Phosphoric Amides

7—Mass Spectrometry of Phosphoric Amido Esters: Fragmentation Patterns and Migratory Aptitudes

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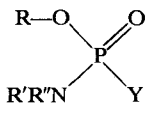
Mass spectra of 38 organophosphorus compounds, containing both phosphate ester (P(O)OR) and phosphoramidate (P(O)NR'R") functional groups, were recorded and discussed. Attention was focused on P—N bond cleavage, which can involve simple fission, fission accompanied by hydrogen migration from the ester group and fission accompanied by the migration of the ester R group to the departing nitrogen atom. Fragmentations characteristic for the *N*(β-chloroethyl) derivatives (phosphorylated nitrogen mustards) are also presented.

INTRODUCTION

Following our interest in the dynamics of the P—N bond under acidic,[1] nucleophilic[2] and electron impact[3] conditions, we decided to examine mass spectra of a series of phosphoric amido esters of the general formula **1**. If the ionization of **1** involves one of the



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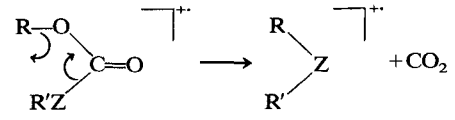
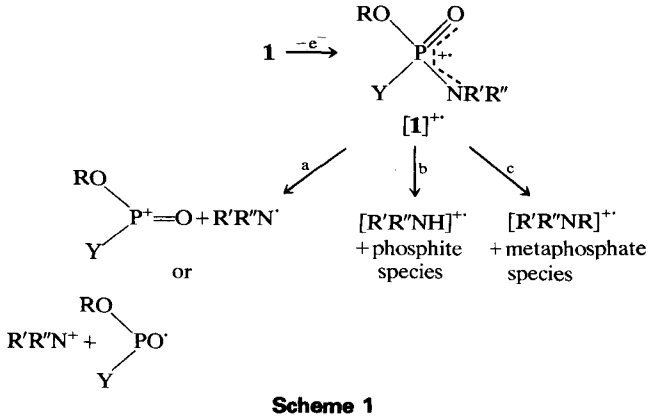
electrons of the phosphoramidate function, the P—N bond in the molecular ion $[\mathbf{1}]^{+\cdot}$ formed would be expected to be much more labile than in the parent substrate. The unimolecular cleavage of the P—N bond could then in principle follow three pathways (a–c), as represented in Scheme 1. Simple cleavage of the P—N bond (pathway a) has been observed before,[3,4] and in this respect substrates **1** parallel the behaviour of carboxylic amides.[5] Pathway b, which involves hydrogen migration to give an amine radical ion, requires release of a neutral phosphite molecule. We had observed this type of fragmentation before,[6] and we wanted to establish the scope of this rearrangement. The P—N cleavage accompanied by O→N migration (pathway c) can be related to the well-known electron impact induced fragmentation of carbonates (**2**)[6] and carbamates (**3**)[7] involving O→O or O→N migration and expulsion of carbon dioxide (Eqn (1)).



(1)

2: Z = O
3: Z = NR"

The objective of this work was to provide more general information on the mass spectrometry of phosphoric amido esters; in particular we were interested in P—N bond cleavage and possible migrations, as well as in structural effects upon these fragmentations. We also report here the mass spectrometry of amidophosphates containing an *N*-(β-chloroethyl) group, which is an essential function in the anticancer drug, cyclophosphamide. Although mass spectrometry has been used in studies of cyclophosphamide metabolites,[8] no detailed analyses of fragmentation patterns have been given.



Scheme 1

RESULTS AND DISCUSSION

The phosphoramidates used in this study can be represented by the general formulae **1A**, **1B**, **1C** and **1D**. The mass spectra of the following compounds were recorded and are discussed in this paper. Selected fragmentation data are listed in Tables 1–4.

Molecular ions derived from substrates **1** are relatively stable since they were observed for all compounds except **1Ad** and **1Cd**, and varied in intensity from 4 to 100%. The simplest mode of the P—N bond

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$(RO)_2P(O)NR'R''$ **1A** $(PhO)_2P(O)NR'R''$ **1B**

$X_2P(O)NHCH_2CH_2Cl$ **1C** $(RO)(MeNH)P(O)NHCH_2CH_2Cl$ **1D**

1A

R = *Me*, *R'* = *H*
a: R'' = Me
b: R'' = Et
c: R'' = iso-Pr
d: R'' = *t*-Bu
e: R'' = Ph
f: R'' = 2-MeC_6H_4
g: R'' = 3-MeC_6H_4
h: R'' = 4-MeC_6H_4
i: R'' = 2-EtC_6H_4
j: R'' = 4-EtC_6H_4
k: R'' = 4-n-BuC_6H_4
l: R'' = 3,4-diMeC_6H_3
m: R'' = 2,6-diMeC_6H_3
n: R'' = 4-$MeOC_6H_4$
o: R'' = 3-$NO_2C_6H_4$
p: R'' = 4-$PhOC_6H_4$
q: R'' = 4-PhC_6H_4
r: R'' = 4-FC_6H_4
R = *R'* = *Me*
s: R'' = Me
R = CD_3, *R'* = *H*
t: R'' = Ph
u: R'' = 4-MeC_6H_4

