JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. **33**, 1104–1108 (1998)

Identification of the C_3H_7 Moiety of Isopropyl- and Propylphosphonates by Electrospray Tandem Mass Spectrometry

Ben L. M. van Baar,* Albert G. Hulst and Eric R. J. Wils
TNO Prins Maurits Laboratory, P.O. Box 45, 2280 AA Rijswijk, The Netherlands

Structure analysis of phosphorus compounds within the framework of the Chemical Weapons Convention requires the specific identification of alkyl substituents on phosphorus. In this work the distinction of the *P*-propyl substituent in propylphosphonic acid derivatives by electrospray tandem mass spectrometry was investigated. *P*-Isopropyl and *P*-*n*-propyl groups were readily distinguished and a mechanistic proposal was given, based on labelling experiments, The findings were applied to the identification of the *P*-propyl moiety of two V-agents, *O*-ethyl *S*-[2-(dialkylamino)ethyl]propylphosphonothiolates, and to a degradation product of a G-agent, propyl propylphosphonate. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: electrospray tandem mass spectrometry; alkylphosphonates; alkylphosphonic acids; deuterium labelling

INTRODUCTION

Mass spectrometry (MS) provides key methods in the analysis of compounds subject to the Chemical Weapons Convention (CWC). General criteria for the identification of chemical warfare agents and related compounds have been specified in detail by the Organisation for the Prohibition of Chemical Weapons (OPCW).[1] Concerning the identification of alkylphosphonic acids (R-P(O)$(OH)_2$, R = C_1–C_3 alkyl) and their esters, typical hydrolysis products of nerve agents, it has been stipulated that an alkyl ester moiety need not be identified beyond the empirical formula, but that the carbon backbone structure of alkyl substituents on phosphorus must be specified. Regarding mass spectrum interpretation, the latter requirement provides some difficulty with respect to the distinction of isomeric *n*- and isopropylphosphonate derivatives. Therefore, the extensive use of MS in CWC-related analysis would be aided by conclusive identification of a C_3-alkyl group on phosphorus.

The scarcity of reports on the electron ionization (EI) MS of alkylphosphonic acids reflects the fact that these compounds are not particularly amenable to EI, with self-protonation accompanying evaporation.[2–4] Therefore, their identification is often achieved by derivatization and subsequent gas chromatographic (GC)/MS analysis. The identification is ideally confirmed by comparison of the data with those for a synthesized, authentic standard, but synthesis is often too laborious when quick identification is required. With the advent of liquid chromatographic (LC)/MS methods, the analysis of underivatized alkylphosphonic acids has become a feasible option. Although good results were initially obtained with thermospray,[5–7] it has been shown recently that electrospray (ES) and atmospheric pressure chemical ionization (APCI) are particularly suited for these acidic compounds.[8–10]

In this paper, we present ES-MS investigations on the distinction of the two C_3-alkylphosphonic acids and some of their deuterated isomers. In addition, the identification was applied to a monoalkyl alkylphosphonate.

EXPERIMENTAL

Materials

Analytical-grade formic acid and acetonitrile were purchased from Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). *tert*-Butylphosphonic acid and *n*-propylphosphonic acid were purchased from Aldrich (Beerse, Belgium). Isopropylphosphonic-d_0 and -d_1 and *n*-propylphosphonic-d_3 acid were synthesized from appropriately labelled 2- and 1-bromopropane and diethyl phosphite. In all cases, the *P*-proton was abstracted from diethyl phosphite with metallic sodium and the bromo compound was added; subsequently, the diethyl ester was saponified. The synthesis products were of >98% purity, as checked by GC/MS of the diethyl esters (before saponification) and by ^{1}H NMR (after

* *Correspondence to*: B. L. M. van Baar, TNO Prins Maurits Laboratory, P.O. Box 45, 2280 AA Rijswijk, The Netherlands
E-mail: baar@pml.tno.nl
Contract/grant sponsor: Netherlands MOD.

