Mass Spectrometry of Nitroazoles

3—*Ortho* Effects: The Loss of OH$^{\cdot}$ and H_2O from Methyl Substituted Nitrodiazoles

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Methyl substituted nitrodiazoles which have the substituents at adjacent positions in the ring are subject to several *ortho* effects. Deuterium labelling of the methyl group and the mobile N-bonded hydrogen show that the loss of OH$^{\cdot}$ originates from the substituents. In some cases the N-bonded hydrogen atom participates also in the loss of OH$^{\cdot}$ and of H_2O.

INTRODUCTION

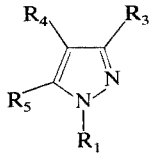
Aromatic compounds having two substituents in adjacent positions may be subject to *ortho* effects. Apart from the diagnostic usefulness such effects can be of considerable value in mechanistic studies. Often, the direct interaction of two substituents enables the *ortho* isomer to be distinguished from the *meta* and *para* substituted isomers.[1] One of the best known *ortho* effects is the loss of a hydroxyl radical, resulting from interaction between nitro and hydrogen containing substituents.[2] A classical example is the loss of OH$^{\cdot}$ from *o*-nitrotoluene, reported by Meyerson.[1a]

Most of the *ortho* effects reported are observed with arenes, and only a few have been found in heterocyclic aromatic compounds. In nitro substituted imidazole derivatives, losses of OH$^{\cdot}$ and H_2O have been attributed to *ortho* effects[3] and an unusual fragmentation was observed in complex imidazole compounds carrying alkyl and nitro substituents.[4]

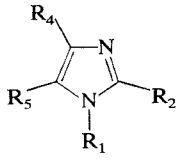
A different kind of interaction between neighbouring substituents is observed when a fragmentation is triggered via migration of an atom or a group of atoms within the molecule.[5] Schwarz and co-workers[5b] investigated the elimination of methyl radicals in *ortho* substituted benzoic acid methyl esters. It was shown that most of the reactions are induced by a hydrogen transfer to the carbonyl function. Generally speaking, fragmentations leading to the expulsion of a radical or a neutral species originating from two directly interacting substituents in, for example 1,2-disubstituted arenes, can be classified as *ortho* effects, while the latter processes are due to proximity effects or, as sometimes called, neighbouring group effects.[5] More examples of these effects are treated in a recent review by Schwarz.[6]

In our studies on the mass spectrometric behaviour of methyl substituted nitropyrazoles[7] and -imidazoles[8] we reported the occurrence of several *ortho* effects. These can be divided into three types: (1) hydrogen atom migration in a fragment ion, to a neighbouring position following elimination of the nitro substituent from this position; (2) hydrogen transfer from the methyl group or the diazole ring to the nitro group followed by losses of OH$^{\cdot}$ and H_2O; (3) oxygen transfer from the nitro group to the methyl substituent followed by loss of CHO$^{\cdot}$ and CH_2O. The first two processes will be treated in this study, the third type will be the subject of an accompanying paper.[9]

The following compounds have been studied.



	R_1	R_3	R_4	R_5
1	H	NO_2	H	H
2	CH_3	H	H	NO_2
3	H	CH_3	H	NO_2
4	H	NO_2	CH_3	H
5	H	CH_3	NO_2	H



	R_1	R_2	R_4	R_5
6	H	NO_2	H	H
7	CH_3	NO_2	H	H
8	CH_3	H	H	NO_2
9	H	H	CH_3	NO_2

A number of labelled compounds have also been studied: the $-CD_3$ and $-^{13}CH_3$ labelled analogues of **2**, **7** and **8** and the $-CH_2D$, $-CHD_2$ and $-CD_3$ analogues of **5**.

RESULTS AND DISCUSSION

H migration after loss of $NO_2^{\cdot}$

Migration of an atom or a group of atoms in a fragment ion to a vacant *ortho* position is not restricted to aromatic compounds carrying a nitro group.[10] In (aromatic) hydrocarbon compounds migration of hydrogen atoms or methyl groups are well known. Quite often only the study of specifically labelled compounds permits the conclusion that a migration precedes a fragmentation.[11]

