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Reactions of Cytosine and 5-Methylcytosine with Osmium(VIII) Reagents: Syntheses and Deamination to Uracil and Thymine Derivatives

C.-H. CHANG, H. FORD and E. J. BEHRMAN*

Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, U.S.A.

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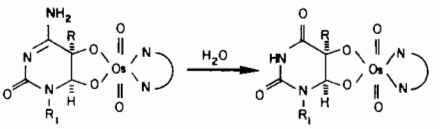
Cytosine and 5-methylcytosine react with osmium tetraoxide in the presence of 2,2'-bipyridyl or N,N,N',N'-tetramethylethylenediamine to form oxo-osmium(VI) esters by addition to the 5,6-double bond. These esters react with water to form the corresponding uracil and thymine derivatives by displacement of the exocyclic amino group.

Introduction

Reactions of osmium(VIII) reagents with uracil, thymine, and their derivatives have been well studied [1]. The corresponding reactions with cytosine and its derivatives are less well-known. Daniel [2] prepared and characterized a cytidine ester. Chang *et al.* [3] partially characterized the product resulting from the reaction between polyC, osmium tetroxide, and 2,2'-bipyridyl. Rosa and Sigler [4] report an osmium derivative of yeast t-RNA^{fMet} in which the osmium atom is localized at C-38. However, the precise chemistry of this interaction is not yet clear. Aside from these reports, the literature contains only kinetic data on the reactivity of cytosine derivatives with various osmium(VIII) reagents [5–9]. We find that these sters can be successfully prepared provided that the conversion to the corresponding 4-oxo derivative (Fig. 1) is recognized and controlled.

Results

We have synthesized the compounds represented by structures I–VIII, Fig. 1. 5-Methylcytosine reacted readily with osmium tetroxide and 2,2'-bipyridyl at neutrality to give the expected osmate ester(III) in about 80% yield. The structure given is consistent with the results of elemental analysis, the IR spectrum, and the proton NMR spectrum taken on the perchlorate salt(IV). The ester formed from the free base is too insoluble for convenient NMR analysis. A sulfate salt was also prepared. It is interesting, however, that treatment of III with HCl yielded an osmium complex which contained bipyridyl and chloride but no 5-methylcytosine**. Solutions of III in phosphate buffer were observed to undergo a slow decomposition. Whereas fresh solutions showed only one spot upon tlc, after standing at 25 °C for two days, two new spots appeared. One of these co-migrated with authentic VII the bipy osmate ester of thymine; the other new spot appears to be *cis*-thymine glycol. Addition of ammonium chloride to these solutions decreased the rate of deamination. The cytosine bipy ester, I, behaved similarly except that the deamination reaction occurred much more rapidly and that a larger number of decomposition products were observed. Multiple spots appeared upon tlc of freshly prepared solutions. Some of these products result from a secondary decomposition of the deamination product, the uracil bipy ester. Figure 2 is a representation of a thin-layer chromatogram showing the osmium-containing products which result from decomposition of the cytosine bipy ester in water and at pH 4 in acetate buffer. The decomposition pattern observed for the uracil bipy ester in water is also shown. The identities of the materials with R_f 0.44 and 0.14 are unknown.



I R=R1 = H; N-N = bipy
II R = H; R1 = ribosyl; N-N = bipy
III R = Me; R1 = H; N-N = bipy
IV the perchlorate salt of III
IVa the bisulfate salt of III
V R = Me; R1 = H; N-N = TMEN
VI R = R1 = H; N-N = bipy
VII R = Me; R1 = H; N-N = bipy
VIII R = Me; R1 = H; N-N = TMEN

Fig. 1. The synthesized compounds.

*Author to whom correspondence should be addressed.

**This complex analyzed for OsO_2Cl_2bipy. It may be related to the complex reported by Ray and Sarkar [10].

TABLE I. R_f Values.

Compound	Solvent A[a]	Solvent B[b]
5-methylcytosine	0.67	–
VIII	0.55	0.18
V	0.45	0.05
VII	0.60	–
III	0.50	–
Cis-thymine glycol	0.52	0.40
Trans-thymine glycol	0.67	0.48
I	0.30	–
VI	0.55	–
Bipy	0.87	–

[a]7:1:2, 2-propanol: conc. NH_4OH:H_2O – tlc on silica gel. [b]75:16:9, ethyl acetate: 2-propanol: H_2O – tlc on cellulose.

