A Study of Radiation-induced Free Radicals in Thymine by Spin-unrestricted Molecular Orbital Calculations

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Two conflicting interpretations of the ESR spectrum of irradiated single crystals of thymine monohydrate have been elucidated by semi-empirical unrestricted Hartree-Fock calculations. Isotropic and anisotropic hyperfine splitting constants for the various radical species proposed have been obtained and compared with experimental values. The result of this comparison is thought to be clearly in favour of one particular of the two alternative interpretations.

The ESR spectrum due to radiation-induced free radicals in single crystals of thymine monohydrate was investigated independently by Henriksen and Snipes¹ and by Hüttermann.² At room temperature the spectrum was dominated by the 5-thymyl radical (radical (I) in Fig. 1) which gives rise to eight lines spread out over approximately 140 G. At 77° K both investigators found a spectrum of six lines superimposed on a very weak 5-thymyl radical pattern. However, their interpretations of the low-temperature spectrum did not agree. Whereas Henriksen and Snipes concluded that the six lines are due to two different radicals, one contributing a quartet and the other a doublet, Hüttermann attributed these lines to only one radical. In both cases specific suggestions were made as to the radical species involved. The 6-thymyl radical and the 2-oxo-4-hydroxy-5-methyl-4-pyrimidinyl radical [radicals (II) and (III) in Fig. 1] were proposed as the origin of the quartet and doublet, respectively. In the alternative interpretation the radical (IV) was made responsible for the sextet.

The main purpose of the present work is to elucidate this discrepancy by molecular orbital

calculations. Two different semi-empirical unrestricted Hartree-Fock (UHF) methods were applied to the radicals (I)–(IV) and to two additional radical candidates: the thymine anion [radical (V)] and the 2,4-dioxo-5-methyl-5-hydroxy-6-pyrimidinyl radical [radical (VI)]. Calculated values of the different elements of

Fig. 1. Labelling of molecules and numbering of atoms.

the hyperfine splitting tensors are compared with the corresponding experimental data. In addition to the attempt to find support for any of the above conclusions it is also of general importance to gain systematic experience with the predictive abilities of the simpler Hartree-Fock methods in cases like the present one.

METHODS OF CALCULATION

The INDO method of Pople and coworkers⁹ was applied to all the radicals and the isotropic as well as the anisotropic components of the principal splitting constants were calculated.

In the INDO method all valence electrons are included and accordingly both π -electron and σ -electron components of the spin density distribution of a π -electron radical are evaluated. Pople and coworkers have developed an explicit procedure for obtaining isotropic hyperfine splitting constants from INDO wave functions before⁴ and after⁵ annihilation of the contaminating quartet spin component. In the present work isotropic splitting constants are obtained by this procedure. Results before and after annihilation will be labelled *sd* (single determinant) and *aa*, respectively.

Beveridge and McIver⁶ have established a method for calculating the anisotropic components of the principal splitting constants from an INDO wave function. The present calculations followed their scheme by use of a computer program written by one of us (A.B.H.).

In addition to the INDO method the Pariser-Parr-Pople (PPP) method^{7,8} for π -electron calculations was used when possible. A combination⁹ of the UHF formalism with and without annihilation^{10,11} and a special scheme for evaluation of semi-empirical parameters in the PPP approximation¹²⁻¹⁷ was employed in these calculations.

In the PPP calculations only π -electron spin densities were obtained, but they could be related to isotropic proton splitting constants by means of the empirical relations

$$A_H = Q \rho_C^\pi \quad (1)$$

$$A_{H(CH_3)} = Q^0 \rho_C^\pi \quad (2)$$

applicable to α - and methyl proton splitting constants, respectively.

In eqn. (1), which is the McConnell relation¹⁸ in its simplest form, A_H is the splitting constant of the α -proton and ρ_C^π the spin density of the $2p\pi$ -orbital of the carbon atom to which the proton is bonded. A value of the proportionality factor Q in the range -20 to -30 G usually gives good agreement between experimental A_H values and theoretical estimates of ρ_C^π .

In equation (2) ρ_C^π is the π -electron spin density on the carbon atom to which the methyl group is attached. $A_{H(CH_3)}$ is the effective methyl proton splitting constant. Hence the formula is applicable only if the methyl group is rotating freely about the C-CH₃ bond. From the ESR spectra one may conclude that this has to be the case for all the radiation-induced thymine radicals.

The relation (2) was calibrated in the present work by a separate PPP calculation on the ethyl radical, leading to $Q^0 = 27.5$ G (π -electron spin density on CH₃: 0.97, observed splitting 26.87 G).

