Reactions of Osmium Tetroxide with Imidazoles

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Abstract

We have studied the reactions of imidazoles with osmium tetroxide both in the presence and absence of ligands other than imidazoles. The pathway involves: (1) osmium tetroxide-ligand complexation (fast), (2) addition of osmium tetroxide to the 4,5-double bond of the imidazole to form the cyclic osmium(VI) ester (slow), and (3) degradation of the osmate ester via ring cleavage of the 4,5-dihydroxydihydroimidazole (very slow).

We report kinetic data for addition to the double bond as a function of imidazole structure. The final product is an osmate(VI) ester of a 4,5-dihydroxydihydroimidazole with two imidazole molecules (or other ligands) occupying the remaining coordination sites. We present evidence, both kinetic and chemical, for an intermediate containing only one such ligand.

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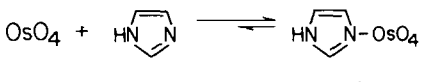

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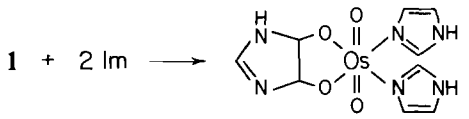

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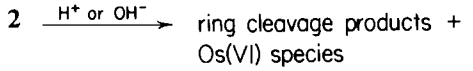

Scheme 1. Reaction of osmium tetroxide with imidazole.

Introduction

The reactions of osmium tetroxide with a number of biologically important heterocycles have been studied in some detail [1–4]. Imidazoles, however, have had little attention.

Rates vary widely, but the common theme is a reaction between osmium tetroxide and an olefinic site, in the presence of tertiary nitrogen ligands, to yield an oxoosmate(VI) ester (Scheme 1). The reaction between imidazoles and osmium tetroxide is a special case for two reasons. First, because the imidazole can function as both the olefin and the ligand, and second, because dissociation of the osmate ester product yields a 4,5-dihydroxydihydroimidazole which is unstable with respect to ring cleavage. Previous work had given examples of imidazoles functioning as ligands (with other compounds as olefins) [5] and of imidazoles functioning as olefins (with other compounds functioning as ligands) [6].

Imidazoles yield various products upon reaction with oxidizing agents. The 4,5-dihydrodiol derivative is reported to be formed by reaction with potassium permanganate [7, 8] and subsequently degraded by ring cleavage. Oxidation involving cleavage of the imidazole ring apparently also occurs with periodate [9], peroxides [7, 10], chromic acid [8], bromo reagents [9] and by electrochemical oxidation [11]. In contrast, nitric acid does not oxidize imidazoles [12].

In competitive reactions of osmium tetroxide with a mixture of amino acids, only the sulfur-containing residues reacted more rapidly than histidine [13]. The reaction of $^{\alpha}N$-acetyl histidine with osmium tetroxide in pyridine yielded small quantities of a cyclic osmate ester [6]. Other investigations [14, 15] have described some aspects of the reactions of osmium tetroxide with histidine and its derivatives.

The kinetics of the oxidation of imidazoles by osmium tetroxide have not been studied although this heterocycle is the nucleus of a number of molecules important in biology. Osmium tetroxide in the presence of imidazole is a good fixative for electron microscopy [16]. For these reasons, and as part of our continued interest in the chemistry of the reactions of osmium tetroxide with proteins, we have studied the oxidation of various imidazoles by this reagent.

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Experimental

Materials

Imidazole, $^{\alpha}N$-acetyl histidine and $^{\alpha}N$-acetyl histamine were obtained from Sigma Chemical Company; imidazole-4-acetic acid was from Calbiochem. 1-Methyl imidazole, 2-methyl imidazole, 4-methyl imidazole, 4-hydroxymethyl imidazole, 4-methyl-5-hydroxymethyl imidazole, pyridine, 2,2′-bipyridine, *N,N,N′,N′*-tetramethylenediamine, sodium deuteroxide and d_3-acetic acid were from Aldrich Chemicals. Deuterium oxide was from the Norell Chemical Company, osmium tetroxide was from Ventron Chemicals and 4,7-diphenyl-1,10-phenanthroline disulfonate was obtained from GFS Chemicals. $^{\alpha}N$-acetyl histidine ethyl ester was synthesized by the procedure of Brenner and Huber [17]. All other chemicals were obtained from the usual sources and were of reagent grade or better.

Instrumentation

All UV and visible spectral determinations were performed using a Cary 15 spectrophotometer. Temperature control (±0.1 °C) was achieved by use of a thermostatted circulating water bath (Forma Scientific Model 2095) and water-jacketed cuvette holders. pH determinations were made with a Radiometer Model 26. NMR spectra were obtained using a Varian T60 (60 MHz) instrument (35 °C). A Beckman IR-2440 instrument was used to obtain IR spectra. Elemental analysis was performed by Galbraith Laboratories. FAB mass spectra were obtained on a VG-70-250S instrument using xenon.

