this also appears to be the case for the imines. The ^{15}N hydrogen-bonding shifts of the various nitrogen compounds studied here are unlike the protonation shifts in that they reflect, to at least some degree, the different basicities of the nitrogens in the various groups of compounds. Thus, we see from the data that the smallest hydrogen-bonding shifts occur for the oximes and the largest for group 2 amines—the weakest and strongest bases, respectively. Within the oximes (Table IV), hydrogen-bonding effects for the aliphatic and aromatic ones are comparable, which is not the case for protonation. The imines **3c** and **3d** and the pyridines **5a** and **5b** have slightly larger hydrogen-bonding shifts than any of the group 1 imines, although their protonation shifts are considerably smaller. But group 1 imines, even those without the nitro substituent, appear to be slightly weaker bases. Within groups 1 and 2, the 4-nitrophenylimines **1d** and **2b** have smaller hydrogen bonding shifts than those of the corresponding methoxyimines **1a** and **2a**. Following the earlier suggestion that, analogous to protonation, the upfield hydrogen-bonding shifts also result from changes in the second-order paramagnetic effect,[6,11] one observes that the relative magnitudes of these shifts largely reflect differences in hydrogen-bond enthalpies. This seems reasonable, because the increases in the $n \rightarrow \pi^*$ transition energies that result from hydrogen bonding and that presumably are responsible for the upfield shifts should be largely dependent on the strength of the hydrogen bond. The epitome of this kind of behavior is exhibited by azobenzene which has only a very small ^{15}N solvent shift in methanol but a protonation shift of about 155 ppm.[2,6] Azobenzene is a rather weak base, and its nitrogens are not hydrogen bonded effectively by a weakly acidic hydroxylic solvent such as methanol, but when protonated by a strong enough acid, the resonances of these nitrogens shift far upfield.

Registry No. **1a**, 836-41-9; **1b**, 2362-77-8; **1c**, 538-51-2; **1d**, 785-80-8; **1e**, 783-08-4; **1f**, 785-81-9; **2a**, 56644-00-9; **2b**, 42974-61-8; **2c**, 6852-56-8; **2d**, 6852-57-9; **3a**, 574-45-8; **3b**, 1749-19-5; **3c**, 1132-38-3; **3d**, 13683-42-6; **4a**, 3717-15-5; **4b**, 5775-72-4; **4c**, 5780-37-0; **4d**, 100-64-1; **5a**, 110-86-1; **5b**, 108-48-5.

Kinetics of the Reaction of Some Tryptophan Derivatives with the Osmium Tetraoxide–Pyridine Reagent

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A series of indole derivatives related to tryptophan reacted with the osmium tetraoxide–pyridine reagent to form bis(pyridine)osmate esters by addition to the 2,3-position of the indole ring. 1-Methyl-α-*N*-acetyl-DL-tryptophan formed a pair of easily separable diastereomeric esters. A study of the kinetics of these reactions showed the expected first-order dependence on osmium tetraoxide and on the indole component. The dependence on pyridine, however, varied with the structure of the indole. Those 3-indolyl derivatives with a three- or four-carbon side chain terminating in a carboxyl group showed an apparent pyridine dependence close to first order. Other indole derivatives approached the normal second-order dependence. We postulate intramolecular carboxylate catalysis to account for these findings. An interesting consequence of the variation in pyridine dependence is that at low pyridine concentrations, a substrate with first-order dependence on pyridine is kinetically favored. At high pyridine concentrations this kinetic selectivity can be inverted to favor the substrate with a square dependence on pyridine.

Osmium tetraoxide is a common tissue fixative and staining reagent. Although it has been used for more than 100 years,[1] the chemistry of its reactions with tissue components is still poorly known. In particular, whereas the reactions with unsaturated fatty acids[2] and nucleic acid components[3] have been investigated fairly thoroughly, the reactions with amino acid residues of proteins have received little attention. Bahr showed in 1954 that certain of the amino acids, including tryptophan, reacted rapidly with osmium tetraoxide.[4]

Hake showed that cysteine was oxidized to cysteic acid and methionine to methionine sulfone.[5] At high temperatures all amino acids are oxidatively deaminated.[6] Ockenden and Schofield[7] showed that several *N*-substituted indoles reacted with the osmium tetraoxide–pyridine reagent to give osmate esters which could be hydrolyzed to the corresponding 2,3-glycols. Indoles which lacked N substitution did not give isolable glycols. Maupin-Szamier and Pollard's interesting study of the reaction of actin with osmium tetraoxide showed reaction of cysteine, methionine, and lysine residues.[8] Since these authors analyzed the reacted protein following acid-catalyzed hydrolysis, tryptophan reactivity could not be studied. Nielson and Griffith[9] have published a valuable study of the reactivity of a variety of blocked amino acid derivatives with osmium tetraoxide. Complexes were isolated from derivatives of histidine, methionine, and cysteine. A blocked tryptophan derivative proved intractable in the sense that isolation of the osmate ester was not successful–but clearly reaction had taken place.

