

Chimia 46 (1992) 130–132  
© Schweiz. Chemiker-Verband; ISSN 0009–4293

# Radiation Damage in Carnidazole: a Single Crystal EPR Study

Théo Berclaz, Gérald Bernardinelli, Michel Geoffroy\*, and Natarajan Rajalakshmi

**Abstract.** Single crystals of hydrated and anhydrous carnidazole have been subjected to X-irradiation at 77 K and studied by EPR. The determination of the EPR tensors shows that  $\text{NO}_2$ , sulfur-centered radicals as well as a radical pair can be generated from this molecule; it appears that the species trapped at 77 K are not the same in the hydrated and in the anhydrous crystal. The results are discussed in the context of the radiosensitizing action of molecules containing a 5-nitroimidazole moiety.

## Introduction

Nitroimidazole compounds are intensively studied in radiation biology because of their potential use in radiotherapy [1], and metronidazole, *e.g.*, has been shown to be an effective radiosensitizer of hypoxic cells [2]. However, the molecular mechanism of such a radiosensitization process is still poorly understood, and more information is needed concerning the nature of the radicals resulting from exposure of these compounds to ionizing radiation. By studying the radiation damage in crystalline *O*-methyl [2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl]thiocarbamate (carnidazole, *Fig. 1*), we show that the radiation behaviour of nitroimidazole compounds is not only determined by their molecular structure but is very dependent upon their environment. For this purpose, we will analyse the EPR spectra due to radiogenic radicals trapped in two slightly different matrices corresponding, respectively, to a hydrated and an anhydrous carnidazole single crystal.

## Experimental

X-rays diffraction showed us that two types of crystals can be simultaneously obtained by slow evaporation of a soln. of carnidazole in a  $\text{H}_2\text{O}/\text{EtOH}$  mixture: 1) crystals having the same parameters as those previously studied by *Blaton et al.* [3] and identified as being crystals of carnidazole·1*H*<sub>2</sub>O, 2) crystals whose structure has been determined [4] in our laboratories, and which appear to be anhydrous carnidazole. A

\*Correspondence: Prof. M. Geoffroy  
Département de Chimie Physique et  
Laboratoire de Cristallographie  
30, quai Ernest Ansermet  
Université de Genève  
CH-1211 Genève 4

reference planes [5], and the resulting curves were analyzed by using second-order perturbation theory [6].

## Results

An ORTEP illustration of the carnidazole molecule, obtained from the crystal structure of the anhydrous compound [4], is shown in *Fig. 1*. Although the unit cell parameters are different, the molecular structure is very similar with that determined from the hydrated crystal; the main conformational difference lies in the torsion angle  $\text{N}(1)\text{--C}(4)\text{--C}(5)\text{--N}(4)$  which is equal to  $175.0^\circ$  in the anhydrous crystal and to  $60.6^\circ$  in the hydrated crystal.

## Hydrated Crystal of Carnidazole

We show, in *Fig. 2*, an example of an EPR spectrum obtained at 77 K with a single crystal of hydrated carnidazole subjected to X-irradiation under liquid  $\text{N}_2$ . Signals marked A are due to a species exhibiting coupling with a spin-1 nucleus, whereas no hyperfine structure can be observed for the signal marked B. Signals

hydrated and an anhydrous crystal of carnidazole were irradiated, at 77 K, during 1 h with a Philips X-ray tube (W anticathode, 30 mA, 30 kV). They were then transferred, without warming, into a finger liquid  $\text{N}_2$  Dewar inserted in the cavity of a Bruker 200D EPR spectrometer. The angular variation of the EPR spectra was studied in the three