Methods

The ionization constants were determined as follows. The imidazole derivatives (except 1-Me-Im[§] and 4-Me-Im) were dried in an oven (100 °C) and all were stored in a desiccator prior to the pK_a determinations. Molecular sieves were utilized to remove water from 1-Me-Im. Solutions (approximately 20 mM) of each derivative were prepared in carbonate-free, deionized, double-distilled water and stored at room temperature. These solutions were titrated with standardized HCl or NaOH at 22 °C. pK_a values were calculated according to equations given in [18] and are the mean of 7 to 11 values.

Solutions of OsO_4 were calibrated prior to each set of determinations using the following λ (ϵ) values: 304(1202), 297(1442), 282(1738) and 275(1733) [19]. Substrate solutions were prepared by dissolving the weighed substrate in buffer and titrating to the desired pH with HCl or NaOH. Sodium carbonate buffers were used at pH 9.5–9.75, sodium borate at pH 9.7 and sodium phosphate in the pH range 7.4–8.2.

The kinetics were run as follows. An aliquot of the substrate solution in buffer, equilibrated at the desired temperature, was used to blank the spectrophotometer. To begin the reaction, an aliquot of temperature-equilibrated OsO_4 solution was added, the contents of the cuvette rapidly mixed by inversion, and absorbance measurements immediately and continuously recorded. The absorbance at 450 nm was monitored as osmate esters of this type exhibit a weak maximum near this wavelength [1]. Reactions were allowed to proceed until a stable infinity value was obtained.

Equations of [20] were used for those reactions in which a stable A_{450} was not obtained. Plots of $\log(A_\infty - A_t)$ against time were linear for at least two half-lives. Slopes of these lines (k_ψ values) were calculated using a linear regression program (Texas Instruments TI-55III) Since these reactions were run under pseudo-first order conditions ([OsO_4] limiting), rate = $k_{app}[OsO_4][S]^n = k_\psi[OsO_4]$. Values of n (the apparent order in substrate) were determined by varying n and fitting plots of k_ψ against $[S]^n$ to the best straight line through zero. Apparent rate constants (k_{app}) were calculated from $k_{app} = k_\psi/[S]^n$, derived from the initial rate expressions above. Rate equations for the reactions of OsO_4 with imidazoles in the presence of tertiary amine ligands are derived in the text (Results and Discussion).

Activation energies were also determined under pseudo-first order conditions ([OsO_4] limiting). The temperature was increased by 5–7 °C increments over the range 10–38 °C using identical reactant concentrations. Plots of $\log k_{app}$ against $1/T$ yielded straight lines (except ImAA) with slopes = $-E_a/2.303R$.

Destruction of the imidazole ring was measured by the reaction with diazotized sulfanilic acid (Pauly) [21]. An aliquot containing 10–100 μg of imidazole (or derivative) was pipetted into a 25 ml volumetric flask and diluted to 5 ml with water. One ml of 1% sulfanilic acid (in 1 N HCl) and one ml of 5% sodium nitrite were added; the reaction was allowed to proceed for 5 min (the flask was occasionally swirled to mix the contents). Three ml of 20% sodium carbonate were added and the solution vigorously mixed for several seconds; 10 ml of 20% ethanol were

[§] Abbreviations used: Im, imidazole; NAH, $^{\alpha}N$-acetyl histidine; NHn, $^{\alpha}N$-acetyl histamine; ImAA, imidazole-4-acetic acid; 1-Me-Im, 1-methyl imidazole; 2-Me-Im, 2-methyl imidazole; 4-Me-Im, 4-methyl imidazole; 4-HM-Im, 4-hydroxymethyl imidazole; 4-Me-5-HM-Im, 4-methyl-5-hydroxymethyl imidazole; NAHOEt, $^{\alpha}N$-acetyl histidine ethyl ester; py, pyridine; bipy, 2,2′-bipyridine; TMEN, *N,N,N′,N′*-tetramethylethylenediamine; BPDS, 4,7-diphenyl-1,10-phenanthroline disodium disulfonate; NaOD, sodium deuteroxide; D_2O, deuterium oxide; CD_3COOH, d_3-acetic acid; OsO_4, osmium tetroxide.

then added. The mixture was allowed to cool for several minutes; the solution was made up to 25 ml with water. The absorbance at 498 nm was measured.

The oxidation state of osmium was monitored by thiosulfate titration [22]. A stock solution of thiosulfate was standardized against potassium iodate [23] using starch as an indicator. Solutions to be used in the titration contained 10 ml of 0.4 M potassium iodide, 5 ml of 2 N sulfuric acid and 25 ml of chloroform; dry ice was added to each solution to remove oxygen. An aliquot of the reaction mixture was added to this solution and the contents of the flask were mixed vigorously, producing a light purple chloroform layer and dark green aqueous phase. The mixture was titrated (with stirring) with thiosulfate to a colorless endpoint in the chloroform layer.