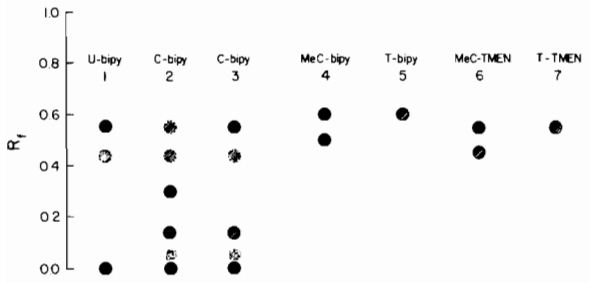


Fig. 2. Thin Layer Chromatography on silica gel, solvent A, 2-propanol: conc. NH_4OH: H_2O–7:1:2. 1 = VI in water, 3 days; 2 = I in water, 3 days; 3 = I, pH 4, 2 days; 4 = III, water, 2 days; 5 = VII, water, 2 days; 6 = V, water, 2 days; 7 = VIII, water, 2 days.

The black spot at the origin is thought to be a reduced osmium species, perhaps OsO_2.

We have studied the kinetics of the decomposition reactions of V. This ester, in which N,N,N′,N′-tetramethylethylenediamine replaces 2,2′-bipyridyl as ligand, is soluble enough in D_2O to make NMR studies easy. The reaction was followed by measurement of the decrease of the 5-methyl resonance of the ester (Fig. 3). The initial product of the decomposition is the corresponding thymine derivative which was identified both by its characteristic methyl resonance and by its chromatographic behavior (Table I). As the reaction proceeds, new methyl resonances appear upfield of those of both the 5-methylcytosine and thymine esters. These were identified as the *cis*- and *trans*-thymine glycols by comparison with the behavior of authentic samples [11] on tlc and by the characteristic color changes which these materials undergo upon spraying with the NaOH-Ehrlich's reagent [12]. Some data on the rate of these conversions are given in Table II. Plots of $\ln C/C_0$ *vs.* time were linear for two to three half-lives. The slopes of these lines yielded first order rate

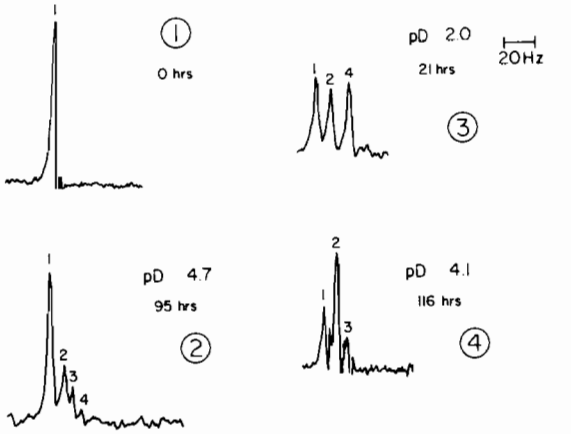


Fig. 3. NMR spectra of the methyl group region for a solution of V in DMSO/D_2O (2:1) at various acidities and times, 35 °C. The resonances marked 1, 2, 3 and 4 correspond to the methyl groups of V, VIII, *cis*-thymine glycol and *trans*-thymine glycol respectively.

constants which are given as half times in Table II. These data show that both the deamination reaction and the secondary isomerization of *cis*-thymine glycol to *trans*-thymine glycol are acid-catalyzed. We could not measure rate constants for the two entries run near neutrality because the methyl resonances of the 5-methylcytosine and thymine esters overlap at these pD values.

Discussion

The hydrolytic deamination of the osmate esters of cytosine and its derivatives is an instance of the well-documented [13] behavior of 5,6-dihydrocytosines of which perhaps the best studied example is the transformation of the cytosine bisulfite addition product [14–16]. The more rapid deamination of the cytosine esters as compared with the corresponding 5-methylcytosine esters is what is to be expected on both steric and electronic grounds for nucleophilic displacement at the 4-position of the pyridimidine ring. The increase in the rate of deamination upon protonation of cytosine derivatives is also expected as well as the inhibition of the rate by ammonia. Our observations also correlate well with those of Wang and Ehrlich who have compared the rates for the bisulfite-catalyzed deamination of cytosine and 5-methylcytosine derivatives [17].