R = *Et*, *R'* = *H*
v: R'' = Me
w: R'' = Ph
R = *Me*, *R'* = *D*
x: R'' = Ph

1B

R' = *H*
a: R'' = Me
b: R'' = Et
c: R'' = iso-Pr
d: R'' = *t*-Bu
e: R'' = CH_2Ph
f: R'' = CH(Me)Ph
g: R'' = $CH_2C_6H_4$-4-Me
R' = *Me*
h: R'' = Me

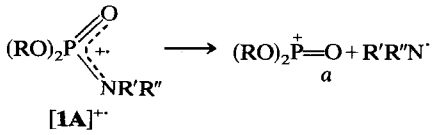
1C

a: X = MeO
b: X = PhO
c: X = Et
d: X = Ph

1D

a: R = Me
b: R = Ph

cleavage of the molecular ion (see Scheme 1, pathway a) is fission yielding a phosphorylium ion (*a*) and an amino radical (Eqn (2)). In agreement with the previous reports,[3,9] we observed formation of dialkylphosphorylium ions (*a*, R = alkyl), in some cases as base



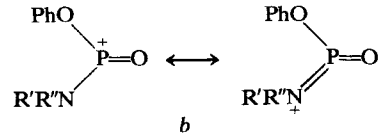
$$[\mathbf{1A}]^{+\cdot} \longrightarrow (RO)_2\overset{+}{P}{=}O \;(a) + R'R''N^{\cdot} \qquad (2)$$

peaks, for all substrates **1A**. For **1Ar** and **1Au** this fragmentation was supported by metastable peaks. However, fragmentation of molecular ions derived from substrates **1B** involved P—OAr, not P—N, bond fission (Eqn (3)); in this respect compounds **1B** behave as aryl phosphates[10] rather than phosphoramidates.

$$[(PhO)_2P(O)NR'R'']^{+\cdot} \xrightarrow{-PhO^{\cdot}} (PhO)(R'R''N)\overset{+}{P}{=}O \qquad (3)$$

$[\mathbf{1B}]^{+\cdot}$ (*b*)

The selectivity in the P—N v. P—O cleavage is certainly a function of the relative stabilities of the possible phosphorylium ions and radical species, reaction (3) being favoured by the resonance stabilization of both the phenoxy radical and the nitrogen-containing phosphorylium ion (*b*). Substrates **1B** also parallel the



b

behaviour of triaryl phosphates[10] by giving rise to the formation of phenol radical ion and phenyl cation.

The other direction possible for pathway a (Scheme 1) is the liberation of a phosphoryl radical and an 'amino cation'. This cleavage, which parallels the behaviour of *N*-substituted carboxamides,[5] was observed

Table 1. Fragmentation data for 1A: *m/z* fragment ion (% relative abundance)

Compound	$[M]^{+\cdot}$	$[(RO)_2P{=}O]^+$	$[M-MeOH]^{+\cdot}$	$[R'R''NH]^{+\cdot}$	$[R'R''N]^+$	$[R'R''NR]^{+\cdot}$	$[R'R''NR-H]^+$	$[M-X]^+$
a	139 (65)	109 (90)	107 (4)	31 (52)	30 (100)	—	—	—
b	153 (6)	109 (60)	—	45 (2)	44 (56)	—	—	138 (100)[a]
c	167 (3)	—	—	—	—	—	—	152 (100)[a]
d	—	109 (12)	—	—	72 (2)	—	—	166 (100)[a]
e	201 (100)	109 (21)	169 (39)	93 (48)	92 (26)	107 (10)	106 (52)	—
f	215 (87)	109 (10)	183 (11)	107 (18)	106 (100)	121 (4)	120 (21)	—
g	215 (100)	109 (12)	183 (45)	107 (55)	106 (50)	121 (7)	120 (36)	—
h	215 (100)	109 (10)	183 (60)	107 (21)	106 (45)	121 (5)	120 (25)	—
i	229 (48)	109 (9)	—	121 (18)	120 (100)	135 (4)	134 (11)	214 (23)[a]
j	229 (43)	109 (6)	—	121 (9)	120 (9)	—	134 (3)	214 (100)[a]
k	257 (32)	109 (9)	—	—	—	—	—	214 (100)[b]
l	229 (100)	109 (11)	197 (50)	121 (11)	120 (21)	135 (2)	134 (10)	—
m	229 (67)	109 (5)	197 (10)	121 (12)	120 (100)	—	134 (4)	—
n	231 (100)	109 (51)	199 (20)	123 (2)	122 (23)	—	—	216 (73)[a]
o	246 (77)	109 (100)	214 (3)	138 (6)	137 (1)	—	—	200 (16)[c]
p	293 (100)	109 (18)	261 (20)	185 (5)	184 (14)	199 (3)	—	216 (9)[d]
q	277 (100)	109 (6)	245 (28)	169 (8)	168 (13)	—	182 (4)	—
r	219 (100)	109 (65)	187 (41)	125 (5)	124 (23)	111 (6)	110 (28)	—
s	153 (34)	109 (48)	—	45 (36)	44 (100)	—	—	—
t	207 (100)	115 (23)	172 (36)	94 (41)	93/92 (32)[e]	110 (9)	109/108 (27)[e]	—
u	221 (100)	115 (13)	186 (65)	108 (17)	107/106 (19)[e]	124 (4)	123/122 (10)[e]	—
v	167 (10)	—	—	31 (72)	30 (100)	59 (6)	—	—
w	229 (67)	137 (6)	—	93 (56)	92 (36)	121 (3)	120 (11)	—
x	202 (100)	109 (24)	169 (28)	94 (44)	93 (31)[f]	108 (9)	107 (43)[f]	—
					92 (10)[g]		106 (18)[g]	

