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Fragmentation and Skeletal Rearrangements of the Protonated Spiro(2H-benzimidazole-2-4′-pyrazole)-5′-one Dyes Studied by Electrospray Ionization and Liquid Secondary Ion Mass Spectrometry

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The mass spectrometric decomposition of the titled compounds was studied by using electrospray ionization (ESI) and liquid secondary ion mass spectrometry (LSIMS) as a methods for $[M+H]^+$ ions generation. Low-energy collision induced dissociation (fragmentation "in source") mass spectra for ESI and B/E linked scan mass spectra of metastable ions for LSIMS were performed. In order to better understand the decomposition of the compounds studied, the mass spectra of isotopically labelled compounds were recorded. The fragmentation pathways of $[M+H]^+$ ions were found to be complex and skeletal rearrangements were observed. It was deduced that subsequent loss of NH_3 and H_2O molecules leads to the formation of ions with polycyclic structures. The fragment ion $[133]^+$ and its complementary fragment ion $[M+H-132]^+$ can be considered as protonated molecules of 3-methyl-1*H*-indazole and 2-hydroxyquinoxaline, respectively. Loss of the CH_3CN molecule also occurs and this is rather simply process. Aniline elimination (H_2N-C_6H_5) and formation of ions at m/z 146 are complex processes and it was difficult to propose plausible mechanisms for these reactions.

Key words: spiro compounds, electrospray ionization, liquid secondary ion mass spectrometry, mass spectrometric fragmentation pathways

Pyrazolone azomethine purple dyes (PAM) are commonly used in color photography. Recently, these dyes in the presence of proper electron donors have been tested as visible light free radical polymerization initiators [1–3]. Similar studies were also performed for selected spiro(2H-arylimidazole-2-4′-pyrazole)-5′(1′H)-one dyes [4]. Spiro(2H-benzimidazole-2-4′-pyrazole)-5′-one (SBP) dyes are used for the synthesis of 1H-pyrazolo[3,4-b]quinoxalines [5], which are a new group of very efficient dyeing photoinitiators [6]. This paper describes the mass spectrometric fragmentation pathways of protonated derivatives (**1–4**). The obtained results can be useful in the determination of by-product structures formed during synthesis of this class of compounds. The compounds studied are shown in Figure 1.

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In order to generate the $[M+H]^+$ ions both liquid secondary ion mass spectrometry (LSIMS) and electrospray ionization (ESI) were utilized.

It is also worth mentioning that there are few publications devoted to mass spectrometric studies of spiro compounds. FAB induced decomposition of bis[N-(diphenylphosphanylselenoyl)-P,P-diphenylphosphanylselenoic amidato-Se,Se′]selenium(II) ([Se{$NSePPh_2$)$_2$-Se,Se′}$_2$]) has been observed by Cea-Oliveras *et al.* [7]. Siebert and co-workers have reported the EI mass spectra of two spiro compounds containing boron [8], some spirooxazines were used to study MALDI process [9]. Ofuji *et al.* have used LC/ESIMS for the quantitative determination of some azaspiracids being marine toxins [10]. Spiro compounds derived from cyclopropane amino acid were studied by APCI multi stage mass spectrometry [11]. Brombacher *et al.* have described in details the mass spectrometric fragmentation pathways of some azaspiracid biotoxins [12]. Liu *et al.* have reported the ESIMS fragmentation of protonated and sodiated molecules of pentacoordinated bisaminoacylspirophosphoranes [13].

In order to confirm that the observed mass spectrometric decomposition of **1**–**4** is a feature of spiro systems, the fragmentation pathways of pyrazolone azomethine dyes (PAM) were analyzed as well (**5a**–**d**). The hydrogens of the methyl group of **1**–**4** were found to be important for the skeletal rearrangement observed. Thus, the 1,3-dihydro-3′-phenyl-1′-phenylspiro(2H-benzimidazole-2-4′-pyrazole)-5′(1′H)-one (**6**) was synthesized and its fragmentation was compared with those observed for **1**–**4**.

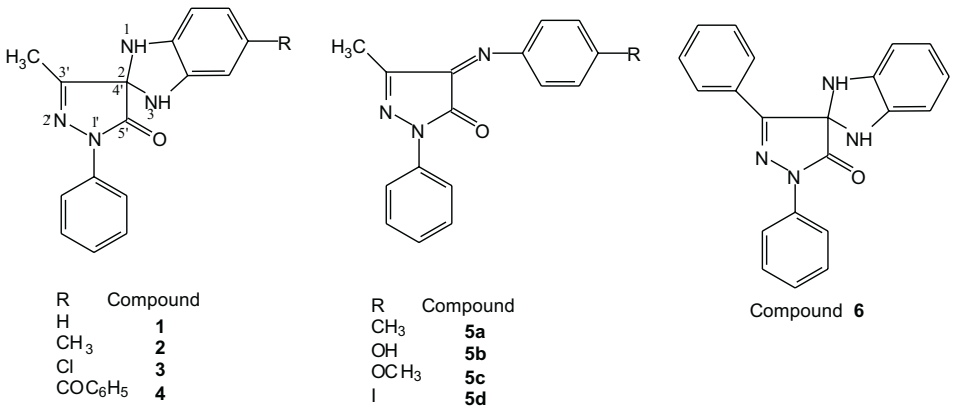

Figure 1. Compounds studied.