Experimental

Chemicals were products of the Sigma or Aldrich Chemical Companies. Thin layer chromatography (tlc) was carried out on glass plates coated with either silica or cellulose and supplied by Analtech. NMR spectra were measured at 35 °C in a Varian Associates

TABLE II. Decomposition of Osmate Esters, 35 °C.

Compound	Solvent	pD[a]	$t_{1/2}$ (hr)	Product Distribution %			
				Cytosine ester	Deamination Product	*Cis*-glycol	*Trans*-glycol
V	D_2O, DCl	1.3	10.6	13	7	trace	80
V	D_2O	4.12	70	32	56	12	0
V	D_2O, phosphate	5.49	119.5	22	57	20	0
V	D_2O, phosphate	6.68	–	86[c]		14	–
V	D_2O, phosphate	7.02	–	89[c]		11	–
V	2:1 DMSO-d_6-D_2O,	4.7	138.6	49	36	14	0
V	2:1 DMSO-d_6-D_2O, DCl	1.98	16.7	26	26	0	48
III	2:1 DMSO-d_6-D_2O, DCl	1.88	26.3	–	–	–	–
III	2:1 DMSO-d_6-D_2O, DCl	1.92	17.6	–	–	–	–
III	2:1 DMSO-d_6-D_2O, DCl	2.95[b]	81.5	–	–	–	–

[a]Measured value using a glass electrode +0.4. [b]The corresponding thymine ester precipitates as the reaction proceeds. [c]The sum of cytosine ester and deamination product. See text.

T-60 instrument (60 MHz). IR spectra were taken on a Perkin-Elmer 237B instrument. Elemental analyses were carried out by Galbraith, Inc.

I. Cytosine(2,2'-bipyridyl)osmate(VI) Ester

Cytosine (220 mg, 2 mmol) was dissolved in 35 ml of an aqueous solution of 2,2'-bipyridyl (164 mg, 1.05 mmol). Osmium tetroxide (250 mg, 0.98 mmol) was then added. The solution was stirred at 25 °C for 10 min. The solution was cooled to 0 °C and the precipitated material collected after 30 min. by filtration. It was washed with water, acetone, and finally dried *in vacuo* over P_4O_{10} overnight. Yield: 330 mg (60%). *Anal.* Calcd. for $C_{14}H_{13}O_5N_5Os$: C, 32.25; H, 2.51; N, 13.43. Found: C, 32.08; H, 2.72; N, 13.29%. IR (KBr): 1651 (C=O), 1615 (NH_2), 823 (O=Os=O) cm^{-1}.

II. Cytidine(2,2'-bipyridyl)osmate(VI) Ester (With F. B. Daniel, ref. 2)

Osmium tetroxide (69 mg, 0.27 mmol) and 2,2'-bipyridyl (42 mg, 0.27 mmol) were dissolved in 5 ml of water. Cytidine (64 mg, 0.27 mmol) was added and dissolved. The reddish-brown solution was stirred at 25 °C for 20 min. The solvent was removed by flash evaporation and finally by drying over P_4O_{10} overnight. Yield was about 90%. *Anal.* Calcd. for $C_{19}H_{21}O_9N_5Os \cdot H_2O$: C, 33.99; H, 3.43; N, 10.43. Found: C, 34.1; H, 3.34; N, 10.67%. IR (KBr): 1650, 1601, 1550, 835 cm^{-1}.

III. 5-Methyl Cytosine(2,2'-bipyridyl)osmate(VI) Ester

5-Methyl cytosine hydrochloride (161 mg, 1 mmol) was dissolved in 35 ml of an aqueous solution of 2,2'-bipyridyl (164 mg, 1.05 mmol). The pH of this solution was adjusted to 7 with NaOH. Osmium tetroxide (250 mg, 0.98 mmol) was added. The solution was stirred for 15 min. at about 25 °C. The precipitate which formed was filtered, washed with water, acetone, and finally dried *in vacuo* over P_4O_{10}. Yield: 390 mg, 70%. A second crop of about 50 mg was obtained by reducing the volume of the filtrate and water washings. Total yield *ca.* 80%. *Anal.* Calcd. for $C_{15}H_{15}N_5O_5Os \cdot 2H_2O$: C, 31.52; H, 3.35; N, 12.25. Found: C, 31.79; H, 3.22; N, 12.38%. IR (KBr): 1657, 1615, 826 cm^{-1}.

