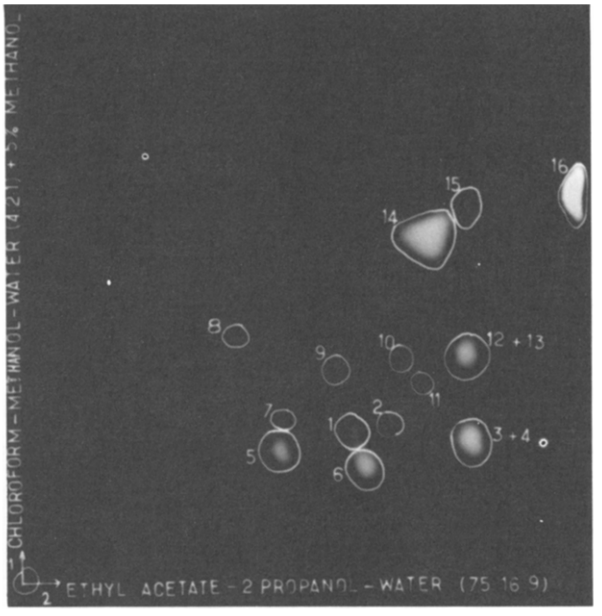

FIG. 1 : Bidimensional thin layer chromatography on Macherey Nagel silica gel MN-S-HR of thymine gamma radiolytic products in aqueous aerated media.
Solvent I : chloroform-methanol-water (4:2:1) (v/v/v), lower phase with 5 % of methanol added.
Solvent II : ethyl acetate-2-propanol-water (75:16:9) (v/v/v)

1 = cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine, 2 = cis 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine ; 3 = trans 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine ; 4 = trans 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine ; 5 = cis 5,6-dihydroxy-5,6-dihydrothymine ; 6 = trans 5,6-dihydroxy-5,6-dihydrothymine ; 7 = 5-hydroxymethyluracil ; 8 = unknown substance ; 9 = 5-hydroxy-5,6-dihydrothymine ; 10 = 6-hydroxy-5,6-dihydrothymine ; 11 = 5-hydroperoxymethyluracil ; 12 = 5-hydroxy-5-methyl barbituric acid ; 13 = 5-hydroxy-5-methyl hydantoin ; 14 = thymine ; 15 = acetylurea ; 16 = N-formyl-N'-pyruvylurea.

of this peroxide which were published earlier (4) were confirmed recently (8).

However, in spite of a recent claim (8), we maintain that the major radiolytic product at neutral pH of thymine is trans 6-hydroperoxy-5-hydroxy -5,6-dihydrothymine **3** .

EXPERIMENTAL PART

Permanently aerated solutions (10 mM) of thymine $^{14}C_2$ were irradiated (6000 rads/min, 7 hours) with gamma rays generated by a ^{60}Co source. The irradiated solution was evaporated to dryness under reduced pressure. The residual syrup was dissolved in the minimal volume of the methanol and then submitted to

RADIATION CHEMISTRY of NUCLEIC ACIDS :

CHARACTERIZATION of THYMINE HYDROXY-HYDROPEROXIDES

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Summary :

The four hydroxyhydroperoxides of thymine produced at pH 6.5 under γ irradiation of thymine in an aerated aqueous solution (10 mM) i.e. cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine **1** *(G = 0.22) ; cis 5-hydroperoxy-6-hydroxy -5,6-dihydrothymine* **2** *(G = 0.04) ; trans 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine* **3** *(G = 0.42) ; trans 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine* **4** *(G = 0.08) have been isolated, synthesized and unambiguously characterized. The cis peroxides gave by H_2S or DMSO reduction cis thymine glycol and, in the same way, trans peroxide yielded trans thymine glycol.*

NMR spectra of **1 , 3 , 4** *enabled us to determine unambiguously the position of the OOH group on the pyrimidic ring. When the hydroxyl group was located at position 6 a doublet JOH_6-H_6 was observed. This doublet collapsed into a singlet when H-6 proton was irradiated in double resonance experiments.*

In a preceeding article (1), we have described the synthesis and the isolation of ten peroxides produced in gamma irradiation of thymine in an aerated aqueous solution. Three out of these ten peroxides i.e. the isomers of (5 or 6)-hydroperoxy-(6 or 5)-hydroxy-5,6-dihydrothymine were determined previously (2).

The original structural assigment of these latter compounds was based on the specific substitution of bromine atom in trans 5-bromo-6-hydroperoxy -5,6-dihydrothymine by the OH group (2). In preceeding papers, we draw attention to the fact that there was no definitive evidence about the position of hydroperoxy group either at C-6 or C-5 on the pyrimidic ring (3).

Mass spectrometry studies with peroxides specifically labeled ^{18}O enabled us to shown that an anionotropic rearrangement took place during the substitution of the halogen (4). These findings allowed us to revise (4-6) the structures of the peroxides generally admitted (1, 7). For example, it was demonstrated that cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine **1** was obtained by the treatment of cis 5,6-dihydroxy-5,6-dihydrothymine with H_2O_2 in a chlorhydric medium (4). Our results concerning the structural assignment

thin layer chromatography system (5) (see legend of fig. 1). The radiolysis products could be located by autoradiography and the peroxides could be detected after the spraying of a potassium iodide solution. As it may be observed in fig.1, the preparation of large quantities of pure peroxides required two successive linear chromatographic runs in systems I and II.