CCC 1076–5174/98/111104–05 $17.50

Received 1 July 1998
Accepted 4 August 1998

saponification). *O*-Ethyl *S*-[2-(dimethylamino)ethyl] *n*-propylphosphonothiolate and *O*-ethyl *S*-[2-(diisopropylamino)ethyl] isopropylphosphonothiolate were synthesized by common procedures, for confirmation of the identified V-agents. *O*-Isopropyl *n*-propylphosphonate was obtained by basic hydrolysis of the corresponding phosphonofluoridate; this phosphonofluoridate was obtained from the Centre d'Études du Bouchet (CEB, Vert-le-Petit, Essonne, France).

Liquid chromatography and mass spectrometry

ES and tandem mass spectra were obtained by flow injection into the standard ES interface of a Quattro II triple-quadrupole instrument (Micromass, Altrincham, UK). The instrument was operated in the positive ion mode and using a 30 V cone voltage. A mixture of water and acetonitrile (1:1, v/v), at a flow-rate of 20 μl min^{-1}, was used as the eluent and the injection volume was 10 or 40 μl. Tandem mass spectra were obtained using argon as the collision gas ($3–4 \times 10^{-3}$ mbar fore-vacuum reading) at an indicated collision energy of 15 eV, unless indicated otherwise.

Experiments involving D_2O as an eluent component were conducted with a glass syringe mounted on an infusion apparatus (Harvard Apparatus, South Natick, MA, USA). All other conditions were as described for flow injection ES-MS and ES-MS/MS.

Energy-resolved product ion spectra of the *m/z* 125 precursors were obtained by varying the collision energy offset from 1 to 25 eV (laboratory frame of reference) and keeping all other conditions identical.

LC/ES-MS was performed, with the Quattro II as a detector, for the identification of *O*-ethyl *S*-[2-(dimethylamino)ethyl]propylphosphonothiolate and *O*-ethyl *S*-[2-(diisopropylamino)ethyl]propylphosphonothiolate, arbitrarily abbreviated to V1 and V2 (*P*-propyl unidentified), respectively; except for the collision energy (20 eV) and the cone voltage (50 V), all MS conditions were as described above. Similar conditions were applied for the synthetically obtained *O*-ethyl *S*-[2-(dimethylamino)ethyl]-*n*-propylphosphonothiolate and *O*-ethyl *S*-[2-(diisopropylamino)ethyl]isopropylphosphonothiolate. A 250 × 4 mm i.d. PRP-1 column (10 μm particles) (Hamilton, Reno, NV, USA) was used for linear gradient separation; the gradient, 30 min from 95:5 to 30:70 (v/v) $H_2O:CH_3CN$ + 0.2% formic acid at 1.0 ml min^{-1}, was provided by Waters M590 and M510 pumps and a Waters M680 gradient controller (Waters, Milford, MA, USA). Loop injection (10 μl loop) was performed with a Valco C6W injector (VICI, Schenkon, Switzerland) and a the column effluent was split 1:20 before the ES probe by a laboratory-made T-piece.

RESULTS AND DISCUSSION

Identification

The ES-MS/MS product ion spectra of protonated *n*-propyl- and isopropylphosphonic acid, *m/z* 125, are shown in Fig. 1. The most notable distinctions in the

