

## Mass Spectrometry of 1,2,5-Oxadiazole *N*-Oxide Derivatives. Use of Deuterated Analogues in Fragmentation Pattern Studies

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Reportamos neste trabalho o estudo sistemático de fragmentação dos derivados de *N*-óxidos de 1,2,5-oxadiazóis por espectroscopia de massa, usando análogos marcados com deutério para identificar algumas fragmentações críticas. Foi confirmada a perda neutra de CH<sub>2</sub>O a partir do *N*<sub>2</sub>-óxido de 3-hidroximetil-4-fenil-1,2,5-oxidiazol, usando o análogo mono-deuterado. A perda de OH, a partir do oxigênio do *N*-óxido, por um rearranjo β-H e δ-H, foi claramente verificada a partir do *N*<sub>2</sub>-óxido de 3-(4-metilpiperazina-1-metil)-4-fenil-1,2,5-oxidiazol, usando-se o análogo tetra-deuterado adequado. O isômero *N*-óxido e análogos desoxigenados foram também usados para confirmar a participação do fragmento óxido no processo de defragmentação.

This paper reported on the study of fragmentation pattern in mass spectrometry of 1,2,5-oxadiazole *N*-oxide derivatives involving deuterium-labeled analogues to identify some critical fragmentations. A neutral CH<sub>2</sub>O loss from 3-hydroxymethyl-*N*<sub>2</sub>-oxide-4-phenyl-1,2,5-oxadiazole was confirmed with the corresponding mono-deuterated analogue. An OH loss, involving the oxygen of *N*-oxide, via β-H and δ-H rearrangement, was clearly revealed from 3-(4-methylpiperazine-1-ylmethyl)-*N*<sub>2</sub>-oxide-4-phenyl-1,2,5-oxadiazole using the adequate tetra-deuterated analogue. *N*-oxide isomer and deoxygenated analogues were also used to confirm the participation of the oxide moiety in the fragmentation process.

**Keywords:** 1,2,5-oxadiazole *N*-oxide, D-labeled furoxan, mass fragmentation, EI/MS

### Introduction

As part of an ongoing research program on the chemistry and biological characterization of *N*-oxide containing molecules, a number of 1,2,5-oxadiazole *N*-oxide, benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide, quinoxaline *N,N'*-dioxide, and 1,2,4-triazine *N*-oxide derivatives were synthesized and evaluated against different biological targets.<sup>1</sup> In the course of our synthetic chemical approach we developed 1,2,5-oxadiazole *N*-oxide derivatives as potential herbicides,<sup>2</sup> bioreductive compounds,<sup>3-6</sup> and anti-trypanosomal drugs,<sup>7,8</sup> using previously described synthetic methods.<sup>9</sup> During the structural elucidation of

these derivatives, we were interested in knowing about the presence and the exact location of the *N*-oxide functionality. Simple and readily available spectroscopic techniques (e.g. IR and <sup>1</sup>H NMR) are not able to unambiguously characterize the presence of an *N*-oxide group. For example, the characteristic IR absorptions of =N<sup>+</sup>-O<sup>-</sup> (1300-1200 cm<sup>-1</sup> and 970-950 cm<sup>-1</sup>) are not considered a conclusive proof because these bands can overlap with the fingerprint bands.<sup>10</sup> Similarly, <sup>1</sup>H NMR spectroscopy can not afford important information about the =N<sup>+</sup>-O<sup>-</sup> system, because these products are heterocycles devoid of protons. However, more recently 2D-HETCOR experiments (sequences of HMQC for one-bond correlation and sequences of HMBC for long distance/carbon correlation) provide us very important data about the presence and position of the =N<sup>+</sup>-O<sup>-</sup> moiety.

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In this context, the initial aim of the present study was to characterize the fragmentation pattern in mass spectrometry of the 1,2,5-oxadiazole *N*-oxide derivatives as an alternative structural determination technique. On the other hand, this work describes the elucidation of the fragmentation patterns of these compounds through the use of deuterated derivatives,<sup>11</sup> *N*-oxide isomer and deoxygenated (without *N*-oxide) analogues.<sup>12</sup>

## Results and Discussion

The spectra for selected derivatives **1-13** (Figure 1) revealed different fragmentation patterns, resulting from different side chains of the 1,2,5-oxadiazole heterocycle at C-3. The most relevant mass spectral data for derivatives **1-13** are presented in Table 1 and Figure 2 rationalizes important fragmentation pathways.

The molecular ion was detected in all cases. However, the abundances were very low for derivatives **4-6**, which contain a residue proven to undergo retro-Diels-Alder-fragmentation (morpholine, thiomorpholine, and methylpiperazine).<sup>13</sup> The same was observed for derivative **12**, that bears an aliphatic side chain on the semicarbazone moiety. The  $[M-16u]^{+}$  ion, corresponding to an oxygen loss, was observed in all cases as a relatively small peak. However, the peak corresponding to  $m/z$   $[M-17u]^{+}$ , became increasingly important for derivatives **4-7** and **11** as result of an OH $\cdot$  loss. This kind of fragmentation has been previously described for similar moieties, e.g., a NO<sub>2</sub> group losses OH $\cdot$  in *o*-nitrotoluenes and *o*-nitroanilines,<sup>13</sup> or for other adequately substituted *N*-oxide heterocycles.<sup>14,15</sup> In these compounds, two different rearrangement processes could explain this radical loss,  $\beta$ -H and/or  $\delta$ -H rearrangement (Figure 3). The high abundance of this fragment ion for

