Grignard reagent obtained from 250 mmol of allyl chloride. The crude product was purified by chromatography on a silica gel column using benzene as the eluent: nmr ($CDCl_3$) δ 7.4–7.0 (m, 5), 5.9–4.7 (m, 6), 2.50 (m, 4), and 2.14 (s, 1). To 2.96 g (15.8 mmol) of this alcohol in 25 ml of acetonitrile was added 18.95 g (34.6 mmol) of CAN in 20 ml of acetonitrile and 5 ml of water at 80°. After 10 min the initially formed deep red color faded to a light yellow. The mixture was cooled and 50 ml of water and 50 ml of ether were added. The ether layer was separated, washed with saturated NaCl solution, dried ($MgSO_4$), and concentrated. Distillation gave 3.7 mmol (23% yield) of a yellowish oil: bp 59–63° (0.04 mm) [lit.[5] bp 100–102° (0.5 mm)]; nmr ($CDCl_3$) δ 8.1–7.8 (m, 2), 7.6–7.2 (m, 5), 6.4–6.9 (m, 3), and 3.60 (m, 2).

Oxidation Procedure.—Typically, 0.625 mmol of the alcohol, 7.50 mmol of acetonitrile, and 1.25 ml of water were added to a flask equipped with a condenser and magnetic stirring bar. A quantity of 1.25 ml of 1.00 *M* CAN was added, the flask was immersed in an oil bath at 80°, and the solution was stirred. In the case of **1** and **2** an initial dark red color formed which faded to a light yellow after 4 min. In the case of **3** the initial color was bright yellow and it faded to a light yellow after 30 min. After the reaction was complete, the flask was cooled in a water bath and 8 ml of water and 8 ml of ether were added to it. The ethereal solution was washed three times with 8-ml portions of water, dried ($MgSO_4$), and concentrated. The products from **1** were determined by nmr analysis by integration of the signals for the methylene protons of **4** (δ 3.55, m), the benzylic protons of **5** (δ 4.15, s), and the benzylic protons of **1** (δ 3.05, s). In several runs, the total recovery was determined by the use of octadecane or *p*-di-*tert*-butylbenzene as standards. The products from **2** were determined by nmr analysis by integration of the signals for the methyl protons of **2** (δ 0.90, s), the methyl protons of **6** (δ 1.22, s), and the methylene protons of **4** (δ 3.55, m). In several cases, the total recovery was determined by the use of mesitylene as a standard. The products from **3** were determined by glpc analysis using benzophenone as a standard and correcting for thermal conductivity and extraction differences as previously described.[2b]

Stability of Benzyl Phenyl Ketone (5) and Pivalophenone (6) to the Oxidation Conditions.—To 0.193 g (1.00 mmol) of **5** and 0.163 g (1.00 mmol) of **6** in 24 ml of acetonitrile and 7.4 ml of water at 80° was added 0.60 ml of a 1.00 *M* CAN solution. After 30 min at 80°, the mixture was cooled and 0.1822 g of standard (benzophenone) was added. Ether and water (20 ml of each) were added and after extraction the ether layer was separated, washed three times with water, dried ($MgSO_4$), and concentrated. Analysis by glpc (correcting for extraction and thermal conductivity differences) showed 97% recovery of **4** and quantitative recovery of **5**.

Registry No.—**1**, 38400-73-6; **2**, 38400-74-7; **3**, 38400-75-8; **4**, 6249-80-5; **5**, 451-40-1; **6**, 938-16-9; allyl chloride, 107-05-1; benzyl chloride, 100-44-7; diallylphenylmethanol, 38400-77-0; cerium, 7440-45-1.

The Reaction of Oxo-Osmium(VI)–Pyridine Complexes with Thymine Glycols

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Received January 22, 1973

We have synthesized *trans*-thymine glycol, *trans*-thymidine glycol, and *trans*-1,3-dimethylthymine glycol by isomerization of the corresponding cis glycols. Both the cis and trans glycols react in aqueous buffer solutions, pH 7–10, with the Os(VI) species, $Os_2O_6py_4$ (formulated by Criegee as OsO_3py_2) to give the corresponding bis(pyridine) *cis*-osmate (VI) esters. The 3-chloropyridine and 3-picoline osmate(VI) esters were also made. Kinetic studies show that the reactions are first order in Os(VI) and in substrate, and inverse first order in pyridine. The rate of reaction increases with increasing pH, but the apparent order in hydroxyl ions is less then one. Labeling experiments with ^{18}O show that ester formation takes place without cleavage of the C–O bond. We also report some observations on the equilibria of the Os(VI) species which suggest a pH-dependent monomer–dimer interconversion and concurrent ligand dissociation.

The radiolysis of nucleic acids, particularly their pyrimidine components, has received a good deal of attention. The recent elegant work of Téoule and Cadet[1] has provided a clearer picture of the course of events for thymine. Twenty-three products of the radiolysis of thymine have been identified. Under typical conditions 25% of the final products are the *cis*- and *trans*-thymine glycols (5,6-dihydroxy-5,6-dihydrothymine). Criegee and his coworkers have shown that the compound then thought to be $OsO_3(pyridine)_2$, among other Os(VI) species, reacts with glycols to form bis(pyridine) osmate(VI) esters.[2] We have shown that these bis(pyridine) esters, in contrast to the uncomplexed esters, are of sufficient hydrolytic stability to allow their easy manipulation in aqueous systems.[3,4] In continuation of our goals of developing selective reactions for the characterization of nucleic acids, we have undertaken this study, which may aid the recognition by electron-microscopic techniques[5,6,7a] of those thymine residues in a DNA molecule damaged by radiolysis. Oxo-osmium species have also been used recently in X-ray diffraction analyses of transfer RNA.[7b]

Results and Discussion

Structure and Equilibria.—Criegee and coworkers[2] formulated the product of the reaction of osmium tetroxide and pyridine in the presence of ethanol as OsO_3py_2. Griffith and Rossetti[8a] have recently presented good spectroscopic evidence which suggests that this compound in the solid state is actually the dimer, $Os_2O_6py_4$, with trans O=Os=O osmyl groups

(1) J. Cadet and R. Téoule, *Biochim. Biophys. Acta*, **238**, 8 (1971); R. Téoule and J. Cadet, *Chem. Commun.*, 1269 (1971).