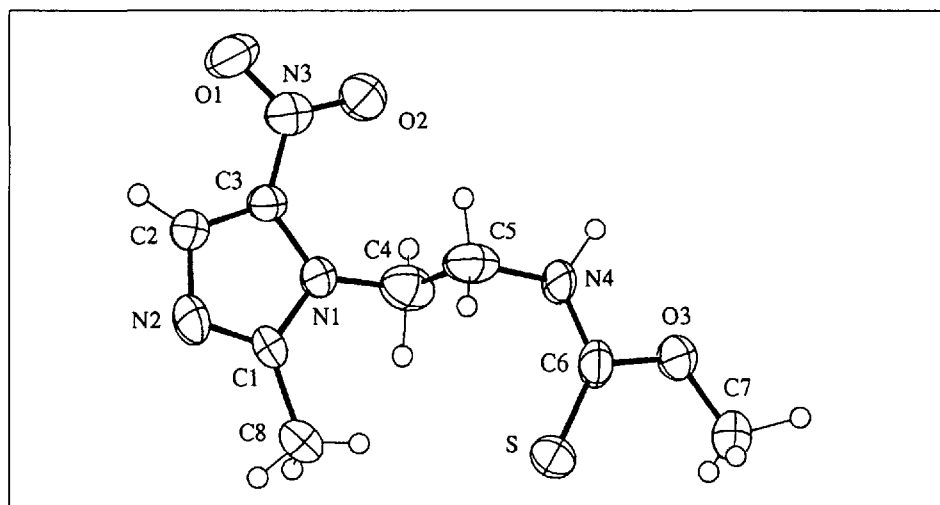


Fig. 1. ORTEP plot of the carnidazole molecule (anhydrous crystal)

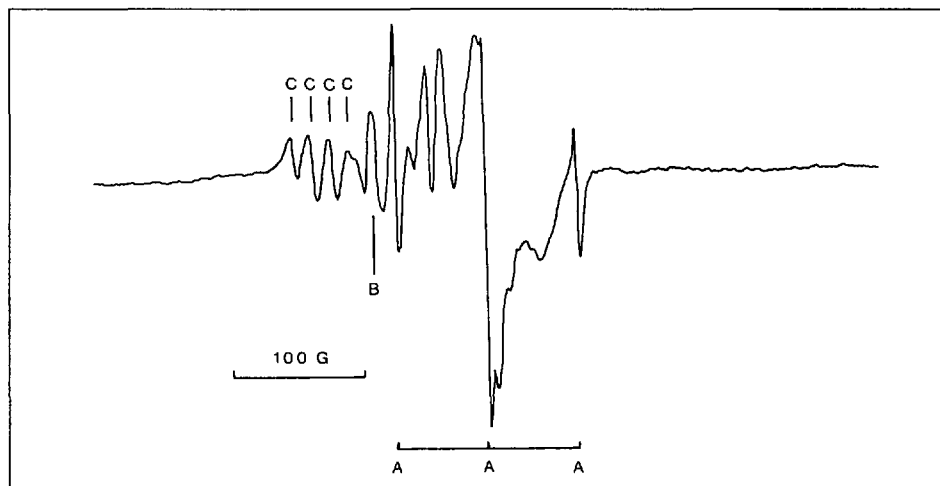


Fig. 2. Example of an EPR spectrum, obtained at 77 K, with an X-irradiated single crystal of hydrated carnidazole

marked C are more difficult to analyze, because, for several orientations of the magnetic field, their hyperfine structure is hidden by overlap with signals due to other species. Nevertheless, the angular variation of this four-line pattern is consistent with hyperfine coupling with both

a spin-1 and a spin-1/2 nucleus, and we have analyzed these signals by assuming an interaction with a <sup>14</sup>N and a <sup>1</sup>H nucleus. A variable-temperature experiment showed that signals B and C decay at 120 K, whereas signals A are still stable up to 240 K. The angular variation of the vari-

ous signals lead to the tensors shown in Table 1.

