MO Calculations of Some Thymine Radicals at the INDO Level

E. Westhof

Institut für Biophysik und physikalische Biochemie, Universität Regensburg

and M. van Rooten

Laboratoire de Chimie Biologique, Université Libre de Bruxelles, Rhode St-Genèse

(Z. Naturforsch. **31 c**, 371 – 376 [1976]; received January 30/March 12, 1976)

MO, INDO, Thymine, Free Radicals

The results of MO INDO calculations of some thymine radicals are presented. These include as well as the hydrogen addition radicals the hydroxyl addition radicals to C5 and to C6 of the C5=C6 double bond of the thymine molecule. The radicals resulting from hydrogen abstraction from the methyl group and from the nitrogen N1 are also studied. Except for radicals where the main spin density is localized on nitrogen atoms, the agreement between experimental and theoretical couplings is satisfactory.

1. Introduction

Our knowledge about the structure of the radicals produced by ionizing radiation in biological molecules is essentially based on electron spin resonance (ESR) studies. Analysis of ESR spectry yields experimental hyperfine splitting constants which can be correlated to spin density distributions. The identification of the radicals is usually only possible when working on single crystals. However, single crystals have two main drawbacks. First, quantitative measurements or reaction kinetics are difficult or even impossible to make. An secondly, some radiochemically important radicals, like OH-addition radicals, have never been observed in such systems. In fact, mainly H-addition radicals have been observed in single crystals. On the other hand, the study of frozen solutions permits to observe such radicals and their reaction kinetics. However, since the ESR spectra obtained from such samples are powder patterns, the identification of the radicals produced may be difficult or inconclusive. Therefore, the need for calculations of spin density distributions together with theoretical reconstructions of powder spectra is increased.

Very recently, the formation of radicals in γ-irradiated frozen aqueous solutions of deoxythymidine-5′-monophosphate has been quantitatively studied by Gregoli and coworkers [1]. In connection with this work, we present the calculations of hyperfine splitting constants in several thymine radicals. These include the H- and OH-addition radicals to C5 and to C6 of the C5 = C6 double bond of thymine. The radicals resulting from H-abstraction from the methyl group and from the nitrogen N1 are analysed in detail together with the cation radical. Next to the anion radical and the radical of H-addition at C6 of the thymine ring, Gregoli *et al.* [1] isolated two other species. One could be identified as the radical of OH-addition at C-6 of thymine on the basis of experimental data and of preliminary results of our calculations. No positive identification of the fourth radical could, however, be made. We present evidence that this radical is the neutral radical resulting from hydrogen abstraction from the methyl group of the thymine molecule.

2. Method of Calculations

The molecular orbital calculations of the spin density distribution were done at the INDO level. The INDO program used was the QCPE program n° 141 [2]. The atomic coordinates necessary for the INDO calculations were obtained either from crystallographic data or by using the QCPE program n° 136 [2]. The proportionality constants relating the spin densities to the isotropic hyperfine splittings were those of Pople and Beveridge [3]. The anisotropic hyperfine splittings of the nitrogen nuclei of some radicals have also been calculated. The radicals calculated are π-radicals – the diagonal elements of the spin density matrix $(2p_x, 2p_x)$ and $(2p_y, 2p_y)$ are only some percent of $(2p_z, 2p_z)$ – and, therefore the in-plane components of the spin density

Requests for reprints should be sent to Dr. E. Westhof, Institut für Biophysik und physikalische Biochemie, Fachbereich Biologie, Universität Regensburg, Postfach 397, *D-8400 Regensburg.*

matrix were neglected. The hyperfine splitting obtained with the magnetic field parallel to the π-orbital is given by $A = a_{\text{iso}} + 2\,b_{\text{aniso}}$. In the molecular plane, the coupling is $A = a_{\text{iso}} - b_{\text{aniso}}$. The proportionality constant for b_{aniso} was calculated with Slater orbitals – since the INDO method uses such orbitals – and was found to be equal to 13.6 G. The calculations were performed on a CDC 6500 at the Brussels Free Universities Computer Center.

