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SELECTIVE HYDROXYLATION OF THYMINE BY 2,2,6,6-TETRAMETHYLPIPERIDINE-1-OXYL BROMIDE IN AQUEOUS SOLUTION

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2,2,6,6-Tetramethylpiperidine-1-oxyl bromide ($TMPO^+Br^-$) was found to mediate selectively the hydroxylation of thymine by water into thymine glycol at room temperature. Almost quantitative yield of thymine glycol was obtained at the molar ratio of $[TMPO^+Br^-]_0/[Thymine]_0 = 2$ in feed under neutral conditions. The reaction mixture showed the ESR spectrum characteristic of 2,2,6,6-tetramethylpiperidine-1-oxyl (TMPO·).

In the previous paper, we characterized the reaction of thymine with hydroxyl radical (·OH) to give thymine glycol, 5- and 6-hydroxy-5,6-dihydrothymine, and 5-hydroxymethyluracil under γ-ray irradiation of the N_2O-saturated aqueous solution.$^{1)}$ The presence of nitro compounds with higher reduction potentials was shown to enhance the thymine glycol formation.$^{1)}$

Recently, Okawara et al. have reported that the immonium oxide salt derived from a stable radical of 4-methoxy-2,2,6,6-tetramethylpiperidine-1-oxyl can oxidize hydroxide ion (OH^-) in alkaline aqueous solution (pH 10.0) to produce hydrogen peroxide in high yield up to 90 %.$^{2)}$ We report here that an analogous salt of 2,2,6,6-tetramethylpiperidine-1-oxyl bromide ($TMPO^+Br^-$) mediates the selective hydroxylation of thymine by water into thymine glycol in neutral aqueous solution.

$TMPO^+Br^-$ was prepared by one-electron oxidation of 2,2,6,6-tetramethylpiperidine-1-oxyl (TMPO·) with Br_2.$^{3)}$ A solution of Br_2 (5.0 mmol) in CCl_4 (10 ml) was added dropwise for 1 h to a CCl_4 solution (56 ml) of TMPO· (10.0 mmol). The resulting mixture was permitted to stand overnight. The reddish brown powder precipitated was

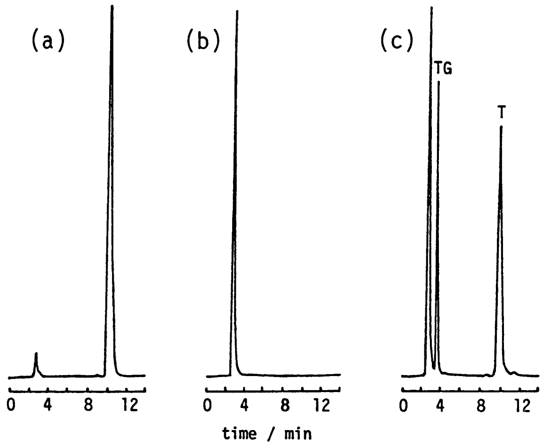


Fig. 1. Liquid chromatograms of (a) 0.49-mM thymine, (b) 0.50-mM $TMPO^+Br^-$, and (c) their mixture: T, thymine; TG, thymine glycol.

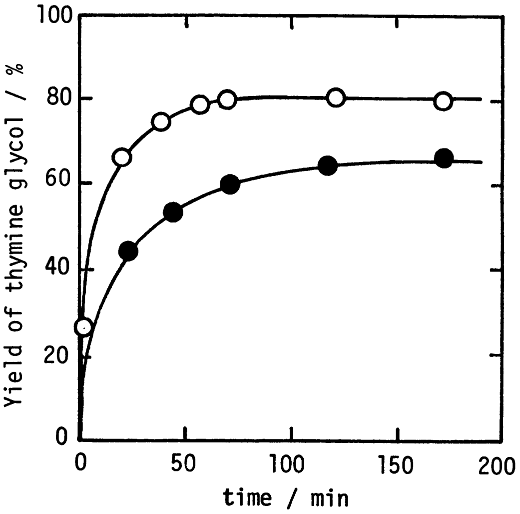


Fig. 2. Variations in thymine glycol yield at (○) pH 7.0 and (●) pH 11.0 as a function of reaction time: $[Thy]_0$ = 1.0 mM; $[TMPO^+Br^-]_0$ = 2.0 mM.

isolated by filtration, dissolved in methanol, and then reprecipitated by adding 10 % v/v ether-hexane; 56 % yield, mp(dec) 76-78 °C.

Figure 1 shows the representative high-performance liquid chromatograms (HPLC; Shimadzu LC-3A, monitored with uv absorption at 210 nm, eluted at a flow rate of 0.6 ml min^{-1}) for aqueous solutions (10 ml, buffered at pH 7.0) of (a) 0.49-mM thymine, (b) 0.50-mM $TMPO^+Br^-$, and (c) their mixture after standing overnight at room temperature. Comparison with the HPLC of authentic samples showed that thymine is converted selectively into thymine glycol (94.7 % based on thymine conversion (46.9 %) in Fig. 1(c)) by $TMPO^+Br^-$ under neutral conditions. The selectivity of the thymine glycol formation decreased in an alkaline solution (pH 11.0) and the complicated product distribution was observed.