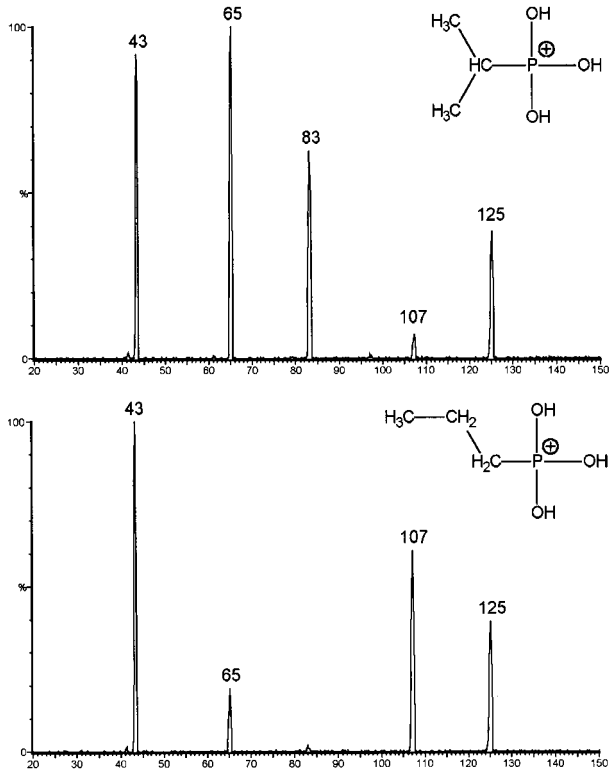
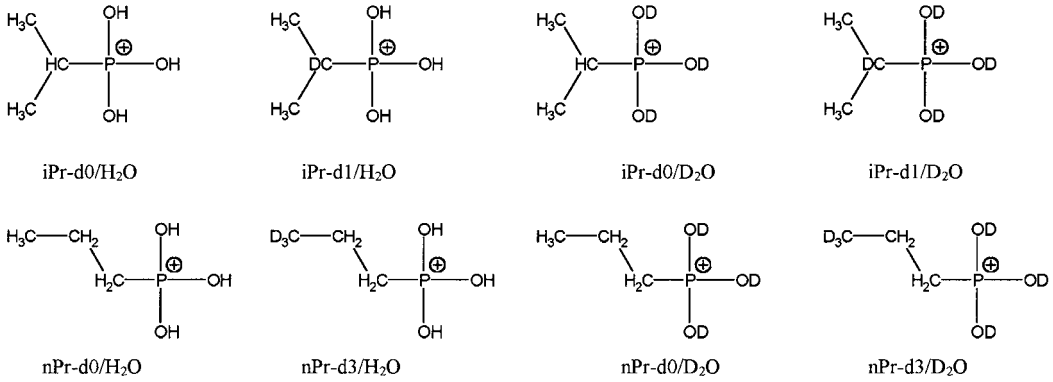


Figure 1. ES-MS/MS product ion spectra of protonated *n*-propyl- (top) and isopropylphosphonic acid (bottom), precursor *m/z* 125; cone 20 V, 15 eV collision energy.

spectra result from the loss of water, *m/z* 107, and the loss of propene, *m/z* 83. In addition, the difference in *m/z* 65 intensity, i.e. for $[M + H - C_3H_6 - H_2O]^+$, largely reflects the reaction consecutive to propene loss. Although the spectra (Fig. 1) were obtained at a collision energy of 15 eV, the energy-resolved spectra (Fig. 2) demonstrate that the loss of propene prevails for protonated isopropylphosphonic acid. Therefore, the observation of distinct product ion tandem mass spectra can straightforwardly be used for identification purposes, just by plain comparison.

The two spectra suggest that there is a competition between loss of water and loss of propene, with the outcome being determined by the nature of the *P*-propyl group. The possibility of a general principle in the behaviour of the *P*-propyl group was further investigated by deuterium labelling experiments. Deuterium was incorporated in the alkyl chain, by synthesis, whereas hydroxy hydrogen was exchanged for deuterium in ES experiments with D_2O as an eluent component ($D_2O:CH_3CN$ 1:1, v/v). The deuterated precursor ions thus generated, isotopomers of *m/z* 125, are depicted in Scheme 1, and the corresponding neutral losses, isotopomers of C_3H_6 and H_2O, are summarized in Table 1. Neutral loss of HDO was never observed, indicating that water loss exclusively involves hydroxy hydrogens and none of the *n*- or isopropyl hydrogens. Hence, water loss does not compete with propene loss in a kinetic sense, i.e. competition for a hydrogen atom, because the alkyl group is not involved in water loss at all.

Propene loss from protonated isopropylphosphonic acid occurs through a 1,4-hydrogen shift, of one of the



Scheme 1.

