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Osmium(VI) Complexes of the 3′,5′-Dinucleoside Monophosphates, ApU and UpA†

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ABSTRACT: The dinucleoside monophosphates, ApU and UpA, react with potassium osmate (VI) and 2,2′-bipyridyl to form the corresponding oxo-osmium(VI) bipyridyl sugar esters in which the osmate group is bonded to the terminal 2′,3′-glycol. Osmium(VIII) tetroxide and 2,2′-bipyridyl react with the dinucleosides to form the corresponding oxo-osmium(VI) bipyridyl heterocyclic esters which result from addition of the tetroxide to the 5,6-double bond of the uracil residue. Although capable of transesterification reactions, these heterocyclic esters are exceptionally stable toward exchange reactions in solution. No apparent exchange was observed after 1 month. This reaction thus seems promising for single-site osmium labeling in polynucleotides.

The chemistry of heavy metal–polynucleotide complexes has important biochemical implications. Certain *cis*-Pt(II) complexes are effective antitumor agents (Rosenberg, 1973; Davidson et al., 1975; Aggarwal et al., 1975). Work on other transition metal complexes should be pursued in this connection (Cleare, 1974). Heavy metal interactions with polynucleotides are interesting in other ways as well (Clarke and Taube, 1974). In particular, oxo-osmium reagents form heavy atom derivatives of polynucleotides which have proved useful for x-ray crystallographic work (Rosa and Sigler, 1974; Suddath et al., 1974; Robertus et al., 1974) and also for the direct visualization approach to the sequencing problem (Salser, 1974; Whiting and Ottensmeyer, 1972).

Oxo-osmium(VI) ligand complexes react with *cis*-glycols to yield the same hexacoordinate monomeric esters which are formed by reaction of the corresponding olefin with an oxo-osmium(VIII) ligand complex (Criegee et al., 1942). The common reactive sites in nucleic acids are the 2′,3′-glycol in a terminal ribose group (Daniel and Behrman, 1975; Conn et al., 1974) for the osmium(VI) reaction and the 5–6 double bond of uracil and thymine residues (Subbaraman et al., 1971; Beer et al., 1966; Highton et al., 1968; Burton, 1967; Burton and Riley, 1966) for the osmium(VIII) system. Osmium(VIII) reagents also oxidize thio bases such as 4-thiouridine (Burton, 1967, 1970) and react rapidly with the isopentenyladenine group (Ragazzo and Behrman, 1976). The interesting chemistry of the reaction discovered by Rosa and Sigler (1974) is not yet clear. The nature of the reaction with olefins is radically affected by the presence of ligands containing tertiary nitrogen such as pyridine. Griffith (Collin et al., 1973, 1974) has shown that in the absence of such ligands, the product is a dimer containing pentacoordinate Os(VI) rather than the tetracoordinate monomer which had been long assumed. The ligand also affects both the rate of formation and the rate of hydrolysis of the product. For example, at pH 9.5, the rate of hydrolysis is increased by about a factor of 30 and the rate of formation decreased by about a factor of 1000 for the ligand-free system as compared with the bis(pyridine) ester (Subbaraman et al., 1972).

We have recently shown (Daniel and Behrman, 1975) that the nature and concentration of ligand species also affect the rate of the ester interchange reaction shown in Figure 1. The rate of the ester interchange reaction is inversely proportional to ligand concentration. For pyridine, the half-time is of the order of minutes. When the ligand is bidentate, however, the half-time for the reaction is greatly increased, particularly in the presence of excess ligand.

All of these factors are of importance for single-site labeling of polynucleotides in aqueous solution; hydrolysis must be prevented and the rate of ester interchange should be minimized. Rosa and Sigler (1974), for example, report that $tRNA^{fMet}$ in solution reacts with both Os(VI) and Os(VIII) species at many sites although only a single site reacts when osmium reagents are allowed to diffuse into crystals. We show in this paper that the 3′,5′ dinucleoside monophosphates UpA and ApU can be selectively labeled with an oxo-osmium(VIII) bipyridyl reagent at the 5–6 double bond of the uracil residue. Exchange to give the sugar ester does not occur to a significant extent.

Results and Discussion

Sugar Esters. Both dinucleoside monophosphates, ApU and UpA, reacted with potassium osmate and 2,2′-bipyridyl to form the expected sugar esters shown in Figure 2A and B. Only one spot was detected by thin-layer chromatography (TLC) in each case. Identification of the product was made by analysis of the low-field nuclear magnetic resonance (NMR) spectra of the products (Figure 3, Table I). The diagnostic resonances are those of the C(1′)-proton for the glycol terminal residue and those of the C(5) and C(6)

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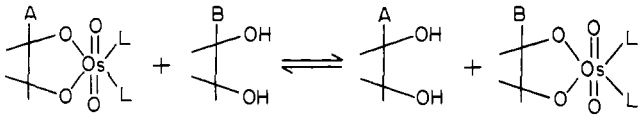

FIGURE 1.

