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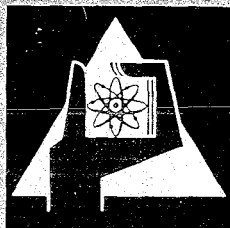
Juli 1969

KFK 1041

Institut für Strahlenbiologie

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5-Bromouracil, 5-Bromodeoxyuridine and 5-Iododeoxyuridine

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(Received 4 December 1968; revision received 28 January 1969)

Electron-spin-resonance spectra of x - or γ -irradiated single crystals of 5-bromouracil (BU), 5-bromodeoxyuridine (BUDR) and 5-iododeoxyuridine (IUDR) were recorded at room temperature as a function of crystal orientation in the magnetic field at 35 GHz (Q-band) and, less extensively, at 10 GHz (X-band). The observed positions of hyperfine lines were fitted to theoretical curves by a computer programme. The results clearly show the existence of two distinct groups of lines, A and B. While the g -factor of group A is close to the free electron value, group B is shifted to lower magnetic fields and is further subject to large variations with changing orientation, both in position and intensity of hyperfine lines.

Both groups are correlated to radicals at the pyrimidine group. It is proposed that one of these radicals is formed by hydrogen addition at the oxygen atom bound to C₄. Two resonance structures are possible, differing in localization of the unpaired spin density at either C₄ or C₆. The observed hyperfine splittings of group A fit completely into this scheme for all three substances investigated, if the unpaired spin is assumed to reside mainly at C₆ in IUDR and at C₄ in BU and BUDR.

Two different radicals are proposed to account for group B having in common the main unpaired spin density at C₅. The radical structure assigned to BU may be formed by abstraction of hydrogen from N₁. It is characterized by two resonance structures with unpaired spin densities at N₁ and C₅ linked by a conjugated double bond. In BUDR and IUDR, the radical representing group B is produced by addition of a hydrogen atom at C₆ resulting in two β -protons at this position.

1. Introduction

Studies of radical production in single crystals of nucleic acid constituents by electron-spin-resonance spectrometry may provide valuable information about primary processes of biological radiation effects. This kind of work is making rapid progress, but has so far been restricted to compounds containing light atoms only. We have extended our investigations to halogenated uracil derivatives for two reasons, one physical and one biological: first, it appeared to be of great interest to study the interactions of nearly free organic radical electrons with nuclear magnetic and quadrupole moments, since very little work has been done in this field. Second, 5-bromouracil is known to increase the biological radiosensitivity, if it replaces the normal constituent thymine in deoxyribonucleic acid. Information on radiation-induced radicals may contribute to an understanding of this radiosensitizing effect.

2. Experimental

2.1. Crystallization and crystal data

Of all three compounds, 5-bromouracil (BU), 5-bromodeoxyuridine (BUDR), and 5-iododeoxyuridine (IUDR), single crystals were grown by slow evaporation of saturated aqueous solutions at room temperature. Compounds of the highest available purity were purchased from various sources and were dissolved without further preparation.

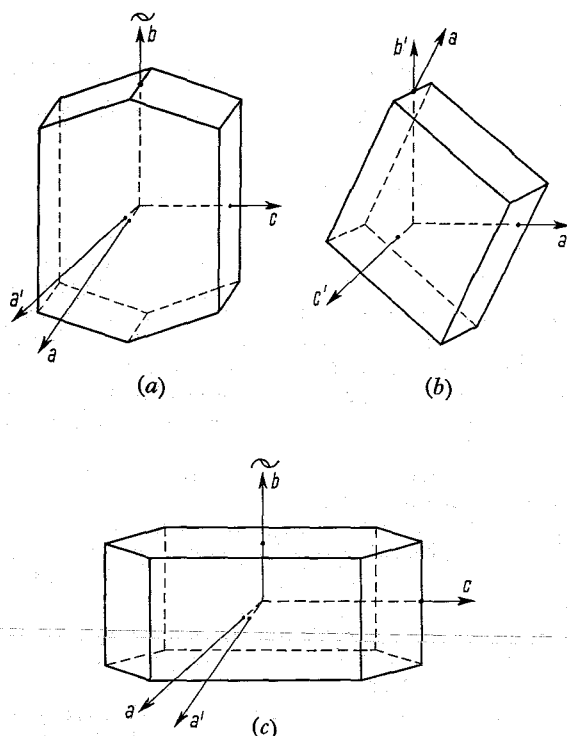


Figure 1. External habit of crystals of (a) BUDR (top left), (b) IUDR (top right), and (c) BU (bottom) showing crystal axes a , b , c and set of E.S.R. reference axes a' , b' , c' employed.

Crystals of BUDR were obtained as flat, colourless platelets, approximately $20 \times 8 \times 2$ mm in size. By x-ray diffraction, they were shown to belong to the monoclinic space group $P2_1$ having two molecules in the unit cell with the dimensions: $a = 9.09$ Å, $b = 5.12$ Å, $c = 12.02$ Å. These data agree exceedingly well with those of Iball, Morgan and Wilson (1966), who determined the crystal-line and molecular structures of BUDR. Crystals grown from D_2O had the same shape as those obtained from H_2O . The external habit of BUDR crystals with axes a , b and c is shown in figure 1 (a), together with the reference system of axes employed in E.S.R. experiments.

Crystals of IUDR were grown as flat, colourless platelets measuring about $6 \times 4 \times 2$ mm. X-ray diffraction gave, within experimental error, the same data as were reported by Camerman and Trotter (1965). The crystals are of the triclinic space group $P1$ and contain one molecule per unit cell. Figure 1 (b) shows the external configuration of IUDR crystals together with the E.S.R. axes. IUDR, too, was grown from D_2O solutions as well.

No data have appeared, to our knowledge, on single crystals of BU. This substance was crystallized in the form of prisms of about $4 \times 2 \times 1$ mm size. These crystals were determined to be of monoclinic space group $C2/m$ by x-ray diffraction. The external habit of BU crystals and the E.S.R. reference system are indicated in figure 1 (c).

