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Anionic rearrangement of deprotonated cyproconazole pesticide in the gas phase

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Abstract

Analysis of the deprotonated cyproconazole pesticide prepared in the gas phase shows that under NICI (negative ion chemical ionization) NH_3 and/or ND_3 chemical ionization conditions, these species are produced by a regioselective proton abstraction pathway. This deprotonation orientation is evidenced from the low energy collision dissociations of $[M - H]^-$ which gives diagnostic product ions that are characteristic of the location of the negative charge. The relative abundance of fragment ions has been investigated using the energy resolved mass spectrometry breakdown. It is demonstrated that these activated dissociations are due to various competitive pathways. Furthermore, the molecular anion isomerization into an ion–dipole complex (by means of a charge-promoted cleavage) takes place prior to direct fragmentations. This isomerization process reflects the close acidity of the neutral partner and competes with other more conventional pathways. The evolution of the relative abundance of both the complementary ions arise from the ion–dipole complex and displayed a complementary dependence trend in the recorded collision-induced dissociation spectra independently of other daughter ions. This shows a competitive formation of the complex ion–dipole *versus* other described fragmentation pathways. The use of high resolution measurements as well as the ND_3 gas phase labeling allowed us to evidence the proposed decomposition mechanisms. (Int J Mass Spectrom 199 (2000) 253–266) © 2000 Elsevier Science B.V.

Keywords: Negative ion chemical ionization; Pesticides; Ion–dipole complex; Ion/molecule reaction

1. Introduction

Fungicides are an important class of compounds used in the phytosanitary field for viticulture and cereal farming against rust, mildew, etc. Tandem mass spectrometry (MS/MS) can be an efficient analytical method to characterize these compounds. This approach requires the production of molecular $M^{+\cdot}$ ions in high yields and when these molecular species show weak abundance, chemical ionization might allow efficient generation of protonated MH^+ molecules (or other quasimolecular species) without extensive fragmentation. Studies of different compounds such as pesticides and warfare agents (e.g. phosphorous compounds) [1–5] as well as nitrogen containing compounds like triazines [6,7] have been done by MS/MS to determine the ion structures. Furthermore, it provides a specific screening strategy for detecting

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Dedicated to Henri Eduoard Audier on the occasion of his 60th birthday.

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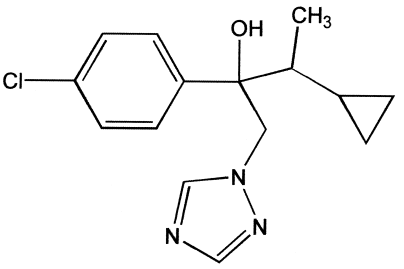
this class of compounds by various collision-induced dissociation (CID) scanning modes. Especially, fixed product ion scan and fixed neutral loss spectra can be advantageously used. However, specificity as well as sensitivity may be seriously reduced, under low energy collision conditions when precursor ions give rise to the formation of a large number of daughter ions. The reason for such behavior can generally be attributed to the presence of many functional sites that are competitively protonated and consequently several isomeric $[MH]^+$ ions coexist, which explains the superimposition of various charge-promoted dissociations patterns [8–12]. Hence two diagnostic ions are buried in the complex CID spectra and specificity of the method is lowered. However, this disadvantage can be overcome if proton migration takes place to yield one specific $[MH^+]$ regioisomer prior to decompositions [13]. In fact, for this class of compounds deprotonation into $[M - H]^-$ species generated under NICI conditions could be an alternative approach since the number of acidic sites is generally lower than that of the basic and nucleophilic groups [14]. Then, only one or two regioisomeric quasimolecular species should be generated. Isomer differentiation from CID $[M - H]^-$ spectra can be possible since proton migration from one site to the charged one occurs more rarely than from positive ions [15]. However in certain cases, molecular isomerization into particular species prior to cleavage can avoid isomeric distinction, particularly stereoisomers. Such isomerization processes can imply the formation of even-electron ion–dipole complexes [15–19] which are often proton-bounded anionic species as represented in

$$[A_1\text{–}A_2]^- \rightarrow [A_1^-, A_2] \Leftrightarrow [A_1^- \cdots H^+ \cdots (A_2\text{–}H)^-] \quad (1)$$

This frequently occurs in protonated molecules [20–22] and in odd-electron $M^{+\cdot}$ species [21,23,24] and their behavior has been well established. Even-electron $A_1A_2^-$ species generated under negative chemical ionization may yield ion–dipole complexes such as $[A_1^- \cdots H^+ \cdots (A_2\text{–}H)^-]$ which competitively dissociate under collision conditions to give rise to the formation of a complementary pair of daughter ions $\{A_1^-$ and $[A_2\text{–}H]^-$ anions from Eq. (1)$\}$. Their relative abundance depends upon the relative acidities of the corresponding A_1H and A_2 neutrals which must be sufficiently similar to allow the formation of a long-lived ion–dipole complex [19]. Earlier, ion–dipole complex formation has been also assumed to occur prior to the decomposition of protonated triazines [16] and as well as from deprotonated molecules from sulfonyl urea [25] in NICI to rationalize the product ions in CID spectra. So far, there is no direct evidence for the formation of such complexes. Particularly, the acidities of neutrals that constitute the proton-bounded dimer are not found in the literature. However, formation of such a complex could be evidenced by energy-resolved mass spectrometry (ERMS) as shown by Kenttämaa and Cooks [3] and Young and Harrison [19].



Scheme 1. Cyproconazole structure.

In the present work, mass spectrometry analysis and ion chemistry of cyproconazole, a substituted triazole, are investigated (see Scheme 1). EI/MS of such hydroxy triazole derivatives show intensive fragmentations which may be very important for rapid identification by means of EI/MS libraries. In environmental applications, EI/MS detection efficiency decreases with the increase of small fingerprint ions which are normally buried in the matrix response. Under PICI (positive ion chemical ionization) conditions, triazole compounds, especially cyproconazole, gives abundant quasimolecular species and under low collision energy conditions gives numerous daughter ions (due to the various possible basic sites) leading to a poor specificity/sensitivity ratio for their detection. But, under NICI conditions quasimolecular species (e.g. $[M - H]^-$) are detected with sufficient sensitiv-

Table 1
Main ions displayed in the NICI resumed mass spectra[a] (with relative ion abundances corrected for natural ^{13}C isotope presence) of the cyproconazole by using ammonia (a) and main peak shifting with labeled ammonia (ND_3) and (b) as a reagent

(a) *m/z*	35	37	68	82	221	222	223	225	272	274	290	291	292	293	326	328	330	359	360	361
Relative intensity	10	3	9	20	25	4	9	...	15	5	55	100	33	35	61	45	5	15	4	5
(b) *m/z*	35	37	68	82	221	222	223	225	272	274	290	291	292	293	294	327	329	331	360	362
Relative intensity	10	3	9	20	7	10	20	5	10	3	56	25	100	20	32	60	45	4	15	4

[a] Ion abundance's relative to the base peak noted as 100%.

ity. Here, decompositions of the deprotonated cyproconazole, prepared under NICI conditions with NH_3/NH_2^- system, have been investigated in order to explore the different paths of decompositions. Particularly, during its dissociation it appears that complementary *m/z* product ions under CID conditions could be explained by classical direct or/and rearrangement processes. Among these cleavages, certain fragmentation may proceed by way of a quasimolecular isomerization into an ion–dipole complex. The formations of such species are investigated in order to explain the dissociation of the diagnostic ions.

