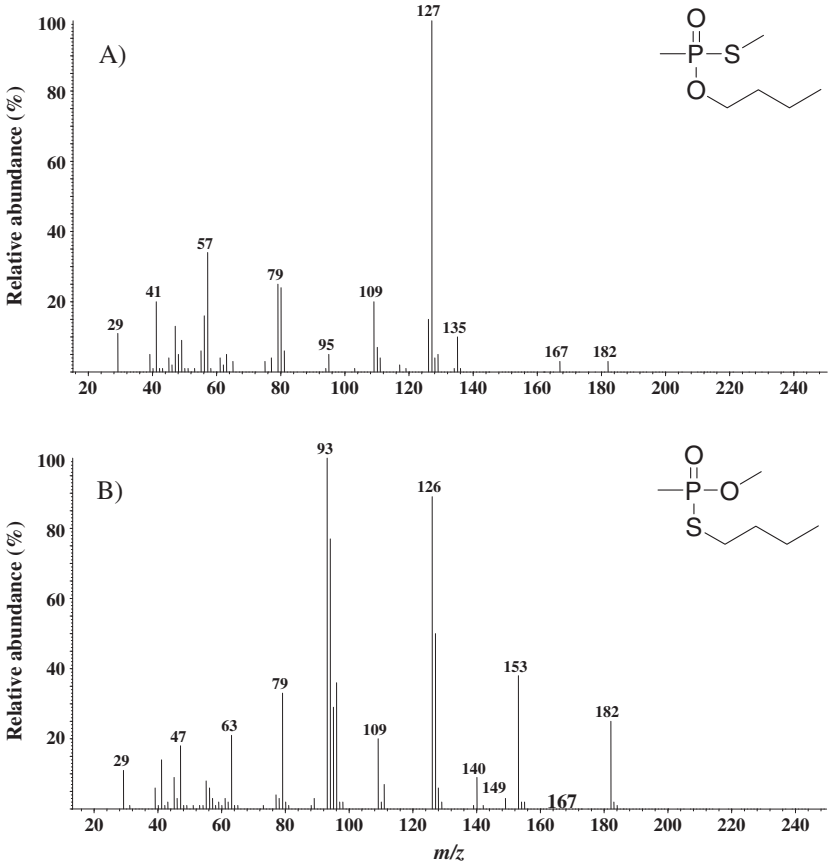


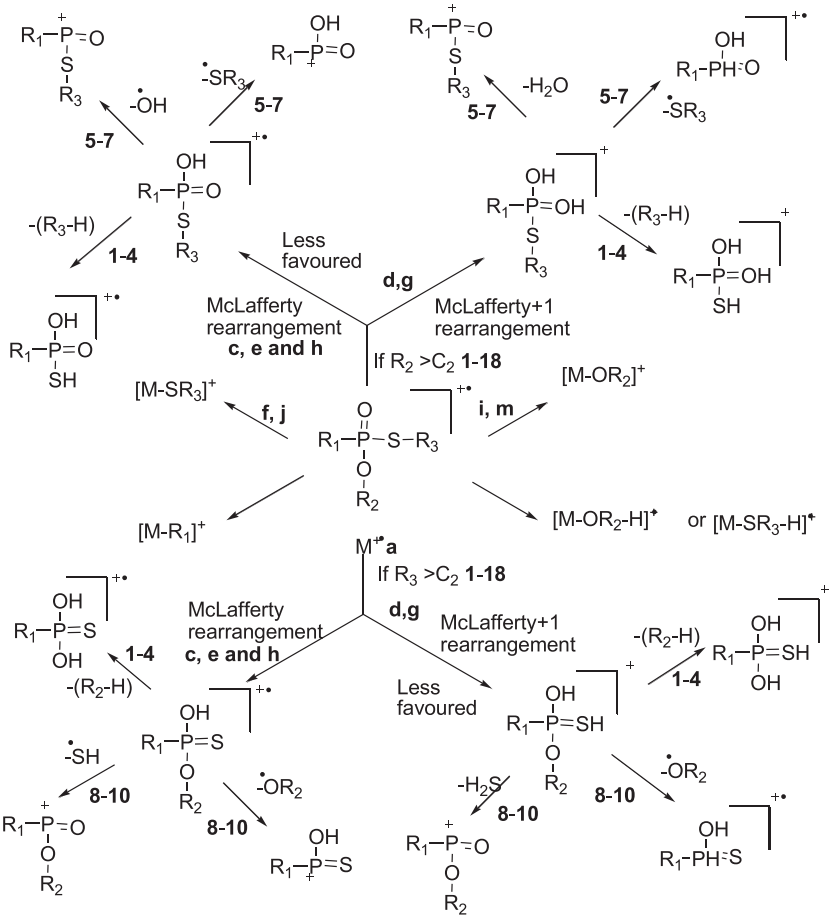
Figure 2. EI mass spectra of (A) compound **5** and (B) compound **8**.

