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Note

Use of the Spin-Trapping Method for the Study of Stable Radicals Produced in Solid Dihydrothymine

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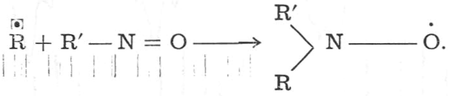
ESR spin-trapping method has been used for the study of radiation-induced radicals in powdered dihydrothymine. Four approximately equal small proton couplings ($A_{H'} = 0.35$ mT), a larger proton coupling ($A_H = 0.195$ mT) and a nitrogen coupling ($A_N = 0.295$ mT) are attributed to the nitroxide radical formed by reaction of the spin trap with 6-dihydrothymyl radical upon dissolving of dihydrothymine. In the pyrimidine bases of nucleic acid and a number of related compounds irradiated in solid state and dissolved, no signs of the trapped radicals were observed.

The electron spin resonance (ESR) spin-trapping method has already been extensively used for the study of short-lived free radicals in solution. The principle of the method is to convert highly reactive, short-lived primary radicals into relatively stable nitroxide radicals[1,2]. A spin trapping compound is usually a nitroso compound, R′ — N = O, which reacts with a reactive radical $\dot{R}$ to form a nitroxide radical according to the following reaction:



$$\dot{R} + R'-N=O \longrightarrow \begin{matrix} R' \\ R \end{matrix}\!\!>N-\dot{O}.$$

The nitroxide radical, $RR'N\dot{O}$, is stable enough to be easily detected by ESR.

It has been demonstrated[3,4] that the spin trapping method can be used for the study of radicals formed in solid state samples. The irradiated samples are dissolved in a solution of the nitroso scavenger which reacts with the radicals from the dissolving solid. In such a way the radicals formed in a solid are studied in solution with the expected advantages of the ESR spectroscopy in solution: multiple increase in resolution and sensitivity.

The present study has been undertaken with the aim of comparing the radicals formed by irradiation of the nucleic acid bases and the related compounds in solid state with the radicals formed in the same compounds in solution. The radicals formed in solution have been quite well understood[5,7]. In our study *t*-nitrosobutane (tNB) was employed as a spin trap. We used various concentrations of tNB in neutral or basic media (nondeuterated or deuterated). Prior to dissolving irradiated powder samples, the tNB solution was stirred for 15 hours in the dark. In such a way a monomer-dimer equilibrium was shifted more toward the monomer[7]. The powder samples of the pyrimidine bases, several halogenated bases (5-Br uracil, 5-Cl uracil, 5-F uracil) and dihydroderivatives (5,6-dihydrouracil, 5,6-dihydrothymine, 5,6-dihydro 6-methyl uracil, 5,6-dihydro orotic acid) were irradiated with gamma rays to a total dose of about 100 kGy. In all of the samples an appreciable concentration of radicals could be detected in powdered samples. After dissolving the samples in the tNB solution, radicals could be detected only in two dihydropyrimidines, dihydrothymine and dihydrouracil, and could be analysed only in one — dihydrothymine. The ESR spectra of dihydrothymine radical in heavy water solution of tNB is shown in Figure 1. The dominant triplet splitting of

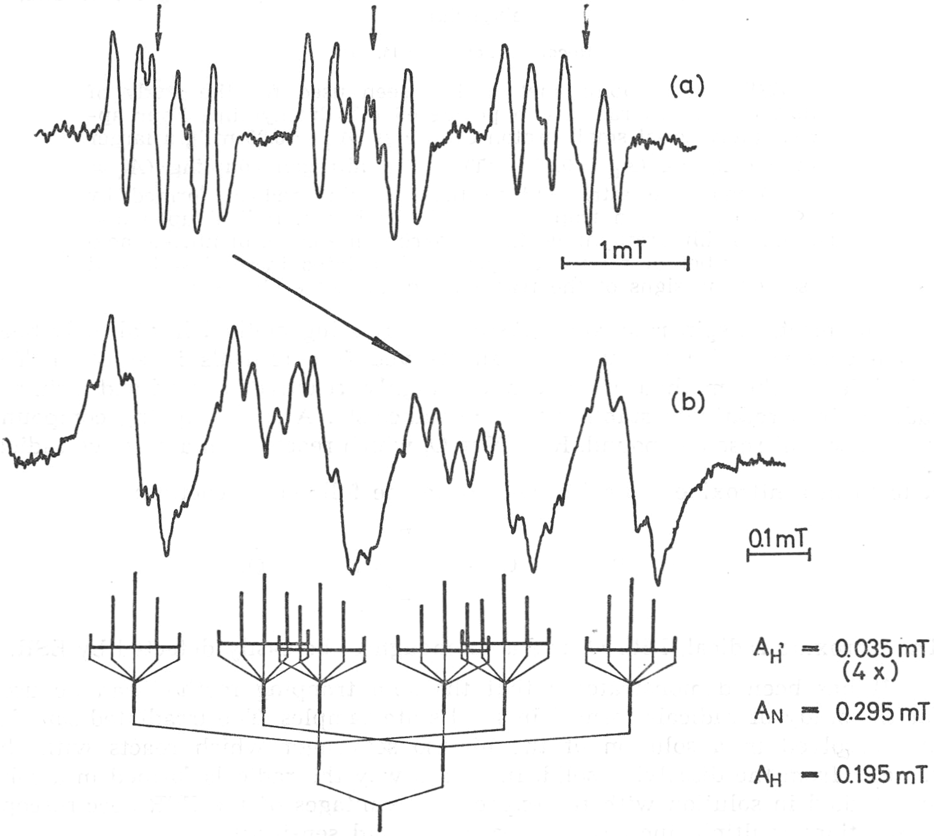
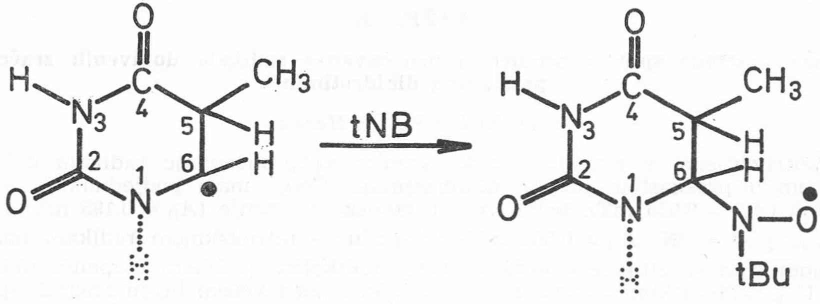


