We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Trends for Pyrazole Fragmentation Determined by Gas Chromatography Coupled with Mass Spectrometry

Clarissa Piccinin Frizzo, Bruno Luís Hennemann,
Bruna Luisa Kuhn, Keli Maiara Wust, Alisson Vasques Paz,
Marcos Antonio Pinto Martins, Nilo Zanatta
and Hélio Gauze Bonacorso

Abstract

In this chapter, we present a review on pyrazole fragmentation by gas chromatography coupled with mass spectrometry, in order to evaluate the substituent effect on pyrazole fragmentation. Our objective was to present a comprehensive study on the fragmentation pattern of substituted pyrazoles, contribute to the systematization of knowledge, and offer support to researchers in the characterization of pyrazoles via a comprehensive and versatile technique such as gas chromatography coupled with mass spectrometry. The pyrazole fragmentation showed two important processes: (i) expulsion of HCN from $[M]^{+\bullet}$ and $[M–H]^+$ and (ii) the loss of N_2 from $[M–H]^+$. Substituents such as D, Me, Br, Cl, and Ph did not influence these two processes; however, the presence of nitro, acetyl, oxime, diphenyl, or methyl and nitro in the *ortho*-position transforms the two processes into secondary fragmentation or results in their absence in the fragmentation of the said pyrazoles.

Keywords: pyrazoles, mass fragmentation, low-resolution spectrum, methyl, nitro

1. Introduction

The pyrazole is one of the most important heterocycles. It is a versatile five-membered heterocycle and a lead molecule in pharmaceutical development, due to its wide range of biological activity (e.g., antimicrobial, anticancer, cytotoxic, analgesic, anti-inflammatory, antihypertensive, antiepileptic, and antidepressant activities) [1]. Some drugs containing pyrazoles (e.g., celecoxib, novalgin, ramifenazone, fipronil, rimonabant, and pyrazofurin) are already in the market. Because of this, research on the synthesis, structural characterization, and properties of pyrazoles is ever increasing. Currently, a search—using pyrazole as topic—on the Web of Science showed 14,000 results, 327 of which are reviews. Early works on the mass spectrum fragmentation pattern of pyrazoles date from the early 1970s. Over approximately one decade, a few scientists—including Thuijl et al. [2–6],

Bowie et al. [7, 8], and Finar and Millard [9]—investigated the mass fragmentation of substituted pyrazoles. In 2005, Santos et al. published a study focused on the mass fragmentation of substituted pyrazoles. Advances in the use, as an analytical tool, of gas chromatography coupled with mass spectrometry—including ionization modes such as electron spray and chemical ionization—and the use of high-resolution mass spectrometry led to novel structural elucidation, fragmentation mechanisms, and other properties for pyrazoles. However, up until 2005, pyrazole fragmentation, as well as the substituent effect, had not been studied from the perspective of these advances. The aforementioned analytical tool brings new insights regarding structural and other properties of pyrazoles. Because of this, and considering that our objective in this chapter is to present a comprehensive study on the fragmentation pattern of substituted pyrazoles, contribute to the systematization of knowledge, and assist researchers in the characterization of pyrazoles via a comprehensive and versatile technique such as gas chromatography coupled with mass spectrometry, only the works published up until 2005 will be discussed here. Thus, in this chapter, we will present the studies on pyrazole fragmentation by gas chromatography coupled with mass spectrometry, in order to evaluate the substituent effect on pyrazole fragmentation and to present a comprehensive study on the fragmentation pattern of substituted pyrazoles.

2. Trends in mass spectrum fragmentation of pyrazoles

Thuijl et al. [2] studied the fragmentation of a series of 10 pyrazoles—substituted at the 2,3,4-, and 5-position—at low resolution. It is worth noting that most pyrazoles are unsubstituted at the 1-position (NH-pyrazole); therefore, they exist as a tautomeric equilibrium of two forms (**Figure 1**).

The molecular structure of the NH-pyrazoles investigated by Thuijl et al. [2] is shown in **Table 1**. In general, the substituent at the 4-position was changed in the pyrazole series, and the compounds are divided into the following two groups in accordance with the substituent: (i) chloro- and bromine-substituted pyrazoles, including mono-, di-, and tri-substituted ones, and (ii) cyano-, nitro-, carboxy-, and
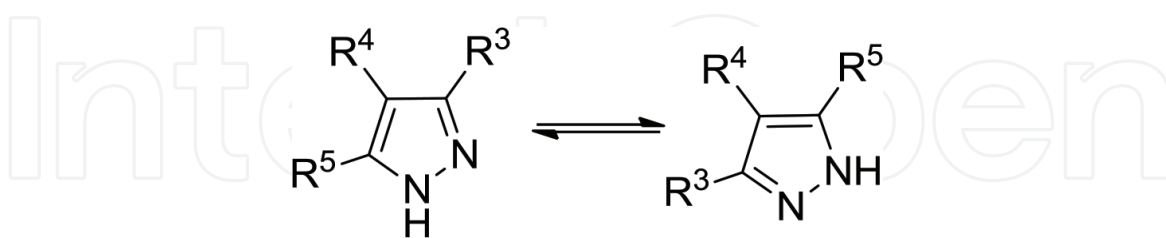
Figure 1.
Representation of the two tautomers of the NH-pyrazoles.

|	Comp.	R^1	R^3	R^4	R^5	Comp.	R^1	R^3	R^4	R^5
	1	H	H	H	H	6	H	H	CN	H
	2	H	H	Cl	H	7	H	H	NO$_2$	H
	3	H	H	Br	H	8	H	H	COCH$_3$	H
	4	H	H	Br	Br	9	H	H	C$_6$H$_5$	H
	5	H	Br	Br	Br	10	CH$_3$	H	H	H

Table 1.
Molecular structure of NH-pyrazoles 1–10.

phenyl-substituted pyrazoles. The authors included an unsubstituted pyrazole and a 1-methyl substituted pyrazole in the series, in order to evaluate the effect that this position has on the fragmentation trend of 4-substituted N*H*-pyrazoles. The simplest pyrazole analyzed was the unsubstituted pyrazole (**1**). The authors showed that the fragmentation follows two distinct routes. The predominant feature is expulsion of HCN (*b*, *m/z* 41, [C_2H_3N]) or H (*a*, *m/z* 67) from the [M]$^{+•}$ ion or expulsion of HCN from [M–H] (*c*, *m/z* 40, [C_2H_2N])—see **Figure 2**.

The second process involves the loss of a nitrogen from [M–H] (*a*, *m/z* 67), which furnishes the cyclopropenyl ion (*e*, *m/z* 39, [C_3H_3]$^+$). The intensity ratio of the ions formed by the loss of HCN (*b* + *c* + *d* + *f*) and loss of N_2 (*e* + *g* + [C_3H]$^+$ + [C_3]$^{+•}$) is 5:1. Elimination of acetylene also occurs but only to a very small extent. Apart from the loss of HCN and H$^•$ from the molecular ion, Khmel'nitskii et al. [10] also reported on the loss of N_2 and HCN from [M–H], which leads to [C_3H_4]$^{+•}$ (*e*, *m/z* 39) and [C_2H_2N]$^+$ (*c*, *m/z* 40), respectively—this was not identified in the study of Thuijl et al. [2]. High-resolution measurements did not reveal the presence of [C_3H_4]$^{+•}$. Metastable defocusing indicated that [C_2H_2N]$^+$ (*c*, *m/z* 40) has only two precursors: *b* and *a*. The transitions 39 → 38 can be assigned to two processes: *e* → *g* and *d* → *f*—see **Figure 2**. The latter process was observed in the spectrum of 3(5),4-dibromopyrazole **4** (see **Figure 4**), in which *e*, *m/z* 39 was absent.

4-Chloro- (**2**) and 4-bromopyrazole (**3**)—see **Figure 3**—have similar fragmentation. Although [M–H]$^{+•}$ is virtually absent, the loss of Br$^•$ (*a*, *m/z* 67) is followed by expulsion of N_2 to give *e*, *m/z* 39. Two successive losses of HCN from [M]$^{+•}$ lead to [CH_3Br]$^{+•}$ (*h*, *m/z* 92, 94) in preference to [CH_2]$^{+•}$ (*h*, *m/z* 14), which is also weak in unsubstituted pyrazoles. The expulsion of HCN and radical bromine was also observed in 3,4-dibromo (**4**). However, for pyrazole **4**, the *a* → *e* process (loss of N_2) was absent. Instead of [$C_3H_2N_2Br$]$^+$ being formed, *a* lost the second Br$^•$ to give [$C_3H_2N_2$]$^{+•}$ (*i*, *m/z* 66), which formed [C_2HN]$^{+•}$ (*d*, *m/z* 39) after elimination of HCN (**Figure 4**).