addition to a $[M–SC_2H_4]^{+\cdot}$ ion (*m/z* 122). Similarly, S-C_3H_7-alkyl- (**3**, **4**, **13** and **14**) and S-C_4H_9-alkyl-group-containing compounds (**8–10**) show ions at *m/z* 107/108 and 93/94, respectively, due to $[M–SR]^+$/$[M–(SR\text{-}H)]^{+\cdot}$ ions. All the S-butyl-containing compounds (**8–10**) show a dominance of McLafferty rearrangement over McLafferty + 1 rearrangement, irrespective of the structure of the butyl group. However, some compounds (**2**, **3**, **11**, **12**, **15**, **16**, and **18**) without a propyl or butyl group on the sulfur atom also show the ions at *m/z* 93, 94 or 107, where these result from other routes of fragmentation as confirmed from the MS/MS and HRMS data.

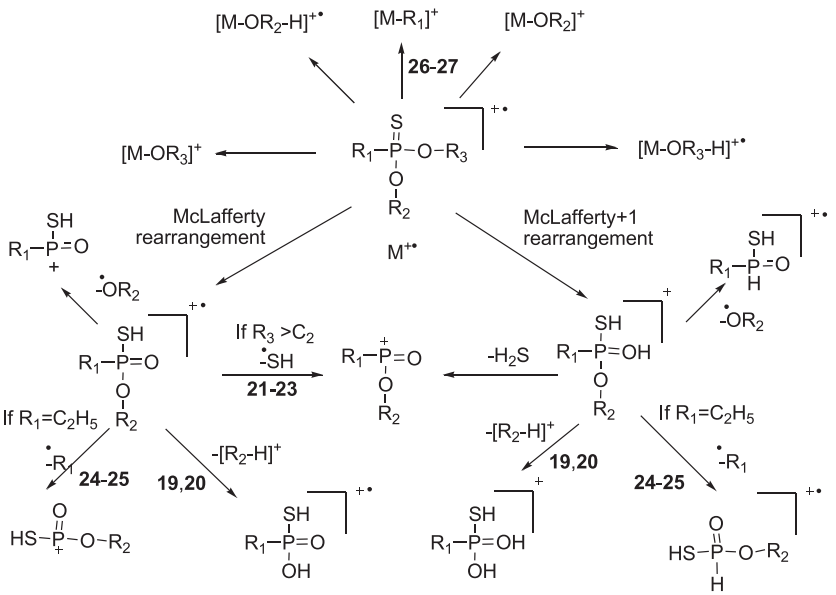
O-Alkyl vs. S-alkyl group

The fragmentation of the S-alkyl group is similar to that of the O-alkyl group, but there are pronounced differences in the McLafferty/McLafferty + 1 rearrangement processes. The compound with a larger O-alkyl group favors the McLafferty + 1 rearrangement, whereas the same alkyl group on the sulfur atom exclusively yields the McLafferty rearrangement ion (spectra of **5** and **8** are shown in Fig. 2 as an example). The lack of a McLafferty + 1 rearrangement in the higher S-alkyl groups (R = C3–C4 alkyl) may be because of the reduced tendency of the sulfur atom to accept the second hydrogen, as similarly reported in the case of O-ethyl S-alkyl methylphosphonothiolates.[31] All the S-methyl-containing compounds show a prominent $[M–SCH_3]^+$ ion (**f**), whereas O-methyl compounds do not show $[M–OCH_3]^+$ ions. This could be due to the higher stability of the leaving $\cdot SCH_3$ radical than of the $\cdot OCH_3$ radical. The dominant $\cdot SCH_3$ radical loss from *O,S*-dimethyl methylphosphonothiolate was reported in theoretical studies.[35,36] In the case of O-ethyl- and S-ethyl-group-containing compounds, the $[M–SC_2H_5]^+$ ion (**j**) is always more abundant in S-ethyl-containing compounds than is the $[M–OC_2H_5]^+$ ion in O-ethyl-containing compounds. The O-C_3H_7-alkyl-group-containing compounds show both the $[M–OC_3H_7]^+$ (**i**) and $[M–OC_3H_6]^{+\cdot}$ ions, but their abundances vary with the propyl/isopropyl groups attached to oxygen; whereas the S-C_3H_7-alkyl-group-containing compounds always show the $[M–SC_3H_6]^{+\cdot}$ ion as dominant over the $[M–SC_3H_7]^+$ ion. This suggests that the stability of the leaving $\cdot SC_3H_7$ radical is less than that of the SC_3H_6 molecule, probably through attaining a stable trimethylene sulfide (or thietane) structure. The $[M–OC_4H_9]^+$ ion is consistently dominant in all O-C_4H_9-alkyl-group-containing compounds over the $[M–SC_4H_9]^+$ ion in the corresponding S-C_4H_9-alkyl-group-containing compounds. As expected, the $[M–SC_4H_8]^{+\cdot}$ ion from S-C_4H_9-alkyl-group-containing compounds is more abundant than the corresponding $[M–OC_4H_8]^{+\cdot}$ ion from O-C_4H_9-alkyl-group-containing compounds, but these ions are not present in compounds where there is a tertiary butyl group on the oxygen/sulfur-atom.

