Reactions of Osmium Tetraoxide with Protein Side Chains and Unsaturated Lipids

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The reactions of OsO_4 with derivatives or model systems (L) for the side chains of tissue proteins have been studied, alone and in the presence of unsaturated lipids (R). The following new complexes have been isolated and their structures studied by vibrational and ^1H n.m.r. spectroscopy: $Os_2O_6L_4$ (L = α-*N*-benzoyl-L-histidine isobutyl ester, imidazole, 1-methylimidazole, 5,6-dimethylbenzimidazole, n-butylamine, or α-*N*-benzoyl-DL-methionine); OsL_2 (L = glutathione or L-cysteine); the mixed species $Os_2O_6L_3L'$ (L = 1-methylimidazole, L' = L-proline methyl ester or α-*N*-benzoyl-L-arginine ethyl ester) and $OsLL'_n$ (L = 1-methylimidazole, L' = α-*N*-benzoyl-L-cysteine or α-*N*-acetyl-L-cysteine); $OsO_2(O_2R)L_2$ (R = cyclohexene, oleic acid, methyl oleate, or cholesteryl acetate; L = 1-methyl- and 5,6-dimethyl-benzimidazole, α-*N*-benzoyl-L-histidine isobutyl ester, or pyridine); $Os_2O_4(O_4R)L_4$ and $Os_3O_6(O_6R')L_6$ (R = methyl linoleate, R' = methyl linolenate; L = 1-methylimidazole). A possible role for OsO_4 in cross-linking proteins and lipids in biological tissue fixation is discussed.

It is generally believed that the fixation of biological tissue by osmium tetraoxide involves unsaturated lipids,[1,2] but the involvement of protein is less certain.[3] As part of our continuing work on the chemistry of tissue fixation [4-6] we have studied the reaction of OsO_4 with some of the side chains of amino-acids commonly found in tissue proteins, to establish whether they constitute reaction sites for the reagent. By using unsaturated lipids in some of the experiments we have sought also to establish whether OsO_4 can cross-link protein and lipid. As far as possible our reaction conditions approximate to those normally used for tissue fixation, *i.e.* reactions in water or aqueous acetone close to pH 7 at room temperatures, with reaction times <1 h.

No amino-acid complexes of osmium have hitherto been characterised; although OsL_4 species (L = aspartic acid, glutamic acid, or glycine) have been said to exist, no analytical data were presented.[7] Stability constants have been measured for osmium(IV) amino-acid complexes,[8] and both cysteine [9] and methionine [10] have been used as colorimetric reagents for osmium. In water [11] and in solutions buffered [12] to pH 7 at room temperatures, OsO_4 has been found to react with most amino-acids to give black intractable solids; at higher temperatures the acids are oxidised.[13] Peptides, however, appear to be relatively unreactive towards OsO_4.[11,12]

RESULTS AND DISCUSSION

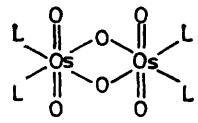
(*a*) *Reactions of* OsO_4 *with Amino-acids and Peptides.*—Since OsO_4 is readily reduced by NH_2 groups [12] but only very slowly by CO_2H groups,[14] the likely reaction sites in amino-acids are either the α-amino-groups or the groups in side chains. In agreement with this we find that amino-acid esters (*e.g.* methyl esters of DL-alanine, -phenylalanine, or α-*N*-benzoyl-DL-lysine, DL-glycine ethyl ester, DL-glutamic acid diethyl ester) give black solids with OsO_4 at approximately the same rates as do the unsubstituted acids at pH 7, whereas amides and other compounds in which the α-amino-group is blocked (*e.g.* α-*N*-benzyloxycarbonylphenylalanine or α-*N*-acetyl-DL-leucine) show no such reactivity. Similarly, there is no reaction between OsO_4 and peptides with blocked terminal groups, *e.g.* α-*N*-benzyloxycarbonyl-L-valyl-L-leucine ethyl ester or glycylglycylglycine methyl ester hydrochloride). There is no colour change when OsO_4 is added to solutions of these, and no change in their vibrational or ^1H n.m.r. spectra.

