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Oxidation of Thymines and Uracils with Sodium Peroxodisulfate

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Reaction of thymines with $Na_2S_2O_8$ in water resulted in selective oxidation of the methyl group at 5-position of thymines. Oxidation of thymines with $Na_2S_2O_8$ 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 $S_2O_8^{2-}$ has been studied by several groups of workers.[1-5] 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 $Na_2S_2O_8$.

A solution of thymine (1a) and $Na_2S_2O_8$ 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 $CHCl_3$-MeOH-H_2O (5:5:3) by the descending method resulted in the isolation of 5-hydroxymethyluracil (2a)[6] and 5-formyluracil (3a).[7] Under similar conditions, the reaction of 1-methylthymine (1b) gave 5-hydroxymethyl-1-methyluracil (2b)[8] and 5-formyl-1-methyluracil (3b)[8] and 3-methylthymine (1c) gave 5-hydroxymethyl-3-methyluracil (2c)[9] and 5-formyl-3-methyluracil (3c).[10] Furthermore, 1,3-dimethylthymine (1d) reacted with $Na_2S_2O_8$ in water to give 5-hydroxymethyl-1,3-dimethyluracil (2d),[9] 5-formyl-1,3-dimethyluracil (3d),[11] and a dimerized compound (4d).

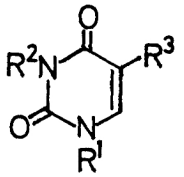
The 5-methyl group of thymines is oxidized to hydroxymethyl, formyl, and carboxyl groups by thymine 2-oxoglutarate dioxygenase and 2a exists in nucleic acids as a minor base. However, no report concerning selective oxidation of the 5-methyl group has been published except for the photo-oxidation of 1a and 1d.[11] Furthermore, the reaction of pyrimidine bases with $S_2O_8^{2-}$ in water was reported to afford the corresponding pyrimidine radicals[2-4] and the cation radicals.[5] Therefore, the oxidation of the 5-methyl group of thymines with $Na_2S_2O_8$ in water may proceed via thymine radicals or cation radicals formed from thymines and $SO_4^{-\cdot}$.

Moschel and Behrman already reported the oxidation of nucleic acid bases with $S_2O_8^{2-}$ in alkaline solution.[1] We further investigated oxidation of thymines with $Na_2S_2O_8$ in acidic solution. Treatment of 1d with $Na_2S_2O_8$ in 1 mol dm^{-3} HCl gave

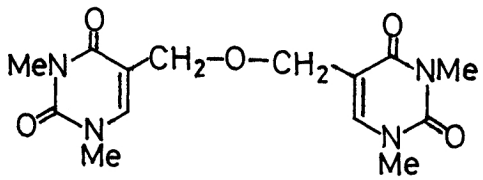
5-chloro-6-hydroxy-1,3-dimethyl-5,6-dihydrothymine (5d) although the oxidation in water containing NaCl gave 2d and 3d but none of 5d. The treatment of 1a with $Na_2S_2O_8$ in 1 mol dm^{-3} HCl gave a somewhat complex reaction mixture but from the reaction mixture 5-chloro-6-hydroxy-5,6-dihydrothymine (5a) was obtained. The compound 5a has been isolated from the reaction of 1a with Cl_2[12] and of DNA with NaClO[13] and the stereochemistry is assigned as the trans-configuration.[14]

Treatment of 1a with $Na_2S_2O_8$ 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.[15] Treatment of 6a with water gave 5a.

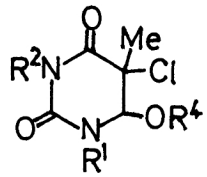
Treatment of uracils with $Na_2S_2O_8$ in AcOH containing alkali halides such as NaCl, KBr, and NaI resulted in a clean halogenation, while the reaction in water gave a complex reaction mixture. Uracil (11a) was reacted with $Na_2S_2O_8$ and NaCl in AcOH and in situ treated with water to give 5-chlorouracil (12a) and 5,5-dichloro-6-hydroxy-5,6-dihydrouracil (13a).[16] 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 $Na_2S_2O_8$ 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.



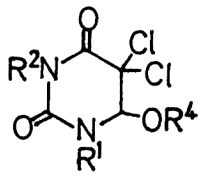
1a: $R^1=R^2=H$, $R^3=Me$
1b: $R^1=Me$, $R^2=H$, $R^3=Me$
1c: $R^1=H$, $R^2=R^3=Me$
1d: $R^1=R^2=R^3=Me$
2a: $R^1=R^2=H$, $R^3=CH_2OH$
2b: $R^1=Me$, $R^2=H$, $R^3=CH_2OH$
2c: $R^1=H$, $R^2=Me$, $R^3=CH_2OH$
2d: $R^1=R^2=Me$, $R^3=CH_2OH$
3a: $R^1=R^2=H$, $R^3=CHO$
3b: $R^1=Me$, $R^2=H$, $R^3=CHO$
3c: $R^1=H$, $R^2=Me$, $R^3=CHO$
3d: $R^1=R^2=Me$, $R^3=CHO$
11a: $R^1=R^2=R^3=H$
11d: $R^1=R^2=Me$, $R^3=H$
12a: $R^1=R^2=H$, $R^3=Cl$
12d: $R^1=R^2=Me$, $R^3=Cl$
15d: $R^1=R^2=Me$, $R^3=Br$
16d: $R^1=R^2=Me$, $R^3=I$



