Unusual Behavior of Pyrazolo[1,2-*a*]pyrazoles in Fast Atom Bombardment (FAB) and Frit-Fast Atom Bombardment (Frit-FAB) Mass Spectrometry. Influence of the Matrix

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The nature of the matrix used in Fast Atom Bombardment (FAB) mass spectrometry analyses of pyrazolo[1,2-*a*]pyrazoles was found to influence significantly their positive and negative ions mass spectra. Indeed the use of glycerol provided an abundant ion corresponding to the protonated molecule $(M+H)^+$ whereas the *meta*-nitrobenzyl alcohol favored the formation of the radical ion $M^{+\circ}$. Such results which are in accordance with the oxidoreduction properties of the matrices studied were also established in Frit-FAB mass spectrometry analyses of pyrazolo[1,2-*a*]pyrazoles.

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Fast Atom Bombardment (FAB) mass spectrometry [1] is commonly employed to characterize thermolabile and polar compounds such as biomolecules and in particular peptides [2] and nucleotides [3]. For the vast majority of molecules, a compound of mass M exhibits in the positive and negative modes an abundant ion corresponding to the protonated molecule $(M+H)^+$ and the deprotonated structure $(M-H)^-$, respectively. Nevertheless, in addition to such proton transfer, some compounds are also identified by the ions $M^{+\circ}$ and $M^{-\circ}$ obtained by charge transfer. The respective contribution of the two ionization processes leading to different $(M+H)^+/M^+$ ratios was studied by Takayama *et al.* [4,5,6] thus establishing the following points.

Firstly, glycerol (G) and *meta*-nitrobenzyl alcohol which are the most common matrices in fast atom bombardment mass spectrometry [7] did afford noticeable differences in the spectra of a series of various biomolecules; as the $(MH)^+/M^+$ ratios were higher when glycerol was used [4]. The specific properties of each matrix influenced the nature of the recorded mass spectra. On one hand, the presence of electrons in glycerol can lead to a reduction process giving rise for instance to dehalogenation [8], dehydroxylation [9], desamination [10], hydrogen fixation producing multiply charged ions of the type $(M+nH)^+$ (n = 2, 3, ...) [11] and electron capture [12]. On the other hand, the use of the oxidative *meta*-nitrobenzyl alcohol matrix inhibited reduction phenomena by electron trapping [13]. The comparison of fast atom bombardment mass spectra recorded for the same structure either in glycerol or in *meta*-nitrobenzyl alcohol enabled us to describe a wide range of reduction reactions [14 and references therein].

Secondly, antioxidant compounds exhibited in their positive ions fast atom bombardment spectra an abundant $M^{+\circ}$ ion [4]. The loss of one electron for an antioxidant structure was in accordance with some results previously obtained in our laboratory [15]. The negative ions fast atom bombardment mass spectrum of the oxidative *meta*-nitrobenzyl alcohol matrix showed an intense $M^{-\circ}$ ion indicating the capture of one electron by this oxidant. Such electron capture capacity provided a possible pathway for ionization by charge transfer according to the two following mechanisms:

$$M + NBA \rightarrow [M+NBA]^* \rightarrow M^{+\circ} + NBA^{-\circ} \quad (1)$$

activated complex

$$M + NBA \rightarrow M^{+\circ} + e^- + NBA \rightarrow M^{+\circ} + NBA^{-\circ} \quad (2)$$

A proof of the feasibility of these pathways has been given by Takayama *et al.* in another paper [6] regarding the positive and negative ions fast atom bombardment mass spectrometry of pyrene. The most abundant ions were $M^{+\circ}$ in the positive mode and $NBA^{-\circ}$ at m/z 153 in the negative spectrum when the *meta*-nitrobenzyl alcohol matrix was used.

Finally, the different properties of glycerol and *meta*-nitrobenzyl alcohol matrices were also described by Takayama *et al.* [5] for the study of the negative ions fast atom bombardment spectrum of α-tocopherol. The detected ion $(M-H)^-$ is formed as shown below:

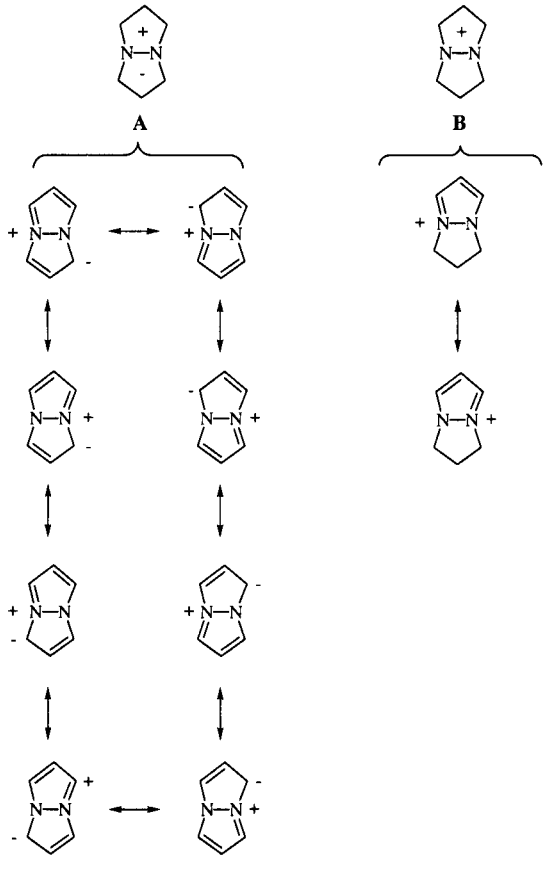
$$M + B \rightarrow [MB]^* \rightarrow (M\text{-}H)^- + (B+H)^+ \quad (3)$$

