Reports

Thymine Addition to Ethanol: Induction by Gamma Irradiation

Abstract. *When thymine in dilute, deaerated, aqueous solution was irradiated with γ-rays in the presence of ethanol, a high yield of products containing both the thymine and ethanol moieties was obtained. These were shown to be isomers formed by the attack of $CH_3\dot{C}HOH$ radicals at the carbon No. 6 position of thymine. A similar reaction was observed with* N,N'*-dimethylthymine, but* O,O'*-dimethylthymine did not react. The reaction may be relevant to the resistance of certain cells to ionizing radiation.*

During a search for the sensitized, radiation-induced dimerization of thymine and the sensitized, radiation-induced dissociation of the thymine dimer, ethanol was used as cosolvent for some of the materials (*1*). Although as yet no sensitized reactions have been seen, a particularly efficient reaction leading to the disappearance of thymine was observed and was traced to the small amounts of ethanol rather than to any of the other constituents. We report here the nature of this reaction to indicate its possible role in biological systems.

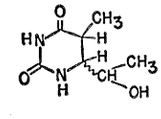
Solutions of thymine-2-C^{14} (1 mg/ml, 1 μc/ml) were deoxygenated in a stream of nitrogen, treated with ethanol (5 μl/ml) and irradiated with γ-rays from a Co^{60} source. The total doses varied from 0.066 to 9.6 megarads at a dose rate of 0.40 Mrad/hr. The resultant solutions were subjected to two-way paper chromatography on Ederol paper. The solvent in the first direction was a mixture of *n*-butanol, formic acid, and water (77 : 13 : 10 by volume), and in the second direction it was propanol, concentrated ammonia, and water (6 : 3 : 1 by volume). Spots were detected by autoradiography, and their radioactivity was determined with a gas-flow proportional counter. Under these conditions thymine was converted into three new compounds (A, B, and C), as shown in Fig. 1. The appropriate R_F values are given in Table 1. The nature of these compounds was investigated after large-scale irradiations.

For the large-scale experiment a solution of thymine (1.35 g) in water (450 ml) was deoxygenated in a stream of nitrogen for at least 1 hour. Ethanol (13.5 ml) and ethanol-1-C^{14} (135 μl, 0.102 μc/μl) were added, and the solution was irradiated (13.2 Mrad). Solvents were removed by rotary evaporation; the residual white solid was dried as much as possible at reduced pressure. The product was treated with hot methanol, and a small insoluble portion was discarded. The solvent was removed and replaced with a mixture of chloroform and methanol (9 : 1 by volume; 50 ml), and the solution was chromatographed on silica gel (280 g). Only one significant fraction was eluted (821 mg, 55 percent); this could not be resolved by thin-layer chromatography. However, even after several recrystallizations from methanol, this material had a wide melting range of 20°C, indicating a mixture of compounds. Two-way paper chromatography revealed the presence of two compounds whose R_F values corresponded to those of compounds A and B in the initial experiment.

The spots were detected by spraying with 0.5*M* sodium hydroxide solution and then adding an acidic solution of *p*-dimethylaminobenzaldehyde. The characterization of compounds A and B as dihydropyrimidines was confirmed by the absence of a band at about 265 mμ in the ultraviolet spectrum. The similarity of the physical properties of A and B suggested that they were isomers; because elemental analysis indicated a 1 : 1 addition product between thymine and ethanol. (Found: C, 49.05; H, 6.94; N, 16.31. $C_7H_{12}N_2O_3$ requires C, 48.83; H, 6.97; N, 16.27 percent). The infrared spectrum revealed the presence of a hydroxyl group (at 3450 cm^{-1}), two carbonyl groups (at 1700 and 1724 cm^{-1}), and amino groups (3280 cm^{-1}), while the nuclear magnetic resonance (NMR) spectrum (in D_2O) showed the presence of several $CC\underline{H}_3$ groups (overlapping bands between τ = 8.75 and 8.98) as well as several unresolved $C\underline{H}$ groups (between τ = 5.5 and 6.9).

The *p*-nitrobenzoyl derivatives of this mixture were prepared by use of *p*-nitrobenzoyl chloride and pyridine. The product was recrystallized from ethanol and gave colorless crystals (141 mg) whose melting point (mp) (207° to 230°C) indicated a mixture of compounds. Elemental analysis indicated that this was a mixture of *p*-nitrobenzoyl derivatives of the above alcohols (found: C, 51.61; H, 4.66; N, 12.92; $C_{14}H_{15}N_3O_6$ requires C, 52.3; H, 4.67; N, 13.1 percent), and the infrared spectrum now lacked the hydroxyl band at 3450 cm^{-1}. Preparative thin-layer chromatography of this material on silica gel in a mixture of chloroform and methanol (95 : 5 by volume) yielded two compounds. The first separated from ethanol as colorless prisms (1.6 mg; mp, 212° to 213°C). The second compound separated from ethanol as colorless needles (3.6 mg; mp, 235° to 237°) whose NMR spectrum (in D_2O) showed two doublets due to $CC\underline{H}_3$ groups at τ = 8.97 and 9.25 (J = 7 cy/sec in both cases). The presence of two $CC\underline{H}_3$ groups, each split by an adjacent $C\underline{H}$ group, confirmed that the two compounds, A and B, are *cis* and *trans* isomers of the addition product of thymine and ethanol (I).