EXPERIMENTAL

Substrates used for the preparation of dyes were purchased from Fluka, Merck or Aldrich. Tested dyes were prepared by the sequences of reactions shown in Figure 2.

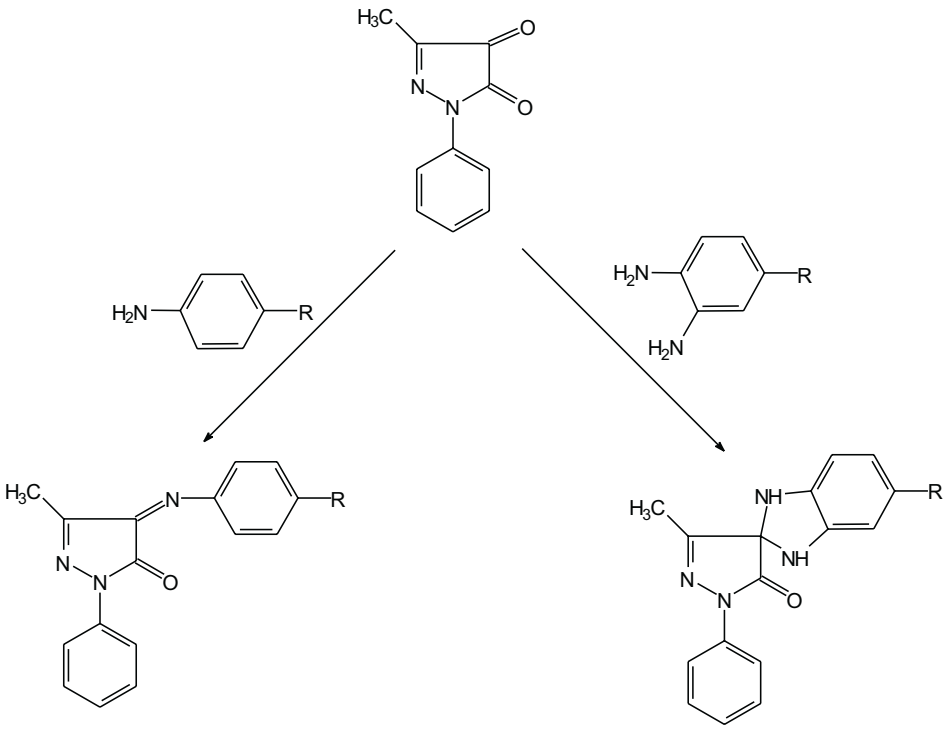

Figure 2. Synthesis of compounds studied.

Substituted derivatives of 4-phenylimino-5-methyl-2-phenyl-2,4-dihydro-pyrazol-3-one and 5-methyl-2-phenyl-2H-pyrazole-3,4-dione were prepared using the method described by Tacconi *et al.* [14]. 1,3-Dihydro-3′-methyl-1′-phenylspiro(2H-benzimidazole-2-4′-pyrazole)-5′(1′H)-one and its derivatives were synthesized by the method given by Metwally *et al.* [5]. A similar procedure was used for the synthesis of compound **6**. The crude products were purified using column chromatography and finally by preparative thin layer chromatography. The final products were identified by ^{1}H NMR and ^{13}C NMR spectroscopy (Varian Gemini 200). The spectra obtained confirmed that the dyes had the desired structures.

The LSI mass spectra were obtained on the AMD 604 two sector mass spectrometer of the reverse B/E geometry, made by AMD Intectra (Germany). A CsI gun supplied the primary ion beam (12 keV, Cs^{+}). The secondary ion beam was accelerated to 8 kV. The compounds were dissolved in NBA (3-nitrobenzyl alcohol) or in DTE/DTT (dithiothreitol/dithioerythritol 1:5); both matrices were obtained from Aldrich. The metastable decays of the $[M+H]^{+}$ ions were analyzed by B/E linked scan mass spectra.