For each radical species one basic set of structural parameters was developed (Table 1) although additional calculations based on variation of geometry were also important in some cases. These basic geometries did all involve planar rings and roughly tetrahedral arrangements (bond angles $108-110^\circ$) around tetravalent carbon atoms. When possible bond lengths were obtained by repeated PPP calculations aiming at bond order-bond length selfconsistency.^{9,19} The results of this procedure are given to three decimal places in Table 1. In the case of radicals (II) and (VI) the values are based on PPP calculations on the open-chain system CHO-NH-CO-NH-CH₃.

Other bond lengths given in Table 1 are simply reasonable values, for instance the standard values recommended by Pople and Beveridge.²⁰ Most bond angles and also a few remaining bond lengths were chosen with some guidance from the results of a crystallographic investigation of thymine monohydrate.²¹ Thus the C5-C9 distance obtained the value 1.503 Å (Table 1) in the INDO calculations. In the PPP calculations the C5-methyl distance was set equal to 1.70 Å and the methylene group of radical (I) was treated as suggested by Flood and Skancke.¹⁷

Table 1. Structural parameters adopted for the basic calculations. The structure of radical (II) was also used for radical (VI) with the addition of: C5—O 1.43 Å, O—H 0.96 Å, $\angle$ COH 109°.

	Radical (I)	(II)	(III)	(V)	(IV)
Bond lengths (Å)					
N1—C6	1.46	1.396	1.402	1.420	1.394
C6—C5	1.52	1.50	1.398	1.418	1.400
C5—C4	1.444	1.50	1.400	1.400	1.463
C4—N3	1.376	1.363	1.402	1.405	1.372
N3—C2	1.385	1.383	1.376	1.360	1.377
C2—N1 ^a	1.353	1.383	1.368	1.376	1.376
C2—O7	1.249	1.244	1.248	1.262	1.245
C4—O8	1.243	1.229	1.384	1.274	1.239
C5—C9	1.503	1.503	1.503	1.503	1.405
C6—H11	1.09	1.08	1.08	1.08	1.08
other C—H	1.09	1.09	1.09	1.09	1.08
N—H	1.01	1.01	1.01	1.01	1.01
O—H			0.96		
Bond angles (degrees)					
N1—C6—C5	108	120	122	122	122
C6—C5—C4	123	110	117	117	118
C5—C4—N3	117	124	118	118	116
C4—N3—C2	126	125	126	126	126
N3—C2—O7	122	122	122	122	122
C6—C5—C9	123		123	123	123
C5—C4—O8	126	126	126	126	126
C5—C9—H	109.5	109.5	109.5	109.5	120
H11—C6—H12	109				
C2—N1—H10	120	120	120	120	120
C4—N3—H16	120	120	120	120	120
N1—C6—H11		120	120	120	120
C4—O8—H12			109		
C9—C5—H12		109			
C2—N1—C6 ^a	131.1	127.6	122.1	120.3	120.3

^a The N1—C2 bond length and the C2—N1—C6 bond angle are both dependent variables. Their values are determined by the values of the other structural parameters.

RESULTS

The principal splitting constants from the INDO calculations are collected in Table 2, separated in their isotropic and anisotropic components. Experimental values are also included for comparison. Before treating each radical species in more detail, we will make some general comments.

Hyperfine splitting constants are usually discussed in terms of their magnitudes only because the signs are normally not obtained experimentally. The signs of the observed data in Table 2 have their basis in the calculated results. However, whenever the value of a

splitting constant is discussed in this paper only the magnitude is considered.

A large part of the isotropic splitting constants obtained from the INDO wave functions has been drastically reduced after annihilation. To be specific this is the case for all the constants which come out with a negative sign in the calculation (constants corresponding to a negative spin density value). Among these are all the α -proton splitting constants but— with the exception of the methyl protons in radical (III)—none of the out-of-plane protons. As can be seen from Table 2 the values before annihilation are close to or lower than the experimental ones. In view of this and in view

Table 2. Isotropic (A^{cont}) and anisotropic (A_1^{dip}) components of the principal splitting constants calculated by the INDO method. Experimental values are included for comparison. In some cases the agreement could be improved by changing the geometry (see text for details). All values are in G.

