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RADIATION-INDUCED RADICALS IN THYMIDINE SINGLE CRYSTALS AT 77°K[1]

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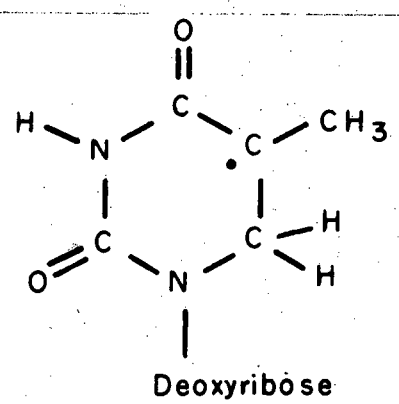
Three different free radicals are formed in detectable quantities in thymidine single crystals irradiated at 77°K. In addition to the spectrum of the previously known 5,6-dihydrothymidin-5-yl radical, a quartet resonance and a doublet resonance are observed. The quartet resonance is attributed to the 5,6-dihydrothymidin-6-yl radical, but the exact nature of the radical giving the doublet remains speculative.

1. Introduction

Thymidine irradiated at room temperature exhibits an electron spin resonance (ESR) spectrum that can be attributed mainly to the 5,6-dihydrothymidin-5-yl radical (I):



(I)

This radical has been characterized previously by single-crystal analysis (Pruden, Snipes, and Gordy 1965). It was evident from the single-crystal spectra that another radical was formed in small quantities, but no definite identification was made.

In this work single crystals of thymidine have been irradiated and observed at 77°K in an attempt to identify and characterize any additional radicals that may be stabilized at that temperature. Two such radicals are observed. The nature of one of these radicals remains speculative, whereas the other was identified as the 5,6-dihydrothymidin-6-yl radical. Details of the identification and characterization of this latter radical are the subject of this paper.

2. Description of the Crystals

Single crystals of thymidine were grown by either slow cooling or slow evaporation of saturated solutions. The crystals are orthorhombic, with space group $P2_12_12_1$ (Furberg, Hordvik, and Taugbøl 1956). The crystals used for the ESR analysis were clear and well developed, with a prominent axis of elongation. When the crystals were placed between crossed polarizers, strong extinctions were observed along the elongated axis and in two mutually perpendicular directions orthogonal to the elongated axis. This served to identify the crystallographic axis system. The individual axes have been assigned different letters by Furberg *et al.* (1956) and by Young, Tollin, and Wilson (1969). The latter authors carried out a complete crystal- and molecular-structure determination for thymidine. According to their axis assignment, which will be used in this ESR analysis, the axis of elongation is the crystallographic a axis.

Based on the single-crystal ESR data at room temperature, Pruden *et al.* (1965) were able to predict the orientation for the thymine ring in the thymidine crystal. These predictions were recently confirmed, within 2 deg, by Young *et al.* (1969). There are four molecules in the unit cell, which has the dimensions (in Ångstroms) of a = 4.860, b = 13.91, and c = 16.32 (Young *et al.*

1969). For each molecule the normal to the plane containing the thymine ring lies in the ab plane, at an angle of 45 deg from each axis. Further details of the crystal structure are discussed in connection with the identification of the 5, 6-dihydrothymidin-6-yl radical.

3. Experimental Procedure

ESR data were taken in each of the three crystallographic planes with a Varian 4502 spectrometer with the X-band rotating cavity. An individual crystal or a piece of a crystal was used for each plane. The crystals were mounted on a thin, stainless steel crystal holder with silicone vacuum grease. (It was essential to keep the amount of grease for mounting small in order to minimize interfering signals from irradiated grease.) The desired orientation was attained by using crossed polarizers. The crystals were slowly cooled to 77^0K and irradiated with 6.5-MeV electrons from a linear accelerator. The dose was approximately 2.5×10^7 rads, given at a dose rate of 0.5×10^6 rad/min. No unpaired spins are stabilized in the metal crystal holder. Following irradiation, the crystal and holder were transferred, without warming, to a liquid nitrogen Dewar that fitted into the ESR cavity.

Second-derivative ESR spectra were recorded at 5-deg intervals in each plane. The rotating-cavity accessory allowed rotation of the magnet about the stationary crystal. The magnetic field sweep was measured with a proton resonance field meter and the microwave frequency with a frequency counter. For many crystal orientations spectra were taken at two or more power levels. This procedure proved to be very efficient in order to distinguish between the induced radicals, since they power-saturate at different levels.

