Radicals Produced in Single Crystals of Thymine Derivatives by UV and X-Rays

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Single crystals of anhydrous thymine (T), thymine monohydrate ($T \cdot H_2O$), 5,6-dihydrothymine (TH_2), 1-methylthymine (mT) and thymidine (dT) were irradiated with X-rays and UV between 77 K and 300 K. Six types of radicals were analyzed by ESR-spectroscopy at 9.5 GHz and 35 GHz after exposure to X-rays. The anion radical occurred only in $T \cdot H_2O$ at 77 K, the 4-yl radical only in TH_2 at 77 K and the 1-yl radical only in T between 77 K and 300 K. The 6-yl radical was found in T, TH_2 and mT. It was converted into the 5-yl radical irreversibly by heat or white light ($\lambda < 600$ nm). The 5-yl radical appeared in all compounds at room temperature. The highest thermal stability was found for the 7-yl radical which was present at room temperature in all compounds except TH_2.

UV-irradiation ($\lambda = 320$ nm) produced radicals only in three crystals (T, TH_2, dT). In T the 5-yl radical was found after exposure at 300 K, and two other radicals, 1-yl and 7-yl, at 77 K. Also at 77 K, the 7-yl radical was present in dT and the 5-yl radical in TH_2 and dT.

Introduction

Thymine was used as a model compound for the analysis of radiation produced radicals by ESR-spectroscopy from the beginning of this field of research. In 1959, Shields and Gordy reported the spectral features of radicals formed by gamma radiation in polycrystalline thymine and thymidine [1]. Eisinger and Shulman in 1963 published similar ESR-spectra that were obtained from UV-irradiated thymine. These authors were the first to attribute the octet spectrum observed earlier to the 5-yl radical [2]. Pioneer work on single crystals of thymidine was again accomplished in Gordy's laboratory by Pruden, Snipes and Gordy in 1965 who confirmed the previous radical assignment [3].

Numerous papers appeared in the wake of this primeval work. In studies on single crystals other radicals at the thymine group were proposed. The following assignments were confirmed by later work: the anion radical [4], the 1-yl radical [5], the 6-yl radical [6] and the 7-yl radical [7]. This, and further work on the occurrence of these radicals in a variety of thymine derivatives has been reviewed earlier [8, 9].

In most of these publications only a single compound or radical was analyzed. Pathways of radical formation were discussed but usually on the small basis of the particular substance studied. We have attempted to gain a broader view by a comparative study of the production of the various base radicals in five different thymine compounds under comparable conditions. As a result, several radicals were identified in some of the substances for the first time completing the general picture. Most important of these is the occurrence of the 6-yl radical not only as a dissociation product of dihydro-thymine but also as an association product in anhydrous thymine and 1-methyl-thymine.

A second purpose of this work was to find out whether light-induced transformation of 6-yl into 5-yl radical is operative in thymine, too. All other types of 6-yl radicals in pyrimidines were found to be susceptible to such treatment previously [10, 11].

We further tried to produce radicals by irradiation of single crystals with ultraviolet radiation. This was indeed possible in three of the five types of crystals. The results fit into the general picture of radical formation via the excitation path.

Experimental

Five types of single crystals were used in this work: anhydrous thymine (T), thymine monohydrate ($T \cdot H_2O$), 5,6-dihydrothymine (TH_2), 1-methylthymine (mT) and thymidine (dT).

The crystals were grown by evaporation of aqueous solutions or, in the case of anhydrous thymine,

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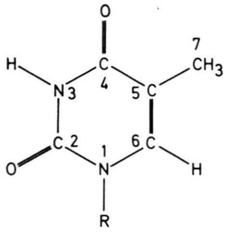
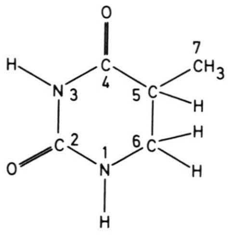
by slow cooling of a hot ethanol solution. The crystal structures were those reported previously for T by Ozeki *et al.* [12], for T · H_2O by Gerdil [13], for TH_2 by Furberg and Jensen [14] for mT by Hoogsteen [15] and for dT by Young *et al.* [16].

Orthogonal axes were used for ESR-measurements. In the monoclinic crystals of T and T · H_2O this was the system a^*, b, c where a^* is normal to the crystallographic axes b and c. In the orthorhombic crystals of TH_2 and dT the crystallographic system a, b, c was used. In the monoclinic crystal mT the system chosen was a^*, b, c^*, with c^* normal to the crystallographic b-axis and parallel to the (102) cleavage plane while a^* was normal to this plane and the b-axis.