(1) M. Schultze, *Verh. Naturhist. Verein. Rheinlande*, **21**, 61 (1864); cited in ref 4.
(2) E. D. Korn, *J. Cell Biol.*, **34**, 627–38 (1967).
(3) L. G. Marzilli, *Prog. Inorg. Chem.*, **23**, 327–33 (1977).
(4) G. F. Bahr, *Exp. Cell Res.*, **7**, 457–79 (1954).
(5) T. Hake, *Lab. Invest.*, **14**, 470–4 (1208–12) (1965).
(6) J. Nyilasi and P. Orsős, *Acta Chim. Acad. Sci. Hung.*, **75**, 405–10 (1973).
(7) D. W. Ockenden and K. Schofield, *J. Chem. Soc.*, 612–8 (1953).
(8) P. Maupin-Szamier and T. D. Pollard, *J. Cell Biol.*, **77**, 837–52 (1978).
(9) A. J. Nielson and W. P. Griffith, *J. Chem. Soc., Dalton Trans.*, 1084–8 (1979).

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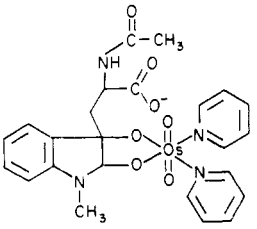


Figure 1. Bis(pyridine)oxoosmium(VI) ester of 1-methyl-α-*N*-acetyl-DL-tryptophan.

Our approach has been a kinetic one. A study of the rates of reaction of various tissue components taken together with stoichiometric work will serve to establish the facts necessary for understanding the chemistry of osmium tetraoxide fixation.

Results

Synthesis and Characterization of the Indole Osmate Esters. The bis(pyridine)oxoosmium(VI) esters of 3-methylindole (skatole), of 1-methyl-α-*N*-acetyl-DL-tryptophan (Figure 1), and of 4-(3-indolyl)butyric acid were synthesized in inert organic solvents by reaction of the indole and osmium tetraoxide in the presence of pyridine. These three osmate esters were characterized by elemental analyses as well as by their electronic, IR, and NMR spectra. The addition of the osmium tetraoxide–pyridine reagent to an olefinic bond proceeds stereospecifically to give the cis adduct. For unsymmetrical olefins, such as the indole derivatives described here, a pair of enantiomers will be produced from this addition unless the indole contains an additional asymmetric center. In this latter case, a pair of diastereomers will be formed. We have good evidence for the formation of a diastereomeric pair when the osmium tetraoxide–pyridine reagent reacts with 1-methyl-α-*N*-acetyl-DL-tryptophan. When the reaction is followed by NMR in D_2O, the singlet due to the acetyl methyl group of the starting material at δ 1.87 is replaced by a pair of singlets at δ 1.70 and 1.97 (Figure 2). These are formed in equal quantity. When the reaction is carried out in deuterated acetone, a pair of singlets is again formed, but as the reaction proceeds, a product crystallizes in the NMR tube. Crystallization of this material also occurs from ethyl acetate. The material giving the high-field singlet remains in solution. When the solid material is filtered and dissolved in D_2O, this solution shows only the low-field acetyl singlet. It is interesting to note that although these two isomers are formed in equal quantity as shown by integration of the NMR spectra, the two products are not of equal stability in aqueous solution. After being allowed to stand for some days, the material giving the low-field acetyl signal can be observed to decompose whereas the spectrum of the other diastereomer remains unchanged. The acetyl signals give the most convenient indication of the presence of the two isomers. However, a doubling of the indole 2-H and *N*-methyl resonances can also be observed in acetone-d_6. We were unable to observe a measurable rotation in 4×10^{-3} M solutions of these compounds in the range 500–370 nm using a Cary 60 instrument. Use of more concentrated solutions was precluded by the absorptivity of the sample.

Molecular models make clear the probable origin of the chemical shift difference between the two diastereomers. In one, isomer A, the acetyl group protrudes into the solution. Presumably it is this isomer which gives the acetyl signal at the normal value of δ 1.97. The other isomer, however, has its acetyl group confined either over the center of the benzene portion of the indole ring or over one of the pyridine rings. It resonates about 0.3 ppm upfield

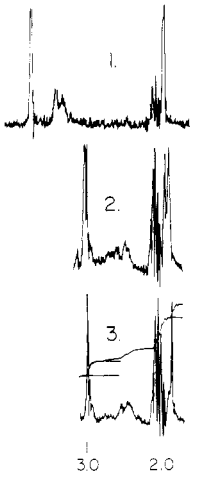


Figure 2. NMR spectra of 1-methyl-α-*N*-acetyl-DL-tryptophan and its bis(pyridine)osmate esters in acetone-d_6 showing the 1-methyl and acetyl regions: panel 1, 1-methyl-α-*N*-acetyl-DL-tryptophan; panel 2, the mixture following reaction of 1-methyl-α-*N*-acetyl-DL-tryptophan with OsO_4–pyridine (note the doubling of both the 1-methyl and acetyl resonances); panel 3, the solution remaining after filtering off crystals of isomer A. For each panel, the assignments are from downfield to upfield: 1-CH_3, α-CH, acetone-d_6, $COCH_3$.

of the normal acetyl singlet. Protons so positioned were shown some time ago to undergo upfield shift of about the same magnitude as we observe here.[10]

α-*N*-Acetyl-L-tryptophan upon reaction with the osmium tetraoxide–pyridine reagent shows a similar NMR pattern, suggesting the formation of a diastereomeric pair in this case as well.