	First proton A^{cont}	A_1^{dip}	A_2^{dip}	A_3^{dip}	Second proton A^{cont}	A_1^{dip}	A_2^{dip}	A_3^{dip}
5-Thymyl radical (I)								
	Ring protons (H11,H12)				Methyl protons (H13, H14,H15)			
Exp.	40.0 ^a				21.7			
Calc., <i>sd</i>	31.6				22.3 ^b			
Calc., <i>aa</i>	26.5				19.2 ^b			
Quartet resonance								
	α -proton (H11)				β -proton (H12)			
Exp.	-28.3	6.2	-8.4	2.2	17.1	-2.4	3.4	-1.1
Calc., rad. (II) <i>sd</i>	-18.3	14.9	-12.0	-2.9	36.6	-2.9	4.1	-1.2
Calc., rad. (II) <i>aa</i>	-7.9	13.5	-11.3	-2.2	32.8	-2.3	4.1	-1.7
Doublet resonance								
	α -proton (H11)				Methyl protons ^b (H13,H14,H15)			
Exp.	-21.1	5.9	-6.1	0.1	small			
Calc., rad. (III) <i>sd</i>	-12.7	10.5	-8.6	-1.9	-7.4			
Calc., rad. (III) <i>aa</i>	-5.5	8.4	-6.9	-1.5	-3.1			
Calc., rad. (V) <i>sd</i>	-13.7	12.1	-9.8	-2.4	1.9			
Calc., rad. (V) <i>aa</i>	-6.0	10.8	-8.9	-1.9	2.9			
Calc., rad. (VI) <i>sd</i>	-17.7	14.7	-11.8	-2.9	0.3			
Calc., rad. (VI) <i>aa</i>	-7.7	13.3	-11.1	-2.2	0.9			
Sextet resonance								
	Ring proton (H11)				Methylene protons (H12,H13)			
Exp.	-11.3	4.0	-3.5	-0.5	-15.4	7.2	-7.0	-0.2
Calc., rad. (IV) <i>sd</i>	-12.1	9.2	-6.9	-2.3	-14.5	11.0	-8.7	-2.3
Calc., rad. (IV) <i>aa</i>	-5.3	7.4	-5.6	-1.8	-15.1	12.1	-10.2	-2.0
					-6.3	8.6	-6.8	-1.7
					-6.6	9.4	-7.9	-1.5

^a Mean value. ^b Mean value calculated by fitting the relation $A_H = B_0 + B_1 \cos^2 \theta$ to the splitting values obtained for one particular methyl proton when $\theta = 0^\circ$ and 90° and then averaging the resultant expression over all angles θ , θ being the dihedral angle of the proton.

of the fact that the α -splitting constants obtained after annihilation correspond to theoretical McConnell Q values that lie substantially lower than the empirical region -20 to -30 G ($A_{\alpha}^{\text{aa}}/eC^{\text{aa}}$ varying from -10 to -12 G), the standard procedure for obtaining isotropic α -proton splitting constants by INDO including annihilation⁵ seems inadequate.

On this basis only single determinant isotropic constants will be compared with experimental values in the following.

The calculated anisotropic components of the splitting constants are characterized by values substantially higher than the observed ones.

Furthermore annihilation only leads to a slight reduction. It therefore seems somewhat dubious to let the eventual rejection of certain radical candidates be too strongly based on the anisotropic components.

In Table 3 the main contributions to the computed spin density distributions are given. All radicals are regarded as π -electron radicals even if radical (II) and (VI) have unsymmetrical side groups. Only the π -part of the INDO spin densities at the conjugated atoms are given.

The 5-thymyl radical (I). This is the only radical that has been identified beyond doubt in irradiated thymine.^{1,2,22} The experimental

Table 3. Calculated π -electron spin densities at carbon, oxygen and nitrogen atoms together with INDO spin densities at hydrogen atoms. Some π densities smaller than 0.05 and hydrogen densities smaller than 0.006 are omitted. The methyl proton spin densities given correspond to a rotational conformation of the methyl group in which the plane defined by the C5—C9 and C9—H13 bonds is perpendicular to the ring plane.