The mass spectrum of pyrazole **5** showed that the loss of HCN is still important for tribrominated pyrazoles. The formation of [C_2NBr_3]$^{+•}$ (*b*, *m/z* 275–281) involves a bromine migration similar to that reported by Bowie et al. [7] for tribromoimidazole. Another example of a bromine migration is the formation of [CBr_2]$^{+•}$
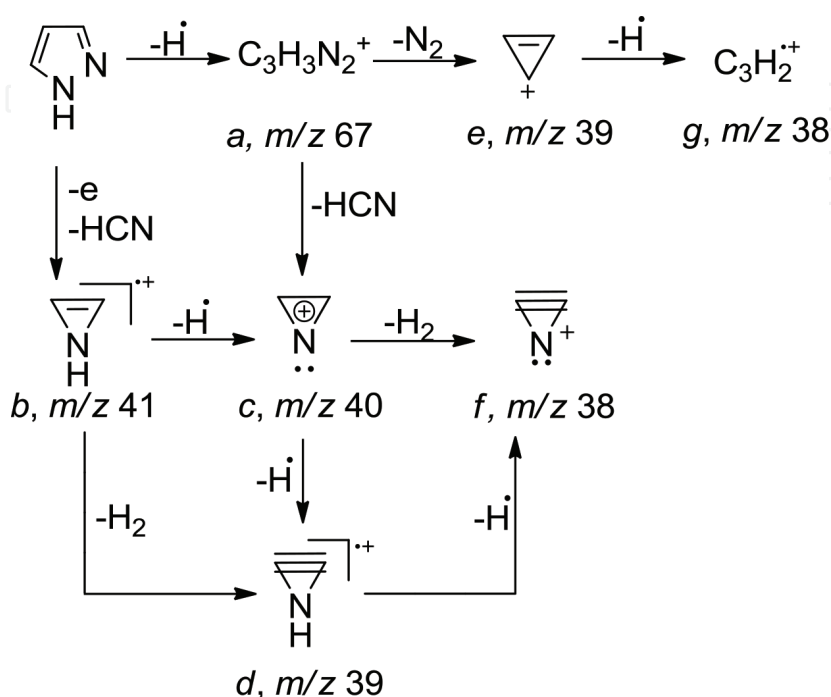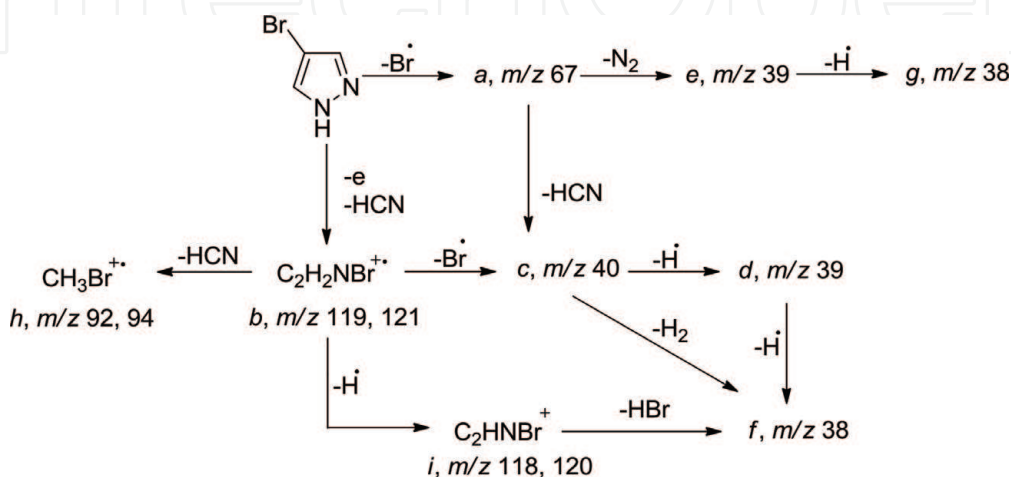
Figure 2.
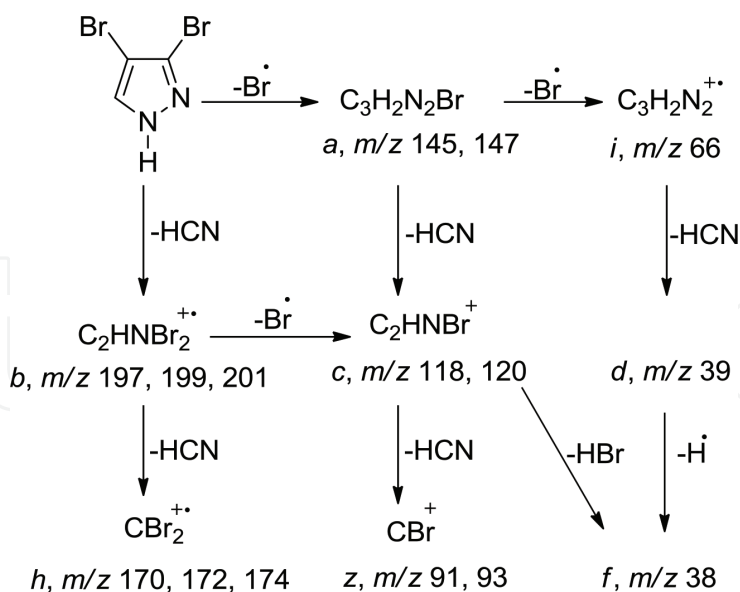*Principal fragmentation of unsubstituted pyrazole **1**.*

(h, m/z 170–174) from $[C_2HNBr_2]^{+\bullet}$ (b, m/z 197–201) in **4**. Principal mass fragmentation of compounds **4** and **5** is shown in **Figure 5**.

In 4-cyanopyrazole **6**, the elimination of HCN plays such an important role that $[M–CN]^{+\bullet}$ (f, m/z 66) is formed, which breaks down in the same way as in **3** and **4**. Principal mass fragmentation of **6** is shown in **Figure 6**.

The mass spectrum of 4-nitropyrazole **7** shows the usual fragments associated with nitro groups in aromatic compounds: $[M–O]^{+\bullet}$ (a, m/z 97), $[M–NO]^{+}$, and $[M–NO_2]^{+}$. Loss of the $-NO_2$ substituent gives a, m/z 67, which forms subsequent fragments—see **Figure 2**. However, another route to c, $[C_2H_2N]^{+}$ was found for **7**—see **Figure 7**.

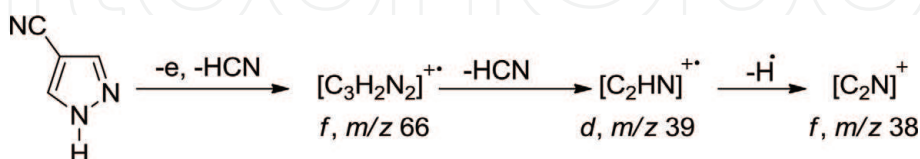**Figure 3.**
Principal mass fragmentation of 4-bromopyrazole 3.

Figure 4.
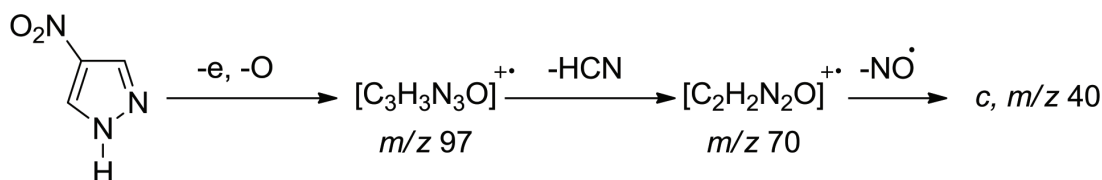*Principal mass fragmentation of 3,4-dibromopyrazole 4.*
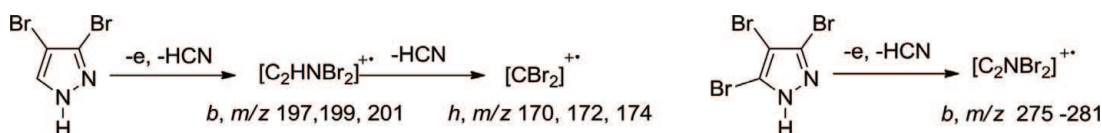
Figure 5.
Principal mass fragmentation of compounds 4 and 5.

The behavior of 4-acetylpyrazole **8** indicates that $[M–CH_3]^+$ (*j*, *m/z* 95) is the base peak and that the loss of the substituent is a two-step process (**Figure 8**). It should be noted that the relative intensity of the unfavorable species $[C_3H_3N_2]^+$ (*a*, *m/z* 67) is small compared to that of $[C_3H_2NO]^+$ (*k*, *m/z* 68), formed by the HCN lost, which is stabler. In contrast to the previous compound, no elimination of HCN from the molecular ions occurs.

The mass spectrum of 4-phenylpyrazole **9** closely resembles that of the unsubstituted pyrazole. Loss of HCN from $[M]^{+•}$ leads to $[C_8H_7N]^{+•}$ (*b*, *m/z* 117) and $[C_8H_6N]^+$ (*c*, *m/z* 116). Alternatively, successive losses of H$^•$ and N$_2$ lead to the phenylcyclopropenyl ion $[C_9H_7]^+$ (*e*, *m/z* 115). Expulsion of HCN from *b* and *c* gives (*f*, *m/z* 90) $[C_7H_6]^{+•}$ and (*d*, *m/z* 89) $[C_7H_5]^+$, respectively (**Figure 9**).
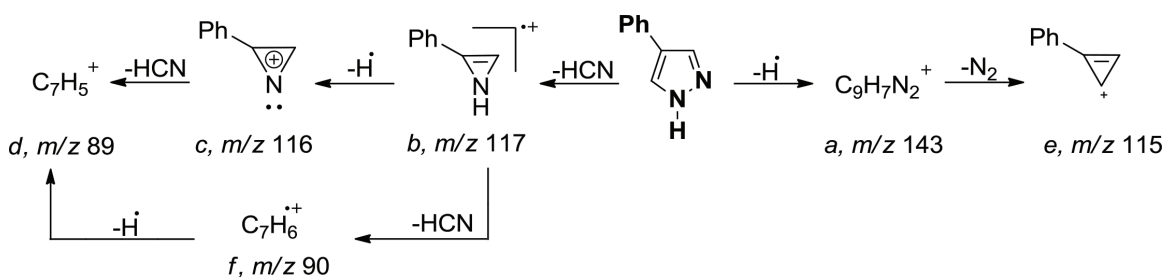
Figure 6.
Principal mass fragmentation of compound 6.

Figure 7.
Principal mass fragmentation of compound 7.
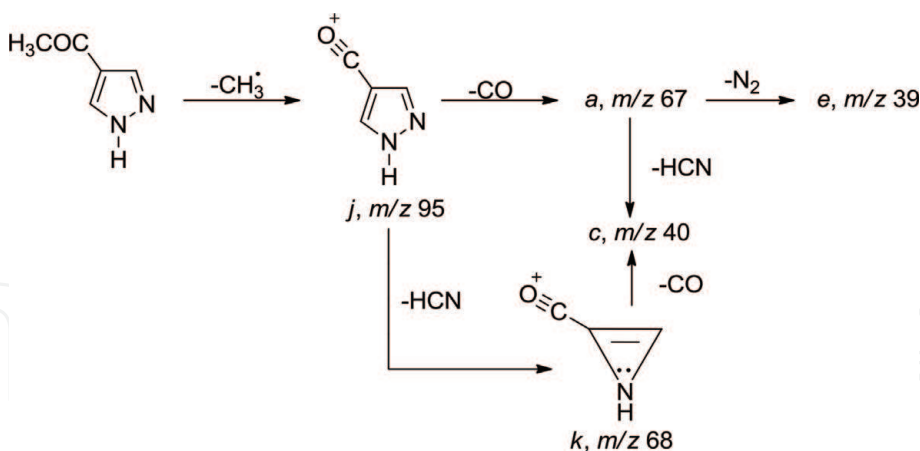
Figure 8.
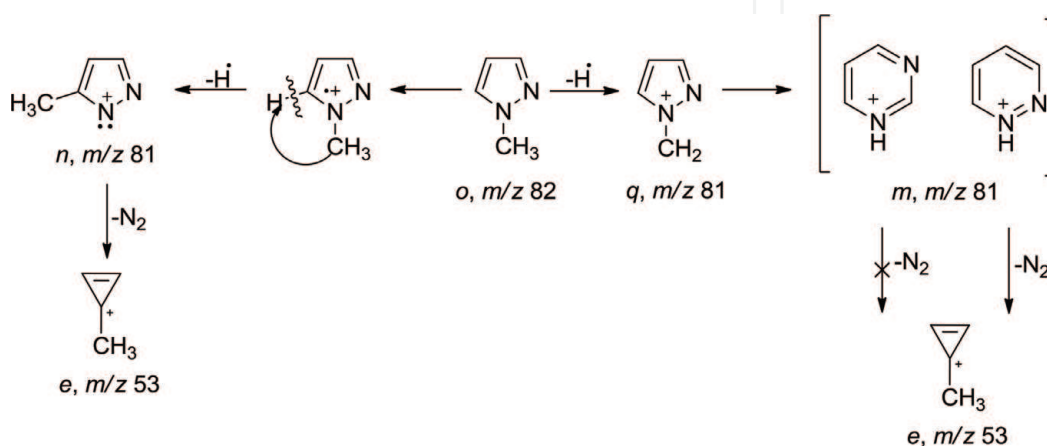*Principal mass fragmentation of compound 8.*

Figure 9.
Principal mass fragmentation of compound 9.

