Characterization and Differentiation of Heterocyclic Isomers. Part 3.# Study of High Internal Energy Ions Produced by Electron Ionization Mass Spectrometry on 3-Methyl-1,2- and 2-Methyl-1,3-Thiazolopyridines

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The eight members of two classes of heterocyclic isomers, namely 3-methyl-1,2-, **1a-d** and 2-methyl-1,3-thiazolopyridines **2a-d** have been characterized by mass spectrometry under electron ionization. High internal energy ions formed in the source have been studied by low and high resolution mass spectrometry.

The data show remarkable differences among the various components of each class depending on the position of the nitrogen in the pyridine ring. Furthermore, by comparing the mass spectra of members of series **1** with those of their corresponding isomers belonging to series **2**, it is still possible to obtain evidence for different behaviors in the fragmentation pathways. It seems to exclude the occurrence of interconversion phenomena from one isomer into another, as well as conversion to a common intermediate before fragmentation. This also suggests that each member of series **1** and **2** retains its own structure after electron ionization.

The data obtained on a double focusing instrument equipped with electrostatic and magnetic analyzers have been compared with those obtained on a mass spectrometer with an ion trap as the analyzer.

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Introduction.

One of the most important problems in many branches of chemistry is the structural characterization of isomers. Different methods are suitable for this aim. In particular, mass spectrometry (ms) can be a valid tool for the identification and structural characterization of isomers of organic and organometallic compounds, both of synthetic origin or naturally occurring. As it operates in the gas phase under high vacuum, mass spectrometry allows the study of intrinsic properties of a given molecule, free of solvating or hydration effects [1].

In addition, mass spectrometry allows one to characterize a given compound by studying the behavior of ions differing in internal energy content. In fact, after the ionization process, the molecular ion, or pseudomolecular ion depending on the ionization technique used, generally has an excess of energy that it tends to dissipate by fragmentation. This produces the formation inside the ion source of fragment ions characterized by a high internal energy. In contrast, ions formed out of this region, the metastable ions, have a low internal energy content [2,3].

Often the study of high internal energy ions does not allow one to distinguish among structural isomers. This can be explained by considering that these ions may easily give rise to rearrangement phenomena, mainly consisting in the conversion of one isomer into another or to a common structure, thus not allowing their characterization and differentiation. In other cases, high internal energy ions may retain their own structure, producing distinctive mass spectra for each isomer.

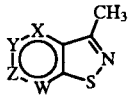
Among positional isomers, those containing heterocyclic rings play an important role in organic chemistry. In fact, owing to different positions of endocyclic groups or type of fusion between rings, several cases are possible. So, from a relatively small structure, many different isomers can be obtained.

For some years on we have been developing a research project consisting in the synthesis and structural characterization of heterocyclic isomers formed by a pyridine ring fused with a five-membered ring [4,8]. Thus the eight members of 3-methyl-1,2- and 2-methyl-1,3-oxazolopyridines have been synthesized and characterized in solution by nuclear magnetic resonance [6] and in the gas phase by mass spectrometry. The study of their mass spectra produced by ions formed in the ion source has allowed only poor distinction among different isomers [7]. On the other hand, a ms/ms investigation on metastable and collision-induced decompositions has provided useful information to differentiate unambiguously each of the eight isomers [8].

Recently our interest has been focused on their thia analogues whose interest is due to the fact that compounds containing the (iso)thiazolo moiety may show analgesic, antipyretic and antiinflammatory properties [9]. Several studies on heterocycles containing nitrogen and sulfur, carried out by mass spectrometry, have been previously reported [10]. In particular, thiazoles, isothiazoles and their derivatives have been the subject of several investigations [11-16].

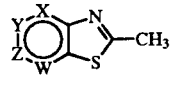
We wish to report here on the structural characterization and differentiation of all the members of the two classes of heterocyclic isomers, namely 3-methyl-1,2-thiazolopyridines **1a-d** and their isomers **2a-d** (Scheme 1), carried out by low and high resolution electron ionization mass spectrometry. The data obtained by a spectrometer equipped with electrostatic and magnet analyzers were compared with those obtained by an ion trap mass spectrometer.

Scheme 1



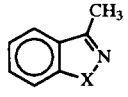
1

a X = N; Y, Z, W = CH
b Y = N; X, Z, W = CH

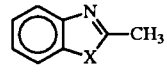


2

c Z = N; X, Y, W = CH
d W = N; X, Y, Z = CH



3 X = S
5 X = O



4 X = S
6 X = O

To evaluate the role of the pyridine nitrogen and that of the heteroatoms in the five-membered ring, the data have been compared with those obtained for 3-methylbenzo-1,2-thiazole (**3**), 2-methylbenzo-1,3-thiazole (**4**), 3-methylbenzo-1,2-oxazole (**5**) and 2-methylbenzo-1,3-oxazole (**6**) (Scheme 1).

The published mass spectra of compounds **3-6** are updated and they had been produced by some laboratories in different experimental conditions [12,16-18]. For these reasons, and to compare them with the mass spectra of compounds of series **1** and **2**, the mass spectra of compounds **3-6** were recorded under the same experimental conditions as those used for compounds **1a-d** and **2a-d**.

EXPERIMENTAL

Synthesis of the Compounds.

3-Methyl-1,2-thiazolo[4,5-*b*]pyridine (**1a**) and 3-methyl-1,2-thiazolo[5,4-*c*] pyridine (**1c**) have been synthesized respectively from 2-cyano- or 4-cyano-3-mercaptopyridine according to a procedure reported in the literature [5]. 3-Methyl-1,2-thiazolo[4,5-*c*] pyridine (**1b**) and 3-methyl-1,2-thiazolo[5,4-*b*]pyridine (**1d**) have been prepared from the appropriated 1,2-oxazolopyridine-4-thiol and $Mo(CO)_6$ according to the procedure reported in ref. 4. 2-Methyl-1,3-thiazolopyridines **2a-d** were obtained by cyclization of the suitable thioacetamidopyridine following published procedures [19-21].