[a] X = $CH_3^{\cdot}$.
[b] X = $C_3H_7^{\cdot}$.
[c] X = $NO_2^{\cdot}$.
[d] X = $Ph^{\cdot}$.
[e] Loss of $H^{\cdot}$ or $D^{\cdot}$; peaks are of similar intensity.
[f] Loss of $H^{\cdot}$.
[g] Loss of $D^{\cdot}$.

Table 2. Fragmentation data for 1B: *m/z* fragment ion (% relative abundance)

Compound	$[M]^{+\cdot}$	$[(PhO)(R'R''N)P{=}O]^+$	$[R'R''NH]^{+\cdot}$	$[R'R''N]^+$	$[R'R''NR]^{+\cdot}$	$[R'R''NR-H]^+$	$[PhOH]^{+\cdot}$	$[Ph]^+$
a	263 (50)	170 (70)	—	30 (28)	107 (21)	106 (18)	(44)	(100)
b	277 (45)	184 (23)	45 (2)	44 (72)	121 (9)	120 (6)	(38)	(100)
c[a]	291 (14)	—	—	—	—	—	(23)	(71)
d[a]	305 (3)	—	—	—	—	—	(14)	(28)
e	339 (57)	245 (8)	107 (21)	106 (100)	183 (7)	182 (43)	(77)	(46)
f[a]	353 (28)	260 (6)	121 (8)	120 (86)	—	—	(29)	(62)
g	353 (28)	260 (4)	121 (10)	120 (100)	197 (3)	196 (16)	(26)	(20)
h	277 (32)	184 (23)	45 (3)	44 (100)	121 (4)	120 (11)	(27)	(46)

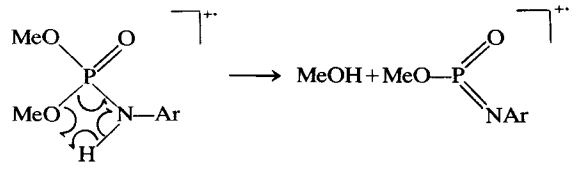
[a] Base peak is $[M-CH_3]^+$.

Table 3. Fragmentation data for 1C: *m/z* fragment ion (% relative abundance)

Compound	$[M]^{+\cdot}$	$[M-CH_2Cl]^+$	$[X_2P{=}O]^+$	$[M-CH_2{=}CHCl]^{+\cdot}$
a	187 (6)	138 (100)	109 (64)	125 (4)
b	311 (35)	262 (100)	—	—
c	183 (4)	134 (100)	105 (47)	—
d	—	230 (53)	201 (100)	217 (3)

in compounds **1Aa**, **b**, **e–r**, **w**, **x** and in **1Bb**, **e–g**, i.e. in secondary substrates which yield upon fragmentation an 'amino cation' $[R''NH]^+$, where R'' = Me, Et, CH_2Ar or Ar, but not where R'' = iso-Pr or *t*-Bu. These amino cations can also be formed via a secondary fragmentation (see below) and, since direct fission $[M]^{+\cdot} \rightarrow [R''NH]^+$ was confirmed by a corresponding metastable peak for only one substrate (**1Am**), this type of fragmentation is probably not the most important mode of P—N bond cleavage.

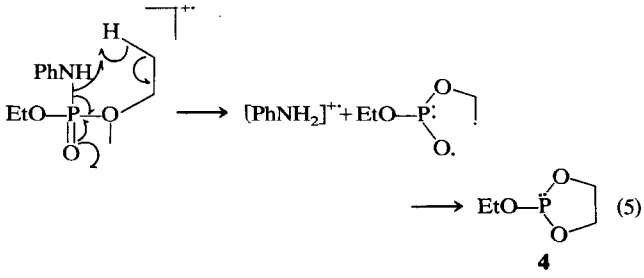
One of the characteristic fragmentations which does not involve P—N fission is elimination of methanol from secondary *N*-aryl dimethylphosphoramidates via a four-membered transition state (Eqn (4)).



(4)

The direct loss of methanol was supported by metastable peaks for most substrates except for compounds (**1Ai–k**), i.e. for those where loss of an alkyl radical from the ring substituent is preferred.[11] The *N*-deuterated-*N*-phenyl derivative (**1Ax**) eliminates a molecule of MeOD and gives the metaphosphate radical ion $[MeOP(O)(NPh)]^{+\cdot}$, thus providing evidence for the mechanism shown in Eqn (4).