As shown in Fig. 2, the yield of thymine glycol increased rapidly with reaction time in aqueous solution of 1.0-mM thymine and 2.0-mM $TMPO^+Br^-$ to attain its ultimate value of 80 % at pH 7.0 after ca. 1 h or 65 % at pH 11.0 after ca. 2 h. On the other hand, when thymine (1.0 mM) was added to an aqueous solution of $TMPO^+Br^-$ (2.0 mM) after standing for 37 h, the yield of thymine glycol decreased to less than 10 % at pH 7.0 and no reaction occurred at pH 11.0. Furthermore, a neutral solution of thymine (1.0 mM) containing equimolar amount of hydrogen peroxide gave no product.[4)]

These results suggest that the selective thymine glycol formation under neutral conditions is due to the direct reaction between thymine and $TMPO^+Br^-$ followed by hydrolytic substitution.[1)]

The ultimate yield of thymine glycol as well as thymine conversion were determined at pH 7.0 after 15-h reaction with varying the molar ratio of $TMPO^+Br^-$ to thymine in feed ($[TMPO^+Br^-]_0/[Thy]_0$). The linear relationship shown in Fig. 3 indicates the overall stoichiometry that thymine reacts with double molar amount of $TMPO^+Br^-$ to be converted into thymine glycol with the selectivity of ca. 84 %.

Figure 4 shows the ESR spectrum of the reaction mixture measured in Ar-purged aqueous solution (pH 7.0), which is characteristic of TMPO· as an one-electron reduction product of the immonium oxide $TMPO^+$.[5)]

On the basis of these results, the selective thymine glycol formation can be represented as follows:

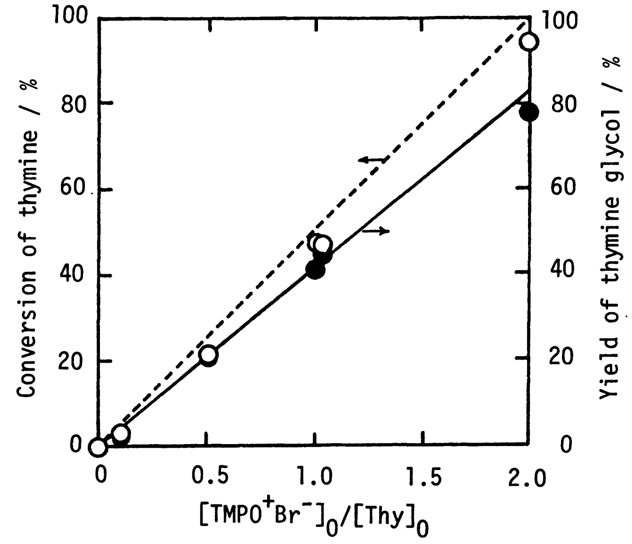


Fig. 3. Influences of the initial molar ratio $[TMPO^+Br^-]_0/[Thy]_0$ on (○) thymine conversion and (●) thymine glycol yield at pH 7.0 after 15-h reaction.

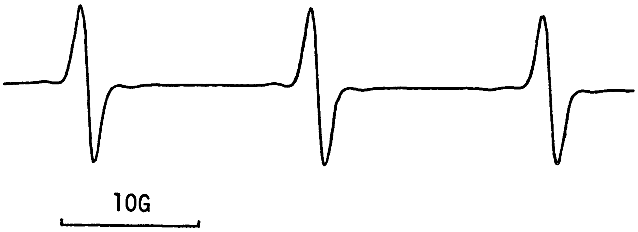
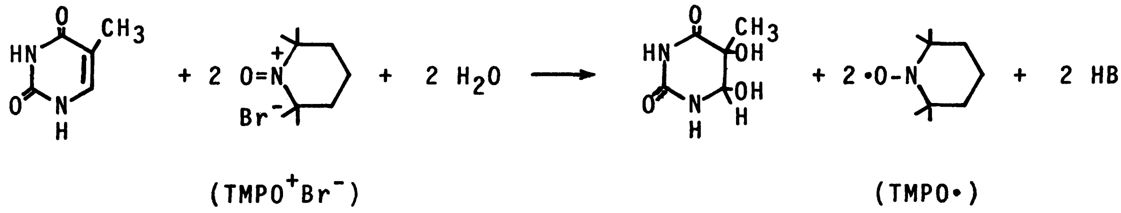


Fig. 4. ESR spectrum of the reaction mixture in Ar-purged aqueous solution (pH 7.0).



Analogous to nitro compounds in the radiation-induced reaction,[1] $TMPO^+Br^-$ is presumed to act as a mediator for promoting the hydroxylation of thymine by water via successive one-electron oxidations of thymine in the first step and certain intermediates[6] in the second step. It is also likely that under alkaline conditions one-electron oxidation of OH^- by $TMPO^+Br^-$ occurs competitively to produce ·OH or hydrogen peroxide, which accounts for the decreased selectivity of thymine glycol formation and complicated product distribution.

Further details will be reported in a subsequent paper.

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References

1) T. Wada, H. Ide, S. Nishimoto, and T. Kagiya, Chem. Lett., 1982, 1041.
2) T. Endo, S. Shiihashi, M. Okawara, and A. Sato, Polym. Prepr. Jpn., 31(3), 523 (1982).
3) M. Okawara, private communication.
4) It was also confirmed that thymine undergoes no reaction in the presence of TMPO· as well as hydrogen peroxide.
5) An identical ESR spectrum was obtained for Ar-purged aqueous solution of TMPO· at pH 7.0.
6) Either hydroxythymyl radicals or their adducts with TMPO· seem to be the possible intermediates undergoing one-electron oxidation to give the corresponding thymyl cations (see also Ref. 1). Similar adduct formation between thymyl radical and N-oxyl radical has been suggested: P. T. Emmerson and R. L. Wilson, J. Phys. Chem., 72, 3669 (1968); R. L. Wilson and P. T. Emmerson, "Radiation Protection and Sensitization", Taylor & Fransis, London (1969), p. 73; S. Kato, M. Kuwahara, and G. Yoshii, Int. J. Radiat. Phys. Chem., 7, 579 (1975).

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