

## Oxidation of Thymines and Uracils with Sodium Peroxodisulfate

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Reaction of thymines with  $\text{Na}_2\text{S}_2\text{O}_8$  in water resulted in selective oxidation of the methyl group at 5-position of thymines. Oxidation of thymines with  $\text{Na}_2\text{S}_2\text{O}_8$  in hydrochloric acid gave 5-chloro-6-hydroxy-5,6-dihydrothymines and in acetic acid containing NaCl gave 6-acetoxy-5-chloro-5,6-dihydrothymines which were converted to 6-alkoxy-5-chloro-5,6-dihydrothymines with alcohols. The reaction of uracils also gave similar products together with 5-chlorouracils.

Damage of nucleic acids by peroxides and superoxides has been received much attention. Reaction of nucleic acid bases and their derivatives with peroxodisulfate ion  $\text{S}_2\text{O}_8^{2-}$  has been studied by several groups of workers.<sup>1-5</sup> However, little attention has been paid to isolation of products. We now describe the selective oxidation of the 5-methyl group of thymines and the formation of 5,6-dihydropyrimidines from thymines and uracils with sodium peroxodisulfate  $\text{Na}_2\text{S}_2\text{O}_8$ .

A solution of thymine (1a) and  $\text{Na}_2\text{S}_2\text{O}_8$  in distilled water was heated at 85-90 °C under nitrogen for 7 h. Rotation Locular Counter-Current Chromatography (Tokyo Rikakikai Co., RLCC) was used for preparative separation of the reaction mixture. RLCC separation of the mixture with  $\text{CHCl}_3$ -MeOH- $\text{H}_2\text{O}$  (5:5:3) by the descending method resulted in the isolation of 5-hydroxymethyluracil (2a)<sup>6</sup> and 5-formyluracil (3a).<sup>7</sup> Under similar conditions, the reaction of 1-methylthymine (1b) gave 5-hydroxymethyl-1-methyluracil (2b)<sup>8</sup> and 5-formyl-1-methyluracil (3b)<sup>8</sup> and 3-methylthymine (1c) gave 5-hydroxymethyl-3-methyluracil (2c)<sup>9</sup> and 5-formyl-3-methyluracil (3c).<sup>10</sup> Furthermore, 1,3-dimethylthymine (1d) reacted with  $\text{Na}_2\text{S}_2\text{O}_8$  in water to give 5-hydroxymethyl-1,3-dimethyluracil (2d),<sup>9</sup> 5-formyl-1,3-dimethyluracil (3d),<sup>11</sup> and a dimerized compound (4d).

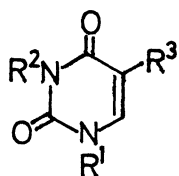
The 5-methyl group of thymines is oxidized to hydroxymethyl, formyl, and carboxyl groups by thymine 2-oxoglutarate dioxygenase and 2a exists in nucleic acids as a minor base. However, no report concerning selective oxidation of the 5-methyl group has been published except for the photo-oxidation of 1a and 1d.<sup>11</sup> Furthermore, the reaction of pyrimidine bases with  $\text{S}_2\text{O}_8^{2-}$  in water was reported to afford the corresponding pyrimidine radicals<sup>2-4</sup> and the cation radicals.<sup>5</sup> Therefore, the oxidation of the 5-methyl group of thymines with  $\text{Na}_2\text{S}_2\text{O}_8$  in water may proceed via thymine radicals or cation radicals formed from thymines and  $\text{SO}_4^{\cdot-}$ .