2. Experimental

Mass spectrometry and tandem mass spectrometry experiments were performed on triple quadrupole mass spectrometer (R30-10 Nermag, France). CI mass spectra were obtained using a direct introduction probe (DCI), where 1 μL of sample solution (0.5 μg μL^{-1} in methanol) was disposed on the heated tungsten filament of the DCI probe which was maintained at 150 °C inside the high pressure source. The source operating conditions were: emission current, 100 μA, repeller voltage, 0 V; source temperature, 100 °C; and NH_3 (or ND_3 purchased from CEA-France) as reagent gas under 1×10^{-4} Torr as pressure measured at the source housing. Cyproconazole was purchased from Promochem (Molsheim, France). In tandem mass spectrometry experiments, CID spectra of deprotonated molecules were performed using argon as collision gas at 4×10^{-5} Torr measured just inside the collision cell housing to yield single-collision conditions. Ion kinetic energy i.e. as E_{lab} was increased from 15 to 85 eV in order to study ERMS breakdowns. The scan rate was 0.4 s for each CID spectrum recorded using EZSCAN-ONYX. Each reported CID spectrum is an average of at least 50 consecutive scans that were selected for optimum signal-to-noise ratio. HRMS measurements were performed on MAT 95Q Finnigan by "peak matching" technique under 8800 as resolution. Tetradecanol and bromotetradecan were used as references for ions at *m/z* 213 deprotonated molecules and *m/z* 79 (Br^-), respectively.

3. Results and discussions

The NICI–NH_3 mass spectrum (Table 1) of cyproconazole (MW 291) shows various quasimolecular species and adduct ions are observed at *m/z* 291, *m/z* 326 and in low abundance at *m/z* 290 and at *m/z* 359. These ions correspond to $M^{-\cdot}$, $[M + Cl]^-$, $[M - H]^-$, and $[M + 68]^-$, respectively. Furthermore, several fragment ions appear at *m/z* 272, *m/z* 221, and *m/z* 68.

3.1. Formation of quasimolecular species and adduct ions

The presence of fragment Cl^- (at *m/z* 35–37) and $C_2N_3H_2^-$ (at *m/z* 68) ions in NICI–NH_3 mass spectra of various chlorinated triazoles (e.g. flusilazole, cyproconazole, tebuconazole, not reported here) can be correlated to the formation of $[M + Cl]^-$ adducts at *m/z* 326–328–330 and the presence of the adduct $[M + 68]^-$ at *m/z* 359–361. If $[M + Cl]^-$ ion is expected, then observation of $[M + 68]^-$ is unusual

Table 2
Relative abundance of the daughter ions of selected $[M + Cl]^-$ and $[M + 68]^-$ ions (i.e. *m/z* 326 and *m/z* 359, respectively) displayed under low energy collision conditions (E_{lab} = 25 eV)

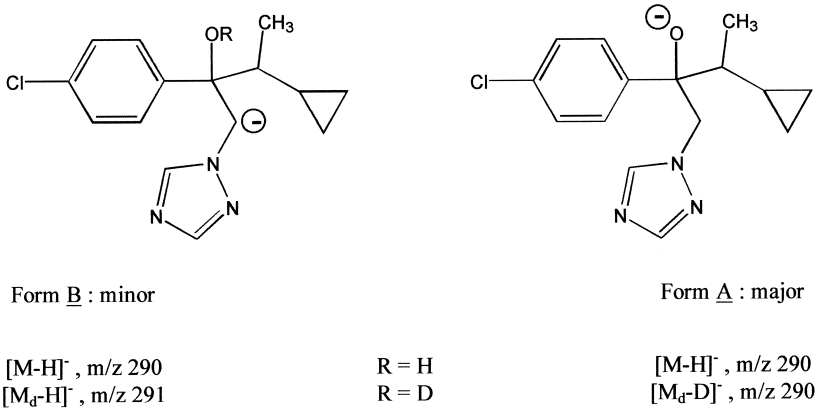
		CID precursor ion spectra[b] (E_{lab} = 25 eV)			
Selected ions[a]	*m/z*	35	68	290	291
$[M_d + Cl]^-$	327	100		0.1 (ε)	...
$[M + Cl]^-$	326	100	...	0.1 (ε)	...
$[M_d + C_2H_2N_3]^-$	360	...	100	11	...
$[M + C_2H_2N_3]^-$	359	...	100	13	...

[a] Ions prepared under NICI/ND_3 or NH_3 conditions.
[b] Abundance related to the base peak of daughter ion (noted at 100%).

[26]. However, its attribution is consistent with the low energy collision spectrum of *m/z* 359 (Table 2) which displays mainly both the complementary ions, i.e.: $[M - H]^-$ at *m/z* 290 and ion at *m/z* 68. It is very likely that the ion at *m/z* 68 is the five membered triazole anion moiety. This assumption is confirmed from the NH_3–NICI mass spectra of several triazole fungicides [27] in which similar adducts that corresponds to the $[M + 68]^-$ and *m/z* 68 ions are observed independent of alkyl side chains.

The odd-electron $M^{-\cdot}$ (*m/z* 291–293) and the even-electron $[M - H]^-$ (*m/z* 290–292) species are competitively produced by resonant electron capture (and/or by charge exchange from Cl^- because neutral chloride is characterized by a relatively high electron affinity value compared to that of the analyte molecule) and by proton transfer to the NH_2^- reagent (or/and to triazole $C_2N_3H_2^-$ anion) and not to Cl^- since neutral HCl is likely to be more acidic than cyproconazole. Actually, thermochemical properties (i.e. electron affinities and gas phase acidities) must be the main factors which orient the deprotonation process in charge exchange reaction assuming that no large change in geometry during the reaction pathway (i.e. absence of intrinsic barrier). Consequently, the presence of chlorine substituant and hydroxyl group must favor the charge exchange and the proton transfer process (form A, Scheme 2). Proton transfer could also take place at the benzylic site of the triazole ring (form B, Scheme 2).