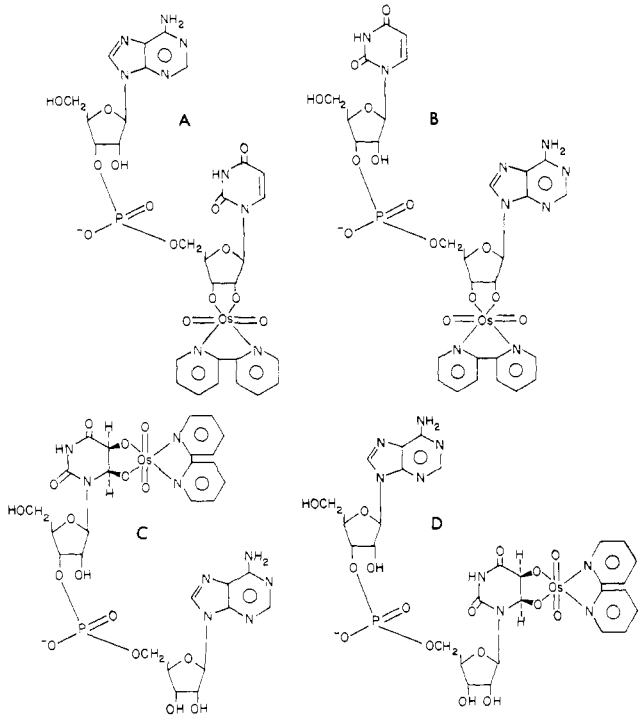

FIGURE 2: (A) ApU sugar ester; (B) UpA sugar ester; (C) UpA heterocyclic ester; (D) ApU heterocyclic ester. In Figure 2C and D, we show, arbitrarily, one of the two possible cis adducts. See Neidle and Stuart (1976), Figure 3.

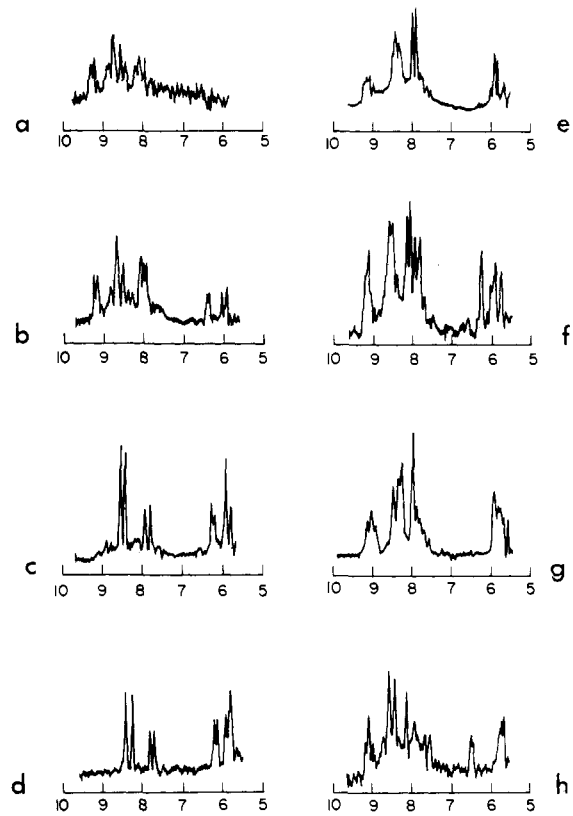

FIGURE 3: Low-field NMR spectra. (a) $Os_2O_6bipy_2$; (b) uridine bipyridyl sugar ester; (c) ApU; (d) UpA; (e) ApU heterocyclic ester, Figure 2D; (f) ApU sugar ester, Figure 2A; (g) UpA heterocyclic ester, Figure 2C; (h) UpA sugar ester, Figure 2B. The concentration range was 4×10^{-3} to 2×10^{-2} M. Each spectrum represents the accumulation of 50–500 scans. The pH range was 7–8.

protons of the uracil residue. The terminal C(1′)-proton resonance shifts downfield by about 0.4 ppm while the C(5) and C(6) proton resonances are unaffected. No exchange is expected (Daniel and Behrman, 1975) or observed for these reactions of the osmium(VI) species with the dinucleosides.

Heterocyclic Esters. When the dinucleosides reacted with osmium tetroxide and 2,2′-bipyridyl, single products were also obtained as shown by TLC and NMR analysis although in this case transesterification reactions are possible (Daniel and Behrman, 1975) (Figure 2C and D). The C(1′)-proton resonances are unaffected. Instead, the olefinic C(6) resonance of the uracil residue shifts from around δ 7.8 to 5.9.