The osmate esters of the other indoles on which kinetic measurements were made were characterized by their spectra only. Our attempts to isolate from aqueous solutions those indole osmate esters which possess a proton at the indole nitrogen were unsuccessful. Only dark intractable material was obtained. However, these esters generally gave no problems in organic solvents. Although insufficiently stable to allow isolation from water, the half-lives of these indole osmate esters in water are sufficient to allow kinetics to be carried out.

Table I gives proton NMR data for some of these indole osmate esters. Saturation of the olefinic bond by reaction with Os(VIII) produces characteristic upfield shifts in the associated protons.[11] The shifts observed for these indole osmate esters correspond well with previous observations. The pyridine protons show the previously observed downfield shifts.

The electronic spectra of all of these complexes show a weak maximum in the visible region around 450 nm in accord with previous data.[12,13] Extinction coefficients are as follows: 200, tryptamine; 190, α-*N*-acetyl-L-tryptophan; 210, 2-(3-indolyl)acetic acid; 190, indole-5-carboxylic acid; 240, 1-methyl-α-*N*-acetyl-DL-tryptophan; 190, 3-(3-indolyl)propionic acid; 180, skatole; 200, 4-(3-indolyl)-butyric acid.

Kinetics of Formation of the Indole Osmate Esters. The kinetics of these reactions were measured under pseudo-first-order conditions with osmium tetraoxide lim-

(10) J. S. Waugh and R. W. Fessenden, *J. Am. Chem. Soc.*, **79**, 846–9 (1957).
(11) L. R. Subbaraman, J. Subbaraman, and E. J. Behrman, *Bioinorg. Chem.*, **1**, 35–55 (1971).
(12) J. A. Ragazzo and E. J. Behrman, *Bioinorg. Chem.*, **5**, 343–52 (1976); *J. Inorg. Biochem.*, **10**, 89 (1979); **11**, 77 (1979).
(13) F. B. Daniel and E. J. Behrman, *J. Am. Chem. Soc.*, **97**, 7352–8 (1975).

Table I. NMR Data (ppm)[a]

compd	solvent	$COCH_3$	β-CH_2	NCH_3	2-H
1-methyl-α-*N*-acetyl-DL-tryptophan (I)	D_2O	1.87 (s)	3.22 (m)	3.75 (s)	nr[b]
	acetone-d_6	1.93 (s)	3.3 (m)	3.70 (s)	nr[b]
	Me_2SO-d_6	1.80 (s)	3.08 (m)	3.75 (s)	nr[b]
bis(pyridine)osmate ester of I, isomer A	D_2O (0.25 M py)	1.97 (s)	2.6 (m)	3.02 (s)	5.43 (s)
	acetone-d_6	1.93 (s)	2.5 (m)	2.95 (s)	5.46 (s)
	Me_2SO-d_6	1.83 (s)	2.4 (m)	2.90 (s)	5.25 (s)
bis(pyridine)osmate ester of I, isomer B	D_2O (0.5 M py)	1.70 (s)	2.6 (m)	3.06 (s)	5.45 (s)
	acetone-d_6	1.85 (s)	2.5 (m)	2.98 (s)	5.84 (s)
α-*N*-acetyl-L-tryptophan (II)	D_2O	1.90 (s)	3.2 (m)		nr[b]
bis(pyridine)osmate ester of II, isomer A	D_2O (0.57 M py)	1.98 (s)	2.7 (m)		5.69 (s)
bis(pyridine)osmate ester of II, isomer B	D_2O (0.57 M py)	1.78 (s)	2.7 (m)		5.74 (s)

[a] All measurements were made at 35 °C and 60 MHz. Me_4Si was used as internal reference for the organic solvents and DSS for aqueous solutions. [b] Not recorded.

Table II. Kinetic Data[a]

substrate	no. of runs	[py], M	n[b]	k', $M^{-(n+1)}$ min^{-1}	k_1, M^{-2} min^{-1}	k_2, M^{-3} min^{-1}
thymidine	3	$(1\text{-}2.5) \times 10^{-1}$	1.96	1974 ± 10	0	1974
indole-5-carboxylic acid	8	$(0.5\text{-}3) \times 10^{-1}$	1.63	3110 ± 125	3000 ± 1500	40 000 ± 6500
tryptamine	4	$(0.5\text{-}3) \times 10^{-1}$	1.54	3050 ± 70	[c]	[c]
2-(3-indolyl)acetic acid	6	$(0.5\text{-}3) \times 10^{-1}$	1.37	6250 ± 450	400 ± 500	19 000 ± 400
α-*N*-acetyl-L-tryptophan	6	$(0.25\text{-}2.5) \times 10^{-1}$	1.19	5300 ± 350	2400 ± 250	7 100 ± 800
1-methyl-α-*N*-acetyl-DL-tryptophan	11	$(0.033\text{-}0.25) \times 10^{-1}$	1.0	5225 ± 500	5600 ± 500	0
3-(3-indolyl)propionic acid	8	$(0.25\text{-}1.0) \times 10^{-1}$	1.19	11 300 ± 700	4900 ± 2500	25 700 ± 2000
4-(3-indolyl)butyric acid	8	$(0.15\text{-}1.0) \times 10^{-1}$	1.05	8000 ± 900	7800 ± 1000	0
skatole	7	$(0.2\text{-}0.9) \times 10^{-1}$	1.14	21 700 ± 3000	10 000 ± 1000	60 000 ± 10 000