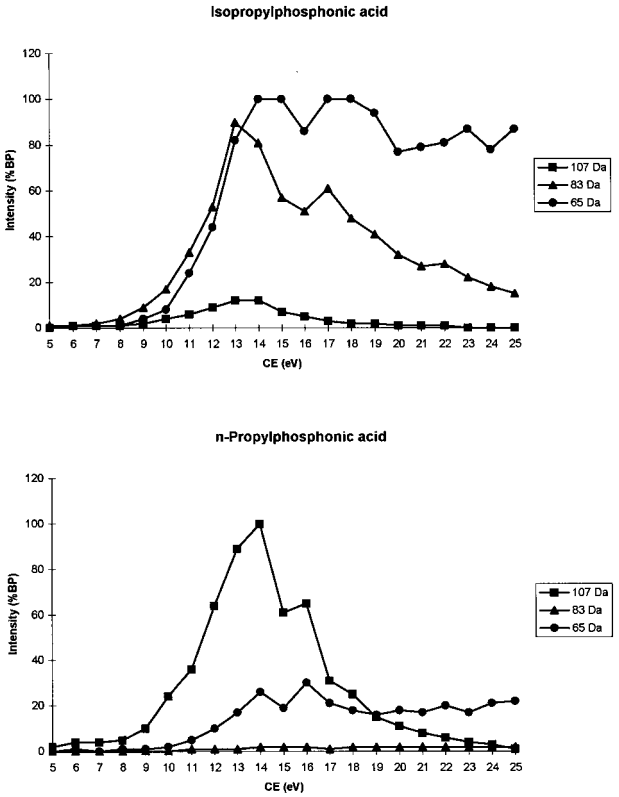


Figure 2. Collision energy (CE) resolved ES-MS/MS product ion spectra of protonated *n*-propyl- (top) and isopropylphosphonic acid (bottom); for clarity only product ions *m/z* 65, 83 and 107 are shown.

Table 1. C_3H_6 and H_2O isotopomer neutral losses in ES-MS/MS product ion spectra[a] (relative intensity,[b] %) of isotopomers of protonated isopropyl- and *n*-propylphosphonic acid

	Neutral					
Isotopomer[c]	$-C_3H_6$	$-C_3H_5D$	$-C_3H_4D_2$	$-C_3H_3D_3$	$-H_2O$	$-D_2O$
iPr-d_0/H_2O	70	–	–	–	7	–
iPr-d_0/D_2O	95	5	–	–	–	7
iPr-d_1/H_2O	–	80	–	–	12	–
iPr-d_1/D_2O	–	95	5	2	–	10
nPr-d_0/H_2O	2	–	–	–	63	–
nPr-d_0/D_2O	3	–	–	–	–	65
nPr-d_3/H_2O	–	–	–	1	85	–
nPr-d_3/D_2O	–	–	–	2	–	65

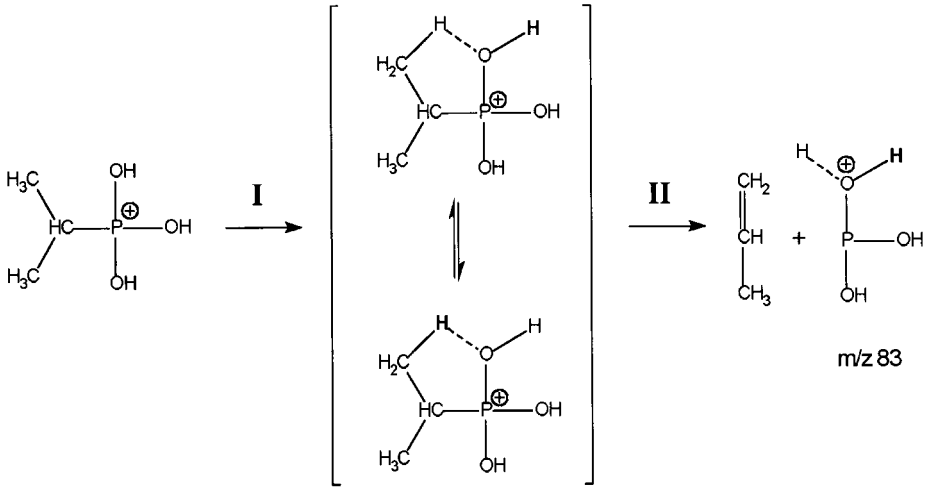
[a] Conditions as in Experimental section, with cone voltage 30 V and collision energy 15 eV.
[b] Relative to base peak.
[c] See Scheme 1 for isotopomer.