(I)



By using a computer of average transients (CAT) further resolution of the NMR spectrum was achieved; the signals confirmed that this structure was correct. Bands were observed at τ = 0.4 ($CON\underline{H}CO$, singlet), 2.1 (aromatic $C\underline{H}$, split), 5.1, 6.75, and 7.5 (three $C\underline{H}$, all split), and 9.0 and 9.3 (two $CC\underline{H}_3$, both doublets).

Because of the small amounts of

Table 1. R_F values of compounds A, B, and C and thymine on Ederof paper, in butanol and formic acid (BF) and in propanol and ammonia (PA).

Compound	R_F	
	BF	PA
A	0.54	0.71
B	.45	.67
C	.71	.75
Thymine	.51	.59

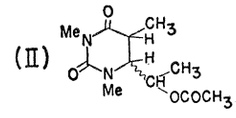
material available, further confirmation of the structures was not possible. However, by use of *N,N'*-dimethylthymine, separation and identification of the reaction products was made easier and information was obtained about the structural requirements for the reaction. Identification of products of the reaction with *N,N'*-dimethylthymine also supported the assignment of structure (I) to the products A and B.

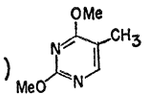
A solution of *N,N'*-dimethylthymine (2.25 g) in water (450 ml) was deoxygenated in a stream of nitrogen. Ethanol (22.5 ml) was added, and the solution was irradiated (24 Mrad); then solvents were removed by rotary evaporation. The resultant colorless oil was dissolved in chloroform, dried, and chromatographed on silicic acid (200 g), on which separation into three components was achieved.

Component I (1.11 g, 31 percent) was recrystallized from carbon tetrachloride to give colorless needles (mp, 153° to 155°C); when it was mixed with the starting material, the melting point was 153° to 154°C. Component III (550 mg, 16 percent) was a colorless gum which could not be caused to crystallize and was not investigated further.

Component II (1.85 g, 53 percent) was shown to contain hydroxyl groups by infrared (band at 3500 cm^{-1}) and NMR spectroscopy (the band at τ = 6.2 was removed by shaking with deuterium oxide). The presence of at least two compounds was suspected from the complex $CC\underline{H}_3$ signals in the NMR spectrum, and it was confirmed by thin-layer chromatography. This material was acetylated with acetic anhydride and pyridine, and the product was rechromatographed on silicic acid in diethyl ether, yielding three fractions.

The first fraction (110 mg, 21 percent) was recrystallized twice from a mixture of petroleum ether and ethanol to give *cis* or *trans* 6-α-acetoxyethyl-5,6-dihydro-1,3-dimethylthymine (II) as colorless needles (mp, 101° to 102°C). Elemental analysis was as follows. Found: C, 54.93; H, 7.44; N, 11.59 percent; mol wt, 248 (Rast method), 242 (mass spectroscopy). Calculated for $C_{11}H_{18}N_2O_4$ requires: C, 54.50; H, 7.30; N, 11.45 percent; mol wt, 242. Spectrographic analysis was as follows: λ_{max}, 222 mμ (log ϵ 3.64); ν_{max}, 1740, 1720, and 1680 cm^{-1}; τ values, 4.9 ($C_7\underline{H}$, quartet), 6.4 ($C_6\underline{H}$, quartet), 6.9 (two $NC\underline{H}_3$, singlet), 7.13 ($C_5\underline{H}$, doublet), 8.0 ($OCOC\underline{H}$, singlet), 8.2 and 8.92 (two $CC\underline{H}_3$, both doublets, J=7 cy/sec).

(II) 

(III) 

The second fraction (225 mg, 43 percent) separated from a mixture of petroleum ether and ether as colorless needles of the isomer (mp, 118°C). Analysis was as follows. Found: C, 54.49; H, 7.31; N, 11.54 percent; mol wt, 240 (Rast method), 242 (mass spectroscopy); λ_{max}, 222 mμ (log ϵ 3.26); ν_{max}, 1750, 1718, and 1675 cm^{-1}; τ values, 4.93, 6.66, 6.86, 6.92 7.2, 8.75, and 8.85.