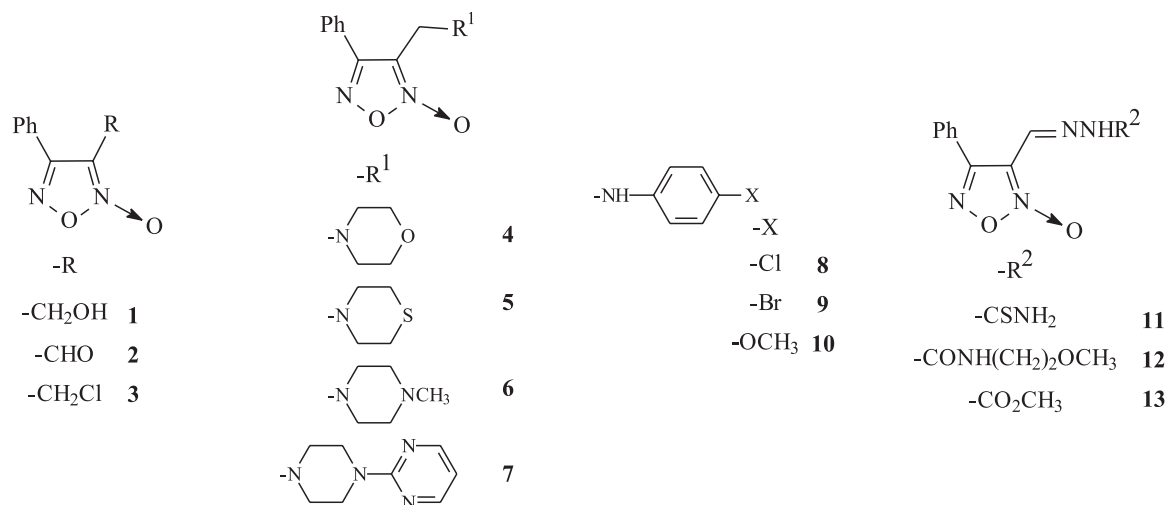
derivatives **4-7** (non-aromatic cyclic amine derivatives) compared to fragment ion abundance's for derivatives **8-10** (phenylamino derivatives) made us to think that a  $\delta$ -H rearrangement was the main process in this kind of structures. With a conventional EI/MS equipment it is not possible to study this fact and others, e.g., to determine whether the  $[M-30u]^{+}$  fragment ion in derivative **1** corresponds to a NO loss (as for the other derivatives) or a CH<sub>2</sub>O loss from the hydroxymethyl substituent in 3-position of the 1,2,5-oxadiazole heterocycle is due to an initial  $\gamma$ -H transference, which produces the stable neutral-product formaldehyde (Figure 4).

In order to explain these critical fragmentations in mass spectrometry, using a EI/MS equipment, we centered our

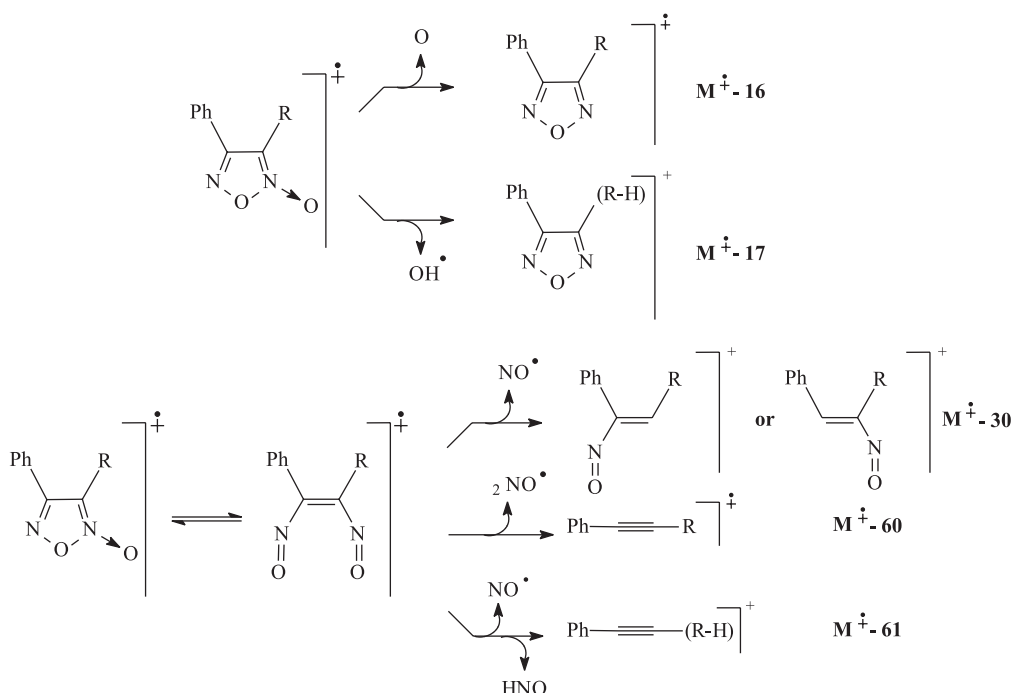
**Table 1.** Abundance of the most characteristic fragment ions in the corresponding 1,2,5-oxadiazole *N*-oxide derivatives EI mass spectrum

Compound No	Abundance (%) <sup>a,b</sup>					
	M <sup>+</sup>	M <sup>+</sup> -16	M <sup>+</sup> -17	M <sup>+</sup> -30	M <sup>+</sup> -60	M <sup>+</sup> -61
<b>1</b>	15.7	0.5	0.2	20.7	58.0	100.0
<b>2</b>	27.6	0.5	0.2	25.5	62.5	100.0
<b>3</b>	12.0	0.3	0.3	10.6	35.3	1.7
<b>4</b>	0.8	0.9	4.1	0.2	0.3	0.7
<b>5</b>	0.8	1.9	11.8	1.3	0.2	0.3
<b>6</b>	0.3	8.5	50.4	0.2	1.0	0.8
<b>7</b>	10.0	7.1	33.8	0.6	0.2	0.3
<b>8</b>	10.6	1.1	0.1	- <sup>c</sup>	1.1	2.6
<b>9</b>	12.1	2.1	-	-	1.1	2.5
<b>10</b>	21.2	3.9	1.7	-	1.6	1.6
<b>11</b>	10.6	3.5	11.3	57.6	55.5	6.8
<b>12</b>	0.2	0.2	0.8	31.8	7.3	0.3
<b>13</b>	2.3	-	-	7.5	13.3	-