Moschel and Behrman already reported the oxidation of nucleic acid bases with  $\text{S}_2\text{O}_8^{2-}$  in alkaline solution.<sup>1</sup> We further investigated oxidation of thymines with  $\text{Na}_2\text{S}_2\text{O}_8$  in acidic solution. Treatment of 1d with  $\text{Na}_2\text{S}_2\text{O}_8$  in 1 mol  $\text{dm}^{-3}$  HCl gave

5-chloro-6-hydroxy-1,3-dimethyl-5,6-dihydrothymine (5d) although the oxidation in water containing NaCl gave 2d and 3d but none of 5d. The treatment of 1a with  $\text{Na}_2\text{S}_2\text{O}_8$  in 1 mol  $\text{dm}^{-3}$  HCl gave a somewhat complex reaction mixture but from the reaction mixture 5-chloro-6-hydroxy-5,6-dihydrothymine (5a) was obtained. The compound 5a has been isolated from the reaction of 1a with  $\text{Cl}_2$ <sup>12)</sup> and of DNA with  $\text{NaClO}$ <sup>13)</sup> and the stereochemistry is assigned as the trans-configuration.<sup>14)</sup>

Treatment of 1a with  $\text{Na}_2\text{S}_2\text{O}_8$  in MeOH containing NaCl gave 5-chloro-6-methoxy-5,6-dihydrothymine (7a). Furthermore, the reaction in AcOH containing NaCl gave 6-acetoxy-5-chloro-5,6-dihydrothymine (6a) which was treated with several types of alcohols such as methyl, cyclopentyl, allyl, and propargyl alcohols to lead to the corresponding 6-alkoxy-5-chloro-5,6-dihydrothymines (7a-10a) in 84-95% yields, although the treatment of 6-acetoxy-5,6-dihydrouracils with alcohols is known to give the corresponding 6-alkoxy-5,6-dihydrouracils.<sup>15)</sup> Treatment of 6a with water gave 5a.

Treatment of uracils with  $\text{Na}_2\text{S}_2\text{O}_8$  in AcOH containing alkali halides such as NaCl, KBr, and NaI resulted in a clean halogenation, while the reaction in water gave a complex reaction mixture. Uracil (11a) was reacted with  $\text{Na}_2\text{S}_2\text{O}_8$  and NaCl in AcOH and in situ treated with water to give 5-chlorouracil (12a) and 5,5-dichloro-6-hydroxy-5,6-dihydrouracil (13a).<sup>16)</sup> The oxidation of 1,3-dimethyluracil (11d) gave 5-chloro-1,3-dimethyluracil (12d) and 6-acetoxy-5,5-dichloro-5,6-dihydrouracil (14d). The yield of 14d increased with increasing amounts of  $\text{Na}_2\text{S}_2\text{O}_8$  and NaCl. The reaction of 11d with KBr and with NaI gave (15d) and (16d), respectively, in good yields. These results are summarized in Table 1.



1a:  $\text{R}^1=\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Me}$

1b:  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Me}$

1c:  $\text{R}^1=\text{H}$ ,  $\text{R}^2=\text{R}^3=\text{Me}$

1d:  $\text{R}^1=\text{R}^2=\text{R}^3=\text{Me}$

2a:  $\text{R}^1=\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{CH}_2\text{OH}$

2b:  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{CH}_2\text{OH}$

2c:  $\text{R}^1=\text{H}$ ,  $\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{CH}_2\text{OH}$

2d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{CH}_2\text{OH}$

3a:  $\text{R}^1=\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{CHO}$

3b:  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{CHO}$

3c:  $\text{R}^1=\text{H}$ ,  $\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{CHO}$

3d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{CHO}$

11a:  $\text{R}^1=\text{R}^2=\text{R}^3=\text{H}$

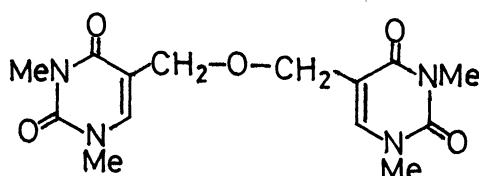
11d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{H}$

12a:  $\text{R}^1=\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Cl}$

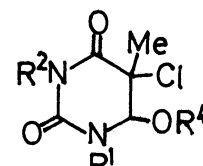
12d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{Cl}$