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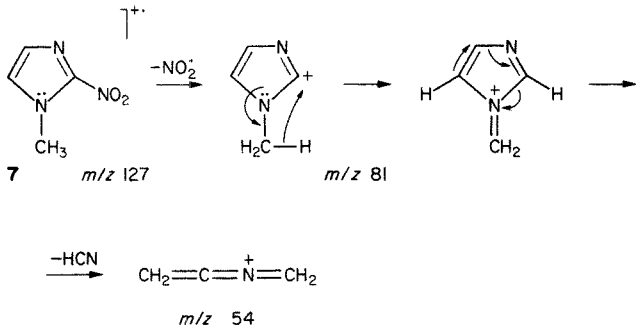
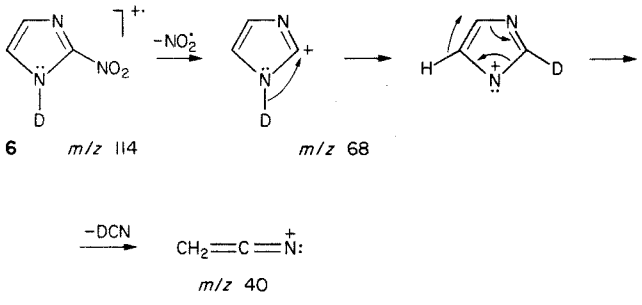


7 *m/z* 127 *m/z* 81 *m/z* 54

Scheme 1. Loss of HCN from the $[M-NO_2]^+$ ion in 1-methyl-2-nitroimidazole (**7**).

1-Methyl-2-nitroimidazole (**7**) expels HCN from the $[M-NO_2]^+$ ion, yielding a fragment at *m/z* 54.[8] Upon labelling with $^{13}CH_3$ the label is completely retained in the ion resulting from the $[M-NO_2]^+$ ion, while the CD_3 analogue shows a specific loss of DCN. The same behaviour can be observed for these decompositions in the field free regions. From various observations[8] it can be concluded that loss of HCN in 1-methylimidazoles involves mainly the C-2 and N-3 atoms of the diazole ring. The mechanism depicted in Scheme 1 is consistent with this view. Similar behaviour is observed for the loss of HCN from the $[M-NO_2]^+$ ion of 2-nitroimidazole (**6**), *m/z* 67 → *m/z* 40, where the N–D labelled compound specifically loses DCN to give *m/z* 40.[12] The exclusive loss of label can only be explained assuming a migration of the N-bonded hydrogen atom to the vacant *ortho* position at C-2 (Scheme 2). The same fragmentations also occur in the field free regions.

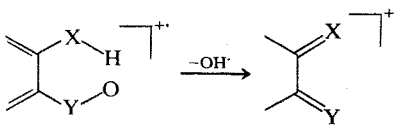


6 *m/z* 114 *m/z* 68 *m/z* 40

Scheme 2. Loss of DCN from the $[M-NO_2]^+$ ion in N-deuterated 2-nitroimidazole (**6**).

Loss of OH·

The migration of a hydrogen atom is often observed when suitable substituents are at such a close distance that rearrangement through a cyclic transition state can occur.[2]



Well known examples are the loss of OH· from *o*-alkyl substituted nitrobenzenes.[1] Noteworthy is the observation that also *meta* and *para* substituted alkyl nitrobenzene derivatives may expel OH·. It has been established that the nitro group abstracts a hydrogen atom from the *ortho* position in, for instance, methyl-*p*-nitrobenzoate. However, a second hydrogen transfer from the substituent to the ring precedes the cleavage of the OH moiety.[13]

Table 1 lists the compounds subject to loss of OH· as a primary process. The CD_3 labelled analogues of **2**, **5** and **8** all lose OD· exclusively. Upon exchange of the mobile hydrogen at N-1 with deuterium **1** and **3** lose OD· while **4**, **5** and **9** still expel OH·.

These results indicate that the hydrogen atom in the OH· loss in the isomers with substituents on adjacent positions (compounds **2**, **4**, **5**, **8** and **9**) originates exclusively from the methyl group. It is very likely that the expulsion of OH· proceeds according to a mechanism similar to the loss of OH· in *o*-nitrotoluene.[1a]

Compound **5**, 3(5)-methyl-4-nitropyrazole, was chosen for a more detailed study involving partially labelled methyl groups: 3(5)-monodeuteromethyl- and 3(5)-dideuteromethyl-4-nitropyrazole were prepared and studied, along with the CD_3 labelled analogue of **5**. The results are presented in Table 2. The kinetic

Table 1. Relative abundances of $[M]^{+\cdot}$ and $[M-OH]^+$ ions (as % of total ion current).