[a] General conditions: 0.1 M phosphate buffer, pH 7, 25 °C, $[OsO_4] = 3.3$ to 10×10^{-4} M, [substrate] = 5 to 30×10^{-3} M. For skatole: $[OsO_4] = 1$ to 2×10^{-4} M, [substrate] = 1 to 2×10^{-3} M. [b] The pyridine dependence, n, was calculated as pyridine activity according to the equation $\gamma = -0.235C_{py} + 0.01C_{py}^2 + 0.064\mu$,[17] where $\mu = 0.44$ M for α-*N*-acetyl-L-tryptophan and 0.22 M for the other substrates. The estimated error in n is ±0.05. [c] Not determined.

iting. All runs showed good first-order dependence on osmium tetraoxide over at least 2 half-lives. Thus the rate is given by eq 1. The dependence on the other compo-

$$v = k\psi[OsO_4] \quad (1)$$

nents was determined by varying their concentrations and observing the effect on $k\psi$. In this way, we found that the dependence on the indole was always first-order. Variation in pyridine concentration, however, gave a dependence on pyridine (expressed as pyridine activity[14]) which varied according to the nature of the indole. Thus the rate can be expressed as eq 2. In eq 2, s represents the substrate,

$$v = k'[OsO_4][s]a_{py}^n \quad (2)$$

a_{py} the activity of pyridine, and n the pyridine dependence. Figure 3 shows van't Hoff plots for thymidine, for 2-(3-indolyl)acetic acid, and for 1-methyl-α-*N*-acetyl-DL-tryptophan. These substrates illustrate the range of pyridine dependence we have observed: from 2 for thymidine to 1 for the tryptophan derivative. Table II summarizes the kinetic results.

The nonintegral order in pyridine activity can most reasonably be accounted for by a three-term rate law of the form shown in eq 3. We had previously postulated

$$v = k_0[OsO_4][s] + k_1[OsO_4][s]a_{py} + k_2[OsO_4][s]a_{py}^2 \quad (3)$$

a three-term rate law for the reaction with thymine.[11] We later showed that these results could be explained more simply by a two-term rate law (omitting the k_1 term) if the effects of pyridine activity were taken into account.[14] Our present results appear to be explainable only on the basis of a three-term rate law.

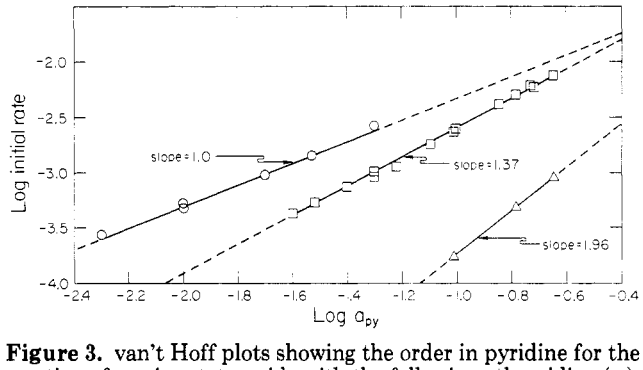


Figure 3. van't Hoff plots showing the order in pyridine for the reaction of osmium tetraoxide with the following: thymidine (△), slope = 1.96; 2-(3-indolyl)acetic acid (□), slope = 1.37; 1-methyl-α-*N*-acetyl-DL-tryptophan (○), slope = 1.0. Reactions were run under the following conditions: 0.1 M phosphate buffer, pH 7, 25 °C, $[OsO_4] = 1 \times 10^{-3}$ M, $[s] = 1 \times 10^{-2}$ M, [py] as indicated.

Combining eq 1 and eq 3 yields eq 4. Plots of $k\psi/[s]$

$$k\psi/[s] = k_0 + k_1a_{py} + k_2a_{py}^2 \quad (4)$$

vs. a_{py} will be nonlinear but will have the y intercept equal to k_0. In the absence of a k_2 term, the plots will be linear. In the absence of a k_1 term, plots of a_{py}^2 vs. $k\psi$ will be linear. Table II and Figure 4a–c record the results of treating the data in this manner. We note that 1-methyl-α-*N*-acetyl-DL-tryptophan and 4-(3-indolyl)butyric acid give linear plots for a_{py} with a y intercept equal to zero. The other substrates all give nonlinear plots when $k\psi/[s]$ is plotted against either a_{py} or a_{py}^2. For these substrates, values for both k_1 and k_2 can be estimated from plots of $(k\psi - k_0)/[s]a_{py}$: these plots should be linear with intercepts equal to k_1 and slopes equal to k_2.[15] Our estimates

(14) R. L. Clark and E. J. Behrman, *Inorg. Chem.*, 14, 1425–6 (1975). We discuss alternative kinetic schemes and the activity dependence in this paper.