The low-resolution spectrum of 1-methylpyrazole **10** showed that a H$^\bullet$ radical is lost preferably from the methyl group, which may be rearranged to give *m* (*m/z* 81) —see **Figure 10**. The fact that the ion [C$_4$H$_5$]$^+$ (*e*, *m/z* 53) is formed by elimination of N$_2$ from [M–H]$^+$ means that it does not exclusively rearrange to the pyrimidinium ion but also, to some extent, to the pyridazinium ion (*m*, *m/z* 81) (**Figure 10**). In the case of H$^\bullet$ abstraction from C-5, another possible explanation for the loss of N$_2$ from [M–H]$^+$ is migration of the methyl group to C-5 (*n*, *m/z* 81) prior to formation of the methylcyclopropenyl ion (*e*, *m/z* 53). Although (*n*, *m/z* 81) might be expected to expel acetonitrile, an *m/z* 81 → *m/z* 40 transition was not observed.

In conclusion, Thuijl et al. [2] showed that pyrazole and its 4-substituted derivate have stable molecular ions, which generally form the base peak. A notable exception is 4-acetylpyrazole, for which the [M–CH$_3$]$^+$ ion is the base peak. Two important processes are observed in unsubstituted pyrazoles: the expulsion of HCN from [M]$^{+\bullet}$ and [M–H]$^+$ and the loss of N$_2$ from [M–H]$^+$—the former process is the most predominant. Substitution influences the relative importance of these two processes. Whereas in 4-bromopyrazole the patterns remain essentially unchanged, introduction of more bromine atoms makes initial loss of HCN less important, due to the competing loss of bromine radicals. However, secondary loss of HCN remains important, resulting in the suppression of the expulsion of N$_2$. The influence of the 4-nitro and 4-acetyl substituents is much more pronounced, because the substituents can be in more than one step, giving rise to the stable ions [M–O]$^{+\bullet}$ and [M–NO]$^+$ in the former and [M–CH$_3$]$^+$ in the latter. In these compounds, the loss of HCN, though still strong as a secondary step, is no longer a primary process. On the other hand, the 4-cyano and 4-phenyl substituents promote the loss of HCN in such a manner that the ion formed by the loss of the substituent is practically absent. In 4-cyanopyrazole, the initial loss of HCN is very strong, resulting in an ion with *m/z* 66, while in 4-phenylpyrazole, two consecutive losses of HCN occur. This marked influence of the substituent makes it difficult, in some cases, to establish the presence of a pyrazole compound from a low-resolution spectrum.

Thuijl et al. [3] reported the fragmentation of the following: monodeuterated pyrazoles at the 1-position (**11**) and 4-position (**12**); dideuterated pyrazoles at the 1,4‿position (**13**) and 3,5-position (**14**); and trideuterated pyrazoles at the 1,3,5-position (**15**) at low resolution—see **Table 2**. The fragmentation of these pyrazoles was similar to the simplest pyrazole (**1**), in that two of the most important fragmentations involved the loss of H$^\bullet$ and HCN from the molecular ion. Similarly, these results can be explained in terms of cleavage of a C–H bond in an α-position relative to a nitrogen atom. According to the authors, this α-cleavage is a frequently
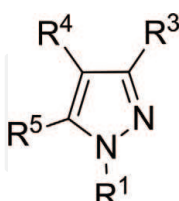

Figure 10.
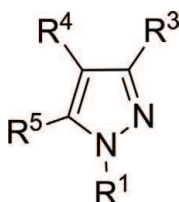*Principal mass fragmentation of compound 10.*

encountered phenomenon in mass spectra of compounds possessing heteroatoms. The loss of HCN from the molecular ion of non-deuterated pyrazoles gives rise to a peak corresponding to the formation of $C_2H_3N^+$ (m/z 41). The peak at m/z 40 ($C_2H_2N^+$) arises because of the subsequent loss of H^+. In a monodeuterated compound, the loss of HCN and DCN forms fragments m/z 42 and m/z 41, respectively. The mass spectra of the 3,5-dideuterated pyrazole (**14**) and 1,3,5-trideuterated pyrazole (**15**) showed that position 3(5) is favored, but the mass spectra of the other three deuterated compounds do not, a priori, exclude contributions from the other two positions. The authors concluded that the loss of HCN from the molecular ion of pyrazole occurs from the 3(5) position with a high specificity. The authors also noted that more quantitative and high-resolution spectra would be useful to enable a complete understanding of the fragmentation of deuterated pyrazoles.

Aldous and Bowie [8] also investigated deuterated 3,5-diphenylpyrazoles at low resolution (**Table 3**). The fragmentation of 3,5-diphenylpyrazoles produced $[C_{13}H_9]^+$ ions through the elimination of $N_2H^{\bullet}$ and $^{\bullet}C_2H_2$ from M^+. This fragmentation differs from other diphenylpyrazoles, imidazoles, and isoxazoles. For example, fragmentation of 4,5-diphenylpyrazole forms $[C_{13}H_9]^+$ ions (m/z 165) from molecular ions, through the $[M–HCN–HCN–H^{\bullet}]$ or $[M–C_2H_3N_2^{\bullet}]$ process. Another usual feature of the 4,5-diphenylpyrazole spectra is the reversible hydrogen rearrangement, which occurs between the N-*H* and the *ortho*-hydrogens of the phenyl rings.

The molecular ions of 3,5-diphenylpyrazoles decompose through the loss of N_2D and N_2H, with N_2H forming at higher intensity. This result parallels the reversible hydrogen rearrangement observed for 4,5-diphenylpyrazoles. The reversible transfer of a phenyl hydrogen to nitrogen followed by back transfer of either $H^{\bullet}$ or $D^{\bullet}$ occurs prior to or during the initial elimination ($N_2D^{\bullet}$ and $N_2H^{\bullet}$ from **16**), and as the lifetime of the decomposing molecular ion increases, H/D scrambling in the phenyl rings competes with the reversible transfer reaction. Proof of the D transfer to the phenyl substituents is substantiated by the observation of the two processes $[M]^{+\bullet} \rightarrow [C_6H_5]^+$ and $[M]^{+\bullet} \rightarrow [C_6H_4D]^+$. The spectra of **16** and **17** show that there is no exchange between the 1- and 4-H/D atoms or between the 4-H (or D) and the

	Comp.	R^1	R^3	R^4	R^5	Comp.	R^1	R^3	R^4	R^5
	1	H	H	H	H	13	D	H	D	H
	11	D	H	H	H	14	H	D	H	D
	12	H	H	D	H	15	D	D	H	D

Table 2.
*Mass spectral investigation of compounds **1** and **11–15**.*

|	Comp.	R^1	R^3	R^4	R^5
	16	D	Ph	H	Ph
	17	D	Ph	D	Ph
	18	H	Ph	H	Ph

Table 3.
*Structure of pyrazoles **16–18**.*

phenyl hydrogens. The metastable decompositions of **16**, **17**, and **18** show that the $[M^+- N_2H^\bullet]^+$ ion from 3,5-diphenylpyrazole eliminates $^\bullet C_2H_2$ after complete carbon and hydrogen scrambling. The other fragmentations shown in **Figure 11** are unexceptional and occur without prior equilibration of the N-*H* and phenyl hydrogens.

Finar and Millard [9] studied the low-resolution mass spectrometry of 1-phenylpyrazole-4-yl-oximes (**19–23**)—see **Table 4**. This is a typical substrate of the Beckmann rearrangement for corresponding 4-amido-1-phenylpyrazoles. If the ions of the anti-oximes were to undergo the Beckmann rearrangement for the corresponding 4-amido-1-phenyl-pyrazole, the $C_{10}H_7N_2O^+$ ion (*m/z* 171) formed by the breaking of the CO–NH bond would be present (**Figure 12**).

Aliphatic aldoximes, such as 4-formyl-1-phenyl-pyrazole oxime (**19**)—**Figure 12**, have relatively intense molecular ions. The spectrum is characterized by the elimination of water from the molecular ion (*a, m/z* 169), as the loss of a hydroxyl radical (*b, m/z* 170). Oxygen is also lost to a small extent (*c, m/z* 171). The base peak represents the oxime lost followed by the formation of the 1-phenylpyrazole ion (*d, m/z* 144). An appropriate metastable ion is formed directly from the molecular ion by a process which must involve the breaking of the 4-substitution and
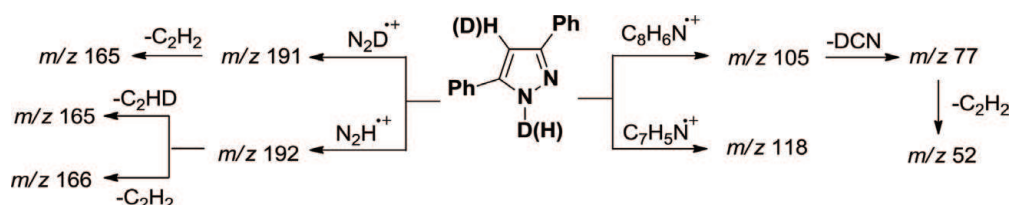
Figure 11.
Fragmentation pattern of compounds 16–18.