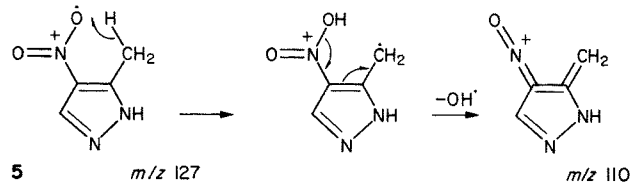
	$[M]^{+\cdot}$	$[M-OH]^+$
1	21	1
2	24	14
3	44	1
4	25	16
5	22	26
8	47	2
9	29	4

Table 2. Loss of OH·/OD· in labelled analogues of 3(5)-methyl-4-nitropyrazole (5)

	-OH·	-OD·[a]	-OH·	-OD·[b]	-OH·	-OD·[c]
$-CH_3$	100	—	100	—	100	—
$-CH_2D$	67	33	79	21	90	10
$-CHD_2$	33	67	53	47	70	30
$-CD_3$	—	100	—	100	—	100

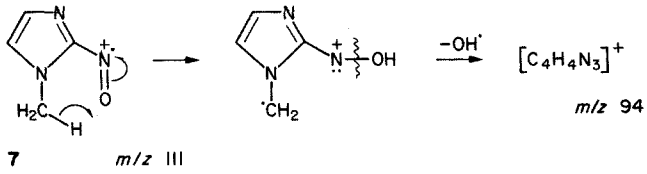
[a] Calculated assuming the methyl group as the exclusive source of hydrogen. [b] source reaction. [c] metastable ion intensities (via *B/E*-scan).

isotope effect in the primary loss of OH· as a source reaction is calculated to be 1.9 for the CH_2D compound and 2.3 for the CHD_2 compound. Both values are in fair agreement with the isotope effect found for the loss of OH· in *o*-nitrotoluene ($k_H/k_D = 2.1$).[1a] The results calculated for the metastable ion intensities yield an isotope effect of 4.6, a value compatible with transfer of a hydrogen atom in the rate-determining step (Scheme 3).



5 *m/z* 127 *m/z* 110

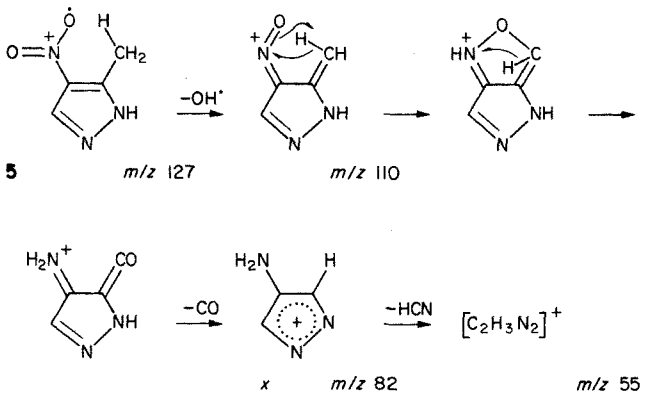
Scheme 3. Mechanism of expulsion of OH·.



Scheme 4. Secondary *ortho* effect in 1-methyl-2-nitroimidazole (**7**).

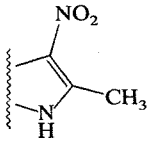
Contrary to what might be expected not all isomers with the methyl and nitro groups in an *ortho* position lose OH· from the molecular ion. Exceptions are 1-nitro-5-methylpyrazole[7] and 1-methyl-2-nitroimidazole (**7**). The latter compound can be seen to lose OH· from the $[M-O]^{+\cdot}$ ion.[8] This is an example of an *ortho* effect in a secondary fragmentation process. The origin of the hydrogen atom in OH· is the methyl group, as can be seen in the CD_3 labelled analogue of **7**. The same fragmentation is found in isomer **8**.

Previously, the loss of CO and HCN was reported,[7] similar to the behaviour of the $[M-OH]^+$ ion in *o*-nitrotoluene.[1a] This reaction occurs from the $[M-OH]^+$ ion of isomers **5** and **9**. The fragmentation sequence can be represented according to Scheme 5.

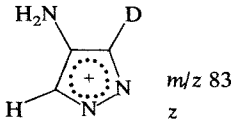


Scheme 5. Secondary losses of CO and HCN in 3(5)-methyl-4-nitropyrazole (**5**).