The presence of mobile protons can be evidenced by H/D exchange experiments [28] from neutral/neutral reactions. Indeed, the A and B isomeric forms can be also formed under ND_3/NICI by efficient OH/OD exchange. The isomer A must be characterized by a shift of the ion at *m/z* 290 contrary to that



Form B : minor

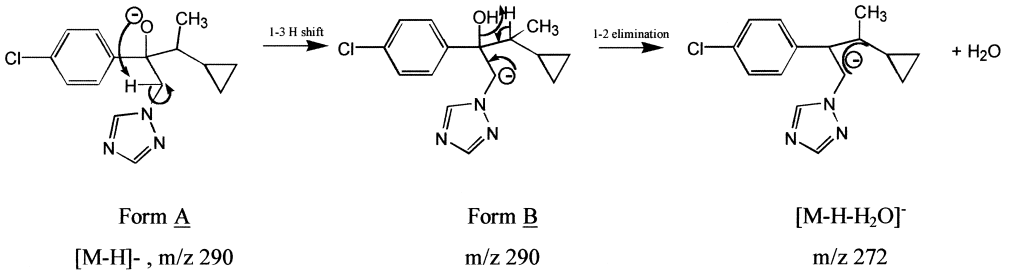
$[M-H]^-$, m/z 290
$[M_d-H]^-$, m/z 291

R = H
R = D

Form A : major

$[M-H]^-$, m/z 290
$[M_d-D]^-$, m/z 290

Scheme 2. Possible isomeric deprotonated A and B molecules.



Scheme 3. Possible loss of water from the $[M - H]^-$ ion via possible A → B isomerization.

occurs for form B from which must be shifted to *m/z* 291 and the labeled $M_d^{-\cdot}$ odd-electron ion be shifted by one thomson to *m/z* 292. In fact, if the latter takes place in the mass spectrum then the labeled $[M_d - H]^-$ (as the B form) is not significantly detected. Consequently, deprotonation induced by NH_2^-/ND_2^- is regioselective and takes place at the hydroxyl site rather than at the benzylic site of the triazole ring which can be considered as less acidic than the hydroxyl site [29]. In addition, the OH/OD total exchange is clearly shown by the labeling incorporation in the adduct ions (i.e. $[M_d + Cl]^-$ and $[M_d + 68]^-$). Note that multiple H/D exchanges at benzylic position are not expected because of the very weak acidity of NH_3 compared to the benzylic site. Through CID investigation of these adducts, it is possible to compare the relative acidity of HCl and $C_2H_3N_3$ neutrals, and further they can be qualitatively related to that of labeled hydroxylic groups. Actually, regioselective deprotonations have been already observed, e.g. for hydroxylic ester compounds [15,17,18] and seem to mainly involve the more acidic site in spite of the large difference in acidity compared to NH_3 reagent (i.e. depending upon the reaction exothermicity). Alternatively, a complete H/D exchange observed under NICI/ND_3 conditions, for the adduct $[M + Cl]^-$ and $[M + 68]^-$ ions is useful to compare the relative acidities of the HCl and $C_2H_3N_3$ neutrals with the hydroxyl site of the substrates under investigation. Indeed, the low energy collision spectra (25 eV) of labeled *m/z* 327 $[M_d + Cl]^-$ and *m/z* 360 $[M_d + 68]^-$ ions (Table 2) are dependent on the order of relative acidity of each reagent.

Results reported in Table 2 indicate that (1) as expected, relative acidity of HCl (1395 kJ mol^{-1}) [30] is higher than the hydroxyl function of cyproconazole (estimated as benzyl alcohol: 1548 kJ mol^{-1}) [30] explaining the very weak $[M - H]^-$ abundance, and (2) the triazole $C_2H_3N_3$ neutral and cyproconazole appear to have closer acidity since abundance of the *m/z* 68 is significant (Table 2). It must be emphasized that the deprotonation process occurs in a regiospecific way at the OH site and not at the benzylic position since the shift to *m/z* 291 was not detected.

3.2. Production of the fragment ions in NICI/NH_3 conditions

As shown in Table 1, the diagnostic ions at *m/z* 68, $[M - H - 69]^-$ at *m/z* 221 and the fragment ions $[M - H - H_2O]^-$ at *m/z* 272 were not extensively produced under high pressure conditions. No deuterium labeling incorporation under NICI/ND_3 conditions was observed in the fragment ions. It appears that from their structure, no position allows the H/D exchanges in the ion source. This indicates that the acidic positions in these ions are either absent or inaccessible to H/D exchanges toward the neutral ND_3 present in the ion source.

3.2.1. Loss of water from $[M–H]^-$ in the ion source

The elimination of H_2O from the $[M - H]^-$ molecular species, requires form B as the common intermediate with intact OH site. This means that the A form (suggested to be a specific produced structure) must isomerize into the form B prior to 1–4 elimination of water induced by the negative charge (Scheme

Table 3
Relative abundances of daughter ions produced under CID (E_{lab} = 60 eV, single collision) conditions for *m/z* 272 and *m/z* 221 fragment ions generated in high pressure ion source under NICI/NH_3

	CID daughter ions (*m/z*)[b]					
Selected parent ions (*m/z*)[a]	26	35	68[c]	111	218	245
272	8.5	12.5	100	5	35	30
221	1	10	100	0.5	...	...

[a] Ions prepared under NICI/NH_3 conditions.
[b] Abundance related to the base peak of daughter ion (noted as 100%).
[c] Confirmed by HRMS ($C_2H_2N_3^-$).

3). Generally, from alkoxide species the mechanism of water loss under CID conditions was not clear [31]. Indeed the multiple H transfers depend upon the ion structure and eventually the stereochemistry [32]. Note that such an elimination is generally unfavored under collision conditions. This can be explained by considering that this process involves a H transfer by means of a skeleton rearrangement having a lower frequency factor than the direct cleavages.

The specific decomposition induced by the collision of the selected $[M - H - H_2O]^-$ (*m/z* 272) ion confirm the structure proposed for it. Indeed, the product ion at *m/z* 68 characteristic of the triazole group is observed along with Cl^- at *m/z* 35, and a less abundant CN^- ion at *m/z* 26 as shown in Table 3. In addition, consecutive loss of HCN (i.e. at *m/z* 245 and at *m/z* 218) is directly produced from $[M - H - H_2O]^-$ which may be due to the opening of the triazole ring as proposed in Scheme 4.