The fragmentation pattern pertinent to the P-alkyl groups is different from that observed in the case of O-alkyl and S-alkyl groups. Moreover, the P-alkyl group fragmentation is almost similar between phosphonothiolates and phosphonothionates and this point is further discussed later.



Scheme 3. Plausible EI fragmentation pathway of dialkyl alkylphosphonothiolates.



Scheme 4. Plausible EI fragmentation pathway of dialkyl alkylphosphonothionates.

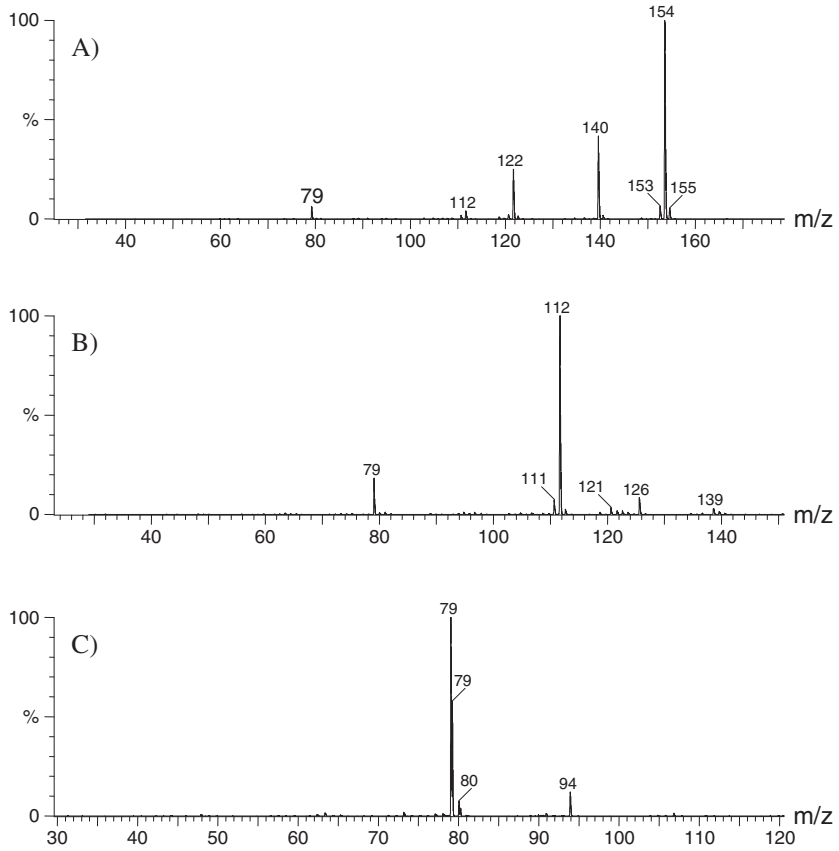


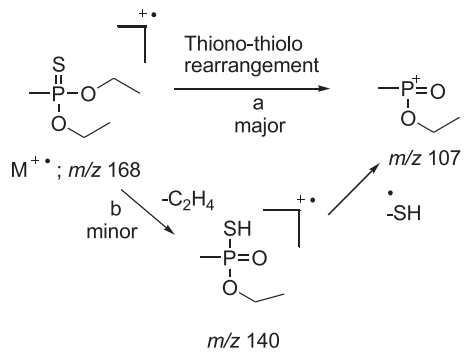
Figure 3. B/E spectra of (A) *m/z* 182, (B) *m/z* 154, and (C) *m/z* 122 of compound **15**.

Phosphonothionates

In *O,O'*-dialkyl alkylphosphonothionates (**19–27**), alkyl groups are attached to both the oxygen and the phosphorus atom, and the other heteroatom (S) exists as P=S. Thus, in this case, it is important to determine the size of the alkyl group attached to oxygen and phosphorus (Scheme 4).