3-Methylbenzo-1,2-thiazole (**3**) and 3-methylbenzo-1,2-oxazole (**5**) have been prepared according to previously reported procedures [22,23]. Compounds **4** and **6** were purchased by Aldrich.

3-Trideuteromethyl-1,2-thiazolo[5,4-*b*]pyridine (**1e**)

To a cooled suspension of 3-cyano-2-mercaptopyridine (0.4 g, 3 m*M*) in anhydrous tetrahydrofuran (10 ml), 1 *M* diethyl ether solution of methyl-d_3-magnesium iodide (6 ml) was added slowly under nitrogen and the mixture was stirred at room temperature overnight. Water was added and the solution was extracted with ether (3 x 10 ml). The ethereal extracts were washed with water, dried and evaporated to give compound **1e** (32%), mp 85-87 (sublimed *in vacuo*); ir (potassium bromide): ν 3020, 1950, 1920, 1570, 1550, 1460 cm^{-1}; pmr (200 MHz, deuteriochloroform, J in Hz): 7.36 (dd, 1H, J = 8.2 and 4.3, H5), 8.22 (dd, 1H, J = 8.2 and 1.6, H4), 8.76 (dd, 1H, J = 4.3 and 1.6, H6) ppm.

Anal. Calcd. for $C_7D_3H_3N_2S$: C, 54.88; H/D 5.91; N, 18.28. Found: C, 55.15; H/D, 5.82; N, 18.40.

Mass Spectrometry.

Mass spectra were measured on a double focusing VG 70-250S mass spectrometer (VG Analytical Ltd., Manchester, UK) operating in the electron ionization mode at 70 eV, emission current 0.2 mA, with a source temperature of 180°C. The accelerating voltage was 8 kV and the resolution was 1000 M/ΔM (10% valley). Owing to the high volatility of the compounds, they were introduced into the mass spectrometer by the coupling with a gas chromatograph Hewlett-Packard 5890 equipped with a 5MS Hewlett-Packard column (30 m x 0.25 mm, film thickness 0.25 μm) by using ultra pure helium (SOL) as a carrier gas. The temperature of the oven was programmed from 100 to 160°C with a gradient of 1°C min^{-1}.

High resolution mass measurements were performed at resolution 10000 M/ΔM (10% valley) against perfluorokerosene standard (Fluka). Data acquisition and analysis of the spectra were performed with a VG 11-250J data system equipped with a Digital PDP 11/83 minicomputer.

For compounds **1a-d**, **2a-d** and **3-6** the mass spectra were also obtained on an ion trap instrument Varian Saturn 4D coupled with a gas chromatograph Varian Star 3400 CX equipped with a column J&W DB5-MS (30μm x 0.25 mm, film thickness 0.25 μm) by using ultra pure helium (SOL) as a carrier gas.

With the aim of limiting experimental variations between different measurements, in all cases solutions containing a couple of corresponding 3-methyl-1,2- and 2-methyl-1,3-thiazolopyridines (*e.g.*, **1a**, **2a**) have been injected, so to record the spectra of the two species in the same experiment. The reported values of relative intensities are the mean of 5-10 scans.

Results and Discussion.

Gas Chromatography-Mass Spectrometry.

The use of the coupling gas chromatography-mass spectrometry (gc-ms) has allowed to confirm the purity of each compound. Furthermore, to evaluate the effects of physico-chemical properties of each isomer, such as boiling point and polarity, on the retention times, some artificial mixtures have been analyzed by

gc-ms. The mass chromatogram of compounds **1a-d** and **2a,c,d** is reported in Figure 1, while in Table 1 their retention times are compared with those of the analogous isomers **3-6**.

From the examination of the data (Table 1) it appears that isomers **5** and **6**, showing the shortest retention times, clearly differentiate from the other compounds. This can be due to the presence of the atom of oxygen that modifies the polarity of the molecules and lowers their boiling points. Compound **6** precedes its 1,2-oxazole isomer **5**.

Table 1

Retention Times (minutes) for Methyltiazolopyridines **1a-d**, **2a-d** and for their Oxa Derivatives **3-6**

1a	1b	1c	1d	2a	2b	2c	2d	3	4	5	6
13:22	14:49	14:21	15:28	22:19	15:24	14:37	12:40	11:31	13:02	6:40	5:12

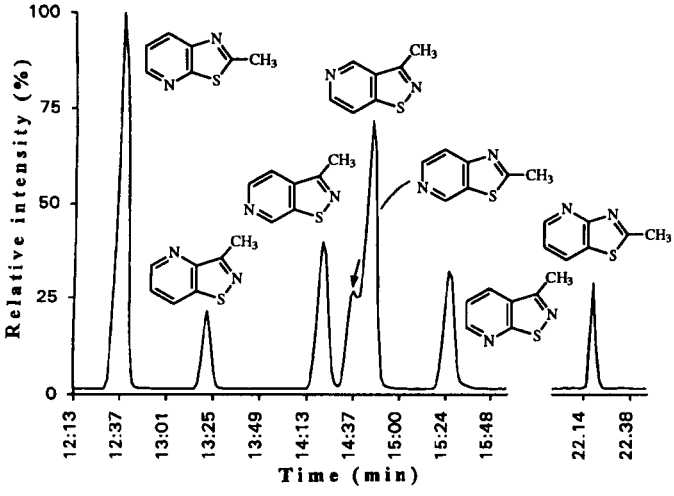


Figure 1. Mass chromatogram of the mixture containing isomers **1a-d** and **2a,c,d**.