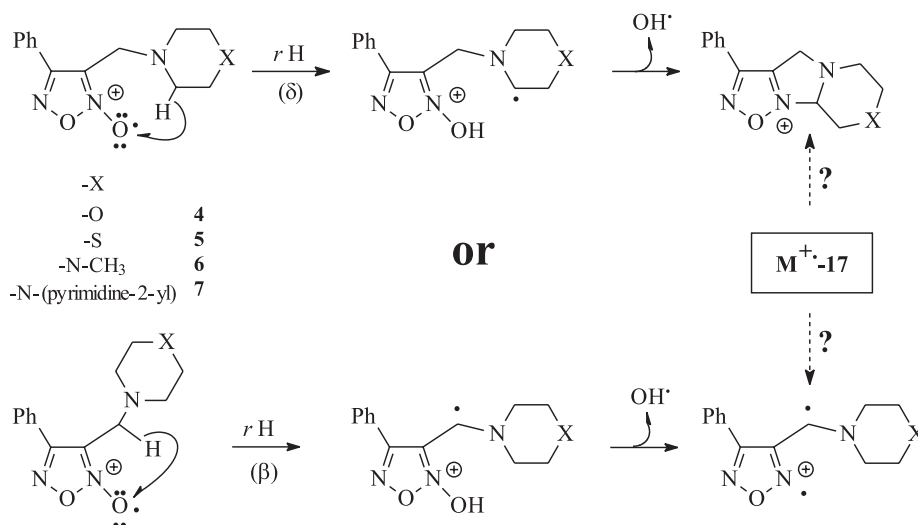
<sup>a</sup> Analytical conditions for EI/MS: direct injection, ion source temperature 250 °C, energy 70 eV. <sup>b</sup> The results are the averages for three independent experiments. <sup>c</sup> The “-” denotes that the fragment ion was not observed.



**Figure 1.** Series of 1,2,5-oxadiazole *N*-oxide developed in order to study their biological activity.



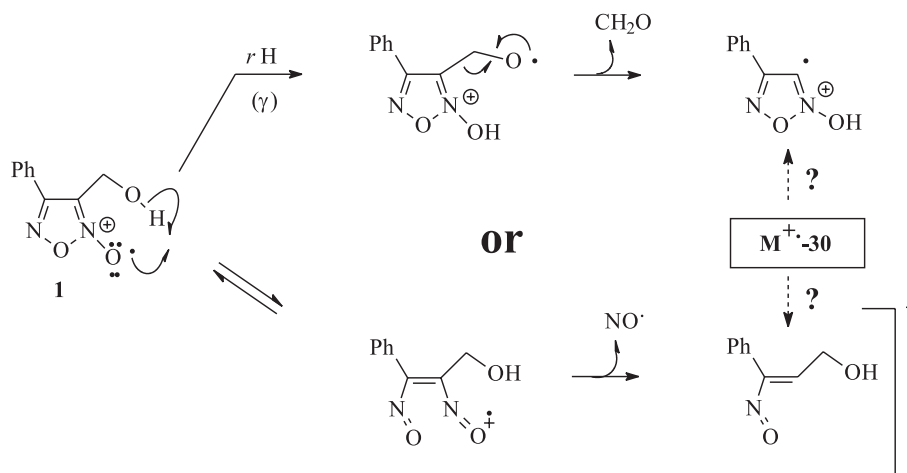
**Figure 2.** Most characteristic fragment ions in the 1,2,5-oxadiazole *N*-oxide derivatives EI mass spectrum.



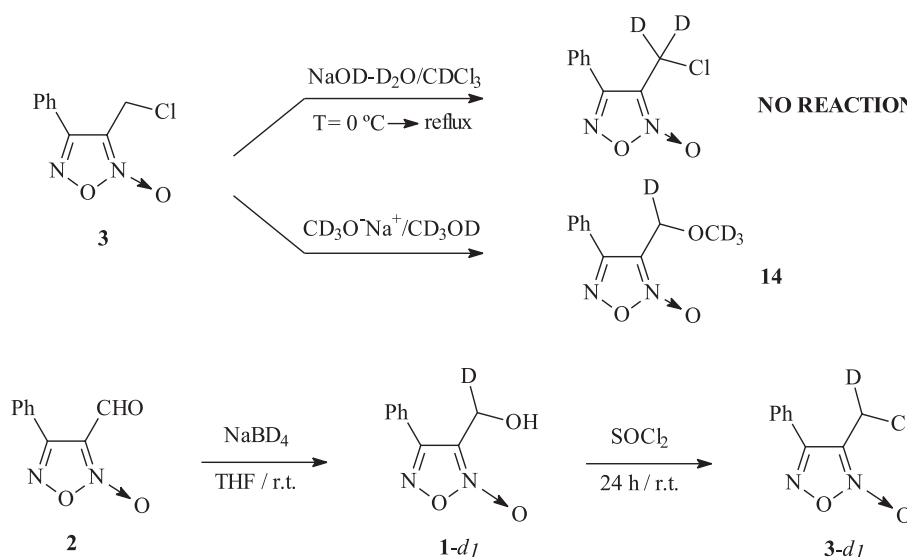
**Figure 3.** Postulated mechanism for the OH $\cdot$  loss in compounds **4**, **5**, **6** and **7** (Note: we are gratefully thank to one of the referee for suggesting the structure of the final product of the  $\delta$ -H rearrangement).