4. Analysis of the Spectra

Figure 1 shows ESR spectra with the magnetic field along the crystallographic a axis. At this orientation, radical (I) contributes only slightly to the absorption, but its outer lines can nevertheless be easily identified (the lines marked by arrows). The remaining prominent portion of the spectrum consists of six lines. Careful consideration of both the g-values and the power-saturation properties of this and other spectra show that these six lines constitute a quartet and a doublet. In figure 1 the quartet is marked by the solid stick-line spectrum whereas the doublet is dashed. The quartet saturates more readily than either the doublet or the signal from radical (I), as can be seen in figure 1 (lower spectrum). The differential saturation properties were used to follow the lines for each radical in the three crystallographic planes.

Radical (I) gave strong, well-resolved spectra at some orientations, especially with the magnetic field near the c axis (see figure 2). In this region it was difficult to determine the line positions for the quartet and impossible for the doublet. Nevertheless, on the basis of the available data, the quartet resonance can be adequately characterized. Some speculations on the origin of the doublet are included.

6. The Quartet Resonance

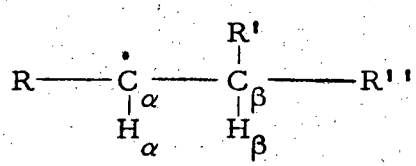
The two outer lines of the quartet could be followed at all magnetic field orientations, and the positions for the two inner lines could be determined for most of the orientations. The results show that the resonance is due to a radical in which the unpaired electron interacts with one α-hydrogen and one β-hydrogen. The β-hydrogen coupling is constant, within ± 1 gauss, for all crystal orientations at which the four lines of the quartet could be measured.

It is therefore assumed that the β-hydrogen has isotropic splitting of 20 gauss. On the basis of the overall quartet splitting and the isotropic β-hydrogen coupling, the α-hydrogen splitting can be determined. The results for the three crystallographic planes are given in figure 3. Although there are four molecules in the unit cell, they all appear identical with regard to the α-hydrogen splitting in the bc and ca planes. In the ab plane, there are two magnetically distinct molecules with regard to this parameter. From the data in figure 3, the principal values for the α-hydrogen coupling can be determined. These are given in table I, along with the direction cosines relating the principal hyperfine axes to the crystallographic abc coordinate system.

For a molecule as complex as thymidine, it would appear, at first, extremely difficult to identify the free radical giving rise to the quartet resonance. There are many different radicals that could be formed which would have an α-hydrogen and a β-hydrogen. These different radicals, however, in general have different principal axis directions for the α-hydrogen coupling. On the basis of the atomic coordinates for the thymidine molecule within the crystal (Young et al. 1969), it is possible to calculate the expected directions for the α-hydrogen coupling for each possible radical. These expected directions can then be compared with the observed ones from Table I. It should be mentioned that this comparison is made under the assumption that the radiation-induced radical has the same orientation as the parent molecule within the crystal. In most ESR studies of irradiated single crystals this has been found to be the case.

Figure 4 shows the thymidine molecule and six different radicals that have been considered as the origin of the quartet resonance. These radicals all have one α-hydrogen and at least one β-hydrogen. Radicals with more

than one β-hydrogen were not rejected as possibilities because the coupling to one of them can be almost zero if it lies in the nodal plane of the p-orbital containing the unpaired electron. For each of these radicals, the general structure is