The crystals were exposed to UV-radiation from a high pressure lamp type XBO or HBO and a filter cutting off wavelengths $\lambda > 295$ nm. Alternatively, X-rays of 100 kV were applied between 77 K and 300 K. ESR-spectroscopy at 9.5 GHz and 35 GHz between 77 K and 500 K was used in order to obtain spectral data. These procedures were complemented by other methods such as annealing by heat and bleaching by visible light at various wavelengths. Differential microwave saturation was also used, along with variation of radiation doses. In addition, crystals deuterated at exchangeable proton positions bonded to N or O were grown from solutions in D_2O. Finally, radical assignments were checked by INDO calculations performed with the QCPE-program No. 146 on a computer TR 440.



THYMINE	R=H
1-METHYLTHYMINE	R=CH_3
THYMIDINE	R=DEOXYRIBOSE



5,6 DIHYDROTHYMINE

Results

Six different types of radicals were identified after the exposure of the five different types of monocrystals to X-rays.

In Table I a survey is given listing ranges of irradiation temperatures between 77 K and 300 K at which the particular radical could be produced. The first two of these, 1-yl and 7-yl, may be classified as dissociation or oxidation radicals, the remaining four radicals as association or reduction radicals in relation to the unsaturated thymine group.

The 1-yl radical

The 1-yl radical resulting from hydrogen dissociation from N 1 has been reported in single crystals of

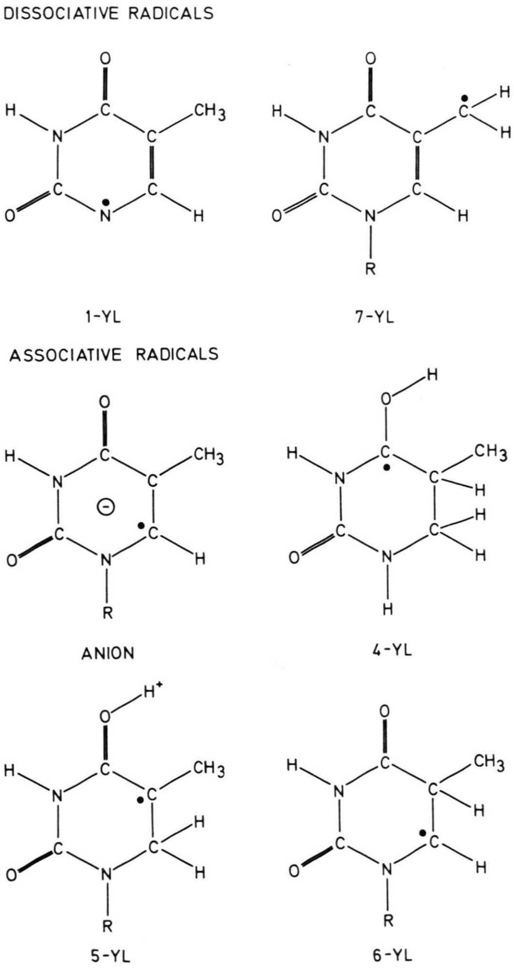


Table I. Temperature range in K of radical formation in different single crystals of thymine derivatives by X-rays between 77 K and 300 K and UV-radiation at 77 K and 300 K. References to earlier studies are included.

Compound Radical		T	$T \cdot H_2O$	TH_2	mT	dT
1-yl	X	77 – 300[5]	–	–	–	–
	UV	77	–	–	–	–
7-yl	X	77 – 300	77 – 300[7]	–	77 – 300[17]	77 – 300[18]
	UV	77	–	–	–	77
anion	X	–	77 – 120	–	–	4.2[4a]
	UV	–	–	–	–	–
4-yl	X	–	–	77	–	–
	UV	–	–	–	–	–
5-yl	X	230 – 300[19]	120 – 300[7]	300[6,20]	300[21]	77 – 300[3,22]
	UV	300	–	77	–	77
6-yl	X	230 – 300	–	77 – 300[6]	77 – 280	–
	UV	–	–	–	–	–

[a] Not analyzed in this work.

anhydrous thymine and thymine monohydrate previously [5]. In contrast to this work we could not detect the 1-yl radical in single crystals of thymine monohydrate but in anhydrous thymine only. There, the radical is produced with a relatively large yield at 230 K. However, it also appears at 77 K or 300 K. Spectra of this radical in three different orientations are shown in Fig. 1. When the magnetic field is parallel to the molecular ring plane, only the quartet of 1 : 3 : 3 : 1 intensity from the methyl group is visible. These lines are further split into anisotropic triplets of nearly equal intensity by the N 1 nucleus in other orientations. The data of the hyperfine tensor derived from the spectra and the direction cosines are listed in Table II.

The radical is stable up to room temperature and against illumination with visible light. Its spectral lines do not show any sign of saturation even at the highest microwave power employed. This property is analogous to that of the 1-yl radical of uracil [23]. Its character facilitates detection of the radical lines when these are hidden among others. Although a search was made under appropriate conditions, no indication of the 1-yl radical as reported [5] could be detected in irradiated $T \cdot H_2O$ nor in any of the other compounds investigated.