Cyclic osmate esters were prepared using the following general procedure. The imidazole derivative (3 mmol) in aqueous solution (10 ml) was reacted with one mmol OsO_4 which was added, with stirring, as an aqueous solution or dissolved in acetone. The reactions were allowed to proceed for 15 to 20 min at 25 °C, after which time the ester was precipitated by addition of 5 to 10 volumes of cold acetone (with stirring) and cooling in a dry ice/ethanol bath for about 15 min. Solvent was decanted (or the precipitate was pelleted by centrifugation) and the brown residue was washed several times with cold acetone. The 1-Me-Im osmate ester was re-precipitated by dissolving this residue in hot water followed by the addition of cold acetone. The precipitate was washed several times with cold acetone and dried at 25 °C under reduced pressure (oil pump, 80 h). NMR analysis indicates that about 4 mol of water and 1/3 mol of acetone remain bound to the product; the NMR spectra of esters of Im and 4-Me-Im could not be obtained due to the insolubility of these compounds in common solvents. Yields: 18% (1-Me-Im ester) and 22% (Im ester) and 63% (4-Me-Im ester). Elemental analysis was obtained for the 1-Me-Im ester. *Anal.* Calc. for $C_{12}H_{18}N_6O_4Os \cdot 5.5H_2O \cdot \frac{1}{3}$acetone; C, 25.22; H, 4.72; N, 13.58. Found: C, 25.14; H, 3.84; N, 13.68%. NMR spectrum: 3.1 (s, 3), 3.9 (s, 6), 6.0 (broad, 2.5), 7.0 (s, 4), 7.8 (s, 2). IR spectrum: 680 cm^{-1} (m), 840 cm^{-1} (s). The UV–Vis spectrum shows a broad peak centered at 450 nm (ϵ = 247 M^{-1} cm^{-1}).

A Xe-FAB spectrum of the complex in a glycerol matrix (introduced from a $CHCl_3$–MeOH solution) showed a cluster of peaks around *m/e* 500 with the most prominent at 503 (=$M + H^+$ for ^{192}Os). There were also smaller clusters at higher values of *m/e*. We can account for most of these by postulating interchange in the matrix of one or both of the two 1-Me-Im ligands for 4,5-dihydroxydihydro-1-methylimidazole and for its 4-keto dehydration product.

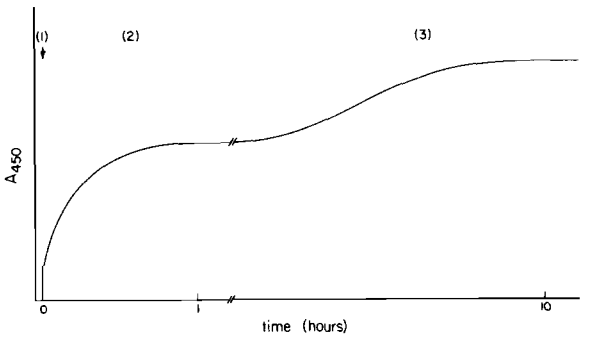

Fig. 1. Typical kinetic run for the oxidation of an imidazole by osmium tetroxide, as measured by the absorbance at 450 nm. The numbered regions of the curve correspond to the reactions of Scheme 1. The time scale shown is relative.

Results and Discussion

Reactions with Pyridine as Ligand

In the presence of a tertiary amine ligand, L (such as pyridine), the reaction of OsO_4 with imidazoles follows the reactions shown in Scheme 1 [3]. The formation of the $OsO_4 \cdot L$ complex is very rapid compared to the other reactions [24], while the third reaction is slow relative to the other two. Thus, reaction 2 can be followed independently. A typical plot of A_{450} *versus* time is shown in Fig. 1. Kinetic data for the addition of OsO_4 to the olefinic site of various imidazoles in the presence of pyridine are given in Table I. The kinetics were run under pseudo-first order conditions; these reactions showed first-order dependence on OsO_4 over at least two half-lives, thus

$$\text{rate} = k_\psi [OsO_4]. \quad (1)$$

The substrate dependence was one for these reactions. Variation in pyridine concentration showed a dependence on pyridine activity (a_{py}) [25] which varied with the structure of the imidazole substrate. Thus,

$$\text{rate} = k' [OsO_4] [S] a_{py}^{\,n} \quad (2)$$

where n is the order in pyridine; then

$$k' = k_\psi / [S] a_{py}^{\,n} \quad (3)$$

As was found for the reaction of OsO_4 with indoles [1] a three-term rate law accounts for non-integral dependence on pyridine:

$$\text{rate} = k_0 [OsO_4] [S] + k_1 [OsO_4] [S] a_{py} + k_2 [OsO_4] [S] a_{py}^{\,2} \quad (4)$$

and

$$k_\psi / [S] = k_0 + k_1 a_{py} + k_2 a_{py}^{\,2} \quad (5)$$

TABLE I. Kinetic Data for the Reaction of Various Imidazoles with Osmium Tetroxide in the Presence of Pyridine[a]

Substrate	pH	T (°C)	No. of runs	[py] (M)	n[b]	k' ($M^{-(n+1)}$ min^{-1})	$10^2 k_0$ (min^{-1})	k_1 (M^{-2} min^{-1})	k_2 (M^{-3} min^{-1})
NAH	7.6	40	7	0.025–0.19	1.70	6.45 ± 0.4	0.22	1.02	[c]
NAH	8.2	40	7	0.014–1.17	1.20	30.30 ± 0.8	0.51	5.30	49.3
NAHOEt	7.4	40	7	0.029–0.22	0.55	3.34 ± 0.4	3.6	4.01	6.0
ImAA	9.5	40	8	0.018–0.59	1.07	11.30 ± 0.2	0.55	7.48	5.9
Im	9.5	30	7	0.019–0.75	1.32	16.70 ± 0.7	1.0	5.01	14.7
NHn	9.5	30	8	0.023–0.68	1.06	16.10 ± 0.4	0	15.80	0