(2) R. Criegee, B. Marchand, and H. E. Wannowius, *Justus Liebigs Ann. Chem.*, **550**, 99 (1942).

(3) L. R. Subbaraman, J. Subbaraman, and E. J. Behrman, *Bioinorg. Chem.*, **1**, 35 (1971).

(4) L. R. Subbaraman, J. Subbaraman, and E. J. Behrman, *Inorg. Chem.*, **11**, 2621 (1972).

(5) M. Beer and E. N. Moudrianakis, *Proc. Natl. Acad. Sci. U. S.*, **48**, 409 (1962).

(6) A. V. Crewe, J. Wall, and J. Langmore, *Science*, **168**, 1338 (1970).

(7) (a) R. M. Henkelman and F. P. Ottensmeyer, *Proc. Natl. Acad. Sci, U. S.*, **68**, 3000 (1971); R. F. Whiting and F. P. Ottensmeyer, *J. Mol. Biol.*, **67**, 173 (1972). (b) R. W. Schevitz, M. A. Navia, D. A. Bantz, G. Cornick, J. J. Rosa, M. D. H. Rosa, and P. B. Sigler, *Science*, **177**, 429 (1972); S. H. Kim, G. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, P. Blattmann, and A. Rich, *Proc. Natl. Acad. Sci. U. S.*, **69**, 3746 (1972); *Science*, **179**, 285 (1973).

(8) (a) W. P. Griffith and R. Rossetti, *J. Chem. Soc., Dalton Trans.*, 1449 (1972). (b) Griffith reports privately that the 640-cm^{-1} band was indeed observed in the ir and that its omission in ref 8a is an error.

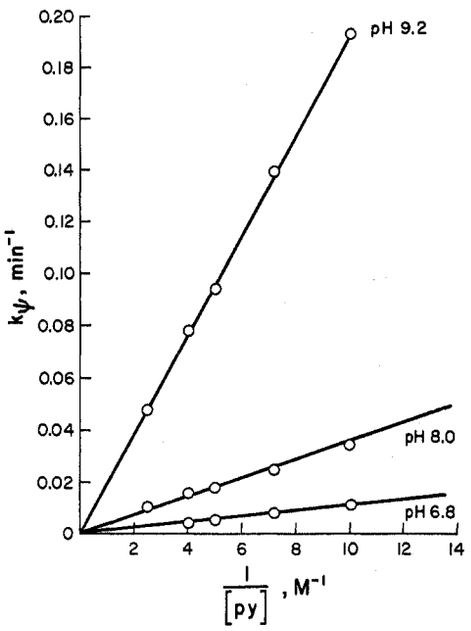
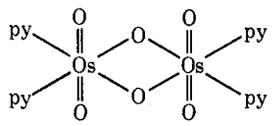


Figure 1.—Variation of k_ψ with pyridine concentration, 15°: [Os(VI)] 4–5 × 10^{-4} M, calculated as OsO_3L_2, λ 304 nm, [*cis*-thymine glycol] 3.2 × 10^{-2} M.

and two oxygen bridges, analogous to the structure demonstrated by X-ray crystallography for $Os_2O_6(NO_2)_4$.[9] We confirm the results of Griffith and



Rossetti. The ir spectrum (KBr) of the complex formed with pyridine is in agreement with their data except that we observe an additional strong band at 645 cm^{-1} which they report only in the Raman spectrum.[8b] The complexes formed with 3-picoline or 3-chloropyridine in place of pyridine are similar in exhibiting strong bands near 830 and near 640 cm^{-1}. We have found, however, evidence for a monomer–dimer equilibrium. The molecular weight of a 0.39 wt % solution in water was 810 ± 40 at 37.5° by vapor phase osmometry (Galbraith Laboratories) and 803 ± 20 and 782 ± 25 at 21.3° by equilibrium ultracentrifugation using a partial specific volume of 0.696. The formula weight for $Os_2O_6py_4$ is 793. Since our kinetic studies were carried out in buffer solutions over the pH range 7–10, equilibrium ultracentrifugation was also carried out in 0.08 M sodium carbonate buffer, pH 9.4. Under these conditions, a 0.39 wt % solution of the Os(VI) species gave a number average molecular weight of 746 ± 25; a 0.0975 wt % solution in the same buffer gave a number average molecular weight of 523 ± 13. Although these data do not yield a consistent equilibrium constant for the dimerization process on the basis of any of the simple assumptions that we have used, they do suggest complete dissociation to the monomeric species at sufficiently low concentrations. Our kinetic work, for example, has been carried out at a concentration of about 0.02 wt %.