efforts on the synthesis of deuterium analogues of some selected furoxan derivatives.<sup>16,17</sup> Initially, we tried to prepare di-deuterium analogue, at the *benzylic* position, of derivative **6**. The synthesis of the di-deuterium chloride **3** was attempted by trying to exchange the “acidic” *benzylic*-protons using a biphasic system NaOD-D<sub>2</sub>O/CDCl<sub>3</sub> at different temperatures (room temperature to reflux for 24 h) (as shown in Scheme 1). Unfortunately, <sup>1</sup>H-NMR monitoring of the reaction mixture showed no significant

exchange under these conditions. When CD<sub>3</sub>O<sup>-</sup>Na<sup>+</sup> / CD<sub>3</sub>OD was used, exchange took place, but the ether **14** was obtained as the result of the substitution by the powerful nucleophile deuterated methoxide (Scheme 1). These synthetic problems led us to undertake the preparation of the mono-deuterium analogue of derivative **6**, at the *benzylic* position, with 3-*d*<sub>1</sub> as starting material (Scheme 1). Using the aldehyde **2**, the mono-deuterium alcohol 1-*d*<sub>1</sub> was produced with more than 95% of

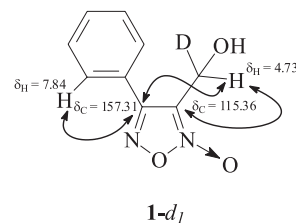


**Figure 4.** Postulated mechanism for the  $\text{NO}\cdot$  and  $\text{CH}_2\text{O}$  loss in compounds **1**.



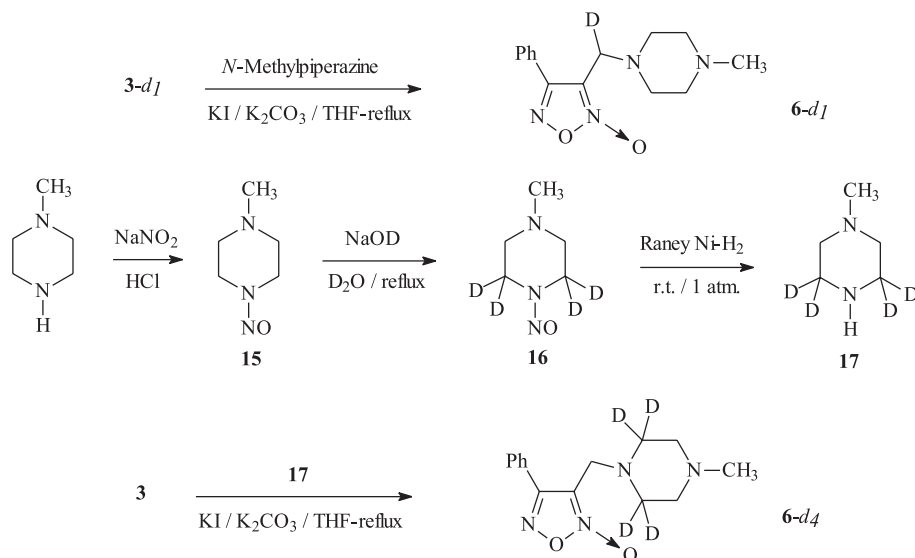
**Scheme 1.**

deuterium incorporation verified by  $^1\text{H-NMR}$  analysis, employing the deuterated-metal reductor- $\text{NaBD}_4$  (Scheme 1). The *N*-oxide moiety was maintained in position  $N_2$  during the reaction of derivative **2** with sodium borodeuteride. This fact was observed through the corresponding 2D-NMR experiments, which showed a quaternary carbon (HMQC experiment) at 115.36 ppm correlating with a proton at 4.73 ppm (HMBC experiment) and another quaternary carbon (HMQC experiment) at 157.31 ppm correlating with protons at 4.73 ppm and 7.84 ppm (HMBC experiment). These HETCOR experiments allowed us to assign unambiguously the heterocyclic carbon atoms ( $d_{\text{C-3}} = 115.36$  ppm,  $d_{\text{C-4}} = 157.31$  ppm) (Figure 5). The product **1-d<sub>1</sub>** was then transformed in a good yield into the corresponding chloride **3-d<sub>1</sub>** using thionyl chloride (Scheme 1).



**Figure 5.** HMBC correlation for derivative **1-d<sub>1</sub>**.

Finally, the amine derivative **6-d<sub>1</sub>** was obtained by the reaction between chloride **3-d<sub>1</sub>** and *N*-methylpiperazine (Scheme 2). The molecular structure of derivative **6-d<sub>1</sub>** has been determined by X-ray diffraction methods (Figure 6).<sup>18,19</sup> In addition, we prepared **6-d<sub>p</sub>**, the tetra-deuterium analogue in the 2,6-position of piperazine ring, through reaction between the chloride **3** and the heterocyclic amine



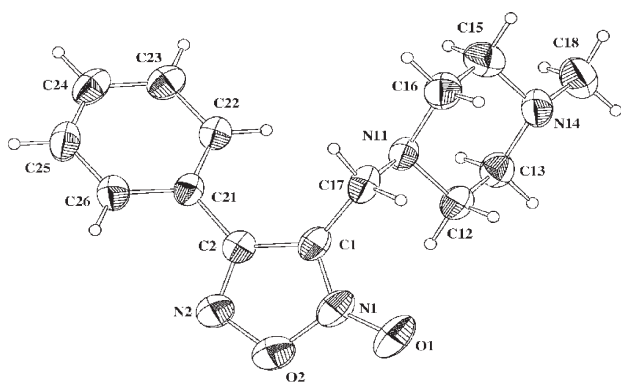
Scheme 2.