Thus, as models for OsO_4–protein reactions, we have studied the reactions of OsO_4 with amino-acids or their derivatives in which the α-amino-group is blocked, the latter simulating the unreactive peptide linkage. The remaining reactive entities will be the side chains: amongst those amino-acids found in tissue protein, the potentially reactive sites are in histidine (amino- and unsaturated nitrogen atoms), tryptophan (amino- and olefinic groups), arginine (amino- and imino-groups), lysine (amino-group), and the sulphur ligands in cysteine, cystine, and methionine. We have confined our attention to these.

(*i*) *Formation of* OsO_4·L *adducts in solution.* Osmium tetraoxide forms bright yellow 1 : 1 adducts OsO_4·L in solution with ammonia,[15] pyridine,[14,16] isoquinoline, phthalazine, pyridazine, and quinuclidine,[17] all with distinctive vibrational spectra (polarised Raman bands near 930 cm^{-1}, strong i.r. bands near 900 cm^{-1} due to stretching vibrations of the OsO_4 moiety).[17] We find that a number of amines (*e.g.* α-*N*-benzoyl-L-histidine isobutyl ester, imidazole and substituted imidazoles, α-*N*-benzoyl-L-lysine methyl ester, or n-butylamine), and also α-*N*-benzoyl-DL-methionine and α-*N*-benzoyl-DL-methionine methyl ester, form similar yellow solutions with the characteristic Raman and i.r. spectra of OsO_4·L adducts. Although the solutions were too unstable for the isolation of solid compounds, it seems likely that they contain such adducts with nitrogen or sulphur atoms acting as donors.

(*ii*) *Formation of* $Os_2O_6L_4$ *species.* A solution of the OsO_4·L adduct formed by α-*N*-benzoyl-L-histidine isobutyl ester ($C_{17}H_{21}N_3O_3$) darkens on standing, and a brown diamagnetic solid of stoicheiometry $OsO_3(C_{17}H_{21}N_3O_3)_2$ can be isolated. As simpler models for the histidine side chain, we have used imidazole ($C_3H_4N_2$), 1-methylimidazole ($C_4H_6N_2$), and 5,6-dimethylbenzimidazole ($C_9H_{10}N_2$). As with $C_{17}H_{21}N_3O_3$, these react with OsO_4 in aqueous acetone to give yellow solutions containing OsO_4·L adducts from which the

brown diamagnetic $OsO_3 \cdot L_2$ species can be obtained. Molecular-weight measurements on an aqueous solution of the brown 1-methylimidazole complex show this to be dimeric, *i.e.* $Os_2O_6(C_4H_6N_2)_4$. The complexes have strong Raman bands near 880 cm^{-1} (polarised in the case of the soluble 1-methylimidazole complex) and i.r. bands near 830 cm^{-1}. Such features are found in the well established diamagnetic complexes $Os_2O_6(py)_4$ (py = pyridine)[4,14,16,18,19] and $Os_2O_6(bipy)_2$ (bipy = 2,2′-bipyridine),[19] which have bands at 880 and 830 cm^{-1} assigned respectively to symmetric and asymmetric stretching vibrations [$\nu_{sym}(OsO_2)$ and $\nu_{asym}(OsO_2)$] of the *trans*-O=Os=O unit. We suggest that the $Os_2O_6L_4$ species formed by α-*N*-benzoyl-L-histidine isobutyl ester and imidazoles have structure (1), as found[16] in $Os_2O_6(py)_4$. Infrared bands near 640 cm^{-1}, partly obscured in some cases by ligand modes, probably arise[5,19] from the Os_2O_2 ring. In addition, i.r. bands[20] typical of imidazole rings co-ordinated *via* the unsaturated nitrogen atoms are observed, and weak bands near 250 cm^{-1} may arise[21] from Os–N stretching vibrations. These $Os_2O_6L_4$ imidazole complexes are the first such species of osmium to be isolated, although osmium(II) and osmium(III) complexes of 2-mercaptobenzimidazole have been reported.[22] In addition to the



(1)

species listed in the Table we have also characterised similar complexes with L = 2-methylimidazole, 1,2-dimethylimidazole, benzimidazole, and 2-phenylimidazole.