The fact that compounds **2**, **4** and **8** do not show this sequence, though they are capable of yielding $[M-OH]^+$ fragments by means of the mechanism in Scheme 5, may depend on the absence of an adjacent mobile hydrogen atom. Compounds **2** and **8** do not have such a hydrogen atom available and are unable to generate a stable aminodiazole *x* via this mechanism. Instead, the $[M-OH]^+$ ions of **2** and **8** lose NO·.[7] In **4** there is no mobile hydrogen atom adjacent to the methyl group. Therefore, no stable aminodiazole can be formed via a hydrogen transfer from the N-1 position to the vacant C-5 (Scheme 6). Apparently the positional requirement for the sequence $[M]^{+\cdot} \rightarrow [M-OH]^+ \rightarrow [M-OH-CO]^+ \rightarrow [M-OH-CO-HCN]^+$ in methyl substituted nitrodiazoles is the following:

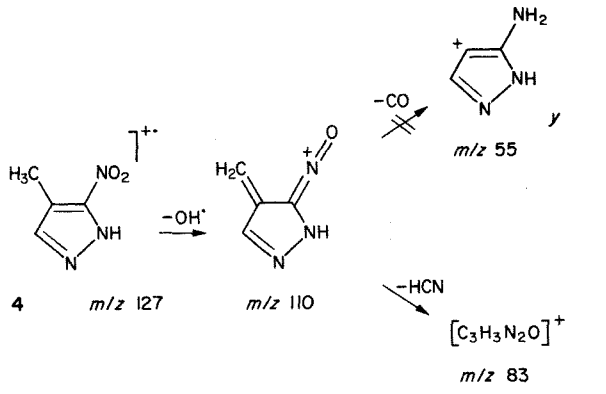


Additional proof comes from the labelling experiments. When the mobile N-bonded hydrogen atom in **5** is replaced by deuterium, the loss of OH· and CO from the molecular ion would yield ion *z*, *m/z* 83, according to the proposed mechanism (Scheme 5):

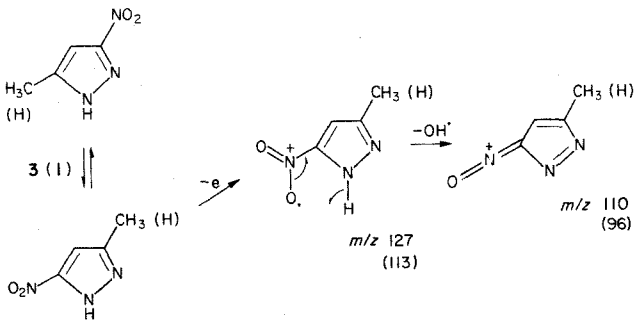


The unlabelled ion *x* (Scheme 5) is capable of expulsion of HCN and, since its proposed structure is symmetrical ion *z* should—apart from a secondary isotope effect—expel HCN/DCN in a 1:1 ratio. For this ratio we found 0.98, in excellent agreement with the mechanism given in Scheme 5 and strongly supporting the mechanism originally put forward by Meyerson for *o*-nitrotoluene.[1a]

In the mass spectra of compounds **1** and **3** a $[M-OH]^+$ fragment of low abundance is observed. Compared with the other isomers subject to loss of OH·, the geometry of **1** and **3** is different: no adjacent substituents are present. Therefore, the mechanism for loss of OH· should be different. The N-deuterated analogues exclusively lose OD·, indicating that the hydrogen atom involved originates from the N-1 position. This also suggests that the reactive tautomers for this process are 5-nitropyrazole (**1**) and 3-methyl-5-nitropyrazole (**3**) respectively (Scheme 7).



Scheme 6. Secondary loss of HCN in 4-methyl-3(5)-nitropyrazole (**4**).



Scheme 7. Loss of OH· in 3(5)-methyl-5(3)-nitropyrazole (**3**) and 3(5)-nitropyrazole (**1**).

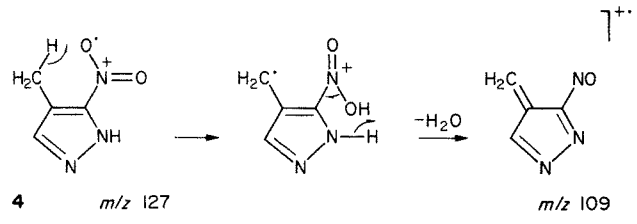
Loss of H_2O

The only compound in the pyrazole series subject to loss of H_2O is **4**, 4-methyl-3(5)-nitropyrazole. In the mass spectrum metastable peaks are found at *m/z* 95.3 and 93.6, due to loss of OH˙ and H_2O respectively. Upon exchange with D_2O the following metastable peaks are observed (Table 3). No metastable peak was

Table 3. Loss of OH˙ and HDO in N-deuterated 4-methyl-3(5)-nitropyrazole (4).