The 7-yl radical

The presence of the 7-yl radical in dT was already suggested by Pruden *et al.* [3]. It was subsequently analyzed in single crystals of $T \cdot H_2O$ [7], mT [17] and dT [18] and in 6-aza-thymine [24]. We also found this species in single crystals of anhydrous thymine.

Table II. Hyperfine tensors for the 1-yl radical in anhydrous thymine and for the anion radical in thymine · monohydrate.

	Principal values		Direction cosines		
	Total	Isotropic	*a* *	*b*	*c*
1-yl Radical (T)					
N1	+ 13.8		0.314	– 0.057	0.945
	0.0	+ 4.6	in the molecular plane		
β-H(C7)	18.0		+ 0.314	– 0.057	0.945
	18.5		0.0	1.0	0.0
	20.0		0.946	– 0.006	– 0.325
Anion-Radical ($T \cdot H_2O$)					
α-H(C6)	– 7.0		– 0.800	0.346	– 0.490
	– 21.0		0.394	0.919	0.007
	– 15.0	– 14.3	– 0.376	0.154	0.914

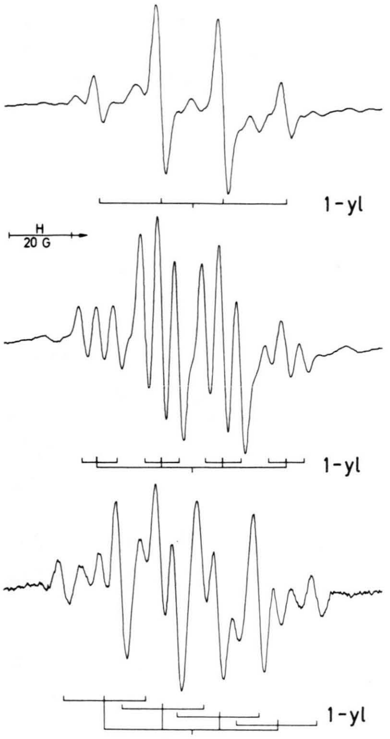


Fig. 1. ESR spectra of single crystals of anhydrous thymine after X-irradiation at 230K, observed at 77K with 9.5 GHz. The incident microwave power is 10.0 mW. In the upper spectrum the magnetic field was oriented parallel to the molecular plane (*i. e.* 18 °C from *a** in the (*a** *c*)-plane. For the middle spectrum the magnetic field is parallel to the *a**-axis, and in the lower spectrum the magnetic field is parallel to the *c*-axis, *i. e.* 18 ° from parallel to the p_z-orbital. The stick diagrams indicate the lines of the 1-yl radical.

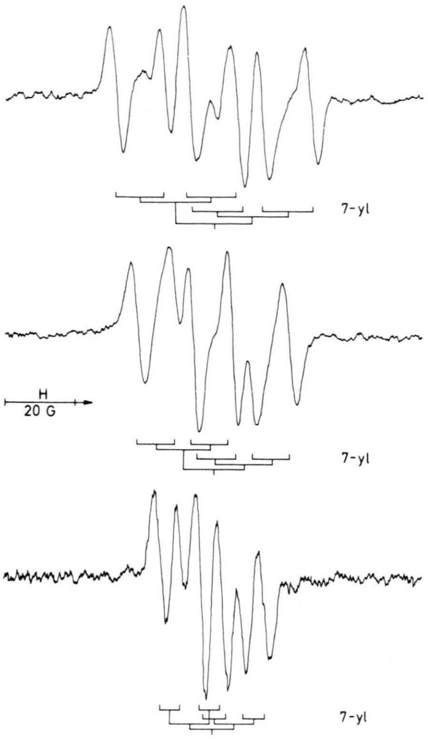


Fig. 2. ESR spectra of single crystals of anhydrous thymine, X-irradiated at 300K and annealed at 485K. The spectra are taken at 300K. Stick diagrams indicate the lines of the 7-yl radical in different orientations of the magnetic field which is oriented along the *a**-axis (top), the *c*-axis (middle) and the *b*-axis (bottom). The lower spectrum is taken at 35.0 GHz, the other two at 9.5 GHz.

Table III. Hyperfine tensors (in Gauss) and direction cosines for the α-protons of the 7-yl radical. The probable errors are estimated to be ± 1.0 G for the hyperfine tensors and ± 6° for the direction cosines.