where B represents the matrix and [MB]* an activated complex. The ionization was obtained by proton transfer and its efficiency depended on the respective proton affinity of the matrix and compound B; the greater the proton affinity of the matrix B, the more abundant the ion $(M\text{-}H)^-$. Under such conditions, glycerol was more suitable to abstract one proton of the molecule under study M (equation (3)) as its proton affinity is higher than the *meta*-nitrobenzyl alcohol one.

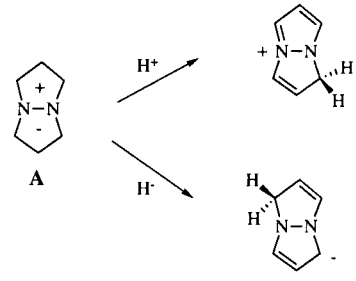
We report in this paper the positive and negative ions fast atom bombardment mass spectra of pyrazolo[1,2-*a*]pyrazoles **A**, also called 3a,6a-diazapentalenes, which belong to a family of mesoionic compounds reviewed several times [16]. Although they are neutral, it was impossible to write down any mesomeric forms without charge separation, as shown in Scheme 1 where eight different structures are represented. In this way, these compounds differ from the positively charged pyrazolium salts **B** already studied in the laboratory by fast atom bombardment mass spectrometry [17, 18] which exhibit only two positively charged resonance forms (Scheme 1).

The fast atom bombardment mass spectrometry study of pyrazolo[1,2-*a*]pyrazoles **A** seemed to us very interesting due to their particular properties. Indeed, such compounds are highly polar owing to their several mesomeric forms implying all charge separations but at the same time they are not basic as protonation and deprotonation destroy their aromaticity as exemplified in Scheme 2.

Scheme 1
Mesomeric Forms of Structures **A** and **B**



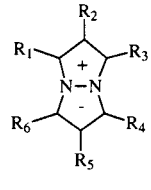
Scheme 2
Protonation and Deprotonation of Pyrazolo[1,2-*a*]pyrazoles



Thus, the polar pyrazolo[1,2-*a*]pyrazoles **A** should exhibit significant fast atom bombardment mass spectra but their ionization by proton transfer commonly obtained for biomolecules should not be the predominant pathway. Besides, the capacity of electron capture by the *meta*-nitrobenzyl alcohol matrix should lead to the easy formation of the $M^{+\circ}$ ion by the loss of one electron. These hypotheses were confirmed by the recorded fast atom bombardment mass spectra and the results are discussed below.

The studied compounds are listed in Table 1. For each of them, the positive and negative ions spectra recorded

Table 1
Structures of the Studied Pyrazolo[1,2-*a*]pyrazoles



Structure	Molecular weight (Daltons)	R_1	R_2	R_3	R_4	R_5	R_6
1	156	H	H	H	CN	H	CN
2	262	CH_3	Br	CH_3	CN	H	CN
3	190	H	H	H	$COCH_3$	H	$COCH_3$
4	268	H	H	H	$COCH_3$	Br	$COCH_3$
5	232	CH_3	CH_3	CH_3	$COCH_3$	H	$COCH_3$
6	314	H	H	H	COC_6H_5	H	COC_6H_5
7	392	H	H	H	COC_6H_5	Br	COC_6H_5
8	356	CH_3	CH_3	CH_3	COC_6H_5	H	COC_6H_5
9	224	CH_3	CH_3	CH_3	C_4HN_2	H	H

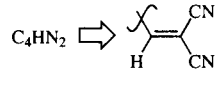


Table 2

Positive Ions Fast Atom Bombardment Mass Spectra [a]

Structure	Molecular weight Daltons	*meta*-Nitrobenzyl Alcohol NBA $M^{+\bullet}$ and related ions	*meta*-Nitrobenzyl Alcohol NBA $(M+H)^+$ and related ions	*meta*-Nitrobenzyl Alcohol NBA Other ions (low abundance)	Glycerol $M^{+\bullet}$ and related ions	Glycerol $(M+H)^+$ and related ions	Glycerol Other ions (low abundance)
1	156	156 (100)	157 (69)	–	–	–	–
2	262	262 (100)	263 (59)	415 $(M+NBA)^+$ 416 $(MH+NBA)^+$	–	–	
3	190	190 (100) 213 $(M+Na)^+$	191 (84) 381 $(2M+H)^+$ 571 $(3M+H)^+$	343 $(M+NBA)^+$ 344 $(MH+NBA)^+$	190 (20)	191 (100) 381 $(2M+H)^+$	283 $(M+H+G)^+$
4	268	268 (100)	269 (90) 537 $(2M+H)^+$	–	268 (30)	269 (100)	–
5	232	232 (100)	233 (79) 405 $(2M+H)^+$	–	232 (41)	233 (100) 465 $(2M+H)^+$	325 $(M+H+G)^+$
6	314	314 (88)	315 (100) 629 $(2M+H)^+$	–	315 (100)	–	–
7	392	392 (88)	393 (100)	–	393 (100)	–	–
8	356	356 (100)	357 (65) 713 $(2M+H)^+$	–	357 (100)	–	–
9	224	224 (100)	225 (63)	377 $(M+NBA)^+$	–	–	–

