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ESR and ENDOR of Free Radicals in γ-Irradiated Single Crystals of Trimethoprim at Room Temperature

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Singe crystals of trimethoprim were examined after γ-irradiation at room temperature by ESR and ENDOR spectroscopy. A radical is formed by abstraction of a hydrogen atom from the CH_2 group of the bridge. The unpaired electron is delocalized mainly onto the trimethoxybenzyl ring. It interacts with one proton of the bridge and with two protons in the trimethoxybenzyl ring.

Trimethoprim, $C_{14}H_{18}N_4O_3$ [full name: 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine], is a well known bacteriostatic drug which inhibits the activity of two different enzymes taking part in biosynthesis of bacterial folic acid. It has been used among others as a metabolic inhibitor in the chemotherapy of malaria and cancer because it affects the synthesis of proteins and nucleic acids. The molecular structure of trimethoprim is shown in Fig. 1. Because of the large number of acceptor and donor sites in the compound molecule, the mechanism of its activity has not been fully determined.

The characteristic structure of the trimethoprim molecule, with an electron donor, a connecting bridge and an acceptor, makes it interesting to investigate the unpaired electron delocalization in radicals derived from trimethoprim and the effect of relative spatial orientation of the donor and acceptor parts on this delocalization. Possible effects of radiation on the biological activity of the compound would be better understood if the free radicals which remain stable after irradiation could be characterised.

This paper aims at identification of the type and electron structure of the radical formed under γ-irradiation of trimethoprim at room temperature.

Experimental

Trimethoprim was obtained from Poznan Pharmaceutical Plant POLFA in the form of a powder. Single crystals of trimethoprim were grown according to the method described in Ref. 1 by crystallization from a saturated solution of trimethoprim in methyl alcohol by slow evaporation at room temperature. The monocrystals were homogeneous, colourless and had well developed surfaces with a maximum size of 3 × 1 × 1 mm. The melting point of the obtained monocrystals was determined to be 196–198 °C.

The monocrystals belong to the triclinic system of $P\bar{1}$ space group symmetry, and their elementary cell parameters are: $a = 10.523$ Å, $b = 11.222$ Å, $c = 8.068$ Å, $\alpha = 101.220°$, $\beta = 112.150°$ and $\gamma = 112.650°$.[1] Each elementary cell comprises two equivalent molecules.

The obtained monocrystals were exposed to γ-irradiation from a cobalt bomb source to a dose of 150 kGy. The ESR spectra measurements were carried out on a spectrometer working in the X-band (9.4 GHz) made by Radiopan, Poland. Electron-nuclear double resonance spectroscopy (ENDOR) measurements of trimethoprim monocrystals were performed at the Department of Physics and Technology of the University in Linköping, Sweden, on a 200D-SRC spectrometer made by Bruker. The ESR and ENDOR signals were recorded by turning the crystal around the three mutually orthogonal axes ***x***, ***y*** and ***z*** at every 5°. The crystal was oriented so that its ***c***-axis was parallel to the ***z***-axis, and its ***b***-axis made an

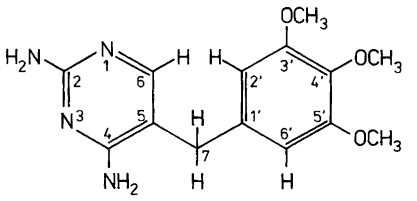

Fig. 1. Molecular structure of trimethoprim with the assumed numbering of the atoms.

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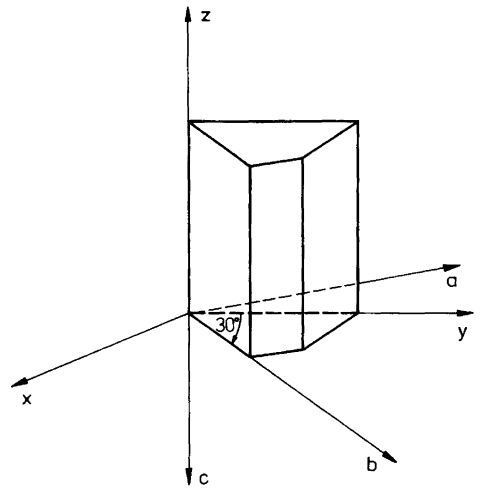

Fig. 2. A monocrystal of trimethoprim in the crystallographic reference frame ***abc*** and the ESR reference frame ***xyz***. The ***c***-axis is parallel to the ***z***-axis, and the ***b***-axis forms an angle of 30° with the ***y***-axis and 62.6° with the ***x***-axis.

angle of 30° with the *y*-axis and 62.6° with the *x*-axis. The relative orientation of the experimental frame axes to the crystal axes is illustrated in Fig. 2.