	Axis	α-H(C6)			α-H′(C7)			α-H″(C7)		
Total		-5.0	-14.0	-10.0	-7.0	-23.5	-15.3	-7.0	-23.5	-15.0
Isotropic			-9.7			-15.2			-15.2	
Anisotropic		+4.7	-4.4	-0.3	+8.2	-8.3	+0.2	+8.2	-8.3	+0.2
anhydrous Thymine (T)	*a**	-0.505	-0.833	-0.314	-0.505	-0.833	0.314	-0.492	0.810	0.314
	b	-0.855	0.461	-0.057	-0.855	0.461	-0.057	0.844	0.533	-0.057
	c	0.116	0.305	0.945	0.110	0.305	0.945	0.215	-0.237	0.945
Thymine · monohydrate ($T \cdot H_2O$)	*a**	-0.800	0.394	-0.376	-0.755	0.535	-0.376	0.816	0.399	-0.376
	b	0.346	0.916	0.154	0.518	0.841	0.154	0.489	-0.862	0.154
	c	-0.490	0.007	0.914	-0.402	0.078	0.914	0.310	0.310	0.914
1-Methyl-thymine (mT)	*a**	0.449	0.894	0.0	0.449	0.894	0.0	1.0	0.0	0.0
	b	0.894	-0.449	0.0	0.894	-0.449	0.0	0.0	1.0	0.0
	*c**	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0
Thymidine (dT)	*a*	0.208	0.674	-0.703	0.208	0.674	-0.703	0.500	-0.519	-0.703
	b	0.120	0.692	0.708	0.120	0.692	0.708	0.529	-0.450	0.708
	c	-0.971	0.258	-0.063	-0.971	0.258	-0.063	0.685	0.726	-0.063

Thus, the 7-yl radical was the only dissociation product present in all compounds except TH_2. We used T for an optimal analysis of the radical since in this case all other species could be annealed by heating to 485 K. Some of the resulting spectra are shown in Fig. 2. One major and two minor α-proton couplings are seen. Analysis was extended from 9.5 GHz to 35 GHz in order to avoid poor resolution due to the nuclear Zeeman splitting.

The tensor data and direction cosines of the three α-protons deduced from the spectra of T are given in Table III. These values have been used to calculate the hyperfine splittings of the same radical in $T \cdot H_2O$ (Fig. 5), mT, and dT (Fig. 7). The fit to experimental data was satisfactory for all three compounds. It was not necessary to assume any molecular disorder, as suggested before [17], if the nuclear Zeeman term was taken into account properly. The tensor data of Table III compare well with those for mT [17], $T \cdot H_2O$ [7] and dT [18]. The latter were obtained with ENDOR and systematically are slightly higher. The lines assigned to the 7-yl radical were reported previously in $T \cdot H_2O$ [25] and in dT [22].

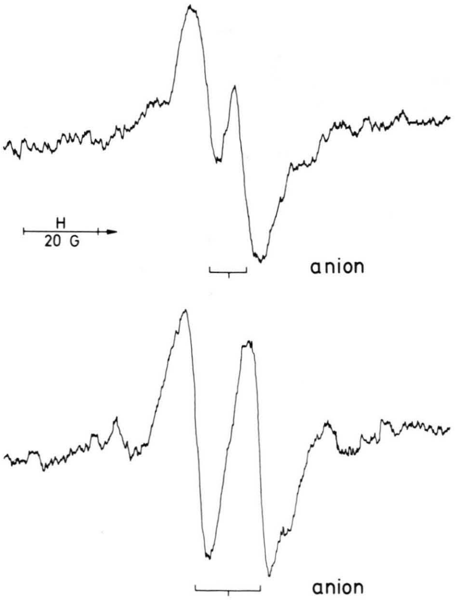


Fig. 3. ESR spectra of single crystals of thymine · monohydrate X-irradiated and observed at 77 K. The incident microwave power is 0.05 mW. The frequency of observation is 9.5 GHz. In the upper spectrum the magnetic field is oriented along the *a**-axis, in the lower spectrum along the *b*-axis. The anion sticks indicate the doublet splitting of the anion radical.

The authors concluded production of the 4-yl and the 6-yl radical. Our data do not support this proposition but indicate on the contrary that both these radicals are absent in the two compounds $T \cdot H_2O$ and dT.

The anion radical

In $T \cdot H_2O$ irradiated with X-rays at 77 K a doublet spectrum was found which we attribute to the anion radical (Fig. 3). This assignment is based on the pronounced instability of these lines against red light ($\lambda > 600$ nm) and heating as well as early saturation of the signal with rising microwave power and irradiation dose (about 5 kGy). All of these features were observed invariably with other ionic radicals [26–28].

The orientational dependence of the doublet splitting is typical of an α-proton and the direction cosines coincide with a proton bonded a C6 (Table II).

The anion radical was reported previously in single crystals of dT after exposure to X-rays at 4.2 K [4]. We could not observe this species in dT at 77 K. The values of the hyperfine tensor reported are slightly different from ours for $T \cdot H_2O$. We attribute this discrepancy to a deviation of nearly 15 °C of the eigenvectors from orthonormality in the earlier work.

The 4-yl radical

Single crystals of TH_2 exposed to X-rays at 77 K show doublet lines in addition to spectral features of other radicals, especially the 6-yl radical. An isotropic splitting of 27.5 G is obtained which is most prominent when the lines of the 6-yl radical are reduced by site splitting (Fig 4). This experimental value is close to an isotropic β-coupling of 25 G calculated by an INDO program for both the anion and the 4-yl radical which may be regarded as a protonated anion.