15d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{Br}$

16d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^3=\text{I}$



4d



5a:  $\text{R}^1=\text{R}^2=\text{R}^4=\text{H}$

5d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^4=\text{H}$

6a:  $\text{R}^1=\text{R}^2=\text{H}$ ,  $\text{R}^4=\text{Ac}$

6d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^4=\text{Ac}$

7a:  $\text{R}^1=\text{R}^2=\text{H}$ ,  $\text{R}^4=\text{Me}$

8a:  $\text{R}^1=\text{R}^2=\text{H}$ ,

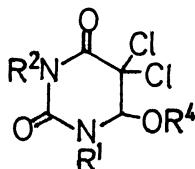
$\text{R}^4=\text{cyclopentyl}$

9a:  $\text{R}^1=\text{R}^2=\text{H}$ ,

$\text{R}^4=\text{allyl}$

10a:  $\text{R}^1=\text{R}^2=\text{H}$ ,

$\text{R}^4=\text{propargyl}$



13a:  $\text{R}^1=\text{R}^2=\text{R}^4=\text{H}$

14d:  $\text{R}^1=\text{R}^2=\text{Me}$ ,  $\text{R}^4=\text{Ac}$

Table 1. Oxidation of Thymines and Uracils with Sodium Peroxodisulfate<sup>a)</sup>

Substrate (mmol)	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub> mmol	Solvent (ml)	Alkali halide (mmol)	Products (Isolated yield/ % <sup>b)</sup> )
<u>1a</u> (1)	2	H <sub>2</sub> O (50)		<u>2a</u> (5), <u>3a</u> (41)
<u>1b</u> (1)	1	H <sub>2</sub> O (50)		<u>1b</u> (25), <u>2b</u> (30), <u>3b</u> (22)
<u>1b</u> (1)	2	H <sub>2</sub> O (50)		<u>2b</u> (6), <u>3b</u> (62)
<u>1c</u> (1)	2	H <sub>2</sub> O (50)		<u>2c</u> (5), <u>3c</u> (58)
<u>1d</u> (1)	1	H <sub>2</sub> O (50)		<u>1d</u> (28), <u>2d</u> (38), <u>3d</u> (22), <u>4d</u> (8)
<u>1d</u> (1)	2	H <sub>2</sub> O (50)		<u>1d</u> (5), <u>3d</u> (6), <u>3d</u> (80), <u>4d</u> (3)
<u>1a</u> (2)	4	1 mol dm <sup>-3</sup> HCl (100)		<u>5a</u> (25)
<u>1d</u> (1)	2	1 mol dm <sup>-3</sup> HCl (50)		<u>1d</u> (17), <u>5d</u> (60)
<u>1d</u> (1)	1	H <sub>2</sub> O (50)	NaCl (2)	<u>1d</u> (22), <u>2d</u> (23), <u>3d</u> (29), <u>4d</u> (5)
<u>1a</u> (2)	4	MeOH (100)	NaCl (8)	<u>7a</u> (77)
<u>1a</u> (2)	4	AcOH (100)	NaCl (8)	<u>6a</u> (86)
<u>1d</u> (1)	1	AcOH (50)	NaCl (2)	<u>1d</u> (20), <u>6d</u> (74)
<u>11a</u> (2)	4	AcOH (100)	NaCl (8)	<u>12a</u> (20), <u>13a</u> (53) <sup>c)</sup>
<u>11d</u> (1)	1	AcOH (50)	NaCl (1.2)	<u>11d</u> (14), <u>12d</u> (72), <u>14d</u> (3)
<u>11d</u> (1)	2	AcOH (50)	NaCl (2.1)	<u>11d</u> (12), <u>12d</u> (45), <u>14d</u> (29)
<u>11d</u> (1)	1	AcOH (50)	KBr (1)	<u>11d</u> (20), <u>15d</u> (71)
<u>11d</u> (1)	4	AcOH (50)	NaI (4)	<u>11d</u> (52), <u>16d</u> (31)

a) The reaction was performed at 85-90 °C in aq. solution or at 100-105 °C in AcOH under nitrogen for 7 h.

b) Yield based on substrate used.

c) After the reaction, the reaction mixture was treated with water.