The next type of P—N bond cleavage in molecular ions derived from substrates **1** is represented by pathway b (Scheme 1) and is accompanied by hydrogen migration. The resulting formation of the amine radical ion involves expulsion of a molecule of neutral phosphite. Such a fragmentation was proposed in the discussion of the mass spectrum of *N*-benzoyl *O,O*-dimethyl-*O*-ethylphosphorimidate.[12] In the present work, this fragmentation occurred in compounds **1Aa**, **b**, **e–r**, **t–x** and **1Bb**, **e–g** yielding the radical ion of the corresponding amine R'R''NH, which then fragmented according to the usual patterns characteristic for this class of compounds. The intense (relative intensity 57%) peak due to the aniline radical ion from the molecular ion of **1Aw** involves the loss of ethyl ethylene phosphite (**4**), probably via the initially formed diradical species (Eqn (5)).



(5)

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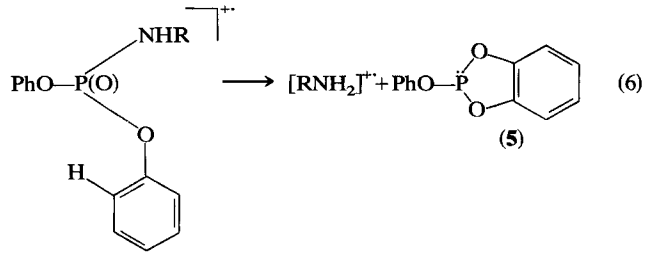
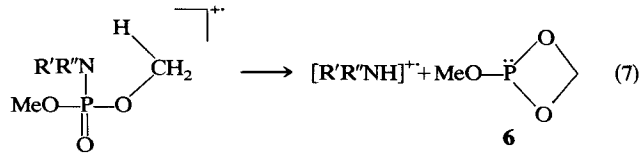
In the phenyl esters **1B** the formation of the amine radical ion (supported in some cases by metastable peaks) indicates expulsion of a well-known[13] type of phosphite—phenyl phenylene phosphite (**5**) (Eqn (6)).



(6)

(**5**)

However, release of the amine radical ion from methyl esters, observed before[12] and supported for **1Am** by a metastable peak, implies the analogous formation of methyl methylene phosphite (**6**) (Eqn (7)).



(7)

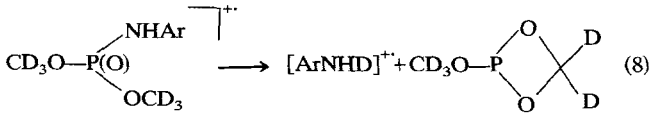
6

Although methylene phosphites of type **6** are not known, it is synthetic difficulties rather than intrinsic

Table 4. Fragmentation data for 1D: *m/z* fragment ion (% relative abundance)

Compound	$[M]^{+\cdot}$	$[M-CH_2Cl]^+$	$[(RO)(MeNH)P{=}O]^+$	$[M-CH_2{=}CHCl]^{+\cdot}$	$[RNHMe]^{+\cdot}$	$[RNMe]^+$
a	186 (4)	137 (100)	108 (94)	124 (5)	—	—
b	248 (34)	199 (94)	170 (32)	186 (10)	107 (65)	106 (19)

instability of such systems that make them inaccessible. When the mass spectra of the two hexadeuterated substrates **1At** and **1Au** were recorded, the corresponding monodeuterated amine radical ions (*m/z* 94 and 108; relative intensity 41 and 17%, respectively) were observed (Eqn (8)).



(8)

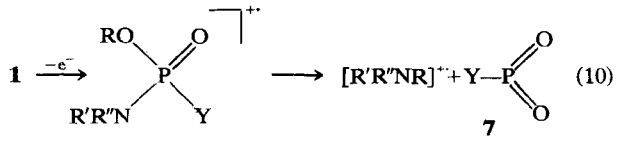
1A t: Ar = Ph
u: Ar = 4-MeC_6H_4

In all cases the amine radical ion $[R'R''NH]^{+\cdot}$ is accompanied by a $[M-1]^+$ peak (often of greater intensity), formed by loss of a hydrogen atom during secondary fragmentation which is typical for various types of amines (Eqn (9)).[14]

$$[R'R''NH]^{+\cdot} \xrightarrow{-H^{\cdot}} [R'R''N]^+ \quad (9)$$

Thus, the fragment $[R'R''N]^+$ can originate from simple cleavage of the P—N bond of $[\mathbf{1}]^{+\cdot}$ (Scheme 1, pathway a) or by fragmentation of the $[R'R''NH]^{+\cdot}$ formed by P—N cleavage of $[\mathbf{1}]^{+\cdot}$ accompanied by hydrogen migration (Scheme 1, pathway b).

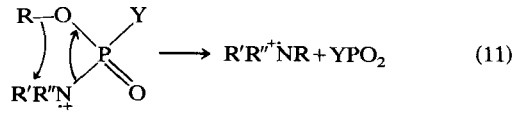
The last, and probably the most interesting, mechanism of the electron impact induced fission of the P—N bonds in compounds **1** is that which involves the migration of the ester group from oxygen to nitrogen (Scheme 1, pathway c). This fragmentation was found for both alkyl (**1A**) and phenyl (**1B**) amido esters and can be represented as shown in Eqn (10).