2.2. Irradiation and E.S.R.-measurements

The crystals were irradiated in air at room temperature either by 100 kV x-rays or by ^{60}Co γ -rays from a 16 kCi source. Essentially the same E.S.R. spectra were obtained with both procedures. All types of crystals on irradiation developed a pale brown colour that grew more intense with increasing doses.

E.S.R. spectra were obtained at room temperature as first derivatives of absorption using commercial spectrometers (AEG, Hilger & Watts). Most measurements were carried out at 35 GHz (Q-band) but some were done at 10 GHz (X-band), mainly for comparisons. Proton resonance was used for measuring magnetic field strength and DPPH for calibrating g -factors.

Spectral data were evaluated following the methods described by Schonland (1959) and Lund and Vännegård (1965). A programme was written for an IBM 7074 computer to determine the parameters giving best fit of experimental data to theoretical curves. Using these values, tensors of g -factor and hyperfine splitting were constructed and diagonalized numerically to obtain the principal values of the tensors and the direction cosines relating the E.S.R. axes to the principal axes of the radicals.

3. Results and discussion

3.1. General remarks

The E.S.R. spectra of all three compounds have in common some unusual properties. Most conspicuously, the presence of two different sets of hyperfine

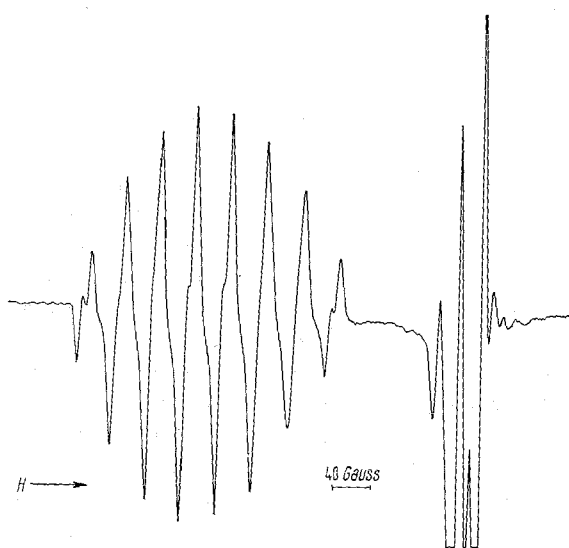


Figure 2. E.S.R. spectrum of IUDR crystal irradiated with 5 Mrads oriented with H parallel to the CI bond showing group A (right) and group B (left) of hyperfine lines.

lines is visible as is demonstrated in figure 2 for a crystal of IUDR oriented to give maximum separation between the two groups of lines designated A and B. Group A is located at the right in figure 2 (high field). Its g -factor is near the free electron value and therefore is characteristic of light atom interaction.

In contrast, the g -factor of group B is much higher on the average, shifting it to the low-field side, but is also subject to a large orientation dependence, as is shown in figure 5. Hence, some lines of this group even appear at the high-field side of group A at certain orientations. This feature can only be due to strong interaction between the radical electron and the halogen atom that gives rise to spin-orbit coupling. In addition to the g -factor, intensities and splittings of group B lines are strongly dependent on crystal orientation, too. Similar effects have been reported for irradiated crystals of chloroacetic acid (Pooley and Whiffen 1962) and dichloroacetamide (Kashiwagi 1966). These were recognized to be due to quadrupole interaction of the chlorine nucleus with the radical electron. Since bromine and iodine have much larger quadrupole moments than chlorine, quadrupole effects are to be expected in the lines of group B leading to the complex spectral features discussed below.

Since identification of individual lines is strongly impeded by overlap, we attempted to discriminate between the two groups by physical means. It was found that the relative intensities of groups A and B varied with irradiation dose and with storage time after several months. For BU, group A decreases with growing doses in accord with earlier quantitative results of Köhnlein (1963) on powdered material. Crystals of BUDR and IUDR exhibit a reversed change. At high doses and after storage group A is much more prominent than group B.

In view of the close correspondence between E.S.R. spectra of BU, BUDR and IUDR, the results for these compounds will be discussed in common, distinguishing between groups A and B only. Whereas the same radical is correlated to group A of all three substances used, group B is assigned to two different radicals, one of which is formed in BU and the other one in the nucleosides BUDR and IUDR.

3.2. The lines of group A (BU, BUDR and IUDR)

For BUDR and IUDR, group A represents at least two different radicals, one of them showing essentially a doublet-type spectrum. At some crystal orientations, the presence of additional less prominent lines overlapping the doublet is revealed. The radical structure giving rise to the former has not been identified. In most crystal orientations, however, the doublet was prominent. By fitting of the experimental g -factor and hyperfine splitting to theoretical curves, it was possible to identify the doublet lines even when there was interference from additional lines. For IUDR this was serious only in the $a'c'$ -plane, whereas for BUDR the doublet could easily be found in nearly all orientations.

BU crystals show a similar doublet-type spectrum, which predominates at low doses. Due to the relatively small size of the crystals, a satisfactory signal-to-noise ratio was obtained only after very high radiation doses. Consequently, only group B lines were visible and, therefore, a complete evaluation of the BU doublet has not been possible. Nevertheless, it could be followed in one crystal plane, and the data obtained will be considered.

The variations of the g -factor and the hyperfine splitting of the IUDR doublet in the three crystal planes are shown in figure 3. The corresponding