3. Addition Radicals

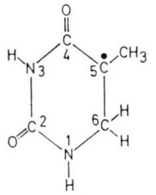
The configuration of the molecules generally changes upon radical formation. INDO energy minimization to obtain the optimum configuration should consequently be applied. However, with such procedures, the agreement between experimental and theoretical couplings is not particularly good. A more pragmatical method has been preferred. This consists in determining the lengths of the bonds between the H-addition site and the rest of the molecule which give best fit between experimental and theoretical couplings of some experimentally well-known radicals. These were the H-addition radicals of benzene[4] and imidazole[5]. The method has already been applied in cases of the H-addition radicals some pyrimidines[6] and purines[13]. All the radicals were calculated planar with the methylene protons symmetrically disposed about the molecular plane. The molecular dimensions were taken from the crystallographic data[22, 23]. For radical **1**, the distance between C5 and N1 was taken from the crystal structure and new coordinates for C6 were calculated with C6 – N1 equal to 1.40 Å and C5 – C6 equal to 1.50 Å. For radical **2**, it is the distance between C4 and C6 which was kept identical to the distance observed in the crystal and new coordinates for C5 and the atoms bonded to it were calculated with C4 – C5 and C5 – C6 equal to 1.50 Å.

3.1. *H-addition radicals*

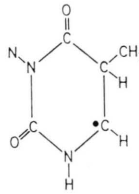
The results obtained for the radical resulting from H-addition of C6 (radical **1**) and giving rise to the famous octet resonance[7, 8] are presented in Table I together with those obtained for the radical resulting from H-addition at C5 (radical **2**).

Table I. Theoretical coupling constants for radicals **1** and **2** as calculated by INDO, together with experimental values (all values in gauss).

Radical	H(C6)	H(C5)	$H(CH_3)$
Radical **1** C–N: 1.40 Å C–C: 1.50 Å	35.0	*	22.1
Experimental (7, 8)	37.0–40.5	*	19.3–21.3
Radical **2** C–C: 1.50 Å	39.2	–17.6	<1.0
Experimental (9)	44.0	–17.4	*



Radical **1**



Radical **2**

The rotation barrier for the methyl group was calculated to be less than 0.5 kcal·mol⁻¹ in radical **1** and less than 3.0 kcal·mol⁻¹ in radical **2**. The calculated β-proton hyperfine coupling constants of the methyl group in radical **1** can be reproduced by the equation:

$$a_{\text{H}}^{\beta}\,(\text{G}) = B_0 + B_2 \cdot \cos^2 \theta$$

with $B_0 = 1.5$ G and $B_2 = 41.2$ G. Since the methyl group is freely rotating, the values for the methyl group in Table I are given in the form $B_0 + 1/2\,B_2$.

The experimental coupling constants of radical **1** depend on its environment and its range of variation is given in Table I. Radical **2** has been positively identified in a single crystal of 5,6-dihydro-thymine[9]. The agreement between experimental and theoretical coupling constants is good. The tendency of the β-proton of radical **2** to be larger than that of radical **1** is reproduced. In pyrimidines with unsubstituted C5 = C6 double bond, the inverse is observed: H-addition radicals at C5 have smaller β-proton coupling constants than H-addition radicals at C6[6, 10].

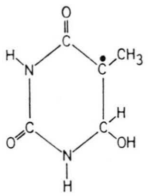
3.2. *OH-addition radicals*

In these radicals, two other structural parameters have to be determined. These are the C – O bond length and the orientation of the hydroxyl proton. In the crystal structure of *cis*-thymine glycol[11], the C – O bond lengths are measured to be 1.418 and 1.430 Å. The calculations were done with 1.40 and 1.43 Å. The orientation of the hydroxyl proton was varied. The extrema of the hydroxyl proton coupling were obtained for the *cis* and *trans* orientations. When looking down the C – O bond, the orientation is called *cis* if the O – H bond is parallel to the C5 – C6 bond (the C5 – C4 bond) in the C6-addition radical (the C5-addition radical) and *trans* if antiparallel. At intermediate orientations, the hydroxyl proton is comprised between these extrema.

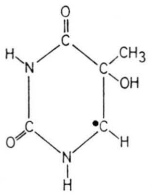
The results for radicals **3** and **4** with the bond lengths for C – O equal to 1.43 Å and for O – H equal to 0.97 Å are presented in Table II.

Table II. Theoretical coupling constants for radicals 3 and 4 as calculated by INDO, together with experimental values (all values in gauss).