[a] For each spectrum, the most intense ion was given an abundance 100. The matrix ions were omitted. The mass of each ion was calculated with the most abundant isotope of each element.

either in glycerol or in *meta*-nitrobenzyl alcohol are given in Tables 2 and 3, respectively. The fast atom bombardment mass spectra of pyrazolo[1,2-*a*]pyrazoles **1-9** exhibited the following features.

Firstly, the most abundant characteristic positive ions were obtained for all compounds when the oxidative *meta*-nitrobenzyl alcohol matrix was used. For instance, compound **3** (190 Da) dissolved in *meta*-nitrobenzyl alcohol exhibited intense ions at m/z 190 Th ($M^{+\circ}$) and 191 Th ($(M+H)^+$) whereas the matrix ions were rather weak (136 Th, 154 Th, 289 Th, 307 Th, ...) as shown in Figure 1. On the other hand, the positive fast atom bombardment mass spectrum of a solution of the same compound in glycerol (Figure 2) showed intense matrix ions (93 Th, 185 Th, 277 Th, 369 Th, ...), the expected protonated structure at m/z 191 Th being less abundant than the ion at m/z 185 Th corresponding to the matrix adduct ion $(2G+H)^+$.

Table 3

Negative Ions Fast Atom Bombardment Mass Spectra [a]

Structure	Molecular weight Daltons	*meta*-Nitrobenzyl Alcohol (NBA) [b] $M^{+\bullet}$ and related ions	*meta*-Nitrobenzyl Alcohol (NBA) [b] Other ions	Glycerol [c] Detected ions
1	156	–	–	–
2	262	–	232 $(Br+NBA)^-$ 385 $(Br+2NBA)^-$	–
3	190	189 (weak)	–	–
4	268	267 (weak)	232 $(Br+NBA)^-$ 385 $(Br+2NBA)^-$	–
5	232	–	–	324 $(M+G)^-$ 416 $(M+2G)^-$
6	314	–	–	–
7	392	–	232 $(Br+NBA)^-$ 385 $(Br+2NBA)^-$	
8	356	355 (weak)	–	–
9	224	223 (weak)	–	–

[a] For each spectrum, the most intense ion was given an abundance of 100. The matrix ions were omitted. The mass of each ion was calculated with the most abundant isotope of each element. [b] In the case of the *meta*-nitrobenzyl alcohol matrix (NBA), no $(M+H)^+$ ions were deteted. [c] In the case of the glycerol matrix, no $M^{+\bullet}$ and $(M+H)^+$ ions were deteted.

Scheme 3

Reduction Reactions Observed in Fast Atom Bombardment and in Frit-Fast Atom Bombardment

$R\text{–}ONO_2 \longrightarrow R\text{–}OH$

$R\text{–}N_3 \longrightarrow R\text{–}NH_2$

$R\text{–}F \longrightarrow R\text{–}H$

$R_2N\text{–}N{=}O \longrightarrow R_2NH$

$R\text{–}NH\text{–}OH \longrightarrow R\text{–}NH2$

Secondly, limited information can be deduced from the negative fast atom bombardment mass spectra whatever the matrix as the ions of the latter were the most abundant as shown in Table 3. To illustrate this point, the most intense ions in the negative fast atom bombardment mass spectra of compound **4** recorded in *meta*-nitrobenzyl alcohol (Figure 3) were matrix related [15,19]:

NO_2^- + nNBA (n = 0, 1, 2,...): 46 Th, 199 Th, 352 Th, ...

$nNBA^{-\circ}$ (n = 1, 2, 3,...): 153 Th, 306 Th, 459 Th, ...

$(nNBA\text{-}H)^-$ (n = 1, 2, 3,...): 152 Th, 305 Th, 458 Th,...