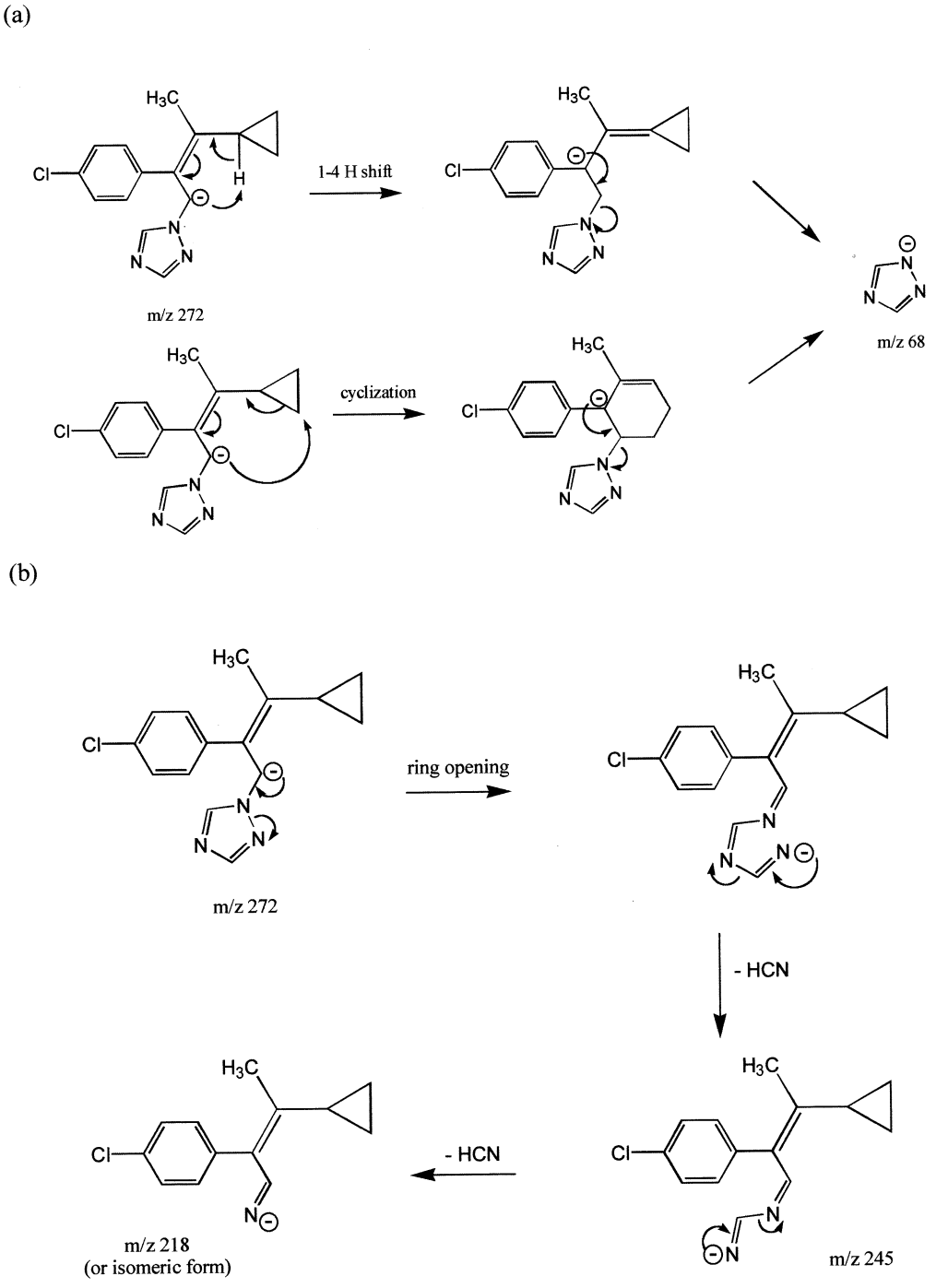
Note that in case of sulfuryl urea herbicides such as metsulfometuron methyl, decomposition of its triazine moiety was not observed. Decomposition orientation favored sulfonylurea cleavage relatively than that of triazine ring. For protonated atrazine [6], decomposition of the heterocyclic skeleton appears to the important cleavage.

3.2.2. Formation of pair of the complementary m/z 221–m/z 68 ions in the ion source

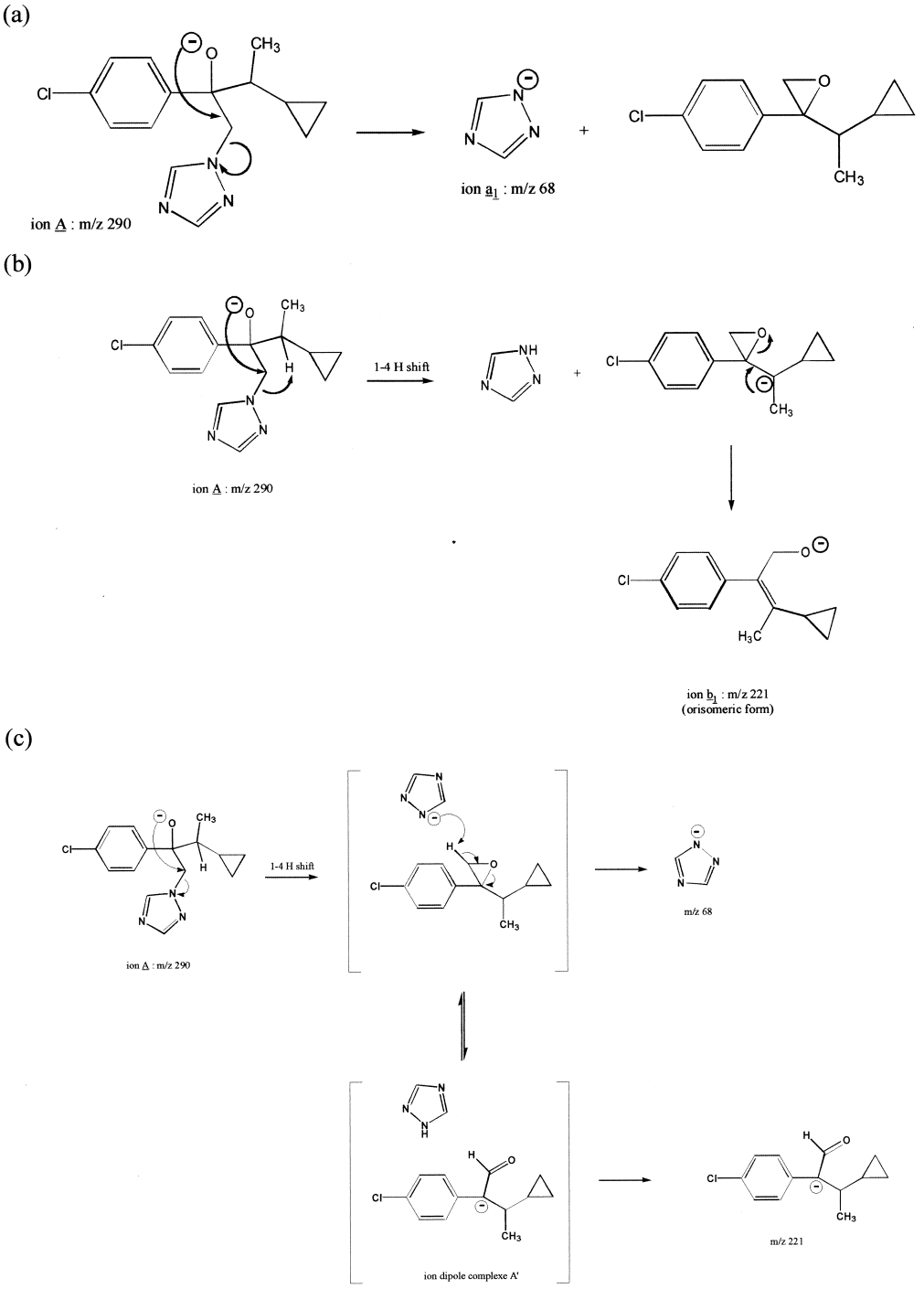
Both the complementary ions (*m/z* 68 and *m/z* 221) could be produced by a competition charge promoted cleavage processes from the A form. This leads either to a direct formation of the $\underline{a_1}$ (*m/z* 68) ion $C_2H_2N_3^-$ [Scheme 5(a)] or by way of the 1,5-H transfer concomitant with the triazole loss yielding $\underline{b_1}$, *m/z* 221 ion $[C_{13}H_{14}OCl]^-$ as proposed in [Scheme 5(b)].