**Anhydrous Crystal of Carnidazole**

An example of an EPR spectrum obtained at 77 K with a single crystal of anhydrous carnidazole is shown in Fig. 3. The angular variation of the signals marked A' and B' could be followed in the three reference planes and will be discussed here. The strong anisotropy of the signals A' suggested that they were due to a radical pair, and this hypothesis was confirmed by observing the forbidden half-field signal ( $\Delta M_s = 2$  transition). The angular variation of the signals A' was, therefore, analyzed by using a *Hamiltonian* which takes the electronic *Zeeman* effect and the electron-electron dipolar interaction (*D* tensor) into account. The signal B' does not exhibit any structure and can be characterized only by its *g* tensor. The various EPR tensors obtained from the anhydrous crystal are shown in Table 2.

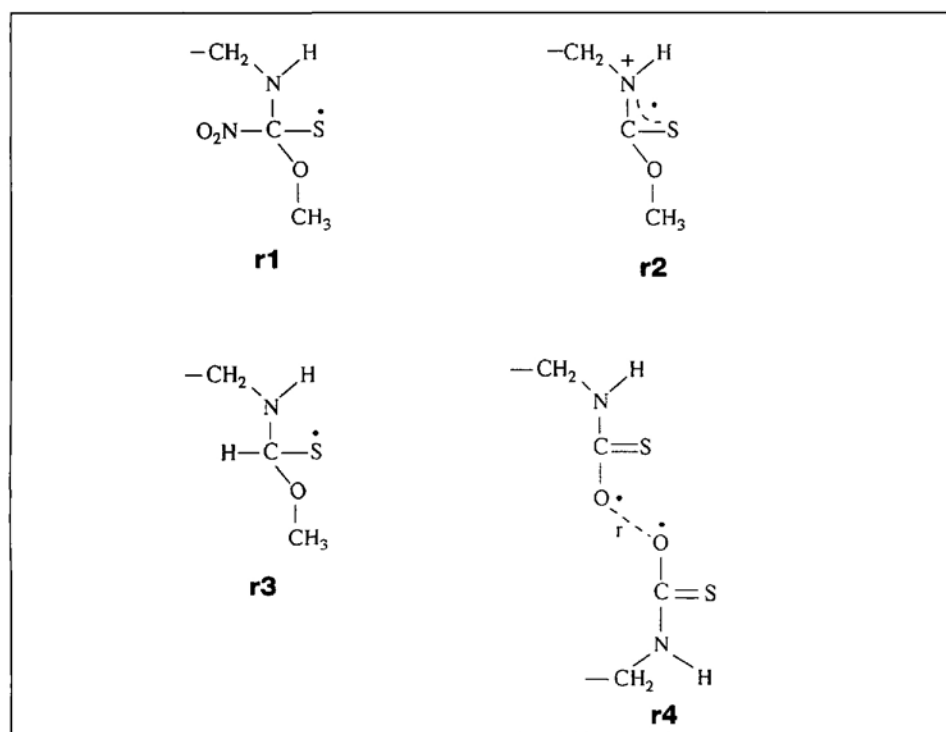
Table 1. EPR Tensors for the Various Radical Species Trapped in an X-Irradiated Single Crystal of Hydrated Carnidazole (A,B,C). The values for NO<sub>2</sub> have been obtained by Ida and Iwasaki from diglycine nitrate [7].