(10)

7

The expulsion of a metaphosphate species (**7**) in this migration parallels the release of CO_2 observed in the mass spectra of carbonates[6] and some carbamates.[7] However, while the release of a thermodynamically stable molecule of CO_2 can provide the driving force for the migration observed for carbonates and carbamates, Eqn (10) illustrates the gas phase formation of the very unstable metaphosphate structure.† Nevertheless, fragmentation represented by Eqn (10) seems to be of a general nature and has been observed for substrates **1Aa**, **e–i**, **l**, **p–r**, **t–x** (metastable peaks observed for **1Ae**, **t**, **u**, **x**) and **1Ba**, **b**, **e**, **g** and **1Db** (metastable peaks observed for **1Bb**, **e** and **1Db**). The spectrum of substrate **1Db** was also recorded at 11 eV, i.e. just above the ionization potential characteristic of carboxylic amides (*c.* 10 eV[16]). Under these conditions, the major fragments observed result from the loss of chloromethyl radical (*m/z* 199, base peak), loss of phenoxy radical (*m/z* 155, relative intensity 14%), and expulsion of the metaphosphate species, $ClCH_2CH_2NH—PO_2$, accompanied by phenyl migration from oxygen to nitrogen $[PhNHMe]^{+\cdot}$ (*m/z* 107, relative intensity 72%). It seems, therefore, that reaction (10) requires the development of the radical cation centre at nitrogen and involves O → N migration similar to other migrations to the electron-deficient nitrogen atom (Eqn (11)).



(11)

The P—N cleavage accompanied by O → N migration, although found for 20 amido esters **1**, was not observed for the remaining 16, theoretically capable of transferring the ester group R to the departing nitrogen. The main reason for the absence of the fragmentation according to pathway c (Scheme 1) is some other preferred collapse of the molecular ion, which occurs faster than reaction (10). One of the major reactions of this type is the loss of alkyl radicals from those substrates which are substituted by an alkyl group higher than methyl, either at nitrogen (**1Ab**, **c**, **d**; **1Bc**, **d**, **f**) or at the *N*-phenyl ring (**1Aj**, **k**). Other specific fragmentations likely to be responsible for quenching the formation of the *N*-methylated amine radical ion are the loss of $NO_2^{\cdot}$ (**1Ao**), $Me^{\cdot}$ (**1An**, *p*-MeO group) or $CH_2Cl^{\cdot}$ (**1Ca**, **b**; **1Da**). However, in three cases the failure to observe products of reaction (10) sheds some light on the structural requirements of this rearrangement. Both tertiary phosphoramidates (**1As** and **1Bh**) do not produce upon fragmentation the molecular ions of the corresponding tertiary amines (trimethyl and phenyldimethyl, respectively). We attribute this result to steric effects of the *two* *N*-methyl groups, hindering the approach of a migrating fragment in the transition state. This conclusion was confirmed by comparison of the mass spectra of the two isomeric *N*-(dimethylphenyl)phosphoramidates (**1Al** and **1Am**). The 3,4-dimethyl derivative (**1Al**) yields in its spectrum two peaks (*m/z* 135 and 134, total relative intensity 19%) resulting from P—N cleavage accompanied by O → N methyl migration, followed by the loss of hydrogen from the amine radical ion formed (Eqn (12)).

$$\mathbf{1Al} \xrightarrow{-e^-} [(MeO)_2P(O)—NH—C_6H_3\text{-}3,4\text{-}Me_2]^{+\cdot} \longrightarrow$$

$$MeO—PO_2 + [3,4\text{-}Me_2C_6H_3NHMe]^{+\cdot} \xrightarrow{-H^{\cdot}}$$

m/z 135

$$[3.4\text{-}Me_2C_6H_2NHCH_2]^+ \quad (12)$$

m/z 134

The fragment of *m/z* 135 is absent in the spectrum of the 2,6-dimethyl derivative (**1Am**); the *m/z* 134 ion can be observed, but occurs with very low intensity (≤5%). Since any electronic effects operating in substrates **1Al** and **1Am** are to a first approximation the same, the observed difference in fragmentation behaviour must stem from the steric hindrance of the two *ortho* methyl groups to the migration of another methyl group from the oxygen atom. Kinetic measurements of the hydrolysis of *N*-aryl-substituted dimethylphosphoramidates, $(MeO)_2P(O)NHAr$,

† It has been recently shown[15] that the elimination of ethanol from diethylphosphate to produce ethyl metaphosphate ($(EtO)_2PO_2H \rightleftharpoons EtO—PO_2 + EtOH$) is characterized by a free energy $\Delta G^0 = c.\ 30$ kJ, and an equilibrium constant, $K = c.\ 10^{-20}$.

showed[1] that the relative reactivities of the 3,4-dimethyl-substituted (Ar = C_6H_3-3,4-Me_2), unsubstituted (Ar = Ph) and 2,6-dimethyl-substituted (Ar = C_6H_3-2,6-Me_2) compounds are 1.63, 1.00 and 0.26, respectively. The low reactivity of the *ortho*-disubstituted substrate was also explained in terms of steric hindrance to the approach of water to the crowded amide function.