Radical	Method	C4	C5	C6	C9	H11	H12	H13	H14	H15	N1	N3	O7	O8
(I)	PPP	<i>sd</i>	-0.06	0.81										0.20
	PPP	<i>aa</i>	0.01	0.79		0.059	0.058	0.080	0.022	0.022				0.13
	INDO	<i>sd</i>	-0.25	0.65		0.038	0.037	0.053	0.013	0.014				0.53
(II)	INDO	<i>aa</i>	-0.07	0.57										0.39
	INDO	<i>sd</i>		0.77		-0.034	0.068	-0.004	0.004	-0.002	0.13		0.07	
	INDO	<i>aa</i>		0.76		-0.011	0.046	-0.001	0.004	-0.001	0.13		0.04	
(III)	PPP	<i>sd</i>	0.54	-0.33	0.57						0.11	0.11		0.04
	PPP	<i>aa</i>	0.43	-0.10	0.47						0.09	0.09		0.03
	INDO	<i>sd</i>	0.47	-0.24	0.53	-0.023	-0.002	-0.025	-0.008	-0.008	0.12	0.11		0.05
(V)	INDO	<i>aa</i>	0.40	-0.07	0.45	-0.008	-0.001	-0.008	-0.003	-0.003	0.10	0.09		0.05
	PPP	<i>sd</i>	0.23	-0.03	0.60						0.06	0.05		0.12
	PPP	<i>aa</i>	0.20	0.07	0.56						0.05	0.04		0.09
(VI)	INDO	<i>sd</i>	0.07	0.03	0.62	-0.025		0.008	0.001	0.002	0.07	0.03		0.18
	INDO	<i>aa</i>	0.09	0.09	0.59	-0.008		0.009	0.002	0.002	0.06	0.03		0.12
	INDO	<i>sd</i>		0.76		-0.033	-0.003	-0.003	0.006	-0.002	0.14		0.08	
(IV)	INDO	<i>aa</i>		0.75		-0.011	-0.000	-0.001	0.005	-0.001	0.14		0.04	
	PPP	<i>sd</i>	0.03	-0.34	0.56						0.08		0.01	-0.07
	PPP	<i>aa</i>	0.01	-0.10	0.44						0.06		0.01	0.02
(IV)	INDO	<i>sd</i>	0.09	-0.23	0.52	-0.023	-0.027	-0.028			0.11		0.05	-0.14
	INDO	<i>aa</i>	0.03	-0.07	0.44	-0.007	-0.009	-0.009			0.09		0.03	-0.04

splitting constants are 36.2 and 43.8 G due to the methylene protons and 21.7 G due to the methyl protons.² The splittings are isotropic.

According to the PPP calculations about 80 percent of the unpaired electron is located at atom C5 (Table 3). The relation (2) (with $Q^0 = 27.5$ G) then gives the following estimates of the methyl splitting constant: 22.2 G (*sd*) and 21.7 G (*aa*), in nice agreement with experiment.

The INDO calculations resulted in a somewhat different spin distribution (Table 3), but the methyl splitting (mean value given in Table 2) came out close to the PPP result.

The calculated methylene splittings are equivalent, as is to be expected from the symmetrical arrangement of the methylene protons. As Table 2 shows the splitting values are substantially smaller than those obtained experimentally. However, further calculations revealed that the methylene proton splittings are sensitive to changes in the conformation of the methylene group. Thus by rotating the fragment $>\text{CH}_2$ around an axis through C5 and N1 a separation of the splitting of the two methylene protons could be obtained. The mean value of the methylene proton splitting constants remained almost constant under this change of geometry. A rotation angle of 5° was needed to achieve the experimental split of 7.6 G.

Furthermore by allowing the HCH angle of the methylene group to decrease the methylene proton splitting could be considerably increased. A bond angle as low as 90° was required to bring the coupling constant up to the experimental value of around 40 G. This does not seem conformationally quite acceptable although a bond angle somewhat smaller than normal might be the result of creating the radical from a thymine molecule without altering its surroundings in the crystal.

The 6-thymyl radical (II). As discussed by Henriksen and Snipes¹ the observed isotropic splitting of the α -hydrogen (28.3 G) was surprisingly large. This surprise is accentuated by a preceding study of irradiated dihydrothymine²³ where the α -proton splitting of the 6-thymyl radical was only 17.4 G, a reasonable value for a conjugated $>\dot{\text{C}}-\text{H}$ fragment. Furthermore the β -proton splitting was also very different in the two cases, 17.1 G in the thymine case and 44.0 G in dihydrothymine.

Only INDO calculations could be performed on the 6-thymyl radical. As is seen from Table 2 the isotropic *sd*-results fit fairly well with the experimental dihydrothymine data, but not with the thymine data. This might indicate that only in the dihydrothymine case the 6-thymyl radical is actually formed. However, Henriksen and Snipes attributed the large differences in splitting constant values to differences in molecular geometry. It seemed important to test this possibility within the INDO calculations.

When the $\text{C}_5\text{H}_{12}\text{CH}_2$ fragment was rotated around an axis through the C4 and C6 positions, the calculated β -proton splitting varied in this way:

-10°	43.7 G
0°	36.6 G
10°	28.1 G
20°	19.3 G

(Positive angle values define the rotation of the plane through C4, C5, C6 such that the β -proton is approaching the plane of the ring). According to these calculations ring puckering will easily be able to account for large variations in the splitting constant. This is in good agreement with the assumptions of Henriksen and Snipes.

