Fragmentations and Reactions of the Organophosphate Insecticide Diazinon and its Oxygen Analog Diazoxon Studied by Electrospray Ionization Ion Trap Mass Spectrometry

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The fragmentations and reactions of Diazinon and related compounds have been studied by electrospray ionization ion trap mass spectrometry. Several novel fragmentation and rearrangements have been observed, including an intramolecular thiono-thiolo rearrangement. The stability, in the gas-phase, of the protomers of 2-isopropyl-4-methyl-6-pyrimidinol has been demonstrated. The complexity of the gas phase ion processes observed suggest that, at present, caution should be exercised in using this approach for the analysis of environmental and other samples until our understanding of these processes increases considerably. (J Am Soc Mass Spectrom 2005, 16, 515–523) © 2005 American Society for Mass Spectrometry

Investigations of the fragmentations and reactions of the ions of simple organophosphate esters produced by electrospray ionization (ESI) in an ion trap (IT) mass spectrometer demonstrated that detailed structural information could be obtained by sequential Collision Induced Dissociation (CID) [1–3]. These studies have been extended to more complex organophosphates such as the chemical warfare agents *O*-ethyl *S*-(2-diisopropylamino)ethyl methylphosphonothiolate (VX) and *O*-isobutyl *S*-(2-diethylamino)ethyl methylphosphonothiolate (RVX) [4], and organophosphate insecticides, one of which Diazinon **I**, together with its oxygen analog Diazoxon **II**, and pyrimidinol side-chain **III**, are the subject of the present paper (see Figure 1 for structures).

Two ESI-MS studies of protonated Diazinon have been reported [5, 6] but tandem quadrupole instruments were used and fragmentation pathways could not be studied as extensively as in the IT mass spectrometer. There are significant differences in the results

of these studies and the present one. Initially in this study, a commercial analytical standard of Diazinon was used. The data obtained suggested that either thiono-thiolo isomerization (Figure 2) had occurred in the fragmentation process or in the sample prior to analysis.

Such isomerizations are well known and have been used in synthesis (for a review of relevant papers, see reference 3). A commercial sample of Diazinon was purified and analyzed to investigate which of these two possibilities was correct. In the event, it was found that isomerization had not occurred in the sample. The method of purification of Diazinon, synthesis of Diazoxon, and results of their analyses are described here.

Experimental

Materials and Analytical Equipment

Diethyl chlorophosphate and 2-isopropyl-4-methyl-6-pyrimidinol were purchased from Aldrich Ltd (Gillingham, UK) and were used as received. Triethylamine was dried by distillation from calcium hydride. Anhydrous solvents were used. Plates for thin layer chromatography (TLC) comprised MK6F silica gel 60A

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Figure 1. Structures of Diazinon I, Diazoxon II, and the side chain pyrimidinol II.

(2.5 × 7.5 cm, layer thickness 250 μg) and were purchased from Whatman International Ltd. (Maidstone, UK). Silica for column chromatography, particle size 40–63 μm, was obtained from BDH Laboratory Supplies (Poole, UK). NMR spectra were recorded on a JEOL Lambda 500 instrument (operating at 500 MHz for ^1H , 125 MHz for ^{13}C and 202 MHz for ^{31}P spectra) as solutions in CDCl_3 , with internal reference SiMe_4 for ^1H and ^{13}C , and external $(\text{MeO})_3\text{P}$ (δ 140 ppm) for ^{31}P spectra. Data are listed as follows: chemical shifts in ppm from reference on the δ scale, integration, multiplicity (s = singlet, d = doublet, t = triplet and sept = septet), coupling constant (J/Hz) and assignment. IR spectra were recorded as liquid films on a Nicolet SP210 instrument using Omnic software (Thermo Electron Corp., Madison, Wisconsin); s after a frequency indicates that the band was strong. Reaction mixtures were monitored by TLC and components visualized using UV light (λ = 254 nm).