Experimental results

ESR spectra of irradiated trimethoprim monocrystals, of which a few are shown in Fig. 3, have a complex structure and moreover are poorly resolved in most orientations, making the analysis difficult. As the two molecules in the elementary cell are magnetically equivalent, the ESR spectra of the monocrystals should be symmetrical. The fact that they are not symmetric may suggest the presence of a radical of a different type. Only one radical has been assigned, however.

The dependence of the *g*-factor on the monocrystal orientation relative to the magnetic field of the ESR spectra is shown in Fig. 4. In this figure and in Fig. 6 the 90–180° angular variation has been folded back on the 0–90° variation to save space. The principal values and the corresponding direction cosines of the *g*-tensor in the laboratory frame are given in Table 1.

Relatively well resolved hyperfine structure of the ESR spectra could be obtained for only a few orientations of the monocrystals (Fig. 3). Thus, identification of the type of the radical formed on the basis of ESR spectra only proved very difficult. In the next step we resorted to ENDOR spectroscopy, which proved beneficial. A typical ENDOR spectrum is presented in Fig. 5.

The analysis of ENDOR spectra provided evidence for the occurrence of three anisotropic interactions between the unpaired electron and hydrogen nuclei in trimethoprim, and indicated the presence of a few weaker hyperfine couplings.

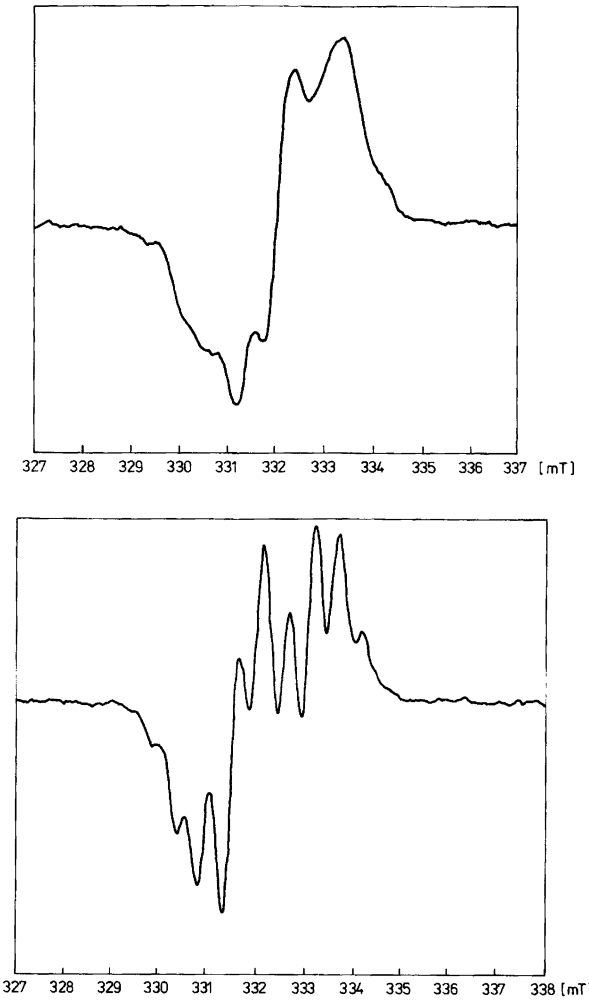

Fig. 3. ESR spectra of γ-irradiated single crystal of trimethoprim at room temperature. The applied field is in the ***yz***-plane and makes an angle of 10° (a) and 115° (b) with the ***y***-axis.

The dependence of the hyperfine splitting values on orientation of the monocrystal with respect to the magnetic field corresponding to the three anisotropic interactions is shown in Fig. 6. The data for the hyperfine splitting tensors are collected in Table 1.

Computational results

In order to investigate the type of radical formed in the irradiated trimethoprim crystal semiempirical MO computations were performed. The radical structure was optimized with the RHF/MNDO method developed by Dewar and Thiel.[2] The geometry of the neutral molecule in the crystal phase[1] was used as an initial trial structure in the geometry optimization. A neutral radical with one hydrogen abstracted from the CH_2 bridge was assumed. Attempts to calculate the molecular anion failed to converge.

