BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 46, 3896—3898 (1973)

The Dimerization of Thymine Glycol and the Splitting of the Dimer by γ-Irradiation in an Aqueous System

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(Received June 11, 1973)

It has been known that the radiolysis of thymine in an aqueous system provides thymine glycol as the main radiolysis product in either the presence¹⁾ or absence of air.²⁾ In our studies of the radiation chemistry of nucleic acid bases, we have found that, in the radiolysis of thymine under anaerobic conditions, there exists a novel reversible reaction between thymine and thymine glycol, and that the reverse reaction from the later is caused by the action of hydrated electrons.³⁾ It has also been observed that the radiolysis of thymine glycol gives several other products with the characteristic UV absorption (260 nm) positive or negative. This paper will deal with the isolation and identification of a novel dimer compound of thymine glycol as the main radiolysis product under N_2O saturation, and also with the dimer splitting to 5-methylbarbituric acid on radiolysis.

Results and Discussion

An aqueous solution of thymine glycol (I) (4 mM) was irradiated with γ-rays in the presence of N_2O to

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1) R. Latarjet, B. Ekert, S. Apergot, and N. Reybeyrotte, *J. Chim. Phys.*, **58**, 1046 (1961).

2) B. Ekert, *Nature*, **194**, 278 (1962).

3) M. Namiki and T. Hayashi, *Int. J. Radiat. Biol.*, **17**, 197 (1970); T. Hayashi and M. Namiki, *Agr. Biol. Chem.*, **36**, 551 (1972).

eliminate the action of hydrated electrons on thymine glycol ($e_{aq}^{-}+N_2O \rightarrow \cdot OH+OH^{-}+N_2$).[4] The irradiated solution was then concentrated and chromatographed on a column of Dowex 50W-H^+; a colorless crystalline product was thus obtained in the eluate. From the results of an elementary analysis ($C_{10}H_{10}N_4O_6$) and from the NMR{(100 MHz, DMSO-d_6 δ1.64 (s, $-CH_3$, 3H), δ11.14 (s, $>$NH, 2H)} and the IR {(KBr disk) 3200 (-NH), 1710, 1725, and 1750 cm^{-1} (C=O)} spectral data, the structure of this compound was assigned to be 5,5′-dimethyl-5,5′-bibarbituric acid (II).

To confirm the (II) structure, an authentic sample was prepared by a modification of the method used by Davidson and Baudisch in the synthesis of 6,6′-biisobarbituric acid,[5] using potassium ferricyanide in alkali as a condensing agent; it was demonstrated that the IR spectrum of the irradiate product agreed perfectly with that of an authentic one, as is presented in Fig. 1. The product (II) is considered to be a novel compound, and it seems to be the main radiolysis product, judging from the glc analysis of the irradiated products silylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Procedure A).

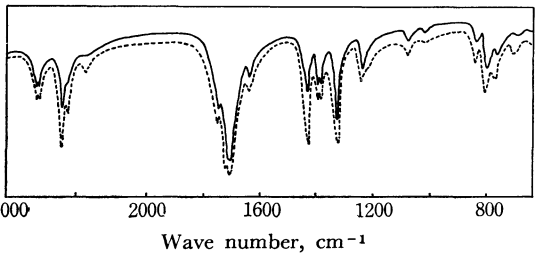
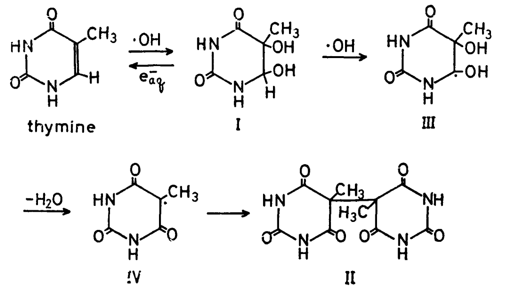


Fig. 1. IR spectra of synthesized (solid line) and isolated (broken line) II.

When its yields under various irradiation conditions were compared with those in air, in N_2 and in N_2O, it was shown that the formation was especially prominent in the case of the irradiation in the presence of N_2O, suggesting that the dimer formation is caused by the reaction with OH radicals. It may thus be proposed that the reaction is initiated by the abstraction of the C-6 hydrogen of I with the OH and/or H radical to form (III) radical, followed by dehydration to give the (IV) radical in a manner similar to that which has been proposed in the case of ethylene glycol;[6] subsequent dimerization then gives the product (II).



Scheme 1.

Judging from this dimer formation from I, it seems reasonable to assume that the dimer may exist in the radiolysis products of thymine in the absence of air, especially in the presence of N_2O. The glc experiments (Procedure A) have been perfomed to try to detect the dimer product (II) among the radiolysis products of thymine, but no appreciable amount of the dimer (II) was detected with the irradiations in air, in N_2, even in N_2O up to 2 Mrad.

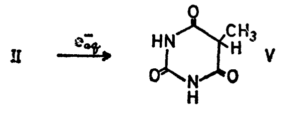
Further investigations of the radiolysis of the dimer (II) were carried out especially in an attempt to ascertain the behavior of the reaction with a hydrated electron.