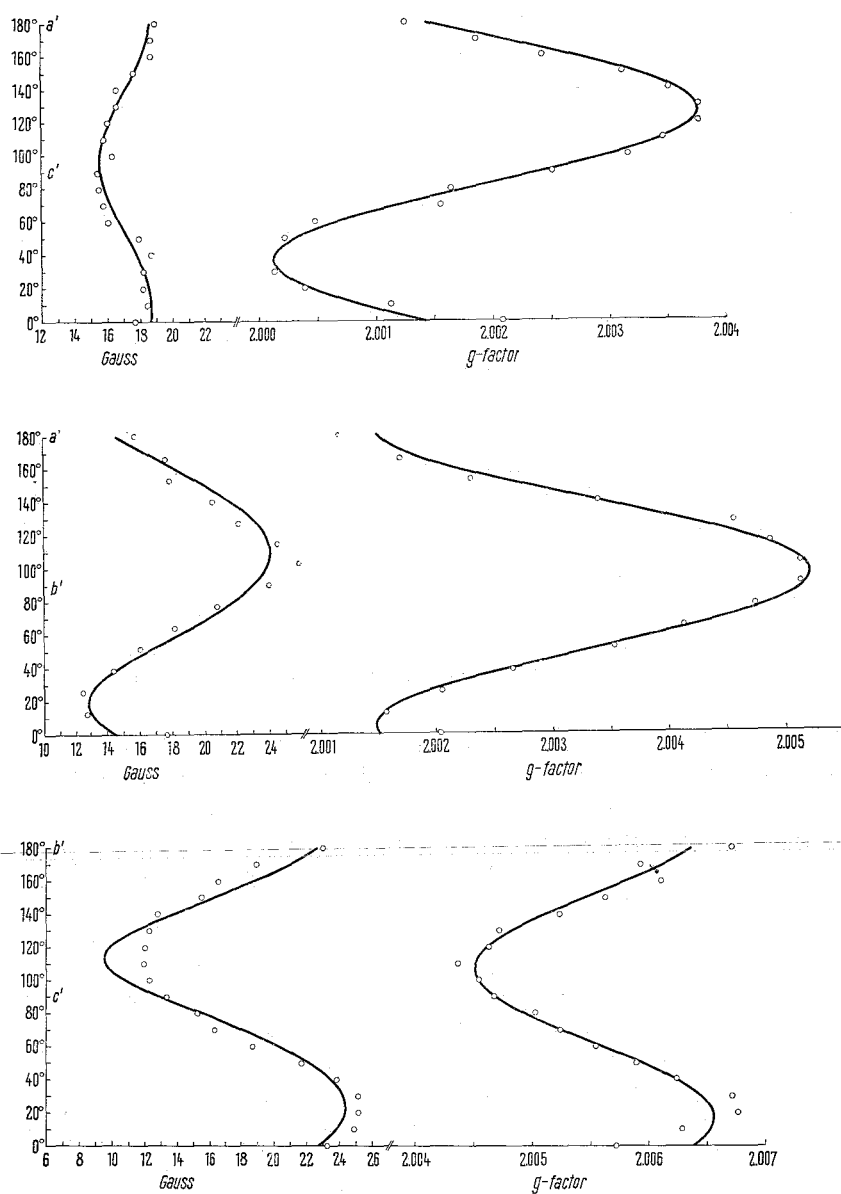


Figure 3. Angular variation of hyperfine splitting in Gauss and of g -factor of the IUDR doublet in three crystal planes formed by the pairs of reference axes, position of which is indicated at the ordinates.

principal values of the hyperfine tensor and the g -tensor are listed in table 1 together with the direction cosines. The elements of the g -tensor are characteristic of a carbon-centred π -electron radical, whereas the hyperfine splitting must be assigned to interaction of the unpaired electron with an α -hydrogen atom. The sign of the splitting cannot be inferred from the spectra but, as usual, is taken to be negative for theoretical reasons (McConnell and Chestnut 1958). The isotropic component of the hyperfine splitting a_{H} due to the

α -proton may be used to calculate the spin density ρ_C on the central carbon atom from the relation of McConnell and Chesnut (1958).

$$a_H = Q \cdot \rho_C$$

where Q is taken to be -28 G. With $a_H = 17.5$ G we arrive at:

$$\rho_C = 0.63.$$

Deuterated IUDR crystals show the same doublet. Therefore, the α -proton must be bound to a carbon atom, since it is not exchanged by deuterium.

	Principal values	Isotropic component	Direction cosines		
			b'	c'	a'
H (α)	(-) 25.48 (-) 12.82 (-) 14.16	(-) 17.49	0.88 -0.42 0.23	0.41 0.91 0.12	-0.26 ≈ 0 0.96
g	2.00604 2.00405 2.00067		0.93 -0.36 ≈ 0	0.29 0.75 0.60	0.21 -0.56 0.80

Table 1. Principal values of hyperfine-coupling, in Gauss, and g -factor for IUDR doublet.

For BUDR crystals, the variations of the g -factor and the hyperfine splitting in the three crystal planes are shown in figure 4. Compared with IUDR, the g -factor is slightly higher but still characteristic of a carbon-centred radical. The most significant difference between BUDR and IUDR, however, is seen in the hyperfine splitting. For BUDR, this is not typical for a CH_α group but rather for a COH group. This assumption fits in with the slightly increased g -factor. Deuterated BUDR crystals show similar spectra in analogous orientations, indicating that the hydrogen in the assumed COH-group is not exchangeable and therefore must originate from a CH bond broken by irradiation.

The principal values of the g -tensor and the hyperfine tensor for the BUDR doublet are given in table 2 together with the related direction cosines. For COH fragments, the isotropic hyperfine splitting a_{OH} of the hydroxyl-proton is assumed to obey a relation similar to that found for CCH groups (Henn and Whiffen 1964):

$$a_{\text{OH}} = (B_0 + B_2 \cos^2 \theta) \rho_C$$

with constants $B_0 = -3.2$ G and $B_2 \approx 30$ G. θ is the dihedral angle of C-O-H. With $a_{\text{OH}} = 4.97$ G and $\theta \approx 60^\circ$, as reported for a hydroxy-radical in 5-nitro-6-methyluracil (Snipes and Benson 1968), we obtain:

$$\rho_C = 0.45.$$

Crystals of BU displayed a doublet analogous to that of BUDR. Data could only be obtained in the ab -plane of the crystal and, therefore, tensors cannot be given. Still, the doublet splitting in this case is again typical for a COH group, the maximum observed being about 7 G compared with 8 G in BUDR. Thus, it is assumed that both doublets represent the same type of interaction, implying that this radical resides on the pyrimidine group. For BUDR this conclusion

