ENDOR Studies of X-Irradiated Single Crystals of Sulphamethoxazole

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Single crystals of sulphamethoxazole were X-irradiated at 273 K. ESR and ENDOR spectra were obtained at 100 K. The free radicals stable at room temperature are formally formed by abstraction of a hydrogen atom from the methyl group of the molecule. The unpaired electron is delocalized in the isoxazole ring. The assignment is supported by comparisons of spin densities obtained experimentally and by semiempirical molecular orbital calculations.

Introduction

Compounds belonging to sulphanilamides are very important drugs in pharmacy and medicine because of their bacteriostatic properties. Therefore, recognition of the type of radicals forming in them under the influence of ionizing radiation as well as their electronic structure seems very interesting. As the sulphamethoxazole molecule includes two rings (isoxazole and benzene) joined through a $-SO_2-NH-$ bridge [1], the question arises which one is the host of stable radicals. The sulphamethoxazole molecule resembles the system A–σ–D, where A and D stand for a ring of acceptor and donor character, respectively, and σ is the joining bridge. The systems of this kind are very interesting from the point of view of molecular electronics.

So far, sulphamethoxazole single crystals have been studied by the ESR method [2, 3]. However, taking into account the complex character of the molecule and its experimental ESR spectra, it seems that the ENDOR method should permit to obtain more accurate and more reliable information about the type of radical formed under irradiation and about its electronic structure.

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Experimental

Single crystals of sulphamethoxazole were grown by slow evaporation from saturated ethanol solution at room temperature. The crystal structure has been determined by Bettinetti et al. [1] to be monoclinic with $a = 1.6062$ nm, $b = 0.5479$ nm, $c = 2.5757$ nm, $\beta = 96.12°$ and with a space group C2/c. The crystallographic axes were localized by the external morphology and identified by Laue and oscillation X-ray methods. To carry out ESR measurements, the orthogonal $x\,y\,z$ system coinciding with the $a\,b\,c^*$ crystallographic axes was chosen, Figure 1. The crystals were irradiated for 15 min at 273 K with an X-ray tube operating at 70 kV and 20 mA. The X-band ESR and ENDOR measurements were made on a Bruker ER-200D spectrometer equipped with a Bruker VT4111 temperature controller. All measurements were performed at 100 K, where the ENDOR signal had maximum strength. The principal values and direction cosines of the hyperfine coupling tensors were calculated by standard procedures [4].

Results

Figure 2 presents selected ESR spectra and the corresponding ENDOR spectra. The X-band ESR spectra

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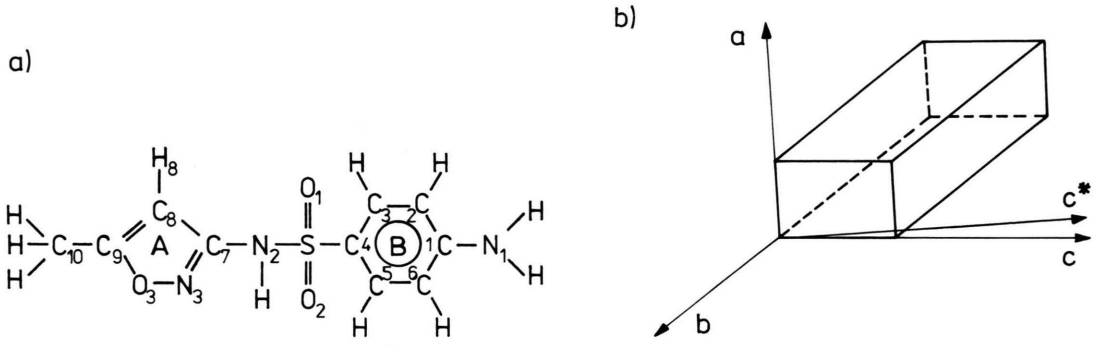

Fig. 1. Structure and numbering of atoms in sulphamethoxazole molecule (a) and the sulphamethoxazole crystals morphology and crystallographic axes arrangement (b).