Equation (11) implies the migration of group R with its bonding electrons to the electrophilic nitrogen. This mechanism could be tested by examining the electronic effects of substituents on the rearrangement. For *N*-aryl dimethylphosphoramidates the fragmentation involves, according to Eqn (11), the migration of the electron-rich methyl group to the nitrogen, whose electrophilicity should be modified by polar effects of ring substituents. Following the elegant approach applied by Djerassi to rearrangements in organic carbonates,[6] we have developed the linear free energy relationship for the fragmentation shown in Eqn (13).

$$\underset{M}{[(MeO)_2P(O)NHAr]^{+\cdot}} \xrightarrow{-MeOPO_2} \underset{F^1}{[ArNHMe]^{+\cdot}} \xrightarrow{-H^{\cdot}} \underset{F^2}{[ArNCH_3]^+} \quad (13)$$

Since the loss of hydrogen from radical ions F^1 to give ions F^2 is a very facile reaction, the abundance of both, F^1 and F^2, fragments was taken as a measure of the efficiency of reaction (10). A function $Z = \sum F/M$ (where F is the sum of the abundance of ions F^1 and F^2 and M is the abundance of the molecular ion) was calculated. In order to minimize the effect of competitive fragmentations on the value of Z, this approach was applied to selected substrates, such as the unsubstituted (**1Ae**, Ar = Ph), the ring methyl (**1Ag**, Ar = C_6H_4-3-Me; **1Ah**, Ar = C_6H_4-4-Me; **1Al**, Ar = C_6H_3-3,4-Me_2) and the fluoro (**1Ar**, Ar = C_6H_4-4-F) derivatives. For these closely related, sterically unhindered substrates any competing fragmentations (loss of MeOH, simple P—N cleavage, etc.) should occur to a similar extent and the value of Z should be directly related to the degree of electron release by ring substituents to the positively charged nitrogen atom. Other ring-substituted phosphoric anilides could not be included in the calculation of Z because of predominant fragmentations involving ring substituents (higher alkyl groups, methoxy and phenoxy groups, nitro group). Estimated values of Z were then correlated with substituent constants σ^+ according to the usual linear free energy relationship (Eqn (14)).

$$\log Z_x = \rho\sigma_x^+ \quad (14)$$

Results for Eqn (14) are presented in Table 5 and illustrated in Fig. 1.

Although the linearity of the plot is rather poor ($r = 0.895$),† the trend is obvious and indicates that, in agreement with the proposed mechanism, electron-releasing substituents inhibit migration to the nitrogen. The reaction constant $\rho = 1.4$ obtained for compounds **1** is higher than that ($\rho = 0.67$) reported by Djerassi[6] for rearrangement in organic carbonates. The greater sensitivity observed for the phosphoramidate structures results most probably from greater localization of the electron deficiency at the nitrogen atom in the $P(O)N^{+\cdot}$ function relative to the carbonate system.

† A similar correlation obtained by Djerassi[6] also showed considerable scatter with standard deviation of the slope of 27%.

Table 5. *z* Values (Eqn 14) for the fragmentation of *N*-aryl dimethylphosphoramidates

Compound	Ring substituent X in Ar	σ_X[a]	Z_X[b]	No. of scans
1Ae	H	0.00	0.62±0.08	12
1Ag	3-Me	−0.07	0.42±0.07	8
1Ar	4-F	−0.07	0.34±0.04	8
1Ah	4-Me	−0.31	0.29±0.04	9
1Al	3,4-Me_2	−0.38	0.12±0.01	10

[a] Taken from Ref. 17.
[b] Average from number of scans given.

In all cases, the secondary amine radical ion, $[R'R''NH]^{+\cdot}$, produced by pathway b or c (Scheme 1), was accompanied by a strong $[M-1]^+$ peak, resulting from loss of a hydrogen atom (Eqn (9)). We were interested in the mechanism of this fragmentation and investigated it for *N*-methylaniline (8a) and related compounds. For the radical ion of **8** the most obvious way of losing hydrogen is α-cleavage,[11] yielding the stable imminium ion (*c*) (Eqn (15)).

$$\underset{\mathbf{8}}{Ph{-}\overset{+\cdot}{N}H{-}CH_2} \longrightarrow H^{\cdot} + \underset{c}{Ph{-}\overset{+}{N}H{=}CH_2} \quad (15)$$

Reaction (15) can be easily verified by labelling experiments, and we recorded the mass spectra of *N*-methylaniline (**8a**), its *N*-deuterated (**8b**) and *N*-trideuteromethyl (**8c**) derivatives, as well as that of *N*,*N*-dimethylaniline **9**. In each of these substrates homolytic cleavage of the molecular ion can involve either the N—X or C—Y bond, giving rise to one of two ions (Scheme 2).