However, it is difficult to rule out the alkoxide promoted cleavage through ion–dipole complex formation [ion A′, *m/z* 290, Scheme 5(c)] [15,17,18] prior to the dissociation. This ion can dissociate competitively through proton transfer isomerization. Such an assumption was made to explain the dissociation of sulfonylurea herbicides [25] and also for larger polyfunctional compounds [15,17]. However, another process could be a priori proposed (Scheme 6) by considering another elemental composition of the *m/z* 221 ion. Indeed, isobaric odd-electron $C_{10}H_8N_3OCl^{-\cdot}$ ion (ion $\underline{b_2}$) as well as $C_{13}H_{14}OCl^-$ (ion $\underline{b_1}$) could be formed in the high pressure NICI source. HRMS measurements allowed us to rule out one elemental composition for *m/z* 221. Indeed, the measured accurate *m/z* value 221.034 84 is related to the *m/z* value 221.035 59 of $C_{10}H_8N_3OCl^{-\cdot}$ (measured at 3.4 ppm) and it strongly differs from the isobaric $C_{13}H_{14}OCl^-$ form (221.073 24 by 173 ppm). Then, odd-electron fragment $\underline{b_2}$ ions must be produced in NICI ion source from dissociation of odd-electron $M^{-\cdot}$ molecular ion rather than $[M - H]^-$.

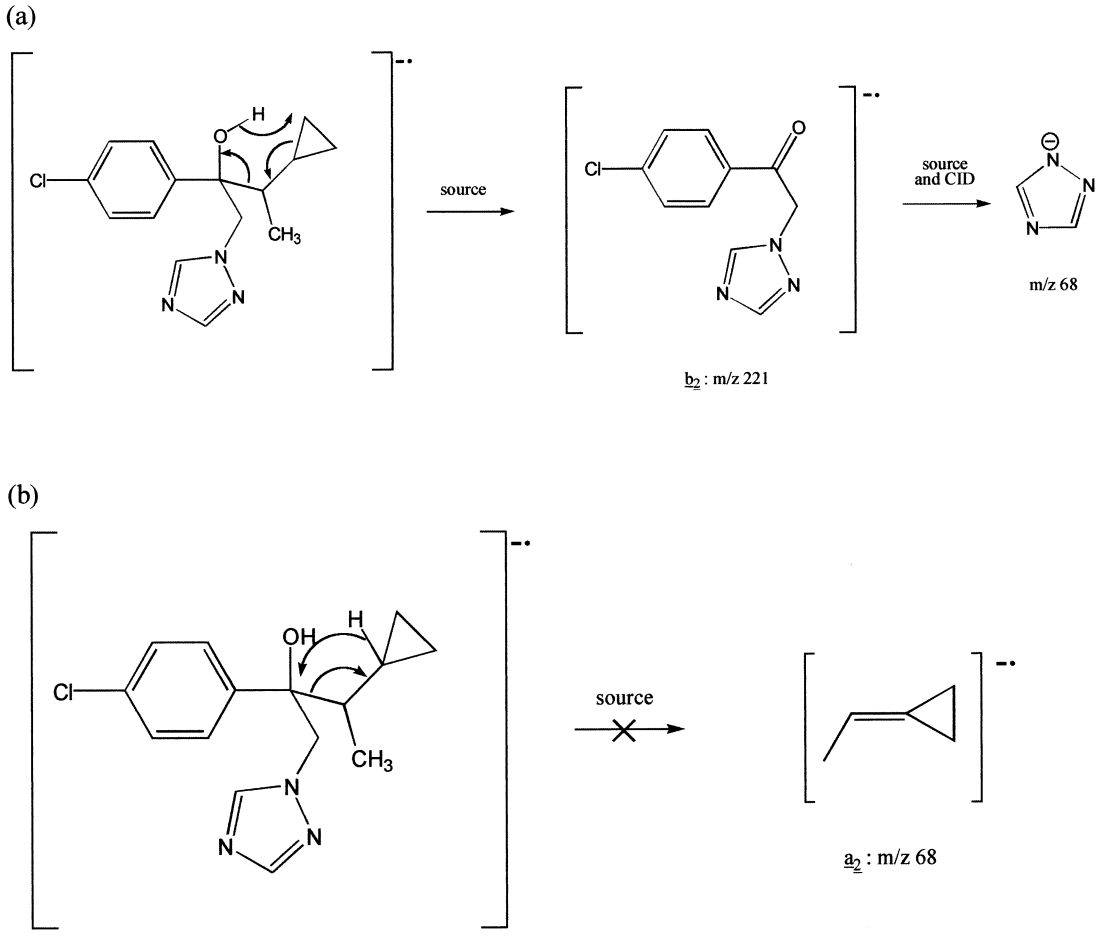
The proposed $\underline{b_2}$ structure [Scheme 6(a)] is consistent with the gas phase labeling experiments because of the partial shift of the *m/z* 221 ion to *m/z* 222 (for 27% of $\underline{b_{2,d1}}$) and *m/z* 223 (for 52% of $\underline{b_{2,d2}}$) under ND_2^-/NICI conditions. Such labeling incorporation in odd-electron species limited to two deuterium can be explained by a possible fast reversible keto-enol process allowing multiple H/D exchanges. Indeed, this behavior from ketone has been shown for carbonyl compounds [33].