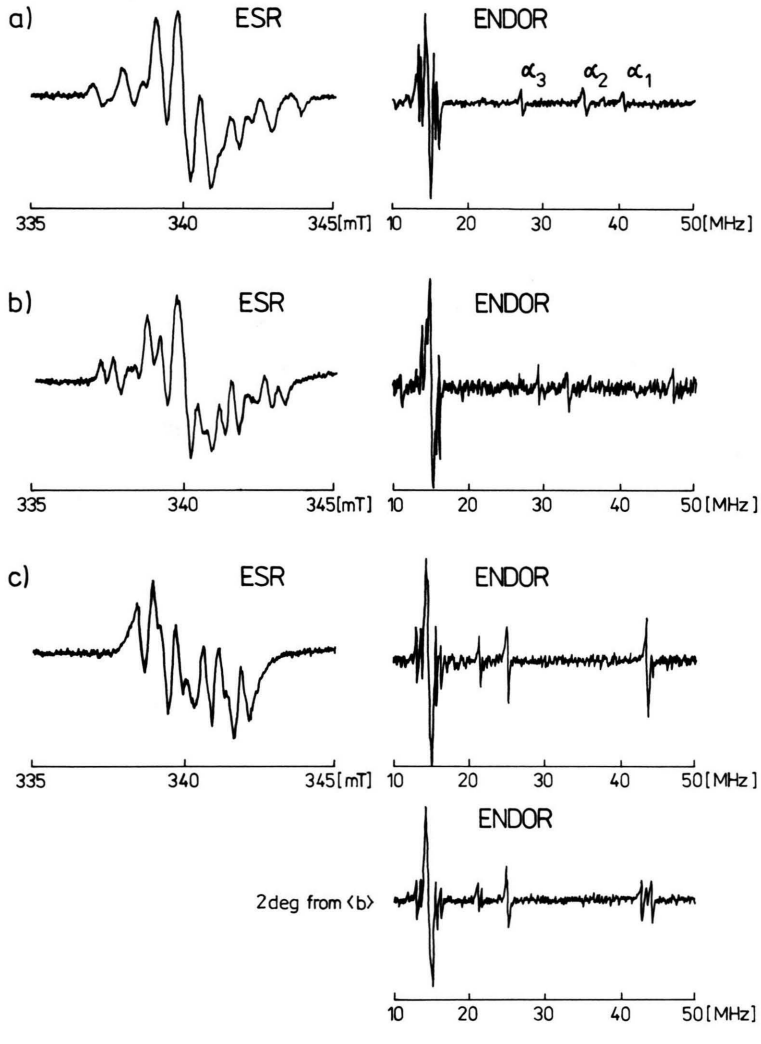

Fig. 2. X-band ESR and ENDOR spectra of X-irradiated sulphamethoxazole single crystal recorded for a few chosen orientations of the crystal in the magnetic B field.
a) $\langle a\,c^*\rangle$ plane – 4 deg. from $\langle a\rangle$,
b) $\langle a\,c^*\rangle$ plane – 54 deg. from $\langle c^*\rangle$,
c) $\langle b\,c^*\rangle$ plane – 0 and 2 deg. from $\langle b\rangle$.