In the case of thia heterocycles **1a-d**, **2a-d**, **3** and **4**, a general trend is that each 1,2-thiazole derivative precedes its 1,3-thiazole isomer, even if large differences in the relative retention times are found. An exception is represented by the couple of isomers **1d**, **2d**, where **2d** shows a shorter retention time than **1d**.

These data seem to indicate that both the nature of the heteroatoms present in the five-membered ring and their arrangement in the ring play a prevailing role in respect to that of the pyridine or benzene in the interaction with the stationary phase of the gas chromatographic column. The extremely long retention time of isomer **2a** may be attributed to the fact that this is the only case in which two nitrogen atoms occur on the same side, free of steric hindrance, of the molecule. Thus, it is reasonably to suppose that both the two nitrogen atoms may interact with the stationary phase of the column, lengthening the retention time of **2a**.

Mass Spectrometry.

The mass spectra produced in the ion source by isomers **1a-d** and **2a-d** are reported in Table 2. Ion compositions have been assigned by accurate mass measurements carried out on high resolution data. The mass measurement errors for the reported compositions were within 6 ppm. As an example the spectra of the 1,2-thiazole isomers **1a** and **1b** are depicted in Figure 2, while those relevant to compounds **1c** and **2c**, differing in the five membered ring, are compared in Figure 3.

Similarly to other analogous heterocycles, such as 1,2- and 1,3-thiazole [24], 3-methyl-1,2- and 2-methyl-1,3-oxazolopyridines [7], all compounds exhibit high stability under electron ionization as shown by the relative intensities of their molecular ions (m/z 150) which in all the spectra are the base peak. The isomers show

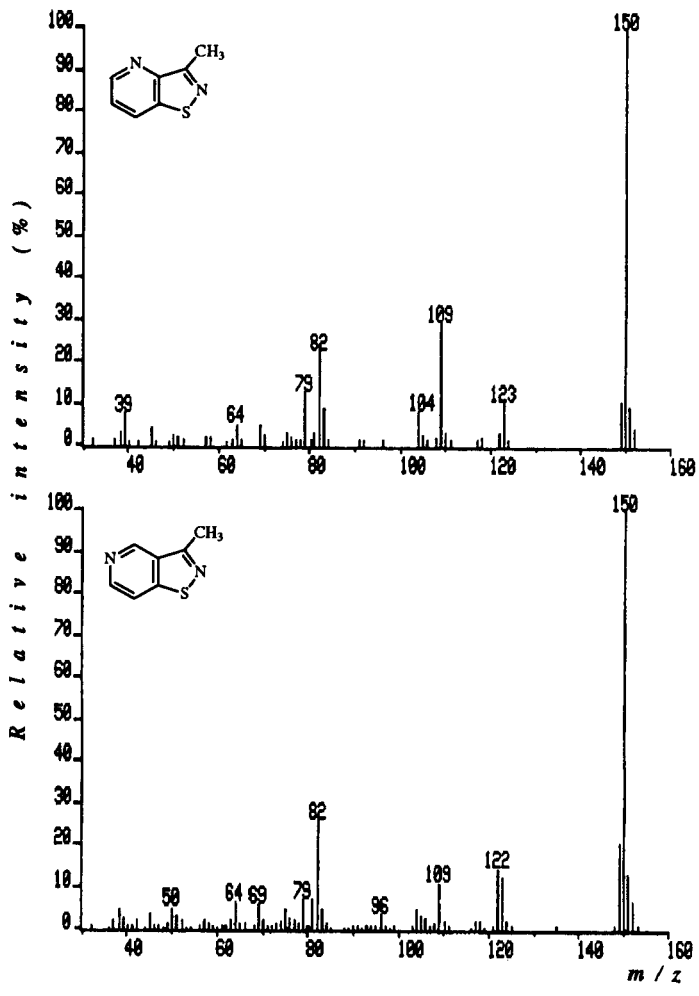


Figure 2. Electron ionization mass spectra of the isothiazole isomers **1a** (top) and **1b** (bottom).

common fragmentation pathways, but remarkable differences in the relative intensities of fragment ions occur. As it appears from the examination of the data (Table 2), while isomers of series **1**

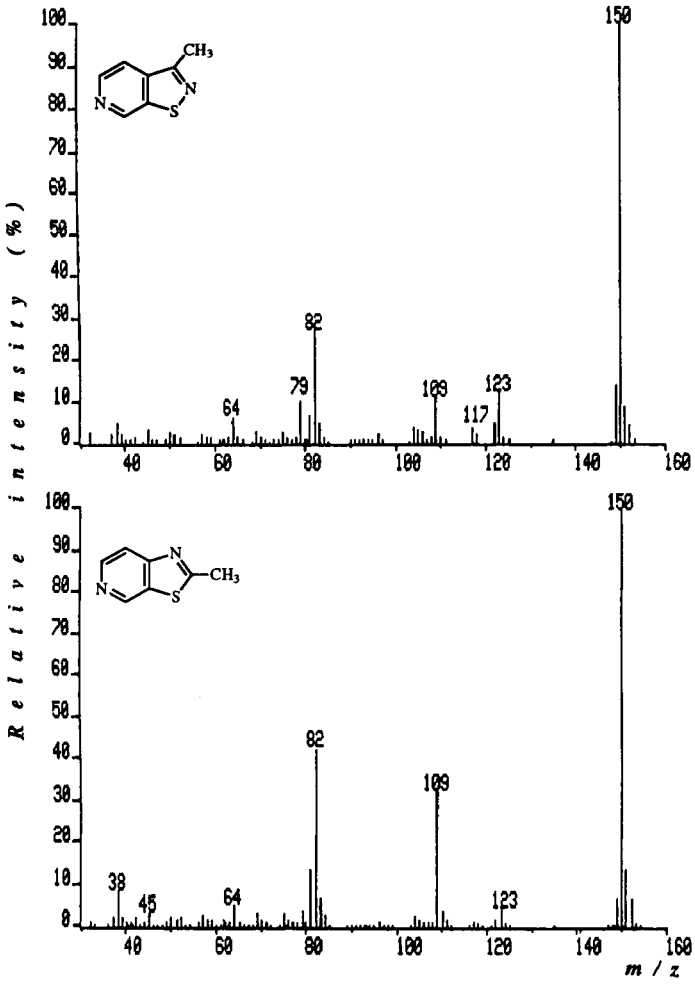


Figure 3. Comparison between the electron ionization mass spectra of isomers **1c** (top) and **2c** (bottom) differing in the five membered ring.