methyl hydrogens to the phosphonate group, because the label in isopropyl-d_1 is always found in the neutral species. This 1,4-hydrogen shift could occur through a covalently bound transition state (see Scheme 2), because there is a minor exchange of hydrogen (<5%) between one of the isopropyl methyl groups and one of the hydroxy groups. Although Scheme 2 distinguishes two steps, the process needs not be a consecutive one. In addition, the involvement of an intermediate ion–molecule complex of propene and (oxygen) protonated trivalent phosphorus acid (not shown) cannot be excluded.

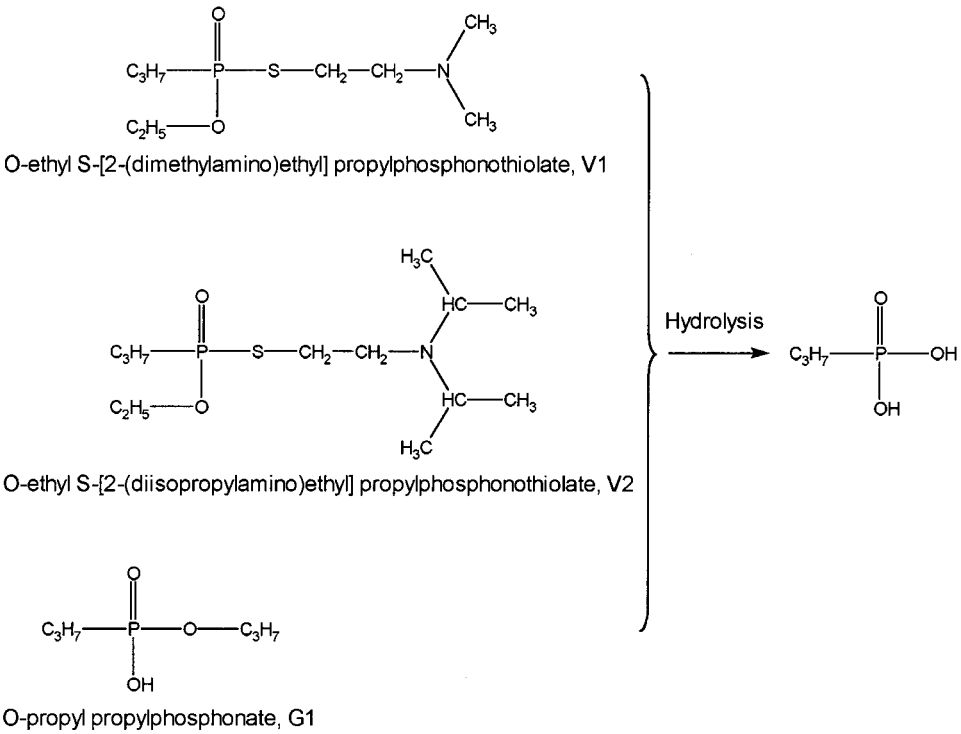
The analogous 1,4-hydrogen shift is not observed with protonated *n*-propylphosphonic acid, although hydrogen is available at the 2-alkyl position. This implies that step I or II (or both) analogous to that in Scheme 2 must be energetically less favourable for the *n*-propyl substituent as compared to the isopropyl substituent. The methyl group on the 1-alkyl position in protonated isopropylphosphonic acid apparently has a sufficient electron-donating capacity to facilitate hydrogen transfer from the 2-alkyl position and/or to facilitate P—C bond cleavage. This effect of 1-alkyl substitution is confirmed by the ES-MS/MS product ion spectrum of protonated *tert*-butylphosphonic acid ($C_4H_9^+$ base peak, $[M + H - C_4H_8]^+$ 27%, $[M + H]^+$ 10%, no $[M + H - H_2O]^+$), where the additional methyl group on the 1-alkyl position shifts the reaction pattern completely to butene loss.