Although α-*N*-acetyl-L-lysine methyl ester gave a yellow solution with OsO_4 in aqueous acetone, the corresponding $Os_2O_6L_4$ reduction product could not be isolated. However, as a model for the ε-amino-group of lysine we used n-butylamine ($C_4H_{11}N$) which gave a yellow solution with OsO_4 and then $Os_2O_6(C_4H_{11}N)_4$. Arginine also has an ε-amino-group, but α-*N*-benzoyl-

Analyses and vibrational spectra of osmium complexes

Complex	Analysis (%) [a] C	H	N	Infrared and Raman spectra (cm^{-1}) [b] $\nu(OsO_2)$	$\nu(Os_2O_2)$		
(*a*) $Os_2O_6L_4$							
$Os_2O_6(C_3H_4N_2)_4$	19.3	2.2	14.8	835vs	580m		
	(19.3)	(2.2)	(15.0)	*887*			
$Os_2O_6(C_4H_6N_2)_4 \cdot H_2O$	22.9	2.9	13.1	832vs	580m		
	(22.9)	(3.3)	(13.3)	*888* [c]			
$Os_2O_6(C_9H_{10}N_2)_4$	40.6	3.9	9.9	842vs	578m		
	(40.7)	(3.8)	(10.5)				
$Os_2O_6(C_{17}H_{21}N_3O_3)_4$	47.3	5.0	9.5	860vs	570m		
	(47.0)	(4.9)	(9.7)				
$Os_2O_6(C_4H_{11}N)_4 \cdot H_2O$	24.0	6.0	6.8	832vs	550m		
	(23.9)	(6.0)	(6.9)				
$Os_2O_6(C_{12}H_{15}NO_3S)_4$ [d]	37.9	3.8	3.6	856vs	630m		
	(38.7)	(4.0)	(3.8)	*900*			
(*b*) $Os_2O_6L_3L'$							
$Os_2O_6(C_4H_6N_2)_3(C_6H_{11}NO_2)$	26.6	3.5	11.0	832vs	578m		
	(25.4)	(3.4)	(11.5)				
$Os_2O_6(C_4H_6N_2)_3(C_{15}H_{23}N_4O_3) \cdot 3H_2O$	29.3	3.8	12.0	835vs	580m		
	(29.9)	(4.5)	(12.9)				
				ν(C–O)	$\nu(OsO_2)$	ν(Os–O)	$\delta(OsO_2)$
(*c*) $OsO_2(O_2R)L_2$							
$OsO_2(O_2C_6H_{10})(C_4H_6N_2)_2$	33.7	4.4	11.0	984m	821m	584w	320w
	(33.6)	(4.4)	(11.2)		*875*		
$OsO_2(O_2C_6H_{10})(C_9H_{10}N_2)_2$	45.2	4.7	8.7	980w	828vs	585w	
	(45.8)	(4.8)	(8.9)		*884*		
$OsO_2(O_4C_{18}H_{34})(C_4H_6N_2)_2 \cdot 2.5H_2O$	42.0	6.1	8.1	1 012m	830vs	578w	280w
	(42.3)	(6.0)	(7.6)		*884*		
$OsO_2(O_4C_{19}H_{36})(C_9H_{10}N_2)_2$	52.1	6.5	6.9	1 000m	830vs	570w	
	(52.7)	(6.7)	(6.6)		*885*		
$OsO_2(O_5C_{29}H_{46})(C_5H_5N)_2$	55.5	7.0	3.4	1 010m	830vs	578w	305w
	(55.7)	(7.0)	(3.3)				
$OsO_2(O_5C_{29}H_{46})(C_4H_6N_2)_2 \cdot H_2O$	51.4	7.0	5.9	1 010m	823vs	580w	312w
	(51.4)	(7.2)	(6.5)				
$OsO_2(O_4C_6H_8N_2)(C_9H_{10}N_2)_2$	40.7	3.9	11.5	1 010m	846vs	581w	284w
	(40.9)	(4.3)	(11.9)		*889*		
$Os_2O_4(O_4C_{19}H_{34})(C_4H_6N_2)_4 \cdot 2H_2O$	35.6	4.8	9.6	1 010m	825vs	580w	292w
	(36.0)	(5.3)	(9.6)				
$Os_3O_6(O_6C_{19}H_{32})(C_4H_6N_2)_6 \cdot 2H_2O$ [e]	32.6	4.3	10.2	1 005m	828vs	583w	280w
	(32.6)	(4.6)	(10.6)		*876*		
				ν(N–H)	$\nu_{asym}(CO_2)$	$\nu_{sym}(CO_2)$	ν(OsS)
(*d*) OsL_2							
$Os(C_{10}H_{15}N_3O_6S)_2$ [f]	30.2	4.2	10.3	3 260vs	1 640vs [g]	1 405m	338m
	(30.0)	(3.8)	(10.5)	3 050s			
$Os(C_3H_6NO_2S)_2$ [h]	16.7	3.0	6.4	3 430s	1 620vs	1 485s	328w
	(16.7)	(2.8)	(6.5)				