We thank Professor Tsunao Hase, Faculty of Science, for his valuable supports throughout the study.

#### References

- 1) R. C. Moschel and E. J. Behrman, J. Org. Chem., **39**, 1983, 2699 (1974).
- 2) K. M. Bansal and R. W. Fessenden, Radiation Res., **75**, 497 (1978).
- 3) S. Fujita and S. Steenken, J. Am. Chem. Soc., **103**, 2540 (1981).
- 4) D. K. Hazra and S. Steenken, J. Am. Chem. Soc., **105**, 4380 (1983).
- 5) O. Neda, K. Yamauchi, and T. Masuda, Bull. Chem. Soc. Jpn., **58**, 227 (1985).
- 6) The compound 2a was identified by direct comparison with an authentic sample obtained commercially.
- 7) E. C. Ressler, A. Kampf, and M. P. Mertes, "Nucleic Acid Chemistry," ed by L. B. Townsend and R. S. Tipson, John Wiley & Sons, New York (1978), pp 89-91.
- 8) T. T. Sakai, A. L. Pogolotti, Jr., and D. V. Santi, J. Heterocycl. Chem., **5**, 849 (1968).
- 9) D. V. Santi and A. L. Pogolotti, Jr., J. Heterocycl. Chem., **8**, 265 (1971).
- 10) All new compounds were fully characterized by <sup>1</sup>H-NMR, IR, and mass spectroscopy and by elemental analyses. The spectral data are given below.  
3c: Mp 202-205 °C: NMR(d<sub>6</sub>-DMSO) δ 3.20(s, 3H), 8.17(s, 1H), 9.87(s, 1H), 12.16 (broad, 1H): IR(Nujol) 3130, 1730(sh), 1685, 1610, 1590 cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 154(M<sup>+</sup>, 12), 126(100), 69(62).