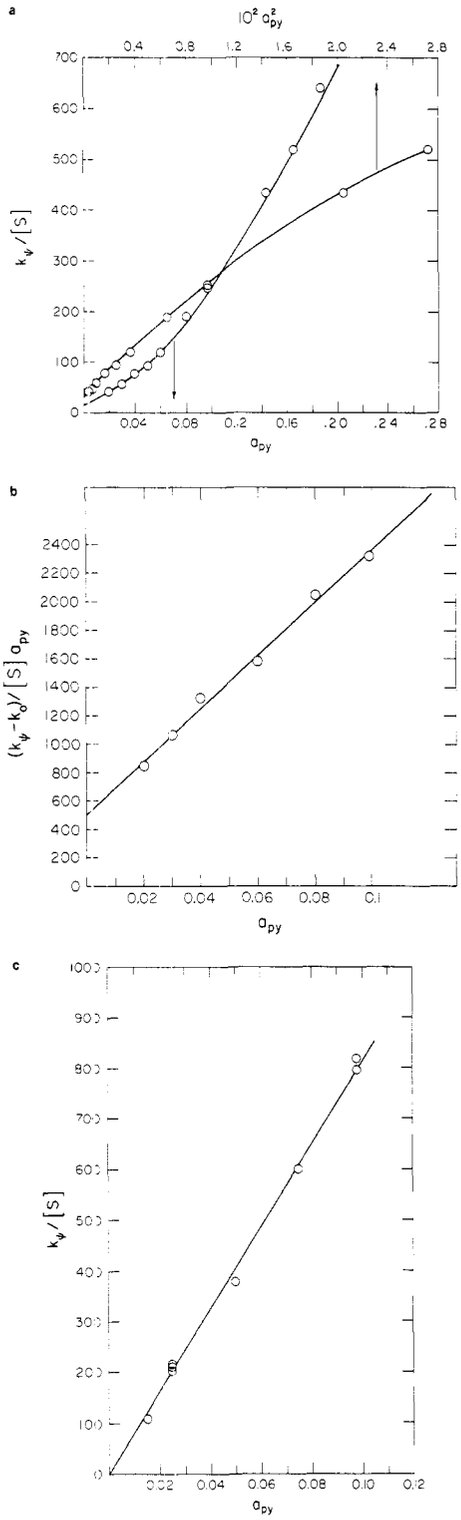
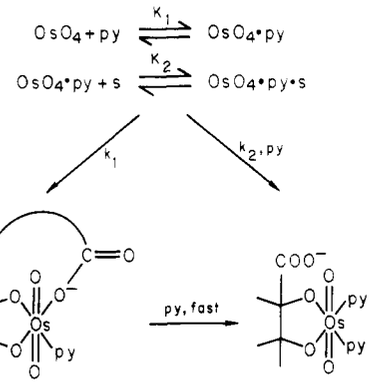


Figure 4. Estimation of k_0, k_1, and k_2. 2-(3-Indolyl) acetic acid: (a) plots of $k\psi/[s]$ vs. a_{py} and a_{py}^2 showing nonlinearity (the y intercept gives k_0); (b) plot of $(k\psi - k_0)/[s]a_{py}$ vs. a_{py} (the y intercept gives k_1, and the slope gives k_2. 4-(3-Indolyl)butyric acid (c), plot of $k\psi/[s]$ vs. a_{py} showing linearity. General conditions: $[s] = 1 \times 10^{-2}$ M, $[OsO_4] = 1 \times 10^{-3}$ M, $T = 25$ °C. 0.1 M phosphate buffer, pH 7.

for k_0 were subject to considerable error due to the nonlinear extrapolation. The values were in the range 0–25 M^{-1} min^{-1} for all of these substrates. We also note that the values for k_1 and k_2 are much less certain than those for k'. This is again due to the indirect method for determination of k_1 and k_2 as compared with that for k'.

Scheme I



Discussion

Two interesting points emerge. First, the data of Table II show a dependence on pyridine concentration which varies with the structure of the indole. Clark and Behrman[14] established that there was a square dependence on pyridine activity for the formation of the bis(pyridine)osmate ester of thymidine derivatives. For these indole derivatives, the apparent pyridine dependence varies from first order to 1.6. We have repeated the thymidine measurements and reconfirmed the square dependence on pyridine activity for the thymidine reaction (Table II, Figure 3). The variation in pyridine dependence with indole structure can be rationalized on the basis of the preferred reaction mechanism for the formation of bis(pyridine)osmate esters discussed by Clark and Behrman[14] (Scheme I). We postulate that a ligand in the indole side chain, such as the carboxylate group, can form the intermediate shown in the k_1 pathway, Scheme I, with a single pyridine molecule. This carboxylate group is then displaced in a subsequent fast step by a second pyridine ligand to form the stable bis(pyridine)osmate ester. The normal k_2 pathway competes with the k_1 pathway to give the same product. The k_1 pathway is first order and the k_2 pathway second order in pyridine. The data of Table II are consistent with these ideas. Models show that the carboxylate group of a three-carbon side chain of 3-indolyl derivatives can coordinate easily with the osmium atom. 1-Methyl-α-*N*-acetyl-DL-tryptophan and 4-(3-indolyl)-butyric acid show only a k_1 term. It is also worth noting that at low pyridine concentrations this postulated carboxylate catalysis increases the rate of reaction significantly. Clark's[16] study of steric and electronic effects on the rate of attack of the osmium tetraoxide–pyridine reagent with a variety of simple olefins supports the conclusion that the rate increase is due, at least in part, to intramolecular carboxylate catalysis. Clark showed that the relative rates for a set of olefins, RCH=C<, as we vary R, is $CH_3 > H > CH_3CH_2$, propyl $\simeq$ pentyl. This suggests that in the absence of other effects, increasing the length of an alkyl side chain at the 3-position of the indole ring should probably not affect the rate much after the chain is two or three carbons long. Table II shows that this is not the case. Table II also shows that the rate increases which accompany the increased length of the carboxylate side chain are also correlated, as expected, with the value of the k_1/k_2 ratio and with the value of n, the overall or apparent pyridine dependence.