The observed features are assigned to the 4-yl radical as the anion radical is excluded by all additional experimental data. Early saturation of microwave power is equally absent as saturation of radiation dose. Also bleaching of the signal by red light is not found. The thermal stability is higher, too. Only at 300 K is the doublet transformed into other lines that are characteristic of the 5-yl radical.

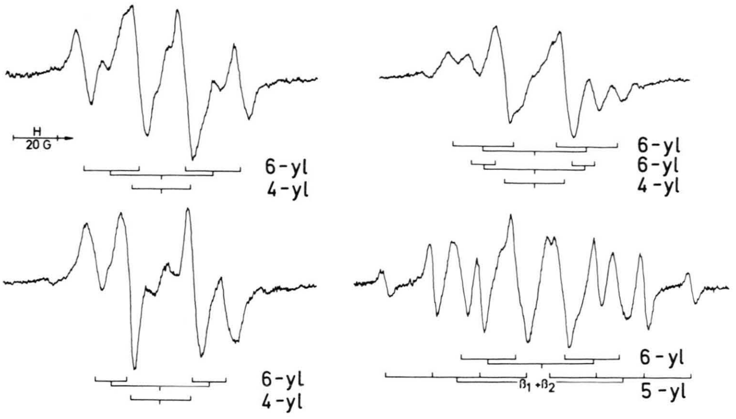


Fig. 4. ESR spectra of single crystals of 5,6-dihydrothymine X-irradiated and observed at 77 K, incident microwave power is 0.1 mW. The magnetic field is oriented along the *b*-axis (upper left), along the *c*-axis (lower left) and 45 ° in the (*bc*)-plane (upper right). Stick diagrams indicate the lines of the 6-yl (upper) and the 4-yl radical. In the upper right spectrum both sites of the 6-yl radical are indicated. The lower right spectrum is obtained after warming the crystal to 300 K. The magnetic field is oriented along the *b*-axis, as it is in the upper left spectrum. Indicated are the lines of the 6-yl and 5-yl radical.

The absence of an additional splitting from the hydrogen added at the carbonyl group is consistent with the INDO calculations. These yield a maximum binding energy for a planar configuration of C 5-C 4-O 4-H giving a vanishing β-coupling. No spectral features of the other thymine derivatives could be attributed to a 4-yl radical but an analogous assignment was given for 5,6-dihydrouracil [29].

The 5-yl radical

The 5-yl radical is the only one observed in all of the five compounds investigated. In TH_2 exposed to X-rays at 300 K it is present as a minor fraction only, whereas it is dominating under similar conditions in the unsaturated compounds. Extensive INDO calculations suggest that the radical is protonated at O 4 [30].

If $T \cdot H_2O$ crystals are grown from D_2O, the anion radical is found at 77 K as a major product which is transformed under illumination with red light ($\lambda >$ 600 nm) into the 5-yl radical by addition of D at C 6 (Fig. 5). When such crystals are illuminated further with white light ($\lambda <$ 600 nm) the HD-adduct disappears in favour of the HH-adduct indicated by a corresponding change of nuclear splittings. The latter product is also obtained by irradiation with X-rays at 300 K. Presumably a reversal of hydrogen association is supported by white light or heat favouring the light isotope which may attach again to give another 5-yl radical.

Transformation of the HD-adduct which is formed in large excess at 77 K into the HH-adduct by heating was also observed in dT [31]. However, 100 °C were required for about 30 min indicating a higher stability than in $T \cdot H_2O$. We were unable to obtain this transformation at 77 K with white light in dT which again may be due to a higher stability.

A positive effect of temperature on the production of the 5-yl radical was also found by us in other crystals. These species appeared in mT after exposure to X-rays at 300 K but not at 77 K. Association of D was not obtained with crystals grown from D_2O in this case.

In single crystals of dT and TH_2 the binomial intensity ratios 1 : 3 : 3 : 1 of the methyl splittings tend to equalize on lowering the temperature down to 77 K. This effect is due to broadening of the inner lines (Fig. 7). The effect was even more pronounced at 4.2 K and was explained by freezing of the methyl rotation [32].

Table IV. INDO calculations and eyperimental results of the 6-yl radical (isotropic splittings in Gauss). Θ is the dihedral angle of the β-proton bonded to C5 with respect to the p_z-orbital of C6. The bond lengths used for these calculations are: C4 – C5: 1,5 Å, C5 – C6: 1,5 Å.