The third fraction (185 mg, 36 percent) could not be made to crystallize. No further resolution was achieved by thin-layer chromatography, and the NMR spectrum showed peaks at τ = 8.9 ($OOOC\underline{H}_3$) and 8.8 ($CC\underline{H}_3$), whose relative intensities (1 : 2) indicated that 1 mole of ethanol had reacted with 1 mole of thymine.

When *O,O'*-dimethylthymine (III) was treated in the same way, thin-layer chromatography revealed that no reaction had taken place.

The above results show that the radiation-induced addition of ethanol to certain pyrimidines is an efficient reaction, G values being in the range normally found for radiation chemistry (2.3 for dimethylthymine, 0.9 for thymine). The reaction appears to proceed by attack of the hydroxyethyl radical on carbon No. 6 of the pyrimidine derivative.

The inability of *O,O'*-dimethylthymine (III) to react is evidently caused by the absence of the carbonyl group at carbon No. 4. Such a group is required for activation of the carbon No. 6 position for subsequent radical attack.

Donnellan and Setlow have recently reported the formation of thymine photoproducts other than thymine dimers in ultraviolet-irradiated bacterial spores (*2*). Since bacterial spores are resistant to the deleterious effects of both ultraviolet light and ionizing radiation, the formation of large amounts of such photoproducts implies either that these products do not interfere with DNA synthesis or that the cells have a very efficient repair mechanism for dealing with them. It follows that those cells which are resistant to ultraviolet light and ionizing radiation should form nonharmful products in preference to lethal thymine dimers. Such a preferential reaction implies an efficient chemical act between substances present in relatively large amounts within a cell.

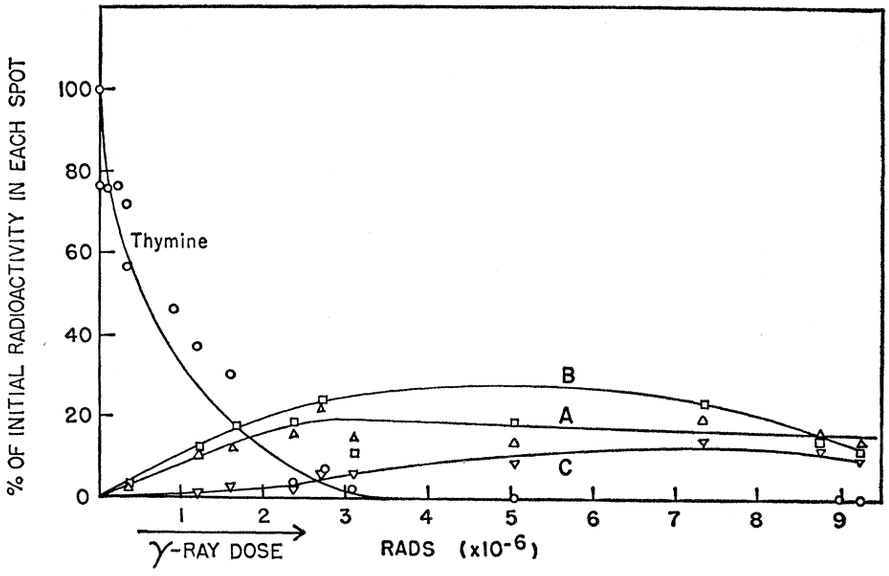


Fig. 1. Disappearance of thymine and appearance of irradiation products A, B, and C as functions of radiation dose. Symbols: ○, thymine; △, A; ▽, B; and □, C.

The foregoing reaction is just such an efficient reaction, and it requires only the presence of hydroxyl groups. Since these groups are present in large amounts within cells (for example, in sugars) and since increased resistance to radiation damage is shown by bacteria grown in a glucose medium (*3*), it is possible that this type of reaction plays a part in the high-dose radiation chemistry of cells.

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References and Notes

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2. J. E. Donnellan, Jr., and R. B. Setlow, *Science* **149**, 308 (1965).
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Mobility of the Hydrated Electron

Abstract: *The transient change in conductivity in diluted barium hydroxide solutions during and immediately after irradiation with single pulses of electrons from a 15-million-electron-volt linear accelerator was measured. Ionic mobility of the radiation-produced hydrated electrons was* 1.84×10^{-3} *cm*^{2} *volt*$^{-1}$ *sec*$^{-1}$ $\pm$ *10 percent. This value corresponds to a diffusion constant of* 4.75×10^{-5} *cm*^{2} *sec*$^{-1}$.

The hydrated electron, one of the prime objects of research in radiation chemistry (*1*, *2*), has been firmly established as the main reducing species in water radiolysis. Its absorption spectrum is now well known, and its yield as well as the rate constants of its chemical reactions with numerous inorganic and organic species have been measured, mostly with spectrophotometric techniques. Also, some other physicochemical properties have been calculated theoretically (*3*) or estimated from experimental data (*1*, *2*).