O,O-Diethyl *O*-[2-Isopropyl-4-Methyl-6-Pyrimidinyl] Thiophosphate (Diazinon)

TLC experiments with commercial Diazinon, a yellow liquid of 90+% purity, were performed using several solvent systems (hexane-acetone, hexane-ethyl acetate, and chloroform), the best separation occurring with 12:1 hexane-acetone. The main spot appeared at R_f 0.2 and an impurity spot at R_f 0.1. Chromatography using a column of silica gel (15 cm long and 5 cm in diameter) and eluting with 12:1 hexane-acetone gave Diazinon as a colorless liquid (3.6 g) of 99% purity. ^1H NMR: δ = 6.66 (1H, s, aromatic CH), 4.38 (4H, dd, J = 8 and 8 Hz, OCH_2), 3.12 (1H, sept, J = 7 Hz, isopropyl CH), 2.47 (3H, s, aromatic CH_3), 1.42 (6H, t, J = 7 Hz, CH_3), 1.31 (6H, d, J = 7 Hz, isopropyl CH_3). ^{13}C NMR: δ = 175.68, 169.97, 164.52, 106.91/106.83 (ring carbons), 65.28/65.21 (OCH_2), 37.42 (CH), 24.07 (isopropyl CH_3), 21.40 (methyl CH_3), 15.78/15.67 (ethoxy CH_3). ^{31}P NMR: δ = 59.5 (consistent with a previous value of 59.5 [7]). IR (film): ν = 2974, 2931, 2906, 2873, 1587s, 1562s, 1471, 1444, 1383, 1352, 1294, 1161, 1099, 1024s, 995, 982, 951, 831. The infrared data parallels earlier data [8]. No evidence for thiono-

thiolo rearrangement during storage was found using the spectroscopic techniques outlined above.

O,O-Diethyl *O*-[2-Isopropyl-4-Methyl-6-Pyrimidinyl] Phosphate (Diazoxon)

A solution of 2-isopropyl-4-methyl-6-pyrimidinol (3.53 g, 23.18 mmol) and triethylamine (3.23 ml, 23.18 mmol) in dichloromethane (30 ml) was added dropwise to a stirred solution of diethyl chlorophosphate (4 g, 23.18 mmol) in dichloromethane (20 ml) at 0–5 °C. After addition, the mixture was allowed to warm to room temperature. A catalytic amount of 4-dimethylaminopyridine was added. After 15 min, the pale brown solution became turbid and a precipitate of triethylamine hydrochloride formed. The solution was heated under reflux for 2 h. After cooling to room temperature, water (20 ml) was added to dissolve the precipitate. The organic layer was separated, washed with 10% w/w sodium bicarbonate solution (20 ml) and dried (MgSO_4). The drying agent was filtered off and the filtrate concentrated. Chromatography of the residue on silica gel, eluting with 4:1 hexane-acetone, gave the title compound as a colorless liquid (4 g, 60%) of >98% purity. R_f in 2:1 hexane-acetone = 0.31 (R_f of starting pyrimidinol = 0.08). ^1H NMR: δ = 6.73 (1H, s, aromatic CH), 4.34 (4H, dd, J = 7 and 7 Hz, OCH_2), 3.12 (1H, sept, J = 7 Hz, isopropyl CH), 2.49 (3H, s, aromatic CH_3), 1.4 (6H, t, J = 7 Hz, CH_3), 1.32 (6H, d, J = 7 Hz, isopropyl CH_3). ^{13}C NMR: δ = 176.24, 170.63, 164.8, 106.5 (ring carbons), 65.27 (OCH_2), 37.64 (CH), 24.45 (isopropyl CH_3), 21.78 (methyl CH_3), 16.25 (ethoxy CH_3). ^{31}P NMR: δ = -9.0. IR (film): ν = 2974, 2931, 2873, 1589s, 1562s, 1446, 1383, 1354, 1290, 1165, 1028s, 997, 985, 948, 867, 835s.