(15) J. F. Bunnett in "Investigations of Rates and Mechanisms of Reactions", E. S. Lewis, Ed., 3rd ed., Wiley, New York, 1974, Part 1, p 148.

(16) R. L. Clark, Ph.D. Dissertation, University of Montana, 1973; University Microfilms International no. 74-11, 629.

(17) J. Byerrum, *Acta Chem. Scand.*, 18, 843–57 (1964).

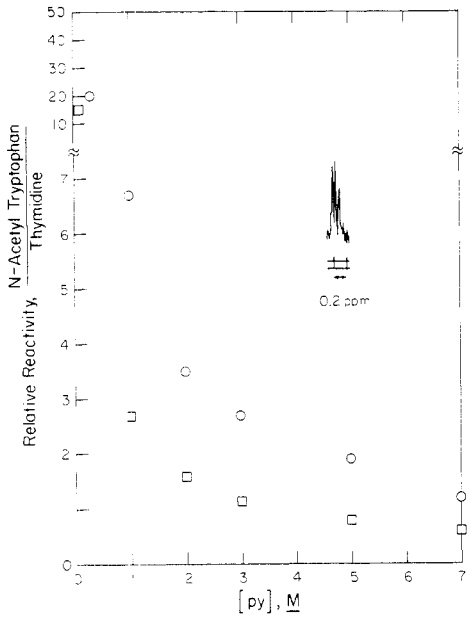


Figure 5. Results of a competitive reaction. Twenty-five micromoles of *N*-acetyl-L-tryptophan and of thymidine were reacted with 22 μmol of osmium tetraoxide in 0.1 M phosphate buffer, pH 7, together with the indicated molarity of pyridine-d_5. For the reaction in 7 M pyridine, the quantities of the other reactants were halved. Volumes were 0.5–1.0 mL. The relative rates were measured following complete reaction by integration of the 6-H singlet of the thymidine ester at δ 5.62 and comparison with the 2-H doublet of isomers A and B of the tryptophan ester at δ 5.69 and 5.74. The insert shows the spectrum of the reaction mixture at [py] = 3.0 M. Symbols: ○, experimental; □, calculated according to eq 2. For this pair of substrates, eq 2 and 3 give almost identical values.

Second, since the total rate depends upon a three-term rate law in which one term is zero order, one term first order, and one term second order in pyridine, the relative rate or rate ratio for a pair of substrates is heavily dependent upon the magnitude of various terms for the substrates in question. For example, thymidine has a very small k_0 term and no detectable k_1 term. *N*-Acetyl-L-tryptophan, on the other hand, has a k_1/k_2 ratio of about 0.3. This means that at low pyridine concentrations the reaction with the tryptophan derivative will be favored; the opposite will be the case at high pyridine concentrations. These ideas were tested directly by the competitive technique. Figure 5 shows the results of a series of competitive experiments in which solutions equimolar in thymidine and *N*-acetyl-L-tryptophan reacted with osmium tetraoxide in the presence of pyridine. The pyridine concentration was varied from 7 to 0.3 M. This figure shows clearly the expected trend toward inversion in relative rate as a function of pyridine concentration. Further, one can see that the measured relative rates agree reasonably well with the calculated values especially when one considers the large change in solvent composition.

The values for n reported in Table II are functions not only of the k_1/k_2 ratio but also of the absolute value of the pyridine concentration. Because of this we emphasize that these n values can be used only over the concentration ranges reported for pyridine in Table II since the relative contributions of the k_0, k_1, and k_2 terms to the total rate are sensitively dependent upon the pyridine concentration. It is important to compare n values for the various substrates for the same pyridine concentration range. The small value of n for skatole is thus only an apparent anomaly. It is due to the low pyridine concentrations at which the rate determinations were made. The k_1/k_2 ratios show that skatole is similar to indole-5-carboxylic acid. For calculation of rates it is probably preferable to use the more accurately known k' values for the pyridine ranges specified in Table II. Outside of those ranges, however, the less accurately known k_0, k_1, and k_2 values must be used.

Our previous work has shown the importance of ligands on the reactivity and selectivity of osmium(VIII) reagents, especially when comparing the monodentate with the bidentate case.[12] Our present work shows an interesting consequence of variation in the concentration of the same ligand upon the relative reactivities of substrates which differ from one another in their kinetic dependence on that ligand.