The same authors based a possible explanation of the large α -proton splitting on a strong dependence of this splitting on the C5–C6–N1 bond angle. However, the present calculations give no support to this view. When the bond angle was shifted from 120 to 115° (compensated by small shifts in other bond angles of the ring), the splitting constant did only change very slightly from 18.3 to 18.9 G. Other variations of geometry were also tested but they did not give any increase of the α -splitting.

A look at the anisotropic constants in Table 2 reveals a rather bad agreement between experiment and calculation for the α -proton whereas the β -proton shows a very nice agreement.

Radical candidates for the doublet resonance: (III), (V) and (VI). From Table 2 radical (III) is seen to be the least satisfactory doublet candidate on the basis of the calculated isotropic splitting, but the best one when the anisotropic components are concerned. Radical

(VI) shows the opposite pattern and radical (V) is intermediate. However, it is seen that the calculated isotropic component is too small in all cases and the anisotropic ones are all too large.

In the doublet case all splitting constants but one have to be sufficiently small to escape detection in the ESR spectrum. The calculated isotropic component of the methyl protons of radical (III) is much too large from this point of view (Table 2) being more than half of the corresponding α -proton value. Table 3 shows how this is connected to a high negative spin population at atom C5. Furthermore the table shows that this population was even more negative in the PPP calculation whereas the spin population at atom C6, that in a sense is responsible for the low α -proton splitting, was about equal to the INDO value.

The Hüttermann radical (IV). When the six lines of the low temperature resonance were interpreted as being due to only one radical, Hüttermann² obtained the experimental splitting constants given in Table 2. As is seen from the table our INDO calculation on the radical postulated by Hüttermann to account for the sextet, radical (IV), gave isotropic *sd*-results in good agreement with experiment. Furthermore Table 3 shows that the PPP calculations gave spin populations at the carbon atoms C6, C5 and C9 that were similar to those from the INDO calculations. The populations at C6 and C9 were large enough for a McConnell *Q* value in the usual range (25–26 G before and 20–21 G after annihilation)¹⁸ and their relative magnitudes corresponded closely to the observed splittings.

The calculated anisotropic components of the principal splitting constants, as shown in Table 2, are larger than the observed values, but there is a qualitative agreement in relative magnitudes. The *aa*-values are slightly better than the *sd*-values.

DISCUSSION

The present calculations do not permit any definite conclusion as to which radical or radicals are actually formed in the thymine monohydrate crystal. There are several reasons for this. The MO methods used are too crude to give anything like really reliable predictions on a quantitative level. Furthermore when

all components of the calculated splitting constants are taken into account, there seems to be no satisfactory overall agreement with experiment for any of the investigated radicals.

Nevertheless, we feel that the present calculations are clearly in favour of the Hüttermann radical. The observed isotropic splittings attributed to this radical are in very good agreement with the INDO values (*sd*) as well as with the PPP spin densities *via* the McConnell relation. A comparison with the alternative quartet-doublet interpretation of Henriksen and Snipes permits us to make a few points that give further support to the Hüttermann radical. Firstly the very high isotropic α -proton coupling constant obtained for the postulated 6-thymyl radical inevitably casts some doubt on the validity of this interpretation. The anomaly of this constant is emphasized by the fact that the α -splitting observed for the 6-thymyl radical in *dihydrothymine* has a quite normal value. Our calculations on the 6-thymyl radical were unable to account for the large α -proton splitting constant. The splitting obtained was in fact very close to the value observed for the 6-thymyl radical in the *dihydrothymine* case. As far as the doublet resonance is concerned the accordance between experiment and calculated splitting constants is not especially good for any of the investigated radical candidates. On the basis of this we feel it justified to say that the quartet and doublet radicals are an unlikely alternative in the interpretation of the thymine monohydrate spectrum.

The anisotropic splitting constants do not support Hüttermann's conclusion in quite the same way. Their agreement with the experimental data is comparable to that found for the doublet radical candidate (III) and somewhat better than for the α -proton in the 6-thymyl radical. Considering the approximate nature of the computational method employed and the lack of experience with this method the discrepancy between observed and calculated values can hardly be said to represent a real argument against the Hüttermann radical. Thus we think it is right to say – as a conclusion – that our calculations point out rather unequivocally Hüttermann's spectral interpretation and radical identification as the most probable of the two alternatives considered.

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