O-Alkyl differentiation

The alkyl groups attached to oxygen atoms are methyl, ethyl, propyl/isopropyl, and butyl/isobutyl/*tert*-butyl. Unlike the phosphonothiolates, the O-methyl-group-containing phosphonothionates (**22**, **23** and **25–27**) show the expected $[M–OCH_3]^+$ ion (*m/z* 151) to confirm the presence of an O-methyl group. In the case of the O-ethyl-group-containing phosphonothionates (**19**, **20**, **26** and **27**), the expected ethylene loss (McLafferty rearrangement) is not observed but they do exhibit a $[M–OC_2H_5]^+$ ion at *m/z* 137. All the O-C_3H_7-alkyl- and O-C_4H_9-alkyl-group-containing phosphonothionates show $[M–OR]^+$ and $[M–(OR-H)]^{+\bullet}$ ions, and the ions due



Scheme 5. Thiono-thiolo rearrangement.

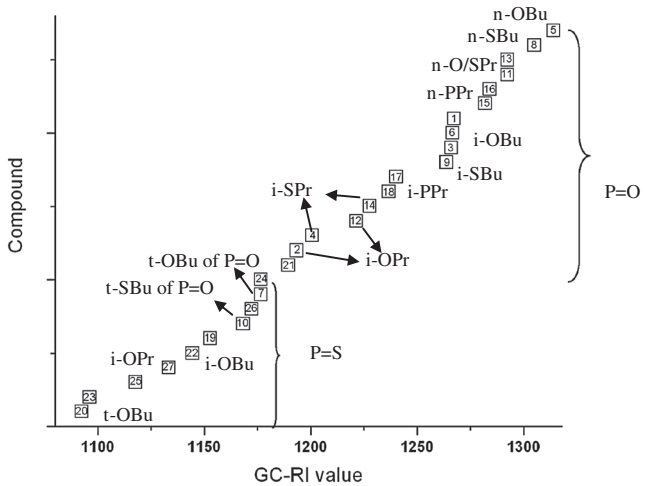


Figure 4. The GC-RI values of compounds **1–27**.

to McLafferty and McLafferty + 1 rearrangements. The McLafferty + 1 rearrangement process is always dominant over the McLafferty rearrangement process, irrespective of the structure of the alkyl group.

P-Alkyl differentiation

It is important to correctly identify the alkyl group attached to the phosphorus atom for all the schedule 2.B.4 chemicals that include dialkyl alkylphosphonothiolates/phosphonothionates, where the alkyl group can be a methyl, an ethyl, or a propyl/isopropyl group. All the P-ethyl-group-containing compounds (**11–14**, **24** and **25**) show loss of an ethyl radical from the molecular ion. A low-abundance but distinct $[M–C_2H_4]^{+\cdot}$ ion (**c**) is found in dialkyl ethylphosphonothiolates, but not in dialkyl ethylphosphonothionates. In addition, the P-ethyl-group-containing compounds consistently exhibit an ion at *m/z* 111 that results from the molecular ion by McLafferty rearrangement followed by the loss of the $^{\cdot}C_2H_5$ radical. The spectra of P-methyl-group-containing compounds include the $[M-CH_3]^+$ ion, but there are other possible routes for the loss of methyl radical from the alkyl groups attached to O/S. The P-C_3H_7-alkyl-group-containing compounds show a $[M–C_3H_6]^{+\cdot}$ ion at *m/z* 140 that is formed by a [1,4] C–P hydrogen shift (propyl) or a [1,3] C–P hydrogen shift (isopropyl).[37] The $[M–C_3H_5]^+$ ion (McLafferty + 1 rearrangement), which is characteristically observed in O/S-C_3H_7-group-containing compounds, is absent in the P-C_3H_7 compounds; however, these compounds show a low-abundance $[M–C_3H_7]^+$ ion at *m/z* 139 formed through α-cleavage (Supplementary Fig. S1, Supporting Information). The above data enables easy recognition of the C_3H_7-alkyl group attached to P; however, the data cannot provide any direct evidence about the structure of the C_3H_7-alkyl group (propyl/isopropyl). However, the spectra of the phosphonothiolates that are isomeric with either P-propyl or P-isopropyl show distinct differences in the relative abundances of other characteristic fragment ions. For example, the ion at *m/z* 126 is more abundant in compound **15** than in compound **17**. The MS/MS spectrum of the $[M–C_2H_4]^{+\cdot}$ ion (*m/z* 154) from **15** showed an abundant peak at *m/z* 126, which suggests that the loss of 28 Da from *m/z* 154 could be from the P-propyl group; this process is insignificant in compound **17** due to the presence of the P-isopropyl group (Fig. 3).