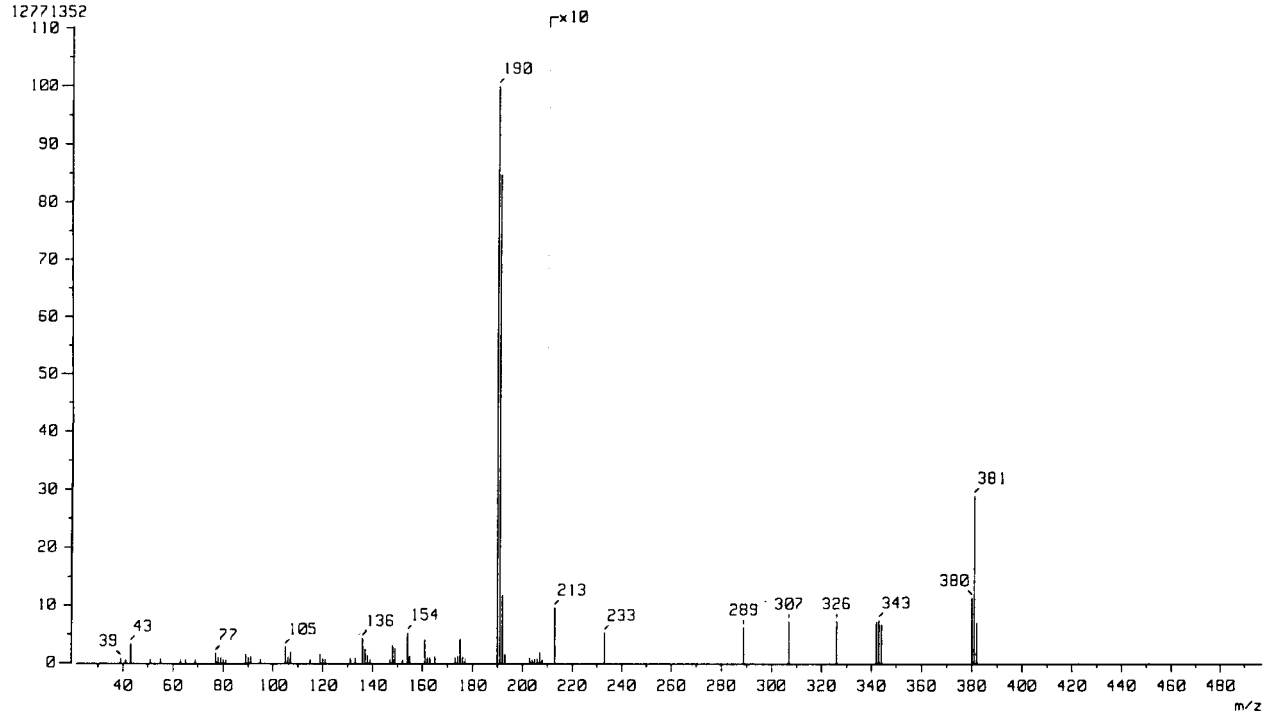


Figure 1. Positive ions Fast Atom Bombardment mass spectrum of compound **3** in *meta*-nitrobenzyl alcohol.

the oxidized ions $(NBA-H+16)^-$: 168 Th, $(2NBA+16)^-$: 322 Th.

Besides, the deprotonated ion (267/269 Th) was weak and a series of bromine adduct ions were also detected: $(nNBA+Br)^-$ (n = 0, 1, 2,...): 79 Th, 232 Th, 385 Th,... showing that the pyrazolo[1,2-*a*]pyrazoles **4** was not stable under the analysis conditions loosing the bromine anion.