	Eigenvalues	Eigenvectors		
		/X	/Y	/Z
<b>Radical A</b>				
<i>g</i>	<i>g</i> <sub>1</sub> =1.991	0.413	-0.909	-0.041
	<i>g</i> <sub>2</sub> =2.001	-0.701	-0.289	-0.651
	<i>g</i> <sub>3</sub> =2.004	-0.581	-0.298	0.757
<sup>14</sup> N-T [MHz]	<i>T</i> <sub>1</sub> =136	0.368	-0.925	0.037
	<i>T</i> <sub>2</sub> =159	0.919	0.376	0.112
	<i>T</i> <sub>3</sub> =188	-0.137	0.038	0.989
<b>Radical B</b>				
<i>g</i>	<i>g</i> <sub>1</sub> =1.953	0.666	0.455	0.589
	<i>g</i> <sub>2</sub> =2.029	-0.745	0.421	0.517
	<i>g</i> <sub>3</sub> =2.121	-0.013	-0.784	-0.620
<b>Radical C</b>				
<i>g</i>	<i>g</i> <sub>1</sub> =1.972	0.678	-0.454	0.577
	<i>g</i> <sub>2</sub> =2.03	-0.734	-0.449	0.509
	<i>g</i> <sub>3</sub> =2.135	0.027	-0.769	-0.638
<sup>14</sup> N-T [MHz]	<i>T</i> <sub>1</sub> =6	-0.773	-0.404	-0.484
	<i>T</i> <sub>2</sub> =47	-0.253	-0.509	-0.759
	<i>T</i> <sub>3</sub> =62	0.581	-0.759	0.292
<sup>1</sup> H-T [MHz]	<i>T</i> <sub>1</sub> =35	0.579	-0.369	-0.726
	<i>T</i> <sub>2</sub> =42	0.630	0.768	-0.113
	<i>T</i> <sub>3</sub> =55	0.516	-0.523	0.677
<b>NO<sub>2</sub> [7]</b>				
<i>g</i>	<i>g</i> <sub>1</sub> =1.9907			
	<i>g</i> <sub>2</sub> =2.0018			
	<i>g</i> <sub>3</sub> =2.004			
<sup>14</sup> N-T [MHz]	<i>T</i> <sub>1</sub> =136			
	<i>T</i> <sub>2</sub> =147			
	<i>T</i> <sub>3</sub> =188			

**Discussion**

The hyperfine tensor reported for the radical A trapped in the hydrated crystal is shown in Table 1 together with the tensor obtained by *Eda* and *Iwasaki* for NO<sub>2</sub> trapped in diglycine nitrate [7]. It appears clearly that the species A can be identified as being the NO<sub>2</sub> radical produced by homolytic scission of a C-N bond. The radical B, trapped in the same crystal, is characterized by a very large anisotropy of the *g* factor which is similar with that observed for R<sup>S</sup> radicals [8]. This species probably results from the addition of a radiogenic radical (*e.g.* NO<sub>2</sub>, structure **r1**) to the C=S bond. As shown in Table 1, the *g* tensor of the radical C is also very anisotropic, and indicates that the corresponding species is also a S-centred radical. However, the hyperfine interaction with both a nitrogen and a proton nucleus suggests for C either a more delocalized structure (like **r2**) or the addition, on the C=S bond, of an H-atom (formed by radiolysis of H<sub>2</sub>O) leading to hyperfine coupling through hyperconjugation. Such a species could be represented by the structure **r3** which assumes that the torsion angle between the axis of the sulfur-p orbital containing the unpaired electron and the C-N bond direction is more suitable for hyperconjugation than in the case of the structure **r1**. The appreciable anisotropy of the hyperfine tensors measured for C, however, makes the structure **r2** more plausible.

The paramagnetic species A' trapped in the anhydrous crystal is a pair formed by two radicals separated by a distance *r*



which can be estimated from the  $D$  tensor: the axially of this tensor makes use of the point dipole approximation applicable and  $D_{\parallel}$  shows that the  $r$  distance is about 5.4 Å. The eigenvector  $D_{\parallel}$  is expected to be aligned along the  $r$  vector and comparison with the crystal structure of the precursor shows that  $D_{\parallel}$  makes an angle of 5° with the vector linking the thiocarboxylate oxygen of two adjacent molecules in the unit cell. This result suggests, therefore, that the two components of the pair are two RC(S)O radicals (structure **r4**) which result from homolytic scission of O-CH<sub>3</sub> bonds and that the two Me fragments recombine to form an ethane molecule. This interpretation is consistent with the  $g$  tensor whose eigenvalues are similar with those previously reported for alkoxy radicals and whose  $g_{\max}$  eigenvector is, as expected, close to the C-O direction (C-O,  $g_{\max} = 7^\circ$ ). It is worth mentioning that this process requires that the distance between the two MeO groups be relatively short in the precursor; this distance is 5.37 Å in the anhydrous crystal (where the pair is indeed observed), whereas it is 6.5 Å in the hydrated crystal (where the pair is not observed). The radical B', which is the second species trapped in the anhydrous crystal, has a  $g$  tensor similar to that measured for the radical B in the hydrated crystal and is also a S-centered radical which has probably a structure of the type **r1**. To summarize, two sorts of radiation damage are observed in carnidazole crystals: the first is due to the formation and reactivity of NO<sub>2</sub>, the second is independent of the 5-nitroimidazole moiety.