The G radiolytic values measured by liquid scintillation counting. The relative importance of peroxides **3** and **4** (*vide supra*) has been established by the measurement of the NMR signals[1] surface.

RESULTS

Cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine was very easily obtained from cis 5,6-dihydroxy-5,6-dihydrothymine (4). HAHN and WANG prepared the same product by this quantitative method (8). The infra-red and NMR spectra of cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine were identical with those reported by these authors.

However there is a discrepancy between the results (Rf and relative amounts) concerning the peroxides obtained by gamma irradiation (4, 8).

Two important points must be emphasized :

- cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine **1** was not the faster hydroxyhydroperoxide in solvent II but the slower one, as could be verified with an authentic sample obtained by chemical synthesis (4, 8) (Table 1).
- cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine **1** was not the major radiolytic product as is shown in the following table.

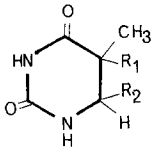
In fact, trans 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine **3** and trans 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine **4** were the faster peroxides in solvent system II. Furthermore compound **3** was the major product obtained by gamma irradiation of thymine (10^{-2} M) at pH 6.

That is demonstrated by the following experiments :

1/ - H_2S reduction : cis or trans configuration

The heterolysis of peroxidic bond under the action of reducting agents such as H_2S took place in a stereospecific way and gave rise to the corresponding alcohol (1, 9). The C-O configuration was retained. Under these conditions the trans peroxides were transformed into trans glycol. The products **3** and **4** gave trans thymine glycol as it could be verified by infrared, NMR and mass spectrometry (1, 10) in perfect agreement with recent results (11). In the other hand, the compounds **1** and **2** gave rise to cis thymine glycol.

N°	Name of the products	G	Rf_I	R_{Th_I}	Rf_{II}	$R_{Th_{II}}$
1	cis 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine	0.22	0.38	0.49	0.43	0.68
2	cis 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine	0.04	0.38	0.49	0.52	0.83
3	trans 6-hydroperoxy-5-hydroxy-5,6-dihydrothymine	0.42	0.36	0.46	0.72	1.14
4	trans 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine	0.08	0.36	0.46	0.72	1.14



1 R_1 = OH R_2 = OOH cis
2 R_1 = OOH R_2 = OH cis
3 R_1 = OH R_2 = OOH trans
4 R_1 = OOH R_2 = OH trans

TABLE 1 : Rf, G values and formula of thymine hydroxy-hydroperoxides

2/ - NMR data : determination of peroxidic group in 5 or 6 position on pyrimidic ring

The values of chemical shifts and coupling constants are summarized in table II.

The NMR spectra in DMSO (method described in ref 1) of the faster peroxides mixture (Rf_1 = 0.36 ; Rf_2 = 0.72) isolated from the radiolytic medium show the occurence in different quantities of two hydroxy-hydroperoxides which were transformed into trans 5,6-dihydroxy-5,6-dihydrothymine and not into the cis isomer. The NMR signal of the hydroxyl function of the compound **4** (the minor one in the mixture) appeared as a doublet which collapsed into a singlet by double resonance experiments (irradiation of H-6 at δ = 4.57 ppm). The coupling between the methinic proton H-6 and the geminal OH allowed us to determine the structure of compound **4** unambiguously i.e. trans 5-hydroperoxy-6-hydroxy-5,6-dihydrothymine. This assignment was confirmed by the singlet relative to the tertiary OH group which characterized compound **3** (the major one in the mixture).

It is worth noting that the singlet observed for the OH group in compound **1** accounted for the 6-hydroperoxy-5-hydroxy structure.

Furthermore, the NMR chemical shift values could be compared. It appears that there were no significant differences for δ CH_3 and δ H-6. The chemical shifts of OH, H-1 and H-3 were in agreement with the proposed structure but it was not sufficient to assign the structures of products **3** and **4** . They were

	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
5	1.26 s	4.36 dd	5.22 s	5.96 d (4.5 Hz)	8.09 d (4 Hz)	9.99 s
6	1.26 s	4.35 dd	5.78 s	6.09 d (4.5 Hz)	7.96 d (4 Hz)	9.85 s

TABLE II : Chemical shifts (δ ppm) and coupling constants (Hz) of (5 or 6)-hydroperoxy-(6 or 5)-hydroxy-5,6-dihydrothymine and thymine glycols

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

5 = cis 5,6-dihydroxy-5,6-dihydrothymine

6 = trans 5,6-dihydroxy-5,6-dihydrothymine

s = singlet d = doublet dd = double doublet

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only valid if the NMR spectra of both products were compared.

3/ - Chemical synthesis

The products **3** and **4** were prepared by the solvolysis of trans 6-hydroxy-5-iodo-5,6-dihydrothymine with hydrogen peroxide in the presence of trifluoracetic acid. Trans 5,6-dihydroperoxy-5,6-dihydrothymine appeared as the intermediate compound. Their structures were demonstrated by mass spectrometry experiments involving selectively labeled ^{18}OH compounds (4, 6). Chromatographic and spectroscopic NMR values, were respectively identical to those of radiolytic products **3** and **4** .

In conclusion, at neutral pH, the initial attack of OH radical preferentially took place in position 5 on the pyrimidic ring in agreement with EPR experiments (12). The trans form of the resulting peroxide was preferentially generated.

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