We have also measured the dissociation of pyridine and 3-picoline from the corresponding Os(VI) species as a function of pH. These data are summarized in Table I. We note that approximately constant values

TABLE I
EQUILIBRIA OF $OsO_3 \cdot L_2$ SYSTEMS, 15°[a]

10^3[$OsO_3 \cdot L_2$], M[b]	L	pH	10^4[L_0], M	10^4[L_a], M	K[c]
5.154	Pyridine	7.45	1.44	1.10	40.46
4.764	Pyridine	8.05	2.77	2.13	43.35
4.764	Pyridine	9.45	10.81	8.32	39.62
4.506	Pyridine	10.15	17.46	13.44	41.50
4.492	3-Picoline	7.9	3.78	1.15	35.46
4.492	3-Picoline	9.15	13.76	4.16	39.20
4.438	3-Picoline	9.95	23.92	7.25	38.40

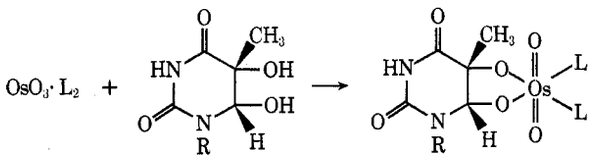
[a] $\mu \approx 0.15$ M, carbonate and phosphate buffer. [b] These concentrations are calculated on the basis of the monomeric species $OsO_3 \cdot L_2$. [c] $K = ([L_a]\{[L_a] + [L_0]\})/(\{[OsO_3 \cdot L_2] - ([L_a] + [L_0])\}[OH^-])$. $[L_0]$ and $[L_a]$ are the free ligand concentrations in the organic and aqueous phases, respectively. These were determined by partitioning the ligand between ether and the aqueous phase (see Experimental Section).

for K are obtained on the assumption of an equilibrium of the type given by

$$OsO_3L_2 + HO^- \rightleftharpoons (OsO_3 \cdot L \cdot OH)^- + L$$

This may be regarded as confirmatory evidence for the model which assumes substantially complete dissociation of the dimer to the monomeric species. We have written the monomer here and elsewhere as OsO_3L_2, but it may well exist as the hydrate $OsO_2(OH)_2L_2$. We have not considered the dissociation of the second ligand to give, for example, a species such as $(OsO_3 \cdot H_2O \cdot OH)^-$ since these solutions undergo no observable decomposition after several days in air at room temperature and since it has been shown[4,10,11] that pyridine-free Os(VI) oxide species are not stable under these conditions.

Kinetics.—$OsO_3 \cdot L_2$, where L represents pyridine, 3-picoline, or 3-chloropyridine, reacts with *cis*-thymine glycol and its derivatives to give the corresponding bis(ligand) osmate(VI) esters in good yield (see Experimental Section). These compounds have already been synthesized by another route.[3]



With limiting concentrations of $OsO_3 \cdot L_2$ and in the presence of added ligand, plots of log $(A_\infty - A_0/A_\infty - A_t)$ *vs.* time were linear for about 80% of the reaction. The slopes of these lines, which give k_ψ, the pseudo-first-order rate constant, were unaffected when the initial concentration of $OsO_3 \cdot L_2$ was varied in the range 2.5–5 × 10^{-4} M. If free ligand was not added, the reaction was complete within the time of mixing. The pseudo-first-order rate constant varied linearly with the first power of the substrate concentration. At constant substrate concentration, k_ψ increased with increasing pH and showed an inverse first-order dependence on ligand concentration (Figure 1). k_ψ was unaffected by halving carbonate buffer

(9) L. O. Atovmyan and O. A. L'yachenko, *J. Struct. Chem.*, **8**, 143 (1967).

(10) J. Périchon, S. Palous, and R. Buvet, *Bull. Soc. Chim. Fr.*, 982 (1963).

(11) J. F. Cairns and H. L. Roberts, *J. Chem. Soc. C*, 640 (1968).

TABLE II

REACTION OF THYMINE AND THYMIDINE GLYCOLS WITH PYRIDINE AND SUBSTITUTED PYRIDINE COMPLEXES OF Os(VI) AT 15° [a]

Registry no.	Substrate	pH[c]	$10^2 k_{obsd}$ min^{-1} [b] 3-Picoline[d] system	Pyridine system	3-Chloropyridine system
1431-06-7	*cis*-Thymine glycol	10.25	69.2	208	
	cis-Thymine glycol	9.25	20.0	60.20	
	cis-Thymine glycol	8.05	2.0	10.70	836.6 (pH 7.9)
	cis-Thymine glycol	6.85		3.30	91.75 (pH 6.5)
1124-84-1	*trans*-Thymine glycol	10.25		13.20	
	trans-Thymine glycol	9.25		3.62	
	trans-Thymine glycol	8.05		0.69	
38645-22-6	*cis*-Thymidine glycol	9.25		18.23	
38645-23-7	*cis*-1,3-Dimethylthymine glycol	10.20		45.67	
	cis-1,3-Dimethylthymine glycol	9.25	5.62	14.36	
	cis-1,3-Dimethylthymine glycol	8.0		2.66	

[a] [Os(VI) complex] = 4–5 × 10^{-4} *M* calculated as the monomer, OsO_3L_2; [L] = 0.08–0.6 *M*; [substrate] = 8–32 × 10^{-3} *M*; λ 304 nm, μ 0.14–0.15 *M*. [b] k_{obsd} = slope of k_ψ *vs.* 1/[L] plots divided by [S], or the slope of k_ψ *vs.* [S] plots multiplied by [L]; see text. [c] The fluctuation in pH in all runs was within ±0.05 pH units. [d] The k_2 values (M^{-1} min^{-1}) evaluated from intercepts of plots of k_ψ *vs.* 1/[3-pic], with *cis*-thymine glycol as substrate were 0.28 (pH 8.05), 0.47 (pH 9.25), and 1.25 (pH 10.25); for *cis*-1,3-dimethylthymine glycol, 0.12 (pH 9.25).

concentration at constant pH and ionic strength. Table II shows that the reactivity for the Os(VI) complexes decreased in the order OsO_3(3-chloropyridine)$_2$, OsO_3(pyridine)$_2$, OsO_3(3-picoline)$_2$ and also gives the numerical results as a function of pH and substrate. *cis*-1,3-Dimethylthymine glycol, which does not ionize appreciably in the pH range investigated, shows the same pH dependence as *cis*-thymine glycol.