NMR Shifts. Examination of the data in Table I shows that the C(2) and C(8) proton resonances of the adenine residue in all of the osmate derivatives of the dinucleoside monophosphates undergo some interesting upfield shifts relative to the parent dinucleoside. We attribute these to stacking interactions with the bipyridyl group. CPK models of these structures show that for both ApU esters conformations exist in which shielding of both the H-2 and H-8 protons can occur. We observe marked upfield shifts of these protons in both esters and, in addition, an upfield shift for the C(1′)-proton of the adenine residue in ApU sugar ester. This is also consistent with the stacking effect (Tso et al., 1969). We also observe upfield shifts for the H-2 and H-8 resonances in the UpA heterocyclic ester again in accordance with the CPK models. The UpA sugar ester provides an interesting test of this hypothesis since models show (see also Conn et al., 1974) that it is impossible to form a structure in which the H-8 proton is shielded by a stacking interaction; H-2 shielding is, however, possible. In accord with model building, we observe no significant shift in the H-8 but only in the H-2 resonance for UpA sugar ester. This hypothesis also predicts that one should observe corresponding upfield shifts in the bipyridyl protons of the dinucleoside esters as compared with those in the uridine and uridylic acid esters. These are also observed (Table I).

Uv Spectra. The uv spectra of the osmate esters of the dinucleoside monophosphates support the assigned structures. All of these compounds have maxima in the vicinity of 260 and 313 nm. The extinction coefficient at 313 nm is approximately constant for all of these compounds as well as for a number of other bipyridyl osmate esters. This band is absent in the corresponding bis(pyridine) esters. We assign this band to complexed bipyridyl (McWhinnie and Miller, 1969). The maximum near 260 nm is due to the sum of the absorptions of the adenine and uracil residues and also a component from the osmate–bipyridyl group. The value of this latter component is given by the difference between a nucleoside and its bipyridyl sugar ester. We have used the approximate value 7600. Thus for the dinucleoside monophosphate sugar esters, ϵ_{260} is given by the sum of the extinction coefficients for the component heterocycles plus 7600; for the heterocyclic esters that component due to uracil is missing. The 260/313 ratio is thus characteristic. These data are summarized in Table II.

Enzymatic Hydrolysis of the Osmate Esters. Treatment of ApU heterocyclic ester with snake venom phosphodies-

Table I: Chemical Shift Assignments, δ

Compound	A_8	A_2	U_6	U_5	U_1'	A_1'	bipy-2	bipy-3	bipy-4
ApU	8.51	8.42	7.80	5.77	5.77	6.14			
UpA	8.41	8.24	7.75	5.80	5.80	6.17			
ApU se[a]	8.15	8.05	7.88	5.78	6.21	5.92	9.13	8.53	7.83
UpA se[b]	8.43	8.12	7.63	5.68	5.68	6.45	9.10	8.58	7.81
ApU he[c]	8.00	7.89	5.91	*e*	5.91	5.91	9.19	8.42	7.81
UpA he[d]	8.28	8.00	5.94	*e*	5.94	5.94	9.06	8.50	7.80
5′-UMP he			5.94	*e*	5.94		9.27	8.67	8.05
Urd se			7.98	5.94	6.33		9.21	8.66	8.05
Os_2O_6 $(bipy)_2$							9.23	8.70	8.05

[a] ApU sugar ester, Figure 2 A. [b] UpA sugar ester, Figure 2B. [c] ApU heterocyclic ester, Figure 2 D. [d] UpA heterocyclic ester, Figure 2 C. [e] Upfield of the region scanned. Integration values are in accord with the assignments.

Table II: Ultraviolet Data.

Compound	λ_{max} (nm)	ϵ_{max}	ϵ_{313}	A_{max}/A_{313}
Ado	258	15 400		
Urd	262	10 100		
Ado se	256	22 900	13 600	1.68
Urd se	257	17 800	12 200	1.46
Ado se-Ado		7 500		
Urd se-Urd		7 700		
Thy he	260 (sh)	6 250	13 200	
ApU se, calc[a]		33 100	13 000	2.54
ApU se, found	257			2.33
UpA se, calcd[a]		33 100	13 000	2.54
UpA se, found	257			2.56
ApU he, calcd[b]		23 000	13 000	1.77
ApU he, found	255			1.67
UpA he, calcd[b]		23 000	13 000	1.77
UpA he, found	254			1.73

[a] $\epsilon_{Ado} + \epsilon_{Urd} + 7600$. [b] $\epsilon_{Ado} + 7600$. se and he refer to sugar ester and heterocyclic ester, respectively. The ligand is 2,2′-bipyridyl. See Figure 2.

terase at pH 7 resulted in cleavage to material which moved with the same R_f as 5′-UMP heterocyclic ester on paper chromatography. Although hydrolysis was very slow (4 days were required for the same degree of hydrolysis as achieved in a few hours for the parent dinucleoside), no hydrolysis was observed in the absence of the enzyme. Incubation of ApU heterocyclic ester in 0.1 M sodium carbonate for 24 h at 25 °C also resulted in partial cleavage.

