Influence of the pH in the $OH^{\cdot}$ Radical Attack on the Pyrimidic Ring

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The attack of OH radical on pyrimidic ring preferentially took place at position 5 at neutral pH and at position 6 at low acidic pH. The *trans* isomers were always the major products. These conclusions arose from studies involving comparison with synthetic peroxides: *cis*-6-hydroperoxy-5-hydroxy-5,6-dihydrothymine (**1**), *cis*-5-hydroperoxy-6-hydroxy-5,6-dihydrothymine (**2**), *trans*-6-hydroperoxy-5-hydroxy-5,6-dhydrothymine (**3**) and *trans*-5-hydroperoxy-6-hydroxy-5,6-dihydrothymine (**4**).

The NMR coupling constants of the OH-6 proton with H-6 allowed us to assign unambiguously the structures of these peroxides in agreement with ^{18}O mass spectrometry results. *G* radiolytic values and NMR data are given.

The discovery and the synthesis of (5 or 6)-hydroperoxy(6 or 5)-hydroxy-5,6-dihydrothymine have been of considerable interest in radiation chemistry of nucleic acids [1,2]. These key compounds provide most of the other radiolytic substances [3]. However the data published on this subject are very disappointing because they seem to be conflicting. In this report, we wish to draw attention to some facts which give a clearer picture of the question and also to describe results concerning NMR coupling constants, *G* values at different pH of these important compounds.

The assignment of the structures required sufficient available quantities and spectroscopic methods. Two corrections [4–6] to the initial structure have been successively made.

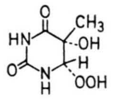
Cis or trans form

Nofre [6] proposed to change the initial structure of trans peroxides into cis peroxides and conversely. This modification was based on the results of Benn *et al.* [7] concerning the structure of cis thymine glycol obtained by permanganic oxidation of thymine. In fact, the glycol resulting from the action of silver oxide on trans 5-bromo-6-hydroxy-5,6-dihydrothymine had the cis configuration.

Position of OOH either in 5 or in 6 position

There were interesting results obtained by recent EPR indirect or flow methods [8,9]. Some doubts could be entertained [10,11] about the position of the OOH on the pyrimidic ring either in position 5 or in 6.

A detailed study has shown that a rearrangement took place during the substitution of the halogen by OH in thymine bromohydrin or 5-bromo-6-hydroperoxy-5,6-dihydrothymine [4]. These conclusions arose from the mass spectra degradation pattern of substances selectively labelled ^{18}OH [4,5]. It appears that all the structures proposed and used until recently [12–16] should be revised *i.e.* the peroxides known as 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine and conversely. The right R_F values of these compounds for thymine and thymidine have been described in recent papers [4,5,17].



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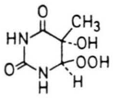

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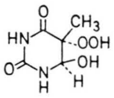

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The mass spectra results have been confirmed unambiguously by the analysis of the proton coupling constants of the OH-6 exo group with the H-6 vicinal. When OOH substituent was present in the 6-position there was no coupling. In double resonance experiments, when the H-6 was irradiated the OH-6 doublet of the compound **4** collapsed into a singlet.

Table I. NMR chemical shifts and coupling constants of peroxides (TMS, DMSO).

	CH_3	H-6	OH or OOH (5)	OOH or OH (6)	H-1	H-3
1	1.35 s	4.62 d (4.5 Hz)	5.29 s	11.57 s	8.30 d (4.5 Hz)	10.02 s
3	1.34 s	4.56 d (4.5 Hz)	6.07 s	11.72 s	8.20 d (4.5 Hz)	9.95 s
4	1.35 s	4.53 dd	11.70 s	6.44 d (4.5 Hz)	8.13 d (4 Hz)	10.13 s

1 = *cis* 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine.
3 = *trans* 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine.
4 = *trans* 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine.

In quantitative analysis the *G* values published are also quite different. It is worthwhile noting that the peroxides are rather unstable products in aqueous neutral or alcaline medium and under radiolytic conditions [2,13,14]. Furthermore we have demonstrated that the site of hydroxyl radical attack on the pyrimidic ring was dependent on the pH of the solution [4].

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Table II. Approximative G values at different pH.

Products	G at different pH 1.5	4	6
cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine	0.14	0.32	0.30
cis 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine	0.19	0.08	0.08
trans 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine	0.30	0.67	0.65
trans 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine	0.41	0.17	0.18

At a pH value below 1.7 and at a concentration 10^{-3} M of thymine, the attack of OH rather took place at position 6. Above this pH value it was in position 5 and 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine was preferentially obtained.

The G values measured from irradiated ^{14}C labeled thymine ($2 \cdot 10^{-3}$ M) are given in the Table II.

The G values proposed in Table II are in perfect agreement, with the analysis of EPR spectra recorded at different pH [8, 9].

In conclusion, it is important to bear in mind the influence of the pH when quantitative studies concerning peroxides are performed.

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