Scheme 4. Collisional competitive and consecutive decomposition of the fragment $[M - H - H_2O]^-$ ions produced in the NICI/NH_3 conditions.



Scheme 5. Possible mechanisms of the complementary *m/z* 68 and *m/z* 221 ion formation.



Scheme 6. Formation of the odd-electron *m/z* 221 and *m/z* 68 ions from the odd-electron $M^{-\cdot}$ molecular ion.

To evidence such ion structure, a CID fixed ion spectrum of the selected *m/z* 221 (Table 3) ion displays at *m/z* 68 as a major daughter ion and in less abundance at *m/z* 111, *m/z* 35, and *m/z* 26 due to CN^-. This again confirms the $\underline{b}_2$ type product ion generated in ion source (Scheme 6). It is necessary to emphasize that if the isobaric even electron $C_{13}H_{14}OCl^-$ anion (form $\underline{b}_1$) was produced in the ion source, its collisional dissociations could yield at *m/z* 68, an odd-electron species with $C_5H_8^{-\cdot}$ (form $\underline{a}_2$, Scheme 6) as elemental composition [Scheme 6(b)]. However, such a negative ion may not be formed because its stability is very low due to fast electron detachment. It should be noted that from an ion source, the HRMS experiments ruled out the corresponding elemental structure expected at 68.062 56 (experimental measurements at 2.4 ppm compared to the theoretical value of $C_2H_2N_3^-$ anion as 68.024 87). Finally, under NICI conditions, production of the *m/z* 68 ion as deprotonated triazole is formed either directly [Scheme 6(a)] or by way of the formation of an ion–dipole complex (form A′) Scheme 5(c). The latter is less probable since the presence of a counter ion of *m/z* 68 (i.e. *m/z* 221 as $C_{13}H_{14}OCl^-$ ion, form $\underline{b}_1$) is ruled out by high resolution measurements. However, the *m/z* 68 ion can be produced by consecutive decomposition of $M^{-\cdot}$ by way of the formation of *m/z* 221 [i.e. $C_{10}H_8N_3OCl^{-\cdot}$, form $\underline{b}_2$, Scheme 6(a)]. The *m/z* 221

Table 4
Relative abundances of daughter ion displayed in CID spectra (E_{lab} = 25 eV, single collision conditions) of the selected $[M - H]^-$ (*m/z* 290–292), $M^{-\cdot}$ (*m/z* 291–293), and $M_d^{-\cdot}$ (*m/z* 292–294) parent ions

Selected species[a]		CID daughter ions intensity[b] (E_{lab} = 25 eV)						
	m/z	35	68	82	111	178	207	221
$[M - H]^-$	290	3	100	32	3	9	50	2
$M^{-\cdot}$	291	100	c	c	...	...	(208)[c]	...
$[M_d - D]^-$ [d]	290	2	100	36	2	6	47	2
$M_d^{-\cdot}$ [d]	292	90	e	e	...	...	(209)[e]	1

[a] Ions prepared under NICI/ND_3 or NH_3 conditions.
[b] Abundance related to the base peak of daughter ion (noted at 100%).
[c] Contribution of the ^{13}C natural isotope of $[M - H]^-$ ions (or $[M_d - D]^-$).
[d] From gas phase labeling conditions.
[e] Contribution of the ^{37}Cl natural isotope.

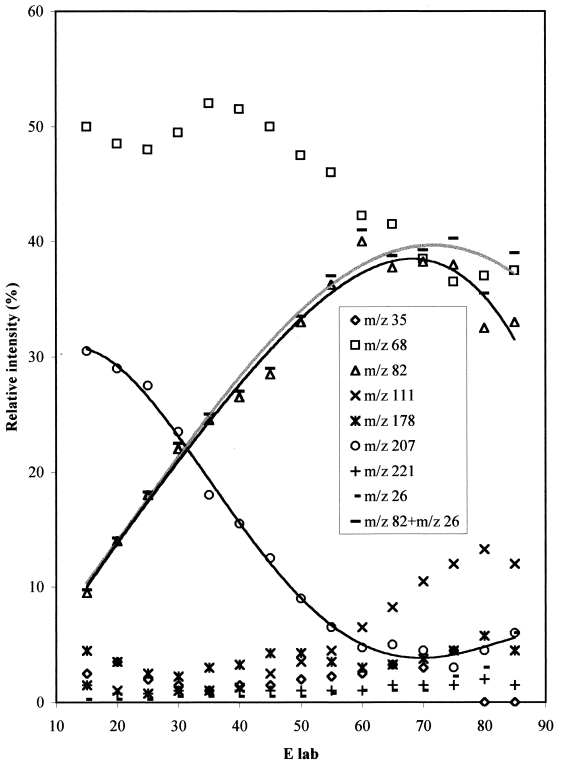
ion is produced as the $\underline{b}_2$form rather than $\underline{b}_1$ form. This excludes the formation of complementary *m/z* 68 and *m/z* 221 ions by means of one common intermediate.

3.3. Orientation of the consecutive decompositions of the long lived $[M - H]^-$ ions under low collision energy conditions

The behavior of the survivor $[M - H]^-$ ions should provide interesting information regarding the different fragmentation pathways occurring from the excited $[M - H]^-$ ions. As shown in the Table 4, CID spectrum of $[M - H]^-$ (*m/z* 290) displays several series of complementary ion pairs such as: *m/z* 68–*m/z* 221, *m/z* 82–*m/z* 207, and *m/z* 111–*m/z* 178.