	Comp.	R	Comp.	R
	19	H	22	*i*-pr
	20	Me	23	*t*-Bu
	21	Et	24	Ph

Table 4.
Mass spectral investigation of compounds 20–24.
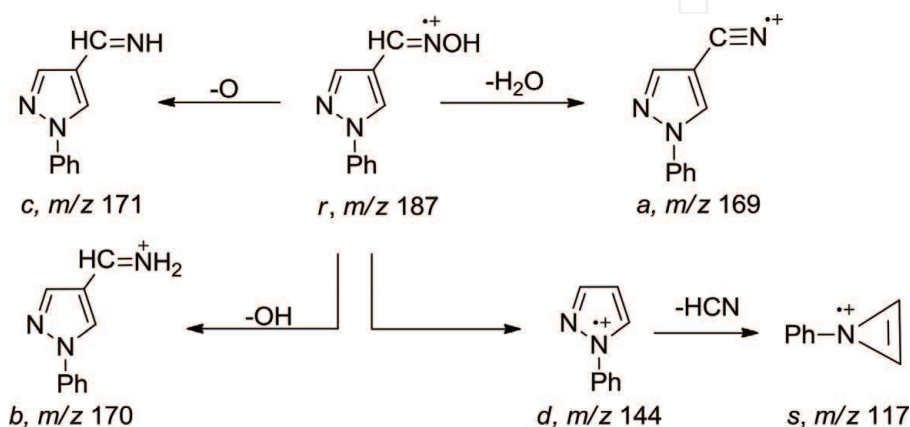
Figure 12.
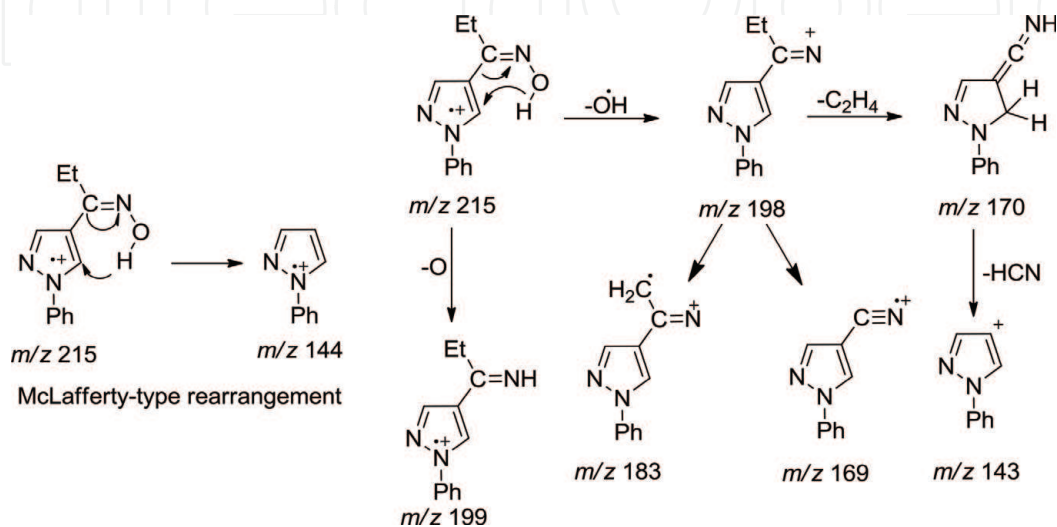*Mass fragmentation of aldoxime pyrazole 19.*

simultaneous transfer of hydrogen to the pyrazole ring. The substitution of this hydrogen by deuterium causes the ion to move to *m/z* 145, thus confirming that the hydrogen is transferred to the pyrazole.

For the ketoxime pyrazoles **20–23**, the one-step elimination of water in **19** is not paralleled by a corresponding one-step loss of the appropriate alcohol, although the elements of alcohol are lost as a hydroxyl radical followed by the alkyl group. Again, exchange of the oximino-hydrogen for deuterium causes this ion to move to *m/z* 145, which indicates that the mechanism described in **Figure 12** is present here. The loss of oxygen from the molecular ion remains a significant process but becomes less so as the substituent increases in size, possibly because of the increased number of fragmentation modes available. **Figure 13** shows how a typical ketoxime—ethyl-1-phenylpyrazole-4-yl oxime **21**—fragments. The ion of *m/z* 170 that arises from the loss of ethylene—perhaps via a McLafferty-type rearrangement—is also present in the spectra, again being formed from M–OH ions. When R = *i*-pr (**22**) and *t*-bu (**23**), it appears that the alkyl substituents undergo rearrangement to the linear-chain isomers in the molecular ions, because **22** eliminates an ethylene molecule and **23** eliminates a propene molecule. Such rearrangements have been noted previously in the mass spectra of branched alkyl compounds [11].

The 4-benzoyl-1-phenyl-pyrazole oxime **24** behaves differently to the other compounds—see **Figure 14**. The molecular ion at *m/z* 263 and the rearrangement ion at 144 are by far the most intense ions in the spectrum. Besides the anticipated loss of oxygen and a hydroxyl radical from the molecular ion, the unexpected ejection of NO, HNO, and H_2NO occurs. The M–H_2NO ion at *m/z* 231 fragments further—due to the loss of HCN—and yields an ion encountered in the mass spectrum of benzophenone oxime. It appears that the molecular ions of these oximes do not undergo the Beckmann rearrangement, but rather a rearrangement of the oximino-hydrogen atom, which has not yet been encountered in the mass spectra of oximes.

Luijten and Thuijl [4] studied how the presence of methyl and nitro groups in the pyrazole ring affects the fragmentation of these compounds in the low-resolution spectrum (**Table 5**).

For compounds **25–27**, the common characteristics are the initial loss of NO$^{\bullet}$ (which leads to the formation of *o, m/z* 97) followed by the loss of O$^{\bullet}$ or the loss of NO$_2^{\bullet}$, which leads to the ion *m/z* 81 (**Figure 15**). The difference between the three compounds occurs due to the fragmentation of the ion *m/z* 81 (*p* to **25** and **27**, and *q* to **26**)—see **Figure 15**. Although all compounds have the *r, m/z* 53 and *s, m/z* 54 ions, their relative abundance is different. At **25** and **27**, the *r, m/z* 53 is the most

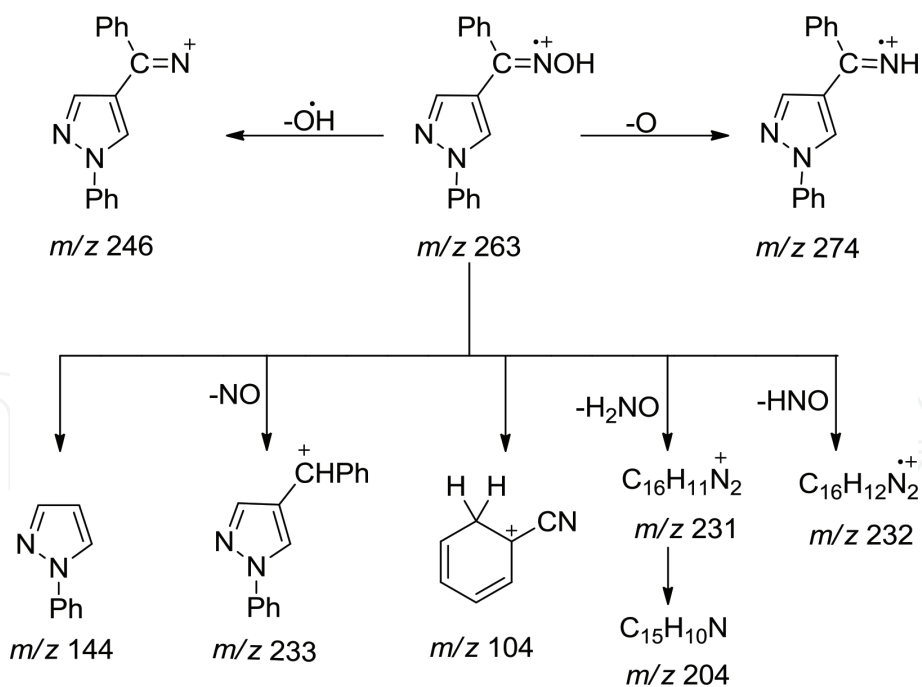**Figure 13.**
Mass fragmentation of ketoxime pyrazoles 20–23.

Figure 14.
4-Benzoyl-1-phenyl-pyrazole oxime 24.

	Comp.	R^1	R^3	R^4	R^5	Comp.	R^1	R^3	R^4	R^5
	25	NO$_2$	CH$_3$	H	H	30	CH$_3$	H	H	NO$_2$
	26	NO$_2$	H	CH$_3$	H	31	H	CH$_3$	H	NO$_2$
	27	NO$_2$	H	H	CH$_3$	32	H	NO$_2$	CH$_3$	H
	28	CH$_3$	NO$_2$	H	H	33	H	CH$_3$	NO$_2$	H
	29	CH$_3$	H	NO$_2$	H					

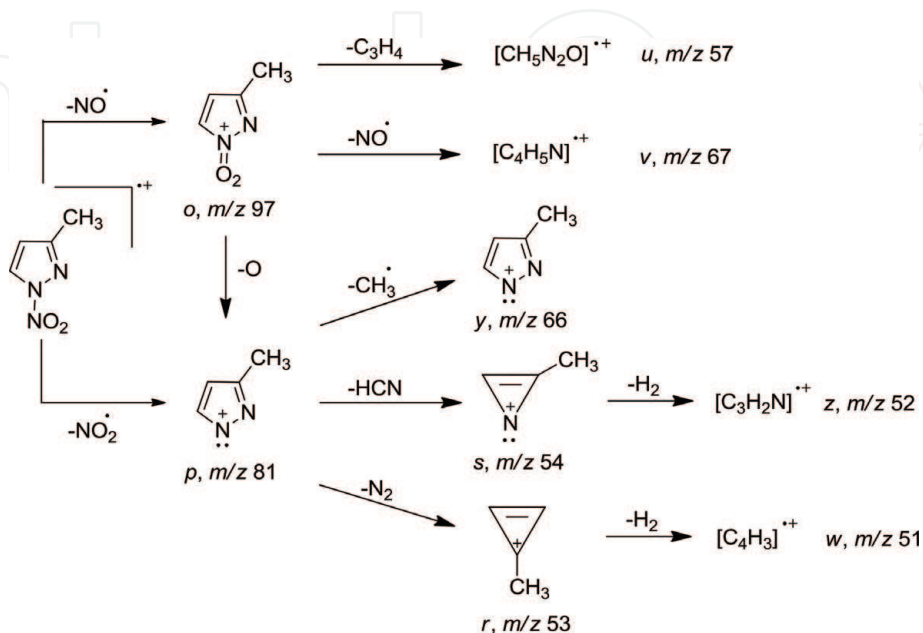**Table 5.**
Structure of pyrazoles 25–33.