	m*	$[M]^{+\cdot}$	Fragment	Neutral lost
m/z	96.5	128 (d_1)	111	OH˙
m/z	95.3	127 (d_0)	110	OH˙
m/z	92.8	128 (d_1)	109	HDO

found at *m/z* 94.5 (*m/z* 128 → *m/z* 110), indicating that neither loss of OD˙ nor H_2O occurs from the labelled compound. This is in agreement with the proposed mechanism for the loss of OH˙ and also with the supposition that loss of OH˙ by this mechanism (Scheme 3) is favoured over a mechanism involving the N-bonded hydrogen atom. Moreover, it shows that in the case of H_2O loss one hydrogen atom originates from the N-1 position and the second one apparently from the methyl group. Therefore it constitutes a *double ortho effect* (Scheme 8). The abundance ratio for the double versus the single *ortho* effect is approximately 1:20. A similar reaction, probably based on the same mechanism, has been reported for 4(5)-methyl-5(4)-nitroimidazole (**9**).[3a] However, here the ratio of H_2O/OH˙ loss is 1:1. In fact this difference can be attributed to the relative abundances of the $[M-OH]^+$ ions in the pyrazole and the imidazole isomers. In the pyrazole compounds **2**, **4** and **5** these ions are five to eight times more abundant compared with the imidazole compounds **8** and **9**. At present this difference in behaviour is not well understood.



Scheme 8. Loss of H_2O in 4-methyl-3(5)-nitropyrazole (**4**).

CONCLUSIONS

Nitroazoles can be subject to *ortho* effects when two substituents are situated on adjacent positions in the ring. Loss of OH˙ from methyl substituted nitrodiazoles is a useful probe in determining the location of the substituents in the ring. In this respect they resemble the fragmentation behaviour of *o*-alkyl substituted nitrobenzene derivatives. Thus, it could be established that when the methyl and nitro groups are adjacent the loss of OH˙ originates exclusively from these substituents. However, not all compounds with adjacently positioned substituents show loss of OH˙, exceptions being 1-nitro-5-methyl pyrazole[7] and 1-methyl-2-nitroimidazole (**7**). The latter compound exhibits the loss of OH˙ as a secondary *ortho* effect.

Use of partially labelled methyl groups in **5**, 3(5)-methyl-4-nitropyrazole provides a means to compare the proposed mechanisms for the loss of OH˙ from **5** and from *o*-nitrotoluene. The two compounds behave quite similarly in the expulsion of OH˙ and in the subsequent reactions from the $[M-OH]^+$ ions.

In addition to the loss of OH˙ the presence of a mobile N-bonded hydrogen atom in some nitrodiazoles leads to the expulsion of a H_2O molecule. The remarkable difference in ratio of OH˙/H_2O loss in compounds **4** and **9** is caused by the relative abundances of the $[M-OH]^+$ ions. It is striking that the isomers with adjacent substituents in the pyrazole series show a more abundant $[M-OH]^+$ ion than the corresponding imidazole isomers.

EXPERIMENTAL

Mass spectral data were obtained with an MS 902 mass spectrometer under the following conditions: ion source temperature, 180 °C; trap current, 100 μA; accelerating voltage, 8 kV; electron energy, 70 eV. Samples were introduced through an all-glass heated inlet system at temperatures of 160–200 °C. Elemental compositions of all ions were determined at resolving powers above 15 000; high resolution measurements of deuterium labelled compounds were performed at a resolving power of 25 000. Fragmentation schemes were derived from accelerating voltage scans at a trap current of 500 μA, using an energy resolving variable monitor slit, set at 80% transmission to obtain symmetrical peak shapes and consequently improved mass determinations. During the course of the investigations the MS 902 was converted to a Kratos MS 9/50, equipped with a metastable scan unit (Mk II). This enabled us to use the *B/E* linked scan technique[14] on the compounds. The results were in complete agreement with those obtained through the high voltage scans.