From these CID spectra, presence of ion pairs at *m/z* 68–221 in triazines [6] and sulfonylurea [25] suggests that a possible isomerization into ion–dipole complex of the $[M - H]^-$ prior to dissociation and it is difficult definitively to rule out such a possibility. In order to verify this hypothesis an ERMS study was carried out. Indeed, a tandem mass spectrometry investigation on the decompositions of molecular ion species proved that when complementary ions are produced, the parent ions could be isomerized into ion–dipole complex prior to decomposition in the ion pair in electron ionization (EI) [21,23,24], in PICI [20–22] and in NICI [15–17,19]. Such a complex formation implies that charge promoted cleavage processes are involved, except in cases where thermal pathways like remote-charge cleavage have been proposed [34,35]. Generally, the process can be accomplished by radical (from odd-electron parent ions), proton, or hydride transfer (from even-electron molecular species) if the complex allows a "slight molecular skeleton reorganization" allowing a low energy reaction pathway, than by direct cleavage. In considering ion–dipole complexes such as proton bounded dimers (i.e. positive $B_1^{\cdots}H^{+\cdots}B_2$ or negative $A_1^{-\cdots}H^{+\cdots}A_2^-$ dimers) composed by neutrals (B_1, B_2, or A_1H and A_2H) that are characterized by similar basicities or acidities which constitute the proton-bounded complex. Such thermochemical properties result in an increase in the lifetime of the complex which allows complete mobility of each partner permitting eventual internal proton transfer.

First, labeling [36–38] experiments, metastable decompositions rationalized by model calculations [39,40] provide some evidences on such molecular isomerisations. Particularly, the ERMS studies were very useful to give decisive proofs concerning the formation of ion–dipole complexes prior to the parent ion dissociation. In particular, relative abundances of fragment ions due to cleavage of complex must be dependent on the kinetic energy (i.e. E_{lab}) of the selected parent ion submitted to low energy collision regime and by increasing the collision energy the direct competitive and consecutive processes are enhanced compared to the ion dipole isomerization. This

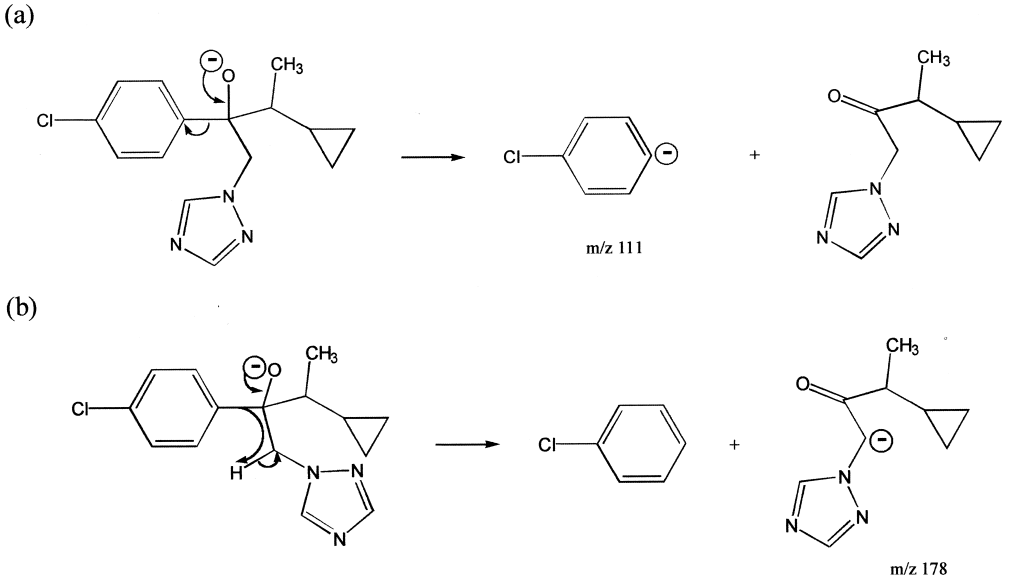


Scheme 7. ERMS of the deprotonated cyproconazole (*m/z* 290) ion.

can be rationalized by considering the low frequency factor of the reaction as well as the low critical energy of the ion–dipole formation [19]. This approach has been applied to study deprotonated cyproconazole. Relative abundances of the daughter ions of $[M - H]^-$ (Table 4) according to the collision energy have been particularly examined to detect which pair of complementary ions could result from direct cleavage or by means of an ion–dipole complex isomerization (Scheme 7).

3.3.1. Direct formation of the m/z 111 and m/z 178 ion pair

Comparison of relative abundances of this ion pair with the collisional energy values show (Scheme 7) different energy dependent dissociation pathways as: (1) the abundance of the fragment ions *m/z* 178 and *m/z* 111 rises with collision energy until 30 eV and (2) at higher collision energy the abundance of *m/z* 178 is remain constant as opposed to ion at *m/z* 111 whose abundance increases. This different behavior suggests that the *m/z* 111 and *m/z* 178 are complementary ions and are generated through direct competitive processes rather than by way of molecular isomerization prior to dissociation (Scheme 8).



Scheme 8. Proposed mechanisms for the formation of the competitive *m/z* 111 and *m/z* 178 ion pairs.

3.3.2. Direct formation of the m/z 68 and m/z 221 ion pair

From ERMS dependences, it appears that both the *m/z* 68 and *m/z* 221 ions abundance (Scheme 7) do not correlate. Such a phenomenon is unexpected from the conclusion arrived by studying NICI mass spectrum (i.e. decompositions directed by high rate constants). Indeed the relative abundances of *m/z* 68 and *m/z* 221 should follow a complementary dependence which is not the case, here. Then, their formation from ion–dipole complex [Scheme 5(c)] cannot be retained under low energy collision conditions.

Formation of the complementary daughter *m/z* 68 and *m/z* 221 from $[M - H]^-$ cannot be compared to those generated from $M^{-\cdot}$. Thus both *m/z* 68 and *m/z* 221 ions generated under collision condition, must enlighten an additional origin for *m/z* 221, here in from $[M - H]^-$. However, from ERMS of the selected $[M - H]^-$, it appears that both the *m/z* 68 and *m/z* 221 ion abundances (Scheme 7) do not correlate. Then, their production from ion–dipole complex [Scheme 5(c)] cannot be retained under low energy collision conditions. Two possibilities can be eventually proposed: the daughter ions are even-electron species [Schemes 5(a) and 5(b)] produced directly from the dissociation of $[M - H]^-$ either by direct cleavage into triazole $\underline{a_1}$ (*m/z* 68) or by 1,4-H proton transfer yielding the *m/z* 221 ylide with opening (or not) of cyclopropane ring.