Many mechanisms of radiosensitization by compounds containing a nitroimidazole moiety have been proposed in the literature [2a]. *E.g.*, these compounds have been suggested to react with radiation-induced free radicals in vital macromolecules and to prevent the repairing of these molecules by H-atoms formed from sulfhydryl groups. It was also proposed that the high electron affinity of nitroimidazole compounds indirectly increases the extent of cationic damage in DNA. In these mechanisms the radiosensitization is thought to result from the properties of the whole nitroheterocycle. However, a previous study [9] on radiogenic radicals produced from a heterocycle containing a NO<sub>2</sub> group showed that the NO<sub>2</sub> radical was easily produced and could lead to secondary radicals. In carnidazole, the NO<sub>2</sub> radical is also produced, since it could be trapped in the hydrated crystal. It could not be stabilized in the lattice of the anhydrous compound, but with both crystals, however, a species was formed (radical B and B') which may be due to the addition of a NO<sub>2</sub> radical on a C-atom of the thio-

Table 2. EPR Tensors Measured for Two Radicals (A' and B') Trapped, at 77 K, in an X-Irradiated Single Crystal of Hydrated Carnidazole

Eigenvalues		Eigenvectors		
		/X	/Y	/Z
<b>Radical A'</b>				
$g$	$g_1=2.0035$	0.576	0.431	0.694
	$g_2=2.0076$	0.223	-0.900	0.373
	$g_3=2.0128$	0.786	-0.059	-0.615
$D(\text{MHz})$	$D_1=-292$	-0.034	0.139	0.989
	$D_2=122$	-0.266	-0.955	0.126
	$D_3=169$	0.963	-0.259	0.069
<b>Radical B'</b>				
$g$	$g_1=1.948$	0.724	0.689	0.004
	$g_2=2.016$	0.534	-0.558	-0.634
	$g_3=2.193$	0.435	-0.462	0.773

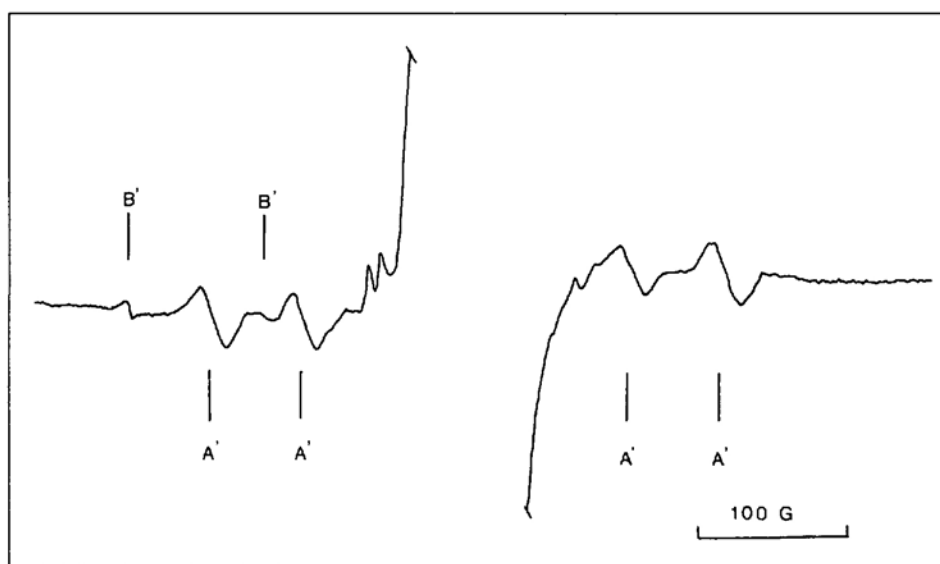


Fig. 3. Example of an EPR spectrum, obtained at 77 K, with an X-irradiated single crystal of anhydrous carnidazole

carbamate group of a neighbour molecule. It is not impossible that similar processes play a role in radiosensitization by nitroimidazole compounds, since the attack on DNA by radiogenic NO<sub>2</sub> could certainly result in biological damage.