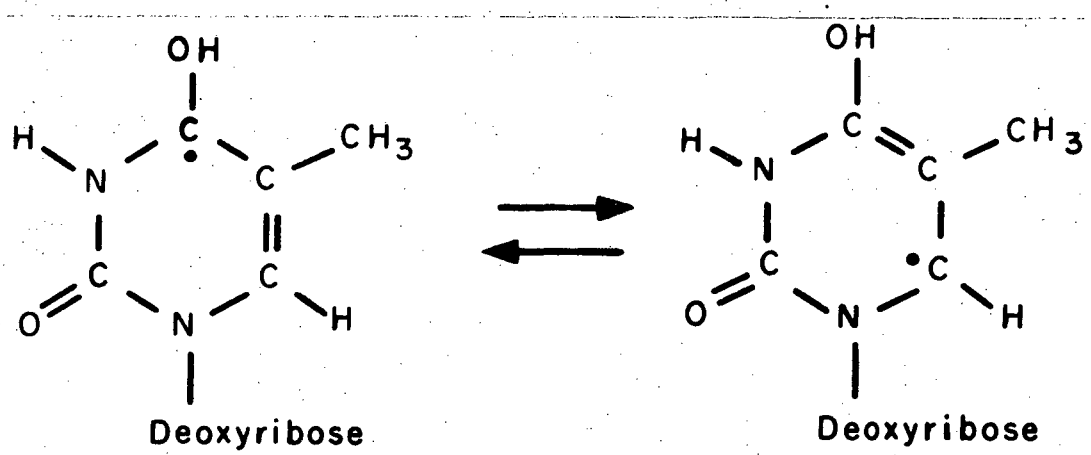
where R, R', R'' are presumed to be groups with no detectable hyperfine coupling. The minimum hyperfine coupling (A_x) for an α-hydrogen is expected to occur with the magnetic field along the C_α — H_α bond (McConnell and Strathdee 1959), and the intermediate coupling (A_z) should occur in the p-orbital direction. In this calculation the C_a — H_α bond direction was taken as the bisector of the R — C_α — C_β angle, and the p-orbital direction as being perpendicular to the plane formed by R, C_α, and C_β. For each of the proposed radicals, the angle between the C_α — H_α bond and the principal axis for A_x, as well as the angle between the p-orbital direction and the principal axis for A_z, was calculated. The results are given in table II. If the angles are large the agreement is poor, and vice versa. It can be seen from this table that the only radical that has expected directions in agreement with those observed is the 5,6-dihydrothymidin-6-yl radical (III). On this basis we conclude that, in the absence of drastic molecular rearrangements, radical (III) (see figure 4) is responsible for the quartet resonance in thymidine irradiated at 77°K.

7. The Doublet Resonance

Strong absorptions from radical (I) in the region where the doublet resonance occurred made it difficult to follow the doublet spectral lines at many orientations. Nevertheless, the data were sufficient to show that the coupling was

anisotropic, with crystal symmetry properties similar to those of radical (III). This suggests that the radical giving rise to the doublet has an unpaired electron with an α-hydrogen coupling and that it is located on the thymine ring rather than on the sugar part of the molecule.

In irradiated single crystals of thymine monohydrate we have recently observed a similar doublet resonance and were able to measure its spectral parameters (Henriksen and Snipes 1970). In that case, the unpaired spin density was shown to reside largely on C(6) of the thymine ring. It was proposed that the radical was formed by an addition of a hydrogen atom to the oxygen bonded to C(4), with subsequent π-orbital resonance producing unpaired spin density at C(6). By analogy, we suggest that the doublet resonance in thymidine irradiated at 77°K is due to a radical of the form



This assignment is consistent with our results, but is not unambiguous, due to the limited amount of data obtainable for the doublet resonance.

In thymine monohydrate, the oxygen atom bonded to C(4) is hydrogen bonded to a water molecule (Gerdil 1961). It was therefore suggested (Henriksen and Snipes 1970) that the radical giving rise to the doublet resonance is formed by electron capture with subsequent proton transfer in the hydrogen bond. Thus, by electron capture the two minima in the potential curve for the

hydrogen bond may be reversed and a neutral radical formed following proton tunneling. In thymidine, the oxygen bonded to C(4) is also hydrogen bonded (Tollin *et al.* 1969). This fact suggests that a similar mechanism operates also in thymidine.

References

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Table I. Principal values for the α-proton hyperfine coupling and the direction cosines relating the corresponding principal axes to the a b c crystallographic axis system.[a]

Principal splitting values (gauss)	Direction cosines		
	l	m	n
$A_x = 14.2$	0	0	1
$A_y = 37.5$	0.71	0.71	0
$A_z = 21.5$	-0.71	0.71	0
$A_\beta = 20$		Isotropic	

(a) Direction cosines for the remaining three molecules in the unit cell are given by (l, -m, -n), (-l, -m, n), (-l, m, -n).

Table II. Deviations of the principal axes for the α-hydrogen splitting from the directions predicted for the different radicals in figure 4.