**17** (Scheme 2). This amine was deuterated via the nitrosamine **15**, prepared following the Ravindran *et al.* procedure,<sup>20</sup> which was converted into the tetra-deuterium analogue **16** following the Keefer-Fodor methodology.<sup>21</sup> To transform nitrosamine **16** into amine **17** we tried a procedure by Kano *et al.* (reduction with  $\text{NaBH}_4 \cdot \text{TiCl}_4$  (2:1) in diglyme) with bad results.<sup>22</sup> The reduction process did not occur and probably, a complex between the methylpiperazine nitrosamine and  $\text{TiCl}_4$  was obtained. The use of  $\text{H}_2$  in Raney-Nickel at room temperature and atmospheric pressure led to compound **17** in an adequate yield.<sup>23</sup> The deuterium incorporation in compound **16** was more than 95% (by  $^1\text{H-NMR}$  analysis).

via the alcohol **1-i**, which was prepared following the Gasco *et al.* methodology (Scheme 3).<sup>10</sup> To study the fragmentation pattern of the molecule “*N*-oxide-free”, we prepared the deoxygenated analogues **1-deoxy** and **7-deoxy** using Zn in  $\text{NH}_4\text{Cl}$  solution as the reduction reagent (Scheme 3).<sup>6,24</sup> These products were clearly confirmed through HETCOR experiments (HMOC and HMBC).

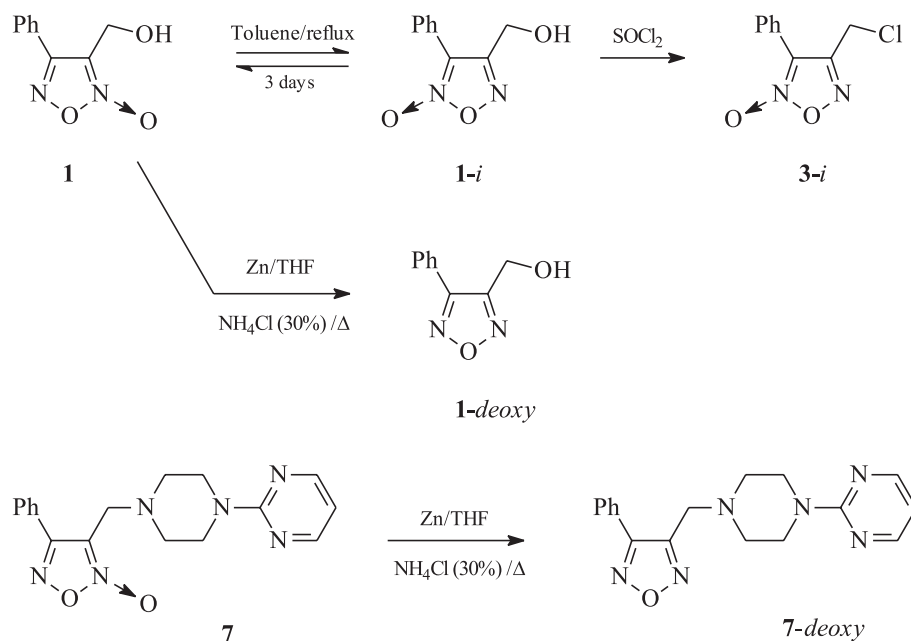
Mass spectrometry was carried out on all the analogues developed. The most characteristic peaks in the mass spectrum (EI/MS) for derivatives **1-d<sub>1</sub>**, **1-deoxy**, **3-d<sub>1</sub>**, **3-i**, **6-d<sub>1</sub>**, **6-d<sub>4</sub>** and **7-deoxy**, together with those for parent compounds **1**, **3**, **6** and **7**, are presented in Table 2. The results clearly indicate that the fragmentation process of compound **1-d<sub>1</sub>** does not occur exclusively through a NO loss, the  $[\text{M}-31\text{u}]^{+}$  ion (not present in the parent compound **1**) probably arise from a CDHO loss. On the other hand, comparing the relative abundance of the  $\text{M}^{+}$  and  $[\text{M}-30\text{u}]^{+}$  ions in compounds **1-deoxy** and **7-deoxy**, 9.9% and 1.6% respectively, we could notice that the  $[\text{M}-30\text{u}]^{+}$  ion was more abundant in the first one, this fact is in accordance with the neutral  $\text{CH}_2\text{O}$  loss fragmentation process in derivative **1**.

The mass spectrum of positional isomer **3-i** did not show the  $[\text{M}-17\text{u}]^{+}$  and  $[\text{M}-31\text{u}]^{+}$  ions, this fact could be indicative that the parent compound **3** losses OH $\cdot$  and HNO as a  $\beta$ -H participation. Deuterium labeling of compound **6** indicated that the  $[\text{M}-17\text{u}]^{+}$  ion of this derivative was the result of  $\beta$ -H and  $\delta$ -H rearrangements (see Table 2). While derivative **6** showed an  $[\text{M}-17\text{u}]^{+}$  ion abundance of 50.4%, derivatives mono- and tetra-deuterated (**6-d<sub>1</sub>** and **6-d<sub>4</sub>**), that could present the  $\beta$ -H and the  $\delta$ -H rearrangement phenomena, showed the abundances of the corresponding



**Figure 6.** Molecular plot of derivative **6-d<sub>1</sub>**. The ORTEP drawing of the molecule shows the labeling of the non-H(D) atoms and their displacement ellipsoids at 30% probability level.