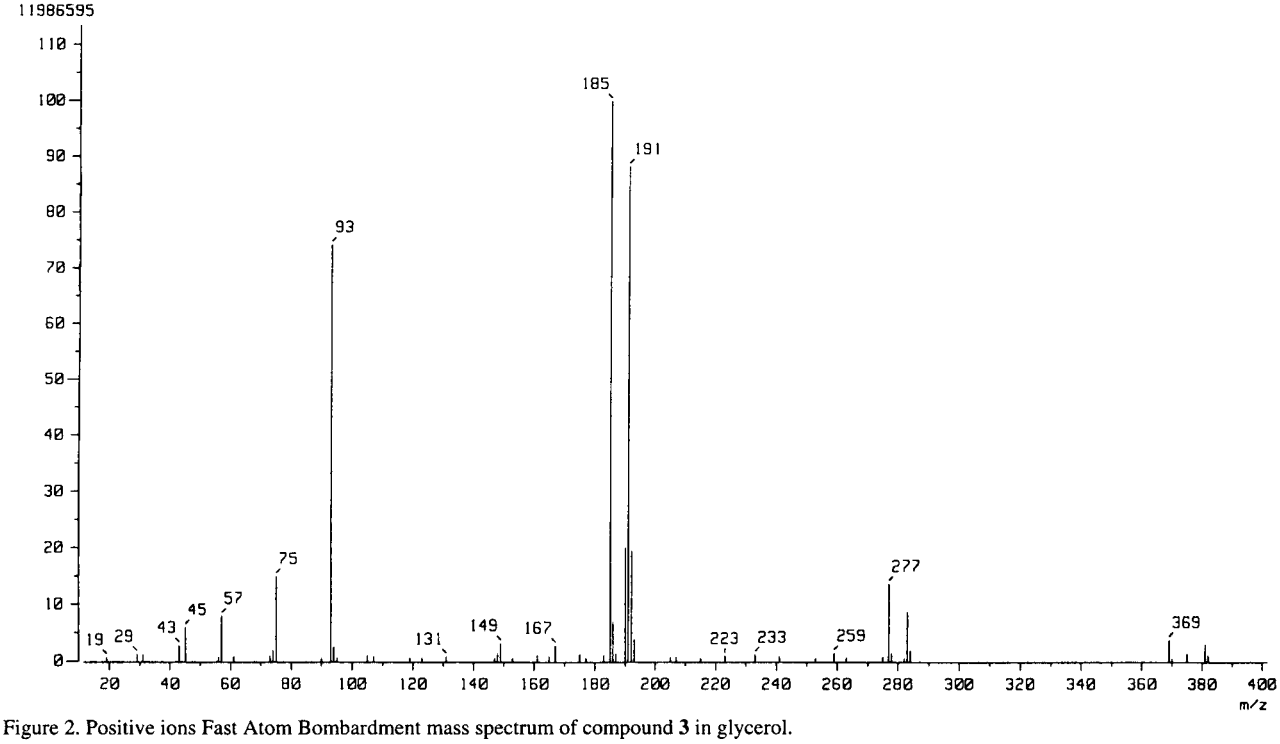


Figure 2. Positive ions Fast Atom Bombardment mass spectrum of compound **3** in glycerol.

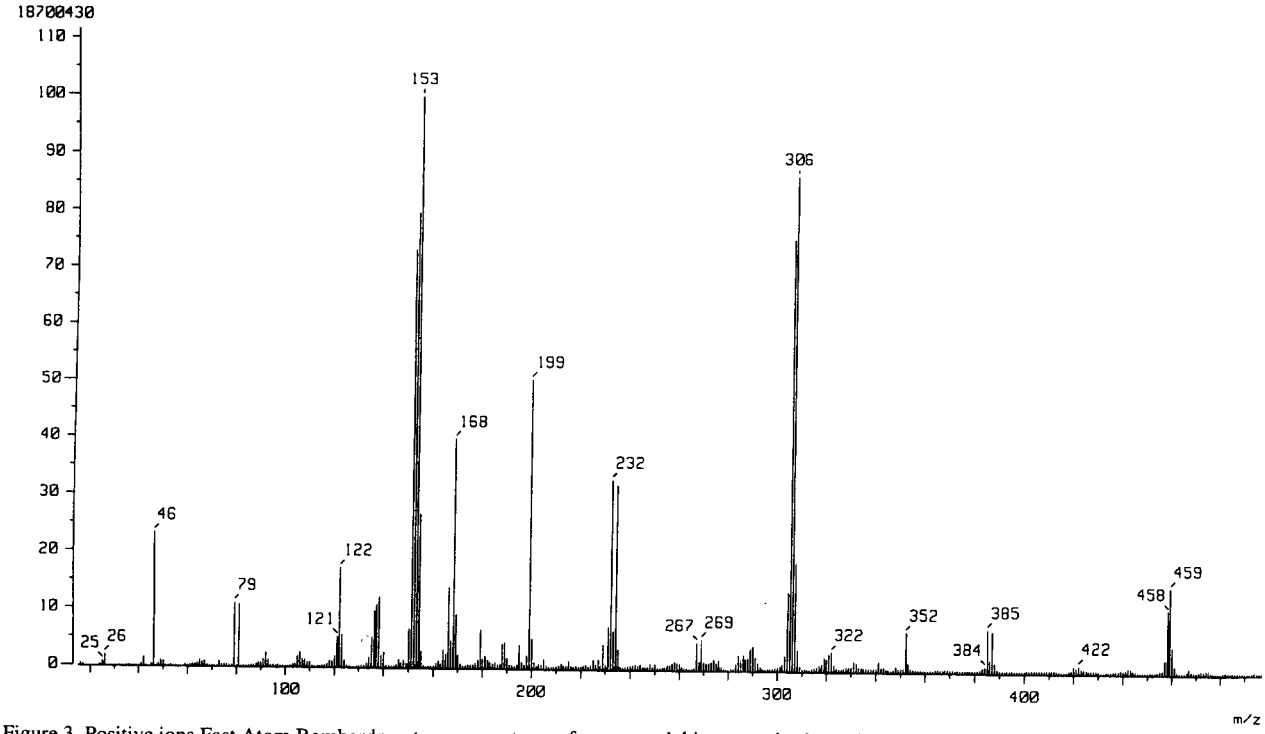


Figure 3. Positive ions Fast Atom Bombardment mass spectrum of compound **4** in *meta*-nitrobenzyl alcohol.

Finally, the positive ions fast atom bombardment mass spectra recorded in *meta*-nitrobenzyl alcohol contained for all compounds an intense radical ion $M^{+\circ}$ formed by charge transfer. In opposition, the same compounds dissolved in glycerol exhibited abundant protonated molecule $(M+H)^+$ produced by proton transfer.