Thiono-thiolo rearrangement

The typical EI-induced gas phase 'thiono-thiolo rearrangement', i.e., conversion of > P(S)-O-R into > P(O)-S-R, has been observed in many classes of phosphorus-containing compounds such as phosphines, phosphates, phosphonates and phosphoramidates, etc.[37–39] Cooks *et al.* studied substituted phosphorothioates

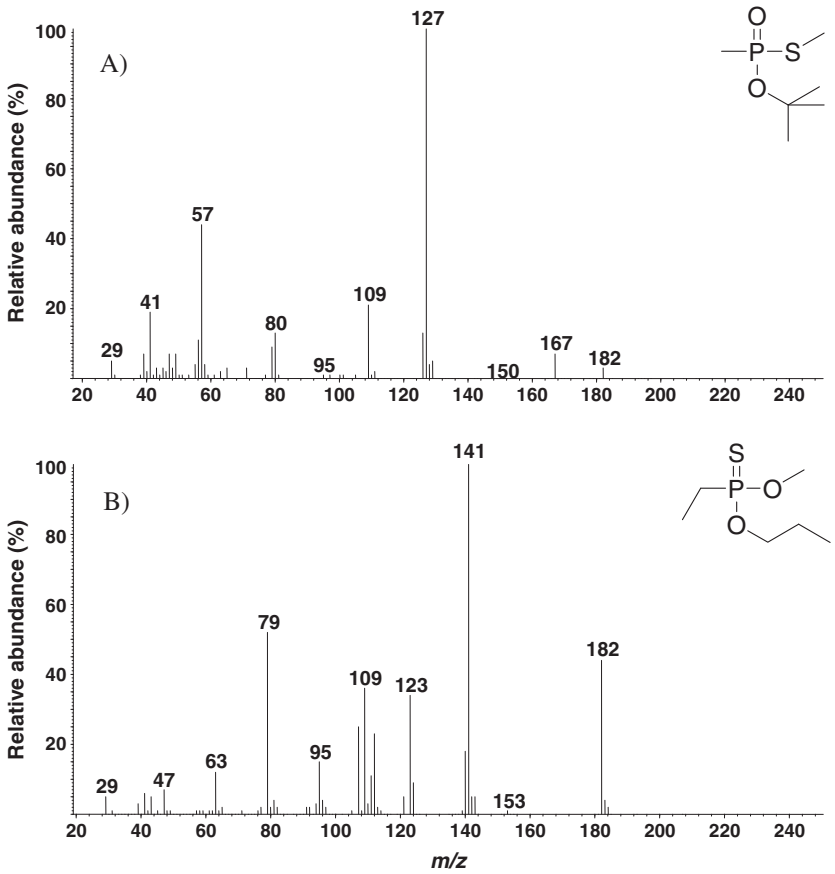
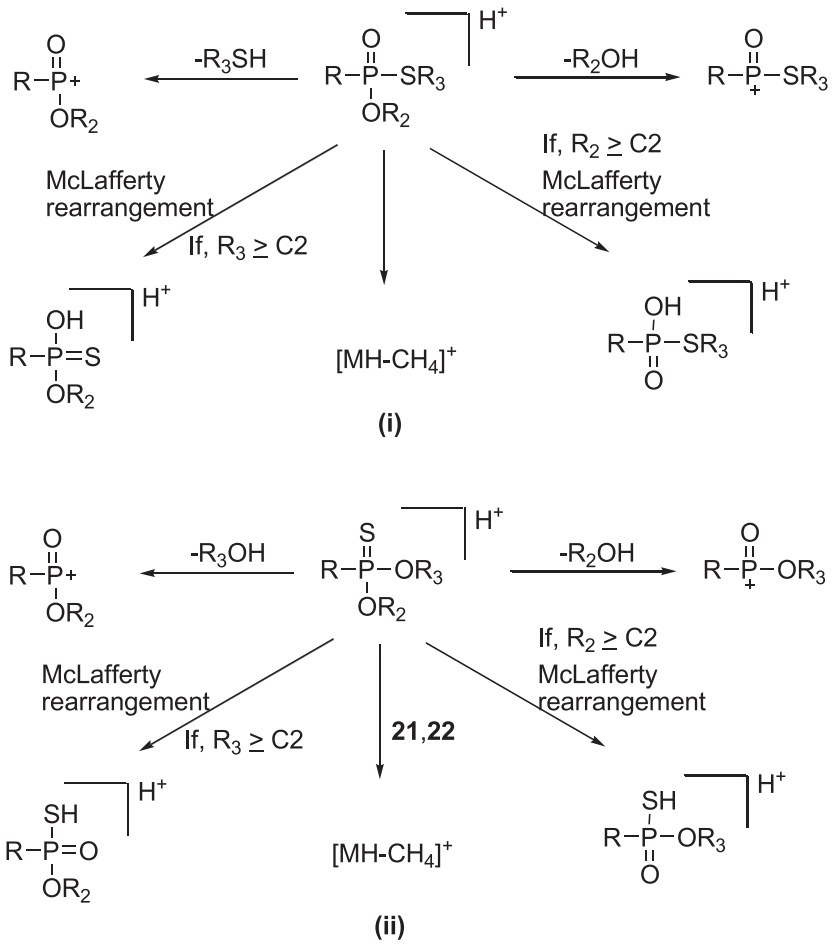