[a]General conditions: reactions were run under pseudo-first order conditions ([OsO_4] limiting, [Substrate] > 10[OsO_4]), 0.1 M sodium phosphate (pH 7.4 to 8.2) or 0.1 M sodium carbonate (pH 9.5). [b]Apparent order in pyridine (n) values were determined from the slopes of van't Hoff plots ($\log k_\psi$ against $\log a_{py}$). [c]Not determined.

A plot of $k_\psi/[S]$ against a_{py} is non-linear, with the y-intercept equal to k_0 (Fig. 2). A plot of $k_\psi/[S]$ against a_{py}^2 is linear in the absence of a k_1 term (this was not the case with any of these imidazoles). The rate constants (k_1 and k_2) were derived from plots of $(k_\psi - k_0)/[S]a_{py}$ (Fig. 3) where the y-intercept equals k_1 and the slope equals k_2 [26].

The variation in pyridine dependence (Table I) with imidazole structure can be rationalized on the same basis as was discussed for corresponding reactions in the indole series [1]. A large k_1/k_2 ratio indicates an intermediate in which one pyridine molecule serves as ligand; the other temporary ligand is a nucleophilic site on the imidazole side-chain. The final product is formed by displacement (in a fast step) of the internal ligand by another molecule of pyridine. $^{\alpha}N$-acetyl histidine is a particularly prominent case of this sort; imidazole-4-acetic acid and $^{\alpha}N$-acetyl histidine ethyl ester also show large k_1/k_2 ratios. A comparison of rate constants (k_2, M^{-3} min^{-1}) for a number of olefinic substrates with the OsO_4–pyridine reagent shows that imidazoles react slowly: isopentenyl adenine [4], 1.5×10^6, 8 °C; cyclohexene carboxylic acid [22], 6×10^4, 8 °C; N-acetyltryptophan [1], 7100, 25 °C; thymidine [1], 1974, 25 °C; imidazole (Table I), 15, 30 °C.

We attempted to measure the activation energy for reaction of the OsO_4–pyridine reagent with a number of imidazole derivatives but the Arrhenius plots were generally curved, probably indicating the presence of two sequential reactions (see Scheme 1) with different activation energies [26].

Reactions with Bidentate Ligands

The kinetics of the reaction of OsO_4 with $^{\alpha}N$-acetyl histidine, in the presence of bidentate ligands (TMEN, bipy and BPDS), also were determined (Table II). These kinetics follow the expression:

$$\text{rate} = k_0[OSO_4][S] + k_1[OsO_4][S][L]^n; \quad (6)$$

combination with eqn. (1) gives

$$k_\psi/[S] = k_0 + k_1[L]^n \quad (7)$$

Plots of $k_\psi/[S]$ against $[L]^n$ yielded straight lines with a y-intercept equal to zero (*i.e.*, the k_0 term is negligible) and the slope equal to k_1.

Reactions of Imidazoles with OsO_4

Imidazole is approximately 100 times more basic than pyridine [27] and so might be expected to serve as an adequate ligand as well as an olefin in these reactions. The stability constants of several M^{2+}·Im species are about 80 times greater than those of the corresponding M^{2+}·py adducts [27, 28]. Because of the rapid subsequent reaction to form the cyclic osmate ester, the stability constant for the OsO_4·Im complex could not be determined. However, the formation of this complex is consistent with the small, very rapid initial rise in the absorbance at 450 nm (see Fig. 1) upon mixing the reactants.

Rate data for the oxidative addition reaction are given in Table III. The order in OsO_4 is one. The apparent order in substrate varies, depending on the structure of the imidazole derivative. 4-Hydroxymethyl, 1-methyl, 2-methyl and 4-methyl-5-hydroxymethyl imidazole exhibit a substrate dependence of two. The kinetics follow the expression:

$$\text{rate} = k_{app}[OsO_4]^2[S]^2 \quad (8)$$

One substrate molecule is the olefinic substrate for the addition reaction; the other is a ligand. A second ligand is subsequently added to complete the formation of the osmate ester. Additional evidence for an intermediate containing only one imidazole as ligand comes from quantitative determination of the imidazole ring in lyophilized reaction mixtures as a function of time, by means of the Pauly reaction. We used 1-Me-Im for these studies because of its relatively low boiling point. The reaction can be stopped by freezing and the unreacted 1-Me-Im and OsO_4 removed by lyophilization. In addition, the oxidation state of osmium in the reaction mixture was determined by thiosulfate titration [22]. The oxidation