One of the aforementioned properties of the hydrated electron is its diffusion constant. This quantity, together with rate constants, is important for the calculation of yields in the diffusion model (*4*), by which method it may be possible to determine the initial spatial distribution of the primary species in the spurs. The diffusion constant D_{e^-} is related to the ionic mobility μ_{e^-} by the expression $D_{e^-} = \mu_{e^-} kT/q_{e^-}$, where k is the Boltzmann constant, T is the absolute temperature, and q_{e^-} is the charge on the electron. We therefore undertook to determine the diffusion constant of the hydrated electron by measuring its mobility, using a conductivity method.

If degassed water is irradiated with a pulse of ionizing radiation, a transient change of conductivity can be observed owing to the formation of hydrated electrons and the ionic species H_3O^+ and OH^-. In alkaline solution, practically all the H_3O^+ ions formed will immediately react with OH^- ions. Now, since the yield (G) of H_3O^+ is larger than that of OH^- [$G_{H_3O^+} = 3.6$ ions per 100 ev, $G_{OH^-} = 1.0$ ions per 100 ev (*5*)], we can expect a transient decrease in OH^- concentration during and immediately after the pulse, corresponding to a "negative yield" of -2.6 and thus approximately equal to electron concentration ($G_{e^-} = 2.6$), and only a very slight increase in H_3O^+ concentration during the pulse. Therefore, the conductivity signal obtained will be strongly dependent on electron mobility.

We used approximately $4 \times 10^{-5} N$ $Ba(OH)_2$ solution (the highest concentration compatible with our technique) prepared by degassing triply-distilled water (*6*) and injecting a small volume of filtered $10^{-2} N$ $Ba(OH)_2$ solution. The exact concentration of the final solution and its carbonate content, the latter being about 5 μM, were determined by a conductometric titration technique. The samples were irradiated with single electron pulses of 4-μsec duration from a 15-Mev linear accelerator, in a quartz cell with 75-μ platinum foils as electrodes. The irradiation assembly and the electric circuit are shown in Fig. 1. The electron beam was collimated in a triple collimator so that a sufficiently homogeneous radiation field of 2.5-cm diameter was obtained. After passing through the cell, the beam was stopped in a Faraday cup. The current from the Faraday cup was integrated in a 10-microfarad capacitor (not shown in the figure), the voltage on which could be measured with a vibrating-reed electrometer. The electrometer reading was used for monitoring the dose; it had been calibrated previously by filling the cell with a Fricke dosimeter solution (*7*, *8*) and establishing the relation between dose and electrometer reading. For measuring the transient conductivity signal, we used a symmetrical circuit (Fig. 1). This was necessary because part of the beam electrons are stopped in the cell, causing strong negative signals from both electrodes. These signals are several times as large as the conductivity signal. By properly adjusting the two channels of the differential amplifier (a Tektronix type CA plug-in unit with a separate type 132 power supply), these "charge-induced" signals could be canceled to a considerable extent. A change in conductance of the cell, however, resulted in a proportional voltage change across the differential amplifier; the voltage change was amplified and fed to the upper beam input of a Tektronix type 555 dual-beam oscilloscope equipped with two type L plug-in units. The lower beam input was connected to the Faraday cup for monitoring the beam pulse shape.

In order to minimize the effect of electrolysis and electrode polarization, the polarity of the batteries was reversed approximately every second by means of an automatic switching device. The resulting square-wave signal at the input of the differential amplifier could be eliminated by adjusting a compensating circuit consisting of two fixed 2-kilohm resistors and two 2-megohm variable resistors (marked "Compensation" in Fig. 1). Guard electrodes on both ends of the cell were kept at the same d-c (and slow a-c) potentials as the adjacent cell electrodes, thus eliminating the possible influence of radiation-induced conductivity in the cell windows. The linear accelerator could be triggered between any two polarity

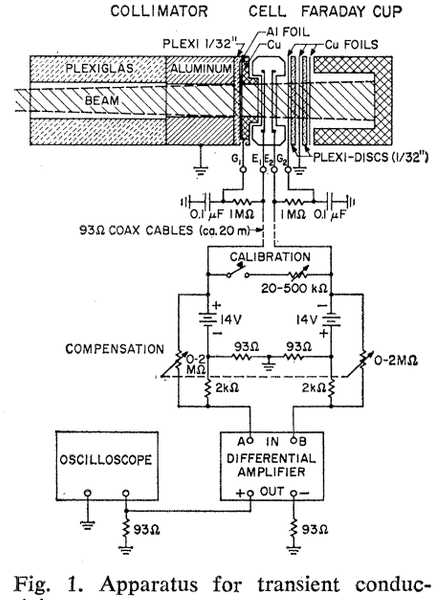

Fig. 1. Apparatus for transient conductivity measurements.