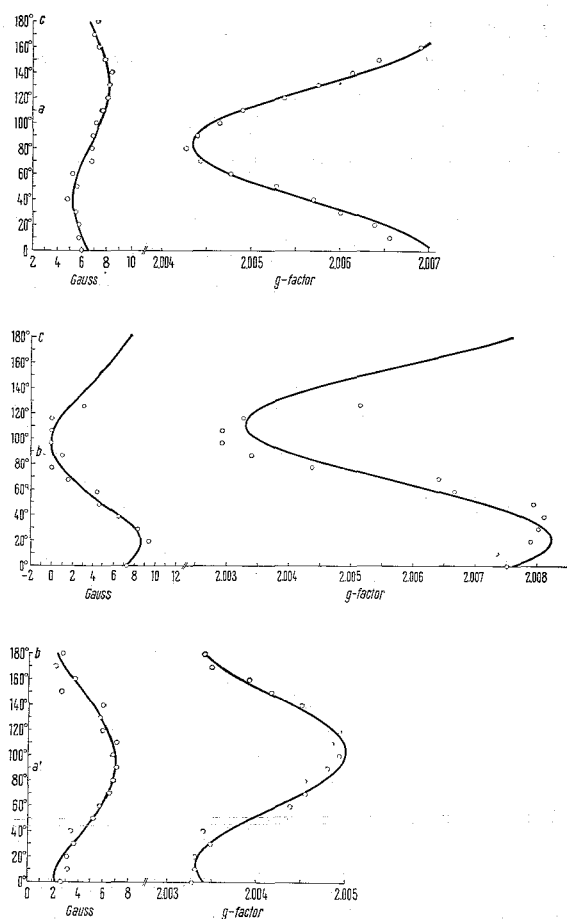


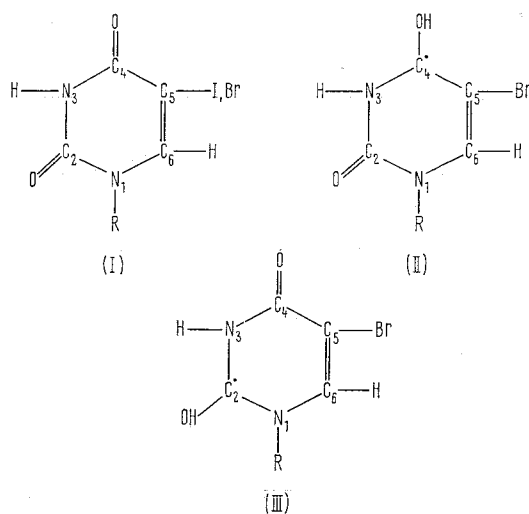
Figure 4. Angular variation of hyperfine splitting in Gauss and of g -factor for the BUDR doublet in crystal planes formed by the pairs of reference axes, position of which is indicated at the ordinates.

	Principal values	Isotropic component	Direction cosines		
			c	b	a'
H (OH)	5.47 0.91 8.53	4.97	0.04 0.99 0.11	0.84 0.02 -0.54	0.53 -0.12 0.83
g	2.00382 2.00761 2.00417		0.08 0.77 -0.62	0.99 -0.12 -0.02	0.09 0.62 0.78

Table 2: Principal values of hyperfine-coupling, in Gauss, and g -factor for BUDR doublet.

is checked by comparison of the E.S.R. data with the orientation of molecules in the crystals determined by Iball *et al.* (1966). The pyrimidine ring meets the crystallographic *ac*-plane under approximately 20° which means that minimum *g*-factors should be obtained in the *ab*- and *bc*-planes whenever H is forming such an angle with the *b*-axis. Figure 4 shows that this check holds perfectly. A comparison of E.S.R. parameters for the hyperfine splitting of the hydroxyl-proton with crystal data is not possible, since the positions of adjacent protons are unknown.

The molecular structure has only been determined for crystals of BUDR and IUDR where the pyrimidine is in the diketo-form of scheme (I). This tautomeric form is also assumed to be present in BU in analogy to uracil (Parry



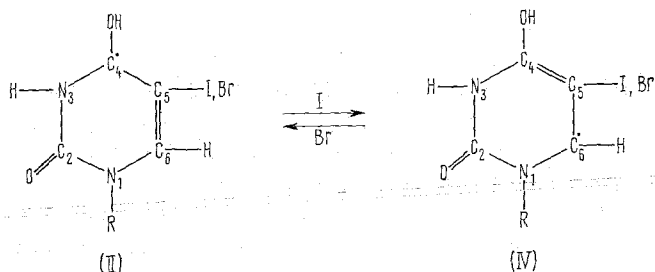
1954). Hydroxy-radicals accounting for the doublet lines of BU and BUDR can readily be generated by hydrogen-addition to the carbonyl-oxygen at C_2 or C_4 accompanied by electronic rearrangement resulting in the radicals of schemes (II) and (III), respectively. A similar reaction has been proposed previously for cytosine (Dertinger 1967). From the E.S.R. results the two radicals cannot be distinguished. Scheme (II) is preferred, however, since it permits a reasonable resonance structure to be drawn, allowing delocalization of spin-density. These features will be discussed in further detail in conjunction with the IUDR radical.

Finding the radical structure for the CH_α doublet of irradiated IUDR is somewhat more difficult. It should also reside on the pyrimidine group, not only due to the close similarity of this compound with BUDR, but also because the deoxyribose group contains too many hydrogen atoms to permit a conformation with only one interacting α -hydrogen. These assumptions could be checked by the crystal data, provided that drastic reorientations do not occur on radical formation. The minimum principal value of the *g*-factor is expected parallel to the π -orbital of carbon-centred CH_α groups. If this carbon atom belongs to the pyrimidine ring, the π -orbital is normal to the ring plane. Figure 3 shows that the minimum *g*-factor is obtained in the $a'c'$ -plane, about 40° away from a' -axis. Since the pyrimidine ring meets the crystallographic *bc*-plane

under about 45° , the plane normal is parallel to the minimum g -factor within experimental limits of error, as may also be inferred from the direction cosines of table 1 for the minimum g -factor.