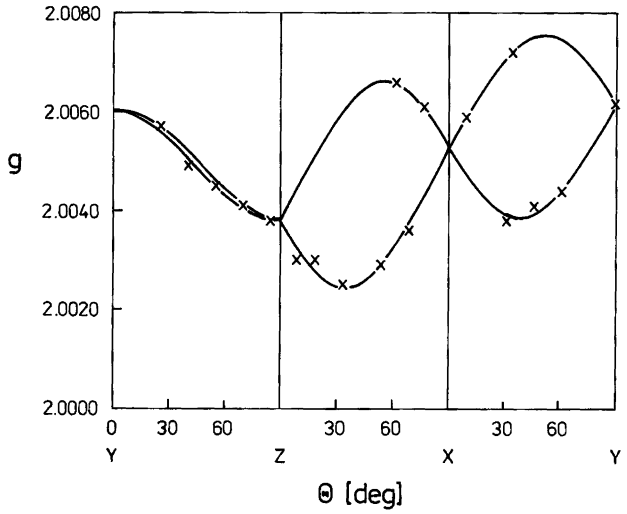

Fig. 4. The *g*-factor dependence on the crystal orientation with the magnetic field in the ***yz***, ***zx*** and ***xy*** planes of the experimental frame, obtained for an irradiated single crystal of trimethoprim at room temperature. The 90–180° angular variation has been folded back on the 0–90° variation to save space.

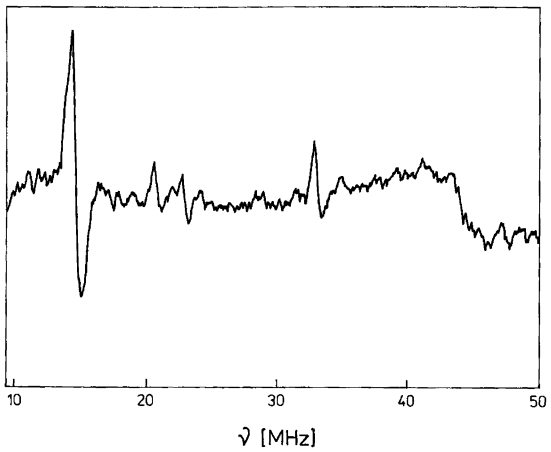

Fig. 5. ENDOR spectrum of a γ-irradiated single crystal of trimethoprim at room temperature. The applied field is in the ***yz*** plane and makes an angle of 10° with the ***y***-axis. The lines at 20.83, 22.98 and 33.31 MHz are attributed to three α-hydrogen atoms.

The optimization of the neutral radical was performed in three steps. First, all geometrical parameters, except the dihedral angles of the atoms of the side groups ($-NH_2$, $-OCH_3$) and hydrogen-bond lengths in these, were optimized. Second, the methoxy groups and the benzyl ring were fully optimized while all other parameters were kept fixed. In the last step all parameters were optimized except those of the methyl part of the methoxy groups as well as the hydrogens in the NH_2 groups. The optimized structure is shown in Fig. 7. Both the pyrimidine and the benzyl rings remain essentially planar. The C–C–C angle of the bridge joining the two rings increases from 117.9 to 130.5°, and the two C–C bond lengths are significantly shortened (Fig. 7). The single hydrogen of the bridge is tilted from its tetragonal position to a position closer to

Table 1. Experimental principal values and direction cosines of the *g*-tensor and of the hyperfine tensors, A_α (in MHz), for the α-hydrogen atoms in a γ-irradiated single crystal of trimethoprim.[a]

Tensor	Principal values	Calculated values	Direction cosines relative to axes		
			x	*y*	*z*
g	2.0021		0.6185	−0.2919	0.7296
	2.0051		−0.3905	0.6915	0.6077
	2.0079		−0.6819	−0.6608	0.3137
A_1	−6.350		−0.1904	0.8343	0.5174
	−14.272		0.5902	−0.3239	0.7394
	−17.237		0.7845	0.4462	−0.4307
A_1^{iso}	−12.620	−11.8			
A_2	−5.015		0.7897	−0.3421	−0.5093
	−14.224		0.4658	−0.2061	0.8606
	−17.200		0.3994	0.9168	0.0034
A_2^{iso}	−12.146	−11.9			
A_3	−17.769		−0.0831	0.7725	0.6296
	−39.259		0.4031	−0.5517	0.7302
	−63.283		−0.9114	−0.3145	0.2655
A_3^{iso}	−40.104	−36.3			

[a] Negative principal values have been assumed for the experimental tensors. The calculated isotropic couplings were obtained by INDO. The orientation of the *xyz*-axes relative to the crystallographic axes is shown in Fig. 2.