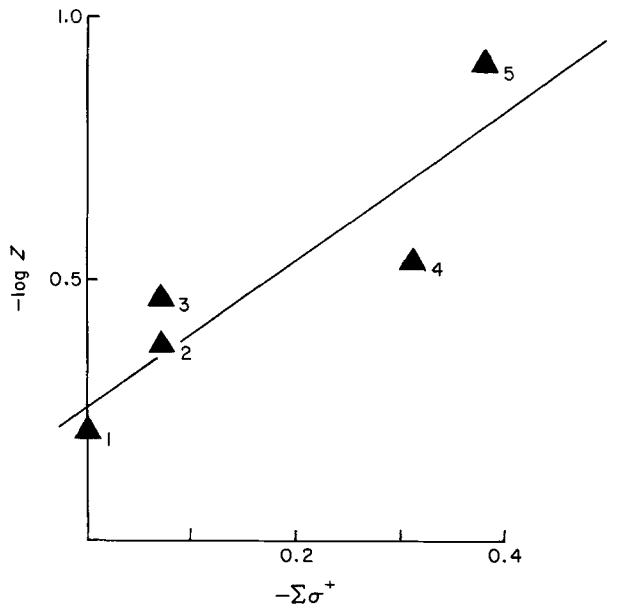
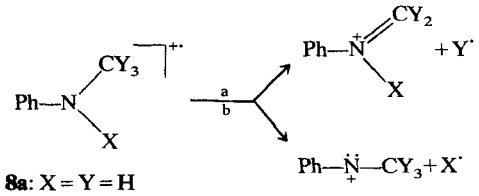


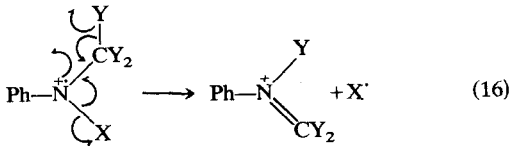
Figure 1. Linear free energy relationship (Eqn (14)). 1: **1Ae**; 2: **1Ag**; 3: **1Ar**; 4: **1Ah**; 5: **1Al**.



8a: X = Y = H
8b: X = D, Y = H
8c: X = H, Y = D
9: X = CH_3, Y = H

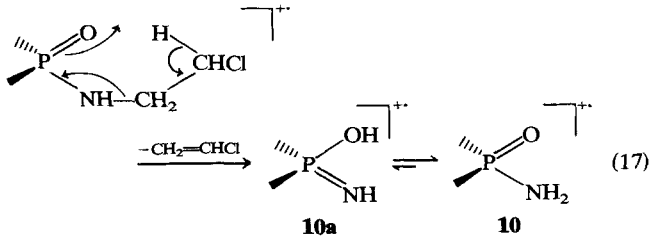
Scheme 2

The mass spectrum of the reference substrate (**8a**) recorded over the temperature range 185–220 °C shows the $[M-1]^+$ fragment (*m/z* 106) as a base peak, together with the molecular ion (*m/z* 107; relative intensity *c.* 80%). Interestingly enough, for the molecular ion derived from **8b**, loss of D· (*m/z* 106, base peak) is slightly preferred over loss of H· (*m/z* 107); after correcting for statistical factors the intensity ratio of fragments *m/z* 106 to *m/z* 107 is *c.* 3.6:1. When the location of atoms H and D is reversed (substrate **8c**), loss of D· and H· occurs to approximately the same extent. Although the $[M-2]^+$ peak (*m/z* 108) is formed from **8c** as a base peak, after statistical correction its intensity relative to the $[M-1]^+$ peak (loss of H·) is 0.9:1. Similarly, in the spectrum of *N,N*-dimethylaniline (**9**), recorded over the temperature range 180–220 °C, ions *m/z* 120 (loss of H·) and *m/z* 106 (loss of Me·) are most abundant and are of almost the same intensity. In conclusion, we found that the two fragmentations indicated in Scheme 2 (loss of Y· or X·) occur with very low intramolecular selectivity. Since the stabilities of the two ions (imminium v. nitrenium) formed must be different, we postulate that the pathway b in fact involves rearrangement yielding another imminium ion (Eqn (16)).



(16)

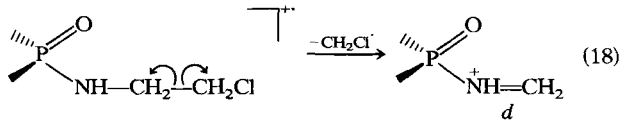
Six substrates (**1C** and **1D**) of the series investigated contain the *N*-(β-chloroethyl) group, which is responsible for the cytostatic alkylating activity of phosphoramidate mustards.[18] Since the mass spectrometry of this class of compounds is virtually non-existent, we were particularly interested in any specific fragmentations resulting from the presence of the β-chloroethyl function. One of the characteristic fragmentations, observed for **1Ca, d** and **1Da, b**, is the extension of the McLafferty rearrangement, involving the elimination of vinyl chloride and formation of the primary phosphoramidate (**10**), probably via its tautomeric form (**10a**) (Eqn (17)).



(17)

10a **10**

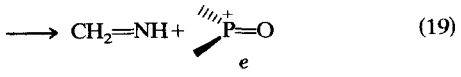
However, the most common fragmentation, observed for all six substrates (and supported by metastable peaks for **1Ca, b, c**), is the C_α—C_β bond cleavage (Eqn (18)).



(18)

d

The highly resonance-stabilized ion *d* is one of the major peaks in the spectra and most secondary fragmentations originate from this ion. One of the interesting fragmentations of *d*, which was observed and supported by a metastable peak for all substrates except **1Cb** (where the loss of PhO· is a competing fragmentation) is loss of the methyleneimine molecule (Eqn. (19)). Thus, in this class of compounds the



(19)

e

phosphorylium ion (*e*) is not formed by direct P—N bond cleavage of the molecular ion, but after initial C—C bond fission of the β-chloroethyl group.