Cis–Trans Isomerization of the Glycols.—Criegee, *et al.*,[2] reported that OsO_3(pyridine)$_2$ as well as $(KO)_2(CH_3O)_4Os$ and $KO(AcO)_3Os{=}O$ react in general only with acyclic 1,2-diols and with cyclic cis 1,2-diols. Other diols, including most trans 1,2-diols, did not react with the exception of *trans*-1,2-cyclohexanediol and *trans*-1,2-cycloheptanediol. Model-building with *trans*-thymine glycol shows that one cannot expect to form a cyclic osmate ester from this rigid compound. Nevertheless, we observed that *trans*-thymine glycol (Table I), *trans*-thymidine glycol, and *trans*-1,3-dimethylthymine glycol all react slowly with OsO_3(pyridine)$_2$. The product is in each case the cis ester as shown by the identity of the ir spectra. Since we have observed that the trans glycols isomerize to the cis glycols in the absence of Os(VI) species (see also Shugar[12]), we interpret the conversion of the *trans*-thymine glycol to the *cis*-osmate ester as the sum of the following reactions.

$$\text{trans glycol} \rightleftarrows \text{cis glycol}$$

$$\text{cis glycol} + OsO_3\cdot py_2 \longrightarrow \text{bis(pyridine) } cis\text{-osmate ester}$$

The isomerization of the *cis*- to the *trans*-thymine glycols has been previously reported,[12,13] but the corresponding reactions of the thymidine and 1,3-dimethylthymine glycols (see Experimental Section) are new.

We have no clues to the mechanism of these interesting transformations aside from our qualitative observation that the reactions are base catalyzed and that 1,3-dimethylthymine glycol is not prevented from undergoing the isomerization. Hahn and Wang have recently suggested a possible pathway for the thymine glycols[14] which is, however, not consistent with our finding of apparently similar isomerizations for thymidine and 1,3-dimethylthymine.

Mechanism.—We outline below a mechanism which fits the data as we have them. We realize, however, that the study of the Os(VI)–pyridine–hydroxyl ion system is incomplete.

$$Os_2O_6L_4 \rightleftarrows 2OsO_3L_2 \tag{1}$$

$$OsO_3\cdot L_2 + HO^- \overset{K}{\rightleftarrows} (OsO_3\cdot L\cdot OH^-) + L \tag{2}$$

$$OsO_3\cdot L_2 + S \xrightarrow{k_2} \text{product} \tag{3}$$

$$(OsO_3\cdot L\cdot HO^-) + S \xrightarrow{k_1} (OsO_3\cdot L\cdot OH\cdot S^-) \xrightarrow[L]{\text{fast}} \text{product} + HO^- \tag{4}$$

Here S is the substrate and the product is the bis(ligand) osmate(VI) ester.

If we assume essentially complete dissociation of the dimer to monomeric species under the kinetic conditions, the rate law for this process is

$$v = k_1[OsO_3\cdot L\cdot HO^-][S] + k_2[OsO_3\cdot L_2][S] \tag{5}$$

The total Os(VI) species is given by

$$[Os(VI)]_{total} = [OsO_3\cdot L\cdot OH^-] + [OsO_3\cdot L_2] \tag{6}$$

We omit any ligand-free Os(VI) species for the reasons already discussed and also because the data show a clean inverse dependence on the first power of the ligand. Substituting for $[OsO_3\cdot L\cdot OH^-]$ from the equilibrium expression (eq 2), we get

$$[Os(VI)]_{total} = \frac{K[OsO_3\cdot L_2][OH^-]}{[L]} + [OsO_3\cdot L_2] \tag{7}$$

$$[OsO_3\cdot L_2] = \frac{[Os(VI)]_{total}[L]}{K[OH^-] + [L]} \tag{8}$$

(12) D. Barszcz, Z. Tramer, and D. Shugar, *Acta Biochim. Pol.*, **10**, 9 (1963).

(13) S. Iida and H. Hayatsu, *Biochim. Biophys. Acta*, **213**, 1 (1970).

(14) B. S. Hahn and S. Y. Wang, *J. Amer. Chem. Soc.*, **94**, 4764 (1972).

This expression may be simplified for the reaction conditions we have used because our data show that the $K[OH^-]$ term (see Table II) is negligible in comparison with the ligand term in the denominator of eq 8. Thus

$$[OsO_3 \cdot L_2] \simeq [Os(VI)]_{total} \quad (9)$$

With limiting concentrations of the Os(VI) species, k_ψ, the pseudo-first-order rate constant, is given by

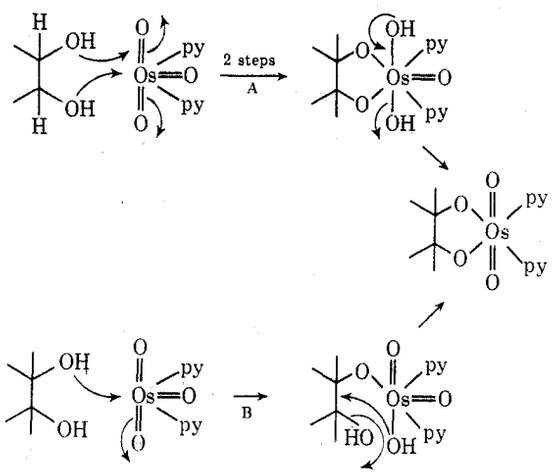
$$v = k_\psi [Os(VI)]_{total} \quad (10)$$

Thus

$$k_\psi = \frac{k_1 K [S][OH^-]}{[L]} + k_2[S] \quad (11)$$

Equation 11 predicts that a plot of k_ψ *vs.* $1/[L]$ will be linear with a slope given by $k_1K[S][OH^-]$ and an intercept equal to $k_2[S]$.