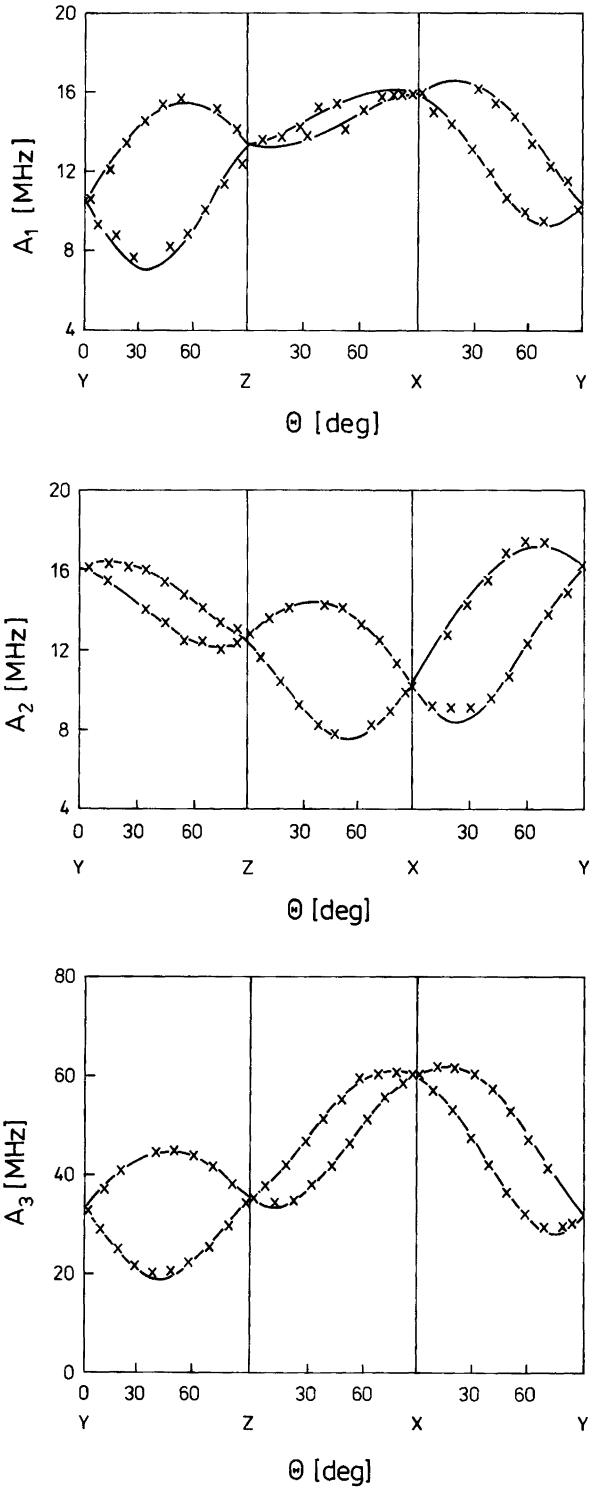

Fig. 6. Orientation dependence of the first-order ENDOR splittings, A_1, A_2 and A_3 of three hydrogens in the ***yz***, ***zx*** and ***xy***-planes of the laboratory frame for an irradiated single crystal of trimethoprim. The 90–180° angular variation has been folded back on the 0–90° variation to save space.

the plane of the trimethoxybenzyl ring. In this ring, two C–C bonds remain essentially unaffected, while four are slightly elongated (Fig. 7). The C–O bonds between ring

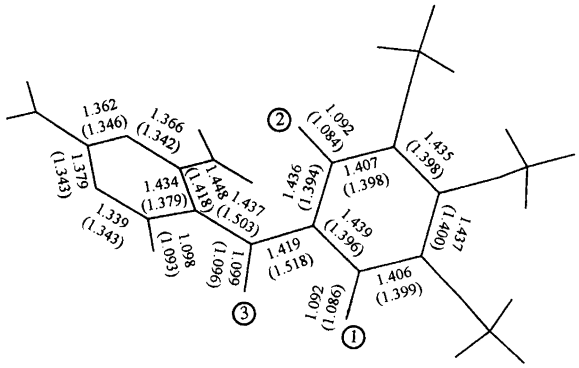

Fig. 7. MNDO-optimized geometry of the radical formed in γ-irradiated single-crystal trimethoprim at room temperature. The trimethoxybenzyl ring is in the plane of the paper. Also shown are optimized bond lengths (in Å) of selected bonds. Values of the neutral molecule[1] are shown in parenthesis. The positions of the three α-protons assigned in Table 1 are also indicated.