- 4d: Mp 182-184 °C: NMR(CDCl<sub>3</sub>)  $\delta$ 3.34(s, 6H), 3.42(s, 6H), 4.35(d, 4H, J=1 Hz), 7.39(t, 2H, J=1 Hz): IR(Nujol) 1700, 1660, 1640 cm<sup>-1</sup>: mass spectrum, m/e (relative intensity) 170(9), 169(M<sup>+</sup>-153, 100), 154(22), 153(46).
- 5d: Mp 110-111 °C: NMR(CDCl<sub>3</sub>)  $\delta$ 1.81(s, 3H), 3.13(s, 3H), 3.17(s, 3H), 4.44(d, 1H, J=5 Hz), 4.79(d, 1H, J=5 Hz). By addition of D<sub>2</sub>O, the signal at 4.79 was changed to singlet and the signal at 4.44 disappeared: IR(Nujol) 3340, 1710, 1680, 1650(sh) cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 208(10), 207(6), 206(M<sup>+</sup>, 29), 149(22), 117(34), 92(48), 90(100).
- 6a: Mp 162-164 °C: NMR(d<sub>6</sub>-DMSO)  $\delta$ 1.64(s, 3H), 2.07(s, 3H), 5.85(d, 1H, J=5 Hz), 9.06(broad, d, 1H, J=5 Hz), 10.89(broad, 1H). By addition of D<sub>2</sub>O, the signal at 5.85 was changed to singlet and the signals at 9.06 and 10.89 disappeared: IR(Nujol) 3300-3100, 1770, 1730, 1710 cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 221(M<sup>+</sup>, 1), 178(81), 163(31), 161(95), 133(31), 117(89), 90(100).
- 6d: Mp 77-78 °C: NMR(CDCl<sub>3</sub>)  $\delta$ 1.74(s, 3H), 2.11(s, 3H), 3.17(s, 3H), 3.27(s, 3H), 5.99(s, 1H): IR(Nujol) 1770, 1730, 1690 cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 249(1), 248(M<sup>+</sup>, 2), 247(1), 206(38), 189(58), 170(42), 153(100).
- 7a: Dec. 205-209 °C: NMR(d<sub>6</sub>-DMSO)  $\delta$ 1.66(s, 3H), 3.32(s, 3H), 4.50(d, 1H, J=5 Hz), 8.83(broad, d, 1H, J=5 Hz), 10.56(broad, 1H). By addition of D<sub>2</sub>O, the signal at 4.50 was changed to singlet and the signals at 8.83 and 10.56 disappeared: IR(Nujol) 3300-3000, 1710(broad) cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 194(2), 193(2), 192(M<sup>+</sup>, 7), 161(17), 160(27), 90(36), 61(100).
- 8a: Mp 233-235 °C: NMR(d<sub>6</sub>-DMSO)  $\delta$ 1.4-1.8(broad, m, 8H), 1.63(s, 3H), 4.05-4.35(broad, m, 1H), 4.56(d, 1H, J=5 Hz), 8.77(broad, d, 1H, J=5 Hz), 10.5(broad, 1H): IR(Nujol) 3300-3000, 1715(broad) cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 248(2), 247(2), 246(M<sup>+</sup>, 4), 211(14), 163(34), 161(100).
- 9a: Mp 188-191 °C: NMR(d<sub>6</sub>-DMSO)  $\delta$ 1.66(s, 3H), 3.96-4.16(m, 2H), 4.63(d, 1H, J=5 Hz), 5.0-6.25(m, 3H), 8.81(broad, d, J=5 Hz), 10.57(broad, 1H): IR(Nujol) 3300-3000, 1700(broad) cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 220(2), 219(3), 218(M<sup>+</sup>, 6), 173(19), 163(34), 161(100), 127(24), 120(23), 118(75).
- 10a: Mp 207-210 °C: NMR(d<sub>6</sub>-DMSO)  $\delta$ 1.66(s, 3H), 3.49(t, 1H, J=2 Hz), 4.25(d, 2H, J=2 Hz), 4.70(d, 1H, J=5 Hz), 8.83(broad, d, 1H, J=5 Hz), 10.63(broad, 1H): IR(Nujol) 3300-3000, 1705(broad) cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 218(7), 217(3), 216(M<sup>+</sup>, 21), 161(36), 160(28), 118(23), 90(51), 84(100).
- 14d: Mp 56-57 °C: NMR(CDCl<sub>3</sub>)  $\delta$ 2.33(s, 3H), 3.17(s, 3H), 3.29(s, 3H), 6.23(s, 1H): IR(Nujol) 1780, 1740, 1710(broad) cm<sup>-1</sup>: mass spectrum, m/e(relative intensity) 270(3), 268(M<sup>+</sup>, 5), 233(14), 230(11), 228(65), 226(100), 209(68).
- 11) R. Alcantara and S. Y. Wang, Photochem. Photobiol., 4, 465, 473 (1965).
  - 12) T. B. Johnson and J. M. Sprague, J. Am. Chem. Soc., 59, 2436 (1937).
  - 13) R. Prat, C. Nofre, and A. Cier, Compt. Rend., 260, 4859 (1965).
  - 14) C. Nofre, M. Murat, and A. Cier, Bull. Soc. Chim. Fr., 1965, 1749; M. Chabre, D. Gagnaire, and C. Nofre, *ibid.*, 1966, 108.
  - 15) D. Cech, L. Hein, R. Wuttke, M. v. Janta-Lipinski, A. Otto, and P. Langen, Nucleic Acids Res., 2, 2177 (1975); Y. Kobayashi, I. Kumadaki, and A. Nakazato, Tetrahedron Lett., 1980, 4605.
  - 16) O. Miyashita, K. Matsumura, H. Shimadzu, and N. Hashimoto, Chem. Pharm. Bull., 29, 3181 (1981).

(Received May 21, 1986)