show fragment ions with similar relative intensities in the range 10-30%, compounds of series **2** generally produce mass spectra characterized by a triplet of intense ions at m/z 150, 109 and 82. An exception is represented by **2d** whose spectrum is quite similar to those of isomers **1a-d**.

All the spectra show an ion at m/z 149 corresponding to the loss of H$^{\bullet}$ from the molecular ion. Its relative intensity is higher for isomers **1b** and **1d** in respect to **1a** and **1c**. In general, compounds belonging to the series **2** show the ion $[M-H]^+$ with a smaller relative intensity than that found for their corresponding 1,2-thiazolo derivatives **1a-d** (Table 2). On the other hand, in the spectrum of **2d** this ion shows a relative intensity higher than that found for **1d**. When the pyridine nitrogen is replaced by a CH group, thus yielding compounds **3** and **4**, the loss of a hydrogen radical still remains highly favored, its relative intensity being 22.2 and 24.1%, respectively. By replacing the sulfur atom with an oxygen, as it occurs for 3-methyl-1,2- and 2-methyl-1,3-oxazolopyridines, the relative intensity of the ion $[M-H]^+$ is in the range 1-5% [7]. The loss of a hydrogen radical is abundant also for the two isomers 4-, and 5-methyl-1,2-thiazole [24]. On the other hand, it is quite scarce for 4-methyl-1,3-thiazole [11].

In compounds **1a-d** and **2a-d**, the loss of a hydrogen radical from the molecular ion might occur from different hydrogen atoms belonging to the pyridine or to the methyl group. To shed light on this and other fragmentations, the study of the 3-trideuteromethyl derivative of **1d** (**1e**) is particularly interesting. Its spectrum has shown the prevailing loss of deuterium with respect to that of hydrogen ($[M-D]^+/[M-H]^+>2$). It indicates the selective loss of a hydrogen radical from the methyl group. It also suggests that, in contrast to that observed in analogous heterocycles, such as benzo-1,2-thiazole and benzo-1,3-thiazole, for which scrambling of hydrogen atoms between different endocyclic positions has been observed [25], in the present case the scrambling phenomena of the methyl hydrogens seem to be very poor.

For all the eight isomers, the loss of a methyl radical is a process of little importance, producing an ion $[M-CH_3]^+$ (m/z 135) whose relative intensity is below 2%.

Other important fragmentations occurring in the ion source consist in the loss of HCN and CH_3CN. The relative intensity of the ion $[M-HCN]^{+\bullet}$ is quite similar for compounds **1a-d**, being in the range 10-12%. In the mass spectra produced by isomers **2a-d**, this fragment ion has very close and small relative intensities for the isomers **2a-c** (4-5%), while the highest intensity for all the eight compounds occurs in the case of **2d** (15.5%).

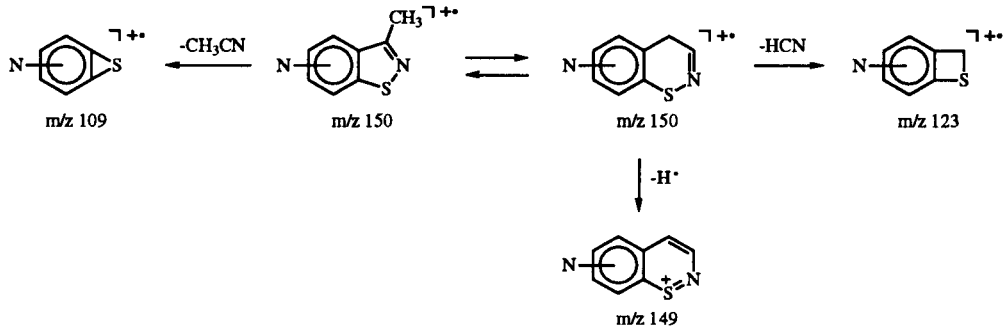
The mass spectra of both the benzoderivatives **3** and **4** also show an ion $[M-HCN]^{+\bullet}$ (m/z 122). In opposition to that observed for compounds of the series **1** and **2**, its relative intensity is about twice for the 1,3-thiazole derivative **4** (6.1%) respect to its 1,2-thiazole isomer **3** (2.7%). On the other hand, for oxa analogues of compounds **1a-d** and **2a-d**, namely 3-methyl-1,2- and 2-methyl-1,3-oxazolopyridines, the fragmentation yielding the loss of HCN does not occur [7]. Similarly, also compounds **5** and **6** do not show loss of HCN.

These data seem to suggest that the pyridine moiety has only a lesser involvement in the loss of HCN from the molecular ion. Furthermore, the comparison between oxa and thia derivatives seems to indicate that the presence of the sulfur in the five-membered ring is an essential requisite to allow the loss of HCN from the molecular ion. On the other hand, the 1,3-thiazolo[5,4-*d*]-pyrimidine shows consecutive loss of two molecules of HCN. It has been supposed that the elimination of HCN from the molecular ion occurs from the pyrimidine ring followed by a further loss of HCN from the thiazole ring [26]. Also the molecular ion of the 2-methyl-1,3-thiazolo[5,4-*d*]pyrimidine shows the loss of HCN from the pyrimidine ring followed by that of CH_3CN [26].