The data show that the k_1 term is large compared with the k_2 term. Indeed, k_2 was measurable only for the 3-picoline system (footnote *d*, Table II). This is also consistent with the reactivity order for the ligand complexes, 3-chloropyridine > pyridine > 3-picoline, and implies rate-limiting nucleophilic attack by the glycol on the osmium species. Two pathways can be written for a mechanism of this type which differ in the nature of the fast ring closure.



Experiments with ^{18}O-labeled glycerol were carried out to distinguish between these pathways. Glycerol labeled (2.2 ± 0.1 atom %) at positions 1 and 2 with ^{18}O was allowed to react with $OsO_3(pyridine)_2$. The bis(pyridine) osmate ester was reductively hydrolyzed. The glycerol was reisolated and found to contain 2.4 ± 0.1 atom % ^{18}O. Pathway A predicts 2.2 ± 0.1 atom % ^{18}O, whereas pathway B predicts no more than 1.4 ± 0.1 atom % ^{18}O. Our finding is thus consistent with pathway A and eliminates pathway B from consideration.

pH Dependence.—Equation 11 predicts first-order dependence on $[OH^-]$. Table I shows, however, that, although the rate of reaction increases with pH, the order in $[OH^-]$ is only about one half. We can account for this in a qualitative way by our observation that the isomerization of the *cis*- to the *trans*-thymine glycol is base catalyzed. Since the trans glycol is converted to the product ester more slowly than the cis glycol (Table I), these reactions will reduce the apparent $[OH^-]$ dependence of eq 10 to some value less than one. We have insufficient data for a quantitative treatment.

Studies at higher pH values are further complicated by ionization of the substrates which have approximate pK_a' values of 10.8[13] (*cis*-thymine glycol) and 10.7[15] (*cis*-thymidine glycol), the base-catalyzed ring cleavage of the glycols,[13,15] and, at very high concentrations of base, cleavage of the esters to the osmate ion.[4]

Experimental Section

Reagents.—Reagent grade pyridine, 3-picoline, and 3-chloropyridine were purified by distillation over KOH. Sources for other chemicals follow: thymine and thymidine, Sigma Chemical Co.; osmium tetroxide, Varlacoid Chemical Co.; dimethyl sulfoxide-d_6, Norell Chemical Co.; tetramethylsilane, Aldrich Chemical Co.; other chemicals were of reagent grade and were obtained from the usual commercial sources. Phosphate buffers were used in the pH range 6–8 and carbonate buffers in the pH range 9–10. Stock solutions of the various pyridines were made up in buffer. Stock solutions of thymine glycol derivatives and of Os(VI) complexes were prepared in double distilled deionized water and stored at 5°

Analyses.—Ultraviolet spectra were measured using a Perkin-Elmer Model 202 instrument, ir spectra on a Perkin-Elmer Model 237B or 457 grating instrument, and nmr spectra on a Varian Associates Model T-60 instrument (60 MHz) at 35° using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. Mass spectra were obtained on a Finnigan Model 1015 S/L instrument. Equilibrium ultracentrifugation was carried out using a Spinco Model E analytical ultracentrifuge equipped with electronic speed control and RTIC temperature control. Runs were carried out at 40,000 rpm using interference optics, a double-sector cell, 12-mm path length with sapphire windows. Partial specific volumes were determined pycnometrically. The pyridine, 3-picoline, and 3-chloropyridine content of the Os(VI) compounds was determined by the method of Ang.[16] Paper chromatography of the osmate esters was carried out on Whatman #1 paper using ethyl acetate:2-propanol:water, 75:16:9 (v/v) (solvent A) and 1-butanol:water, 86:14 (v/v) (solvent B). Standard osmate(VI) esters were prepared by the reaction of OsO_4 with thymine or thymidine in the presence of various ligands as previously described.[3] Elementary analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Kinetics.—Kinetic runs were conducted by spectrophotometry in 1-cm capped silica cells. Reactions were started by the addition of substrate and were followed by the increase in absorbance at 304 nm where we observed the maximum difference between $OsO_3 \cdot L_2$ and the osmate(VI) esters. Reactions were run under pseudo-first-order conditions with the substrate in at least tenfold excess over the Os(VI) species. The ionic strength was between 0.14 and 0.15 *M*.

Os(VI) Complexes with Pyridine and Substituted Pyridines.—Complexes of the general formula $Os_2O_6 \cdot L_4$, where L represents a monodentate ligand such as pyridine, were prepared by a slight modification of Criegee's method.[2] Osmium tetroxide (0.5 g) was dissolved in 8 ml of CCl_4. Pyridine (2 ml), 3-picoline (2.2 ml), or 3-chloropyridine (2.5 ml) was added followed by 1.9 ml of absolute ethanol as reducing agent. The mixture was allowed to stand at room temperature for 18 hr. The precipitated material was filtered, washed several times with CCl_4, and dried under vacuum. Ir: (KBr) $Os_2O_6(pyridine)_4$, 830, 645 cm^{-1}; $Os_2O_6(3\text{-picoline})_4$, 835, 635 cm^{-1}; $Os_2O_6(3\text{-chloropyridine})_4$, 837, 640 cm^{-1}. Compare ref 8.

$Os_2O_6(pyridine)_4$ is identical with the material prepared by Badger's method[17] and reported to be $OsO_4(pyridine)_2$.