Figure 5. EI mass spectra of compounds with the same RI values: (A) compound **7** and (B) compound **24**.

and demonstrated the effect of electronegativity of the substituent on this rearrangement.[39] Santoro reported this rearrangement occurring under EI fragmentation of some alkyl thiophosphate esters.[40] Recently, Saeidian *et al.* reported the thiono-thiolo rearrangement in *O*-alkyl-*N*,*N*-dimethyl alkylphosphonothionoamidates, based on observation of unexpected loss of the ·SR radical, which was equal to or more abundant than the expected ·OR radical loss. They further supported the occurrence of the rearrangement with theoretical calculations.[41] Barr *et al.* studied a few dimethyl methylphosphonothionates (isotopically labelled) under ESI conditions and found that their protonated molecules undergo thiono-thiolo rearrangement.[32] Although many examples of thiono-thiolo rearrangement have been reported, it has not been explored in the case of dialkyl alkylphosphonothionates.

In the present study, the EI spectra of all the phosphonothionates (**19–27**) show the expected ·OR radical loss from the molecular ion; however, the spectra also include the ion due to the loss of ·SR radical from the molecular ion, $[M–SR]^+$, which is always of lower abundance (<5%, except for **24**) than the $[M–OR]^+$ ion. Formation of the $[M–SR]^+$ ion can be explained by a thiono-thiolo rearrangement. There is, however, another plausible fragmentation pathway that can lead to the same ion, i.e., ·SH radical loss from $[M–(R\text{-}H)]^{+\cdot}$, the McLafferty rearrangement ion. Both pathways result in the same structure (elemental composition); thus HRMS data alone is not sufficient to confirm the fragmentation pathway for formation of the $[M–SR]^+$ ion. We thus carried out linked-scan experiments on a model compound, diethyl methylphosphonothionate, and found a relatively abundant $[M–SC_2H_5]^+$ ion (*m/z* 107). The precursor ion spectrum of the ion at *m/z* 107 shows two peaks corresponding to $M^{+\cdot}$ (*m/z* 168) and $[M–C_2H_4]^{+\cdot}$ (*m/z* 140), where the former ion is dominant. The product ion spectrum of *m/z* 140 showed a small peak at *m/z* 107. This reveals that direct loss of $\cdot SC_2H_5$ from the molecular ion is the preferred fragmentation that can be explained by thiono-thiolo rearrangement (Scheme 5, Supplementary Fig. S2, Supporting Information).

Theoretically, the reverse of the thiono-thiolo rearrangement, which may be named as the 'thiolo-thiono' rearrangement, i.e., conversion of >P(O)-S-R into >P(S)-O-R, is possible in dialkyl phosphonothiolates. In such a case, the presence of the $[M–OR]^+$ ion is expected in the EI spectra of dialkyl phosphonothiolates in addition to the expected $[M–SR]^+$ ion. Among the studied dialkyl alkylphosphonothiolates (**1–18**), the $[M–OR]^+$ ion appeared only in compounds where a propyl or a butyl group was attached to sulfur (**3–4**, **8–10**, **13–14**). This reveals that the dialkyl phosphonothiolates undergo a 'thiolo-thiono' rearrangement, especially when a higher alkyl group is attached to sulfur. Although the studied compounds undergo thiono-thiolo or thiolo-thiono



Scheme 6. Plausible CI fragmentation pathways of (i) dialkyl alkylphosphonothiolates (**1–18**) and (ii) dialkyl alkylphosphonothionates (**19–27**).

rearrangement, the resulting fragment ions from the rearranged products are found to be of low abundance. Consequently, this process does not affect the regular fragmentation pathways of the studied compounds that provide structural information.