In the case of compounds of series **1** or **2** the loss of HCN from the molecular ion might involve the pyridine or the methyl group. The mass spectrum of **1e** shows the formation of an only ion at m/z 125, corresponding to the loss of 28 u from the molecular ion. It indicates that the selective loss of DCN involves the methyl moiety, excluding both the scrambling of the deuterium atoms on the heterocyclic system, and the involvement of the pyridine.

On the other hand it is difficult to hypothesize the loss of HCN from the five-membered ring if this preserves the structure of the parent neutral molecule. Thus it is reasonable to suppose that, consequently to the ionization process, the methyl(iso)thiazole moiety gives rise to a ring expansion yielding a thiazine-like structure, from which the loss of HCN may easily occur. This is also supported by the mass spectrum of 5,6-dihydro-4*H*-1,2-thiazine whose molecular ion fragments by loss of HCN [27]. Furthermore the occurrence of a ring-expanded structure for compounds **1a-d** and **2a-d** might explain also the loss of a hydrogen radical, that, as

Scheme 2



above reported, is a significant fragmentation process occurring in the ion source. The fragmentation pathway proposed for 1,2-thiazolo derivatives is depicted in Scheme 2. An analogous pathway may be also proposed for 1,3-thiazolo isomers **2a-d**.

The loss of HCN may also occur from the $[M-H]^+$ ion, yielding the second generation fragment ion at m/z 122. Its intensity is much higher for compounds of the series **1**, where the highest abundance is observed for **1b**, than for 1,3-thiazole derivatives for

Table 2

Mass Spectra Data Obtained with a Double Sector and with an Ion Trap (data in italics) Spectrometers for Compounds **1a-d**, **2a-d**. Ion Compositions have been Obtained by Accurate Mass Measurements of High Resolution Data

	Ion	Compound							
m/z	Composition	**1a**	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**
150	$[C_7H_6N_2S]^{+\cdot}$	100	100	100	100	100	100	100	100
		100	*100*	*100*	*100*	*100*	*100*	*100*	*100*
149	$[C_7H_5N_2S]^+$	10.3	19.8	13.7	21.8	6.0	10.4	6.5	26.6
		11.5	*19.8*	*14.6*	*20.8*	*6.2*	*11.5*	*7.3*	*21.9*
123	$[C_6H_5NS]^{+\cdot}$	10.1	12.1	12.0	10.6	4.7	5.0	4.0	15.5
		16.7	*18.7*	*20.8*	*15.6*	*8.3*	*9.9*	*8.8*	*18.9*
122	$[C_6H_4NS]^+$	3.8	14.0	4.8	7.0		1.9		1.3
		4.2	*14.6*	*9.4*	*8.3*	*1.6*	*3.1*	*2.0*	*2.0*
118	$[C_7H_6N_2]^{+\cdot}$	1.7	1.5	1.5	1.6		1.0		3.0
		3.1	*2.0*	*2.0*	*2.6*	*2.0*	*1.0*	*1.0*	*3.1*
117	$[C_7H_5N_2]^+$	1.3	2.0	3.2	1.9				3.3
		2.0	*2.0*	*4.2*	*2.6*		*1.0*	*1.0*	*3.1*
109	$[C_5H_3NS]^{+\cdot}$	29.0	10.4	10.6	14.1	63.1	21.5	31.8	11.7
		24.0	*7.3*	*10.4*	*10.4*	*47.9*	*13.5*	*25.0*	*7.3*
108	$[C_5H_2NS]^+$	1.5	1.4	1.3	1.4	2.0			1.6
106	$[C_6H_6N_2]^{+\cdot}$	1.0	2.3	2.3	1.6		1.2		4.1
		3.1	*4.2*	*4.2*	*3.1*	*2.0*	*3.1*	*2.0*	*5.2*
105	$[C_6H_5N_2]^+$	2.2	2.7	2.8	4.7	1.2	2.4	1.0	7.6
		3.1	*4.2*	*3.1*	*5.2*	*2.0*	*4.7*	*2.0*	*6.9*
104	$[C_6H_4N_2]^{+\cdot}$	7.8	4.4	3.2	9.8	2.4	3.0	2.0	9.4
		8.3	*4.2*		*9.4*	*2.0*	*3.6*	*3.6*	*6.2*
96	$[C_5H_4S]^{+\cdot}$	1.4	3.5	1.6	1.5		1.4		
		3.1	*5.2*	*3.6*	*3.1*	*1.0*	*2.0*	*1.0*	*1.6*
83		8.7	4.3	4.4	6.5	15.0	6.8	6.3	10.3
		14.9	*5.2*	*5.2*	*7.3*	*13.9*	*7.3*	*7.3*	*10.4*
82	$[C_4H_2S]^{+\cdot}$	22.2	25.4	26.9	16.3	33.2	42.4	41.9	23.3
		32.3	*26.0*	*36.5*	*18.8*	*37.5*	*38.5*	*43.8*	*22.9*
81	$[C_4HS]^+$	2.7	7.0	6.3	3.2	3.8	11.8	12.9	5.9
		6.2	*10.4*	*14.6*	*6.2*	*6.2*	*12.5*	*18.8*	*9.4*
79	$[C_5H_5N]^{+\cdot}$	12.7	6.9	9.5	11.7	8.8	5.1	3.6	17.7
		21.2	*10.4*	*14.6*	*15.6*	*20.8*	*11.8*	*7.3*	*20.8*
75		3.1	4.8	2.5	4.4	4.6	4.0	3.0	4.7
69	$[C_3HS]^+$	4.8	5.8	2.4	3.0	3.6	10.9	3.1	4.3
		9.4	*9.4*	*5.2*	*5.2*	*11.6*	*12.5*	*5.2*	*5.2*
64	$[C_4H_2N]^+$	4.8	6.2	5.9	5.5	8.0	5.0	4.4	6.8
		9.4	*8.3*	*10.4*	*8.3*	*10.4*	*6.2*	*6.2*	*10.4*
45									
		10.4	*7.3*	*8.3*	*6.2*	*10.4*	*7.3*	*7.3*	*6.8*

