Radiolysis of Thymine in Aerated Aqueous Solution

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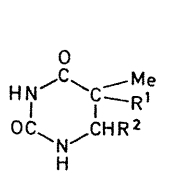
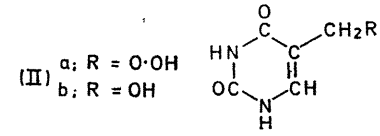
Summary Twenty-three products of radiolysis of thymine in aerated aqueous solution have been identified and classified in a coherent scheme.

WE report the determination of the structure of fifteen new radiolysis products from aerated aqueous solutions of thymine, which has enabled us to establish a coherent scheme of radiolytic degradation for this base—a compound of interest in the field of radiation chemistry of nucleic acids.[1]

[^{14}C]Thymine (2×10^{-3}–2×10^{-2} M) in aerated aqueous solution was irradiated with ^{60}Co-γ-rays (2 kCi). The radiolysis products were separated by chromatography (Table 1),[2] and were identified by their spectroscopic properties (i.r., u.v., n.m.r., and mass spectra) and chemical properties.[3] The structures were confirmed by independent syntheses. The main products of γ-radiolysis of thymine (2×10^{-3} M) in aerated aqueous solution are shown in the Scheme, over 90% of the starting material is thus accounted for.



(I) a; R^1 = O·OH, R^2 = H
b; R^1 = OH; R^2 = H
c; R^1 = H; R^2 = O·OH
d; R^1 = H, R^2 = OH
e, R^1 = O·OH; R^2 = OH
f; R^1 = OH; R^2 = OH
g; R^1 = OH, R^2 = O·OH
h; R^1 = OH; R^2 = OH



(II) a; R = O·OH
b; R = OH

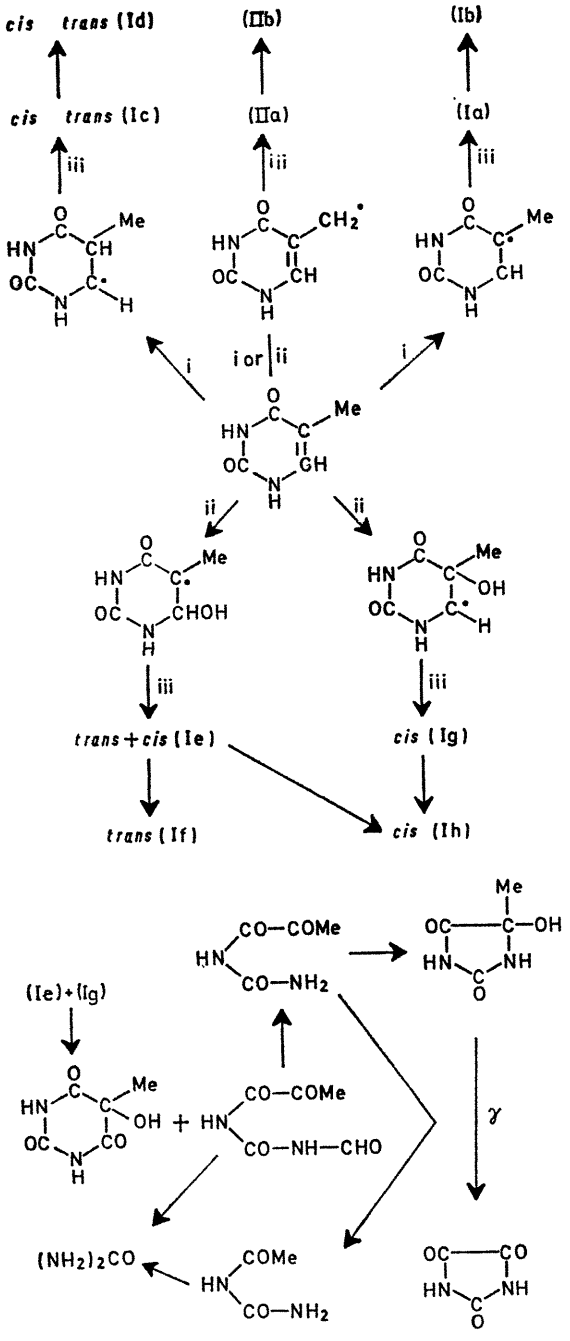
Reaction of OH radicals (produced by radiolysis of water) with thymine gave 6-hydroxy-5,6-dihydro-5-thymyl and 5-hydroxy-5,6-dihydro-6-thymyl radicals, while reaction of thymine with hydrogen atoms [or e^-(aq.); H^+] gave (5-uracyl)methyl, 5,6-dihydro-5-thymyl, and 5,6-dihydro-6-thymyl radicals in low yield.