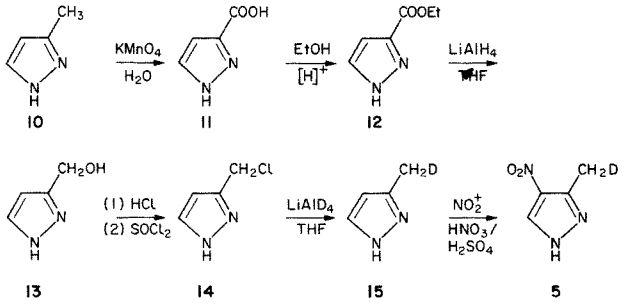
Preparation of compounds

The syntheses of the methyl substituted compounds has been described earlier.[7,8]

3(5)-Nitropyrazole (1). A solution of 3 g 1-nitropyrazole[15] in 30 cm^3 benzonitrile was refluxed for 2 h. After being cooled the mixture was poured into 150 cm^3 *n*-hexane. A voluminous white solid precipitated; after filtration and drying the solid was heated in boiling benzene to remove all of the benzonitrile. Filtration and drying under reduced pressure afforded 2.4 g of **1** (79%). M.p. 173 °C (lit.[16] 174–175 °C).

2-Nitroimidazole (6). **6** was prepared by a diazotization reaction in the presence of nitrite ions[17] from 2-aminoimidazole.[18]

3(5)-Monodeuteromethyl-, 3(5)-dideuteromethyl- and 3(5)-trideuteromethyl-4-nitropyrazole. These compounds were prepared according to Scheme 9. 3(5)-Methylpyrazole (**10**)[19] (4 g) was dissolved in 200 cm^3 H_2O. To this solution 16 g $KMnO_4$ was added and the mixture was allowed to stand at room temperature. After 30 min the mixture was gently heated to reflux for about 2 h. The mixture was filtered off after cooling and the solution evaporated to a smaller volume under reduced pressure. Recrystallization from acidified H_2O (pH 2) yielded 3.4 g **11** (63%).



Scheme 9. Synthesis of the partially deuterated analogues of 3(5)-methylpyrazole.

The corresponding ethyl ester (**12**) was prepared by refluxing 3.4 g **11** in 50 cm^3 absolute ethanol, to which was added 1 cm^3 concentrated sulphuric acid. The reaction mixture was concentrated by evaporation and dissolved in H_2O. The solution was neutralized with sodium bicarbonate (pH 8). Extraction with ethyl acetate and recrystallization yielded 3.1 g **12** (75%), m.p. 158 °C (lit.[20] 158 °C).

Reduction to 3(5)-hydroxymethylpyrazole (13). To a solution of 1.1 g $LiAlH_4$ (Merck) in 75 cm^3 THF were added 1.8 g **12** in 80 cm^3 THF. The mixture was kept at room temperature whilst stirring for 1 h. The excess hydride was decomposed with 20 cm^3 diluted sulphuric acid and the solvent was removed by evaporation under reduced pressure. Repeatedly carried out extractions of the slurry with hot absolute methanol and evaporation of the filtrate afforded 900 mg of crude **13** (71%), which is a colourless viscous liquid. The product was used in the subsequent reactions without further purification. After converting **13** to the hydrochloric salt the product was dissolved cautiously in 5 cm^3 $SOCl_2$. After this addition the mixture was refluxed for 20 min and allowed to cool. Evaporation of the thionyl chloride and repeated washings with dry ethyl ether afforded 991 mg **14** (96%), isolated as a white crystalline solid, m.p. 153–155 °C (lit.[20] 155–156 °C).

Reduction to 3(5)-monodeuteromethylpyrazole 15. To a stirred solution of 500 mg $LiAlD_4$ (Aldrich, 98 atom % D) in 60 cm^3 THF was added 900 mg **14.** The reaction mixture was allowed to stand at room temperature for 1 h whilst stirring. After decomposition of the excess deuteride with dilute sulphuric acid and filtration, the filtrate was evaporated to a small volume and dissolved in aqueous alkali (pH 10). Extraction with diethyl ether yielded 370 mg of crude **15** (75%) as a colourless liquid. Without further purification **15** was nitrated following the procedure for **3.**

3(5)-Dideuteromethylpyrazole was synthesized by using $LiAlD_4$ in the first and $LiAlH_4$ in the second reduction step.

3(5)-Trideuteromethylpyrazole was prepared using $LiAlD_4$ in both reduction steps. Melting points were the same as reported for unlabelled **5**. Measurements at 10 eV revealed the following D-contents: $-CH_2D$ compound, 0.4% d_2, 99.0% d_1 and 0.6% d_0; $-CHD_2$ compound, 0.2% d_3, 99.3% d_2 and 0.5% d_1; $-CD_3$ compound, 99.6% d_3, 0.3% d_2 and 0.1% d_1.

The exchange of the mobile N-bonded hydrogen atom by deuterium was performed by introducing D_2O and the sample via the all-glass heated inlet system simultaneously. Exchange of H by D varied from 60–90% depending on the compound. Ion abundances given are corrected for incomplete deuteration.

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