Mass Spectrometry

The mass spectrometric techniques and experimental conditions used in the present work have been described in detail elsewhere [2] but in this work only the Esquire LC, Bruker Daltonics, GmbH (Bremen, Germany) was used. All ions were isolated before fragmentation, thus removing ambiguity in the precursor-fragment ion relationships.

Electronic Structure Calculations

The geometries of the pyrimidinol systems were fully optimized using the DFT approach with B3LYP pa-

Figure 2. The thiono-thionolo rearrangement.

Figure 3. Fragmentation of protonated Diazinon, IH^+ (m/z 305).

parameterization [9]. A triple σ valence plus diffusion and polarization functions basis set, 6-311 + G(2d,2p), was chosen for calculations. Energies were corrected by the zero-point vibrational contribution. All calculations were done by using the Gaussian 03 package of programs [10].

Results

Diazinon I

Isolation and fragmentation of protonated Diazinon, IH^+ , produced four ions with m/z values of 277, 249, 169, and 153 (Figure 3). These ions were isolated and fragmented, as were their fragment ions, and the results are summarized in Figure 4.

The ions with m/z 277 and 249 are formed by sequential loss of ethene from the ethoxy groups as expected [1]. The ion with m/z 153a is attributed to protonated pyrimidinol, IIH^+ , and that with m/z 169a

to the corresponding protonated pyrimidinethiol, presumably formed by thiono-thiolo rearrangement of IH^+ . The designator a in m/z 153a and 169a distinguishes these ions from tautomer ions produced by fragmentation of m/z 249. The ions with m/z 153 and 169 isolated after fragmentation of protonated Diazinon are not pure m/z 153a and 169a, but contain small amounts of m/z 153b and 169b. It is not immediately apparent whether the ions with m/z 153b and 169b result solely from fragmentation of m/z 249 or also from fragmentation of m/z 305. The ions m/z 153a and 169a are distinguished from the isomeric ions m/z 153b and 169b by their characteristic fragmentations, the ions with the designator a losing 69 Da attributed to isobutyronitrile and those with the designator b losing either H_2O or H_2S . Fragmentation of m/z 277 gives only traces of m/z 153a and 169a, the majority of the ion fragmenting to m/z 249 and its fragment ions. No m/z 153a and 169a are produced on fragmentation of m/z 249. The results obtained when using D_2O are shown in Figure 5.

The fragmentation of m/z 250 gives the surprising result that only HDO and not H_2O is lost whereas the product ions from the alternative fragmentation routes show a partial retention of the deuterium.

Diazoxon II

The isolation and fragmentation of protonated Diazoxon, IIH^+ , and its product ions are very similar to that of protonated Diazinon except that, of course, the sulphur-containing ions are absent with the corresponding oxygen analogs appearing in their place. They are summarized in Figure 6.

Unlike the product ion formed from elimination of ethene from IH^+ , the analogous ion from protonated Diazoxon, IIH^+ , m/z 261, loses ethene to give m/z 233 but also m/z 153a. Similarly, fragmentation of m/z 233 gives traces of m/z 153a in addition to the expected m/z 153b and 215.

2-Isopropyl-4-Methyl-6-Pyrimidinol III

Fragmentation of IIH^+ , m/z 153, obtained from a pure sample of **III** gave a 20:1 ratio of the ions with m/z 84

Figure 4. Fragmentation of protonated Diazinon, IH^+ and its product ions.

Figure 5. Fragmentation of ID^+ and its product ions.

Figure 6. Fragmentation of protonated Diazonox and its product ions.

and 135 which suggests that IIIH^+ is a 20:1 mixture of m/z 153a and 153b.

Electronic Structure Calculations

The results of the present calculations together with some earlier ones are given in Table 1.

Discussion

Sites of Protonation

It is appropriate to consider the structure of protonated Diazonox before discussing its fragmentation. Heterocycles with hydroxyl or thiol groups positioned α or γ to a ring nitrogen can exist as a mixture of tautomers [11]. The position of equilibrium depends upon the compound, the medium, and whether it is in the gas phase or in solution. The equilibrium position for 4-hydroxypyrimidine (Figure 7) has been studied in the gas phase and the keto and enol forms are almost equally stable [12]. Unfortunately keto-enol tautomer-

Figure 7. Tautomers of 4-hydroxypyrimidine.

isms of protonated species relevant to this study do not appear to have been studied in the gas phase.