RI values

The RI values were calculated for all the studied compounds (Supplementary Table S1, Supporting Information). It is known that lower homologs (fewer carbon atoms) will elute faster than the higher ones (methyl < ethyl < propyl < butyl).[42] For the propyl and butyl groups, the elution order is isopropyl < propyl and t-butyl < isobutyl < butyl, respectively. The studied compounds, however, contain different alkyl groups on the heteroatoms (O, S and P) and hence show combined effects in their RI values (Fig. 4). The phosphonothionates eluted faster than the corresponding isomeric phosphonothiolates. Thus, the RI values are important for the differentiation of phosphonothiolates from phosphonothionates.

Two pairs of the studied isomers, i.e., **15** and **17**; **26** and **27**, displayed similar EI spectra. When the spectrum of one compound is searched in the in-house library (that includes the spectra of **1–27**), the other compound of the pair appears in the search result with a close match factor value. In such cases, the RI value is crucial for assigning the correct structure. On the contrary, the RI values of a few isomers are the same or very close (±1), but they show distinct EI spectra. For example, the RI value of **7** and **24** is 1176.8, whereas the EI spectrum of **7** is different from that of **24** (Fig. 5).

GC/CIMS analysis

All the compounds (**1–27**) were analyzed under GC/CIMS conditions. Methane was chosen as the reagent gas because methane-CI is likely to yield more fragment ions than isobutane; the fragment ions can provide additional structural information. The positive ion methane-CI spectra of all the studied compounds showed an abundant $[M+H]^+$ ion at *m/z* 183 in addition to $[M+C_2H_5]^+$ (*m/z* 211) and $[M+C_3H_5]^+$ (*m/z* 223) ions (Supplementary Table S5, Supporting Information). The CI data is particularly useful for the butyl group compounds, which showed low-abundance molecular ions in their EI mass spectra. In addition to $[M+H]^+$ ions, the CI spectra included a few fragment ions that were characteristic for the O- and S-alkyl groups. The general CIMS fragmentation is depicted in Scheme 6. The major fragment ions of dialkyl alkylphosphonothiolates are due to McLafferty rearrangement from the $[M+H]^+$ and $[M+C_2H_5]^+$ ions, in addition to low-abundance $[MH–R_2OH]^+$ and $[MH–R_3OH]^+$ or $[MH–R_3SH]^+$ ions. The CI data reveals that the McLafferty rearrangement is more feasible through the O-alkyl group than through the S-alkyl group; for

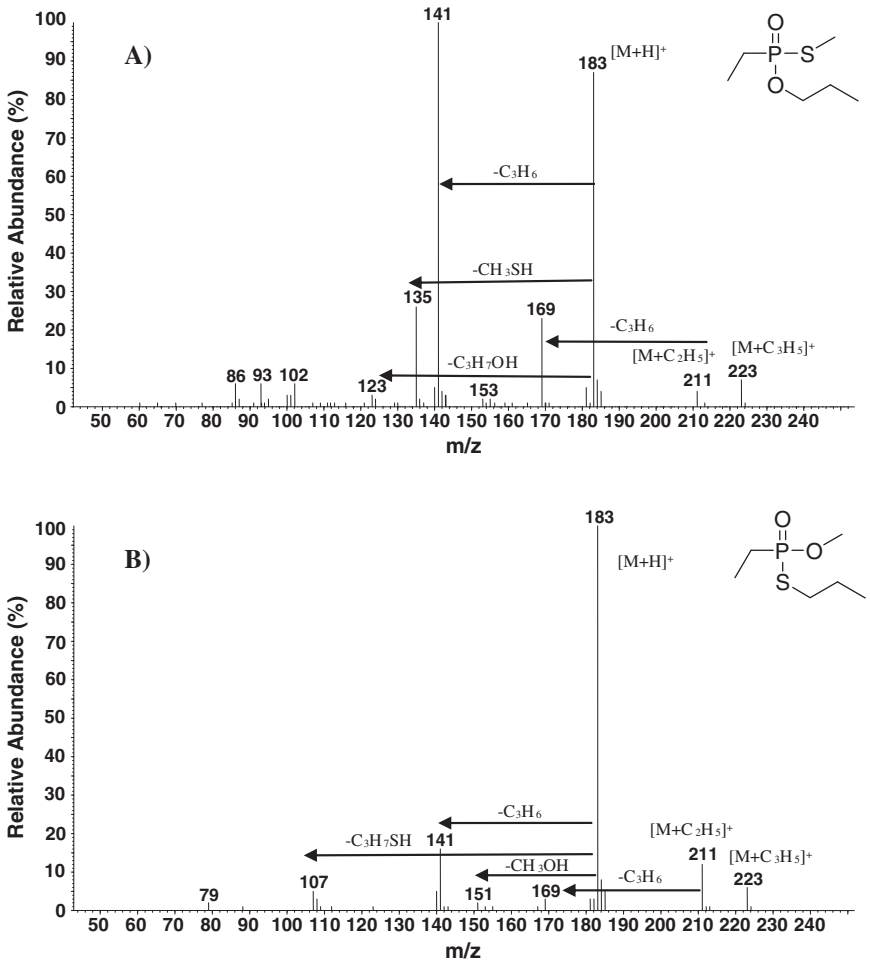


Figure 6. Methane-CI mass spectra of (A) compound **11** and (B) compound **13**.