Although accurate positions of protons at C_6 and N_3 were not determined by x-ray crystallography, the symmetry of the pyrimidine ring structure permits the assumption that they are directed along the diagonal connecting N_3 and C_6 . This orientation is almost parallel to the c -axis of the crystal. Both figure 3 and the cosines given in table 1 show that the minimum hyperfine splitting is found along this direction. Since nitrogen splitting is not seen in the spectra, the interacting α -hydrogen must reside at C_6 .

The arguments advanced clearly show that the IUDR doublet is due to a base radical localized at C_6 containing one interacting α -proton. This conclusion implies that no atomic, but simply electronic, rearrangements have occurred at these atoms during radical formation. A quite similar case has been reported for irradiated 5-nitro-6-methyluracil (Snipes and Benson 1968). Adopting the model proposed by Snipes and Benson, we are able to account for the BU and BUDR doublets as well as for the IUDR doublet by the same proportion of radical formation shown in scheme (II). As a consequence of electronic rearrangement, an equilibrium between two different structures exists, depending mainly on the halogen substituent at C_5 as shown below:



The model proposed is very attractive, since it assigns similar radical structures to closely related compounds. Furthermore the remaining spin densities of 0.35 for BUDR and of about 0.3 for IUDR are easily understood by the resonance structures. This implies that, e.g. the IUDR spectra should exhibit an additional strongly anisotropic hyperfine interaction due to the hydroxyl-proton at C_4 . This may, in fact, be seen in some IUDR spectra where the line widths of the CH_α doublet are sufficiently small to allow observation of a small splitting of about 5 G maximum appearing as rather anisotropic. On the other hand, additional hyperfine structure due to the CH_α group at C_6 has not been detected in the spectra of BU and BUDR.

3.3. The lines of group B (BUDR and IUDR)

Generally, group B of E.S.R. spectra shows a very complex pattern and not all of its lines can be analysed in a satisfactory way, owing to the large quadrupole interactions of bromine and iodine. For BUDR, interpretation of spectra is even more involved than for IUDR for three reasons: (1) Bromine contains two isotopes in about equal abundances that differ in nuclear magnetic moments and, hence, also in interaction energies with radical electrons. (2) Although the two molecules of BUDR in a unit cell of monoclinic crystals appear to be

arranged in equivalent positions, site splitting was observed for the lines of group B in BUDR which suggests that the planar structure of the pyrimidine ring is puckered on radical formation. Such an effect is not unlikely, since even very slight deviations from planarity may result in large site splitting owing to the large magnetic moment of the bromine nuclei. (3) The magnetic moment of iodine considerably exceeds that of bromine and thus gives a better separation of groups A and B in most orientations of IUDR crystals compared with BUDR.

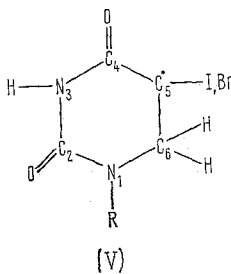
These features explain why IUDR is much more amenable to interpretation of group B and we shall therefore start the discussion with this compound returning to the spectrum of figure 2. In this particular orientation group B consists of eight lines of about equal spacing with an approximate intensity ratio of 1 : 3 : 4 : 4 : 4 : 4 : 3 : 1. The spectrum is obtained when H is approximately parallel to the CI bond of the IUDR molecule as has been established by comparison with the data of Camerman and Trotter (1965). If a CI fragment is treated in analogy to a CH group, which can only be true very roughly, the minimum iodine hyperfine splitting should be observed if the magnetic field has the same direction. The minimum splitting is seen in the $a'b'$ -plane at exactly the same orientation which yields the spectrum of figure 2, i.e. when H is parallel to the CI bond.

The spin Hamiltonian accounting for the halogen nucleus neglecting nuclear Zeeman-terms may be written as:

$$\mathbf{S} \cdot \mathbf{T} \cdot \mathbf{I} + \mathbf{I} \cdot \mathbf{P} \cdot \mathbf{I},$$

where the tensor T represents the magnetic interaction of the radical electron with the halogen nucleus, and the tensor P the nuclear quadrupole interaction.

Obviously, the CI bond of IUDR is a principal axis of the quadrupole tensor P , which is taken to be traceless and of cylindrical symmetry. Since this direction coincides with a principal axis of the hyperfine tensor T , the spectrum of figure 2 may be treated to first order, assuming the selection rule $\Delta M_I = 0$. On this basis the observed hyperfine structure is attributed to interaction of the radical electron with the iodine nucleus of spin 5/2, each iodine line being further split into a 1 : 2 : 1 triplet by two β -protons with separations equal to the iodine coupling. This interpretation implies that the radical accounting for group B is formed by hydrogen addition to C_6 and simultaneous reshuffling of the $C=C$ double bond according to scheme (V) in complete analogy to radical production in thymidine (Pruden, Snipes and Gordy 1965).



The hyperfine splitting of the two methylene β -protons at C_6 should very nearly be isotropic. Figure 2 shows that its value is about 40 G, in good

agreement with the methylene coupling observed with the thymidine radical. It is therefore concluded that the spin-density ρ_C at C_5 of radical (V) also is:

$$\rho_C \simeq 0.7.$$

An evaluation of the spin density by means of the iodine coupling is not a very straightforward procedure, since (1) its tensor elements are not easily obtained from the spectra, and (2) the validity of McConnell's relation has not been established for a CI group. On the contrary, as was stated explicitly by Pooley and Whiffen (1962), a CCl fragment cannot be treated in the same manner as a CH group, especially with respect to the sign of the isotropic hyperfine splitting of chlorine, which apparently is positive owing to dipole-dipole contributions of excited states to the ground state of the radical. Nevertheless, we shall try to give a rough estimate of the components of the iodine hyperfine tensor T^I assuming the β -proton couplings to be exactly isotropic. Then, the total hyperfine splitting of group B is a measure of the iodine interaction, assuming implicitly the selection rule $\Delta M_I = 0$ and equal spacing between individual lines. The principal T^I value that is parallel to the CI bond, is obtained from the spectrum in figure 2 as:

$$T_1^I = 40 \text{ G.}$$

This value is the only reliable one since, in this direction, quadrupole moments constitute a small perturbation only. The maximum value should be found along the π -orbital of the radical, i.e. along the normal of the pyrimidine ring. This is indeed observed as the direction of maximum total hyperfine splitting. The T_2^I -value thus obtained is:

$$T_2^I \simeq 80 \text{ G.}$$

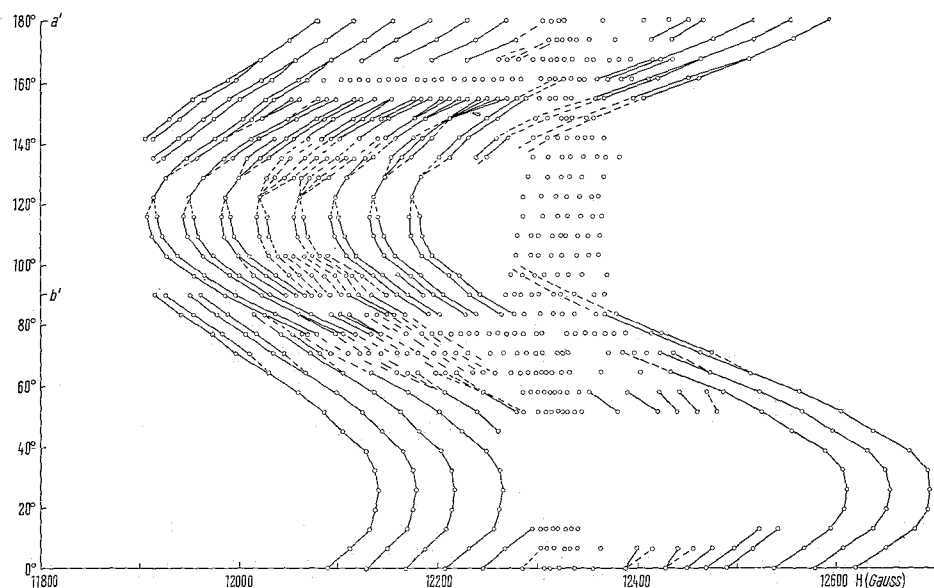


Figure 5. Total gamut of hyperfine lines obtained from IUDR in the $a'b'$ -plane. Lines of group B have been linked by solid or, if ambiguous, dotted lines.

The intermediate value, evaluated from a direction normal to those of T_1^I and T_2^I is approximately:

$$T_3^I = 60 \text{ G.}$$

The treatment just applied to the hyperfine splitting of iodine is a rather crude one since even an equal spacing of the iodine lines is not found for all orientations. These deviations are due to quadrupole interaction of the radical electron with the halogen nucleus which obviously is comparable to the hyperfine term $\mathbf{S} \cdot \mathbf{T} \cdot \mathbf{I}$. A rough estimate of the quadrupole coupling may be given using the weak extra lines appearing at small angular deviations from the direction of the CI bond (Pooley and Whiffen 1962). Figure 5 shows that these lines appear about 15° off the CI bond direction. The spacing of the weak transitions is of about the same magnitude as the iodine splitting at this orientation, i.e. about 45 G. This means that

$$3eqQ/4I(2I-1) \simeq 45 \text{ G.}$$

With an iodine nuclear spin of $I=5/2$, we arrive at a quadrupole coupling expressed in terms of eqQ :

$$eqQ = 1800 \text{ MHz.}$$

This value is in remarkably good agreement with that of 2000 MHz measured for iodine bound covalently (Slichter 1964).

To illustrate the complexity of IUDR spectra, all lines obtained in the $a'b'$ -plane are plotted in figure 5. This may be taken to demonstrate that exact assignments of iodine couplings, or of any other parameters of the hyperfine terms comprising the spin Hamiltonian, are necessarily crude and incomplete estimates at present. Nothing definite can be said, for example, about the equivalence of the methylene protons, though the authors believe that some inequivalence prevails owing to puckering of the pyrimidine ring on radical formation.

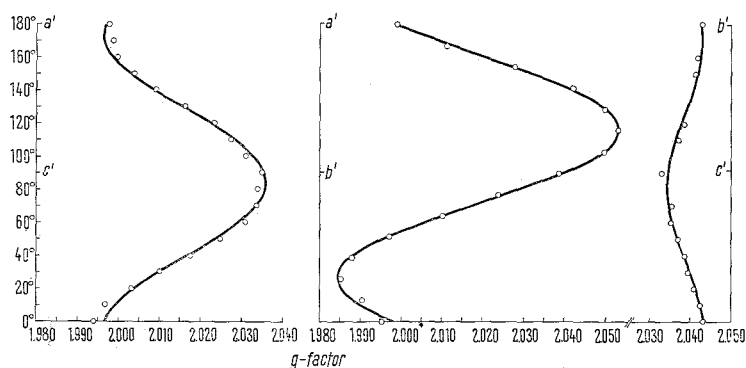


Figure 6. Angular variation of g -factor of group B of IUDR in three crystal planes.

Variations of the g -factor of radical (V) in IUDR are shown in figure 6 for three crystal planes. The experimental data fit the theoretical curves very well indeed, as may be expected at 12 kG, since at this high magnetic-field strength, field-independent terms of the spin Hamiltonian have negligible effect on the g -factor, which is seen to be strongly anisotropic due to large spin-orbit coupling. The principal values of the g -factor listed in table 3, together with the direction

cosines, are consistent with those expected for a radical localized on the base group of the IUDR molecule, as has been verified by comparison with crystallographic data.

	Principal values	Direction cosines		
		b'	c'	a'
g (IUDR)	1.99847	0.93	0.14	0.32
	2.03872	-0.04	0.94	-0.32
	2.08482	-0.35	0.29	0.88
g (BUDR)		c	b	a'
	2.00967	-0.06	0.99	≈ 0
	2.01072	0.01	0.05	0.99
	2.02292	0.99	0.06	≈ 0

Table 3. Principal values of g -factor for radical (V) in IUDR and BUDR.