An aqueous solution of II (4 mM) was irradiated with 2 Mrad under the following conditions: a) in N_2 as an anaerobical one, b) in the presence of KSCN to scavenge OH radicals, and c) saturated with N_2O to scavenge hydrated electrons. The irradiated solutions showed the UV spectra shown in Table 1. On the tlc (cellulose) of the irradiated solutions, both a) and b) provided a prominent spot at R_f 0.3 (*n*-PrOH: H_2O) or 0.6 (*t*-BuOH: Methyl ethyl ketone: Formic Acid: H_2O), which coincided well with that of the authentic V, which c) did not show any appreciable changes in the chromatogram, suggesting a further degradation of V. In addition, the main peak (R_f 10 min) in the gas chromatograms of the products obtained with a) and b) also agreed with that of authentic V, and the $G(+V)$ values as estimated by the glc analysis (Procedure B) were 1.5, 1.3, and 0.2 for the conditions of a), b), and c) respectively. These results indicate that II reacts readily with hydrated electrons to give V as the prinicpal product, while it hardly reacts at all with OH radicals to form V.

Table 1. UV max. (nm) of irradiated II and related compounds

	In 0.1 M NaOH	In 0.1 M HCl
Irrad. II (a)[a]	270	263
Irrad. II (b)[a]	270	263
Irrad. II (c)[a]	no	no
Unirrad. II	238	no
Authentic V	270	263
Thymine	290	265

a) Irradiation condition, see text.



Scheme 2

In summary, we could elucidate the further radiolysis processes of thymine glycol (I) involving a new dimer formation; that is, I reacts mainly with the OH radical form II *via* III and IV, and then V is formed by the

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[4] E. J. Hart and M. Anbar, "The Hydrated Electron," John Wiley & Sons, Inc., New York, N. Y. (1970), p. 90.

[5] D. Davidson and O. Baudisch, *J. Biol. Chem.*, **64**, 619 (1925).

[6] F. Seidler and C. V. Sonntag, *Z. Naturforsch.*, **24b**, 780 (1969).

initial reactions of the hydrated electrons with II.

Experimental

Materials. I (*cis*-type) was obtained by the method of Baudisch and Davidson[7] and was recrystallized from water until it gave a single spot on tlc. V was prepared according to the procedure of Doumas and Biggs.[8]

Irradiation of I. A solution of I (1.28 g) in triply-distilled water (200 ml) was bubbled with purified N_2 gas for 5 min and subsequently with N_2O gas for 15 min; then it was irradiated up to 2 Mrad with γ-rays of Co-60 at room temperature. The irradiated solution was concentrated to 100 ml under reduced pressure at 50 °C, and was chromatographed over a column (diameter of 4 cm) of Dowex 50W-X8, 100—200 mesh, H^+-type (250 ml) by elution with distilled water. After the elution of the UV-absorbing and yellow fractions about 120 ml, the following fractions, containing unchanged material, easily provided a colorless crystalline product. The product was recrystallized by dissolving it in alkali and making acid, thus giving II as white prismatic crystals (37 mg); mp above 300 °C; Found: C, 42.59; H, 3.49; N, 19.62%. Calcd for $C_{10}H_{10}N_4O_6$: C, 42.56; H, 3.57; N, 19.85%. Molecular weight as determined by the vapor-pressure method: 276±28 in DMF. UV: λ_{max} 238 nm in alkali, end absorption in neutral and acid.

Irradiation of Dimer (II). A solution of II (4 mM) in 0.01 M NaOH with triply-distilled water (final pH 10—11) was irradiated with 2 Mrad under the following conditions: a) bubbling with N_2 gas for 15 min before irradiation, b) in the presence of 25 mM KSCN, bubbling with N_2 gas for 15 min before irradiation, and c) bubbling with N_2 gas for 5 min and then with N_2O gas for 15 min before irradiation.

Glc Analysis. The analyses were carried out on a Shimadzu GC-4BMPF apparatus.

Procedure A: The freeze-dried samples were treated with BSTFA in acetonitrile (50%) and then heated at 140 °C for 1 hr. A glass column (1 m) packed with 1.5% Silicone OV-17 was used. The retention time of II was 8 min at 240 °C with 40 ml/min of N_2.

Procedure B: To the solution of V or the irradiated solution of II we added uracil as the internal standard, and then the mixture was freeze-dried. These samples were treated with bis(trimethylsilyl)acetamide in acetonitrile (25%) and then heated at 100 °C for 30 min. The column utilized was 1.5% OV-1 in a 2-m Pyrex glass tube. The column conditions were a 2-min initial hold, followed by a 7 °C/min temperature program from 130 °C to 220 °C.

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7) O. Baudisch and D. Davidson, *Ber.*, **58**, 1680 (1925).

8) B. Doumas and H. G. Biggs., *J. Biol. Chem.*, **237**, 2306 (1962).