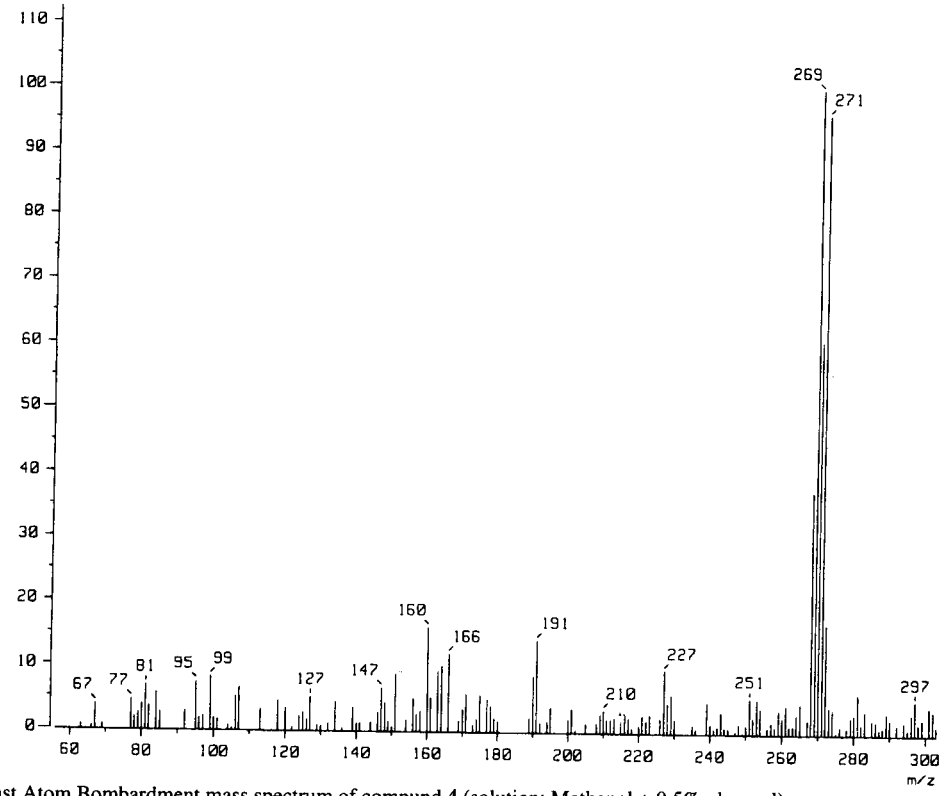


Figure 4. Frit-Fast Atom Bombardment mass spectrum of compund **4** (solution: Methanol + 0.5% glycerol).

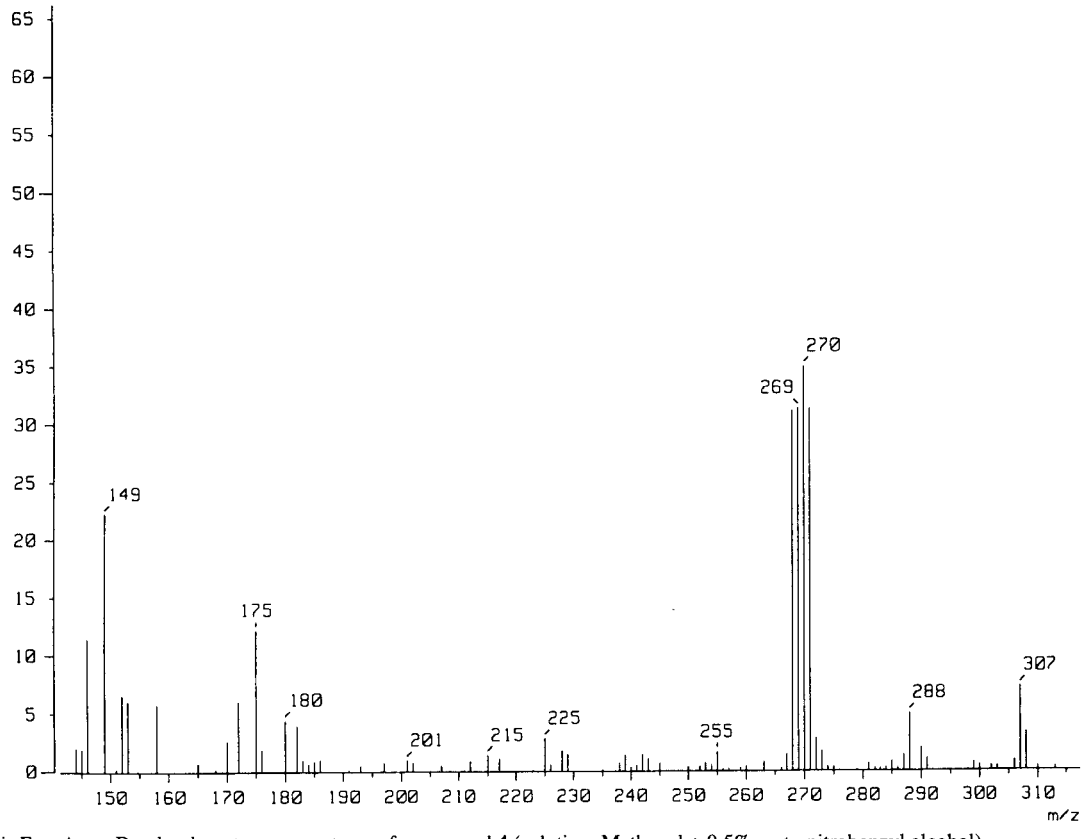


Figure 5. Frit-Fast Atom Bombardment mass spectrum of compound **4** (solution: Methanol + 0.5% *meta*-nitrobenzyl alcohol).