which this fragmentation is quite poor and detectable only in the mass spectra of **2b** and **2d** with relative intensities lower than 2% (Table 2). The ion at m/z 122 might also derive from the molecular ion by direct elimination of the radical H_2CN , as observed in the mass spectrum of 5,6-dihydro-4*H*-1,2-thiazine [27], further supporting the hypothesis for a ring-expansion for the molecular ion of compounds **1a-d** and **2a-d**. The study of the ratios of the ions at m/z 123 and 122 is also a good way to distinguish each isomer from the others. A significant example is constituted by **1b** where, differently from all the other isomers, the ions at m/z 123 and 122 have similar intensities, with the latter slightly prevailing.

A further loss of HCN is observed from the ion at m/z 123 corresponding to $[M\text{-}HCN]^{+\bullet}$ yielding the second generation fragment ion $[(M\text{-}HCN)\text{-}HCN]^{+\bullet}$ at m/z 96. It suggests that in compounds of series **1** and **2** the loss of HCN may occur both from the five membered ring and from the pyridine moiety. As already observed, while the loss of HCN from the (iso)thiazole ring is highly favored from the molecular ion, that from the pyridine ring occurs only after that a molecule of HCN has been eliminated by the five membered ring. The ion at m/z 96 has very low abundances (<2%) that only in the case of isomer **1b** reaches 3.5% (Table 2).

Another characteristic fragmentation of compounds **1a-d** and **2a-d** regards the elimination of CH_3CN from the molecular ion, yielding the fragment ion at m/z 109. Its relative intensity shows wide variations depending on the isomer considered, ranging from 10.4 (**1b**) to 63.1% (**2a**) (Table 2). Contrarily to what observed for the loss of HCN, the relative intensities of the ion $[M\text{-}CH_3CN]^{+\bullet}$ are in general higher for the 1,3-thiazolo derivatives **2a-d** than their corresponding 1,2-thiazolo isomers **1a-d**. Inside isomers of series **2**, this loss is highly favored for isomer **2a** in respect to compounds **2c** and **2b**. An exception is represented by the couple **1d**, **2d** for which **1d** shows a higher abundance for this process. Inside each series constituted by 1,2- and 1,3-thiazolo derivatives, this loss prevails from isomer **a** (**1a**, 29.0; **2a**, 63.1%) with respect to the others.

The mass spectrum of **1e** shows an ion at m/z 109 due to the contribution of two isobaric ions that have been resolved by high resolution mass spectrometry. One is produced by the selective loss of CD_3CN and the other is due to the loss of CS from the molecular ion. The very low intensities of the ion at m/z 112 due to $[M\text{-}CH_3CN]^{+\bullet}$, and of those due to $[M\text{-}CH_2DCN]^{+\bullet}$ (m/z 111) and $[M\text{-}CHD_2CN]^{+\bullet}$ (m/z 110), again suggest very little scrambling of the hydrogens of the methyl group over the heteroaromatic system.

The loss of CH_3CN constitutes an important process occurring in the ion source also for the molecular ions of 3-methyl-1,2- and 2-methyl-1,3-oxazolopyridines [7], as well as for the methylbenzothiazoles **3** and **4**. It represents the most intense fragment ion for **3** (31.8%), while in the mass spectrum of **4** its intensity is about one third (12.3%). Both compounds **3** and **4** show the prevailing loss of CH_3CN with respect to that of HCN.

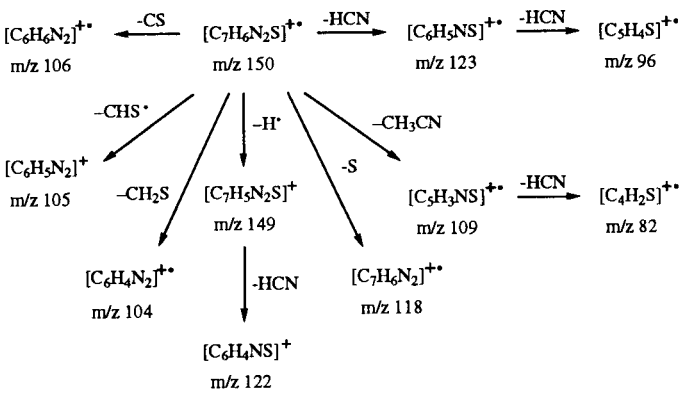
Direct losses of sulfur-containing species from the molecular ion involve the elimination of S, $^{\bullet}CHS$ and CH_2S yielding ions at m/z 118, 105, 104, respectively (Table 2). All these processes are generally more favored from compounds **1a-d** than from their isomers **2a-d**. While the elimination of sulfur is a very poor fragmentation producing ions with relative intensity less than 2% for compounds of series **1**, that of CH_2S has a higher abundance, more pronounced for isomers **1a** (7.8%), **1d** (9.8%) and **2d** (9.4%) (Table 2).

Another important fragmentation pathway for compounds **1a-d** and **2a-d** yields the ion at m/z 82. High resolution measurements have allowed us to determine its elemental composition attributable to $[C_4H_2S]^{+\bullet}$ (Table 2). This suggests that this ion is produced by losses of CH_3CN and HCN, the last occurring in this case from the pyridine. The presence of the ion at m/z 82 also in the mass spectrum of **1e** confirms this hypothesis. Each 1,2-thiazole derivative shows lower relative intensities than those relevant to its corresponding 1,3-thiazole isomer. As an example, the relative intensity of the ion at m/z 82 is 25.4% in the mass spectrum of **1b**, while it is 42.4% for **2b**. Among isomers of series **2**, compounds **2b** and **2c** show similar relative intensities and higher than that relevant to isomers **2a** and **2d** (Table 2).