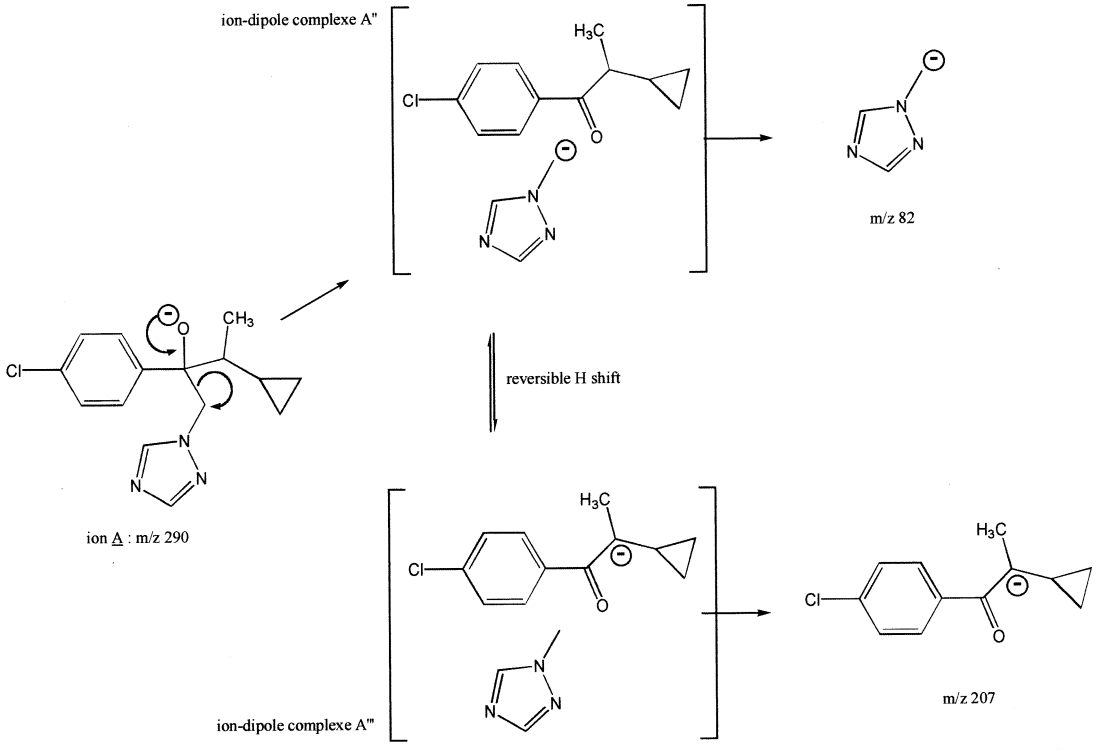
3.3.3. Evidence on the m/z 82–m/z 207 formation via dissociation of an ion–dipole complex

The relative abundance of *m/z* 82 and *m/z* 207 daughter ion pair from $[M - H]^-$ exhibits the same behavior as seen under varying collision energy regimes (Scheme 7). Indeed, with increasing collision energy, the relative abundances of the *m/z* 207 and *m/z* 82 ions evolves, a reverse trend promoted by the alkoxide site in which a benzylic cleavage is produced leading to A″ ion dipole complex which dissociates directly into triazol methylid, *m/z* 82, or isomerized into A‴ form to produce enolate *m/z* 207 ions. Consequently, these complementary ions must very likely be generated by means of the ion–dipole complex prior to dissociation as shown in the following Scheme 9.

Indeed, low energy collision, a tight transition state must be favored, i.e. proton transfer from the enolic position to the anim ylide site must be enhanced leading to methyl-1-triazole neutral loss (i.e. formation of *m/z* 207). This is in contrast with the direct formation of deprotonated methyl triazole which is favored at higher collision energy by direct ion cleavage characterized by a loose transition state. Furthermore, at high collision energy, the decrease of the *m/z* 82 abundance could be attributed to its consecutive dissociation into CN^- (*m/z* 26). Indeed, the complementary {[*m/z* 82] + [*m/z* 26]} abundance correlates well with the *m/z* 207 abundance. This phenomenon described previously, gives evidence on the existence of an ion–dipole complex as an intermediate during or prior to dissociation before entering the collision cell. The stability of such an ion–dipole complex depends upon the similarity of the relative acidity of neutral (i.e. methyl-1-triazole and 4-chlorophenyl-1 propanone-1 cyclopropyl-2) related to both the anions which solvate the proton in the complex. This favors a reversible proton transfer and the tight transition state is not really a limitative step as bottleneck of the reaction pathway.

4. Conclusion

The $[M - H]^-$ species prepared under NICI conditions in the gas phase from cyproconazole pesticide are regioselectively charged at a less number of sites than it is in PICI. This was evidenced by using labeled gas phase ND_3 reagent which allows gas phase neutral/neutral H/D exchange. Actually, this labeling enlightens the presence of only one acidic site. The regioselective location of the negative charge permits us to decrease the number of various product ions under low collision excitation conditions. Consequently, this results in an enhancement of the structural effects for helping investigation of the ion structure. On the other hand, observed orientation of fragmentations of $[M - H]^-$, promoted by a negative charge evidences the charge location on hydroxy



Scheme 9. Putative dissociation of the deprotonated cyproconazole, via an ion–dipole complex, into the *m/z* 82–*m/z* 207 ion pair.

group. Further, from the fragment Cl^- and $C_2H_2N_3^-$ ions, formation of noncovalent adduct $[M + Cl]^-$ and $[M + C_2H_2N_3]^-$ ions may be consistent with a hydrogen bonding at the acidic OH site rather than at the eventual benzylic methylene which is a less acidic group. Alternatively, this assumption was confirmed from the H/D exchange experiments in NICI–ND_3 conditions. Indeed, the latter lead to only one D atom incorporated in the produced adduct ions and not in the deprotonated ion. This behavior reflects that the benzylic CH_2 group is more acidic relative to ND_3 to undergo H/D exchanges from $(M - H)^-$. However, it is less acidic relative to HCl (or DCl) and $C_2H_3N_3$ ($C_2H_2DN_3$) to allow proton mobility from the methylen site. This is shown by the absence of $[M_{d3} - D]^-$.

The relative abundances of fragment ions investigated from the ERMS breakdown provides information on the mechanism of the ion formation that involves various competitive pathways. The origin of the pair of complementary ions was established using these breakdown dependences. Especially, a fragment ion such as deprotonated methyl triazole is competitively produced with an enolate complementary fragment ion through an anion isomerization into an ion–dipole complex (by way of a charge-promoted cleavage) which takes place prior to the direct fragmentations of the intact $[M - H]^-$ ion. With other pairs of ions such an assumption has been ruled out. Furthermore, from the CID spectra of $[M - H]^-$ it is considered that the fragment ion at *m/z* 221 to be an even-electron species. In fact, in the ion source it (*m/z* 221) exists as an odd-electron species, which is formed by the cleavage of the odd-electron $M^{-\cdot}$ as shown by high resolution measurements.

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