The ESI mass spectra of compounds studied were obtained on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer equipped with a Harvard Apparatus syringe pump. The sample solutions were prepared in methanol/water (1:1) at a concentration of approximately 10^{-5} M. The ESI source potentials were capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage 45 V. The last parameter is important because at 30 V the "in source" fragmentation was not observed. The source temperature was 120°C and the desolvation temperature was 300°C. Nitrogen was used as the nebulizing and desolvating gas at flow-rates of 100 and 300 l h^{-1}, respectively. The liquid chromatography/mass spectrometric analyses

(HPLC/MS) were performed on the same instrument, which was coupled with a Waters model 2690 HPLC pump (Milford, MA USA). The samples were injected by using an autosampler onto a Nova Pak C_{18} RP column (150×3.9 mm id., Waters). The flow rate was 0.5 ml min^{-1}. A gradient of acetonitrile and 5% formic acid in water was applied. The linear gradient started from 0% CH_3CN reaching 95% CH_3CN after 15 minutes and the latter concentration was maintained for 5 minutes.

In order to obtain the ESI mass spectra of deuterium labelled compounds the sample solutions were prepared in CH_3OD/D_2O (1:1). For LSI mass spectra the matrix solution was mixed with an excess of CH_3OD/D_2O and then evaporated. Both CH_3OD and D_2O were obtained from Aldrich.

RESULTS AND DISCUSSION

In the synthesis of **2–4** (but not **1**, Figure 1) two geometric isomers are formed. These isomers are distinguishable by HPLC/MS analyses on the basis of total ion chromatograms (TIC) and single ion chromatograms (SIC) of the $[M+H]^+$ ions, however, their fragmentation pathways were found to be identical.

The fragmentation patterns of **1–4** observed under LSI (metastable decays) and ESI (fragmentation "in-source") conditions were found to be similar, regardless of differences in relative intensities (ri) of the observed peaks. In Figure 3 the LSI B/E mass spectrum and the ESI mass spectrum of **2** are shown as the representative examples.

Subsequent loss of NH_3 and H_2O molecules: In the ESI mass spectra, loss of mass 17, NH_3 molecule, and loss of mass 35, NH_3 and H_2O molecules, were observed. Elimination of NH_3 was also observed in LSI B/E mass spectra and in LSI mass spectra as the result of "in source" fragmentation as shown in Figure 4a for **2** (m/z 276). In Figure 4b the B/E mass spectrum of the $[\mathbf{2}+H-17]^+$ ion is shown, and the loss of water is observed (m/z 258).

In order to understand how the loss of NH_3 and H_2O proceeds from the protonated compounds, the mass spectra of the labelled compounds were recorded.

Dissolving of compounds **1–4** in CH_3OD/D_2O leads to H/D exchange at N(1) and N(3) atoms. In the mass spectra recorded for these isotopically labelled compounds we observed $[M(D_2)+D]^+$ ions with m/z 3 units higher than the respective $[M+H]^+$ ions. The mass shifts of the fragment ions depend on how many deuterium atoms are eliminated. In Figure 5 the LSI mass spectrum of the labelled **2** is shown.

For the deuterium derivatives, the NH_2D molecule is eliminated rather than the NH_3 molecule as indicated by the m/z value of the fragment ions (*e.g.* 278 for **2**, Figure 5). This fragmentation was also confirmed by B/E mass spectra of the $[M(D_2)+D]^+$ ions. It seems likely that the hydrogen atoms originate from the methyl group and eliminated nitrogen atom is N(1) or N(3). A plausible mechanism for ammonia elimination is shown in Figure 6.

The fragment ion formed can exist in two tautomeric forms and for both tautomers there are several possible resonance structures, some of which are shown in Figure 6. The existence of these tautomeric forms leads to H/D exchange between the nitrogen and carbon atoms of newly formed six-membered ring (Figure 6).