The continuous Flow-Fast Atom Bombardment methodology was introduced by Caprioli *et al.* [20] as one of the most convenient interface for the coupling of liquid chromatography to mass spectrometry thus enabling the analysis of complex mixtures of polar compounds. Under such dynamic conditions, 0.5% of the matrix (glycerol, *meta*-nitrobenzyl alcohol) is added to the hplc eluent. A derived method named Frit-Fast Atom Bombardment mass spectrometry is used in our laboratory where a frit is mounted at the end of the silica tubing [21]. It was interesting to verify if the reactivities observed in neat glycerol or *meta*-nitrobenzyl alcohol or with only 0.5% of the matrix present in the studied solutions were identical. We have already described in a previous paper [14] various reduction reactions which were occurring in Fast Atom Bombardment as well as in Frit-Fast Atom Bombardment experiments (Scheme 3). Comparison of Fast Atom Bombardment mass spectra of the same compound recorded with glycerol and *meta*-nitrobenzyl alcohol showed that reduction was only occurring with glycerol either pure (Fast Atom Bombardment) or at a concentration of 0.5% in methanol (Frit-Fast Atom Bombardment).

The reactivity of pyrazolo[1,2-*a*]pyrazoles under Fast Atom Bombardment and Frit-Fast Atom Bombardment conditions was checked and found to be similar. Indeed, the Frit-Fast Atom Bombardment mass spectra of compound **4** (Figures 4 and 5) and **8** (Figures 6 and 7) were recorded using either 0.5% of glycerol or 0.5% of *meta*-nitrobenzyl alcohol in methanol. The ion formed by charge transfer, $M^{+\circ}$, was present with a noticeable abundance when *meta*-nitrobenzyl alcohol was used (268 Th for compound **4**, Figure 5; 356 Th for compound **8**, Figure 7). On the other hand, the protonated molecule *i.e.* the $(M+H)^+$ ion produced by proton transfer was the most intense ion with glycerol (269 Th for compound **4**, Figure 4; 357 Th for compound **8**, Figure 6).

The similarities between Fast Atom Bombardment and Frit-Fast Atom Bombardment mass spectra which have been encountered with various types of structures [14 and this work] could be explained by a rapid evaporation of the hplc eluent on the frit leaving solely the matrix and the studied compound thus recreating the static Fast Atom Bombardment conditions.

The unusual behavior of the non basic but polar pyrazolo[1,2-*a*]pyrazoles in Fast Atom Bombardment and Frit-Fast Atom Bombardment mass spectrometry was established.

First of all, structural information was deduced from the positive ions spectra as characteristic ions were always present with noticeable abundances.

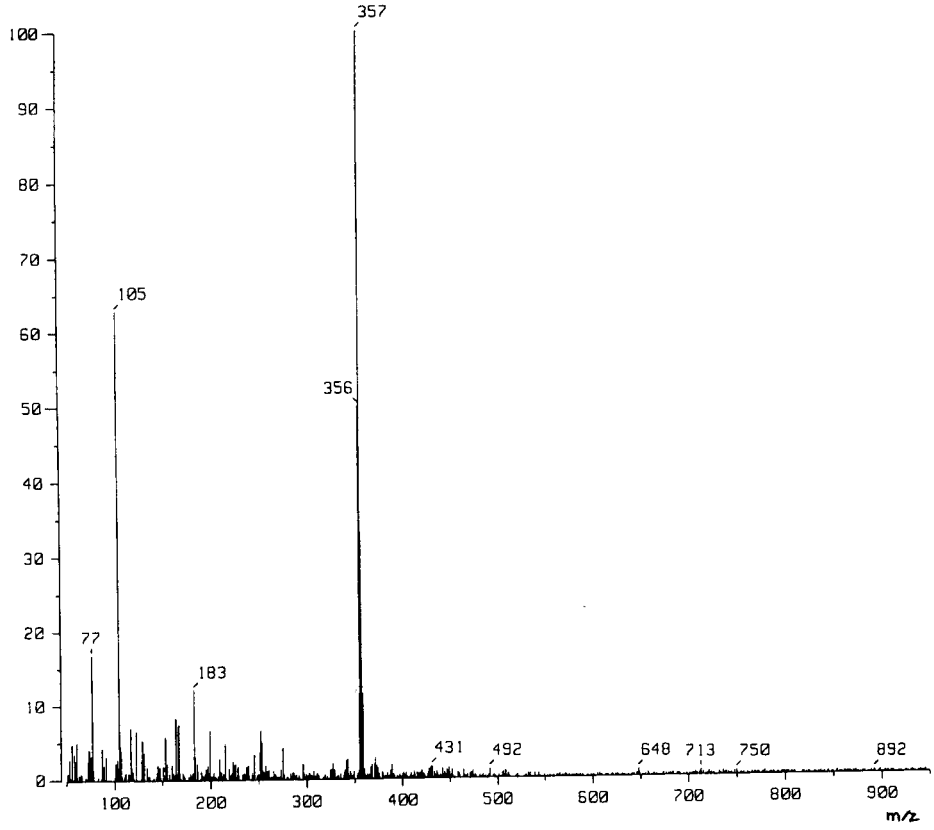


Figure 6. Frit-Fast Atom Bombardment mass spectrum of compound **8** (solution: Methanol + 0.5% glycerol).