carbons and methoxy groups also stay essentially unchanged. The central C_4–O bond decreases from 1.371 to 1.363 Å, while the $C_{3'}$–O and $C_{5'}$–O bonds increase from 1.363 and 1.360 Å to 1.371 and 1.371 Å, respectively.

Single-point RHF/INDO + CI calculations[3] using the MNDO optimized structures were employed to extract hyperfine splittings. The calculated hyperfine splittings (HFS) for the optimized structure are shown in Table 1. Three major hydrogen splittings are predicted, in agreement with experiment, as well as a smaller coupling (ca. 5.5 MHz) of the single hydrogen directly bonded to the pyrimidine ring. The isotropic part of each α-hydrogen hyperfine coupling was estimated from the INDO-calculated unpaired π-electron spin density using the McConnell equation:[4]

$$A_\alpha^{iso} = Q_{CH}^{H}\,\rho \qquad (1)$$

where ρ is the unpaired π-electron density and Q_{CH}^{H} is an empirical constant dependent on the type of radical. Commonly the constant Q_{CH}^{H} takes values in the range −64.4 to −73.4 MHz, but in the present case this range seems too small. This has also been found for other systems, e.g. the biphenyl radical cation,[5,6] which requires a value of −86 MHz to give a good agreement with experimental data. This value of Q_{CH}^{H} gives the isotropic HFS presented in Table 1. Although slightly underestimated, the agreement with the experimental values is satisfactory. The INDO calculated densities of the unpaired electron on individual carbon atoms are: $\rho(C7) = 0.43$, $\rho(C_{6'}) = \rho(C_{2'}) = 0.14$, $\rho(C_{4'}) = 0.17$, $\rho(C_{1'}) = -0.06$, $\rho(C_{3'}) = \rho(C_{5'}) = -0.04$, $\rho(C_2) = (C_4) = 0.06$, $\rho(C_6) = 0.07$. Only densities exceeding 0.025 are listed. Thus, the unpaired electron is almost completely localized on the bridge and the benzyl ring, and only slightly delocalized onto the pyrimidine ring. Such a pronounced asymmetry

in the electron delocalization is most probably due to the considerable difference of the electronic properties of the 2,4-diaminopyrimidine and 3,4,5-trimethoxybenzyl rings. The former shows strong electron donor properties, while the latter has electron acceptor properties.

Discussion

On the basis of the ESR and ENDOR spectral analysis and the MO calculations it is concluded that in a monocrystal of trimethoprim the ionizing radiation induces the formation of a radical by abstraction of a hydrogen atom from the CH_2 group in the bridge joining the two rings of the molecule. The inferred structure of this radical is presented in Fig. 7.

The unpaired electron interacts with the hydrogen atom at C_7, and as it delocalizes mainly onto the trimethoxybenzyl ring (3,4,5-trimethoxybenzyl) it also interacts with the hydrogen nuclei at $C_{2'}$ and $C_{6'}$. Thus, in this radical three hyperfine interactions of the α-type occur. Two of them are almost of the same strength. The eigenvectors of the *g*- and hyperfine coupling tensors are in mutual agreement with this interpretation, i.e. (a) The eigenvectors for the intermediate hyperfine coupling principal values (which should be along the unpaired electron orbital) are all almost parallel, indicating a planar radical fragment. (b) The eigenvector for the minimum principal *g*-value is nearly parallel to the above directions. (c) The eigenvectors for the minimum hyperfine coupling principal values (which should be along the C–H bond direction) are almost parallel for (1) and (3), and make an angle of about 130° with (2). This is consistent with the suggested structure.