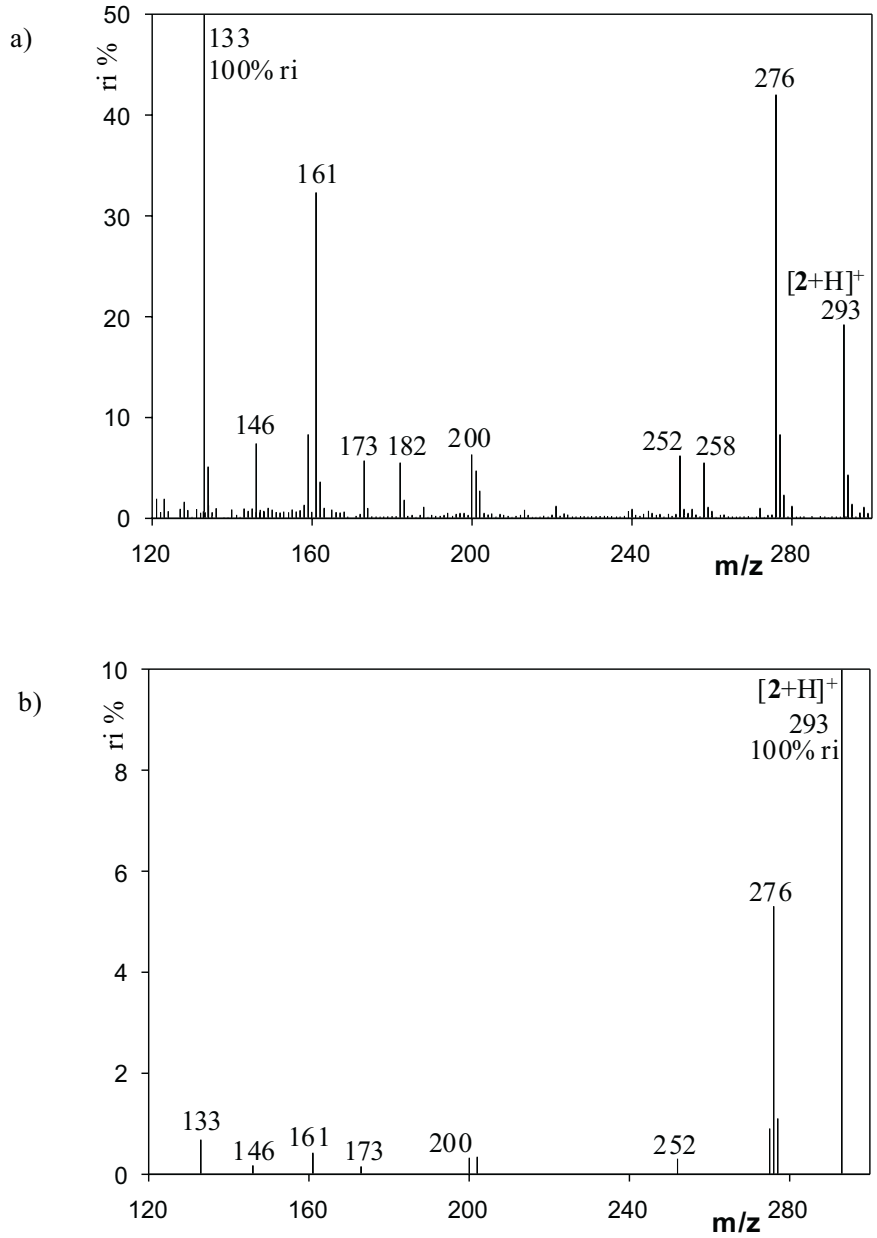

Figure 3. The ESI mass spectrum of **2** (a) and the B/E mass spectrum of the $[2+H]^+$ ion (b).

Loss of the water molecule for the isotopically labelled compounds **1–4** occurs as HDO or H_2O elimination, but not as D_2O elimination. This is clearly seen in the B/E mass spectrum of fragment ions formed after ammonia loss from the $[\mathbf{2}(D_2)+D]^+$ ion (Figure 7, ions at m/z 259 and 260).