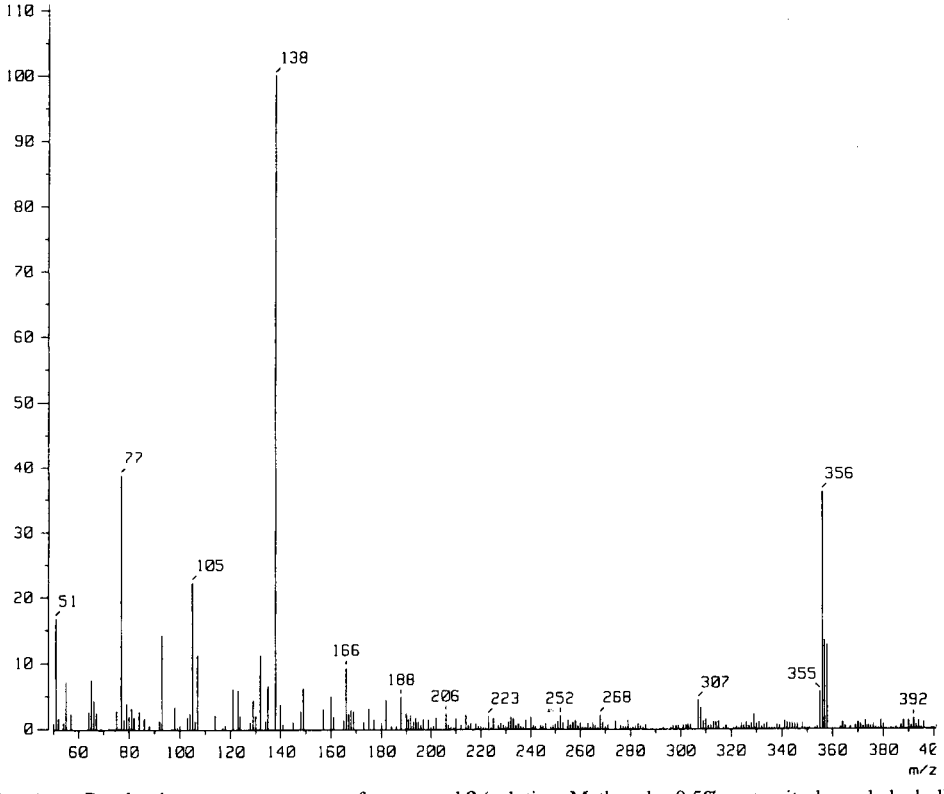


Figure 7. Frit-Fast Atom Bombardment mass spectrum of compound **8** (solution: Methanol + 0.5% *meta*-nitrobenzyl alcohol).

Furthermore, the ionization process was strongly dependent on the nature of the matrix as demonstrated previously by Takayama *et al.* [4,5,6]. Electron transfer leading to the radical ion $M^{+\circ}$ and proton transfer producing the protonated molecule $(M+H)^+$ were observed with *meta*-nitrobenzyl alcohol and glycerol, respectively.

Such differences in the spectra recorded under different experimental conditions (positive/negative modes, glycerol/*meta*-nitrobenzyl alcohol) could complicate or even mislead unknown compound identification thus implying that perfect knowledge of the various ionization pathways, of the matrices properties and finally the features of the compound studied is mandatory.

EXPERIMENTAL

The Fast Atom Bombardment mass spectra were recorded on a SX102 type spectrometer, Jeol Ltd, Tokyo, Japan. The energy of the neutral atom beam was 3 keV (emission current: 20 mA). Calibration was accomplished using Ultramark 1621, Heraeus, Karlsruhe, Germany, as a reference. The Fast Atom Bombardment mass spectra were measured at a resolution of 1000. The data were acquired and processed with an HP Apollo series 400 using the Jeol complement software.

Samples were placed on a target by dissolving them directly in the matrix, *meta*-nitrobenzyl alcohol or glycerol obtained from commercial suppliers, Aldrich, Milwaukee, WI, USA.

The Frit-Fast Atom Bombardment mass spectra were recorded on a LX 2000 type spectrometer, Jeol Ltd, Tokyo, Japan. The energy of the neutral atom beam was 3 keV (emission current: 20 mA). Calibration was accomplished using Ultramark 1621, Heraeus, Karlsruhe, Germany, as a reference. A flow rate of 1 ml/minute of the solvent (methanol + 0.5% glycerol or methanol + 0.5% *meta*-nitrobenzyl alcohol) was obtained by a Waters pump (Model 510). The compounds studied were dissolved in a minimum amount of methanol and introduced through a Rheodyne 7125 loop into the flow.

The syntheses of compounds **1** to **9** have been described elsewhere [22].

Acknowledgments.

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