Radiation Chemistry of Nucleic Acids. Isolation and Characterization of Thymine Glycols.¹

MASTER

Sir:

Since the discovery by Muller² in the late twenties that x-rays cause mutagenic effects, the radiation chemistry of nucleic acids has received considerable attention.³ Radiation effects on thymine have been in the forefront, and 5,6-dihydroxy-5,6-dihydrothymine of trans (I) and cis (II) configurations have been indicated as products.⁴ However, the actual isolation and identification of I has not been previously accomplished. We now wish to report the successful characterization of I and the chemistry of I and II.

Thymine, dissolved in triply distilled water, was saturated with oxygen. The solution (375 ml per run, 3×10^{-2} M, pH ~6) was irradiated with ¹³⁷Cs-Gammator⁵ at a dose-rate of 2 krad/min⁶ for a total of 10 hr, and resaturated with oxygen at 2 hr intervals. The irradiated solution was evaporated (35°) until dry and the residue was extracted with methanol. The combined methanolic extract was concentrated, applied on Whatman No. 3 Paper, and eluted with n-butanol saturated with water. Materials with R_f values of 0.47 and 0.40 were cut out and rechromatographed twice. The purified material was recrystallized from methanol.

The R_f 0.40 material was found to have the identical ir, uv, and nmr spectra as the cis thymine glycol (II)^{4b,7} prepared by permanganate oxidation of thymine⁸ at pH 6.8.

The trans isomer (I), R_f 0.47, has a m.p. 145-147° (decp); shows only end absorption in the uv region; has peaks for CH₃ (s, δ 1.60), C(6)H (d, δ 4.70, J 2.0 Hz), C(5)OH (s, δ 6.15), C(6)OH (m, δ 6.60) N(1)H (d, δ 8.32, J 2.0 Hz) and N(3)H (s, δ 9.65) in the nmr spectrum [in (CD₃)₂SO at 100 MHz with internal standard (CH₃)₄Si]; and displays bands at 3430 (sh) and

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3370 cm^{-1} for OH groups in its ir spectrum [KBr pellet]. A mass spectrum of I gives no parent ion, but an M-18 peak. However, after silylation with Regisil No. 27002 in pyridine, a molecular ion peak at m/e 448 corresponding to a tetra-trimethylsilyl derivative of I was evident. Compound I slowly decomposes when stored in a vacuum desiccator at ambient temperature. When paper chromatography with n-propanol-water (10/3) was carried out with this material, another compound (R_f 0.38) was detected in addition to the unchanged I. This new compound was shown to be 2,3,4,5-tetrahydro-2,4-dioxy-5-hydroxypyrimidine (III). It shows only end absorption in the uv region; has peaks for CH_3 (s, δ 1.60), C(6)H (s, δ 7.57), C(5)OH (broad δ 7.18) and N(3)H (broad δ 9.60) in the nmr spectrum in $(\text{CD}_3)_2\text{SO}$ (addition of D_2O eliminates the signals for OH and NH protons); and it displays an ir band at 3360 cm^{-1} for the OH group. A mass spectrum of III gives the parent ion at m/e 142.

With the purified I on hand, the interrelation of I and II could then be examined. When a solution of II (20 mg/ml H_2O) was heated at 90° for 4 hr, four products with R_f 0.27, 0.32, 0.39 and 0.57 (I) were detected together with II (0.47) by Fink's reagent on tlc [Eastman 6065, cellulose, with fluorescent indicator nPrOH/HOH]. The formation of I was also followed chromatographically by examining samples withdrawn at 30 min intervals. Although a very weak spot of I appeared for the 30 min sample, it never exceeded 2% at the end of 4 hr. When the solution of II was allowed to stand at 37° for 10 hr, only I and II could be detected. A time study revealed that I began to appear only after standing for 5 hr, however the amount seemed to be very minor at all times.

The major product (R_f 0.39) from II when heated at 90° was isolated and identified as 1,2,3,4-tetrahydro-2,4-dioxy-5-methyl-6-hydroxypyrimidine [5-methylbarbituric acid, (IV)] by uv and ir

spectra. Thus, the results suggest that the conversion of II to IV is a facile process, but that conversion of II to I is inefficient at best, in contrast to earlier reports.^{4a,4e}

On the other hand, when a solution of freshly prepared I (100 mg/15 ml H₂O) was heated at 90° for 30 min, or allowed to stand at 37° for 3 hr, six and five spots, respectively, appeared on tlc. The major products were II (~4%) and III (~4%) in each case with ~85% of I unchanged.

Based on stereochemical considerations, a mechanistic explanation of the above discussed reactions may be given. The most predominant conformer of II was suggested to have an equatorial C(5)OH.^{4c} Also, the same C(5)OH configuration may be assigned to I by examining the molecular structure of its analogue, thymine-thymine adduct.⁹ Thus, the major conformer for I and II may be I_{eq} and II_{ax} with equatorial and axial C(6)OH, respectively, as shown. Since the stereorequirement for a trans elimination process is favored by a trans planar conformation,^{10,11} I_{ax} and II_{eq}, the minor conformers, are the species favored for dehydration. Thus, in neutral aqueous solutions, II possibly dehydrates^s at elevated temperature to give IV as the major product. By the same consideration, elimination of water from I should be difficult between C(5)OH and C(6)H, but is possible for C(6)OH and N(1)H to give III.¹² In aqueous solutions, addition of water to III would result in the formation of I or II. Thus I → III → I + II provides a route for the conversion of I to II. This is a minor process since I, although unstable when dry, is stable in neutral aqueous solutions. On the other hand, II may also form III in the absence of its formation of IV. Although II → III → I is expected, II to IV is a favored process. Thus the conversion of II to I occurs only in trace quantities. The above results may rule out the possibility of efficient cis and trans

isomerization of I and II via a common intermediate,^{4a,4b} by ring-chain tautomerization.

These findings are probably of significance to our understanding of the initial steps of the radiation chemistry of thymine in aqueous solution.

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