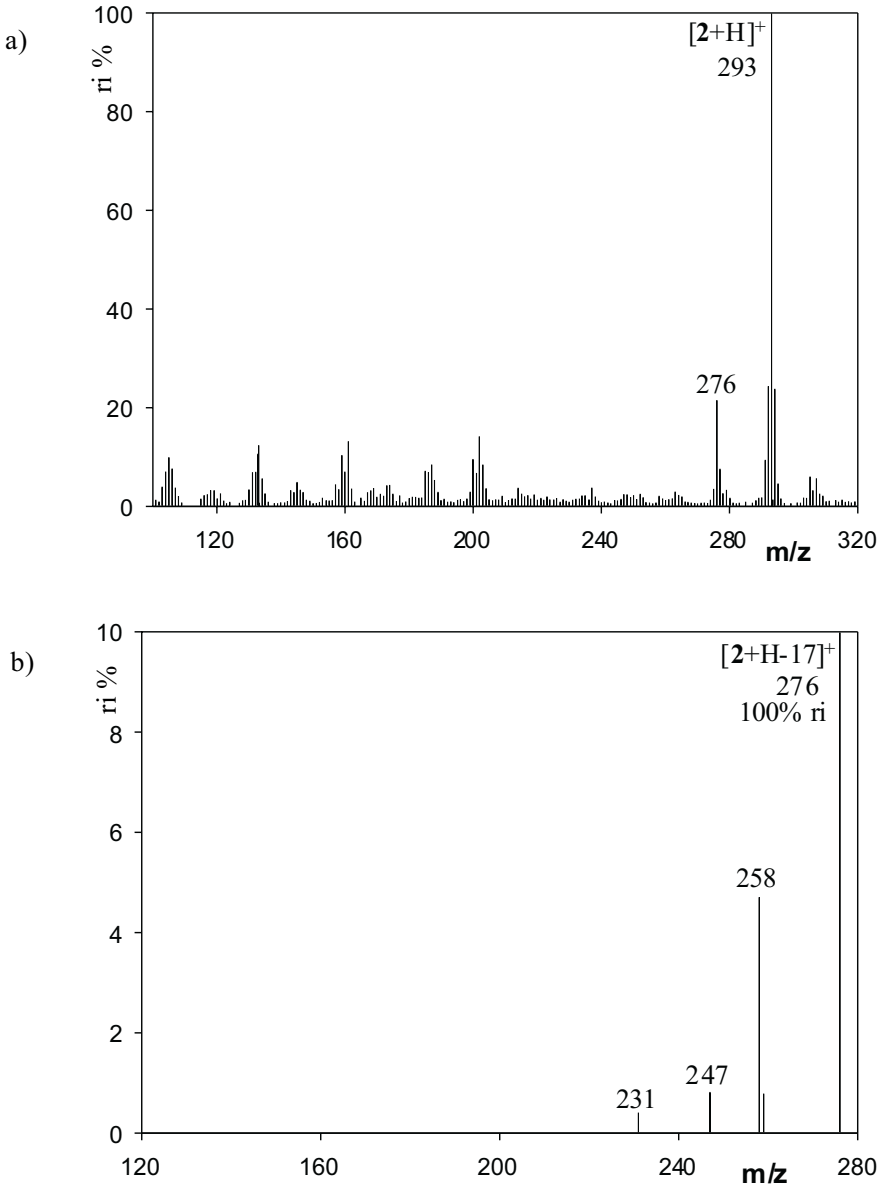

Figure 4. The LSI mass spectrum of **2** (a) and the B/E mass spectrum (b) of the fragment ion formed after ammonia loss from protonated **2**.

As mentioned above, after ammonia loss tautomerization in the resulting ion is possible, leading to H/D exchange. This explains, why the loss of HDO and H_2O for deuterium derivatives occurs with approximately equal efficiency. A plausible mechanism for this process is shown in Figure 8. Also in this case it is possible to propose a number of resonance structures for the ions formed; two of them are shown in Figure 8.

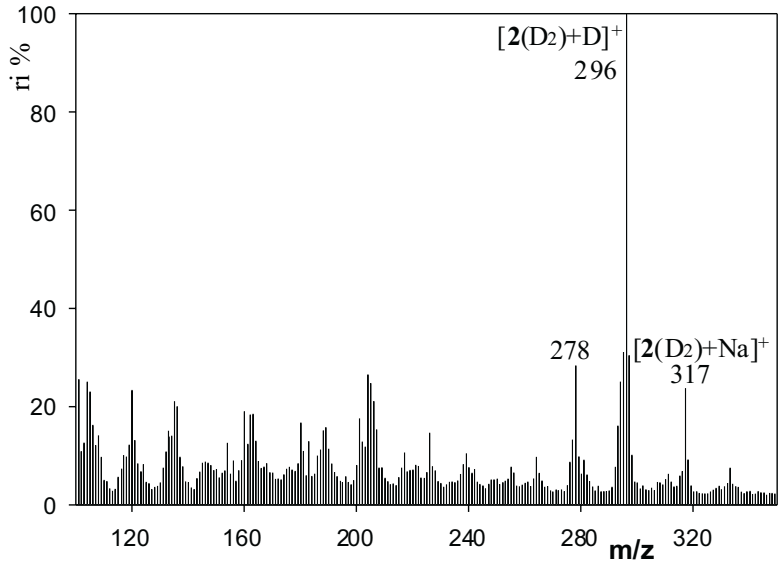

Figure 5. The LSI mass spectrum of deuterium labelled **2**.

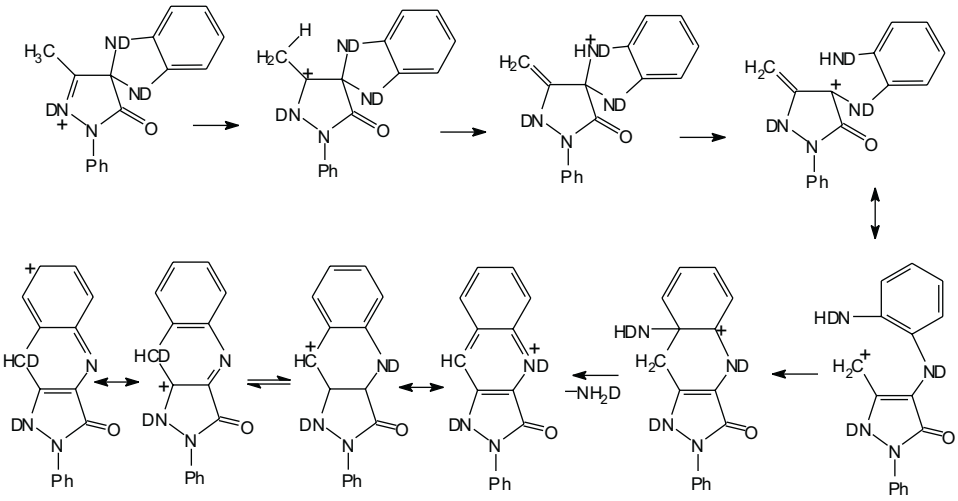

Figure 6. Plausible mechanism for ammonia loss from protonated **1–4**.

