Mass Spectrometric Behaviour of 1-Imino-substituted Pyrazolo[1,2-*a*]-[1,2,4]Triazoles under Electron and Chemical Ionization

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The spectra of five pharmacologically interesting substituted pyrazolo[1,2-*a*][1,2,4]triazole hydroiodides were measured under electron and chemical ionization. In the electron ionization spectra, in addition to the intense molecular ion peak of the free base ($M^{+\bullet}$), there was also a relatively intense molecular ion peak of the hydroiodide form, which is unusual since the hydroiodides are rarely so stable. The phenylimino and phenylamino substituents of the triazole ring affected the fragmentation behaviour of the compounds very much. The chemical ionization reagent gases used in this work were methane, isobutane, deuterated ammonia and acetone. In all the cases practically only $[M+H]^+$ ions were observed, the only exception being acetone which also gave rise to intense $[M+C_2H_3O]^+$ and $[M^+C_3H_7O]^+$ adduct ions. None of the reagent gases used was able to cause any fragmentation.

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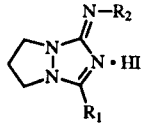
Introduction.

The variously substituted pyrazolo[1,2-*a*][1,2,4]triazoles such as compounds **1-5** (Diagram) are interesting compounds due to their biological activity [1-3].



Compound	R_1	R_2
1	H	H
2	CH_3	H
3	CH_3	C_6H_4-4-Br
4	NHC_6H_5	H
5	NHC_6H_5	C_6H_5

In this work, the mass spectrometric behaviour of compounds **1-5** (Diagram) under electron and chemical ionization has been studied. The purpose of the study was to clarify the fragmentation of the pyrazolo[1,2-*a*][1,2,4]triazoles in the gas phase and also to find out how the hydroiodide salt affected the fragmentation behaviour. The possible effects of the substituents were also considered.

All compounds **1-5**, isolated as the hydroiodide, were derived from the related (un)substituted 1-amidinopyrazolidine by cyclization reactions using different reactants. Compounds **1** and **2** were synthesized by heating the unsubstituted 1-amidinopyrazolidine with formic acid and acetic acid anhydride, respectively. Treatment of the related starting compound with acetyl chloride under mild conditions led to the open chain form of **2** and **3**, which cyclocondensed under heating to yield the final products. Compounds **4** and **5** were prepared *via* the 1-amidino-2-thiocarbamoylpyrazolidines synthesized from the requisite 1-amidinopyrazolidines and phenylisothiocyanate.

Some of the fragmentations were verified through metastable ion analysis and the collision induced dissociation (CID) technique. The elemental compositions of the principal fragment ions under electron ionization (EI) were confirmed by exact mass measurements. Chemical ionization mass spectra were recorded using methane, isobutane, acetone and deuterated ammonia as the reagent gas.

Results and Discussion.

EI Mass Spectra.

The 70 eV electron ionization mass spectra of the compounds **1-5** are presented in Table 1. All the compounds studied in this work were isolated as hydroiodides as previously mentioned. Both the free bases and the hydroiodides appeared to be relatively stable under electron impact accordingly as evidenced by the intense molecular ion peaks. Usually the free bases were more stable than the hydroiodides, except for **3** whose molecular ion peak of the hydroiodide salt gave rise to the base peak of the spectrum. It is unusual that the molecular ion peaks of the hydroiodides were so stable, as they often do not exist at all [4,5].

The hydroiodides decomposed under electron ionization through the loss of HI giving rise to the molecular ion of the free bases ($M^{+\bullet}$). The molecular ions of the hydroiodides most probably also decomposed thermally in the probe by eliminating HI as a neutral molecule. This can be seen from the presence of the noticeably intense $HI^{+\bullet}$ and I^+ ions at m/z 128 and m/z 127, respectively. There was also an intense $[M+H]^+$ ion in every spectrum, especially those of **1-3**. According to the collision induced