obtained were identical to those presented by previous investigators [2, 3]. ENDOR spectra at a frequency $\nu_{rf} > 20$ MHz are composed of three lines, Figures 2a, b. Only when the crystal is rotated in a magnetic field about the c^* and a axes, individual ENDOR lines are split into two due to magnetic inequivalence of molecules in an elementary cell (site splitting), Figure 2c. In the range $10\ \text{MHz} < \nu_{rf} < 20$ MHz a few ENDOR lines were recorded. Most probably, in this range additional ENDOR lines appear due either to interactions with protons at neighbouring molecules or to an unknown type of radical. The presence of another type of radical is evidenced by the asymmetry of the ESR spectra observed for such orientations of the crystal for which site splitting does not occur, Figures 2a, b. Since individual ENDOR lines overlap in the range 10–20 MHz, they have not been fully analyzed. The three lines in the strong coupling region $\nu_{rf} > 20$ MHz were equally prominent, regardless of the portion of the ESR spectrum probed. The angular variation of the position of the three ENDOR lines points to the fact that hyperfine couplings corresponding to those lines originate from the interaction of an unpaired electron with three hydrogen nuclei at α position. Figure 3 presents angular variations of the three ENDOR transitions denoted by α1, α2, α3. Table 1 gives the calculated principal values and direction cosines of the hyperfine coupling tensors. On the basis of the obtained ENDOR results we can conclude that the stable radical is formed by abstraction of one of the hydrogen atoms from the CH_3 group (Fig. 4) and is identical to that postulated in [3]. The unpaired electron is then delocalized onto the isoxazole ring. The greatest densities of the unpaired electron can be expected on the atoms C10, C8 and N3. In such a case the unpaired electron would interact mainly with the three nuclei of hydrogen atoms denoted in Fig. 4 as $H_{\alpha 1}$, $H_{\alpha 2}$, and $H_{\alpha 3}$ as well as with the nucleus of nitrogen N3. The isotropic hyperfine couplings $H_{\alpha 1}$ and $H_{\alpha 2}$ would be approximately of the same magnitude. These predictions are consistent with the experimental results collected in Table 1. Moreover, according to this table not only are the isotropic and principal values of the hyperfine interaction of $H_{\alpha 1}$ and $H_{\alpha 2}$ very close, but they are (considerably) greater than the values for $H_{\alpha 3}$. No ENDOR signals corresponding to the interaction of the unpaired electron with the N3 nitrogen nucleus were observed.

In the above considerations we have taken into account the possibility of formation of other types of

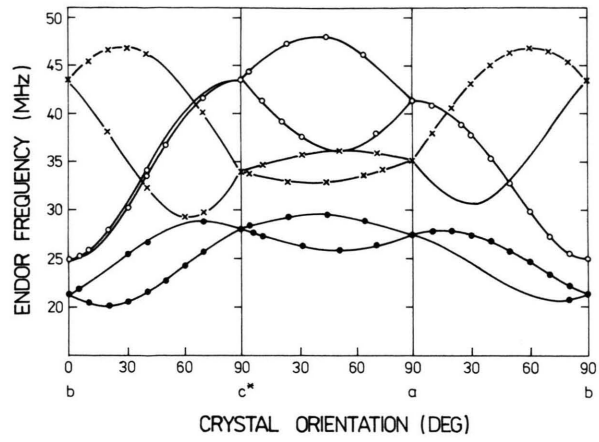

Fig. 3. Angular variations of the ENDOR transitions associated with radical R1 in X-irradiated sulphamethoxazole single crystals; o stands for $H_{\alpha 1}$, × for $H_{\alpha 2}$, and • for $H_{\alpha 3}$.

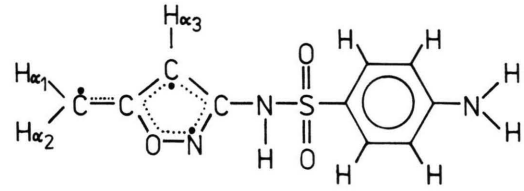

Fig. 4. The structure of R1 formed by abstraction of a hydrogen atom from the CH_3 group.

Table 1. Principal values and direction cosines of the hyperfine tensors (in MHz) for α-hydrogen atoms in a X-irradiated single crystal of sulphamethoxazole.

Tensor	Principal values	Isotropic values	Direction cosines relative to a, b, c^* axes		
$H_{\alpha 1}$	20.94	43.80	−0.0162	−0.9996	−0.0223
	43.35		−0.7690	0.0268	−0.6387
	67.11		0.6391	0.0068	−0.7691
$H_{\alpha 2}$	20.90	44.16	0.5568	−0.5153	−0.6515
	43.39		0.7447	−0.0376	0.6663
	68.18		0.3678	0.8562	−0.3628
$H_{\alpha 3}$	11.02	21.94	−0.1686	0.9464	0.2754
	22.95		0.7878	−0.0385	0.6147
	31.85		0.5923	0.3206	−0.7392

radicals. One of them could be R2, see Fig. 5b, in which the unpaired electron would interact with three nuclei of hydrogen atoms at α positions (with two of them interacting equally) and with a nitrogen nucleus. This type of radical would be formed by abstraction of a hydrogen atom from the NH_2 group, and the unpaired electron would be delocalized onto the B ring