To know how the *N*-oxide group position affect on the furoxans' mass spectrometry behavior, we prepared the *N*-oxide-positional isomer **3-i**. This compound was obtained



Scheme 3.

**Table 2.** Abundance of the critical fragment ions in the corresponding 1,2,5-oxadiazole *N*-oxide analogues EI mass spectrum

Compound No	M <sup>+</sup>	Abundance (%) <sup>a,b</sup>							
		M <sup>+</sup>	M <sup>+</sup> - 16	M <sup>+</sup> - 17	M <sup>+</sup> - 18	M <sup>+</sup> - 30	M <sup>+</sup> - 31	M <sup>+</sup> - 61	M <sup>+</sup> - 62
<b>1</b>	192	15.7	0.5	0.2	0.6	20.7	- <sup>c</sup>	100.0	0.8
<b>1-d<sub>1</sub></b>	193	25.6	1.7	0.6	1.1	27.8	3.0	100.0	20.4
<b>1-deoxy</b>	176	100.0	0.2	1.2	0.3	9.9	3.5	8.2	6.0
<b>3</b>	210	12.0	0.3	0.3	-	10.6	0.1	1.7	0.1
<b>3-d<sub>1</sub></b>	211	12.2	0.2	0.3	0.1	11.4	1.2	4.7	0.3
<b>3-i</b>	210	8.6	0.1	-	-	6.9	-	1.6	-
<b>6</b>	274	0.3	8.5	50.4	0.4	0.2	0.2	0.8	0.3
<b>6-d<sub>1</sub></b>	275	1.2	7.7	45.6	23.0	0.3	-	0.7	0.5
<b>6-d<sub>4</sub></b>	278	0.3	8.7	46.2	17.0	-	-	0.4	0.1
<b>6-d<sub>4</sub><sup>d</sup></b>	278	4.6	17.1	100.0	35.5	0.3	-	2.6	0.9
<b>7</b>	338	10.0	7.1	33.8	0.2	0.6	0.2	0.3	-
<b>7-deoxy</b>	322	12.7	0.4	1.7	0.1	0.2	0.1	0.5	1.1

<sup>a</sup> MS experiments were performed using a Shimadzu MS QP 1100 EX equipment, with EI at 70 eV, with direct insertion probe, the ion source temperature 250 °C and the mass range was 40-500 amu. <sup>b</sup> The results are the averages for three independent experiments. <sup>c</sup> The “-” denotes that the fragment ion was not observed. <sup>d</sup> At 20 eV, ion source temperature 150 °C. These conditions were used in order to increase the abundance of the peak of the molecular ion, other conditions and other products were not studied.

ion ([M-18u]<sup>+</sup>) near to 20% (23.0 and 17.0%, respectively). Unfortunately, the mono-deuterium analogue, **6-d<sub>1</sub>**, did not allow us to conclude about the kind of the initial H-rearrangement, because the *benzylic* position contain one hydrogen and one deuterium. On the other hand, the tetra-deuterium analogue, **6-d<sub>4</sub>**, permitted us to confirm both initial rearrangement pathways, yielding two possible stable radical cations. The high abundance of the [M-18u]<sup>+</sup> ion in derivative **6-d<sub>4</sub>**, and the very low abundance in the parent compound, indicated the participation of the deuterium atom in a fragmentation process.

Further the deoxygenated derivatives **1-deoxy** and

**7-deoxy** allowed us to establish that in the parent compounds the loss of oxygen, as expected, principally occurs from the *N*-oxide moiety. The deoxy-derivatives showed the [M-16u]<sup>+</sup> ion in a very low abundance, *i.e.* abundance ([M-16u]<sup>+</sup>) / abundance (M<sup>+</sup>) = 0.002 for **1-deoxy** and 0.031 for **7-deoxy** (compare with the corresponding abundance ([M-16u]<sup>+</sup>) / abundance (M<sup>+</sup>) values for **1** and **7**, 0.032 and 0.71 respectively), probably due to an oxygen loss from the heterocycle system 1,2,5-oxadiazole. In the deoxy-analogues the [M-17u]<sup>+</sup> ion could be the result of a further H<sup>+</sup> loss.

## Experimental

All starting materials were commercially available research-grade chemicals and used without further purification. All solvents were dried and distilled prior to use. All the reactions were carried out in a nitrogen atmosphere. The typical work-up included washing with brine and drying the organic layer with sodium sulphate. Compounds **1-3**, **1-i**, **1-deoxy**, **7**, **7-deoxy**, **15** and **16** were prepared as previously described.<sup>4,6,9,20,21</sup> Elemental analyses were obtained from vacuum-dried samples (over phosphorous pentoxide, 24 h at room temperature) and performed on a Fisons EA 1108 CHNS-O analyzer, and were within  $\pm 0.4\%$  of theoretical values. Infrared spectra were recorded on a Perkin Elmer 1310 apparatus, using potassium bromide tablets; the frequencies are expressed in  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  spectra and HETCOR experiments were recorded on a Bruker DPX-400 (at 400 MHz and 100 MHz) instrument, with tetramethylsilane as the internal reference; the chemical shifts are reported in ppm. MS experiments were performed using the Shimadzu MS QP 1100 EX equipment, with EI at 20 or 70 eV, with direct insertion probe, the ion source was set a 150 °C or 250 °C and the mass range was 40-500 amu.