Radical configuration/ experiment	H(C5)	H(C6)	(C6)	H(N1)
Puckered pyrimidine ring as 5,6-dihydrothymine	32.8	– 9.3	0.68	+ 1.3
Planar pyrimidine ring, $\Theta = 30°$	38.1	– 17.6	0.77	– 3.0
Planar pyrimidine ring, $\Theta = 20°$	53.2	– 17.4	0.76	– 3.0
Planar pyrimidine ring, $\Theta = 10°$	66.0	– 17.0	0.75	– 2.6
Experimental results (5,6-dihydrothymine, anhydrous thymine, 1-methylthymine)	48.0 (77 K)	– 17.4		

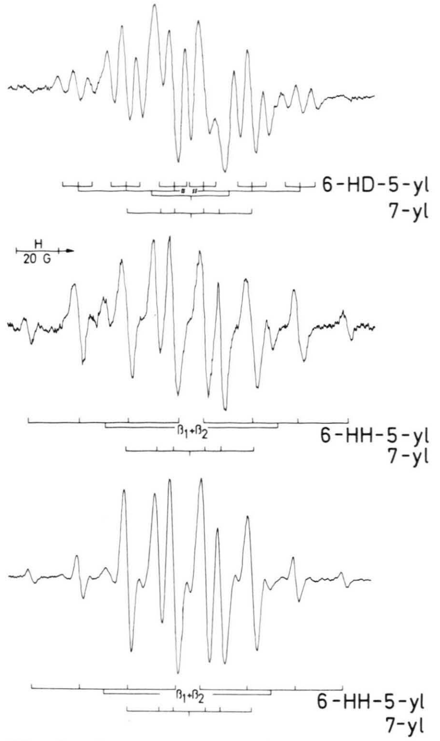


Fig. 5. ESR spectra of single crystals of thymine · monohydrate grown from D_2O X-irradiated and measured at 77 K. The magnetic field is oriented along the *b*-axis. The upper spectrum is obtained after illuminating the crystal with red light. The upper stick diagram shows the lines of the resulting HD-adduct of the 5-yl radical, while the lower stick diagram represents the 7-yl radical in the three spectra. The middle spectrum is obtained after subsequent illumination with light of $\lambda > 400$ nm. Here, the lines of the resulting HH-adduct of the 5-yl radical are indicated. The lower spectrum is obtained after warming a X-irradiated crystal at 300 K showing the same stick diagrams as above.

The 6-yl radical

The 6-yl radical was first analyzed in detail by Henrikson and Snipes in a single crystal of TH_2 exposed to X-rays [6]. It was characterized by an anisotropic α-proton and an isotropic β-proton interaction. We could confirm the assignment of these authors and obtained the same results for the isotropic splitting values (Table IV). We also identified the lines of the 6-yl radical in T irradiated at 230 K and in mT irradiated at 77 K. In Fig. 6 (upper right) the lines of the 6-yl radical in T are indicated. They are in good agreement with those calculated for this orientation (18 °C from the direction of the p_z-orbital) from the tensor data of TH_2. The same is true for all other orientations where the α-splitting could be seen as well as for mT. Due to overlapping of other lines especially at the minimum splitting we could not extract the complete tensor data for T and mT. The 6-yl radical could not be detected in $T \cdot H_2O$ and dT. We consider the lines attributed to this radical previously [22, 25] as clearly due to the 7-yl radical (vide supra). The tensor data reported [22, 25] strongly differ from those obtained with TH_2 [6].

The 6-yl radical could be transformed into the 5-yl radical by heat. An apparent activation energy of 23.1 kcal · mol^{-1} was reported for this process from work with powdered TH_2 [33]. We could induce the same transformation by illumination with white light ($\lambda < 600$ nm) at 77 K in all three compounds (Fig. 6). This process was found to be irreversible in all cases.

A reversible variation of the β-coupling of the 6-yl radical was found to be the same for the three crystals of T, TH_2 and mT. It increases from 44 G at 300 K to 48 G at 77 K.

INDO calculations show in agreement with the direction cosines of the proton bonded to C6 that the pyrimidine ring in the 6-yl radical is planar. A molecular rearrangement from the puckered ring conformation occurs in TH_2 [6]. In the unsaturated molecules the ring of the parent molecules remains planar; but the methyl group must move out of this plane on association of hydrogen at C5 in order to obtain satisfactory sp_3 hybridization of C5 (Table IV). The identical β-coupling and its variation with temperature in TH_2 indicate, however, that the conformation cannot be hindered substantially in the unsaturated crystals although these are packed much more closely.

Ultraviolet-induced radicals

Radicals were produced also by UV-radiation. Using cut-off filtering the maximum yield was found at about 320 nm but generally the conversion of absorbed energy into radicals was rather inefficient. The quantum yield was not determined. Sufficient radical concentrations were obtained after irradiation for about 30 minutes. Three of the six types of radicals produced with X-rays were identified in three of the five types of crystals investigated. The temperatures at which they were obtained are included in Table I.