[a] Calculated values are given in parentheses. [b] Only the strongest bands are quoted; Raman modes are italicised. [c] At 893 cm^{-1} (polarised) in water. [d] S, 9.0 (8.6%). [e] O, 16.7 (17.1%). [f] S, 7.9 (8.0%). [g] ν(C=O) at 1 720m cm^{-1}. [h] S, 14.9 (14.9%).

L-arginine ethyl ester gave no reaction with OsO_4; the ε-amino-group is probably deactivated by the adjacent C=N, just as the amino-groups in amides are deactivated by adjacent C=O groups. The blocked tryptophan derivative α-*N*-benzyloxycarbonyl-L-tryptophan *p*-nitrophenyl ester gave a dark purple intractable material. However, it is known that hydrolysis of OsO_4–pyridine mixtures with tryptophan give the corresponding 1,2-glycol,[23] so it appears that the olefinic double bond in tryptophan does react with OsO_4. Although both α-*N*-benzoyl-DL-methionine ($C_{12}H_{15}NO_3S$) and its methyl ester appear to give OsO_4·L solutions, only the former gave a precipitate, of $Os_2O_6(C_{12}H_{15}NO_3S)_4$.

(*iii*) *Formation of* OsL_2 *species* (L = *glutathione or* L-*cysteine*). The peptide glutathione ($C_{10}H_{15}N_3O_6S$) with OsO_4 in aqueous acetone yields a brown complex of stoicheiometry $Os(C_{10}H_{15}N_3O_6S)_2$. The paramagnetism of the complex ($\mu_{eff.}$ 2.62 B.M. at room temperature) * suggests that it contains Os^{IV}, and we propose the formulation $Os(C_{10}H_{15}N_3O_6S)_2$ with co-ordinated $[C_{10}H_{15}N_3O_6S]^{2-}$ as found in the copper(II) glutathione complex.[24] As with other complexes of this ligand, the absence of the S–H stretch in the i.r. spectrum (2 515 cm^{-1} in the free ligand [24]) suggests formation of a metal–sulphur bond, and the bands at 1 640 and 1 405 cm^{-1} of the complex [24] indicate strong co-ordination from an ionised $[CO_2]^-$ group. We suggest that each ligand is terdentate, with co-ordination of one oxygen atom from $[CO_2]^-$ and of the sulphur and amide nitrogen atoms, the last two giving a five-membered ring as found [24] in palladium glutathione complexes.

With L-cysteine ($C_3H_7NO_2S$), aqueous OsO_4 yields a brown insoluble product $Os(C_3H_7NO_2S)_2$. The observed diamagnetism of the complex suggests that it contains Os^{II}. The i.r. spectrum (tentative assignments in the Table) is very similar to that found for $Pt(C_3H_6NO_2S)_2$, for which co-ordination from sulphur and oxygen in $[C_3H_6NO_2S]^-$ has been proposed; [25] in the osmium complex the CO_2H group may function as a bidentate or a bridging ligand, giving in either case octahedral co-ordination to the osmium.