**3-(1-Deuterio-1-hydroxymethyl)-*N*<sub>2</sub>-oxide-4-phenyl-1,2,5-oxadiazole (1-d)**. A solution of **2** (1.0 g, 5.3 mmol) in THF (5 mL) was stirred at 0 °C. Sodium borodeuteride (220 mg, 5.3 mmol) was then added, and the resulting solution was stirred for 3 h at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc. After the work-up process the residue was purified by column chromatography ( $\text{SiO}_2$ , petroleum ether:EtOAc (10 to 30%)), to yield 600 mg (59%), mp 65.0-67.0 °C; IR  $\nu_{\text{max}}/\text{cm}^{-1}$ : OH 3400, 1560, 1410, 1050, 770, 680  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (deuteriochloroform):  $\delta$  2.30 (bs, 1H, OH), 4.73 (s, 1H, -CDHOH), 7.57 (m, 3H, phenyl protons), 7.84 (m, 2H, phenyl protons);  $^{13}\text{C NMR}$  (HMQC and HMBC experiments) (deuteriochloroform):  $\delta$  53.40 (-CDH), 115.36 (-C=N<sup>+</sup>-O<sup>-</sup>), 126.55 (-C-phenyl), 128.18 (-C-phenyl), 129.80 (-C-phenyl), 131.76 (-C-phenyl), 157.31 (-C=N). Anal. Calc for  $\text{C}_9\text{H}_7\text{DN}_2\text{O}_3$ : C, 55.96; H, 3.63; N, 14.51%. Found: C, 56.00; H, 3.92; N, 14.35%.

**3-(1-Chloro-1-deuteriomethyl)-*N*<sub>2</sub>-oxide-4-phenyl-1,2,5-oxadiazole (3-d<sub>1</sub>)**. A mixture of **1-d<sub>1</sub>** (300 mg, 1.6 mmol) and  $\text{SOCl}_2$  (0.19 mL) was stirred at room temperature for 24 h. The reaction mixture was treated with ice, sodium bicarbonate saturated solution (until basic pH), and extracted three times with EtOAc (20 mL). Then of the work-up process the residue was purified by chromatography ( $\text{Al}_2\text{O}_3$ , petroleum ether:EtOAc (0 to 5%)) to yield 300 mg (89%), colorless oil; IR  $\nu_{\text{max}}/\text{cm}^{-1}$ : 1601, 1462,

1435, 772, 696  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (deuteriochloroform):  $\delta$  4.57 (s, 1H, -CDHCl), 7.56 (m, 3H, phenyl protons), 7.80 (m, 2H, phenyl protons);  $^{13}\text{C NMR}$  (HMQC and HMBC experiments) (deuteriochloroform):  $\delta$  32.94 (-CDH), 113.31 (-C=N<sup>+</sup>-O<sup>-</sup>), 126.20 (-C-phenyl), 127.84 (-C-phenyl), 129.81 (-C-phenyl), 131.51 (-C-phenyl), 156.40 (-C=N). Anal. Calc for  $\text{C}_9\text{H}_6\text{ClDN}_2\text{O}_2$ : C, 51.06; H, 2.84; N, 13.24%. Found: C, 50.69; H, 2.99; N, 13.00%.

**4-(Chloromethyl)-*N*<sub>2</sub>-oxide-3-phenyl-1,2,5-oxadiazole (3-i)**. A mixture of **1-i** (150 mg, 0.8 mmol) and  $\text{SOCl}_2$  (0.1 mL) was stirred at room temperature for 24 h. The reaction mixture was treated with ice, sodium bicarbonate saturated solution (until basic pH), and extracted three times with EtOAc (20 mL). Then of the work-up process the residue was purified by chromatography ( $\text{Al}_2\text{O}_3$ , petroleum ether:EtOAc (0 to 5%)) to yield 110 mg (65%), colorless oil; IR  $\nu_{\text{max}}/\text{cm}^{-1}$ : 1595, 1450, 1415, 770, 690  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (deuteriochloroform):  $\delta$  4.72 (s, 2H, -CH<sub>2</sub>Cl), 7.58 (m, 3H, phenyl protons), 7.83 (m, 2H, phenyl protons). Anal. Calc for  $\text{C}_9\text{H}_7\text{ClN}_2\text{O}_2$ : C, 51.31; H, 3.33; N, 13.30%. Found: C, 51.07; H, 3.05; N, 12.92%.

**3-[1-Deuterio-1-(4-methylpiperazine-1-yl)methyl]-*N*<sub>2</sub>-oxide-4-phenyl-1,2,5-oxadiazole (6-d<sub>1</sub>)**. A mixture of **3-d<sub>1</sub>** (150 mg, 0.7 mmol), *N*-methylpiperazine (65 mg, 0.7 mmol),  $\text{K}_2\text{CO}_3$  (100 mg, 0.7 mmol), KI (10 mg, 0.07 mmol) and THF as solvent was heated at reflux until absence of chloride ( $\text{Al}_2\text{O}_3$ , 20% EtOAc in petroleum ether). The solvent was removed in vacuum and the residue was purified by column chromatography ( $\text{Al}_2\text{O}_3$ ,  $\text{CH}_2\text{Cl}_2$ ) to yield 100 mg (51%), mp 97.5-99.5 °C; IR  $\nu_{\text{max}}/\text{cm}^{-1}$ : 2926, 2797, 1595, 1574, 1456, 768, 700  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (deuteriochloroform):  $\delta$  2.29 (s, 3H, CH<sub>3</sub>-N), 2.45 (m, 4H, -CH<sub>2</sub>N), 2.58 (m, 4H, -CH<sub>2</sub>N), 3.54 (s, 1H, -CDHN), 7.52 (m, 3H, phenyl protons), 7.92 (m, 2H, phenyl protons);  $^{13}\text{C NMR}$  (HMQC and HMBC experiments) (deuteriochloroform):  $\delta$  46.27 (CH<sub>3</sub>-N), 50.04 (-CDH), 53.10 (-CH<sub>2</sub>-N), 55.23 (-CH<sub>2</sub>-N), 113.04 (-C=N<sup>+</sup>-O<sup>-</sup>), 127.35 (-C-phenyl), 128.52 (-C-phenyl), 129.47 (-C-phenyl), 131.45 (-C-phenyl), 158.00 (-C=N). Anal. Calc for  $\text{C}_{14}\text{H}_{17}\text{DN}_4\text{O}_2$ : C, 61.09; H, 6.18; N, 20.36%. Found: C, 60.96; H, 6.30; N, 20.22%.