The 5-yl radical appeared at 77 K in TH_2 and copiously in dT (Fig. 7). The production in dT showed a quadratic increase with intensity indicating a biphotonic process. The only case where radicals

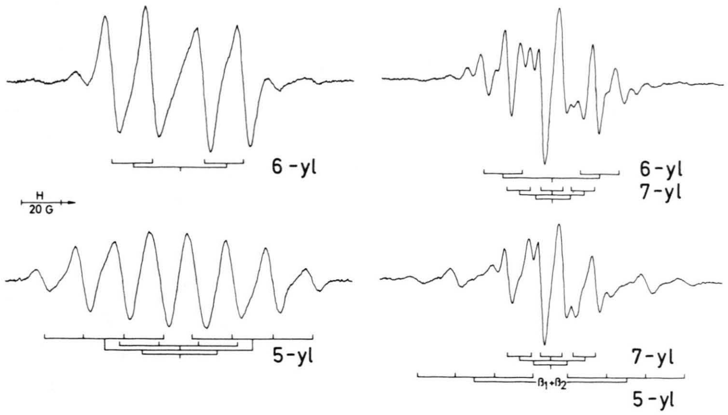


Fig. 6. ESR spectra of single crystals of 5,6-dihydrothymine (left) and anhydrous thymine (right). The upper left spectrum is recorded after X-irradiation at 300 K. The stick diagram indicates the lines of the 6-yl radical. The lower left spectrum is obtained after illuminating the crystal with light of $\lambda < 600$ nm at 77 K. The stick diagram shows the lines of the 5-yl radical. Both spectra are taken at 300 K with the magnetic field oriented along the *a*-axis. The upper right spectrum is recorded at 77 K after X-irradiation at 230 K. Indicated are the lines of the 6-yl and of the 7-yl radical. After illuminating the crystal with light at 77 K the spectra bottom right is obtained. There the lines of the 7-yl and the 5-yl radicals are indicated by the stick diagrams. For both spectra on the right the magnetic field was oriented along the *c*-axis. Frequency of observation for all spectra was 9.5 GHz.

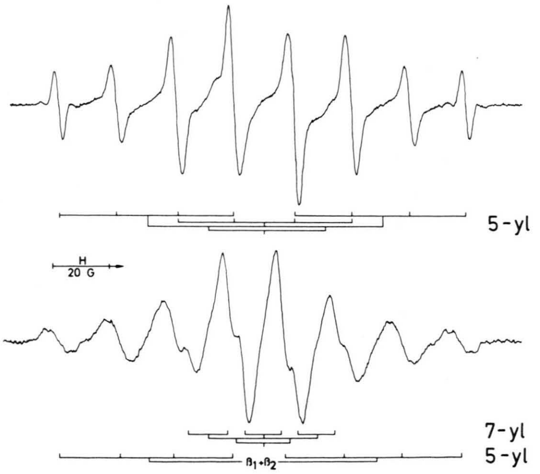


Fig. 7. ESR spectra of single crystals of thymidine irradiated with light of $\lambda > 295$ nm and observed at 77 K. In the upper spectrum the magnetic field is parallel to the *c*-axis, in the lower spectrum parallel to the *b*-axis. The receiver gain is increased by a factor 10 for the lower spectrum. Stick diagrams show the lines of the 5-yl and the 7-yl radical.

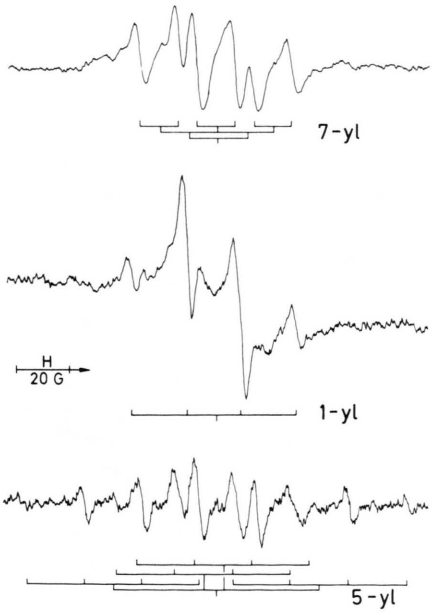


Fig. 8. ESR spectra of single crystals of anhydrous thymine after irradiation with light of $\lambda > 295$ nm. The upper spectrum is obtained after irradiation at 77 K. The magnetic field is oriented along the *a**-axis. The incident microwave power is 0.05 mW. Indicated are the lines of the 7-yl radical. The spectrum in the middle is also obtained after illumination at 77 K. The magnetic field is parallel to the molecular plane (*i. e.* 18 ° from *a** in the (*a** *b*)-plane), but the microwave power is 10.0 mW. The lines of the 1-yl radical are indicated. The spectrum at the obtained after illumination at 300 K. The magnetic field is parallel to the *a**-axis. The lines of the 5-yl radical are indicated.

were produced by UV at 300 K was found for the 5-yl radical in T (Fig. 8). In crystals of dT grown from D_2O a large fraction of the 5-yl radical was produced as the HH-adduct by ultraviolet light at 77 K. This result is different from that obtained with X-rays where the HD-adduct was found to predominate.

The 7-yl radical was visible at 77 K in T (Fig. 8) and in dT (Fig. 7). In addition a small amount of the 1-yl radical was present in T (Fig. 8).

