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Oxidation of Thymidine by Peroxomono- and Peroxodisulfate Ions

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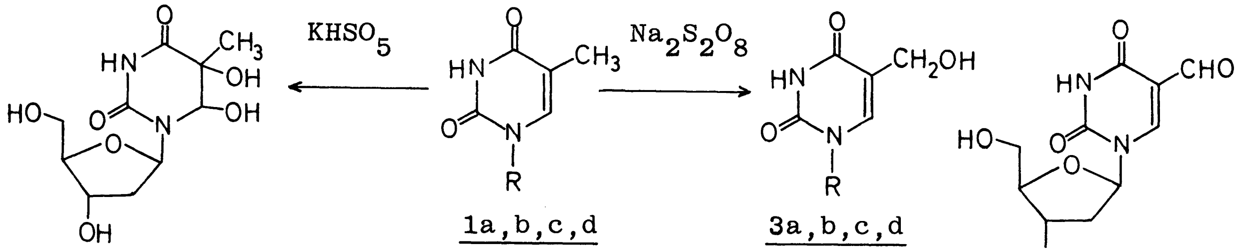
Oxidation of thymidine by $KHSO_5$ gave thymidine glycols, while a similar treatment with $Na_2S_2O_8$ gave 5-hydroxymethyl-2'-deoxyuridine.

The γ-radiation of DNA and its components as a solid state or in a frozen solution is known to result in the formation of those cation radicals,1) although the damage of DNA on γ-radiation has been considered to be mainly caused by the reaction of DNA with hydroxyl radical.2) Oxidation of nucleic acid related compounds by peroxodisulfate ion ($S_2O_8{}^{2-}$) has been investigated as a model for the formation of cation radicals of DNA3) since heating or photo-radiation of $S_2O_8{}^{2-}$ affords sulfate anion radical ($SO_4{}^{\bar{\cdot}}$) which undergoes removal of one electron from aromatics4) and alkenes.5) However, little attention has been paid to isolation of the products except for our reports. On the other hand, oxidation of alkenes by peroxomonosulfate ion ($HSO_5{}^-$) has been reported to give the corresponding epoxides6) and the reaction of thymine epoxides has been of interest in connection with a damage of DNA.7) These observations led us to study a comparison of products on the oxidation of thymidine (1a) between by $KHSO_5$ and by $Na_2S_2O_8$.

A solution of 1a (1 mmol) and $KHSO_5$ (2 mmol) in water (50 ml) was heated at 75 °C for 4 h under argon atmosphere. The reaction mixture was concentrated to ca. one-fifth to its original volume with an aspirator at room temperature and submitted to chromatography on ODS-silica gel8) with low pressure pump. A mixture of thymidine glycols (2) (0.62 mmol) was obtained as main products together with 5-hydroxymethyl-2'-deoxyuridine (3a) (0.02 mmol), thymine (4) (0.06 mmol), and recovered 1a (0.17 mmol). Further separation of 2 by reversed phase8) and gel filtration (Tosoh TSK-gel HW-40) chromatography with water led to the isolation of three isomers of 2; ^{1}H-NMR spectral data and melting point of the main compound (ca. 60% of the mixture9) by NMR spectroscopy) was similar to those of cis-thymidine glycol reported.10) When 0.1 mol dm^{-3} sodium phosphate buffer solution at pH 7.0 was employed as the solvent, the yield of 2 was decreased but that of 3a was increased; the oxidation gave 2 (0.17 mmol), 3a (0.08 mmol), 4 (0.02 mmol), and recovered 1a (0.60 mmol). A similar treatment of 1a (1 mmol) with $Na_2S_2O_8$ (1 mmol) instead of $KHSO_5$ (2 mmol) in water (50 ml) gave 3a (0.38 mmol) as a main product together with 5-formyl-2'-deoxyuridine (5)[11] (0.06 mmol), 4 (0.04 mmol), and recovered 1a (0.37 mmol) but none of 2 was obtained. The oxidation of 1a in the buffer solution at pH 7.0 gave 3a (0.28 mmol), 4 (0.01 mmol), 5 (0.03 mmol), and recovered 1a (0.54 mmol). The reaction with $Na_2S_2O_8$ was further applicable to the selective oxidation of 5-methyl group of α-thymidine (1b), 3'-deoxythymidine (1c), and 5'-deoxythymidine (1d); the oxidation gave the corresponding 5-hydroxymethyluridines such as 3b in 59% yield based on 1b consumed (Conv. 41%), 3c (Conv. 38%, Yield 64%), and 3d (Conv. 38%, Yield 62%). The structures of 3b, 3c, and 3d were determined on the basis of their ^1H-, ^{13}C-, ^1H-^1H COSY, and ^1H-^{13}C COSY spectral data.[12]



2

5

R =
a: 2'-deoxy-β-D-ribofuranosyl
b: 2'-deoxy-α-D-ribofuranosyl
c: 2',3'-dideoxy-β-D-ribofuranosyl
d: 2',5'-dideoxy-β-D-ribofuranosyl

DNA in γ-irradiated cells is known to contain 2 and 3a. The thymine glycol and 5-hydroxymethyluracil residues in DNA are eliminated by thymine glycol DNA glycosylase and 5-hydroxymethyluracil DNA glycosylase, respectively. In spite of the fact that the reaction of thymine with hydroxyl radical mainly leads to the formation of adducts at its 5,6-double bond,[13] the amount of 5-hydroxymethyluracil in human urine is ca. twice of thymine glycols,[14] indicating that the conversion of 1a into 3a in DNA may be caused by not only the reaction with hydroxyl radical but also the other mechanism. Therefore, the oxidation of 1a by $KHSO_5$ and by $Na_2S_2O_8$ is of interest as a model for the oxidative damage of thymine residue in DNA. Furthermore, our results suggest a possibility of formation of cation radicals of DNA on oxidative damage in vivo.