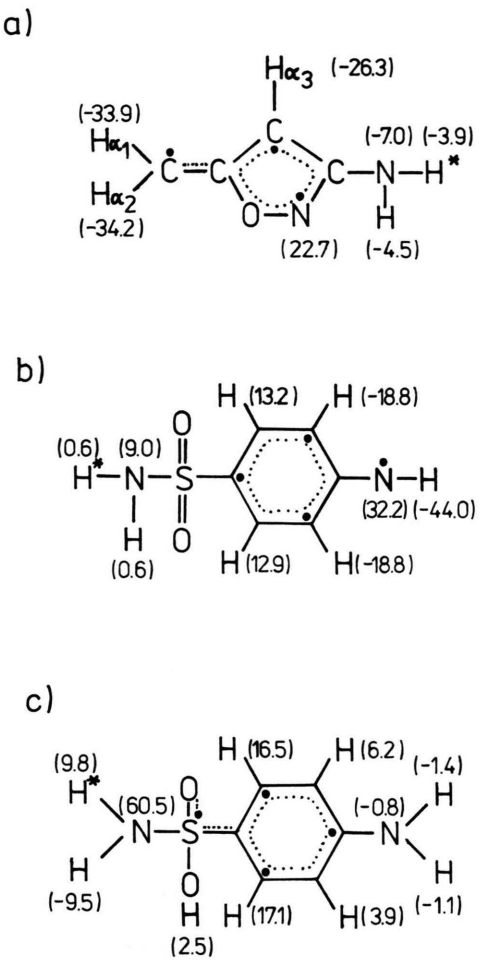

Fig. 5. The results of MNDO and INDO calculations for different types of sulphamethoxazole radicals: a) R1, b) R2, c) R3. The values of hyperfine interactions in MHz are given in parentheses. H* represents the hydrogen atom replacing the rest of the molecule.

of the molecule with high density at the positions: N1, C2, C4 and C6 (Figure 5 b).

The other possible radical of R3 type could be formed by addition of a hydrogen atom to one of the oxygen atoms O1 or O2, while the unpaired electron would also interact with two nuclei of hydrogen atoms at α positions and could delocalize onto the nitrogen atom. Then, one of the hydrogen atoms of the NH_3 group would be responsible for the third hyperfine interaction denoted by $H_{\alpha 3}$.

In order to verify that the stable radical formed under X-irradiation in a sulphamethoxazole single crystal is of R1 type, we compared the directions of the

Table 2. Angles ϕ between the directions of the parent molecule and the tensor axes of the radical.

Bond/plane directions (X-ray data)	Direction cosines	Principal values (ENDOR data)	Direction cosines	ϕ
C(8)–H(8)	−0.1728	$H_{\alpha 3\,\min}$ *	−0.1686	1.8°
	0.9417		0.9464	
	0.2873		0.2754	
C(10)–H2(10)	−0.3929	$H_{\alpha 2\,\min}$	0.5568	62.6°
	−0.1763		−0.5153	
	−0.9031		−0.6515	
C(10)–H3(10)	0.0057	$H_{\alpha 3\,\min}$	−0.0162	6.5°
	0.9909		−0.9996	
	0.1326		−0.0223	
Normal to the plane				
C(9)C(8)C(7)	0.8205	$H_{\alpha 3\,\text{int}}$ *	0.7878	3.9°
	0.0010		−0.0385	
	0.5716		0.6147	
C(2)C(1)C(6)	−0.2378	$H_{\alpha 3\,\text{int}}$	0.7878	71.8°
	0.4827		−0.0385	
	0.8429		0.6147	

* Minimum and intermediate principal values in Table 1, respectively.

principal values of the hyperfine interaction tensors with the corresponding directions in the molecule in the crystal. A comparison between the directions of the principal values of hyperfine interaction tensors and the directions in a sulphamethoxazole molecule can only be made for the R1 type radical fragment including C9C8H8C7 (Table 2). This fragment is supposed not to undergo significant changes during the radical formation whereas the conformation of the CH_3 group is very likely to change considerably after abstraction of one of the hydrogen atoms. As follows from the data given in Table 2, the direction of the intermediate value of the $H_{\alpha 3}$ tensor is almost parallel to that of the normal to the C9C8C7 plane (the angle they make is about 4 deg.) and at the same time makes a large angle (72 deg.) with the direction of the normal to the benzene ring. The above data undoubtedly confirm the presence of R1 type radical in the X-irradiated single crystal of sulphamethoxazole.