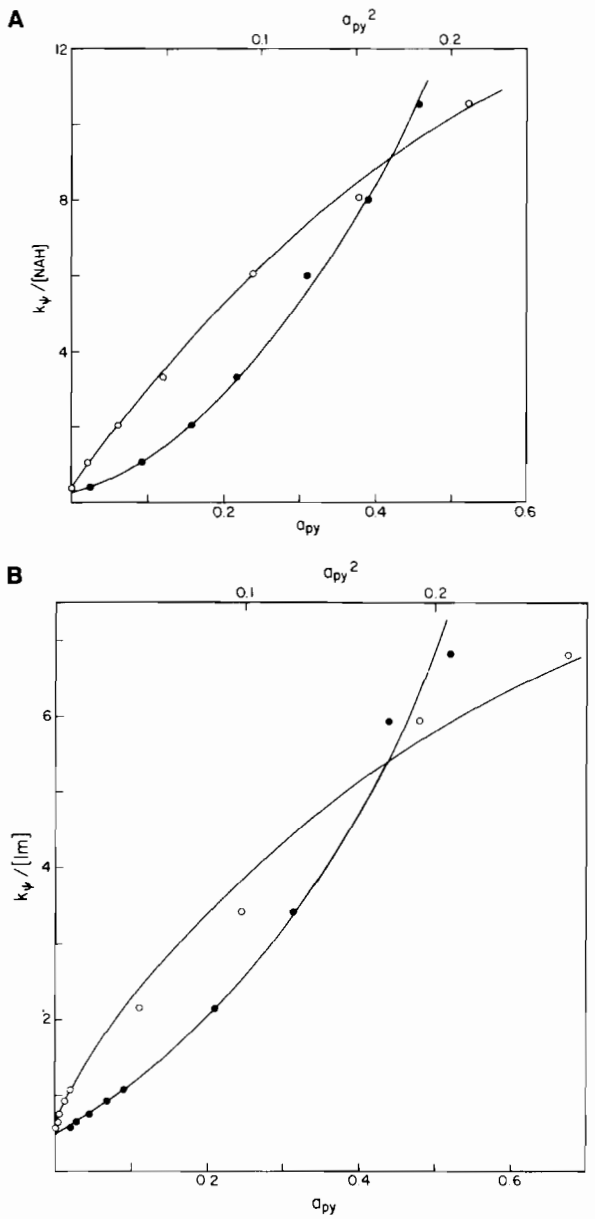

Fig. 2. Determination of k_0 for reaction of osmium tetroxide with imidazoles in the presence of pyridine. (A) [NAH] = 1.36 × 10^{-2} M, [OsO_4] = 1.35 × 10^{-3} M, [py] = 0.023–1.05 M, 0.1 M sodium phosphate, pH 8.2, 40 °C. (B) [Im] = 2.17 × 10^{-2} M, [OsO_4] = 2.15 × 10^{-3} M, [py] = 0.02–0.8 M, 0.1 M sodium carbonate, pH 9.5, 30 °C. • = a_{py}, ○ = a_{py}^2.

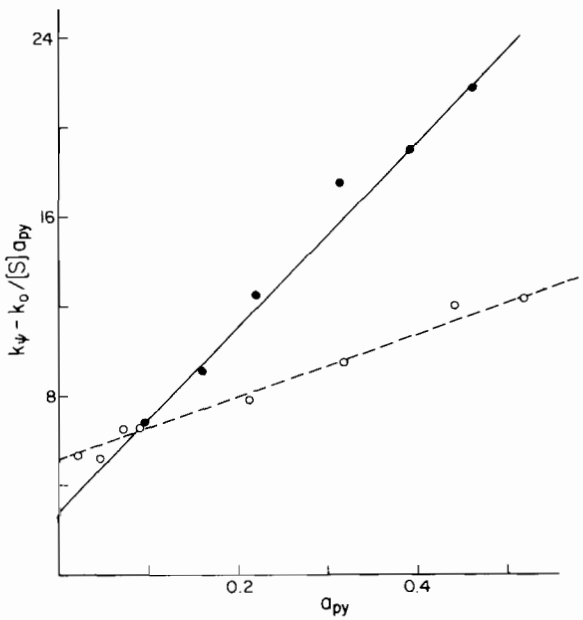

Fig. 3. Determination of k_1 and k_2 for reaction of osmium tetroxide with imidazoles in the presence of pyridine. General conditions are the same as those for Figure 2. • - - - • = $^{\alpha}N$-acetyl histamine, ○ - - - ○ = imidazole.

state of osmium decreases from +8 to +6 expected [29–31] for osmate ester formation (Fig. 4); the reduction is complete in about 20 minutes. The Pauly assay (on lyophilized material) shows that only two 1-Me-Im molecules are present after 20 minutes of reaction (Fig. 4). The half-time of this reaction is about three minutes; this is consistent with the half-time of the reaction monitored conventionally by the increase in absorbance at 450 nm. A third mole of 1-Me-Im is added to form the final product; this is a slow step ($t_{1/2}$ about 30 minutes). In both the intermediate and final product the dihydroimidazole ring appears to be Pauly reactive. We explain this by noting that the Pauly assay is carried out first in acid and then in basic solution. These conditions are apparently sufficient to lead first to hydrolysis of the ester [22] and then to dehydration of the 4,5-dihydroxydihydro imidazole [32] to yield a Pauly-positive 4-keto imidazole.