The lines of group B obtained from BUDR crystals may be treated in full analogy to those of IUDR. The arrangement of molecules in the monoclinic crystals of BUDR used in this work precludes registration of E.S.R. spectra with CBr bonds of all molecules oriented parallel to the magnetic field as long as the principal axes of the crystal are used as E.S.R. axes. The minimum principal value of the hyperfine coupling tensor T^{Br} of bromine is obtained as:

$$T_1^{\text{Br}} \approx 33 \text{ G.}$$

An estimate of the remaining tensor elements and the quadrupole coupling will not be attempted for BUDR in view of the complicating conditions stated at the beginning of §3.3.

The principal values of the g -tensor listed in table 3 are slightly smaller than those of IUDR in accord with the ratio of spin-orbit coupling constants of bromine and iodine.

3.4. The lines of group B (BU)

In comparison with BUDR and IUDR, rather different properties were observed in the lines of group B of BU, which lead to the assumption that a different type of radical is formed in this compound. Four lines of equal intensity are representative of group B under these conditions. The observed splitting is attributed to the bromine nucleus.

When H is nearly parallel to the a -axis, the spectrum of figure 7 is obtained. The lines of group B are now centred at about the g -factor of the free electron, i.e. are superimposed on group A and consist of four groups of lines. The outermost groups show a well-separated 1:1:1 triplet of about 15 G spacing, whereas the inner two groups of lines appear to be strongly disturbed by second-order transitions. The only assumption that is consistent with the observed triplet is a nitrogen-centred radical. The additional quartet structure is again related to the hyperfine coupling of bromine. Spectra of both these particular orientations were also taken at 10 GHz, confirming that the features described result from hyperfine terms only.

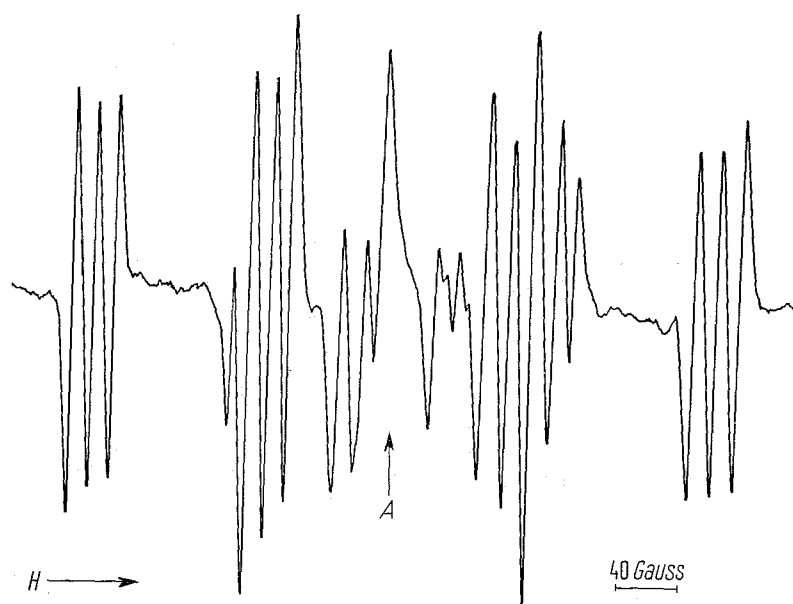
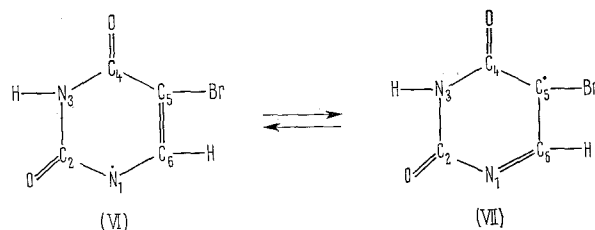


Figure 7. E.S.R. spectrum of BU crystal oriented with H parallel to the *a*-axis. Group B consists of four groups of lines the outer ones being split into triplets by nitrogen coupling.

A detailed analysis of group B lines from irradiated BU crystals will be the subject of a later publication. It is hoped that the molecular structure of this compound will be determined in the meantime. Nevertheless, the main conclusions to be drawn from our work are not affected and may be given here.



It is proposed that the radical under consideration is formed by hydrogen abstraction from the nitrogen atom N_1 of the pyrimidine group leaving an unpaired electron at this site. Via resonance the unpaired spin is delocalized over the pyrimidine ring, which may be visualized by the two resonance structures shown below. It is seen that in scheme (VII) the unpaired electron at C_5 strongly interacts with the bromine atom bound to this atom. Interaction with the hydrogen atom at C_6 is not observed, since it is lying in the pyrimidine plane at a right angle with respect to the orbital of the radical electron, and hence gives negligible splitting. A radical similar to that proposed here has previously been assigned to irradiated cytosine (Cook, Elliot and Wyard 1967).

4. Conclusions

Of the three different types of radicals concluded from the E.S.R. spectra of irradiated single crystals of BU, BUDR and IUDR two are assumed to be

generated by addition of atomic hydrogen. One site of attachment is the oxygen atom at C₄. The ensuing radical can be described by two resonance structures (schemes (II) and (IV)) the unpaired spin density residing at either C₄ or C₆. It is concluded that the former prevails in BU and BUDR, while the latter is assigned to IUDR. A second radical is produced by hydrogen addition to C₆ in BUDR and IUDR (scheme (V)).

The third radical, which is also represented by two resonance structures (schemes (VI) and (VII)), is found only in BU. This species is assumed to arise from hydrogen abstraction at N₁. Since in BU hydrogen addition and abstraction radicals are present in comparable concentrations at low doses, it may be concluded that the primary process of radical formation is removal of atomic hydrogen from N₁ which subsequently adds on to the CO group at C₄.

For the deoxyribosides BUDR and IUDR, such a simple balance cannot be made up, as all radicals proposed are produced by hydrogen addition. This situation, however, is not without precedent, since it also prevails for thymidine. If this substance is irradiated at room temperature the only type of radical found arises from hydrogen attachment (Pruden *et al.* 1965). It was later shown that these H-atoms originate from the deoxyribose group (Müller 1968). The question of why no deoxyribose radicals have been detected has not been answered so far. However, a recent study on radical formation by irradiation of single crystals of deoxyribose (Hüttermann and Müller 1969) may be used as a basis for the following tentative explanation for the preponderant occurrence of hydrogen addition radicals in the deoxyribosides studied and possibly other nucleic acid constituents.