Table 1

Principal Fragment Ions (Intensity ≥ 6) in the Mass Spectra of the Compounds **1-5**. Data are Corrected for ^{81}Br Contributions, Otherwise Uncorrected, m/z (% Relative Intensities)

Compound	m/z (relative intensity)
1	1-Imino-6.7-dihydro-1*H*,5*H*-pyrazolo[1,2-*a*][1,2,4]triazole Hydroiodide 253 (6), 252 (68) $M_1^{\bullet}$ $HI^{+\bullet}$, 128 (53), 127 (27), 126 (6), 125 (84), 124 (100) $M_1^{+\bullet}$, 123 (51), 98 (93), 97 (78), 96 (10), 84 (10), 71 (11), 70 (49), 69 (14), 68 (9), 56 (8), 55 (33), 54 (8), 44 (6), 43 (40), 42 (23), 41 (65), 40 (6), 39 (15), 29 (7), 27 (21)
2	1-Imino-3-methyl-6,7-dihydro-1*H*,5*H*-pyrazolo[1,2-*a*][1,2,4]triazole Hydroiodide 267 (6), 266 (70) $M_1^{\bullet}$ $HI^{+\bullet}$, 156 (24), 140 (6), 139 (71), 138 (84) $M^{+\bullet}$, 137 (35), 128 (78), 127 (37), 114 (29), 113 (11), 112 (100), 111 (80), 110 (9), 98 (11), 96 (6), 85 (18), 84 (17), 83 (8), 72 (48), 71 (35), 70 (64), 69 (44), 68 (6), 57 (6), 56 (8), 55 (7), 44 (27), 43 (81), 42 (76), 41 (65), 40 (11), 39 (12), 30 (9), 29 (6), 27 (19), 15 (13)
3	1-(4-Bromophenyl)imino-3-methyl-6,7-dihydro-1*H*,5*H*-pyrazolo[1,2-*a*][1,2,4]triazole Hydroiodide 421/423 (16), 420/422 (100) $M_1^{\bullet}$ $HI^{+\bullet}$, 296 (6), 293/295 (36), 292/294 (33) $M_1^{+\bullet}$, 265/267 (19), 266 (6), 252/254 (8), 224/226 (17), 214 (9), 196/198 (25), 182/184 (7), 181 (7), 172 (6), 171 (9), 155/157 (9), 147 (7), 146 (7), 145 (15), 128 (18), 127 (8), 117 (8), 102 (14), 96 (10), 91 (7), 90 (13), 76 (15), 75 (14), 69 (28), 63 (7), 55 (11), 50 (7), 42 (48), 41 (36), 39 (8), 27 (9)
4	1-Imino-3-phenylimino-6,7-dihydro-1*H*,5*H*-pyrazolo[1,2-*a*][1.2.4]triazole Hydroiodide 343 $M_1^{\bullet}$ $HI^{+\bullet}$, 216 (14), 215 (100) $M_1^{+\bullet}$, 214 (15), 187 (6), 186 (9), 156 (9), 128 (19), 127 (10), 119 (6), 118 (11), 94 (8), 77 (16), 51 (6), 41 (10)
5	1-Phenylimino-3-phenylamino-6,7-dihydro-1*H*,5*H*-pyrazolo[1,2-*a*][1,2,4]triazole Hydroiodide 419 (8) $M_1^{\bullet}$ $HI^{+\bullet}$, 292 (25), 291 (100) $M_1^{+\bullet}$, 221 (17), 220 (97), 146 (6), 128 (29), 127 (14), 119 (7), 118 (28), 117 (18), 104 (11), 103 (6), 91 (9), 77 (40), 71 (6), 65 (8), 51 (10), 44 (11), 42 (10), 41 (9)

dissociation (CID) mass spectra of the hydroiodides they only eliminate hydrogen iodide and no iodine elimination was observed. These results show that the existence of the $[M+H]^+$ ion was caused by either self chemical ionization or more probably $HI^{+\bullet}$ may act as a protonating agent.