Radical	H(C6)	H(OH)	H(CH_3)
Radical **3** *cis*	25.9	3.8	22.5
Radical **3** *trans*	29.0	0.0	22.6
Experimental			
(12)	14.6	*	22.2
(1)	19.0	*	20.5
Radical **4** *cis*	−17.2	−0.3	<3.0
Radical **4** *trans*	−17.0	−2.8	<3.0
Experimental (12)	−18.9	*	*



Radical 3



Radical 4

Other values for the bond lengths change only slightly the absolute values of the coupling but not the main trends. The couplings due to the methyl protons are identical to those in the H-addition radical at C6 whereas the coupling of the β-proton at C6 is calculated to be smaller than that of the methylene protons in the H-addition radical at C6. The coupling caused by the hydroxyl proton varies with its orientation and is maximum in the *cis* orientation. With the OH bond length equal to 0.97 Å it is at most 5.0 G. Despite the too large value for the proton bonded to C6 in radical **3**, the calculations give satisfactory agreement with the experimental couplings.

Concerning the couplings due to nitrogen nuclei, one may add the following. In radicals **1** and **3**, only nitrogen N3 gives significant coupling ($a_{\text{iso}} = -0.9$ G; $b_{\text{aniso}} = -0.6$ G). On the other hand, in radicals **2** and **4**, it is nitrogen N1 which contributes coupling ($a_{\text{iso}} = 1.4$ G; $b_{\text{aniso}} = 1.9$ G). These values are slightly smaller for the H-addition radicals than for the OH-addition radicals.

4. Abstraction Radicals

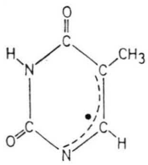
Two types of abstraction radicals have been studied. The one resulting from hydrogen abstraction from the nitrogen N1 (radical **5**) and that resulting from hydrogen abstraction from the methyl group (radical **6**). Both radicals can be the result of deprotonation of the cation radical. Therefore, the cation radical is also included. The cation radical has already been calculated by Bergene and Melo [14] as well as Adams and Box [15]. The last authors have in addition calculated the cation radical deprotonated at N1, which is identical to the radical resulting from hydrogen from nitrogen N_1 (radical **5**). We recalculated both, as well as the cation radicals of 1-CH_3-thymine and thymidine in order to see if the substitution at N1 would change the couplings. Table III contains the results for the thymine, 1-

Table III. Theoretical coupling constants for the cation radicals of thymine [H(x) = H(N1)], 1-CH_3-Thymine [H(x) = H(CH_3); $B_0 + \frac{1}{2} B_2$], and thymidine [H(x) = H(C1′)] as calculated by INDO. The results for the deprotonated cation (radical 5) and from experiment are also given.

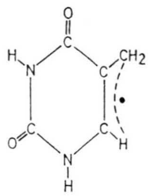
	N1		N3		H(C6)	H(CH_3)	H(x)	H(N3)
	a	b	a	b		$B_0 + \frac{1}{2} B_2$		
Thymine	10.3	6.4	−2.6	−1.7	2.8	13.1	−11.6	2.0
1-CH_3-Thymine	11.0	7.2	−2.1	−1.0	2.8	11.0	10.0	1.2
Thymidine	11.8	9.5	−2.0	−1.5	3.1	11.0	4.7	0.6
Experimental thymine								
(16)	4.0–5.3	3.3–4.0	<2	*	<4	20.4	*	*
(17)	6.1–6.4	3.5–3.8	*	*	*	19.1	*	*
Thymine Radical **5**	10.1	8.0	−2.3	−1.3	4.8	11.7	*	1.1

CH_3-thymine, and thymidine cation radicals together with those for the thymine cation deprotonated at N1. The experimental couplings obtained from polycrystalline samples[16] and single crystals[17] of thymine are also presented.

As already noted by Adams and Box[15], the results are qualitatively but not quantitatively correct. It has often been remarked that INDO calculations overestimate nitrogen couplings. However, one can say that the cation radicals of thymine and its N1 substituted derivatives should have similar ESR spectra, since the calculations give roughly the same couplings. Furthermore, the absence of the large coupling due to the hydrogen bonded to N1 of thymine in the spectra of single crystal as well as of alkaline ice indicates that the species observed experimentally is the deprotonated cation (radical **5**). We also calculated cation radicals deprotonated at N3 and at both N1 and N3. The calculations show that such radicals should be of σ symmetry rather than π symmetry with large spin density on N3 and none at all on the methyl group. A change in molecular geometry can easily induce a transformation from π to σ symmetry. Therefore, further study with geometry optimization and annihilation of higher spin state contaminants is necessary before making conclusions about these radicals.