The other imidazoles (imidazole, 4-methyl imidazole, $^{\alpha}N$-acetyl histidine, $^{\alpha}N$-acetyl histamine and

TABLE II. Kinetic Data for Reaction of OsO_4 with $^{\alpha}N$-acetyl Histidine in the Presence of Various Bidentate Ligands[a]

Ligand	pH	T (°C)	[L] (M)	n[b]	k_1 ($M^{-(n+1)}$ min^{-1})
bipy	7.43	30	0.2–1.6 × 10^{-2}	0.68	172 ± 1.1
BPDS	7.40	20	0.1–1.8 × 10^{-2}	0.75	567 ± 2.0
TMEN	7.70	20	0.01–0.33	0.80	27 ± 0.9

[a]General conditions: reactions were run under pseudo-first order conditions, [OsO_4] limiting, [NAH] greater than 10[OsO_4], 0.1 M sodium phosphate buffer. [b]Values of n (apparent order in ligand) were determined from the slopes of van't Hoff plots (log k_ψ against log[ligand]).

TABLE III. Kinetic Data for Reaction of OsO_4 with Various Imidazoles in the Absence of Additional Ligand[a]

Substrate	pK_a[b]	n[c]	k_{app}[d] (M^{-n} min^{-1})	E_a (cal/mol)
4-Me-Im	7.50	3	3911	−373
IM	7.10	3	1319	−880
NHn	7.00	3	563	+2533
ImAA	7.41	3	420	[e]
NAH	7.25	3	345	+3019
2-Me-Im	7.82	2	492	+5790
4-Me-5-HM-Im	6.95	2	179	+14758
1-Me-Im	7.01	2	94	+16091
4-HM-Im	6.47	2	22	+15660

[a]General conditions: reactions were run under pseudo-first order conditions ($[S]/[OsO_4] \geq 30$) in 0.1 M sodium carbonate, pH 9.75, 20 °C (except the activation energy determinations). [b]pK_a values were determined at 22 °C in carbonate-free, deionized, distilled water; the ionic strength was not adjusted. [c]n = apparent order in substrate. [d]k_{app} values were determined from the slopes of plots of k_ψ against $[S]^n$. [e]The Arrhenius plot was non-linear.

imidazole-4-acetic acid) show kinetics which follow the expected rate law:

$$\text{rate} = k_{app}[OsO_4][S]^3. \quad (9)$$

The rate data (Table III) follow an approximately linear Brönsted relationship [33] (Fig. 5) where

$$\log k_{app} = {}^{\beta}\text{nucleophile}(pK_a) + \text{constant} \quad (10)$$

with $^{\beta}$nuc values of 0.91 and 1.61 for the series n = 2 and n = 3, respectively. These data show that the olefin reacts as a weak nucleophile with osmium tetroxide to produce the osmate ester. This is in accord with previous findings [34–37].

Table III also shows activation energies for a number of these reactions. The values are quite variable as might be expected for reactions which represent a composite of an equilibrium and a rate step (reactions 1 and 2, Scheme 1). The ionic strength effect for a number of imidazoles is generally positive, except in the case of 4-hydroxymethyl imidazole, which shows a decrease in rate with increasing salt concentration [38].

Degradation, with Ring Cleavage, of Osmate Esters of Imidazoles

4,5-Dihydroxydihydro imidazoles, in addition to the dehydration reaction leading to 4-keto imidazoles [32], also easily undergo ring cleavage by reversal of the synthetic route from glyoxals and formamidines. Ekeley and Ronzio found that 2-phenyl 4,5-dihydroxydihydroimidazole dissociated in water to form glyoxal [39]. We find that the osmate esters of imidazoles likewise suffer a slow degradation involving ring cleavage. This reaction was observed

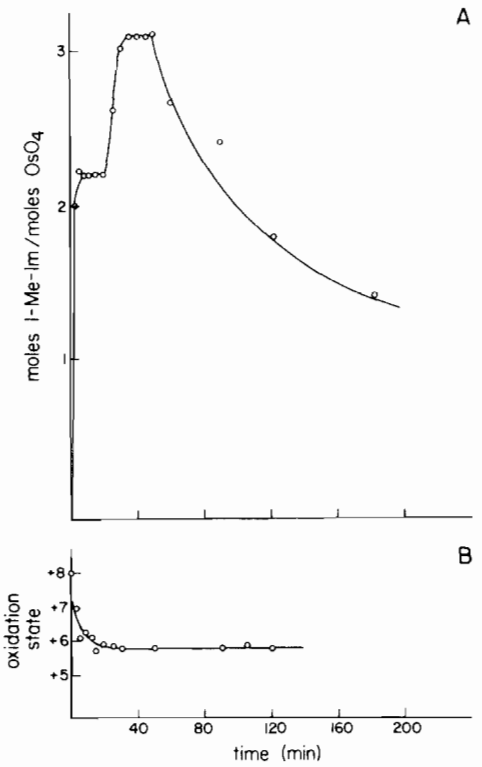

Fig. 4. The reaction of osmium tetroxide with 1-methyl imidazole followed by Pauly assay and thiosulfate titration. The reaction was run in 0.1 M sodium carbonate, pH 9.75, 10 °C; $[OsO_4] = 2.28 \times 10^{-3}$ M, $[1\text{-Me-Im}] = 7.0 \times 10^{-2}$ M. Panel A: the formation of the osmate ester as determined using the Pauly reaction; Panel B: determination of osmium oxidation state by the thiosulfate titration method. The values for the Pauly determination are the average of two runs under identical conditions (± 10% error).