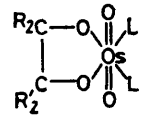
(*b*) *Cross-linking Reactions with* OsO_4.—(*i*) *With amino-acid side-chain groups: formation of* $Os_2O_6L_3L'$ *and* $OsLL'_n$. We find that amino-acid side-chain derivatives react with OsO_4·L adducts in aqueous acetone. Thus, the 1-methylimidazole adduct OsO_4·$C_4H_6N_2$ with α-*N*-benzoyl-DL-methionine methyl ester gives $Os_2O_6(C_4H_6N_2)_4$, while $Os_2O_6(C_4H_6N_2)_3L'$ is precipitated if a similar reaction is carried out in the presence of other donors L' [L' = L-proline methyl ester ($C_6H_{11}NO_2$) or α-*N*-benzoyl-L-arginine ethyl ester ($C_{15}H_{23}N_4O_3$)]. These complexes are diamagnetic.

Reactions of OsO_4·L [L = 1-methylimidazole or α-*N*-benzoyl-L-histidine isobutyl ester ($C_{17}H_{21}N_3O_3$)] with α-*N*-acetyl-L-cysteine ($C_5H_9NO_3S$) give brown complexes of stoicheiometry $OsL(C_5H_9NO_3S)_2$, while L-cysteine reacts with OsO_4·$C_4H_6N_2$ to give cystine and a product of stoicheiometry $Os(C_4H_6N_2)(C_3H_7NO_2S)_3$·$H_2O$.

* Throughout this paper: 1 B.M. ≈ 9.27 × 10^{-24} A m^2.

The observed diamagnetism of these complexes suggest that they contain octahedral Os^{II}. The i.r. spectra (see Experimental section) are too complicated to allow useful structural information to be deduced from them; we note, however, the absence of the S–H stretching vibration near 2 550 cm^{-1} of the free cysteine ligands, suggesting formation of metal–sulphur bonds.

(*ii*) *With amino-acid side chains and lipids: formation of* $OsO_2(O_2R)L_2$. We find that reaction of OsO_4 in aqueous acetone with α-*N*-benzoyl-L-histidine isobutyl ester, 1-methylimidazole, or 5,6-dimethylbenzimidazole and unsaturated lipids R gives $OsO_2(O_2R)L_2$ [R = cyclohexene (C_6H_{10}), oleic acid ($C_{18}H_{34}O_2$), methyl oleate ($C_{19}H_{36}O_2$), or cholesteryl acetate ($C_{29}H_{48}O_3$)]; excess of amine L is necessary for the reaction. The Raman spectra of these $Os(O_2R)L_2$ species have strong bands near 880 cm^{-1} and strong i.r. bands near 830 cm^{-1} typical of *trans*-O=Os=O moieties [4,26] but no i.r. bands near 640 cm^{-1} associated [5,19] with Os_2O_2 rings. In addition to bands due to co-ordinated L and to R, there are new i.r. bands near 1 010 cm^{-1} typical [4] of C–O stretches in oxo-osmium(VI) esters. In the ^1H n.m.r. spectra the protons adjacent to the ester linkage occur in the vicinity of δ 4.2—4.5 p.p.m. Similar spectral features are observed for established $OsO_2(O_2R)L_2$ species of structure (2),[4,17] and we propose that the present complexes have this structure also.



(2)

Species of this type are known with a wide variety of alkenes R (including those used here) with L = pyridine,[4,14] isoquinoline,[4,14,17] and ½ bipy; [27] the presence of such *N*-donors greatly accelerates the reactions of OsO_4 with alkenes probably *via* OsO_4·L or OsO_4·2L intermediates.[27,28] With 1,3-dimethyluracil ($C_6H_8N_2O_2$) we obtained the solid $OsO_2(C_6H_8N_2O_4)_2L_2$ species (L = 1-methylimidazole or 5,6-dimethylbenzimidazole). The 1-methylimidazole complex was unstable in air but was characterised by ^1H n.m.r. spectroscopy, the protons adjacent to the ester linkage occurring as two doublets (*J* 5 Hz) at δ 5.58 and 4.57 p.p.m. Analogous complexes of other nucleotides with L = py or ½ bipy have been established.[29,30] Each double bond in the dialkene methyl linoleate ($C_{19}H_{34}O_2$) and the trialkene methyl linolenate ($C_{19}H_{32}O_2$) reacts with OsO_4 to give a cyclic ester structure as in (2), the protons adjacent to the ester linkage appearing as a broad resonance near δ 4.4 p.p.m. in the ^1H n.m.r. spectra. In the absence of nitrogen donors, 1 mol of methyl linolenate reacts with only 2 mol of OsO_4, due perhaps to an inter- or intra-molecular diester bridge between two of the double bonds.[31]