In the region of m/z 80-30, an intense ion is at m/z 79 that, from high resolution data is attributable to $[C_5H_5N]^{+\bullet}$, formally corresponding to the molecular ion of the pyridine. Different fragmentation processes might produce this ion. In the mass spectrum of **1e** it is shifted to m/z 81, indicating the presence of two deuterium atoms. It is therefore reasonable to suppose that the ion at m/z 79 is produced by the loss of HCN from the methylisothiazolo moiety followed by the elimination of CS. For the eight isomers relative intensities for this ion range from 3.6 (**2c**) to 17.7% (**2d**). Compounds **1a-d** show relative intensity ranging from 6.9 (**1b**) to 12.7% (**1a**).

The fragmentation pathways occurring in the ion source for compounds **1a-d** and **2a-d** are summarized in Scheme 3.

Scheme 3



Doubly-charged Ions.

The mass spectra of the eight isomers **1a-d** and **2a-d**, as well as those of their benzo derivatives **3-6** are characterized not only by the presence of mono charged ions, but also by doubly charged species. This may be explained by considering that the large aromatic system and the presence of at least two heteroatoms can well delocalize two positive charges. Most of the spectra show the species $[M]^{2+}$, $[M\text{-}HCN]^{2+}$ and $[M\text{-}CH_3CN]^{2+}$ (Table 3). As observed for mono charged species, also for bipositive ions the molecular ion shows the highest abundance.

Ion Trap Mass Spectra.

The same experiments described above have been carried out also on an ion trap mass spectrometer. Basically this kind of instrument differs from the double sector mass spectrometer because in the latter ion separation occurs in space, while in the ion trap it occurs in time. Nevertheless the double sector and the ion trap mass spectrometers are based on deeply different architectures, the mass spectra of compounds **1a-d** and **2a-d** obtained with the two

Table 3

Doubly Charged Ion Mass Spectra for Compounds **1a-d**, **2a-d**. The Relative Intensity of the Most Intense Doubly Charged Ion has been Assigned Arbitrarily to 100

	Compound							
Ion	**1a**	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**
M^{2+}	100	100	100	100	100	100	100	100
$[M-H]^{2+}$		2.1			2.4			
$[M-HCN]^{2+}$	7.8	9.1	33.3	19.0	7.1	11.7	33.4	12.5
$[M-CH_2N]^{2+}$		13.7	17.0	9.5		10.0	7.4	10.4
$[M-C_3H_3N]^{2+}$		4.4				3.3		
$[M-C_3H_4N]^{2+}$	1.2	4.6		4.8		6.7		
$[M-CH_3CN]^{2+}$	4.5	16.7		2.4	2.4	3.1		8.3

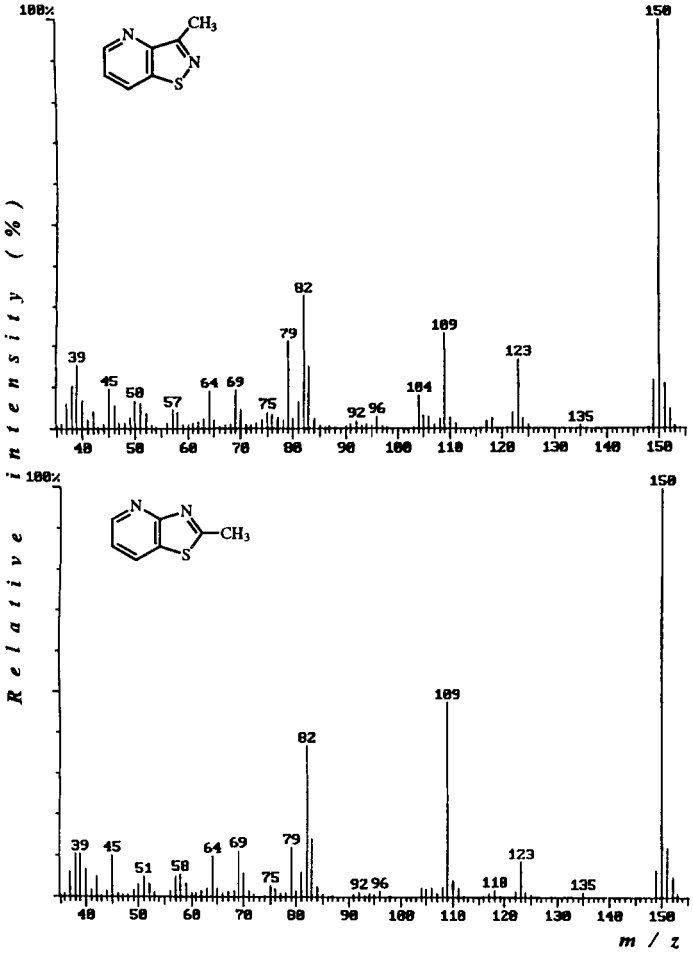


Figure 4. Electron ionization ion trap mass spectra of isomers **1a** (top) and **2a** (bottom).

instruments are quite similar (Table 2). As an example, the ion trap mass spectra for compounds **1a** and **2a** are depicted in Figure 4.

Also the ion trap mass spectra of the eight isomers are dominated by the molecular ion as the base peak. Fragmentation pathways are the same than those occurring in the ion source of the double sector instrument. Generally by using an ion trap the mass spectra show slightly more intense fragment ions. As an example, in the ion trap mass spectrum of **1c** the fragment ion at m/z 123 has relative intensity equal to 20.8% compared to 12.0% found by using the double sector spectrometer.

Conclusions.