Figure 15.
Fragmentation of 1-nitro-3-methylpyrazole 25.

abundant ion, which is expected, because the two compounds have equivalent structures, as can be seen in **Figure 16**, while for compound **26**, the most abundant ion is *s*, *m/z* 54. **Figure 16** explains the production of *r*, *m/z* 53 and *s*, *m/z* 54 for nitropyrazoles. The formation of the *s*, *m/z* 54, occurs due to the loss of HCN, whereas the formation of *r*, *m/z* 53 is due to the loss of N_2. Luijten and Thuijl [4] attributed these differences to the position of the methyl groups in pyrazoles, because for **25** and **27**, *r*, *m/z* 53 is stabler, while for **26**, *s*, *m/z* 54 is stabler. Fragmentation of compound **25** is explained in **Figure 15**.

Of note in the mass spectrum of compound **28** is the very stable molecular ion. Different from pyrazoles **25–27**, the ion *g*, *m/z* 81 has low abundance, which suggests the formation of ion *h*, *m/z* 80 through the loss of HNO_2 or a loss of H$^{\bullet}$ from the $[M–NO_2]^+$ fragment. The presence of ions *b*, *m/z* 97 and *f*, *m/z* 111 indicates the presence of the nitro group in the molecular ion. Upon fragmentation, the ion *b*, *m/z* 97 generates the ion *c*, *m/z* 69, which can fragment in two distinct ways—one generating the ion *d*, *m/z* 43 through the release of $-C_2H_2$ and the other generating the ion *e*, *m/z* 42 through the release of -HCN. Another interesting fragmentation generates the ion *d*, *m/z* 43 directly from the molecular ion, through the loss of $-C_3H_2NO_2{}^{\bullet}$. The fragments are summarized in **Figure 17**.

The fragmentation of 1-methyl-4-nitropyrazole **29** (see **Figure 18**) was different from the 3-isomer **28**. Starting from ion *b*, *m/z* 97, Luijten and Thuijl [4] observed only the formation of ion *e*, *m/z* 42, whereas in isomer **28**, starting from the same fragment, they observed the formation of ions *m/z* 42 and *m/z* 43. The formation of the ion *e*, *m/z* 42 was also observed from ion fragmentation *d*, *m/z* 111 for compound **29**; however, compound **28** did not indicate this fragmentation pathway.

The authors draw attention to the difference in fragmentation of ion *m/z* 81 for compounds **28** and **29**. In compound **28**, ions *g*, *m/z* 81; *h*, *m/z* 80; and *l*, *m/z* 79 lose -HCN to generate the fragments *k*, *m/z* 54; *i*, *m/z* 53; and *m/z* 52, respectively (see **Figure 17**), at similar abundances, while **29** produces an abundant ion at *m/z* 52. The fact that the *m/z* 80 ion in **29** (*g*, **Figure 18**) releases N_2, but the same ion in **28** (*h*, **Figure 17**) does not, is related to the expansion of the ring structure for the formation of a pyrimidine fragment at **60** and a pyridazine at **29**. Compared to its isomers **28** and **29**, compound **30** has a low abundance of the molecular ion *m/z* 127. The fragmentation pattern of compound **30** (see **Figure 19**) indicates that the nitro group is absent; however, the presence of two adjacent groups gives rise to an *ortho* effect, which is responsible for two breaking patterns in this compound. The first is
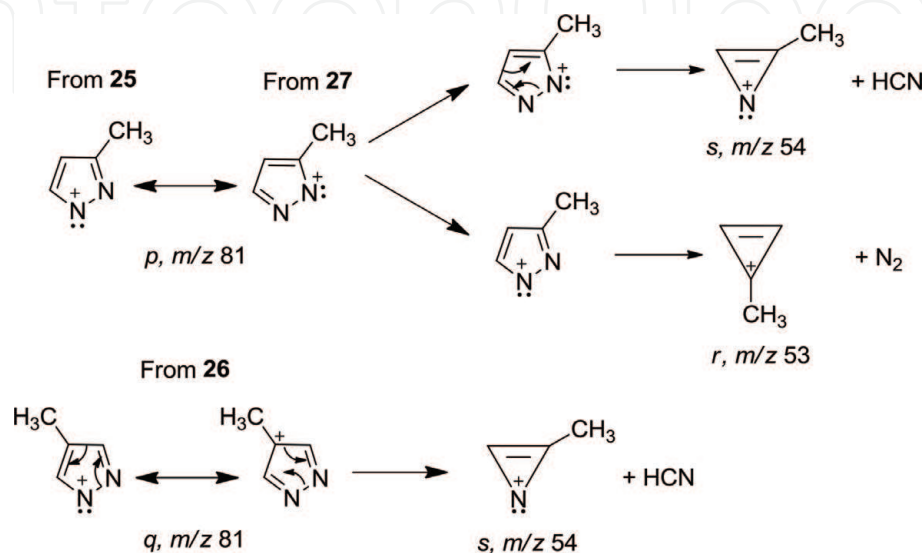
Figure 16.
Fragmentation of the $[M\,NO_2]^+$ of methyl-1-nitropyrazoles 25–27.
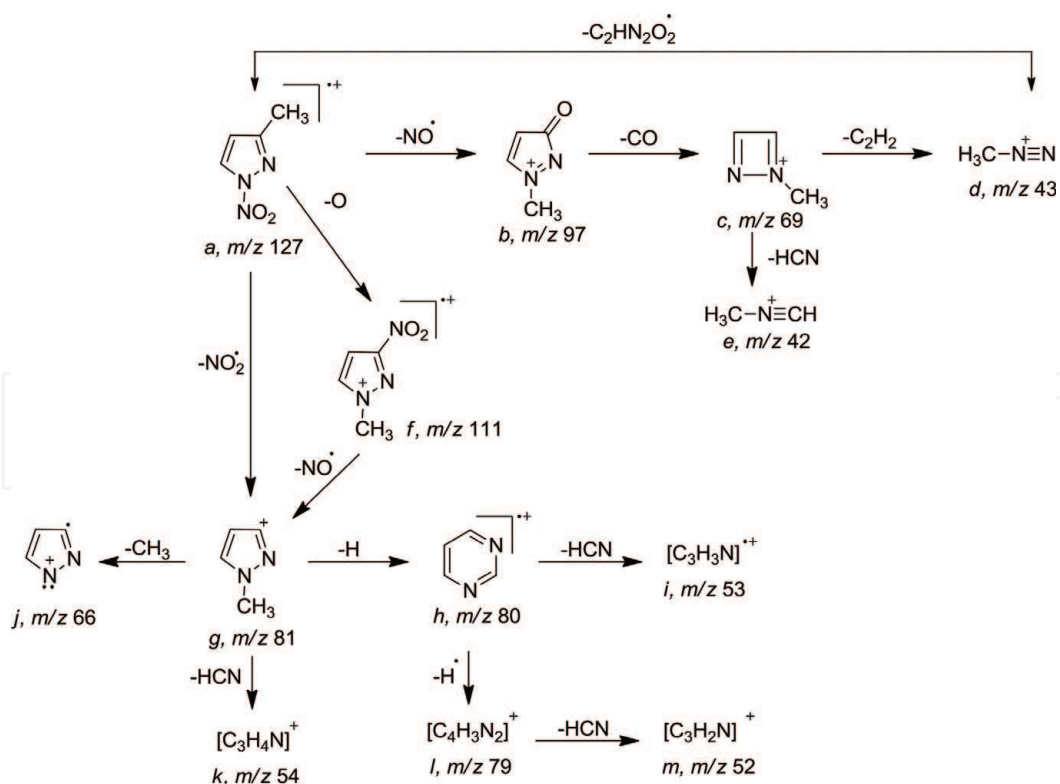
Figure 17.
Fragmentation of 1-methyl-3-nitropyrazole 28.
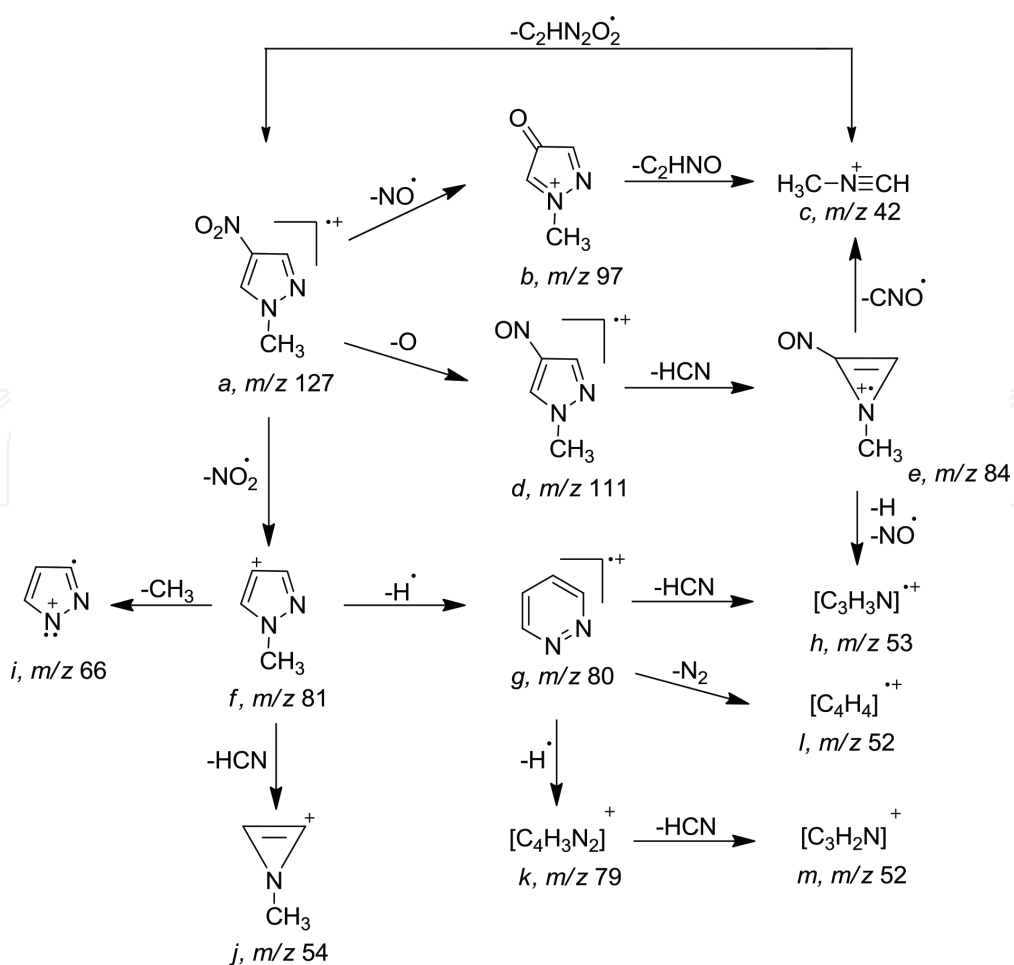
Figure 18.
Fragmentation of 1-methyl-4-nitropyrazole 29.