Received: January 21, 1992

- [1] a) D.W. Whillans, G.E. Adams, *Radiat. Res.* **1975**, *62*, 407; b) A.R.J. Silver, P.O'Neill, T.C. Jenkins, *Biochem. Pharmacol.* **1985**, *34*, 3537; c) M.V.M. Lafleur, E.J. Pluijmakers-Westmijze, H.Loman, *J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1211.
- [2] a) R.L. Willson, W.A. Cramp, R.M.J. Ings, *Int. J. Radiat. Biol.* **1974**, *26*, 557; b) D.E. Moore, B.J. Wilkins, *Radiat. Phys. Chem.* **1990**, *36*, 547; M.V.M. Lafleur, H. Loman, *Radiat. Envir. Biophys.* **1986**, *25*, 159; M.C.R. Symons, W.R. Bowman, P.F. Taylor, *Tetrahedron Lett.* **1990**, *31*, 3221
- [3] N.M. Blatan, O.M. Peeters, C.J. DeRanter, *Acta Crystallogr., Sect. B* **1979**, *35*, 753.
- [4] Crystal structure determination: C<sub>8</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>S,  $M_r = 244.3$ , monoclinic,  $P2_1/n$ ,  $a = 7.304(1)$ ,  $b = 15.437(3)$ ,  $c = 10.650(4)$  Å,  $\beta$  = 109.47(1)°,  $V = 1132.1(5)$  Å<sup>3</sup>,  $Z = 4$ ,  $D_x = 1.43$  g cm<sup>-3</sup>,  $\mu = 0.272$  mm<sup>-1</sup>,  $F_{000} = 512$ . 1477 measured reflections at room temperature (Philips PW1100 diffractometer), 890 observed ( $|F_o| > 4\sigma(F_o)$ ),  $R = 0.067$ ,  $\omega R = 0.032$  ( $\omega = 1/\sigma^2(F_o)$ ) 181 variables with non-H-atoms refined with anisotropic displacement parameters. All coordinates of the H-atoms have been observed and refined. Full details of the crystal structure are submitted for publication (G. Bernardinelli, T. Berclaz, M. Geoffroy, N. Rajalakshmi, *Acta Crystallogr., Sect. C*).
- [5] For the hydrated crystal of carnidazole, the coordinates of the unit cell vectors  $a, b, c$  in the cartesian EPR reference frame XYZ are respectively:  $a = 0.23, 0.02, -6.47$  Å;  $b = 8.50, -18.56, 0.09$  Å;  $c = -8.55, -3.83, 0.82$  Å. For the anhydrous crystal, the EPR reference axes are defined as follows: Y is aligned along  $-b$ , X makes an angle of 40° and 110.5° with  $a^*$  and  $c^*$ , respectively.
- [6] M. Iwasaki, *J. Magn. Reson.* **1974**, *16*, 417.
- [7] B. Eda, M. Iwasaki, *J. Phys. Chem.* **1982**, *86*, 2084.
- [8] R. Franzi, M. Geoffroy, G. Bernardinelli, *Mol. Phys.* **1984**, *52*, 947; E. Sagstuen, C. Alexander, *J. Chem. Phys.* **1978**, *68*, 762.
- [9] M. Geoffroy, T. Berclaz, A. Celalyan-Berthier, N. Rajalakshmi, M.V.V.S. Reddy, *Radiat. Phys. Chem.* **1991**, *38*, 519.