Principal splitting axis	III	IV	V	VI	VII	VIII
A_x (minimum) [a]	15	73	64	53	30	17
A_z (intermediate) [b]	0	80	86	50	55	75

(a) The minimum splitting constant is along the C — H bond in the >C — H fragment (McConnell and Strathdee 1959). In this calculation it is assumed that this direction is the bisector of the two other bonds in the >•C — H fragment. The direction for this bisector has been calculated from the undamaged molecule based on the data given by Young et al. (1969). The deviations, which are given in deg, are taken from the scalar product of the direction for A_x (given in table I) and the bisectors for the different radicals in figure 4.

(b) The intermediate splitting constant is along the p-orbital containing the unpaired electron. This direction is perpendicular to the plane containing the >•C — H fragment.

FIGURE LEGENDS

Figure 1. Second-derivative ESR spectra of a thymidine single crystal irradiated and observed at 77^0K. The external magnetic field is along the crystallographic a axis. Arrows indicate the outer components of the 5,6-dihydrothymidin-5-yl radical. The bars beneath the upper spectrum represent the absorption lines for the quartet resonance (solid bars) and the doublet (dashed bars). Relative power levels for the two spectra are indicated. Observation frequency 8997 MHz.

Figure 2. Second-derivative spectra for three crystal orientations in the bc plane, showing the different orientations. Observation frequency 8984 MHz.

Figure 3. The splitting (in gauss) for the α-hydrogen of the quartet resonance. The data were calculated from the overall quartet splitting, assuming an isotropic coupling of 20 gauss for the β-hydrogen.

Figure 4. The molecular structure of thymidine (II) and six radicals considered for the origin of the quartet resonance.