the loss of OH$^\bullet$ (*j*, *m/z* 110) from the molecular ion, with subsequent losses of NO$^\bullet$ (*k*, *m/z* 80) and HCN (*l*, *m/z* 53). The $^{13}CH_3$ indicated that no ring expansion occurs and that the remaining oxygen atom—[M–OH]$^+$ ion, *m/z* 110—occupies its original position [5]. The second breaking pattern is less common and involves the loss of CHO$^\bullet$ from the molecular ion, leading to the formation of *b*, *m/z* 98 (**Figure 19**). In a previous study, the authors showed that the carbon and hydrogen atoms that were lost originate from the methyl group [5]. The fragmentation of ion *b*, *m/z* 98 leads to the formation of ion *e*, *m/z* 68, which, according to the authors, must be identical to the pyrazole's molecular ion, since its subsequent fragmentation is analogous. Ion *b*, *m/z* 98 also shows a fragmentation path in which, after two successive losses of hydrogen radicals, it produces ion *g*, *m/z* 96, which can still suffer two losses—one forming ion *h*, *m/z* 68, through the loss of N_2, and the other forming ion *i*, *m/z* 66, through the loss of NO$^\bullet$. Unlike its isomers **28** and **29**, compound **30** does not show fragmentation of the molecular ion *a*, *m/z* 127, which leads to the formation of the *m/z* 42 or *m/z* 43 ions.

The mass spectrum of 3(5)-methyl-5-(3)-nitropyrazole (**31**) indicated a stable molecular ion—ions *g*, *m/z* 111; *e*, *m/z* 97; and *h*, *m/z* 81 confirmed the presence of the nitro group (**Figure 20**). One of the fragmentation pathways of the molecular ion *a*, *m/z* 127 generates ion *b*, *m/z* 110 through the release of $^\bullet$OH. Ion *b*, *m/z* 110 can still fragment in two ways: through the release of N_2 to form ion *c*, *m/z* 82, which, when it loses NO$^\bullet$, forms the ion *d*, *m/z* 52, and through losing NO$^\bullet$ to form ion *k*, *m/z* 80. It is important to note that, similar to compounds **28** and **29**, ion *f*, *m/z* 41 is formed directly from the molecular ion by the loss of $C_2H_2N_2O_2$. Due to the contiguous substituents in compound **32** (**Figure 21**), two *ortho* effects are observed: (*i*) a loss of $^\bullet$OH, leading to the formation of ion *e*, *m/z* 110, which, after losing HCN, leads to ion *f*, *m/z* 83, and (*ii*) the loss of a H_2O, which gives a fragment of *m/z* 109, which can lose N_2 or HCN fragments. Other direct fragmentations of the molecular ion are the losses of $NO_2^\bullet$ and NO$^\bullet$. As for pyrazole **28**, the loss of CO$^\bullet$ from the *m/z* 97 ion to form ion *j*, *m/z* 69 is observed for compound **32**. This suggests that it is different from the *m/z* 69 ion of compound **28** (**Figure 17**), because for pyrazole **32**, the formation of the [CH$_3$–C≡N$^+$H] of *m/z* 42 through the loss of HCN is different from the *m/z* 42 ion formed in **28** ([CH$_3$–N$^+$≡CH]). The mass spectrum of pyrazole **32** has a second fragmentation at *m/z* 69 (*h* ion)—a possible route is the loss of the ion of H$^\bullet$ and HCN from the [M–NO]$^+$ ion, *m/z* 97.
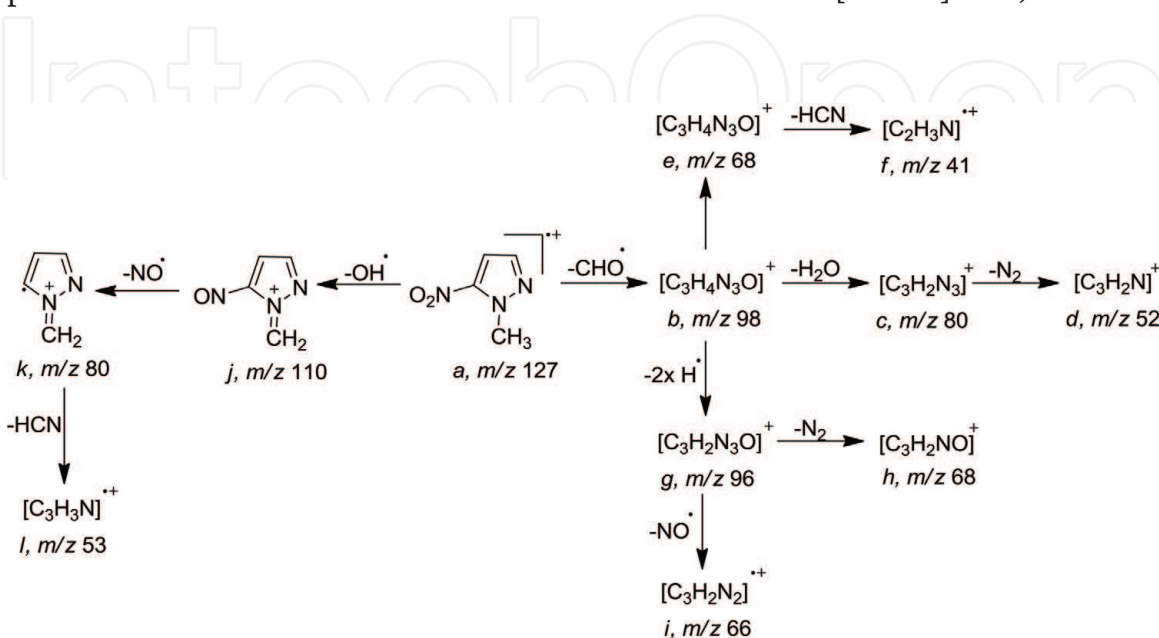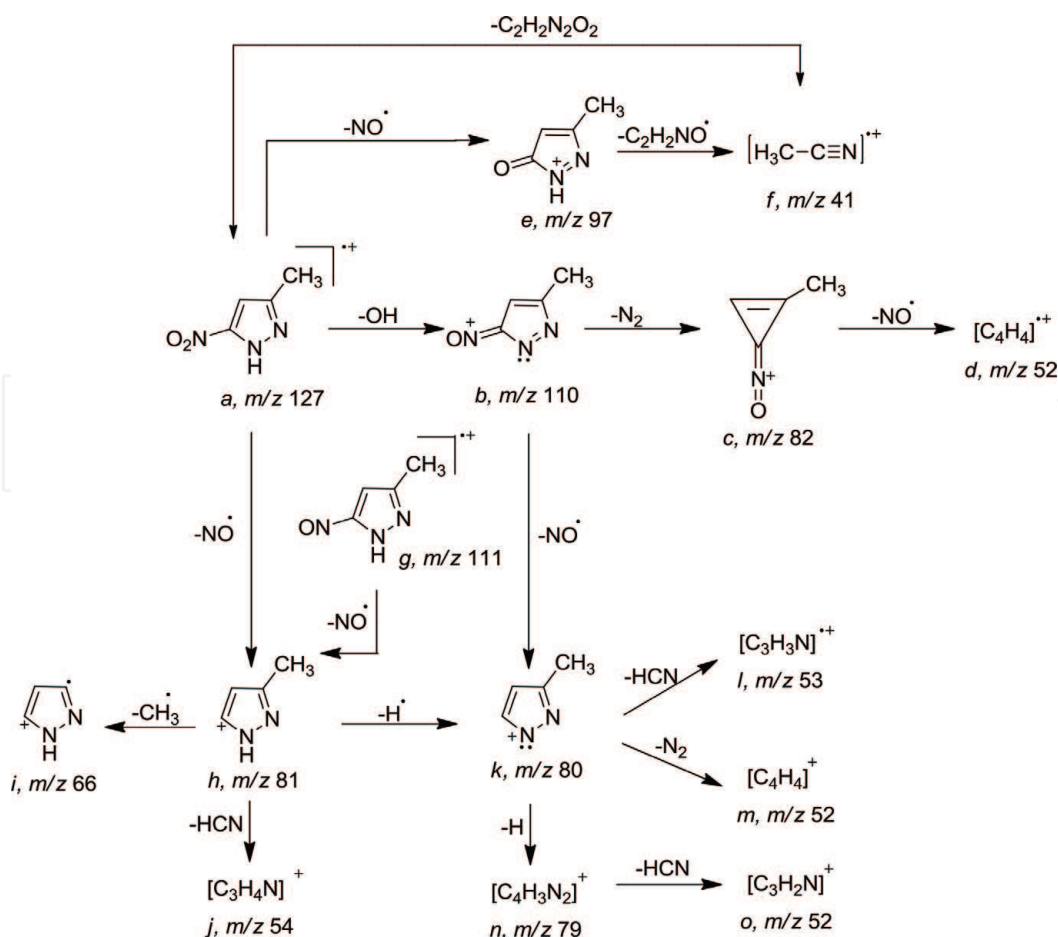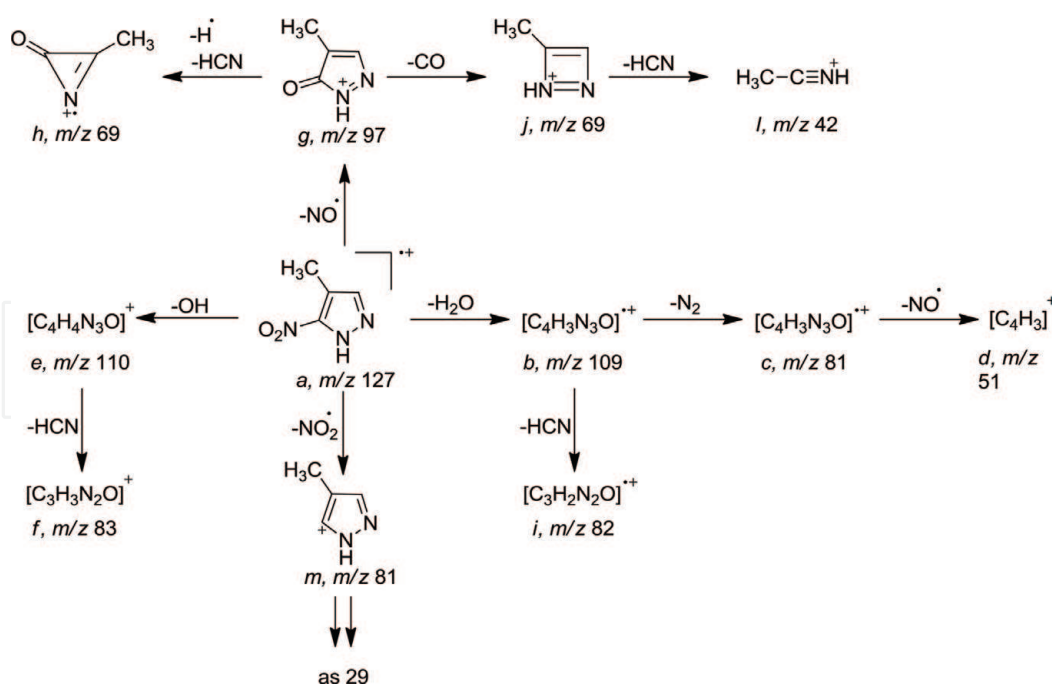