The ring structure in the compounds studied is relatively stable even though in some cases there were, however, a few intense fragment ions. The $[M-27]^+$ ion appeared in every spectrum but especially in those of **1-3** it was worth of notice. There were two possible formation pathways for the $[M-27]^+$ ion. One was the radical-site-initiated decomposition of the pyrazolidine ring with respect to the nitrogen atom leading to the cleavage of the C_5-C_6 bond. This was followed by the C_7-N_8 bond rupture with simultaneous rearrangement of one hydrogen giving rise to the $[M-C_2H_3]^+$ ion. It is also possible that the formal $[M-C_2H_3]^+$ ion was in actual fact formed from the $[M+H]^+$ ion through the elimination of C_2H_4. The other formation pathway for the $[M-27]^+$ ion was the elimination of HCN. According to the accurate mass measurements the former decomposition was more favourable than the latter, which was observed only for **1** and **2**. The cleavage of the triazole ring and the following elimination of HCN or RCHN is a common and a known feature of 1,2,4-triazoles [6,7]. The elimination of HCN through the cleavage of the C_1-N_2 and C_3-N_4 bonds is possible only for compound **1**, instead with compound **2** the exocyclic imino group must be lost. The cleavage of the triazole ring also took place with compounds **4** and **5** leading to the formation of the ion $[C_7H_6N_2]^{+\bullet}$ at m/z 118, which means that their charge remains on the nitrile part of the molecule instead of the $[M-R_1CN]$ fragment. The stability of the $[C_7H_6N_2]^{+\bullet}$ ion can be explained by the conjugation between the phenyl and cyano groups in the molecule. Both for **1** and **2** the formation of the $[M-C_2H_4]^{+\bullet}$ ion was also favourable. With compound **3** the phenylimino substituent at position 1 caused an additional fragmentation to take place directly from the molecular ion ($M^{+\bullet}$). The $[C_7H_5N_2Br]^+$ ion at m/z 196 was formed, which means that phenylimino group with one of the ring nitrogen was lost.

Compared with the previous study on triazole containing heterocycles [8], one could easily predict that the triazole ring would decompose first. Since this did not take place in this case, it is possible that the conjugation between the double bond of the triazole ring and the C_1=N- group inhibited this from happening.

For compounds **4** and **5** the existence of the phenylamino group at position 3 seemed clearly to diminish the intensity of the molecular ion peak of the hydroiodide, whereas the $M^{+\bullet}$ was the base peak in both cases. In addition to the noticeably minor $[M-C_2H_3]^+$ ion, also the $[M-C_2H_5]^+$ ion appeared in the case of **4** and **5**. In the spectrum of **4** there were many fragment ions but they all were rather weak. In **5** the most abundant fragment was the ion $[C_{14}H_{10}N_3]^+$ at m/z 220, which formed through a cleavage of the N_8-C_1 and C_3-N_4 bonds with a simultaneous migration of one hydrogen. The presence of two phenyl rings in the ion formed, making it noticeably stable. Compound **5** decomposed between N_8-C_1 and N_2-C_3 bonds, as also did compound **3**, leading to the $[C_7H_5N_2]^+$ ion at m/z 117, which formation indicates at least a partial transformation of the compound into the bisimino form.

CI Mass Spectra.

Chemical ionization (CI) is often used to determine the molecular mass of organic compounds, but sometimes it is also possible to obtain useful structural information. In this work deuterated ammonia, isobutane, methane and

acetone were used as reagent gases. The first three reagent gases mentioned above, gave rise only to the protonated molecules of the free base and also the protonated molecule of the hydroiodide appeared in most of the spectra. None of the three gases were able to produce fragmentations, not even methane.

However, methane was able to form adducts with the molecular ion of the free base. The $[M^+C_2H_5]^+$ ion appeared for every compound. Isobutane produced the rather weak $[M^+C_3H_7]^+$ and $[M+C_4H_9]^+$ adducts of the molecular ion peak of the free base, whereas there were no adducts in the deuterated ammonia CI spectra. Deuterated ammonia was used mainly routinely to check the active hydrogens of the molecule. The hydrogens attached to the nitrogen and the hydrogen in the hydroiodide were replaced with deuterium.