Figure 1. — ESR spectrum of dihydrothymine, gamma-irradiated at room temperature and dissolved in the heavy water solution of tNB, with 0.25 M NaOH: (*a*) complete ESR spectrum; the arrows indicate the positions of the di-*t*-butyl nitroxide lines, (*b*) the expanded $M_I = -1$ nitroxide nitrogen line. The stick diagram shows the positions of the resonance lines calculated with the use of the indicated coupling parameters.

1.57 mT originates from the interaction of the unpaired electron with ^{14}N of the nitroxide. That coupling gives only modest information on the trapped radical. More informative are the secondary hyperfine splittings. The sextet of any of the three groups of lines (upper spectrum in Figure 1) is superimposed with a weak signal of the di-*t*-butyl nitroxide spectrum[7], indicated by the arrows in Figure 1. It is the presence of this spectrum that gives rise to a slight difference in appearance of the $M = -1$, $M = 0$ and $M = +1$ lines in Figure 1a.

The relative intensity of the six lines is very close to 1 : 1 : 1 : 1 : 1 : 1; the intensity ratio is not changed by varying the conditions of sample treatment or recording the spectra. As seen from Figure 1b, the structure of all six lines is identical (except when they are superimposed). We conclude that we deal with a single type of radicals.

The 1 : 1 : 1 : 1 : 1 : 1 sextet of any of the three groups of lines comes from a proton coupling, $A_H = 0.195$ mT, and a ^{14}N nucleus coupling, $A_N = 0.295$ mT. Further splitting of each of the six lines into approximately a 1 : 4 : 6 : 4 : 1 quintet may be brought about by approximately equal coupling of four protons with $A_{H'} = 0.035$ mT.



(I) (II)

The radical giving rise to such a hyperfine ESR spectrum must be the radical formed from the 6-dihydrothymyl radical (I) by reaction with tNB. The triplet splitting of 0.295 mT is assigned to N1. The 0.195 mT doublet comes from the C6-H proton. The hydrogen bound to N1 does not give any observable coupling, because the ESR spectra are the same in regular and heavy water. The radical is probably deprotonated in the basic medium. The remaining four small proton couplings must originate from the interaction of the three methyl protons and the hydrogen atom bound to C5. Although in this scheme the δ protons (methyl protons) give rise to a rather high hyperfine splitting (0.035 mT), we do not see any other possibility of explaining the observed ESR pattern.

Radical **II** formed from primary radical **I** that has been formed directly in solution gives rise to a quite different resonance pattern[6]. It is possible that the difference comes from different conformations of radical **II**, as a consequence of possible different conformations of the primary radical, radical **I**, formed in two different environments.

Unfortunately, we have been unable to extend this study to other systems, because we could not detect any nitroxide radicals in other dissolved samples that had been irradiated in solid state. It is possible that some other spin trap would be more reactive toward the primary radicals upon dissolving the host solid, to yield higher concentration of the secondary, nitroxide radicals. However, such a trap should be a simple compound which does not give any internal hyperfine couplings in the nitroxide radical. On the other hand, the unpaired electron in the nitroxide radical must be close enough to the skeleton of the primary radical to sense the paramagnetic nuclei in the vicinity of the trap reaction site. At present we have been unable to find such a trap.

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SAŽETAK

Primjena metode spinske stupice u proučavanju radikala dobivenih zračenjem praškastog dihidrotimina

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Upotrijebljena je metoda spinske stupice za proučavanje radikala dobivenih zračenjem u praškastom uzorku dihidrotimina. Četiri mala podjednaka protonska cijepanja ($A_{H'} = 0.035$ mT), jedno veće protonsko cijepanje ($A_H = 0.195$ mT) i jedno cijepanje jezgre ^{14}N ($A_N = 0.295$ mT) pripisuju se nitroksidnom radikalu nastalom reakcijom spinske stupice s 6-dihidrotimil radikalom prilikom otapanja dihidrotimina. U pirimidinskim bazama nukleinskih kiselina i većem broju srodnih spojeva, ozračenih u čvrstom stanju, nisu opaženi nikakvi znakovi stabilnih radikala nakon otapanja u otapalu sa stupicom.

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