example, the $[MH–C_3H_6]^+$ ion is more abundant in compound **1** (O-propyl) than in compound **3** (S-propyl). As expected, compound **19** that has O-ethyl and O-propyl groups shows a dominant $[MH–C_3H_6]^+$ ion over the $[MH–C_2H_4]^+$ ion. The n-alkyl groups (propyl and butyl) can easily be differentiated from their branched isomers (isopropyl and isobutyl/*tert*-butyl) because the McLafferty rearrangement ion is always of higher abundance from a branched alkyl group than from a n-alkyl group. The ion at *m/z* 155 is found in all the O-/S-butyl-group-containing compounds (**5–10**, **21–23**), and it appears to be formed by the loss of C_2H_4 from $[M+H]^+$, although this ion also corresponds to $[(M+29)–C_4H_8]^+$. The presence of $[MH–R_2OH]^+$ and $[MH–R_3OH]^+$ or $[MH–R_3SH]^+$ ions, although of low abundance, gives information about the alkyl group attached to the heteroatoms. As expected, the $[MH–RSH]^+$ ion is absent in dialkyl alkylphosphonothiolates. The isomeric compounds, which have similar EI spectra, show a better differentiation under CI conditions; for example, compounds **11** and **13** show distinct CI spectra (Fig. 6). The McLafferty rearrangement ions formed from the $[M+H]^+$ and $[M+C_2H_5]^+$ ions (*m/z* 141 and 169, respectively) are more dominant in compound **11** than in compound **13**. The ion at *m/z* 135 ($[MH–CH_3SH]^+$) in **11** and the *m/z* 151 ion ($[MH–CH_3OH]^+$) in **13** confirm the presence of S-methyl and O-methyl groups, respectively. In a similar manner, the ion at *m/z* 123 in **11** reveals the presence of an O-propyl group and the ion at *m/z* 107 in **13** confirms the presence of an S-propyl group. Thus, the methane-CI data clearly divulges the alkyl groups attached to the heteroatom (O or S).

CONCLUSIONS

A series of isomeric dialkyl alkylphosphonothiolates (**1–18**) and dialkyl alkylphosphonothionates (**19–27**), which belong to schedule 2.B.4 of the CWC, was synthesized in-house and then analyzed by GC/MS under EI and CI conditions. The study mainly emphasizes the differentiation of phosphonothiolates and phosphonothionates (connectivity between P and S) and also the identification of the alkyl group attached to the sulfur, oxygen and phosphorus atoms. There are vast differences among the EI spectra that reflect the structure of the alkyl group attached to the oxygen, sulfur and phosphorus atoms and also the connectivity between P and S. The overall EI fragmentation is primarily affected by the alkyl group attached to the heteroatoms (O, S and P). The $[M–SR]^+$ and $[M–(SR-H)]^{+\bullet}$ ions are characteristically found in phosphonothiolates, confirming the presence of a S-alkyl group, while these ions are absent or of low abundance in phosphonothionates. The McLafferty + 1 rearrangement process due to presence of a higher alkyl group (propyl/butyl) is predominant in the case of O-alkyl compounds, while this process is less favored in those compounds where the same alkyl group is attached to the sulfur atom. The phosphonothiolates and phosphonothionates that have similar O-alkyl groups are distinguishable by their distinct $[M–R]^+$ and $[M–OR]^+$ ions. The fragmentation of P-alkyl groups is different from that of O/S-alkyl groups. All the phosphonothionates eluted faster than the corresponding phosphonothiolates; thus the GC-RI values can also be used for their discrimination. In cases where the spectral differences are poor, the GC-RI values are crucial. This study also provide an EI mass spectral database for the scheduled compounds (2.B.04) that is useful for their unambiguous identification during OPCW proficiency tests. The methane-CI mass spectra show $[M+H]^+$ ions for all the studied compounds. The CI fragment ions are characteristic of the alkyl groups attached to O and S, thus making structural characterization easy. The studied compounds undergo thiono/thiolo rearrangements to some extent, but the fragment ions resulting from the rearranged products are of low abundance, and do not interfere with the structure-indicative fragment ions.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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