When acetone was used as a reagent gas, there were, as with other gases, remarkably intense protonated molecules of the free base as well as of the hydroiodide salt. The formation of the adduct ions under acetone CI was especially favourable; both the $[M+C_2H_3O]^+$ and $[M+C_3H_7O]^+$ adducts of the free base were relatively intense. A typical acetone spectrum is shown in Figure 1. Furthermore, in some cases the proton bound dimer was also shown in the spectra. The formation of the adducts mentioned above is typical for acetone [9]. The favoured adduct formation for the compounds studied, can be explained with the large number of heteroatoms; the more heteroatoms there are, the more there are possible sites for hydrogen bridges.

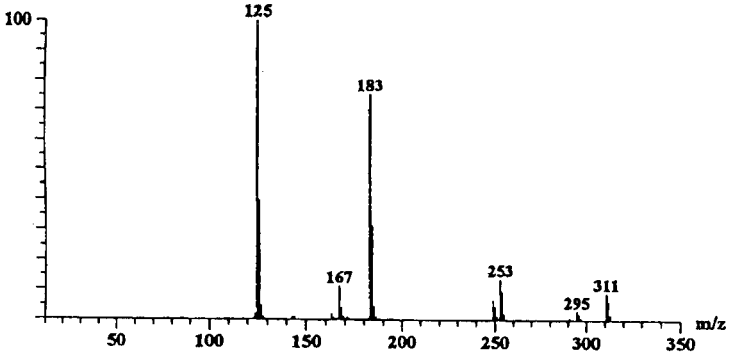


Figure 1. The acetone CI mass spectrum of 1-imino-6,7-dihydro-1*H*,5*H*-pyrazolo[1,2-*a*][1,2,4]triazole hydroiodide (**1**).

The fragmentation of the ions formed was studied by collision induced dissociation (CID), because no fragmentations took place in the ion source. Since all the compounds behaved similarly under acetone CI, compound **1** is used as an example. Even the CID technique did not provide many fragmentations. The acetone CI CID spectra of the $[MHI+59]^+$ adduct ion indicated that HI was attached rather tightly to the molecule, because only the elimination of acetone was observed. The CID spectra showed that the $[M+C_2H_3O]^+$ adduct decomposed either losing the acetyl as a neutral ketene, $CH_2=C=O$, giving rise to the protonated molecule at m/z 125, or acetyl may also leave as CH_3CO^+, leading to the ion at m/z 124. The ketene elimination was more favourable than the latter elimination. The $[M+C_3H_7O]^+$ ion mainly lost the acetone as a neutral molecule leading to the ion at m/z 125. This can be easily understood, since due to the several nitrogen atoms, the proton affinities of the compounds studied must be noticeably higher than the proton affinity of acetone. The corresponding adducts of the hydroiodide decomposed similarly.

The only fragmentation that was observed for the protonated molecule, was the cleavage of 28 mass units leading to the ion at m/z 97. This result confirms that in the corresponding EI spectrum for **1**, the ion at m/z 97 originated at least in part from the protonated molecule.

EXPERIMENTAL

Measurements were made with a Jeol D300 mass spectrometer equipped with a combined EI/CI source and connected to a personal computer equipped with Windows based software: The Schrader System. Samples were introduced through a direct inlet probe at temperatures 200-320°. Typical source conditions were: temperature 170°, electron energy 70 eV for EI and 200 eV for CI, accelerating voltage 3kV and ionization current 300 μA. Under chemical ionization the reagent gas flow was adjusted by controlling the source housing pressure (8×10^{-6}). The reagent gases were: acetone, deuterated ammonia, methane and isobutane. Accurate mass measurements were made at resolution 5000 using the data system. Metastable ion analysis was performed with linked-scans at constant B/E. In CID experiments, helium was added to the first field-free region until the transmission of the main beam was 33%.

The synthesis and identification of the compounds have been described elsewhere [10,11].

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