in two ways. First, it can be followed by the slow increase in absorbance at 450 nm subsequent to ester formation (reaction 3, Scheme 1; Fig. 1); the solution changed in color from gold to dark brown. Under typical conditions, half-times for these reactions (although dependent on the ligand) are of the order of several hours; the degradation is sufficiently slow that it does not interfere with measurement of kinetics of ester formation.

The ring cleavage reaction can also be followed by proton NMR spectroscopy. This was carried out in both dilute NaOH and acetic acid for the osmate ester formed from 1-Me-Im (the structure of this ester is shown in Fig. 6 with the ^{1}H NMR assignments derived from 60 MHz spectra). In 1×10^{-3} M NaOD, new resonances appeared at δ 2.3 and 8.2, with a half-time of about two hours. We attribute these to formation of *N*-methyl formamidine because *N*-methyl formamide resonates at δ 7.9 and the difference between the resonances of formamidine and formamide is 0.3 ppm. Integration shows about 70% conversion. We have not definitely identified the

other product(s). In 1×10^{-3} M CD_3COOH, these same resonances appear but are accompanied by others at δ 2.4, 2.7 and 7.9 which we attribute to *N*-methyl formamide and methylamine (Fig. 6). Some dehydration of the 4,5-dihydroxydihydro imidazole (to the 4-keto derivative) may also occur. These reactions are much slower than degradation in basic solution (half-times approximately ten hours).

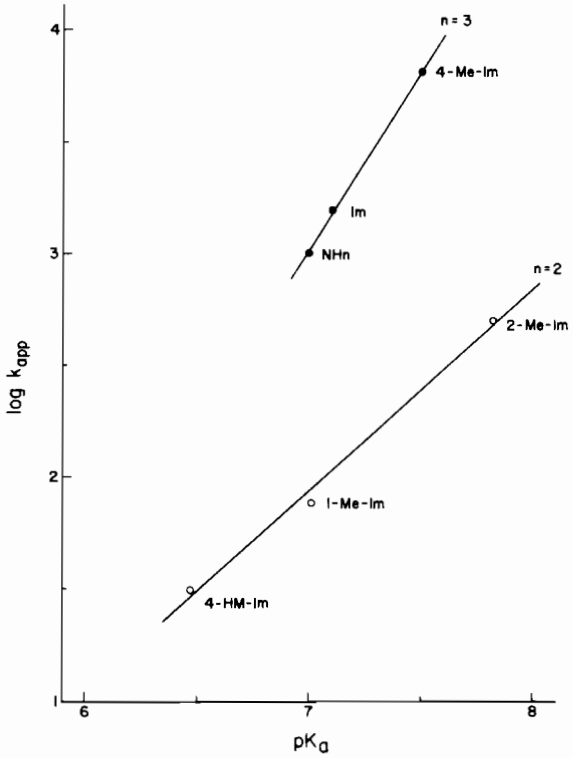

Fig. 5. Brönsted plots for the reaction of osmium tetroxide with imidazoles. The reactions were run under pseudo-first order conditions ([OsO_4] limiting), in 0.1 M sodium carbonate buffered to pH 9.75, at 20 °C. The log k_{app} values are the *y*-intercepts of plots of log k_{app} *vs.* [NaCl] [38], *i.e.* at zero ionic strength. *n* is the apparent order in substrate (●, $n = 2$ and ○, $n = 3$). pK_a values are taken from Table III.

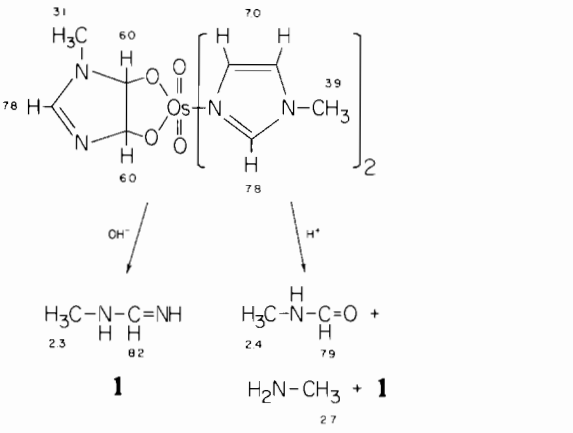

Fig. 6. Structure of $[(1\text{-Me-Im})_3Os(VI)O_4]$ ester and the proton NMR assignments for this compound and some of its degradation products. The assigned numbers are the chemical shifts (ppm) determined from 60 MHz spectra, 35 °C, acetone internal standard.