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8) Fuji-gel packed column RQ-2, 24 mmø x 360 mm.
9) NMR spectral data of 2 are as follows. The main compound: mp (dec.) 191-193 °C; ^{1}H-NMR (D_2O) δ 6.22 (dd, J=6 Hz and J=8 Hz, H-1'), 5.08 (s, H-6), 4.43 (m, H-3'), 3.95 (m, H-4'), 3.76 (m, H-5'), 2.41 and 2.23 (m, H-2'), 1.46 (s, Me) (d_6-DMSO) δ 10.31 (s, NH), 6.13 (d, J=4 Hz, OH-6), 6.04 (dd, J=6 Hz and J=8 Hz, H-1'), 5.38 (s, OH-5), 5.03 (d, J=4 Hz, OH-3'), 4.74 (d, J=4 Hz, H-6), 4.73 (t, J=4 Hz, OH-5'), 4.15 (m, H-3'), 3.63 (m, H-4'), 3.44 (m, H-5'), 2.19 and 1.83 (m, H-2'), 1.21 (s, Me);

^{13}C-NMR (D_2O) δ 177.89, 154.99, 88.32, 86.94, 81.59, 75.24, 73.92, 64.61, 39.21, 24.64 (DMSO) δ 174.78, 151.98, 86.19, 83.56, 78.77, 72.31, 71.42, 62.53, 37.20, 23.49. A minor compound (ca. 20% of the mixture by ^{1}H-NMR): mp (dec.) 196-200 °C; ^{1}H-NMR (D_2O) δ 6.22 (t, J=7 Hz, H-1'), 5.06 (s, H-6), 4.45 (m, H-3'), 3.95 (m, H-4'), 3.80 (m, H-5'), 2.30 (m, H-2'), 1.48 (s, Me); ^{13}C-NMR (D_2O) δ 177.93, 155.51, 88.24, 87.58, 82.36, 75.13, 73.19, 64.00, 40.01, 24.35. Another minor compound (ca. 20 % of the mixture by ^{1}H-NMR) could not be satisfactorily purified: ^{1}H-NMR (D_2O) δ 6.44 (d, J=8 Hz, H-1'), 5.08 (s, H-6), 4.54 (d, J=6 Hz, H-3'), 4.43 (s, H-4'), 4.23 and 3.84 (d, J=13 Hz, H-5'), 2.57 (dd, J=6 Hz, J=15 Hz, H-2'), 2.35 (dd, J=8 Hz and J=15 Hz, H-2'), 1.48 (s, Me); ^{13}C-NMR (D_2O)δ174.72, 153.89, 92.64, 90.59, 89.06, 77.38, 76.06, 73.66, 45.68, 21.97. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 and TMS were used as internal standard in D_2O and in DMSO, respectively.

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12) NMR data of 3b, 3c, and 3d are as follows. 3b: mp 215-220 °C; ^{1}H-NMR (D_2O) δ 7.97 (s, H-6), 6.21 (d, J=7 Hz, H-1'), 4.41-4.47 (m, H-3' and H-4'), 4.40 (s, CH_2O), 3.73 (dd, J=13 Hz and J=3 Hz, H-5'), 3.64 (dd, J=13 Hz and J=5 Hz, H-5'), 2.74 (ddd, J=15 Hz, J=7 Hz, and J=6 Hz, H-2'), 2.22 (d, J=15 Hz, H-2'); ^{13}C-NMR (D_2O) δ 168.13, 154.40, 143.09 (C-6), 115.65 (C-5), 91.83 (C-3' or C-4'), 90.24 (C-1'), 73.57 (C-3' or C-4'), 64.39 (C-5'), 59.50 (CH_2O), 42.44 (C-2'). 3c: mp 137-139 °C; ^{1}H-NMR (D_2O) δ 7.96 (s, H-6), 6.11 (dd, J=7 Hz and J=4 Hz, H-1'), 4.38 (s, CH_2O), 4.25 (m, H-4'), 3.90 (dd, J=13 Hz and J=3 Hz, H-5'), 3.74 (dd, J=13 Hz and J=5 Hz, H-5'), 2.47 (m, H-2'), 2.16 (m, H-2'), 2.09 (m, H-3'), 1.89 (m, H-3'); ^{13}C-NMR (D_2O) δ 167.98, 154.50, 142.65 (C-6), 115.84 (C-5), 89.27 (C-1'), 85.00 (C-4'), 65.23 (C-5'), 59.42 (CH_2O), 34.15 (C-2'), 27.50 (C-3'). 3d: mp 157-159 °C; ^{1}H-NMR (D_2O) δ 7.71 (s, H-6), 6.22 (t, J=7 Hz, H-1'), 4.39 (s, CH_2O), 4.24 (dd, J=10 Hz and J=4 Hz, H-3'), 4.11 (dq, J=7 Hz and J=4 Hz, H-4'), 2.43 (m, H-2'), 1.38 (d, J=7 Hz, H-5'): ^{13}C-NMR (D_2O) δ 167.89, 154.41, 142.14 (C-6), 116.58 (C-5), 88.35 (C-1'), 85.70 (C-4'), 77.57 (C-3'), 59.35 (CH_2O), 40.95 (C-2'), 20.86 (C-5').

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