Experimental Section

The following chemicals were obtained from the sources indicated: α-*N*-acetyl-L-tryptophan, Sigma; 3(3-indolyl)propionic acid, Tridom; 1-methyl-α-*N*-acetyl-DL-tryptophan, by Schotten–Baumann acetylation of 1-methyl-DL-tryptophan [mp 171 °C (lit.[18] 171–172 °C)]; all others, Aldrich. 2-(3-Indolyl)acetic acid and 3-(3-indolyl)propionic acid were recrystallized from water, mp 167–168 and 134 °C, respectively; tryptamine was recrystallized from benzene (mp 116–117 °C). The extinction coefficients for the following compounds were used to determine the concentrations in water at pH 7 (compound, λ,ε): OsO_4, 250 nm, 2760; 290 nm, 1640; 1-methyl-α-*N*-acetyl-DL-tryptophan, 292 nm, 5230; α-*N*-acetyl-L-tryptophan, 280 nm, 5580; pyridine, 250 nm, 2750; thymidine, 267 nm, 9770.

The kinetics of formation of the indole osmate esters were followed in aqueous buffered solutions by the increase in absorption of the weak maximum or shoulder in the electronic spectrum at 450 nm. Reactions were run under pseudo-first-order conditions with osmium tetraoxide limiting. Infinity values were constant for at least 1 h although, as noted above, the indoles bearing a proton on the indole nitrogen were not indefinitely stable in aqueous solution. Indole-5-carboxylic acid was an exception. Its osmate ester was stable for only a few minutes. It breaks down to a bright blue material, λ_{max} = 625 nm. The dependence on the other components was determined by varying their concentrations and observing the effect on the pseudo-first-order rate constant, $k\psi$. Carbon, hydrogen, and nitrogen analyses were carried out by Galbraith Laboratories.

Bis(pyridine)oxoosmium(VI) Ester of 1-Methyl-α-*N*-acetyl-DL-tryptophan, Isomer A. A solution of 0.049 g (1.93 × 10^{-4} mol) of OsO_4 in 5 mL of ethyl acetate was added to a solution of 0.05 g (2.03 × 10^{-4} mol) of 1-methyl-α-*N*-acetyl-DL-tryptophan and 0.06 mL (7.43 × 10^{-4} mol) of pyridine in 5 mL of ethyl acetate. The solution turned golden. The solid product was collected by filtration after 4 h at 25 °C. The product was washed with ethyl acetate and ethyl ether and dried. The yield was 0.039 g (29.5%), but more product can be obtained by concentration of the filtrate. Anal. Calcd for $C_{24}H_{26}O_7N_4Os$: C, 42.85; H, 3.89; N, 8.33. Found: C, 43.01; H, 4.00; N, 8.13.

Isomer B. This material has not been obtained as a solid. A clean solution was prepared as follows. A solution of 0.0293 g of OsO_4 (1.153 × 10^{-4} mol) in 0.5 mL of acetone-d_6 was added to a solution of 0.030 g of 1-methyl-α-*N*-acetyl-DL-tryptophan (1.153 × 10^{-4} mol) and 0.056 mL of pyridine (6.918 × 10^{-4} mol) in 1.5 mL of acetone-d_6. After 2 h at 25 °C, the mixture was placed at 5 °C for 10 h. Crystals of isomer A were filtered, washed with acetone, and dried; yield 36 mg (46%). Isomer B was recovered quantitatively in the initial filtrate. The NMR spectrum showed no contamination by isomer A (see Figure 2).

Bis(pyridine)oxoosmium(VI) Ester of 4-(3-Indolyl)butyric Acid. Osmium tetraoxide (0.1 g, 3.93 × 10^{-4} mol) dissolved in 5 mL of anhydrous diethyl ether was added to an ether solution containing 0.2 mL of pyridine (2.58 × 10^{-3} mol) and 0.08 g of 4-(3-indolyl)butyric acid (3.93 × 10^{-4} mol). The solution immediately turned a cloudy yellow. After 3 h the solid product was filtered and washed with diethyl ether. This product was then dried in vacuo at room temperature for 2 h: yield 0.15 g (62%); NMR (D_2O) δ 5.65 (s, 2-H). Anal. Calcd for $C_{22}H_{23}O_6N_3Os$: C,

(18) H. R. Snyder and E. L. Eliel, *J. Am. Chem. Soc.*, **70**, 3855–6 (1948).

42.92; H, 3.77; N, 6.83. Found: C, 43.03; H, 3.95; N, 6.84.

Bis(pyridine)oxoosmium(VI) Ester of Skatole. Osmium tetraoxide (0.1 g, 3.93×10^{-4} mol) dissolved in 5 mL of anhydrous diethyl ether was added to an ether solution containing 0.18 mL of pyridine (2.32×10^{-3} mol) and 0.052 g of skatole (3.96×10^{-4} mol). After 1 h of reaction the precipitate was filtered and washed with diethyl ether. This product was then air-dried for 2 h: yield 0.132 g (62%); NMR ($CDCl_3$) δ 1.87 (s, Me), 5.50 (s, 2-H). Anal. Calcd for $C_{19}H_{19}N_3O_4Os$: C, 41.98; H, 3.52; N, 7.73. Found: C, 42.02; H, 3.66; N, 7.62.