Radical **5**



Radical **6**

For radical **6**, the bond lengths C3 – C5, C5 – C6 and C5 – CH_2 were set equal to 1.45 Å and the C – H bonds of the CH_2-group were assumed to make an angle of 120°. The neutral radical has been calculated as well as the radicals obtained after deprotonation at N1, N3, and at both positions. The results are contained in Table IV. The main difference between the experimental couplings obtained in single crystals[18] and in solution[19], where the proton bonded to N1 is absent, is the decrease of the coupling of the proton bonded to C6. The calculations reproduce this trend. Furthermore, the ratio of the calculated isotropic couplings of the nitrogen N1 and N3 is in agreement with the experimental results of Neta[19] only for radical **6** deprotonated at N1.

In their analysis of the radicals produced by γ-irradiation of frozen aqueous solutions of dTMP, Gregoli *et al.*[1] could isolate four radicals and identify three of these. The fourth radical (the spectrum of which is essentially a quartet and therefore called T_4) was tentatively assigned to a cationic species

Table IV. Theoretical coupling constants for different structures of the hydrogen abstraction radical from the methyl group as calculated by INDO for thymine and 1-CH_3-thymine. The experimental values taken from single crystal spectra (18 and 20) and from solution spectra (19) are also given.

	H(C6)	H′($\dot{C}H_2$)	H″($\dot{C}H_2$)	H(N1)	N1		$\frac{N1\ iso}{N3\ iso}$
					a	b	
Thymine Radical **6**	−12.1	−16.1	−16.0	−2.4	1.2	1.9	8.8
Radical **6** Deprotonated at N1	− 3.8	−15.6	−15.5	*	2.3	2.3	2.3
Radical **6** Deprotonated at N3	−10.3	−15.5	−14.6	*	2.3	2.3	11.5
Radical **6** Deprotonated at N1 and at N3	− 2.6	−13.3	−12.7	*	3.9	3.4	3.2
Experimental thymine (18)	−11.3	−15.4	−15.4	*	*	*	*
(19)	− 7.79	−16.06	−15.38	*	0.83	*	2.2
1-CH_3-thymine Radical **6**	−12.0	−15.6	−16.0	*	1.3	1.7	7.7
Radical **6** Deprotonated at N3	−10.5	−14.5	−15.4	*	2.2	2.0	14.5
Experimental 1-CH_3-thymine (20)	− 9.9	−16.1	−15.7	*	*	*	*

of the thymine molecule but all possible structures of the radical of H-abstraction from the methyl group could not be excluded. Our purpose is to show that this fourth radical is radical **6**. Since the cation radicals of the thymine derivatives should all give similar spectra (exemplified in Fig. 3 of ref. 16), cationic species can be excluded. The powder spectrum of radical **6** in the deprotonated form has been reported by Sevilla and coworkers (Fig. 1 of ref. 21). This spectrum presents a larger number of hyperfine lines than T_4. The calculations reflect this fact since, in the deprotonated form, the proton bonded to C6 has a much lower coupling than both methylene protons. Thus, the spectrum of the deprotonated form of radical **6** should present more hyperfine lines than that of radical **6** itself, where all three α-protons have roughly identical couplings.

We have now to compare the spectrum T_4 with that of radical **6** in single crystals[20]. For this purpose, we used the following experimental result. Schmidt[20] has shown that after warming to 200 °C an irradiated single crystal of 1-CH_3-thymine, only radical **6** is present. In Fig. 1 is shown the powder spectrum of X-irradiated 1-CH_3-thymine obtained by us after warming for 20 min to 200 °C. Except for a singlet component in the centre of the spectrum, this spectrum is identical to that of the fourth radical isolated by Gregoli and co-workers, T_4 [1]. The INDO results for radical **6** of 1-CH_3-thymine are also contained in Table IV. It can be seen that the substitution at N1 does not change the spin density distribution in such radicals. In support of this identification, there is the fact that the spectra attributed to the deprotonated forms of the cation radical and of radical **6** are only observed in γ-irradiated alkaline ice of thymine, where the molecule is known to be deprotonated at N1. In such ice, the nucleoside and nucleotide display the spectrum of T_4. In neutral aqueous solutions of thymine and its derivatives, again T_4 is observed[1].