**2,2,6,6-Tetradeuterio-4-methylpiperazine (17)**. A mixture of *N*-nitrosopiperazine tetradeuterated **16** (190 mg, 1.4 mmol) in MeOH (5 mL) was stirred at room temperature, Raney nickel (170 mg) was then added, and the air in the flask was replaced by hydrogen. The reaction mixture was vigorously stirred for 6 hours at room temperature. Raney nickel (170 mg) was then added, and the system was re-saturated with hydrogen and stirred for 1 hour at room temperature. The solution was decanted and filtered through Celite. The solvent was removed in vacuum and

the residue was the product **17** (60 mg, 40%), which was used in the next reaction without further purification. <sup>1</sup>H NMR (deuteriochloroform): δ 2.00 (s, 1H, NH), 2.35 (s, 3H, CH<sub>3</sub>-N), 2.36 (m, 4H, -CH<sub>2</sub>N).

3-[(4-Methyl-2,2,6,6-tetradeuteriopiperazine-1-yl)methyl]-*N*<sub>2</sub>-oxide-4-phenyl-1,2,5-oxadiazole (**6-d<sub>4</sub>**). A mixture of **3** (121 mg, 0.57 mmol), **17** (60 mg, 0.57 mmol), K<sub>2</sub>CO<sub>3</sub> (80 mg, 0.57 mmol), KI (8 mg, 0.06 mmol) and THF as solvent was heated at reflux until absence of chloride (Al<sub>2</sub>O<sub>3</sub>, 20% EtOAc in petroleum ether). The solvent was removed in vacuum and the residue was purified by column chromatography (Al<sub>2</sub>O<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>) to yield 40 mg (25%), colorless oil; IR ν<sub>max</sub>/cm<sup>-1</sup>: 2917, 2849, 1599, 1575, 1456, 767, 699cm<sup>-1</sup>; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 2.30 (s, 3H, CH<sub>3</sub>-N), 2.51 (m, 4H, -CH<sub>2</sub>N), 3.63 (s, 2H, Ar-CH<sub>2</sub>N), 7.59 (m, 3H, phenyl protons), 8.02 (m, 2H, phenyl protons); <sup>13</sup>C NMR (HMQC and HMBC experiments) (acetone-*d*<sub>6</sub>): δ 45.90 (CH<sub>3</sub>-N), 50.00 (Ar-CH<sub>2</sub>-N), 51.80 (-CD<sub>2</sub>N), 54.00 (-CH<sub>2</sub>-N), 113.50 (-C=N<sup>+</sup>-O<sup>-</sup>), 126.00 (-C-phenyl), 128.50 (-C-phenyl), 129.50 (-C-phenyl), 131.50 (-C-phenyl), 157.50 (-C=N). Anal. Calc for C<sub>14</sub>H<sub>14</sub>D<sub>4</sub>N<sub>4</sub>O<sub>2</sub>: C, 60.43; H, 5.04; N, 20.14%. Found: C, 60.08; H, 5.44; N, 20.02%.

### Crystallography

Suitable needles shaped single crystals of **6-d<sub>4</sub>** were obtained by slow evaporation from AcOEt. Derivative **6-d<sub>4</sub>** crystallizes in the monoclinic P2<sub>1</sub>/c space group with a = 10.906(3), b = 14.581(3), c = 10.100(2) Å, β = 114.83(2)° and Z=4. The structure were solved from 1455 reflections with I>2s(I) and refined to agreement R1-factors of 0.044. Most H-atoms were detected in a difference Fourier map. However, they were positioned stereochemically and refined with the riding model. The program used to solve and refine the structure was SHELXS.<sup>25</sup> The program used to generate the ORTEP graphics was ORTEP-II.<sup>19</sup>

### Conclusions

In summary, by using labeling experiments and adequate analogues, we were able to interpret EI/MS data to elucidate the fragmentation patterns in mass spectrometry of 1,2,5-oxadiazole *N*-oxide derivatives.

### Electronic Supplementary Information

Listings of interatomic bond distances, selected angles, atomic anisotropic displacement parameters, hydrogen atoms positions and isotropic displacement parameters for derivative **6-d<sub>4</sub>**, X-ray studies. Available at <http://jbcbs.sbg.org.br> as a PDF file.

### Acknowledgement

We thank the financial support from CONICYT-Uruguay (Grants 347/95 and 1019/95), Comisión Honoraria de Lucha contra el Cáncer (Uruguay), CONICET-Argentina and by FAPESP-Brazil. Part of the X-ray diffraction experiments were carried out at the National Diffraction Laboratory (LANADI), La Plata, Argentina. We also thank Dr. Ana Denicola and Dr. Alvaro Diaz for critical reading of the manuscript.

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18. As expected, the 1,2,5-oxadiazole heterocycle is planar to within experimental accuracy, with the oxygen atom of *N*-oxide on the ring plane. N-O bond distances in the heterocycle clearly reflect the asymmetry in the bonding structure imposed by the *N*-oxide moiety. In fact, the single N1-O2 bond is appreciably longer (1.434(3) Å) than the other, partially double, N2-O2 bond (1.371(3) Å). The phenyl ring subtends a dihedral angle of 39.20(9)° with the heterocycle.
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Received: January 30, 2003

Published on the web: February 27, 2004

FAPESP helped in meeting the publication costs of this article.