Molecular Orbital Calculations

Applying the quantum-mechanical methods MNDO [5] and INDO [6] we also calculated values of the hyperfine interactions of the unpaired electron for all

the three possible types of radicals R1, R2, and R3 as well as for sulphamethoxazole cation and anion. The MNDO method was used to optimize the radical geometry. Then, for the so optimized geometry the unrestricted Hartree-Fock variant of the INDO method was employed to calculate the hyperfine interactions. Spin annihilation was not applied.

Because of time limitations and program restrictions, the calculations were carried out for chosen fragments of the molecule. For the R1 radical the calculations were performed for the structure shown in Fig. 5a, where H* is a hydrogen atom replacing the remaining part of the molecule. A similar procedure was used for the radicals R2 and R3 (Figure 5b, c). Computed values of the hyperfine interactions, in MHz, are given in parentheses. Comparing the results of quantum-mechanical calculations with experimental data we find that they agree only in the case of the R1 radical. Moreover, only for this radical the interaction of two hydrogen atoms with the unpaired electron was found to be stronger than that of the third one. The fact that the calculated values of $H_{\alpha 1}$ and $H_{\alpha 2}$ hyperfine splittings were lower, whereas that of $H_{\alpha 3}$ was higher than the corresponding experimental ones, can be accounted for by the difference between the theoretical conformation of this fragment of the radical and its real conformation in the crystal. Since the INDO calculations were carried out for the conformation of the radical optimized by the MNDO method, treating the radical as a free molecule, the atoms $H_{\alpha 1}$, $H_{\alpha 2}$, C9, C8, C7 lie practically in one plane. However, if the INDO calculations are carried out for the conformation in which the planes $H_{\alpha 1}$ C10 $H_{\alpha 2}$ and C9C8C7 are twisted relative to each other by a certain angle, the results show an increase in the hyperfine splitting for $H_{\alpha 1}$ and $H_{\alpha 2}$ above the experimental one whereas for $H_{\alpha 3}$ a decrease below the experimental value occurs. A detailed analysis of this problem would, however, require additional computation. In the case of sulphamethoxazole anion and cation the unpaired electron is mainly localized on the bridge connecting the two rings, and in particular on the SO_2 group, and it is only insignificantly delocalized on the isoxazole A and benzene B rings.

Discussion

Allylic type radicals were thoroughly studied by Huttermann and Bernhard [7, 8]. These papers present an analysis of the problem of planarity of the radical and the delocalisation of the unpaired electron onto individual atoms as well as the influence of the substituents on the delocalisation. The comparison between our experimental data and theirs, in conjunction with the theoretical calculations, shows that the free radical R1 trapped at room temperature in X-irradiated sulphamethoxazole has the structure of Figure 4. The delocalization of the unpaired electron through the bridge onto the benzene ring in the case of R1 is very small and the value of the hyperfine coupling with any proton of the benzene ring or the connecting bridge does not exceed 6 MHz. This small spin delocalization, evident from experiments as well as from calculations, can be qualitatively understood from the simple valence bond structures of R1, which can be regarded as a delocalized Π-electron radical. Structures can be drawn with the unpaired electron located on C_{10}, C_8, and N_3 but not on N_2. This and the presence of the SO_2 bridging group hinders the delocalization of the unpaired electron to the benzene ring. As indicated above, it was impractical to compare the stability of R1 with that of isomeric radicals obtained by hydrogen abstraction at other positions by quantum chemical calculations. It is reasonable, however, that the delocalization over the isoxazole ring is one explanation for the stability of the R1 radical.

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