TABLE 1

R_F *and* G *values of thymine radiolysis products* (MN-S-HR *silica gel*)

Products	Solvents[a] (A)	(B)	G values[b]
Thymine	0·78	0·63	−2·6
Urea	0·34	0·20	0·075
5-Hydroxydihydrothymine	0·54	0·43	0·016
cis- and *trans*-6-Hydroxydihydrothymine	0·57	0·55	0·008
cis-Thymine glycol	0·34	0·30	0·125
trans-Thymine glycol	0·30	0·45	0·123
Dihydrothymine	0·80	0·57	—
5-Hydroxymethyluracil	0·42	0·32	0·017
5-Hydroxy-5-methylbarbituric acid	0·55	0·68	0·149
5-Hydroxy-5-methylhydantoin	0·53	0·74	
Acetylurea	0·79	0·70	0·010
Formylurea	0·73	0·69	0·065
Formylpyruvylurea	0·77	0·94	0·460
Parabanic acid	0·69	0·94	—
trans-5-Hydroperoxy-6-hydroxydihydrothymine	0·36	0·72	0·830
cis-5-Hydroperoxy-6-hydroxydihydrothymine	0·38	0·43	0·314
cis-6-Hydroperoxy-5-hydroxydihydrothymine	0·38	0·52	0·081
5-Hydroperoxymethyluracil	0·50	0·58	0·047
5-Hydroperoxydihydrothymine	0·60	0·65	0·062
cis-6-Hydroperoxydihydrothymine	0·63	0·69	0·027
trans-6-Hydroperoxydihydrothymine	0·65	0·75	0·028
5-Hydroperoxy-5-methylhydantoin	0·56	0·81	0·005
5-Hydroperoxy-5-methylbarbituric acid	0·59	0·87	0·011
trans-5,6-Dihydroperoxydihydrothymine	0·40	0·83	0·009

[a] (A) = Chloroform–methanol–water (4 : 2 : 1) lower layer with 1% of methanol (two successive runs); (B) = Ethyl acetate–isopropyl alcohol–water (75 : 16 : 9). [b] Obtained in aerated acidic medium.



SCHEME

Reagent: i, H• ; ii •OH ; iii, O_2, red. [i may be e^-(aq) H^+]

Under optimum conditions, a yield of more than 50% of peroxides was obtained. For example, γ-irradiation of a solution of thymine (2×10^{-3} M) in acidic medium (9000 rad/min; 1 h) gave *trans*- (32%) and *cis*-5-hydroperoxy-6-hydroxydihydrothymine (12%); *cis*-6-hydroperoxy-5-hydroxydihydrothymine (3%); 5-hydroperoxymethyluracil (1·8%); 5-hydroperoxydihydrothymine (2·4%); and *cis*- (1·06%) and *trans*-6-hydroperoxydihydrothymine (1·1%). The spray reagent used did not always reveal the following products: *trans*-5,6-dihydroperoxydihydrothymine (0·35%); 5-hydroperoxy-5-methylbarbituric acid (0·45%); and 5-hydroperoxy-5-methylhydantoin (0·20%).

Decomposition of these peroxides gives rise to alcohols, along with other products. In the case of 5-(or 6-)hydroperoxy-6-(or 5-)hydroxydihydrothymine, a β-ketol was also obtained, together with the corresponding diureide resulting from ring-opening. The diureide was converted into monoureide and five-membered-ring products.

The alcohols that have been identified are *cis*- and *trans*-5,6-dihydroxydihydrothymine, 5-hydroxydihydrothymine, *cis*- and *trans*-6-hydroxydihydrothymine, and 5-hydroxymethyluracil.

From 5-(or 6-)hydroxy-6-(or 5-)hydroperoxydihydrothymine, 5-hydroxy-5-methylbarbituric acid was also obtained. The ring may also be opened, producing formylpyruvylurea. The latter was converted into acetylurea and formylurea.[4] Pyruvylurea was not identified among the radiolysis products, since on ring-closure it gives 5-hydroxy-5-methylhydantoin.[5] Parabanic acid is formed on γ-radiolysis of the latter compound.

TABLE 2

Decomposition products (%)†	(a)	(b)	(c)
cis-5,6-Dihydroxydihydrothymine	0·3	43	73
trans-5,6-Dihydroxydihydrothymine	59	0·2	3
5-Hydroxy-5-methylbarbituric Acid	4	traces	4·2
Acetylurea	1·5	—	—
Formylpyruvylurea	27	25	15
5-Hydroxy-5-methylhydantoin	3·5	7·5	3·9

† (a) = *trans*-5-Hydroperoxy-6-hydroxydihydrothymine.
(b) = *cis*-5-Hydroperoxy-6-hydroxydihydrothymine.
(c) = *cis*-6-Hydroperoxy-5-hydroxydihydrothymine.

Table 2 lists the products of decomposition in aqueous medium of the three most important thymine glycol hydroperoxides.

We thank Mrs. Georges, Mrs. Voituriez, and Miss Pouchot for technical assistance.

(*Received, November 27th*, 1970; *Com.* 2053.)

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[5] R. Teoule and J. Cadet, *Compt. rend.*, 1969, **268D**, 2501.