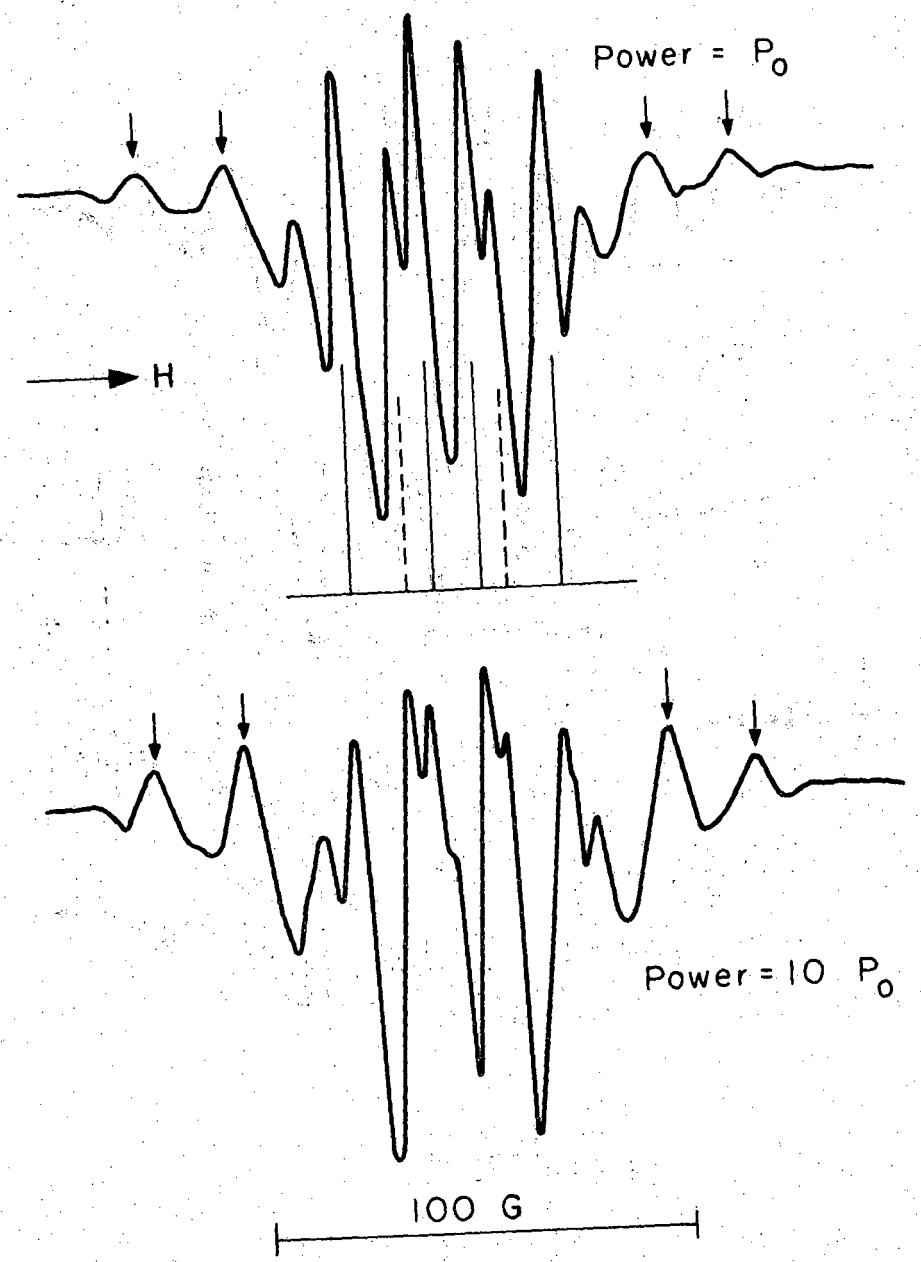

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Fig. 1

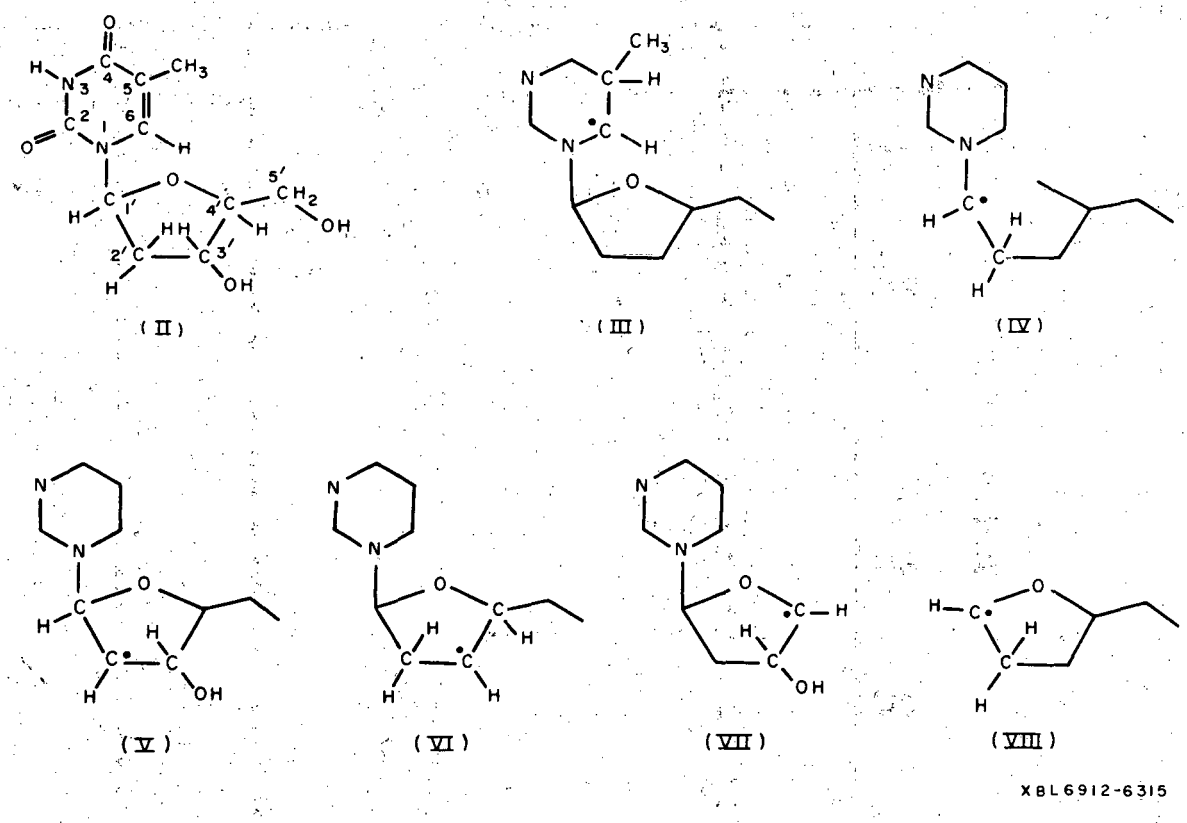

Fig. 4

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