In addition to keto-enol tautomerism of the protonated pyrimidine, proton tautomerism between the two nitrogen atoms is possible. In the gas phase movement of the proton between the nitrogen atoms will presumably be prevented by a high energy barrier because of the rigidity of the ring, in contrast to systems with peptide bonds where the proton is mobile [13]. Consideration of proton affinities of the heteroatoms in Diazonox suggests that three sites are important: the thiono sulphur atom and the two pyrimidine nitrogen atoms (Table 1).

Placement of the proton on the thiono sulphur atom is unlikely to lead to the fragmentations observed without its migration presumably to N-1 of the pyrimidine ring. Thus only two placements of the proton on Diazonox need to be considered. These are as shown in Figure 8; oxonium forms are drawn but the positive charge could equally reside on NH. The structures are designated m/z 305_(P=S 1H) and m/z 305_(P=S 3H), the P=S indicating the thiono structure (and, more importantly, the P—O—pyrimidine bonding) and the number prefixing H denoting the nitrogen bearing the proton.

At this juncture it is necessary to consider the structures of m/z 153a and 153b (and by implication, the structures of m/z 169a and 169b for, as mentioned earlier, they fragment along parallel pathways). The simplest explanation for the difference between these ions is that they are protomers of protonated pyrimidinol, IIIH^+ . Movement of the proton between the ring nitrogen atoms is unlikely to occur in the gas phase, as suggested earlier, and was confirmed by stable isolations of m/z 153a and m/z 153b. That these ions are protomers and not structural isomers is supported by the fragmentation of m/z 153 from a pure sample of the pyrimidinol **III**. But which protomer is which? This can be deduced from the observation that m/z 153b loses H_2O on fragmentation. This is most likely to occur in the protomer with the proton on the nitrogen atom adjacent to the hydroxy group. The structures appear in Figure 9. Assuming the production of m/z 153a and 153b

Table 1. Calculated proton affinities of the basic groups in Diazonox

Group or compound	Proton affinity (kJ mol ⁻¹)	Reference
P=S	880	3
P-SR	820	3
P=O	890	3
P-OR	780	3
2-Isopropyl-4-methyl-6-pyrimidinol	945 (N-1) 956 (N-3) enol 957 (N-3) keto ^a	this work

^aThe neutral keto tautomer is 8 kJ mol⁻¹ more stable than the enol tautomer.

Figure 8. Protomers of protonated Diazonox.

Figure 9. Protomers and tautomers of the pyrimidinol side chain.

in the gas phase occurs at approximately the temperature of the drying gas (300 °C), their observed ratio of 20:1 deduced from the fragmentation of m/z 153, is in good agreement with that calculated from the proton affinities given in Table 1.

The sulphur analogs m/z 169a and 169b will have corresponding structures designated m/z 169_(3H) and 169_(1H).

Thiono-Thiolo Rearrangements

An intramolecular thiono-thiolo rearrangement of protonated simple organophosphorus esters was not observed in the gas phase, and electronic structure calculations indicated a TS energy exceeding 500 kJ mol⁻¹ [3], much greater than the TS energies for the rearrangements and fragmentations normally observed with organophosphate esters [3,614]. Thus, the facile thiono-thiolo rearrangement observed with IH^+ can be attributed to the strong positive charge on the C-6 of the pyrimidine ring in the structures shown in Figure 6. That both protomers rearrange confirms that isomerization results from direct nucleophilic attack of the sulphur on C-6 rather than via some more complex TS involving the nitrogens (with or without a proton) of the pyrimidine ring. Attack at the 5-position, although

perhaps favored for steric reasons, is not possible on electronic grounds. Rearrangement to the 6-position is supported by common pathways of fragmentation of m/z 305_(P=S) and 305_(P=O).