Application

The identification of *n*- and isopropylphosphonic acid was used for the determination of the *P*-propyl substituent in three nerve agent related compounds from OPCW Proficiency Tests (see Scheme 3). Two compounds, V1 and V2, are V-type nerve agents, whereas the other, *O*-propyl propylphosphonate (G1), is a hydrolysis product of the corresponding G-type nerve agent (all compounds: *P*-propyl unspecified). Most of the structure of the compounds could be resolved from EI and CI GC/MS data, but the nature of the *P*-propyl substituent could not be established. The two V-agents were, at least partially, hydrolysed to the corresponding propylphosphonic acids, under the ES conditions used (Fig. 3), whereas G1 was already (partially) hydrolysed in aqueous solution (pH around 7). It is noted that



Scheme 2.



Scheme 3.

V-type compounds generally hydrolyse to the corresponding *O*-alkyl alkylphosphonic acids,[5,11] thus losing the sulphur atom. For the V-type compounds it was established that hydrolysis occurs inside the API source, because the propylphosphonic acids (sulphur free) were observed at the same LC/ES-MS retention time as their respective V-agent precursors, with the ion signal depending on the cone voltage applied. *O*-propyl propylphosphonate was subjected to flow injection experiments only, but it readily produced a propylphosphonic acid. The spectra of the three hydrolysis products are shown in Fig. 4; the *P*-propyl substructure is *n*-propyl for V1 and G1 and isopropyl for V2. This identification was confirmed by synthesis of V1 and V2 with the identified *P*-propyl groups and of *O*-isopropyl *n*-propylphosphonate; the spectral data of synthesized, authentic compounds and identified unknowns were identical.

CONCLUSION

The *P*-propyl group of propylphosphonates and propylphosphonothiolates can rapidly and unequivocally be identified as an *n*-propyl or isopropyl moiety by ES-MS/MS experiments on the propylphosphonic acid generated by hydrolysis. Distinct fragmentation, with protonated isopropylphosphonic acid showing abundant propene loss, occurs via a 1,4-hydrogen shift; the distinction is probably due to the effect of methyl substitution on the 1-alkyl position on hydrogen transfer and/or P—C bond cleavage.

Acknowledgements

We thank Mr G. Moes, Ing. A. Fidder and Mrs Ing. S. de Kant for the synthesis of labelled compounds and V-agents. The continued support of the Netherlands MOD is acknowledged.