(*c*) *Osmium Tetraoxide and Tissue Fixation*.—It has been suggested [1] that, if the OsO_4–protein reaction is

significant in tissue fixation, the likely sites are at SH groups,[2,32] at histidine,[33] and at tryptophan[34] side chains. The present work has confirmed that, under reaction conditions similar to those used in tissue fixation, OsO_4 does indeed react with such groups. The isolation of $Os_2O_6L_3L'$ and $OsLL_n'$ mixed species suggests that OsO_4 could act as an inter- or intra-protein cross-linking agent, and there is cytological evidence for such a cross-linking action for OsO_4 in membranous protein.[1,35] The formation of $OsO_2(O_2R)L_2$ species from OsO_4, L, and unsaturated lipids R suggests a possible protein–lipid cross-linking role for OsO_4 where such substrates are found together (*e.g.* in membranes), again in accord with cytological evidence.[36] Indeed, the initial formation of OsO_4·L adducts between OsO_4 and donor sites in proteins could well accelerate the reaction producing $OsO_2(O_2R)L_2$, since reaction between OsO_4 and alkenes is known to be greatly accelerated by amines such as py and bipy.[28] The recent evidence[37] from *X*-ray photoelectron spectroscopy that OsO_4-fixed tissue contains osmium-(VI), -(IV), and possibly -(III) species is in partial accord with the formation of osmium-(VI), -(IV), and -(III) species in this work using model systems.

EXPERIMENTAL

(*a*) OsO_4·L *Solutions and* $Os_2O_6L_4$ *Complexes.*—The yellow solutions of OsO_4·L were prepared by adding the amine L to OsO_4 (1 : 1) in acetone; their Raman and i.r. spectra were recorded immediately.

The $Os_2O_6L_4$ complexes with imidazole, 2,3-dimethyl- or 2-phenyl-imidazoles, benzimidazole, 5,6-dimethylbenzimidazole, and α-*N*-benzoyl-L-histidine isobutyl ester were generally prepared by adding the ligand (2 equivalents) in $CHCl_3$ or acetone to OsO_4 in the same solvent. The solids precipitated from the initial yellow solution were filtered off and dried *in vacuo*. In the case of 5,6-dimethylbenzimidazole, ethanol was added to facilitate reduction. For the other $Os_2O_6L_4$ complexes the following procedures were used.

Di-μ-oxo-bis[bis(1-methylimidazole)dioxo-osmium(VI)], $Os_2O_6(C_4H_6N_2)_4$·H_2O. To stirred 1-methylimidazole (0.4 g) was slowly added OsO_4 (0.1 g) in small portions. **CAUTION:** in one instance an explosion resulted. The resulting brown solution was stirred for 30 min and acetone (*ca.* 10 cm³) added, giving a solid which was washed several times with acetone and dried *in vacuo*; *M* (osmometrically in water) 691 (calc. for $C_{16}H_{26}N_8O_6Os_2$: *M* 818).

Di-μ-oxo-bis[bis(n-butylamine)dioxo-osmium(VI)], $Os_2O_6(C_4H_{11}N)_4$. Osmium tetraoxide (50 mg, 0.2 mmol) in acetone (0.5 cm³) was added to n-butylamine (0.2 g, 2.7 mmol) in water (5 cm³) to give a yellow solution from which the complex precipitated. The solid was washed with acetone and diethyl ether and dried *in vacuo*. The reverse addition of reactants gave an intractable black material.

Di-μ-oxo-bis[bis(α-N-*benzoyl*-DL-*methionine)dioxo-osmium* (VI)], $Os_2O_6(C_{12}H_{15}NO_3S)_4$. To a solution of α-*N*-benzoyl-DL-methionine (0.1 g, 0.4 mmol) in acetone (5 cm³) was added OsO_4 (0.05 g, 0.2 mmol) in acetone. The complex, which precipitated as a grey-brown solid from an initially yellow solution, was washed with acetone and dried *in vacuo*. Infrared spectrum: 3 380 [ν(OH)], 1 640 (amide C=O), 856 [$\nu_{asym}(OsO_2)$], and 712 cm⁻¹ (monosubstituted aromatic).