Anal. Calcd for $Os_2O_6(pyridine)_4$: pyridine, 39.92. Found: 39.56. Calcd for $Os_2O_6(3\text{-picoline})_4$: 3-picoline, 43.88. Found: 43.79. Calcd for $Os_2O_6(3\text{-chloropyridine})_4$: 3-chloropyridine, 48.79. Found: 48.32. These complexes consumed 2 equiv of iodide per atom of osmium corresponding to reduction of Os(VI) to Os(IV) when titrated according to the method described for osmate(VI) esters.[4]

Osmate(VI) Esters.—$Os_2O_6(pyridine)_4$ (2.5×10^{-4} mol) and *cis*-thymine glycol (5×10^{-4} mol) were mixed in 10 ml of a 1 *M*

(15) S. Iida and H. Hayatsu, *Biochim. Biophys. Acta*, **228**, 1 (1971).
(16) K. P. Ang, *Anal. Chem.*, **38**, 1411 (1966).
(17) G. M. Badger, *J. Chem. Soc.*, 456 (1949).

aqueous solution of pyridine. The dark brown solution was allowed to stand overnight and then evaporated to dryness. The brown powder was washed several times with diethyl ether and dried under vacuum. The same product was obtained when Os_2O_6(pyridine)$_4$ and *cis*-thymine glycol were allowed to react in water. The 3-picoline and 3-chloropyridine esters were prepared in the same way. In all cases the yield was about 98%. These esters were identical with the esters prepared from osmium tetroxide, the ligand, and thymine.[3]

***cis*-Thymine Glycol.**—This material was synthesized by the procedure of Baudisch and Davidson.[18] It was recrystallized from 95% ethanol to yield white crystals: mp 214–215° (lit.[13,18,19] 216, 220, 215°); ir (KBr) 3350, 3425 (OH), 3238 (NH), 1738, 1700, 1668 (C=O), 1230, 1172, 1088, 1052, 987, 933 cm^{-1}; nmr (DMSO-d_6) δ 1.27 (s, 3, 5 -CH_3), 4.32 (t, 1, J = 5 Hz, 6 -H), 5.23 (s, 1, 5 -OH), 5.97 (d, 1, J = 5 Hz, 6 -OH), 8.07 (d, broad, 1, J = 5 Hz, 1 -NH), and 9.93 (s, broad, 1, 3 -NH). The nmr data are in good accord with those reported by Chabre, *et al.*,[20] except that the quartet for H-6 is not sufficiently resolved in our work and appears as a triplet. The uv spectrum in carbonate buffer, pH 9.85, showed a broad peak between 220 and 230 nm (ϵ 2350). The cis glycol was also prepared by performic acid oxidation of thymine. There are precedents[21] for the formation of the cis glycol by peracid oxidation, although the usual product is the trans glycol. A mixture of 5 g of powdered thymine, 45 ml of 90% formic acid, and 9 ml of 30% H_2O_2 was kept at 40° until all of the peroxide had been consumed (about 66 hr). The solution was evaporated to dryness under reduced pressure; 100 ml of water was added to the solid residue; and the mixture was again taken to dryness. This residue was then heated with 100 ml of water at 98° for 1 hr. The solution was cooled in an ice bath, whereupon 1.5 g of unreacted thymine separated. The filtrate, containing the thymine glycol, was evaporated to dryness under reduced pressure and the residue was recrystallized from ethanol to yield 3.5 g (55%) of *cis*-thymine glycol identical with the material prepared by the Baudisch and Davidson procedure.[18] *cis*-Thymine glycol was also prepared for comparison in small amounts by hydrolysis of the bis(pyridine) osmate ester of thymine.

***trans*-Thymine Glycol.**—The trans glycol was prepared by isomerization of the cis glycol. One gram of the cis glycol was refluxed in 80 ml of water for 8 hr. Chromatography in solvent A showed only two spots corresponding to the two glycols. Heating beyond 9 hr resulted in the appearance of two additional spots, which are ring-cleavage products as shown by their reaction with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) in the absence of alkali. These appear on paper chromatograms at R_f values corresponding to the additional spots reported by Shugar.[12] The trans glycol was separated by preparative chromatography in solvent A on Schleicher and Schull Orange Ribbon paper (thick, high capacity). The R_f ratio of the trans to cis isomer was 1.5 ± 0.05. The glycols were located on chromatograms by their uv absorption and by their characteristic color changes following the NaOH–Ehrlich's reagent spray (yellow to pink to blue).[22] The isomers were distinguished from one another by the fact that the cis but not the trans reacted with the periodate–benzidine reagent[23] and by the fact that the cis isomer had zero mobility in solvent A if the paper was impregnated with borate.[24] It was eluted with water, crystallized from 95% ethanol, and rechromatographed to remove traces of the cis isomer to give a white solid in about 14% yield: mp softens 166–168°, 218–219° dec; ir (KBr) 3351, 3412 (OH), 3202 (NH), 1744, 1714, 1695 (C=O), 1280, 1163, 1097, 970 cm^{-1}; nmr (DMSO-d_6) δ 1.25 (s, 3, 5 -CH_3), 4.39 (t, 1, J = 5 Hz, 6 -H), 5.84 (s, 1, 5 -OH), 6.15 (d, 1, J = 5 Hz, 6 -OH), 8.05 (d, broad, 1, J = 5 Hz, 1 -NH), and 9.95 (s, broad, 1, 3 -NH). These data are virtually identical with those reported by Cadet and Téoule[1] (nmr) and by Téoule[25] (ir). The nmr data, published by Hahn and Wang[14] are in error; all δ values should be reduced by 0.32. Compare also the ir assignments of Nofre, *et al.*,[26] and the nmr assignments of Rouillier, *et al.*[27] The uv spectrum in carbonate buffer, pH 9.85, showed a peak at 221 nm (ϵ 1635 at 230 nm).