The radicals observed in pure deoxyribose at room temperature are formed by removal of atomic hydrogen, followed by electronic rearrangement and shifting of at least one other proton between two neighbouring carbon atoms. In the presence of pyrimidine groups containing unsaturated bonds, the second proton may also be removed from the pentose as a hydrogen atom and react with a pyrimidine ring to give another hydrogen addition product. Thus, two H-atoms would be liberated per primary event of energy absorption in the deoxyribose, leaving this group in a non-radical state. This hypothesis should be open to experimental proof, e.g. by E.S.R. spectroscopy of irradiated single crystals at low temperatures.

Concerning the possible contribution of the present results to an understanding of the radiosensitizing action of BU substitution, these can only be regarded as a small part of a whole system of processes and thus would appear not to permit far-reaching conclusions. One statement to the contrary may be advanced, however. Under the conditions of the present experiments free halogen atoms definitely were absent in significant amounts after irradiation, since such species should easily be detected, if present. It cannot be excluded entirely that halogen atoms are generated but vanish rapidly by undergoing further reactions. The observed radical products do not lend support to this notion, though.

ACKNOWLEDGMENTS

We wish to thank Professor Dr. K. G. Zimmer for his continuous and encouraging interest in this work. Furthermore, we thank Drs. W. Schneider

and G. Weitz for x-ray diffraction measurements and Frau Gisela Kammerer for patient preparation of the manuscript.

Les spectres de résonance paramagnétique électronique de monocristaux de 5-bromouracile (BU), 5-bromodéoxyuridine (BUDR) et 5-iododéoxyuridine (IUDR) soumis à l'irradiation x ou γ , ont été enregistrés à température ambiante en fonction de l'orientation du cristal dans le champ magnétique à 35 GHz (bande Q) et, secondairement, à 10 GHz (bande X). Les positions observées des lignes hyperfines ont été ajustées avec les courbes théoriques à l'aide d'une calculatrice. Les résultats montrent clairement l'existence de deux groupes distincts de lignes, A et B. Alors que le facteur g du groupe A est voisin de la valeur de l'électron libre, le groupe B est déplacé vers des champs magnétiques inférieurs et, par suite, se trouve soumis à d'importantes variations selon l'orientation du cristal: variation de la position et variation de l'intensité des lignes hyperfines.

Aux deux groupes correspondent des radicaux dans le groupement pyrimidine. L'un de ces radicaux peut être formé par fixation d'hydrogène à l'atome d'oxygène lié au C_4 . Deux structures de résonance sont alors possibles, différant par la localisation de la densité du spin célibataire: en C_4 ou en C_6 . Les lignes hyperfines du groupe A trouvent parfaitement, pour les trois substances étudiées, leur explication dans ce schéma, si l'on suppose le spin célibataire localisé principalement en C_6 pour l'IUDR et en C_4 pour le BU et le BUDR.

Pour le groupe B on propose la formation de deux radicaux différents ayant pour point commun la plus grande densité de spin célibataire en C_5 . La structure radicalaire assignée au BU peut se former par abstraction de l'hydrogène du N-1. Elle se caractérise par deux structures en résonance, dont les densités de spin célibataire sont en N_1 et C_5 , liées par une double liaison conjuguée. Pour le BUDR et l'IUDR, le radical correspondant au groupe B se forme par addition d'un atome d'hydrogène en C_6 ; on a alors en position 6 deux protons β .

Die Elektronen-Spin-Resonanz-Spektren von röntgen- oder γ -bestrahlten Einkristallen von 5-Bromuracil (BU), 5-Bromdesoxyuridin (BUDR) und 5-Ioddesoxyuridin (IUDR) wurden bei Zimmertemperatur in Abhängigkeit von der Kristallorientierung im Magnetfeld bei 35 GHz (Q-Band) und, weniger häufig, bei 10 GHz (X-Band) aufgenommen. Die experimentell gefundenen Positionen der Hyperfein-Linien wurden durch Computer-Programme an theoretische Kurven angepaßt. Die Ergebnisse zeigen deutlich das Vorhandensein zweier verschiedener Liniengruppen A und B. Während der g -Faktor der Gruppe A nahe bei dem Wert der freien Elektronen liegt, ist die Gruppe B zu kleineren Magnetfeldern verschoben und zudem großen Veränderungen bezüglich der Lage und der Intensität der Hyperfein-Linien bei Veränderungen der Kristallorientierung unterworfen.

Beiden Gruppen werden Radikale in der Pyrimidin-Gruppe zugeordnet. Es wird vorgeschlagen, daß eines dieser Radikale durch Wasserstoffanlagerung an den an C_4 gebundenen Sauerstoff gebildet wird. Es sind dann zwei Resonanzstrukturen möglich, die sich in der Lokalisierung der Spindichte unterscheiden, die entweder an C_4 oder an C_6 konzentriert ist. Die beobachteten Hyperfein-Linien der Gruppe A aller drei untersuchter Substanzen passen völlig zu diesem Schema unter der Annahme, daß der ungepaarte Spin bei IUDR hauptsächlich an C_6 und bei BU und BUDR vor allem an C_4 lokalisiert ist.

Für die Gruppe B werden zwei verschiedene Radikale vorgeschlagen, denen die Konzentration des Hauptteils der ungepaarten Spindichte an C_5 gemeinsam ist. Das in BU gebildete Radikal kann durch Abstraktion des Wasserstoffs an N gebildet werden. Es wird durch zwei Resonanzstrukturen charakterisiert, die den ungepaarten Spin an N_1 und C_5 haben, welche durch eine konjugierte Doppelbindung miteinander verbunden sind. Das Radikal, welches der Gruppe B in BUDR und IUDR entspricht, wird durch Wasserstoffanlagerung an C_6 gebildet, wodurch dann an dieser Stelle zwei β -Protonen gebunden sind.

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