(*b*) $Os_2O_6L_3L'$ *Complexes.*—The preparation of $Os_2O_6(C_4H_6N_2)_3(C_6H_{11}NO_2)$ is typical. To the yellow solution obtained from OsO_4 (50 mg, 0.2 mmol) and 1-methylimidazole (32 mg, 0.4 mmol) in acetone (20 cm³) was added an excess of L-proline methyl ester in acetone–dichloromethane (1 : 1) (10 cm³). The resulting cloudy brown solution was added to light petroleum giving the product which was filtered off and dried *in vacuo*.

(*c*) $OsO_2(O_2R)L_2$ *Complexes.*—The preparation of $OsO_2(O_2C_6H_{10})(C_4H_6N_2)_2$ is typical. To the yellow solution obtained from OsO_4 (50 mg, 0.2 mmol) and 1-methylimidazole (32 mg, 0.4 mmol) in acetone (25 cm³) was added cyclohexene (16 mg, 0.2 mmol) in acetone (10 cm³). The brown solution was stirred for 30 min and the solid filtered off. More product was obtained from the filtrate by addition of light petroleum. The combined solids were washed with diethyl ether and dried *in vacuo*. With methyl linoleate and methyl linolenate, 4 and 6 mol equivalents respectively of 1-methylimidazole and 2 and 3 equivalents of OsO_4 respectively were used. The complexes were sensitive to moisture and were handled under a dry nitrogen atmosphere.

(*3-Acetoxycholestane-5,6-diolato)dioxobis(pyridine)osmium*-(VI). A solution of OsO_4 (0.1 g, 0.4 mmol) and pyridine (0.2 cm³) in carbon tetrachloride (6 cm³) was added to cholesteryl acetate (0.17 g, 0.4 mmol) in carbon tetrachloride (5 cm³). After 2 h, light petroleum was added to give the complex as a brown precipitate; ν(C=O) at 1 720 cm⁻¹. Hydrogen-1 n.m.r. spectrum in $CDCl_3$: δ 1.92 (s, 3, CH_3), 3.8 (t, 1, CH), 7.45 (d, 4, β-protons, py), 7.75 (d, 2, γ-protons, py), and 8.81 p.p.m. (d, 4, α-protons, py).

(*d*) OsL_2 *Complexes.*—Glutathione or L-cysteine (*ca.* 0.1 g) was dissolved in water (10 cm³) and acetone added until the solution became cloudy. Water was added dropwise to this solution to dissolve the solid, and the mixture added to OsO_4 (*ca.* 0.05 g) in acetone (10 cm³). The resulting solid was filtered off, washed with acetone and diethyl ether, and dried *in vacuo*.

(*e*) *Other Cysteine Complexes.*—$OsL(C_5H_9NO_3S)_2$. To the yellow solution obtained from OsO_4 (50 mg, 0.2 mmol) and α-*N*-benzoyl-L-histidine isobutyl ester (69 mg, 0.2 mmol) in acetone (20 cm³) was added excess of α-*N*-acetyl-L-cysteine ($C_5H_9NO_3S$) in acetone (10 cm³). The solid was filtered off, washed with acetone and diethyl ether, and dried *in vacuo*; $\mu_{eff.}$ = 0.56 B.M. at 298 K (Found: C, 38.5; H, 4.3; N, 8.2. $C_{27}H_{37}N_5O_9OsS_2$ requires C, 38.0; H, 4.5; N, 8.4%). Infrared spectrum: 3 290s, 3 140s, 1 725s, 1 645vs, 1 525s, 1 215m, 1 175m, 1 100w, 860w, and 710w cm⁻¹.