Formation of the fragment ions $[133]^+$ and $[M+H-132]^+$. Rationalization of the formation of $[133]^+$ and its complementary partner $[M+H-132]^+$ ions is shown in Figure 9. The peaks corresponding to $[133]^+$ ion were most intense ions in the ESI mass spectra and the $[M+H-132]^+$ ions were also abundant.

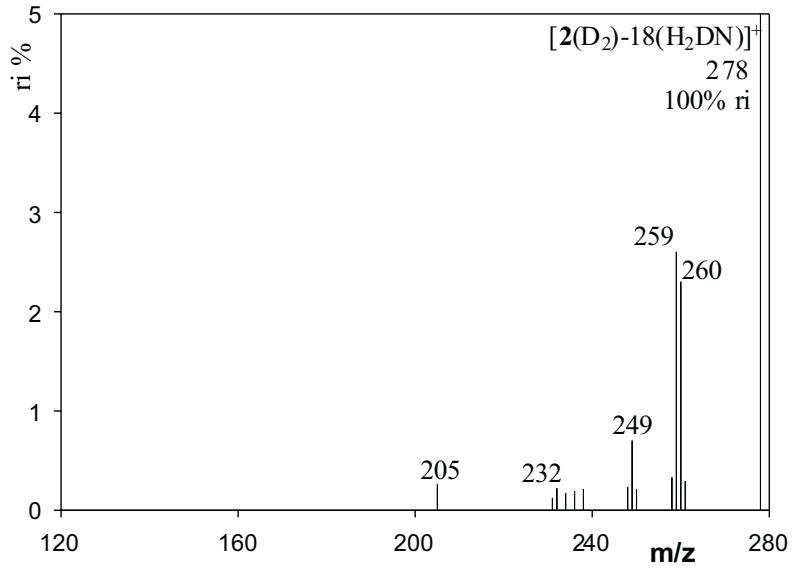

Figure 7. The B/E mass spectrum of the fragment ion formed after ammonia loss from deuterium labelled **2**.

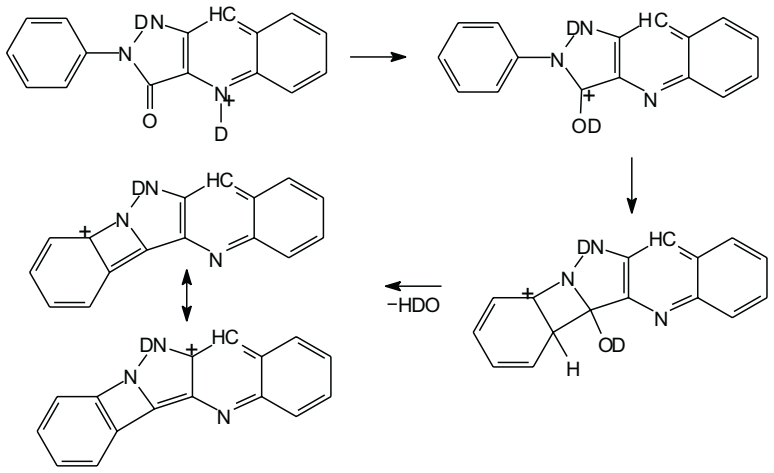

Figure 8. Plausible mechanism for water loss from fragment ions formed after ammonia loss from protonated **1**–**4**.

For isotopically labelled derivatives these fragment ions have masses of one or two units higher, as shown in Figure 10 for **1** (m/z 134 and 135 instead of 133, and m/z 148 and 149 instead of 147). Thus, analogously to the case of NH_3 and H_2O loss, H/D exchange between the methyl group and the imino group (N(1) or N(3), Figure 1) occurs during the formation of these ions.

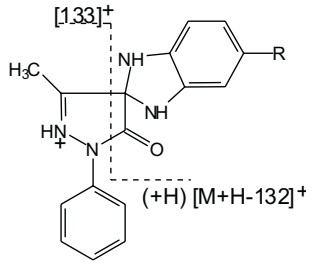

Figure 9. Rationalization for formation of $[133]^+$ and $[M+H-132]^+$ fragment ions from protonated **1**–**4**.