Discussion

The survey given in Table I immediately shows the occurence of the 5-yl and 7-yl radicals in the four unsaturated compounds investigated. The 5-yl radical, which was the first to be identified in pyrimidines (2,3), was analyzed previously in all substances studied including TH_2, the only saturated compound.

The 7-yl radical has the highest thermal stability up to about 500 K. This radical is the most prominent one after irradiation at room temperature besides the 5-yl radical. This result indicates a high dissociation probability of hydrogen from the saturated side group.

Two further radicals, 1-yl and 4-yl, were only observed in one compound each. The second dissociative radical besides 7-yl, the 1-yl radical, shows considerably higher stability against transformation by heat or illumination than ionic products. The 4-yl radical resulting from addition of hydrogen at the C 4 carbonyl oxygen was solely found in TH_2 irradiated at 77 K. This radical may result from protonation of an anion. When the temperature is raised it is transformed into the 5-yl radical and has vanished completely at room temperature. Its stability against visible light and its saturation characteristics are quite different from the anion radical which was not found in this crystal. Ths anion radical was only seen in $T \cdot H_2O$ at 77 K. It disappeared rapidly on illumination with red light ($\lambda > 600$ nm) or heating above 77 K. An anion was also reported by Box and Budzinski in dT at 4.2 K [4].

The last species to be discussed is the 6-yl radical. Its identification proves its occurrence as an association product in unsaturated thymine derivatives besides the 5-yl radical. The spectral features of the 6-yl product were clearly separated from those of the 7-yl radical in the less polar compounds T and mT. It was also present as a dissociation product in TH_2 which has been reported previously [6].

The 6-yl radical could be converted into the 5-yl radical by visible light ($\lambda < 600$ nm) in all pyrimidines studied [10, 11]. In this respect thymine compounds were no exception. On illumination these radicals were converted in all of the three compounds where they were detected.

The same reaction as with light could also be accomplished by heating. This feature, however, is in contrast to other apolar pyrimidines where heating induced the reverse reaction. For this reason an admixture of 5-yl radicals present after irradiation could not be removed as in other compounds rendering a quantitative pursuit of 6-yl into 5-yl conversion by light rather difficult. INDO calculations indicate that the transfer of H from C 5 to C 6 is accompanied by proton attachment at O 4 [29].

There is one essential deviation from the results obtained with other pyrimidines. That is the shift of thermal stability from the 6-yl to the 5-yl radical even in the apolar compounds studied. The presence of the methyl group at C 5 obviously does not preclude the attachment of hydrogen at this position in the unsaturated compounds T and mT but may be regarded as the cause of the observed instability. However, this phenomenon is also found for the 6-yl radical in TH_2. In this case, the radical is formed by dissociation of H from the puckered ring structure situated in a loosely packed lattice. Hence, the observed shift must have other causes. There is no indication of intermolecular conformational strains acting on the 6-yl radical in T and mT since the same couplings are found as in TH_2. In addition, a small but significant temperature dependence of the H_β-splitting is found that is identical for the 6-yl radical in the three compounds. In view of the large differences of crystal structure this parallelism is attributed essentially to intramolecular forces of the 6-yl radical.

Radical production in single crystals by UV-light of about 300 nm was possible in three compounds only. With the exception of T where the 5-yl radical was present after irradiation at 300 K, all other products were found after exposure at 77 K only. The 5-yl radical was also formed by UV at 77 K in TH_2 and dT and the 7-yl radical in dT. The 6-yl radical was found to be absent although it should be a favourite addition product of the excitation path. This is not surprising, however, since this radical is transformed into the 5-yl radical by UV with high efficiency. The absence of the anion radical is consistent with its role in the ionization path which may also be true for the 4-yl radical. All radicals identified after UV treatment were also found after irradiation with X-ray but not in all compounds.

The main radical products that were stable at room temperature were formed by dissociation of hydrogen from the methyl group and by association of hydrogen to the C5–C6 double bond. Addition at C6 was found to be more stable against heat or visible light than addition at C5.

Radical formation in pyrimidines was classified previously under two different types of reactions, the ionization path and the excitation path [11]. All thymine derivatives studied so far comply with this scheme. Anions were detected exclusively in the two compounds T · H_2O and dT (4). In T · H_2O these were converted into 5-yl radicals by red light demonstrating radical formation via the ionization path. At the same time, the 6-yl radical was found to be absent only in these two compounds. In deuterated crystals irradiated with X-rays at 77 K, deuterons were added at C6 presumably again via the ionization path. An exchange with H occured only subsequently when the temperature was raised sufficiently. In accord with the classification proposed previously [11], T · H_2O and dT are regarded as predominantly polar crystals in contrast to the others which are more apolar. In several cases experimental results clearly show radical production via the ionization path. These are the formation of anions and the association of D at C6. These products were not formed by UV-radiation which yielded addition of H at C6 only. This result supports the conclusion that only the excitation path leads to radical production by UV.

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