The corresponding 1-methylimidazole complex was made in a similar manner; $\mu_{eff.}$ = 0.69 B.M. at 298 K (Found: C, 30.6; H, 4.0; N, 10.2; S, 11.7. $C_{14}H_{22}N_4O_3OsS_2$ requires C, 30.6; H, 4.0; N, 10.2; S, 11.8%). Infrared spectrum: 3 290s, 3 140s, 3 070s, 1 720s, 1 640vs, 1 525vs, 1 410m, 1 365s, 1 220s, 1 174m, 1 100m, 1 045w, 860w, 750w, 650w, 580w, 535w, and 365vw cm⁻¹.

$Os(C_4H_6N_2)(C_3H_7NO_2S)_3$·$H_2O$. To a solution of OsO_4 (0.25 g, 1.0 mmol) and 1-methylimidazole ($C_4H_6N_2$) (0.1 g, 1.2 mmol) in aqueous acetone (1 : 1, 20 cm³) was added excess of cysteine ($C_3H_7NO_2S$) in water (20 cm³). The solution turned rapidly brown and cystine was precipitated. Acetone was added to the filtrate and on standing a brown complex precipitated; $\mu_{eff.}$ = 0.76 B.M. at 298 K (Found: C, 23.6; H, 3.9; N, 10.2; S, 14.8. $C_{13}H_{26}N_5O_7OsS_3$ requires C, 23.9; H, 4.0; N, 10.7; S, 14.8%). Infrared

spectrum: 3 400s, 3 010vs, 1 625vs, 1 580vs, 1 520s, 1 400s, 1 380m, 1 345m, 1 300m, 1 200m, 845m, 780m, 665w, 600w, 530m, 465w, 380s, and 315w cm^{-1}.

α-N-*Benzoyl*-L-*histidine Isobutyl Ester*.—The preparation of this ligand has not been described in the literature previously. Dry hydrogen chloride gas was bubbled through a solution of α-*N*-benzoyl-L-histidine (1.5 g) in isobutyl alcohol (30 cm^3) until all solid had dissolved. The solution was heated under reflux for 2 h followed by solvent removal *in vacuo*. The solid obtained was dissolved in methanol (40 cm^3), sodium methoxide in methanol was added to pH 7.5, and the solvent removed. The residue was extracted with methylene dichloride and the solvent removed. Recrystallisation of the solid from benzene–methylene dichloride gave α-*N*-benzoyl-L-histidine isobutyl ester as prisms (yield 89%), m.p. 119—120 °C (Found: C, 65.0; H, 6.7; N, 13.1. $C_{17}H_{21}N_3O_3$ requires C, 64.7; H, 6.7; N, 13.3%). Infrared spectrum: 3 280 (NH, amide), 1 730 (C=O, ester), 1 640 (C=O, amide), and 715 cm^{-1} (monosubstituted benzene). N.m.r. spectrum in $CDCl_3$: δ 0.75, 0.85 (d, 6, $2CH_3$), 1.84 (m, 1, CH), 3.25 (m, 2, CH_2), 3.80 (d, 2p, $CO-CH_2$), 4.95 (b, 1, CO–CH–N), 6.32 (s, 1, C=CH), 7.4 and 7.82 (2m, 5, aromatics), 7.56 (s, 1p, CH=N), 8.58 (m, 1, NH), and 10.48 p.p.m. (m, 1, NH).

Analytical data were obtained by the Microanalytical Department, Imperial College, oxygen analyses by F. Pascher (Bonn). Molecular weights were determined osmometrically on a Perkin-Elmer–Hitachi 115 instrument. Infrared spectra were recorded from 200 to 4 000 cm^{-1} on a Perkin-Elmer 457 instrument on KBr discs or Nujol mulls between caesium iodide plates, and in chloroform or methylene dichloride using KBr cells. Raman spectra were obtained on a Spex Ramalog 5 instrument with a DPC-2 detector using a krypton-ion laser (6 471 Å excitation); solids were scanned as 5% sample–95% KBr spun discs, and solutions of OsO_4·L adducts in aqueous acetone were examined in a spinning solution cell. Hydrogen-1 n.m.r. spectra were recorded on a 60-MHz Perkin-Elmer R-12 spectrometer, and magnetic-susceptibility measurements were made on solids by the Gouy method [and on solutions of $Os_2O_6(C_4H_6N_2)_4$ by the Evans' method [38]].

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