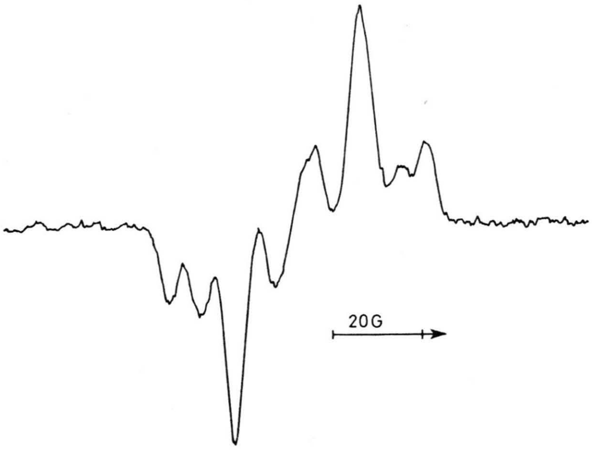


Fig. 1. ESR spectrum of polycrystalline 1-CH_3-thymine X-irradiated at room temperature and heated to 200 °C for 20 min showing the powder spectrum of radical **6**.

5. Conclusions

For the addition radicals, the agreement between experimental and theoretical couplings is good. INDO calculations can therefore be used with a predictive value if applied, with our geometrical parameters, to addition radicals of aromatic molecules. However, the spin density distribution in nitrogen-centrered radicals seems to be more difficult to reproduce. Simple changes in bond lengths induce a transformation of π to σ symmetry in such radicals. A more detailed study based on geometry optimization is needed.

The calculations support the identification made by Gregoli *et al.*[1] of the OH-addition radical to C6 in γ-irradiated aqueous solutions. Concerning the spectrum T_4 left unidentified by these authors but hypothetically assigned to a cationic species, the calculations together with some experimental investigations suggest that it is due to the radical resulting from hydrogen abstraction from the methyl group. Since Gregoli and coworkers[1] have shown that T_4 decays into the OH-addition radical at C6 upon warming, a new reaction mechanism must be considered. This can be some hydration reaction by which radical **6** adds a water molecule to transform into radical **3**.

Note added in proof:

After acceptance of this paper, Sevilla sent us a preprint of a work on "N1-substituted thymine π-cation radicals".

The ESR parameters of all N1-substituted thymine derivative cation radicals are similar as predicted by the calculations. Further, their spectra have no similarity with thes pectrum T_4.

---

[1] S. Gregoli, M. Olast, and A. Bertinchamps, Radiation Res. **65**, 202 [1976].

[2] Quantum Chemistry Program Exchange, Department of Chemistry, Bloomington, Indiana 47401.

[3] J. A. Pople and D. L. Beveridge, Approximate Molecular Orbital Theory, MacGraw-Hill, New York 1970.
[4] E. Westhof, W. Flossmann, and A. Müller, Mol. Phys. **28**, 151 [1974].
[5] E. Westhof and W. Flossmann, J. Amer. Chem. Soc. **97**, 6622 [1975].
[6] W. Flossmann, E. Westhof, and A. Müller, J. Chem. Phys. **64**, 1688 [1976].
[7] M. G. Ormerod, Int. J. Radiat. Biol. **9**, 291 [1965].
[8] A. Müller and J. Hüttermann, Ann. Acad. Sci. N.Y. **222**, 411 [1973].
[9] T. Henriksen and W. Snipes, J. Chem. Phys. **52**, 1997 [1970].
[10] W. Flossmann, E. Westhof, and A. Müller, Int. J. Radiat. Biol. **28**, 105 [1975].
[11] J. L. Flippen, Acta Crystallogr. **B 29**, 1756 [1973].
[12] M. G. Ormerod and B. B. Singh, Int. J. Radiat. Biol. **10**, 538 [1966].
[13] W. Flossmann, E. Westhof, and A. Müller, Int. J. Radiat. Biol. **25**, 437 [1974].
[14] R. Bergene and T. B. Melø, Int. J. Radiat. Biol. **23**, 263 [1973].
[15] S. M. Adams and H. C. Box, J. Chem. Phys. **63**, 1185 [1975].
[16] M. D. Sevilla, J. Phys. Chem. **75**, 626 [1971].
[17] A. Dulcic and J. N. Herak, J. Chem. Phys. **57**, 2537 [1972].
[18] J. Hüttermann, Int. J. Radiat. Biol. **17**, 249 [1970].
[19] P. P. Neta, Radiat. Res. **49**, 1 [1972].
[20] J. Schmidt, J. Chem. Phys. **62**, 370 [1975].
[21] M. D. Sevilla, C. van Paemel, and G. Zorman, J. Phys. Chem. **76**, 3577 [1972].
[22] R. Gerdil, Acta Crystallogr. **14**, 333 [1961].
[23] K. Ozeki, N. Sakabe, and J. Tanaka, Acta Crystallogr. **B 25**, 1038 [1969].