Fragmentation of m/z 305_(P=S 3H) and m/z 305_(P=O 3H)

These ions fragment to give m/z 153_(3H) and 169_(3H) as the respective sole ionic products. Their stoichiometry requires a hydrogen from one of the ethyl groups to be transferred to the protonated pyrimidino moiety. Thus, concerted elimination of ethene and a metaphosphorothionate or metaphosphate is the most likely mechanism and is illustrated in Figure 10, the curved arrows referring to the eliminations and not to the thiono-thiolo rearrangement.

Fragmentation of m/z 305_(P=S 1H) and m/z 305_(P=O 1H)

These ions lose ethene to give m/z 277_(P=S 1H) and m/z 277_(P=O 1H) which, in turn, also lose ethene to give m/z 249_(P=S 1H) and m/z 249_(P=O 1H), respectively. While loss of ethene might be expected to follow the

Figure 10. Fragmentation of m/z 305_(3H).

Figure 11. Fragmentation of m/z 305_(P=S).

mechanism illustrated for m/z 305_(P=S) [when no subscript is attached to a m/z value, no distinction is made between the various possible structures, i.e., whether the ion has the P=S or P=O form or the location of the proton on the pyrimidine ring cannot be discerned] in Figure 611, protonation of an ethoxy oxygen atom is unlikely, given the greater proton affinity of the pyrimidine nitrogen atoms. However, when using D₂O, in the final product ion following the sequential ethene elimination, m/z 250, the deuterium remains on the pyrimidine ring (scrambling with hydroxyl hydrogens on the phosphorus moiety does not occur as if it did both H₂O and HDO would be expected to be eliminated – see later). The ³H tautomers are not involved as m/z 153_(1H) and 169_(1H) and not their ³H tautomers are formed. When the proton (or deuterium) is on N-1, it can interact directly with the phosphorus moiety in two ways depending on the conformation. Two conformers, designated a and b, are shown in Figure 612.

Since the proton affinity of N-1 is significantly greater than that of the thiono sulphur atom in m/z 305_(P=S 1H), the proton will be attached to N-1. Conformer a is unreactive to fragmentation, but Conformer b can fragment via the mechanism illustrated in Figure 613 to produce ethene and m/z 277_(P=S 1H) [the designator P=S is still used as the P—O—pyrimidine bond is intact] which can fragment similarly.

The ions m/z 305_(P=O 1H) and 277_(P=O 1H) can fragment similarly. In addition to the ions with m/z 153_(1H) and 169_(1H) being formed on fragmentation of m/z 249_(1H), an ion with m/z 231 formed by loss of water is observed. Loss of H₂S to give an ion with m/z 215 is not observed.

Figure 12. Conformers of the _(1H) protomer of IH⁺.

On using D₂O the isotopomeric ions m/z 250_(1D) are isolated which on fragmentation gives m/z 169_(1H), 170_(1D), 6153_(1H), 6154_(1D), and 6231_(1D) (see Figure 614). The ratio of the isotopomers is unusual with that ratio m/z 169_(1H): m/z 170_(1D) being ca. 1:1, that of m/z 153_(1H): m/z 154_(1D) being ca. 2:1 and no deuterated isotopomer of m/z 231. (It is interesting to note that when fragmenting the deuterated isotopomer m/z 234 from the oxygen analog of Diazinon the ratio m/z 153_(1H): m/z 154_(1D) is also ca. 2:1 and again only HDO is lost). It was expected, and indeed partially confirmed by these results, that a proton/deuteron in the 1 position of the pyrimidine ring can exchange with a proton/deuteron on the phosphorus moiety. It is difficult to rationalize the isotopomer ratios in the product ions, and in particular the sole loss of HDO rather than H₂O, unless there is an energy barrier to H/D exchange and fragmentation.