Anal. Calcd for $C_5H_8N_2O_4$: C, 37.52; H, 5.04; N, 17.50. Found: C, 37.55; H, 5.23; N, 17.41.

***cis*-Thymidine Glycol.**—This compound was synthesized by the permanganate oxidation of thymidine.[15] The glycol was separated from the other components of the oxidation mixture by preparative chromatography on Schleicher and Shull Orange Ribbon paper using Solvent B. The R_f ratio of thymidine to its cis glycol was 2.75. The glycol was eluted from the paper with water and crystallized from methanol–ether mixtures to give a white solid: mp softens 134–138°, 190–191° dec (lit.[15,28] mp 191–193°, 189–190°); ir (KBr) 1742, 1694 cm^{-1} (C=O); nmr (DMSO-d_6) δ 1.23 (s, 3, 5 -CH_3), 4.75 (s, 1, 6 -H), 5.46 (s broad, 1, 5 -OH), 6.05 (multiplet, 1, 6 -OH), and 10.00 (broad, 1, 3 -NH). (Compare ref 15, 28.)

***trans*-Thymidine Glycol.**—*cis*-Thymidine glycol (200 mg) was dissolved in 16 ml of water and refluxed for 8 hr. The trans and cis isomers were separated by preparative paper chromatography in solvent B. The compounds were located by their uv absorption and by the NaOH–Ehrlich's reagent spray. The yield of trans glycol was about 5%. Both isomers reacted with the periodate–benzidine reagent. The R_f ratio of the trans to the cis isomer was 1.8; mp 195–197° dec; ir (KBr) 1700 cm^{-1} (C=O); nmr (DMSO-d_6) δ 1.31 (s, 3, 5 -CH_3), 4.70 (s, 1, 6 -H), 5.76 (s broad, 1, 5 -OH), 5.93 (multiplet, 1, 6 -OH), and 10.25 (broad, 1, 3 -NH).

The mass spectrum (75 eV) gave peaks at m/e 259 (M − OH)$^+$, 160 (MH − deoxyribosyl)$^+$, 117 (deoxyribosyl)$^+$.

1,3-Dimethylthymine.—This compound was prepared in 96% yield following the procedure described by Davidson and Baudisch[29] for the synthesis of 1,3-dimethyluracil. Crystallization from 95% ethanol gave white needles: mp 152° (lit.[30] mp 153°); ir (KBr) 1705, 1670, 1645 cm^{-1} (C=O); nmr (DMSO-d_6) δ 1.82 (s, 3, 5 -CH_3), 3.2–3.3 (two s, 6, 1- and 3 -CH_3), and 7.6 (s, 1, 6 -H).

***cis*-1,3-Dimethylthymine Glycol.**—The glycol was synthesized from 1,3-dimethylthymine following the procedure of Baudisch and Davidson[18] for thymine glycol. The material was obtained in 84% yield as an uncrystallizable gum after drying over P_2O_5. The material did not react with periodate: ir (neat) 3375 (OH), 1725, 1662 (C=O), 1185, 1137, 1050 cm^{-1} (CO); nmr (DMSO-d_6) δ 1.33 (s, 3, 5 -CH_3), 3.0 (two s, 6, 1- and 3 -CH_3), 4.5 (d, 1, J = 5 Hz, 6 -H), 5.15 (broad, 2, 5- and 6 -OH).

Anal. Calcd for $C_7H_{12}N_2O_4$: C, 44.67; H, 6.43; N, 14.89. Found: C, 44.44; H, 6.53; N, 14.97.

***trans*-1,3-Dimethylthymine Glycol.**—*cis*-1,3-Dimethylthymine glycol (420 mg) was dissolved in 34 ml of water and refluxed for 5.5 hr. The trans and cis isomers were separated by preparative paper chromatography in solvent B. The compounds were located by their uv absorption. The R_f ratio of the cis to the trans isomer was 1.2. The yield of trans glycol was about 54% and it was obtained as a gum after drying over P_2O_5: ir (neat) 3400 (OH), 1650 (C=O), 1185, 1056 cm^{-1} (CO); nmr (DMSO-d_6) δ 1.28 (s, 3, 5 -CH_3), 3.0 (two s, 6, 1- and 3 -CH_3), 4.55 (s, 1, 6 -H), 3.76 (broad, >2, 5- and 6 -OH, H_2O).

Anal. Calcd for $C_7H_{12}N_2O_4 \cdot {}^1/_2H_2O$: C, 42.64; H, 6.99; N, 14.21. Found: C, 42.52; H, 6.70; N, 14.32.

Oxygen-18 Measurements.—Osmium tetroxide (1.18×10^{-4} mol) was mixed with 8.83×10^{-4} mol of allyl alcohol in 2.5 ml of 3.3 ± 0.1 atom % $H_2{}^{18}O$ in a closed container under air. Under these conditions, the osmate ester initially formed hydrolyzes with 100% Os–O bond cleavage to give glycerol and an Os(VI) species which is reoxidized to osmium tetroxide by oxygen.[4] The osmium thus cycles until all of the allyl alcohol is consumed. After 11 days at room temperature, the black precipitate was coagulated by warming and then filtered. The precipitate was discarded. The filtrate containing glycerol was evaporated under a stream of air to about 0.1 ml. Water was added and the evaporation was repeated twice in order to remove any remaining

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traces of allyl alcohol. A sample was analyzed by mass spectrometry as previously reported.[4] The remainder was diluted to 2 ml with ordinary water and then treated with excess Os_2O_6(pyridine)$_4$ and a trace of free pyridine. After standing overnight, the solution was filtered. The filtrate was dried under a stream of air to give the brown bis(pyridine) osmate ester of glycerol. This ester was reductively hydrolyzed to glycerol in water in the presence of a 2–3-fold excess of $NaHSO_3$ at room temperature for 30 min. The mixture was analyzed by measurement of the mass spectrum at m/e 61, 62, and 63 using the calculations described by Biemann.[31] Suitable blanks were run with $NaHSO_3$ alone.