This study, applied to all the members of the two classes of thiazolopyridine derivatives **1a-d** and **2a-d**, revealed that each isomer produces a distinctive mass spectrum. It is supposed that the one-electron removal yields the formation of molecular ions with *composite* structures. In particular, a ring expansion of the methyl(iso)thiazole moiety to a thiazine ring should allow us to explain the loss of HCN from the molecular ions of the eight isomers.

Main fragmentations occurring in the ion source involve the losses of HCN and CH_3CN. These are competitive processes whose abundances depend upon both the position of the nitrogen in the pyridine ring and on the nature of the five-membered ring.

The data have shown that it is possible to characterize and differentiate isomers belonging to the same series (*i.e.*, **1** or **2**) depending on the position of the nitrogen in the pyridine ring. Furthermore it is also possible to distinguish the corresponding isomers differing in the nature of the five-membered ring (for example **1a** from **2a**). Comparison between the data allows us to evaluate the role of the position of the nitrogen in the pyridine ring as well as that exerted by the five-membered ring on the fragmentation pathways followed by high internal energy ions produced in the ion source of the mass spectrometer.

The characterization of low internal energy ions formed out of the ion source is actually in progress.

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REFERENCES AND NOTES

For part 2 see G. Giorgi, M. Anzini, A. Cappelli, F. Corelli, and S. Vomero, *J. Am. Soc. Mass Spectrom.*, **7**, 653 (1996).

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[1] J. W. McKiernan, C. E. A. Beltrame, and C. J. Cassady, *J. Am. Soc. Mass Spectrom.*, **5**, 718 (1994).

[2] R. G. Cooks, J. H. Beynon, R. M. Caprioli, and G. R. Lester, Metastable Ions, Elsevier Scientific Publ. Co., Amsterdam, 1973.

[3] K. L. Bush, G. L Glish, and S. A. McLuckey, Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry; VCH, New York, 1988.

[4] S. Chimichi, R. Nesi, F. Ponticelli, and P. Tedeschi, *J. Chem. Soc., Perkin Trans. 1*, 1477 (1990).

[5] S. Chimichi, D. Giomi, P. Tedeschi, and F. Ponticelli, *Synth. Commun.*, **23**, 73 (1993).

[6] S. Chimichi, R. Nesi, F. Ponticelli, and P. Tedeschi, *Magn. Reson. Chem.*, **23**, 86 (1985).

[7] F. Ponticelli, D. Giomi, S. Papaleo, and P. Tedeschi, *Org. Mass Spectrom.*, **28**, 451 (1993).

[8] G. Giorgi, F. Ponticelli, G. Czira, and K. Vékey, *J. Am. Soc. Mass Spectrom.*, **6**, 962 (1995).

[9] T. Y Shen, R. L. Clark, A. A. Pessolano, and T. J. Lanza, Merck & Co. Inc., *German Patent* 2, 330, 109, 1974, *Chem. Abstr.*, **80**, 95916 (1974).

[10] Q. N. Porter, Mass Spectrometry of Heterocyclic Compounds, 2nd Ed, John Wiley & Sons, New York, 1985, pp 893-941 and references therein.

[11] G. M. Clarke, R. Grigg, and D. H. Williams, *J. Chem Soc. (B)*, 339 (1966).

[12] B. J. Millard, and A. F. Temple, *Org. Mass Spectrom.*, **1**, 285 (1968).

[13] G. Salmona, and E. J. Vincent, *Org. Mass Spectrom.*, **13**, 119 (1978).

[14] E. Jeanneau-Nicolle, C. Bosso, M. Benoit-Guyod, and G. Leclerc, *Org. Mass Spectrom.*, **28**, 37 (1993).

[15] G. Karminski-Zamola, V. Tralic-Kulenovic, L. Fiser-Jakic, M. Bajic, and D. W. Boykin, *Rapid Commun. Mass Spectrom.*, **9**, 1345 (1995).

[16] A. Selva, and E. Gaetani, *Org. Mass Spectrom.*, **7**, 327 (1973).

[17a] M. Ohashi, K. Tsujimoto, A. Yoshino, and T. Yonezawa, *Org. Mass Spectrom.*, **4**, 203 (1970); [b] W. Reichen, *Helv. Chim. Acta*, **60**, 186 (1977).

[18a] M. H. Palmer, E. R. R. Russell, and W. A. Wolstenholme, *Org. Mass Spectrom.*, **2**, 1265 (1969); [b] T. Doppler, H. Schmid, and H.-J. Hansen, *Helv. Chim. Acta*, **62**, 314 (1979).

[19] T. Takahashi, T. Yatsuka, and Y. Onuma, *J. Pharm. Soc. Japan*, **63**, 153 (1943).

[20] A. Couture and P. Grandelaudon, *Heterocycles*, **22**, 1383 (1984).

[21] A. Couture and P. Grandelaudon, *J. Heterocyclic Chem.*, **24**, 1765 (1987).

[22] T. Vitali, E. Gaetani, F. Ronchini, M. Nardelli, and G. Pelizzi, *J. Chem. Soc., Perkin Trans. 2*, 1114 (1977).

[23a] G. Casini, F. Gualtieri, and M. L. Stein, *J. Heterocyclic Chem.*, **2**, 385 (1965); [b] G. Casini, F. Gualtieri, and M. L. Stein, *J. Heterocyclic Chem.*, **6**, 279 (1969).

[24] , B. J. Millard, *J. Chem. Soc. (C)*, 1231 (1969).

[25] A. Selva, U. Vettori, and E. Gaetani, *Org. Mass Spectrom.*, **9**, 1161 (1974).

[26] A. Tatematsu, S. Inoue, S. Sugiura, and T. Goto, *Nippon Kagaku Zasshi*, **87**, 66 (1966).

[27] W. M. Leyshon, and D. A. Wilson, *J. Chem. Soc. Perkin I*, 1925 (1975).