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References

1 J. S. Deetz and E. J. Behrman, *J. Org. Chem.*, *45*, 135 (1980).
2 L. G. Marzilli, *Prog. Inorg. Chem.*, *23*, 255 (1977).
3 L. R. Subbaraman, J. Subbaraman and E. J. Behrman, *Bioinorg. Chem.*, *1*, 35 (1971).
4 J. A. Ragazzo and E. J. Behrman, *Bioinorg. Chem.*, *5*, 343 (1976); *11*, 77 (1979).
5 A. J. Neilson and W. P. Griffith, *J. Chem. Soc., Dalton Trans.*, 1084 (1979).
6 J. S. Deetz, *Ph.D. Dissertation*, The Ohio State University, 1980, p. 75 (University Microfilms International #80-22256).
7 M. M. Botwinnik and M. A. Prokofjev, *J. Prakt. Chem.*, *256*, 191 (1937).
8 A. Pinner and R. Schwartz, *Chem. Ber.*, *35*, 2441 (1902).
9 G. L. Schmir and L. A. Cohen, *Biochemistry*, *4*, 533 (1965).
10 B. Radziszewski, *Chem. Ber.*, *17*, 1289 (1884).
11 Y. Takayama, *Bull. Chem. Soc. Jpn.*, *8*, 189 (1933).
12 F. L. Pyman, *J. Soc. Dyers Colour.*, *36*, 107 (1920).
13 J. S. Deetz and E. J. Behrman, *Int. J. Peptide Protein Res.*, *17*, 495 (1981).
14 G. F. Bahr, *Exp. Cell Res.*, *7*, 457 (1954).
15 T. Hake, *Lab. Invest.*, *14*, 1208 (1968).
16 S. Angermüller and H. Fahimi, *Histochem. J.*, *14*, 823 (1982).
17 M. Brenner and W. Huber, *Helv. Chim. Acta*, *36*, 1109 (1953).
18 A. Albert and E. P. Serjeant, 'Ionization Constants of Acids and Bases', Wiley, New York, 1962, pp. 16–42.
19 A. A. Waldrop, *Ph.D. Dissertation*, The Johns Hopkins University, 1978, p. 27 (University Microfilms International #78-06279).
20 L. M. Schwartz and R. I. Gelb, *Anal. Chem.*, *50*, 1592 (1978).
21 M. Pesez and J. Bartox, 'Colorimetric and Fluorometric Analysis of Organic Compounds and Drugs', Marcel Dekker, New York, 1974, pp. 383–384.
22 L. R. Subbaraman, J. Subbaraman and E. J. Behrman, *Inorg. Chem.*, *11*, 2621 (1972).
23 I. M. Kolthoff and E. B. Sandell, 'Textbook of Quantitative Inorganic Analysis', MacMillan, New York, 1948, pp. 614–624.

24 S. F. Kobs and E. J. Behrman, *Inorg. Chim. Acta, 128*, 21 (1987)*.
25 R. L. Clark and E. J. Behrman, *Inorg. Chem., 14*, 1425 (1975).
26 J. F. Bunnett, in E. S. Lewis (ed.), 'Techniques of Chemistry', Vol. VI, Part 1, Wiley, New York, 1974, pp. 367–488.
27 R. J. Sundberg and R. B. Martin, *Chem. Rev., 74*, 471 (1974).
28 D. W. Margerum, G. R. Cayley, D. C. Weatherburn and G. K. Pagenkopf, in A. E. Martell (ed.), 'Coordination Chemistry', Vol. 2, American Chemical Society, Washington, D.C., 1978, pp. 1–220.
29 M. Schröder, *Chem. Rev., 80*, 187 (1980).
30 E. J. Behrman in J.-P. Revel, T. Barnard and G. H. Haggis (eds.), 'Science of Biological Specimen Preparation', SEM Inc., 1984, pp. 1–5.

*The ordinate in Fig. 3 of this ref. should be in units of 10^{-1} k.

31 R. A. Sheldon and J. K. Kochi, 'Metal-Catalyzed Oxidations of Organic Compounds', Academic Press, New York, 1981, pp. 152–188.
32 J. W. Cornforth and H. T. Huang, *J. Chem. Soc.*, 731 (1948).
33 J. Shorter, 'Correlation Analysis of Organic Reactivity', Wiley, New York, 1982, pp. 173–204.
34 K. B. Sharpless, A. Y. Teranishi and J.-E. Backvall, *J. Am. Chem. Soc., 99*, 3120 (1977).
35 S. G. Hentges and K. B. Sharpless, *J. Am. Chem. Soc., 102*, 4263 (1980).
36 K. A. Jørgensen and R. Hoffmann, *J. Am. Chem. Soc., 108*, 1867 (1986).
37 M. Tokles and J. K. Snyder, *Tetrahedron Lett., 27*, 3951 (1986).
38 S. F. Kobs, *Ph.D. Dissertation*, The Ohio State University, 1986, pp. 91–92 (University Microfilms International #87-03573).
39 J. B. Ekeley and A. R. Ronzio, *J. Am. Chem. Soc., 57*, 1353 (1935).