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Registry No. I, 732-10-5; I, bis(pyridine)osmate ester, isomer 1, 72161-24-1; I, bis(pyridine)osmate ester, isomer 2, 72203-28-2; II, 1218-34-4; II, bis(pyridine)osmate ester, isomer 1, 72161-25-2; II, bis(pyridine)osmate ester, isomer 2, 72203-29-3; 1-methyl-DL-tryptophan, 26988-72-7; thymidine, 50-89-5; indole-5-carboxylic acid, 1670-81-1; tryptamine, 61-54-1; 2-(3-indolyl)acetic acid, 87-51-4; 3-(3-indolyl)propionic acid, 830-96-6; 4-(3-indolyl)butyric acid, 133-32-4; skatole, 83-34-1; thymidine bis(pyridine)osmate ester, 35582-04-8; indole-5-carboxylic acid bis(pyridine)osmate ester, 72161-26-3; tryptamine bis(pyridine)osmate ester, 72161-27-4; 2-(3-indolyl)acetic acid bis(pyridine)osmate ester, 72161-28-5; 3-(3-indolyl)propionic acid bis(pyridine)osmate ester, 72161-29-6; 4-(3-indolyl)butyric acid bis(pyridine)osmate ester, 72161-30-9; skatole bis(pyridine)osmate ester, 72161-31-0; osmium tetraoxide, 20816-12-0; pyridine, 110-86-1.

Interaction of Cyclopropane with Platinum-Metal Chlorides under Carbon Monoxide Pressure in Benzene

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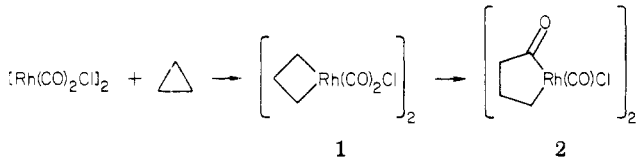
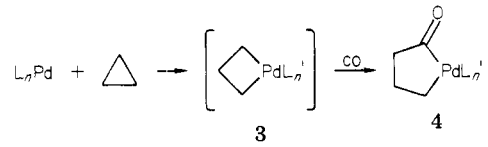
The six platinum-metal chlorides were reacted with cyclopropane in benzene under carbon monoxide pressure to produce propylbenzenes and, in three instances, chlorobutyrates. The isomer of propylbenzene obtained ranged from pure *n*-propylbenzene for $PdCl_2$ to pure isopropylbenzene for $OsCl_3$ and $IrCl_3$. The other three platinum-metal chlorides gave mixtures of the two propylbenzenes. The reaction was shown to be specific for benzene and cyclopropane as chlorobenzene and toluene failed to enter into the reaction. Likewise, methylcyclopropane did not react with benzene to produce butylbenzenes.

Platinum and rhodium complexes have been shown to undergo reaction with cyclopropanes to form platinacyclobutanes[1] and rhodiacyclopentanones,[2] respectively. Palladium chloride was reported[3] to react with cyclopropane in benzene under carbon monoxide pressure to produce, upon workup with methanol, a mixture of the three possible methyl chlorobutyrates and *n*-propylbenzene. The rhodiacyclopentanone **2**, obtained by the interaction of $[Rh(CO)_2Cl]_2$ with cyclopropane, was thought to occur via CO insertion into the rhodiacyclobutane **1**.[2]



If palladium underwent a similar set of insertions as was found for rhodium, then one could possibly envision the formation of the 4-chlorobutyrate as arising from **4** and *n*-propylbenzene as arising from **3**. As this possibility



intrigued us and offered the potential of being characteristic for the platinum metals, we decided to undertake a study of the interaction of cyclopropane with platinum-metal chlorides under carbon monoxide in benzene.

Results

We first reexamined the reaction of cyclopropane with palladium chloride in benzene under 100 atm of carbon monoxide at 90 °C. We found that the ratio of chlorobutyrates (73%) to *n*-propylbenzene (27%) was similar to that reported earlier.[3] While the formation and ratio of these products was not too dependent upon carbon monoxide pressure and temperature, we did find that the ratio of products was very dependent upon the molar ratio of cyclopropane to palladium chloride. When the molar ratio of cyclopropane to palladium chloride was 1.5:1, *n*-propylbenzene represented about 27% of the product composition. However, when we increased the molar ratio of cyclopropane to palladium chloride to 30:1, we found that *n*-propylbenzene now represented greater than 90% of the product mixture. The chlorobutyrates made up the remaining part of the product mixture, and their relative ratios are given in Table III. We found that similar results were obtained by using 15 atm of carbon monoxide at 90 °C, and the remaining experiments reported here were done at this lower pressure.

The yield of *n*-propylbenzene (based on $PdCl_2$) was 40–50%. Palladium was converted to a mixture of $[Pd(CO)Cl_2]_2$ and $[Pd_2Cl(CO)_2]_n$. These products are the same as those reported for the palladium-mediated carbonylation of ethylene under similar reaction conditions.[4] These palladium complexes could be filtered, washed with benzene, and reused to effect the formation of *n*-propylbenzene from a fresh charge of benzene, cyclopropane, and

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