There are a few more features of the experimental data which need to be considered. First, the *g*-factor variation is unusually large for a carbon-centred radical. This might imply delocalization of spin density on oxygen or nitrogen atoms. Under these conditions the maximum *g*-factor should be along the $C_{4'}$–O bond, which is roughly parallel to the bisector of the $C_{2'}$–H and $C_{6'}$–H directions (Figs. 1 and 7). As is well known, these latter directions are close to the minimum values of the hyperfine splittings of the hydrogens denoted A1 and A2 in Table 1. Performing the arithmetics gives the direction (−0.7727, −0.6351, −0.0105), which deviates 19° from the direction for the maximum *g*-factor. This is considered satisfactory in view of the crudeness of the estimate. However, the MO calculations do not predict this delocalization. The calculated π-electron spin density on the oxygen is only 0.01. Probably the geometry of the bridge and the angle between the two ring planes can play an important role in the calculated spin distribution, but this has not been investigated further.

Secondly, the anisotropic parts of the hyperfine splittings A1 and A2 obtained by subtracting the isotropic coupling from the principal values of Table 1 deviate from the form expected for a π-electron carbon-centred radical. For unity spin density one usually finds (30, 0, −30) MHz along the C–H bond, parallel to the π-orbital, and perpendicular to these directions, respectively.[7] In the present case the spin density is delocalized. This has been taken into account in a method to calculate dipolar couplings from UHF/INDO theory.[8] Another method applicable to delocalized π-electron radicals involves an extension of the theory in Ref. 7 to include contributions from spin densities at several atoms. The results show that the form of the dipolar coupling is sensitive to the spin density distribution.[6] Calculations with the latter method give principal dipolar components of A1: 5.7, −2.0, −3.7 MHz and of A2: 5.9, −1.9, −4.0 MHz. This should be compared with the measured dipolar components of A1 and A2; 6.3, −1.7, −4.6 and 7.1, −2.0, −5.1 MHz, respectively. The agreement between theory and experiment is quite good considering the difficulty of estimating the spin density at the C, N and O positions. In the calculation the RHF/INDO + CI calculated spin densities on C_7 and the benzyl ring carbons were used. Calculated principal directions are as expected where the intermediate value in each dipolar tensor is found in the direction perpendicular to the plane of the benzyl ring and the two large values are found in the plane. Because of the contribution from spin densities at several atoms the large, positive component is shifted +7° from the C–H bond direction and the large, negative component +97° from the C–H bond direction. The calculated directions are consistent with the properties of the experimentally observed principal directions discussed above.

Finally, it might have been advantageous to compare directly directions obtained from ESR and ENDOR with those from crystallographic data, by expressing the crystallographic (*a*,*b*,*c*) coordinates in the experimental frame (*x*,*y*,*z*). This has not been carried out, since the internal consistency of the ESR and ENDOR data was considered satisfactory, as described above, and since the geometry of the radical differs from that of the trimethoprim molecule.

A delocalization of the unpaired electron from the $-C_7H_7-$ bridge onto the pyrimidine ring is less likely in view of the computational results. From the ESR point of view, it would bring about only one α-type hyperfine interaction with the hydrogen atom at C_7 and a few β-type interactions with the hydrogens of the NH_2 groups at C_2 and C_4. It is difficult to think of other radicals which would be formed in the pyrimidine ring by e.g. abstraction or attachment of a hydrogen atom, in which three α-type hyperfine interactions would be present. All in all the most probable type of radical is the one in which the hydrogen atom is abstracted from the carbon atom C_7, and the unpaired electron is delocalized onto the trimethoxybenzyl ring.

Delocalization of the unpaired electron through a bridge connecting two rings has already been considered by many authors.[9–12] For example, Voevodskii *et al.*,[9] based on the studies of Tuttle and Weissman[10] and McConnell and McLachlan,[11] have analyzed the ESR

spectra of ion radical of aromatic compounds in which two phenyl rings are linked by a saturated hydrocarbon bridge. In all the cases studied the electron transfer between the rings occurs at a rate higher than 10^7 Hz. Harriman and Maki[13,14] also investigated two aromatic rings connected by different bridging groups: CH_2, S, O and CH_2CH_2. The hyperfine structure was found to be especially sensitive to the intramolecular electron transfer rate when the latter is of the same order of magnitude as the hyperfine intervals when expressed as frequencies. They determined the rate of electron transfer through the bridge, obtaining values ranging from 10^6–10^8 Hz for different compounds. In our case the rate of electron transfer between the pyrimidine and the trimethoxybenzyl ring is lower than 3×10^6 Hz. Such a small rate is probably due to a large difference in electronegativity between these rings and to a delocalization of the unpaired electron onto the trimethoxybenzyl group. Assessment of the temperature dependence of this delocalization requires further investigation.

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