Stability of the Bipyridyl Esters toward Exchange. All of these esters appear to be extremely stable toward exchange at neutral pH and room temperature. There is no change in the NMR spectrum after 12 h at 35 °C in D_2O. Samples stored at 25 °C, pH 7, in water show only one spot upon TLC after 1 month. This is in contrast to the corresponding pyridine esters discussed below. This result was somewhat unexpected in view of our previous work on the exchange behavior of bipyridyl osmate esters in 80% Me_2SO–20% D_2O (Daniel and Behrman, 1975). In this solvent system, although the bipyridyl esters were much more stable than the corresponding pyridine esters, the half-time for exchange in the absence of excess bipyridyl was only about 10 h at 35 °C. Part of the increased stability which we observe for the dinucleoside monophosphate osmate esters appears to be due simply to the solvent change since Midden (unpublished work in this laboratory) has found that 5′-dTMP heterocyclic bipyridyl ester exchanges with 5′-UMP in H_2O at 35 °C, pH 7, with a half-time of about 10 days. In addition to the solvent effect, the stacking interactions with bipyridyl discussed above may also contribute to the increased stability of the osmate esters of the dinucleosides.

Stability of the Corresponding Pyridine Esters. In contrast to the results with 2,2′-bipyridyl as ligand, when UpA reacted with osmium tetroxide in the presence of approximately 1 M pyridine–0.01 M phosphate buffer (pH 7.35), multiple osmium-containing products resulting from ester interchange are formed. At least two spots were observed upon TLC of the reaction mixture after 12 h. This behavior is expected and is analogous to the results observed with uridine itself (Daniel and Behrman, 1975) where three out of four of the possible products were tentatively identified.

Experimental Section

Proton magnetic resonance spectra were measured on a Varian Associates T-60 (60 MHz) instrument equipped with a T-6055 frequency lock and operated at 35 °C. All measurements were made in D_2O. The instrument was locked on the HDO band at δ 4.67 (or on an acetone capillary) and spectra were accumulated using a Varian C-1024 computer of average transients. The frequency lock permitted the accumulation of as many as 500 scans with no apparent loss of resolution. Chemical shifts were measured relative to the HDO band at δ 4.67 which was in turn referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate at zero. We estimate the accuracy of our chemical shift measurements at ±0.02 ppm. Note that our shifts differ from those reported by Ts'o et al. (1969) because of different referencing and temperature.

Ultraviolet spectra were measured in 0.02 M sodium phosphate buffer (pH 7.0) using a Perkin-Elmer Model 202 instrument.

Thin-layer chromatography was carried out on Eastman silica sheets using 0.02 M phosphate buffer (pH 7)–pyridine, 20:1 (v/v) as solvent. Osmium-containing spots were revealed with 2% thiourea in 2 N HCl. Paper chromatography was carried out using ethanol–1 M ammonium acetate (pH 7.5), 7:3 (v/v). Purified snake venom phosphodiesterase (*Crotalus adamanteus*) was purchased from Worthington.

The dinucleoside monophosphates were obtained from P-L Biochemicals, Miles, or Boehringer-Mannheim. The NMR and ultraviolet spectra of each batch were carefully checked before use as some samples were grossly contaminated.

Synthesis of the Dinucleoside Monophosphate Osmate

Esters. (a) Formation of the Heterocyclic Esters. The dinucleoside monophosphate (1–5 mg, 2–10 μmol) was reacted with an equimolar quantity of OsO_4 and a threefold molar excess of 2,2′-bipyridyl for about 30 min at a pH between 7 and 8 in water. The excess bipyridyl was then extracted with CCl_4. The aqueous layer was passed through a 10-cm Bio-Gel P-2 column and the eluate evaporated to dryness in vacuo. The residue was taken up in D_2O for NMR analysis.

(b) Formation of the Dinucleoside Monophosphate Sugar Esters. Reaction mixtures were made up as described under (a) except that a twofold molar excess of potassium osmate (Lott and Symons, 1960) replaced OsO_4. After mixing, the alkaline solution was rapidly adjusted to pH 7 with HCl. Following a 30-min reaction time, the mixture was filtered through a fine glass frit, desalted on Bio-Gel P-2, evaporated in vacuo, and taken up in D_2O for analysis.

$Os_2O_6bipy_2$, if present, is separated by the Bio-Gelreatment; it appears on TLC with R_f 0.33.

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