Figure 19.
*Fragmentation of 1-methyl-5-nitropyrazole **30**.*

Figure 20.
Fragmentation of 3(5)-methyl-5(3)-nitropyrazole 31.
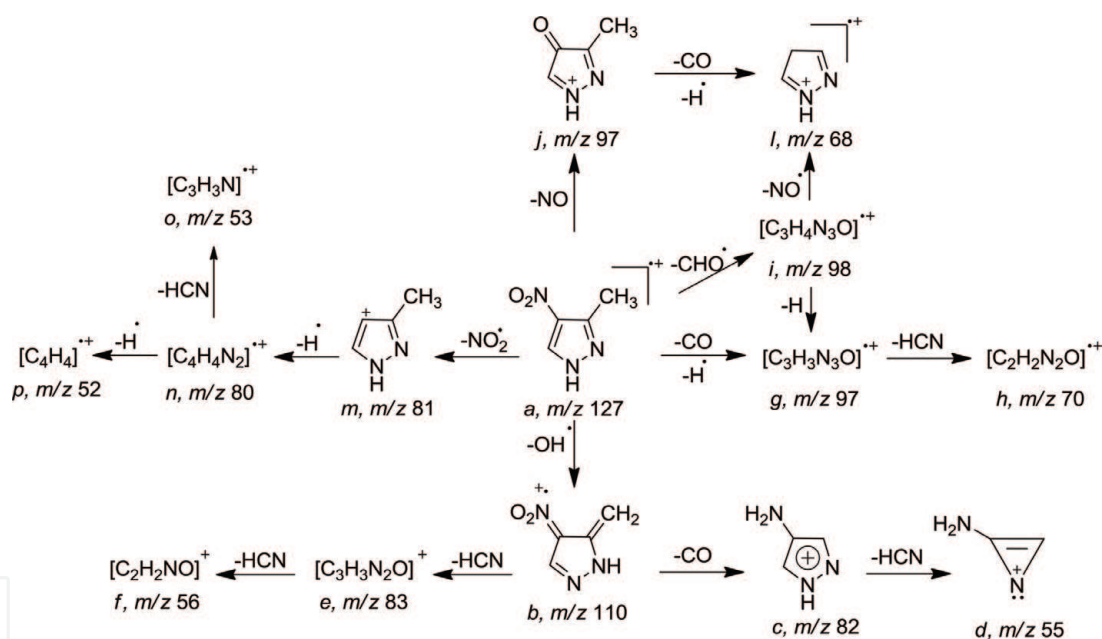

Figure 21.
Fragmentation of 3(5)-nitro-4-methylpyrazole 32.

Similar to its isomers (**30** and **31**), pyrazole **33**—see **Figure 22**—had breaks due to the *ortho* effect: the primary losses of OH, CHO, and CH_2O. Ion *b*, *m/z* 110— which is formed by the loss of OH—is capable of releasing CO, thus forming ion *c*, *m/z* 82, which, through a loss of HCN, leads to the formation of ion *d*, *m/z* 55. Another interesting primary loss is NO·, which leads to the formation of ion *j*, *m/z*

97. Ion *j* is capable of releasing CO and, following the loss of a hydrogen radical, ion l, *m/z* 68 is formed. The predominant breakdown process ($[M–NO_2]^+$) to form *m*, *m/z* 81 followed the loss of a hydrogen in **31**, **32**, and **33**; therefore, the formation of the pyridazine molecular ion as in **29** would be expected. In all three cases (**31–33**), the product ion *m/z* 80 indicates the fragmentation behavior of this species: the loss of H•, HCN, and N_2. Two other primary losses are those of CHO• and CH_2O. The $[M–CHO]^+$ ion, *i*, *m/z* 98, loses a hydrogen radical, followed by expulsion of HCN, to yield $[C_2H_2N_2O]^{+•}$, *h*, *m/z* 70, in contrast to the $[M–CHO]^+$ ion of **30**, which has an entirely different breakdown pattern (**Figure 19**).

The first fragmentation for pyrazoles substituted with NO_2 shows the rings intact, whereas the methyl substitution leads to the loss of a hydrogen radical with ring expansion. It is interesting to note that pyrazoles with $R^1 = NO_2$ (**25**, **26**, and **27**) lose this grouping in the first fragmentation, whereas the pyrazoles with $R^1 = CH_3$ do not suffer fragmentation of this group in the first breaks.

Tilborg and Thuijl [6] studied compounds **34–36**, **37–43**, and **44–47** (**Table 6**) using collision-induced dissociation (CID) mass spectrometry at low resolution, in order to understand how fragmentation occurs when pyrazoles are substituted at the 4-position. There are large differences between the ions derived from **34** and

Figure 22.
Fragmentation of 3(5)-methyl-4-nitropyrazole 33.

	Comp.	R^1	R^3	R^4	R^5	Comp.	R^1	R^3	R^4	R^5
	34	NO_2	H	H	H	42	D	Br	D	H
	35	H	Br	H	H	43	D	Br	D	D
	36	H	H	Br	H	44	D	H	Br	H
	37	H	Br	D	H	45	H	D	Br	H
	38	H	Br	H	D	46	H	D	Br	D
	39	D	Br	H	H	47	D	D	Br	H
	40	D	Br	H	D	48	D	D	Br	D
	41	H	Br	D	D					

**Table 6.**
*Structure of pyrazoles 32–48.*

those from **35** and **36**. In 1-nitropyrazole **34**, the comparatively low abundance for hydrogen loss might be caused by the absence of hydrogen on the nitrogen rings.

The authors used the CID's corrected spectra of the deuterium analogs **35** and **36**, and they suggested that these—in addition to the **43** and **48** spectra—can be used to correct the spectra of the other compounds for the multiple losses of H (D). For each spectrum, the intensities were normalized for a total abundance of fragmentation equal to 100. From these data, approximate preference factors could be calculated for the collision-induced process $[C_3H_3N_2]^+ \rightarrow [C_3H_3N_2]^{+\bullet} + H^\bullet$. According to the authors, the losses are not specific to **35** or **36**, which suggests that a small amount of energy is required for the process to occur. The most abundant peak for the $[C_3H_3N_2]^+$ ion generated from **34** is *m/z* 39. The authors suggested that this may be due to $[C_3H_3]^+$ and/or $[C_2HN]^{+\bullet}$, formed by the loss of N_2 and/or HCN + H$^\bullet$, respectively. However, based on previous studies [11], it is indicated that $[C_3H_3]^+$ is more likely, due to the loss of N_2 being easier. These losses are compatible for the cyclic 1-pyrazolyl cation *a*, in which the positive charge is shared by all ring atoms **(Figure 23)**.