This is perhaps not unreasonable as exchange can be envisaged to involve the transformation shown in Figure 615, with the right-hand structure representing a transition state in H/D scrambling. Before excitation, the deuterium will be sequestered on the ring nitrogen N-1. It is suggested that elimination of H₂O, or HDO when using D₂O, occurs from the left-hand structure before H/D exchange takes place. Also, fragmentation to produce m/z 169_(1H) only occurs via the high-energy right-hand structure. During this fragmentation, a proton or deuterium will have to return to the ring nitrogen, but this will have to be a concerted process (if reversion to the left-hand structure took place, some H₂O would be expected to be eliminated, and this was not observed). The different isotope ratios between m/z

Figure 13. Fragmentation of m/z 305_(P=S 1H), Conformer b.

Figure 14. Fragmentations of m/z 306_(1D) and m/z 250_(1D).

$153_{(1H)}/154_{(1D)}$ and m/z 169_{(1H)}/170_(1D) are attributed to kinetic isotope effects.}

That there is no discernable loss of H_2S from m/z 249_(1H) can be rationalized by m/z 249_(1H) being a mixture of m/z 249_(P=S 1H) and m/z 249_(P=O 1H) with the latter dominating as indicated by the relative intensities of product ions m/z 153_(1H) and m/z 169_(1H). As m/z 231 only loses PO_2SH as the neutral fragment, it is not possible to say which subscript ($\text{P}=\text{S}$ 1H) or ($\text{P}=\text{O}$ 1H) or both is appropriate. Isolation and fragmentation of both m/z 154_(1D) and m/z 170_(1D) gives m/z 135. On isolation, m/z 135 can be fragmented further with sequential loss of 42 Da (C_3H_6) and 27 Da (HCN) giving ions m/z 93 and m/z

66. It also loses HCN to give a trace of m/z 108. The fragmentations of m/z 305 are summarized in Figure 6.

Previous Studies on Diazinon

As mentioned in the introduction, there have been two studies on the ESI-MS-MS of Diazinon [5,6] but using tandem-in-space rather than the present tandem-in-time instrumentation. The earlier study [5] found the ions m/z 169 and 153 to be the dominant fragment ions from IH^+ . Ions attributed to sequential loss of ethene were present only in trace amounts. This situation is the converse from the present study where loss of ethene is

Figure 15. Proton shift from the pyrimidinol side chain to the phosphorus moiety prior to the elimination of water.

Figure 16. Fragmentations of m/z 305 and its product ions.

the dominant fragmentation pathway. The authors of the early study also reported an ion with m/z 125 which was not observed in the present study. While their attribution of m/z 153 to IIIH^+ agrees with our results, their assignment of m/z 169 to $(\text{EtO})_2\text{P}(\text{O})(\text{S})^+$ does not; such an assignment ignores the possibility of thiono-

thiolo rearrangement and requires a most unlikely fragmentation. The more recent study [6] (which does not refer to the earlier one) described dominant fragment ions with m/z 169 and m/z 153, but only traces of ions from sequential loss of ethene, and no ion with m/z 125. The authors of this study attributed ion m/z 169 to

the sulphur analog of IIIH^+ resulting from a thiono-thiolo rearrangement, although they were undecided as to where and how this took place. Neither of the two reported studies provided more detail on the fragmentations. In the absence of a more detailed comparison, the differences between the previous studies and the present one are tentatively ascribed to the differences in energies and time scales of the fragmentations, 50 and 12/25 eV for the two earlier studies on μs time scales and <3 eV and 30 ms for the present.

Conclusions

This study illustrates how the use of electrospray ionization ion trap mass spectrometry can reveal detailed structural information on the fragmentations and reactions of organophosphate esters. Study of the pesticide Diazinon under such conditions has revealed the importance of thiono-thiolo rearrangements and tautomeric equilibria in the gas phase; both phenomena are poorly understood at present. Future studies will examine the thiono-thiolo rearrangements of structurally simpler analogs of Diazinon. The observed complexity of the gas-phase ion processes indicates that considerable care and understanding is necessary if this approach is to be used in analyzing environmental samples.

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