4d



5a: $R^1=R^2=R^4=H$
5d: $R^1=R^2=Me$, $R^4=H$
6a: $R^1=R^2=H$, $R^4=Ac$
6d: $R^1=R^2=Me$, $R^4=Ac$
7a: $R^1=R^2=H$, $R^4=Me$
8a: $R^1=R^2=H$, $R^4=$cyclopentyl
9a: $R^1=R^2=H$, $R^4=$allyl
10a: $R^1=R^2=H$, $R^4=$propargyl



13a: $R^1=R^2=R^4=H$
14d: $R^1=R^2=Me$, $R^4=Ac$

Table 1. Oxidation of Thymines and Uracils with Sodium Peroxodisulfate[a)]

Substrate (mmol)	$Na_2S_2O_8$ mmol	Solvent (ml)	Alkali halide (mmol)	Products (Isolated yield/ %[b)])
1a (1)	2	H_2O (50)		2a (5), 3a (41)
1b (1)	1	H_2O (50)		1b (25), 2b (30), 3b (22)
1b (1)	2	H_2O (50)		2b (6), 3b (62)
1c (1)	2	H_2O (50)		2c (5), 3c (58)
1d (1)	1	H_2O (50)		1d (28), 2d (38), 3d (22), 4d (8)
1d (1)	2	H_2O (50)		1d (5), 3d (6), 3d (80), 4d (3)
1a (2)	4	1 mol dm^{-3} HCl (100)		5a (25)
1d (1)	2	1 mol dm^{-3} HCl (50)		1d (17), 5d (60)
1d (1)	1	H_2O (50)	NaCl (2)	1d (22), 2d (23), 3d (29), 4d (5)
1a (2)	4	MeOH (100)	NaCl (8)	7a (77)
1a (2)	4	AcOH (100)	NaCl (8)	6a (86)
1d (1)	1	AcOH (50)	NaCl (2)	1d (20), 6d (74)
11a (2)	4	AcOH (100)	NaCl (8)	12a (20), 13a (53)[c)]
11d (1)	1	AcOH (50)	NaCl (1.2)	11d (14), 12d (72), 14d (3)
11d (1)	2	AcOH (50)	NaCl (2.1)	11d (12), 12d (45), 14d (29)
11d (1)	1	AcOH (50)	KBr (1)	11d (20), 15d (71)
11d (1)	4	AcOH (50)	NaI (4)	11d (52), 16d (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.

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6) The compound 2a was identified by direct comparison with an authentic sample obtained commercially.
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10) All new compounds were fully characterized by ^{1}H-NMR, IR, and mass spectroscopy and by elemental analyses. The spectral data are given below. 3c: Mp 202-205 °C: NMR(d_6-DMSO) δ3.20(s, 3H), 8.17(s, 1H), 9.87(s, 1H), 12.16 (broad, 1H): IR(Nujol) 3130, 1730(sh), 1685, 1610, 1590 cm^{-1}: mass spectrum, m/e(relative intensity) 154(M^+, 12), 126(100), 69(62).

4d: Mp 182-184 °C: NMR($CDCl_3$) δ3.34(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^{-1}: mass spectrum, m/e (relative intensity) 170(9), 169(M^+-153, 100), 154(22), 153(46).
5d: Mp 110-111 °C: NMR($CDCl_3$) δ1.81(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_2O, the signal at 4.79 was changed to singlet and the signal at 4.44 disappeared: IR(Nujol) 3340, 1710, 1680, 1650(sh) cm^{-1}: mass spectrum, m/e(relative intensity) 208(10), 207(6), 206(M^+, 29), 149(22), 117(34), 92(48), 90(100).
6a: Mp 162-164 °C: NMR(d_6-DMSO) δ1.64(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_2O, 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^{-1}: mass spactrum, m/e(relative intensity) 221(M^+, 1), 178(81), 163(31), 161(95), 133(31), 117(89), 90(100).
6d: Mp 77-78 °C: NMR($CDCl_3$) δ1.74(s, 3H), 2.11(s, 3H), 3.17(s, 3H), 3.27(s, 3H), 5.99(s, 1H): IR(Nujol) 1770, 1730, 1690 cm^{-1}: mass spectrum, m/e(relative intensity) 249(1), 248(M^+, 2), 247(1), 206(38), 189(58), 170(42), 153(100).
7a: Dec. 205-209 °C: NMR(d_6-DMSO) δ1.66(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_2O, 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^{-1}: mass spectrum, m/e(relative intensity) 194(2), 193(2), 192(M^+, 7), 161(17), 160(27), 90(36), 61(100).
8a: Mp 233-235 °C: NMR(d_6-DMSO) δ1.4-1.8(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^{-1}: mass spectrum, m/e(relative intensity) 248(2), 247(2), 246(M^+, 4), 211(14), 163(34), 161(100).
9a: Mp 188-191 °C: NMR(d_6-DMSO) δ1.66(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^{-1}: mass spectrum, m/e(relative intensity) 220(2), 219(3), 218(M^+, 6), 173(19), 163(34), 161(100), 127(24), 120(23), 118(75).
10a: Mp 207-210 °C: NMR(d_6-DMSO) δ1.66(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^{-1}: mass spectrum, m/e(relative intensity) 218(7), 217(3), 216(M^+, 21), 161(36), 160(28), 118(23), 90(51), 84(100).
14d: Mp 56-57 °C: NMR($CDCl_3$) δ2.33(s, 3H), 3.17(s, 3H), 3.29(s, 3H), 6.23(s, 1H): IR(Nujol) 1780, 1740, 1710(broad) cm^{-1}: mass spectrum, m/e(relative intensity) 270(3), 268(M^+, 5), 233(14), 230(11), 228(65), 226(100), 209(68).

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