The differences between the correct CID spectra for $[C_3H_3N_2]^+$ ions generated from **37** and **38** were more difficult to understand. For these data, compounds **39–45** and **46–48** were labeled for further interpretation. These results allowed the authors to identify compositions of various fragment peaks. The ions in the regions between *m/z* 64 and *m/z* 68 do not provide any information on structural differences, but should be responsible for the loss of one or more hydrogens. From the behavior of the labeled compounds, the ions in the *m/z* 50–54 region must be formed by the loss of CH_2, $CH_3^\bullet$, $NH_2^\bullet$, and NH_3, respectively. The structurally significant differences in the *m/z* 67 ions for these compounds are in the *m/z* 36–42 region. The *m/z* 42 ion in **36** retains its marker when it is connected to the nitrogen atom. When the hydrogen's atoms attached to the carbons are labeled, one of them is retained, and this leads to the formation of *m/z* 44 at **40**, whereas in **48** this is absent. Thus, it is possible to conclude that the 4-bromopyrazole ion (**36**) can lose $C_2H^\bullet$. The *m/z* 41 and 40 fragments in **35** and **36** are due to the losses of CN$^\bullet$ and HCN, respectively. In the fragmentation of **35** and **36**, there is a predominant loss of $CH_2N^\bullet$. For compound **36**, the ion $[C_3H_3N_2]^+$ is five times more abundant than when generated from *m/z* 67 in **34** and **35**. This ion only retains its marker when it is attached at the nitrogen—it is not found if *m/z* is greater than 30. Thus, the authors assumed it should be $[NH_2]^+$. The *m/z* 26 and the *m/z* 28–24 fragments were exceptions and have similar abundances in **35** and **36**: *m/z* 28 $[N_2]^{+\bullet}$, *m/z* 27 $[HCN]^{+\bullet}$, *m/z* 25 $[C_2H]^{+\bullet}$, and *m/z* 24 $[C_2]^{+\bullet}$. Thus, the structure of the *m/z* 67 from **35** and **36** can be described as shown in **Figure 24**.
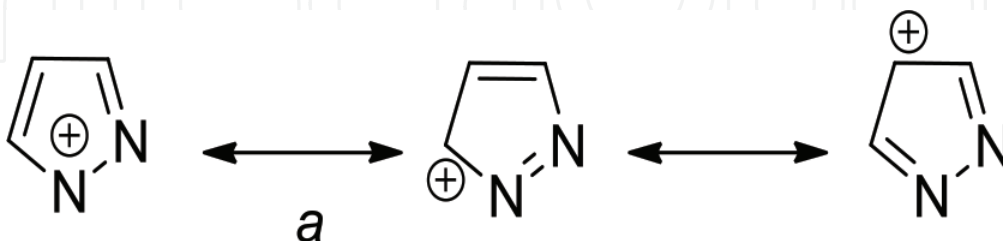
Figure 23.
Resonance structures from pyrazole ion.
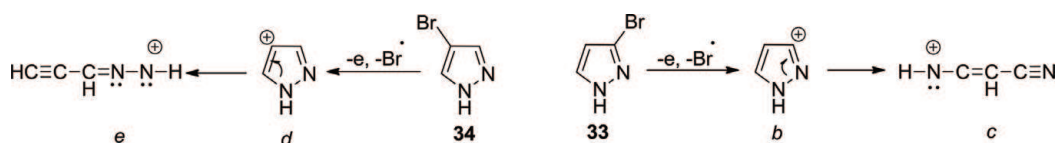
Figure 24.
Linear structure formed from fragmentation of 3(5)bromopyrazole.
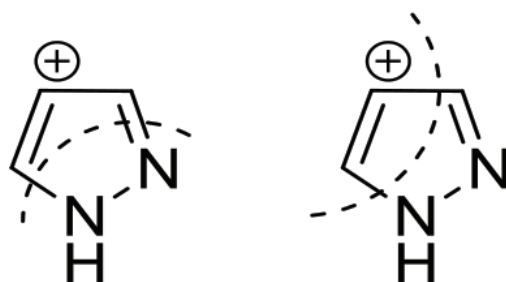
Figure 25.
Two possible cross sections for compound 34, based on the d ion.

The authors suggested that the peaks between *m/z* 41 and *m/z* 38 are formed due to the losses of CN•, HCN, and $CH_2N^\bullet$ and that the generation of $[C_2N]^+$ can be understood from ions *b*, *c*, and *d*; however, it cannot be formed from *e*. Therefore, the majority of $[C_3H_3N_2]^+$ ions formed from **36** have the cyclic structure of *d* rather than the linear structure of *e*. In contrast, most ions formed from **33** have structure *c*. The abundances of the fragments $[C_3]^{+\bullet}$, *m/z* 36 and $[C_3H]^{+\bullet}$, *m/z* 37 decrease in accordance with the following order: **34** > **35** > **36**. The formation of these ions can be explained by ions *a*, *b*, and *c* (**Figure 2**). The ions formed from **34** have the cyclic structure of *a*, whereas those generated from **36** mostly have the structure of *d*. Thus, the authors concluded that only a tiny fraction of the ions formed from **35** are cyclic, which is consistent with the presence of fragments *m/z* 29, $[N_2H]^+$ and *m/z* 42 $[CH_2N_2]^{+\bullet}$, in the corrected CID spectrum of $[C_3H_3N_2]^+$ ions generated from **36**. These fragments are readily formed from *d* (according to **Figure 25**) or *b* (**Figure 24**), but not *c* (**Figure 24**). The level of ion loss can be used advantageously to distinguish isomeric ions from different geometries (e.g., between cyclic and linear species). To achieve a given attenuation of the main beam, more target gas is needed for an apparently smaller cyclic ion than for a linear species.

3. Conclusions

In conclusion, unsubstituted, deuterated 4-chlorine, 4-bromine, phenyl, and methyl pyrazoles have stable molecular ions that generally form the base peak. The tandem fragmentation of these pyrazoles showed two important processes: (i) expulsion of HCN from $[M]^{+\bullet}$ and $[M–H]^+$ and (ii) loss of N_2 from $[M–H]^+$—the former process is the most predominant. However, substitution influences the relative importance of these two processes. While the patterns remain essentially unchanged in 4-bromo(chloro)pyrazoles, the introduction of more bromine atoms makes the initial loss of HCN less important because of the competing loss of bromine radicals. Secondary loss of HCN remains important, however, as it suppresses the expulsion of N_2. The influence of the 4-nitro and 4-acetyl substituents is much more pronounced, because the substituents can be in more than one step, giving rise to the stable ions $[M–O]^{+\bullet}$ and $[M–NO_2]^+$ in the former and to $[M–CH_3]^+$ in the latter case. In these compounds, the loss of HCN, though still strong as a secondary step, is no longer a primary process. When methyl- and nitro- groups are *ortho*-positioned, the pyrazole fragmentation sequence is affected. On the other hand, the 4-cyano and 4-phenyl substituents promote the loss of HCN in such a manner that the ion formed by the loss of the substituent is practically absent. In 4-cyanopyrazole, the initial loss of HCN is very strong, giving an ion with *m/z* 66, while in 4-phenylpyrazole, two consecutive losses of HCN occur. The fragmentation of monodeuterated, dideuterated, and trideuterated pyrazoles was similar to unsubstituted pyrazole but showed that the H• is lost from C–H bond cleavage in an

α-position relative to a nitrogen atom. The fragmentation of deuterated 3,5-diphenylpyrazoles differs from other diphenylpyrazoles; however, two similarities were found with the fragmentation of 4,5-diphenylpyrazoles: (i) the loss of N_2D and N_2H and (ii) the reversible transfer of a phenyl hydrogen to nitrogen, which, in the case of 1,3-diphenyl, occurs prior to or during the initial elimination of N_2D and N_2H. The fragmentation of 1-phenylpyrazole-4-yl-oximes depends on the substituent. The aldoximepyrazole suffered the loss of the following: water, a hydroxyl radical, and aldoxime radicals. In turn, the ketoximes suffered the loss of alkyloxime via a McLafferty-type rearrangement. Benzyl oxime pyrazoles behave differently from other compounds. Besides the anticipated loss of oxygen and a hydroxyl radical from the molecular ion, the unexpected ejection of NO, HNO, and H_2NO occurs. It appears that the molecular ions of these oximes do not undergo the Beckmann rearrangement, but rather a rearrangement of the oximino-hydrogen atom, which has not yet been encountered in the mass spectra of oximes. Finally, it is important to note that unfortunately the experimental gas chromatographic conditions were not informed by most of authors. Only Luijten and van Thuijl [4] informed the purity of compounds and it was checked on a GCMS system, using a 5% SE-30 packed column.

Acknowledgements

Conflict of interest

There are no conflicts to declare.

Author details

Clarissa Piccinin Frizzo*, Bruno Luís Hennemann, Bruna Luisa Kuhn,
Keli Maiara Wust, Alisson Vasques Paz, Marcos Antonio Pinto Martins,
Nilo Zanatta and Hélio Gauze Bonacorso
Department of Chemistry, Núcleo de Química de Heterociclos (NUQUIMHE),
Federal University of Santa Maria, Santa Maria, Brazil

*Address all correspondence to: clarissa.frizzo@gmail.com

IntechOpen

References

[1] Faria JV, Vegi PF, Miguita AGC, Dos Santos MS, Boechat N, Bernardino AMR. Recently reported biological activities of pyrazole compounds. Bioorganic and Medicinal Chemistry. 2017;**25**:5891-5903

[2] van Thuijl J, Klebe KJ, van Houte JJ. The mass spectra of some pyrazole compounds. Organic Mass Spectrometry. 1970;**3**(12):1549-1559. DOI: 10.1002/oms.1210031213

[3] van Thuijl J, Klebe KJ, Van Houte JJ. The mass spectra of some deuterated pyrazoles. Journal of Heterocyclic Chemistry. 1971;**8**(2):311-312. DOI: 10.1002/jhet.5570080227

[4] Luijten WCMM, van Thuijl J. Mass spectrometry of nitroazoles. 1—The mass spectra of methyl substituted nitropyrazoles. Journal of Mass Spectrometry. 1979;**14**(11):577-584. DOI: 10.1002/oms.1210141102

[5] van Thuijl J, Klebe KJ, Van Houte JJ. Preference factors and isotope effects in the loss of H. (D.) and HCN (DCN) from deuterated pyrazoles upon electron-impact. Journal of Mass Spectrometry. 1971;**5**(9):1101-1105. DOI: 10.1002/oms.1210050910

[6] van Tilborg MWEM, van Thuijl J. Structure determination of [C3H3N2]+ ions, formed from monosubstituted pyrazoles C3H3N2R, by collision-induced dissociation mass spectrometry. Journal of Mass Spectrometry. 1984;**19**(11):569-572. DOI: 10.1002/oms.1210191108

[7] Bowie JH, Cooks RG, Lawesson S, Schroll G. Electron impact studies. XII. Mass spectra of substituted imidazoles. Australian Journal of Chemistry. 1967;**20**:1613-1624. DOI: 10.1071/CH9671613

[8] Aldous GL, Bowie JH. Electron-impact studies XCV-skeletal rearrangement fragments in the mass spectra of 3,5-diphenylisoxazole and 3,5-diphenylpyrazole. Journal of Mass Spectrometry. 1975;**10**(1):64-68. DOI: 10.1002/oms.1210100110

[9] Finar IL, Millard BJ. High-resolution mass spectrometry. Part VI. A hydrogen rearrangement in the mass spectra of pyrazole oximes. Journal of the Chemical Society C. 1969;**0**(19):2497-2499. DOI: 10.1039/J39690002497

[10] Khmel'nitskii RA, Krasnoshchek AP, Polyakova AA, Grandberg II. Mass spectra and structure of organic compounds. XX. Mass spectra of pyrazole and methylpyrazoles. Zhurnal Organicheskoi Khimii. 1967;**3**(9): 1540-1546 CA 68, 34199r (1968)

[11] Grubb HM, Meyerson S. In: McLafferty FW, editor. Mass Spectrometry of Organic Ions. New York: Academic Press; 1963. pp. 516-519 eBook ISBN: 9780323142779