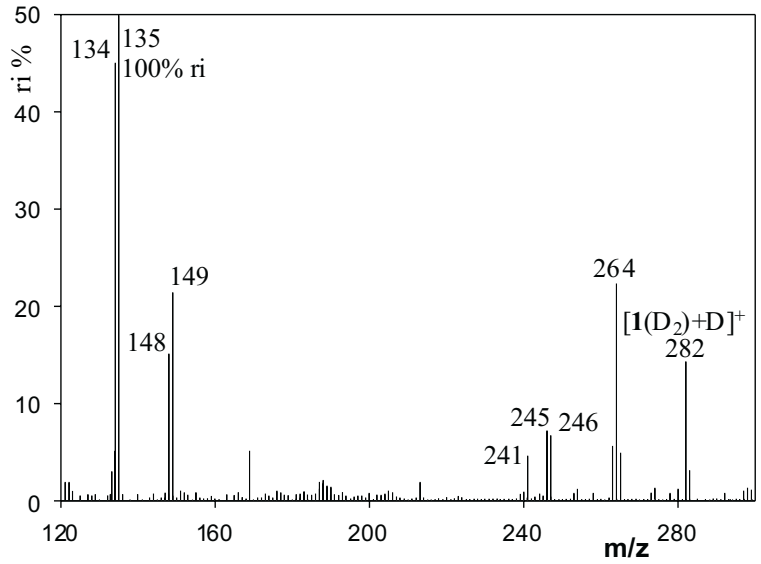

Figure 10. The ESI mass spectrum obtained for deuterium labelled **1**.

The $[133]^+$ ion contains either a three-membered ring (structure a) or, more likely, a five-membered ring (tautomeric structure **b**, **c** and **d**) as shown in Figure 11a. Although the formation of three-membered structures was already proposed for mass spectrometric decomposition of five-membered nitrogen heterocycles [15], the structure **d** corresponding to the protonated molecule of 3-methyl-1*H*-indazole seems to be most stable.

Plausible structures for the $[M+H-132]^+$ ion are shown in Figure 11b. The structures containing six-membered ring seem to be favored and especially tautomer **d**, which can be considered as a protonated molecule of 2-hydroxyquinoxaline, since it is a fully aromatic bicyclic system.

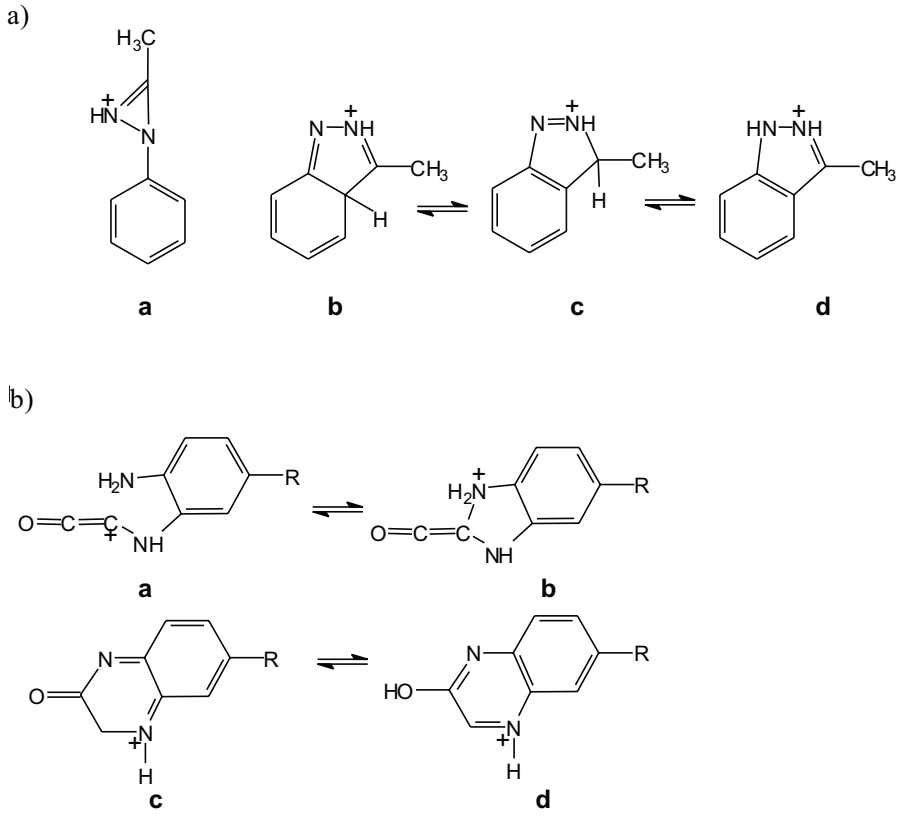

Figure 11. Possible structures of the $[133]^+$ fragment ion (a) and possible structures of the $[M+H-132]^+$ fragment ion (b).

Other fragmentations of the protonated molecules. Loss of mass 41, the CH_3CN molecule, is observed for both labelled and unlabelled **1–4**. This process seems to be rather simple, in this case H/D exchange does not occur.

It is difficult to rationalize the loss of mass 93, probably aniline elimination (H_2N-C_6H_5), and formation of an ion at m/z 146. Since these are complex processes it is difficult to propose plausible mechanisms for these reactions.

Mass spectrometric fragmentation of $[5+H]^+$ and $[6+H]^+$ ions. The ESI mass spectra of **5a** and **6** and the fragmentation patterns observed are shown in Figure 12.