Dissociation Constants for $OsO_3 \cdot L_2$.—The distribution coefficient of pyridine and 3-picoline between buffer and diethyl ether was determined at 15°. The pyridine concentration in the organic phase was measured at 256 nm after transfer to 0.1 N H_2SO_4 using ϵ 5200. 3-Picoline was measured at 263 nm (ϵ 5560). The distribution coefficient, $D = [L_0]/[L_a]$, where the subscripts refer to the organic and aqueous phases, was found to be 1.3 ± 0.02 (pyridine) and 3.3 ± 0.05 (3-picoline). When Os_2O_6(pyridine)$_4$ was equilibrated between equal volumes of buffer and ether, it was found that no detectable quantities of Os(VI) species were extracted into the organic phase, as shown by the lack of absorption in the 300–350-nm region. The degree of dissociation of the ligand from the Os(VI) species could thus be measured from the quantity of ligand in the ether phase and the distribution coefficient. The dissociation constants were calculated from the relationship

$$K = \frac{[OsO_3 \cdot L \cdot OH^-][L_a]}{[OsO_3 \cdot L_2][HO^-]}$$

using $K_w = 5 \times 10^{-15}$.[32] If we can assume no dissociation of the second ligand (see text), then

$$[OsO_3 \cdot L \cdot OH^-] = [L_a] + [L_0]$$

$$[OsO_3 \cdot L_2] = [OsO_3 \cdot L_2]_{initial} - ([L_a] + [L_0])$$

in the absence of added ligand.

Registry No.—Os–3-picoline dimer, 38641-67-7; Os–3-picoline monomer, 38669-79-3; Os–pyridine dimer, 38641-68-8; Os–pyridine monomer, 38669-80-6; Os–3-chloropyridine dimer, 38677-68-8; Os–3-chloropyridine monomer, 38669-81-7; *trans*-thymidine glycol, 38645-24-8; 1,3-dimethylthymine, 4401-71-2; *trans*-1,3-dimethylthymine glycol, 38645-26-0.

Acknowledgment.—We thank the National Science Foundation for support (GB-21267), Dr. George Serif for his help in determining the mass spectra, Mr. John Ragazzo for some of the ir spectra, and Dr. Kirk Aune for the ultracentrifugal data.

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A Comparison of Lithium Aluminum Hydride and Diborane in the Reduction of Certain 3-Indolylglyoxamides

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Received December 13, 1972

The utility of lithium aluminum hydride ($LiAlH_4$) and diborane for the preparation of tryptamines from 3-indolylglyoxamides, including certain 4-trifluoromethyl derivatives, has been studied. Three distinctions in the behavior of these reducing agents toward the glyoxamides have been observed. (1) Diborane allows elaboration of the tryptamine side chain without concomitant reduction of trifluoromethyl substituents, whereas these groups are converted into methyl substituents by $LiAlH_4$ when reducing conditions are sufficiently vigorous to give the tryptamine. (2) Reduction of the glyoxamides with diborane may be accompanied by reduction of the indolic enamine triad to give indolines, an event not seen with $LiAlH_4$. (3) 1-Alkyl-3-indolylglyoxamides are converted into the corresponding tryptamines by diborane, whereas $LiAlH_4$ reduction gives 1-alkyl-3-indolylglycolamines. The formation of a 3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indole (**4**) was observed in the $LiAlH_4$ reduction of 5-methoxy-*N*,*N*,2-trimethyl-4-(trifluoromethyl)-3-indolylglyoxamide (**3c**). Diborane reduction of 3-indolecarboxylic acid (**16b**) and its ethyl ester **16a** gave skatole (**17**) as the major product.

Application of the Nenitzescu reaction[1] to 2-trifluoromethyl-1,4-benzoquinone and alkyl 3-aminocrotonates constitutes a convenient preparation of certain 4-trifluoromethylindoles.[2] The availability of these last substances prompted us to prepare the 4-trifluoromethyl congeners of biologically significant tryptamines, and the procedure of Speeter and Anthony[3] seemed to be the most direct way to achieve this objective. In this method an indole which is unsubstituted at the 3 position is converted into a 3-glyoxamide, reduction of which gives the tryptamine. Lithium aluminum hydride ($LiAlH_4$) is the usual reagent for this reduction, but the use of borane has been reported on one occasion.[4] In this paper we compare the effect of these two reducing agents on certain 3-indolylglyoxamides, including the 4-trifluoromethyl derivatives.

The required amides of Table I were prepared readily from 5-methoxy-2-methyl-4-trifluoromethylindole (**1**)[2] by the usual technique (see Scheme I).[3] Reduction of the N^b,N^b-dimethylglyoxamide **3c** with $LiAlH_4$ in boiling tetrahydrofuran (THF) for 48 hr gave the 4-methyltryptamine **2** and the 3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indole **4**. The former product is identical with that obtained by $LiAlH_4$ reduction of 5-methoxy-2,4,N^b,N^b-tetramethyl-3-indolylglyoxamide,[5] and its formation constitutes another example of the conversion of a trifluoromethyl substituent into a methyl group by $LiAlH_4$. Such conversions were observed earlier for a 6-trifluoromethylindole,[6] another 4-trifluoromethylindole,[2] and a benzotrifluoride.[7] A

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