EXPERIMENTAL

Methanol-d_4 99.5% D Min Wilmad glass Co. was used as supplied. Melting points and boiling points are uncorrected and Merck Kieselgel was used for thin layer and column chromatography. ^{1}H NMR spectra were determined on a Varian XL 100 spectrometer using tetramethylsilane as an internal standard. All solvents and reagents were AnalaR grade and were purified before use by conventional methods.

Substrates

Most of the substrates were prepared according to the procedures reported before: **1AA, v**;[3] **1Ae–m**;[1] **1An–r**;[19] **1Ca** and **1Da, b**;[20] **1Ad** and **1Bc–g**;[21] **1As**[22] and **1Aw**.[23]

1Ab, c were prepared from dimethylphosphorochloridate and two equivalents of the corresponding amine in ether. **1Ab**: 78%, b.p. 88 °C/0.5 mm. (Found: C, 31.2; H, 7.75; N, 9.0. $C_4H_{12}NO_3P$ requires C, 31.37; H, 7.90; N, 9.15%.) **1Ac**: 56%, m.p. 36–38 °C. (Found: C, 35.75; H, 8.5; N, 8.3. $C_5H_{14}NO_3P$ requires C, 35.93; H, 8.44; N, 8.38%.)

1At, u were prepared from dimethyl-d_6 phosphorochloridate (synthesized from PCl_3 and methanol-d_4 according to Ref. 24; 78%, b.p. 34 °C/0.5 mm) and the corresponding amine.[1] **1At**: 54%, m.p. 85–87 °C. (Found: C, 45.95; H+D, 8.93; N, 7.0. $C_8H_6D_6NO_3P$ requires C, 46.39; H+D, 8.72; N, 6.76%.) **1Au**: 55%, m.p. 114–115 °C. (Found: C, 48.9; H+D, 9.36; N, 6.4. $C_9H_8D_6NO_3P$ requires C, 48.88; H+D, 9.07; N, 6.34%.)

1Ax was prepared by dissolving **1Ae** in the excess of methanol-d_4 and this solution was used for the mass spectral determination.

1Ba, b were prepared from diphenyl phosphorochloridate and an excess of the corresponding amine in

ether. **1Ba**: 80%, m.p. 93–94 °C. (Found: C, 59.15; H, 5.3; N, 5.3. $C_{13}H_{14}NO_3P$ requires C, 59.32; H, 5.36; N, 5.32%.) **1Bb**: 91%, m.p. 49–50 °C. (Found: C, 60.85; H, 5.9; N, 5.15. $C_{14}H_{16}NO_3P$ requires C, 60.65; H, 5.82; N, 5.05%.)

1Bh was prepared by *N*-methylation of **1Ba**: A solution of 0.009 mole of **1Ba** and 0.015 mole of MeI in THF was added to a stirred suspension of NaH in THF. The mixture was stirred at room temp. for 2 hours, filtered and the solvent evaporated in vacuum. The residue was dissolved in chloroform, filtered and the solvent evaporated under reduced pressure. The remaining oil was purified by column chromatography (silica gel, chloroform/acetone, 95:5). Yield 35%. (Found: C, 60.1; H, 5.75; N, 4.75. $C_{14}H_{16}NO_3P$ requires C, 60.65; H, 5.82; N, 5.05%.)

1Cb: Trimethylamine (0.144 mole) was added to a refluxing mixture of diphenylphosphorochloridate (0.06 mole) and β-chloromethylammonium chloride (0.072 mole) in 250 ml of ether. The mixture was refluxed for 5 hours, then stirred at room temperature overnight. After filtration and evaporation of the solvent a pale yellow oil, which crystallized on standing, was obtained. Yield 96%, m.p. 34–37 °C. (Found: C, 53.85; H, 4.95; N, 4.7. $C_{14}H_{15}ClNO_3P$ requires C, 53.94; H, 4.85; N, 4.47%.)

1Cc was prepared from diethylphosphinic chloride in the manner analogous to that for **1Cb**. The crude product was purified by column chromatography (silica gel, acetone). Yield 31%. (Found: C, 39.55; H, 8.15; N, 6.9. $C_6H_{15}ClNOP$ requires C, 39.24; H, 8.23; N, 7.63%.)

1Cd was prepared and purified as above. Colourless waxy solid; 31%. (Found: C, 60.3; H, 5.45; N, 4.5. $C_{14}H_{15}ClNOP$ requires C, 60.12; H, 5.40; N, 5.01%).

8c: Equimolar amounts of aniline and iodomethane-d_3 (Merck) were stirred together at room temp. for 2 hours and then refluxed for a further 2 hours. After filtration the liquid product was separated by preparative TLC (silica gel, petroleum ether/ethyl acetate, 9:1). A fraction which showed a peak at *m/z* 110 (molecular ion) in the mass spectrum was identified as **8c**.

Mass spectra

Mass spectra were recorded on a VG Micromass 16F spectrometer operating at 70 eV and an ion source temperature between 180 and 220 °C. A direct probe introduction system operating at ambient temperature was used. Samples of compounds were chromatographically (TLC) homogeneous and all gave satisfactory ^1H NMR spectra.

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