In the case of **5b–d** the decompositions are analogous to these of **5a**, and appropriate shifts of m/z values of the fragment ions were observed. The skeletal rearrangements discussed above do not take place for **5a–d**. This observations confirms that these processes are strongly related to the spiro system of **1–4**.

As shown in Figure 12, the fragmentation pathway of **6** is similar to those of **1–4**, *e.g.* the most abundant ion of m/z 195 corresponds to the ion of m/z 133 for **1–4**.

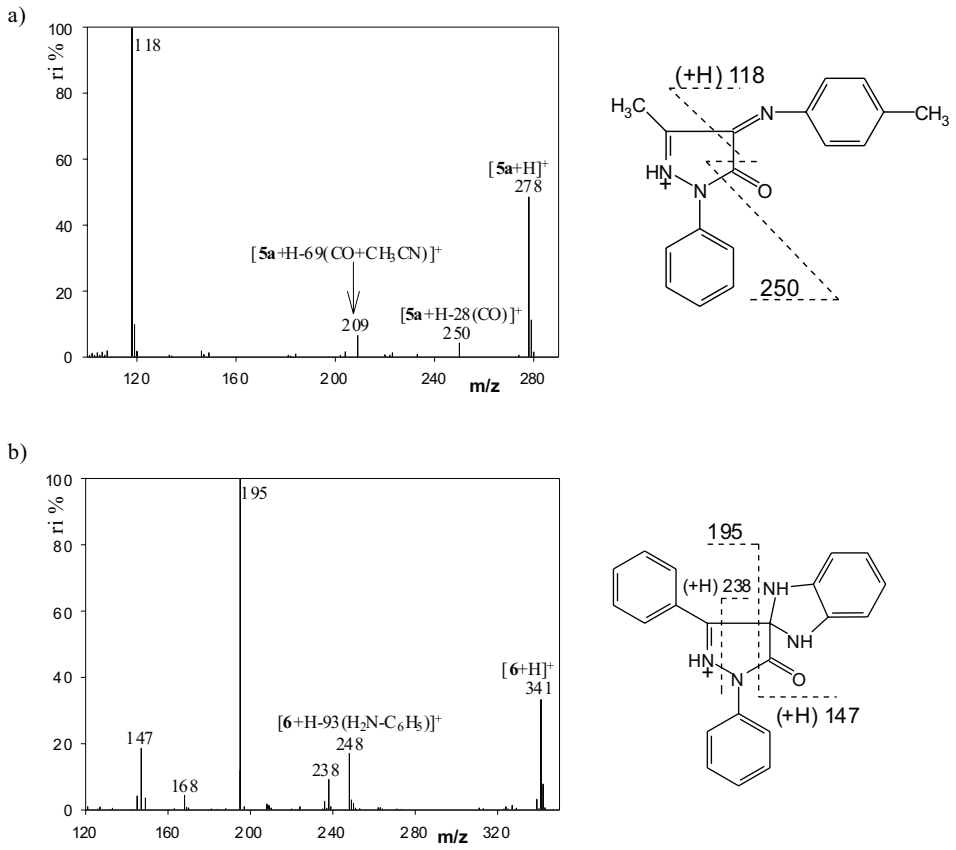

Figure 12. The ESI mass spectra obtained for **5a** (a) and **6** (b) and the fragmentation patterns observed.

However, the rearrangement, which requires interaction of the spiro system with the methyl group, namely subsequent loss of NH_3 and H_2O molecules, does not occur. Interaction of the spiro system with the methyl group for **1–4** also proceeds with formation of the $[133]^+$ ion and its complementary partner ion $[M+H-132]^+$. The occurrence of analogous processes for **6**, formation of $[195]^+$ ion and its complementary partner ion $[M+H-194]^+$, leads to the conclusion that for these processes interaction with the methyl group is not necessary (although this interaction occurs when possible).

In the case of **5a–d** there is an interesting decomposition, consisting of the subsequent losses of CO and CH_3CN molecules. This process has been already observed for such compounds during electron ionization and has been discussed in detail [16].

CONCLUSIONS

Mass spectrometric decomposition of compounds containing a spiro system is complex, however, the mass spectra of isotopically labelled compounds enable us to draw plausible mechanisms for the fragment ions formation and their structures. Subsequent loss of NH_3 and H_2O molecules occurs through a charge induced bond ruptures and due to the presence of a methyl group in the neighborhood of the spiro system. This process leads to the formation of the polycyclic structures. Formation of the fragment ion $[133]^+$ and its complementary partner $[M+H-132]^+$ (or $[195]^+$ and $[M+H-194]^+$ for **6**) is an important feature of the spiro system for **1–4**. In these cases, bicyclic ions are probably formed. Loss of the CH_3CN molecule (C_6H_5CN for **6**) is also a significant process.

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