IV. 5-Methyl Cytosine(2,2'-bipyridyl)osmate(VI) Ester Perchlorate

Perchloric acid (1 ml, 1.5 mmol) was added to 100 mm (18 mmol) of III. The osmate ester first dissolved followed by rapid precipitation of the perchlorate salt. The precipitate was filtered, washed with a little dilute perchloric acid, then with acetone, and finally air-dried. Yield: 98 mg (88%). *Anal.* Calcd. for $C_{15}H_{15}N_5O_5Os \cdot HClO_4$: C, 28.33; H, 2.54; N, 11.01. Found: C, 28.27; H, 2.61; N, 10.83%. IR (KBr); 1148, 1111, 1087 (perchlorate), 834 cm^{-1}. NMR (DMSO-d_6, TMS): δ 1.76 (s, CH_3); δ 5.05 (d, J = 6 Hz, H-6); δ 9.34 (d, J = 6 Hz, H-1).

IVa. A bisulfate salt was prepared using 6 *N* H_2SO_4 in a manner similar to that described for the perchlorate salt. IR (KBr): 1215, 1190, 1049 (bisulfate), 834 cm^{-1}.

V. 5-Methylcytosine(N,N,N',N'-tetramethylethylenediamine)osmate(VI) Ester Perchlorate

5-Methylcytosine hydrochloride (160 mg, 1 mmol) was dissolved in 10 ml water; TMEN (0.2 ml, 154 mg, 1.33 mmol) was added followed by osmium tetroxide

(250 mg, 0.975 mmol). The mixture was stirred at 25 °C for 20 min and then lyophilized. Dilute perchloric acid (3 ml, 1.5 *M*) was added to the lyophilized powder. Crystals appeared after cooling in ice-water and scratching the flask. This material was filtered, washed with dil. $HClO_4$, ether, and dried *in vacuo* over P_4O_{10}. Yield: 360 mg, 60%. *Anal.* Calcd. for $C_{11}H_{23}N_5O_5Os \cdot HClO_4$: C, 22.17; H, 4.06; N, 11.75. Found: C, 21.99; H, 4.14; N, 11.52%. IR (KBr): 1140, 1125, 1079 (perchlorate), 845 cm^{-1}. NMR (DMSO-d_6/D_2O 2:1, TMS): δ 1.82 (s, CH_3), δ 4.97 (s, H-6). A water-insoluble precipitate was also obtained from the mother liquor after standing overnight. Yield: 50 mg. *Anal.* Found: C, 17.4; H, 4.04; N, 6.81%.

VI. Uracil(2,2'-bipyridyl)osmate(VI) Ester

Uracil (56 mg, 0.5 mmol) and 2,2'-bipyridyl (78 mg, 0.5 mmol) were dissolved in 20 ml of water. Osmium tetroxide (125 mg, 0.5 mmol) was added. The mixture was stirred for 25 min at 25 °C. The precipitate which formed was collected by filtration, washed with cold water, acetone, and finally dried over P_4O_{10} *in vacuo*. Yield: 150 mg (60%). *Anal.* Calcd. for $C_{14}H_{12}O_6N_4Os \cdot H_2O$: C, 31.11; H, 2.61; N, 10.37. Found: C, 31.25; H, 2.66; N, 10.52%. IR (KBr): 1737, 1701 (C=O), 829 (O=Os=O) cm^{-1}.

VII. Thymine(2,2'-bipyridyl)osmate(VI) Ester

This ester was prepared in a procedure analogous to that described for VI. Yield: *ca.* 80%. IR (KBr): 1732, 1707, 829 cm^{-1}. NMR (DMSO-d_6, TMS): δ 1.58 (s, CH_3), 4.93 (d, J = 5 Hz, H-6).

VIII. Thymine (N,N,N',N'-tetramethylethylenediamine)osmate(VI) Ester

This ester was prepared according to ref. 18.

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