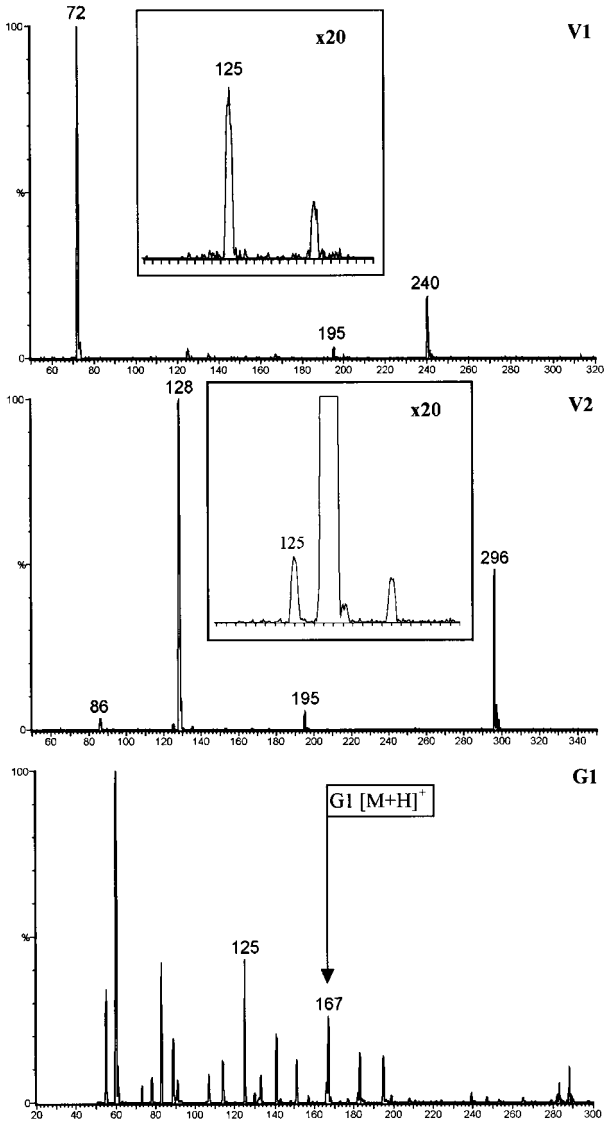


Figure 3. ES mass spectra of compounds V1, V2 (cone 50 V) and G1 (cone 30 V), obtained by LC/MS (V1 and V2) and by flow-injection (G1) of Proficiency Test sampes; spectra show the *m/z* 125 hydrolysis product.

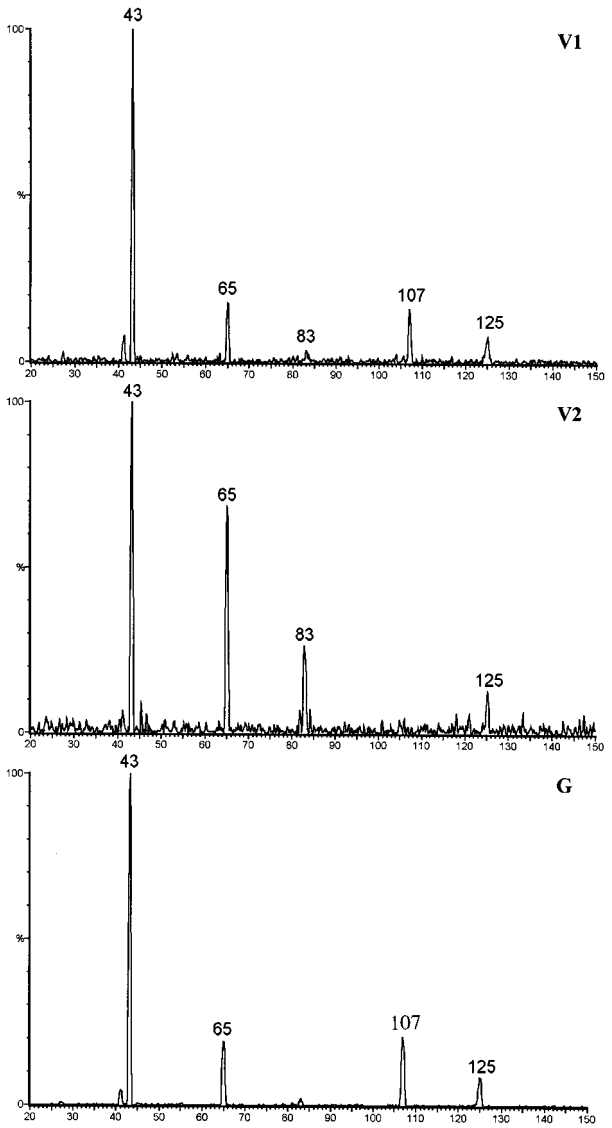


Figure 4. ES-MS/MS product ion spectra of precursor ions of *m/z* 125 from compounds V1, V2 (cone 50 V, collision energy 20 eV) and G1 (cone 30 V, collision energy 15 eV).

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