FREE RADICALS OF SOME AZA-URACIL DERIVATIVES HAVING ANTI-VIRAL ACTIVITY: AN ESR AND INDO STUDY

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Abstract: The free radicals obtained during γ and UV irradiation of powder aza-uracil derivatives, having high anti-viral activity are analysed by using EPR spectrometry and INDO Molecular Orbitals calculus. A comparison with uracil ESR is used in order to identify the various radicals.

1. Introduction

There is considerable interest to analyse the free radicals obtained by the irradiation of pyrimidine bases and their derivatives for the understanding of the biological damage of the irradiation process [1, 2,3]. It is known that aza-derivatives of thymine and uracil bases have biological activity too and for this reason it is important to know the free radicals obtained during their irradiation [4]. In the present work we analyse the free radicals obtained by the γ and UV irradiation of aza-uracil compared with those of uracil by using ESR spectrometry and we calculate their spin density by semi-empirical INDO method [5].

2. Experimental details

The aza-uracil derivatives and uracil were irradiated by using γ radiation (14.4 and 8.8 MeR) and UV (5 min). Samples for EPR measurements were measured as powders without any further treatment, after the irradiation. EPR spectra were obtained with a Jeol JES-ME 3X, X band spectrometer with 100 kHz modulation frequency, at room temperature. Optimisation of
spectral resolution was achieved by making multiple accumulations and using fast Fourier transformation with a Gaussian profile for subsequent noise reduction. The geometry optimisations of free radicals' structures were calculated using MM$^+$ molecular mechanics force-field for the spin density were obtained by using INDO semi-empiric MO, with UHF formalism.

2. Results and discussion

The uracil ESR analysis was carried out in order to compare the ESR parameters with those of aza-uracil and to identify the various free radicals yield by irradiation. This comparison is based on a similar analysis of thymine and aza-thymine [1, 6] which mention that in both molecules the same types of radicals were formed, but in different ratio. The uracil spectra (Fig.1) were analysed by using the single crystal data [7] and two free radicals are identified, their ESR parameters being shown in Tab. 1.

![Uracil EPR spectrum at room temperature (3380 G ±100 G).](image1)

![Aza-uracil EPR spectrum at room temperature (3180 ±100 G).](image2)
The difference between the powder and the single crystal spectra is due to different ratio of the generated free radicals; in the present study the ratio between radicals I and II [7] is 5:1 and not 1:1. The aza-uracil ESR spectra are shown in Fig. 2 and it can be seen that they have larger line shape than uracil connected with a mixture of different paramagnetic species.

Table 1. The ESR parameters for uracil and aza-uracil powder samples, at room temperature

<table>
<thead>
<tr>
<th>Uracil ESR parameters</th>
<th>Aza-uracil ESR parameters</th>
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<tbody>
<tr>
<td><strong>I:</strong> $g_1=2.0021$ $g_μ=2.0051$</td>
<td><strong>I:</strong> $g_1=2.0034$ $g_μ=2.0062$</td>
</tr>
<tr>
<td>$a_{\perp}^{N(1)}=14.5$ $a_{\perp}^{N(1)}=0.5$ $a_3^{N(1)}=0.5$</td>
<td>$a_{\perp}^{N(1)}=4.2$ $a_{\perp}^{N(1)}=1.2$</td>
</tr>
<tr>
<td>$a_{\perp}^{H(5)}=8.0$ $a_{\perp}^{H(5)}=29.0$ $a_3^{H(5)}=13.0$</td>
<td>$a_{\perp}^{N(2)}=19.0$ $a_{\perp}^{N(2)}=3.0$</td>
</tr>
<tr>
<td><strong>II:</strong> $g=2.0055$</td>
<td><strong>II:</strong> $g=2.0058$</td>
</tr>
<tr>
<td>$a_{\perp}^{H(6)}=8.0$ $a_{\perp}^{H(6)}=30.0$ $a_3^{H(6)}=18.0$</td>
<td>$a_{\perp}^{H(6)}=18.4$</td>
</tr>
<tr>
<td>$a_{\perp}^{H(6)}=13.0$ $a_{\perp}^{N(1)}=0.8$</td>
<td>$a_{\perp}^{H(6)}=22.3$ $a_{\perp}^{H(5)}=14.4$</td>
</tr>
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The EPR parameters for aza-uracil are presented in Tab.1. and they characterise the radicals I and II (Fig. 3a, b), the first one being the major radical.

Figure 3. Free radicals structures obtained by $\gamma$ irradiation of aza-uracil:

a] radical I
b] radical II
These radicals are different compared with the radicals that appear during the irradiation of uracil. The UV irradiation does not change the shape of the spectra and for this reason we suppose the presence of the same type of free radicals.

We assume an axial symmetry of the tensors and not an orthorhombic one, like in uracil analysis. Due to the width of the line shape we suppose that both radicals are present in tautomeric forms, situation that will enlarge the line shape.

Molecular mechanics calculus (MM*) version was used in order to obtain the optimum geometry foraza-uracil free radicals. The spin density in the regions where we suppose that the unpaired electron is localised is obtained by INDO calculus, in UHF formalism. The results support our assumptions of spectra assignments: the radical I is the major one, because the binding energy is lower (\( E_{\text{bind}}^I = -2995 \text{ Kcal/mol} \)) and therefore is easier to be obtained by irradiation (\( E_{\text{bind}}^II = -3253 \text{ Kcal/mol} \)). After irradiation, the net charge density and the spin density on N atoms where the unpaired electron is located is growing on radical I (N(6): -0.081/-0.173) and radical II (N(1): -0.020/-0.132).

4. Conclusion

The effect of the aza-uracil powder samples irradiation is the formation of two tautomeric free radicals, one of them being as major component. The INDO MO calculus confirms these radicals, based on the values of the calculated spin density.