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BIOMIMETIC METAL ENCAPSULATION

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I. INTRODUCTION

The genesis of the field of bioinorganic chemistry was a result of recognizing that the metal center of metalloproteins and metalloenzymes are conventional complexes with sometimes unconventional coordination environments imposed by a protein structure. In this sense coordination chemistry entered the field of biology with the ability to explain redox, spectroscopic, or other properties of metalloproteins and to make predictions of new properties based on low molecular weight metal complexes that are analogs of a biogenic macromolecule. However in the last 20 years it has become increasingly clear that low-molecular-weight natural products also play critical roles in life processes, particularly in metal ion transport. The most important and well characterized of such compounds are the siderophores (refs. 1-4). These are complexing agents that have a very high affinity for Fe(III), solubilize this otherwise hydrolytically insoluble ion, and facilitate its transport into microbes such as bacteria and fungi. They are essentially classic coordination complexes and the study of their structures and physical properties represents both an exercise in fundamental coordination chemistry and a key to understanding the biological role such compounds play.

As we (refs. 1, 3) and others (refs. 2, 4-6) have noted, iron is the most abundant transition metal in the earth's crust and yet is highly inaccessible under ambient conditions because of the insolubility of $Fe(OH)_3$. And yet, for all plants and animals, and for virtually all microbes, life without iron is impossible. Essential iron-containing enzymes and proteins are found in species ranging from bacteria to man. In response to the challenge posed by iron requirement and unavailability, microorganisms secrete high-affinity iron-binding compounds called siderophores (from the Greek, "iron carrier") (ref. 6). More than a hundred naturally-occurring siderophores have been isolated and characterized to date (ref. 1). Examples are shown in Figures 1 and 2. As will be developed, the ongoing search for new siderophores is driven by several factors, including their potential for clinical use.

Siderophores solubilize ferric ion and enable it to be transported into cells, usually via specific membrane-bound uptake systems. This process involves a highly specific recognition of the ferric complex by receptor proteins at the cell surface. Iron uptake and inhibition studies with a series of modified siderophores, semisynthetic siderophores, and synthetic siderophore analogs have demonstrated remarkable stereospecificity and enantioselectivity in the receptor-siderophore

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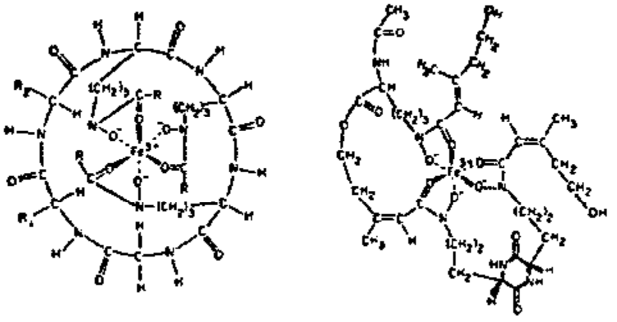
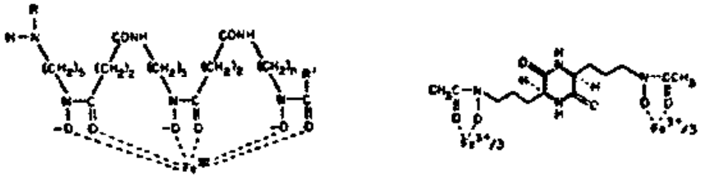


Ferrichrome Family

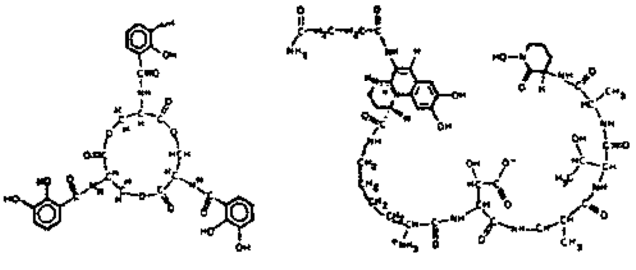
Coprogen



Ferrioxamine Family

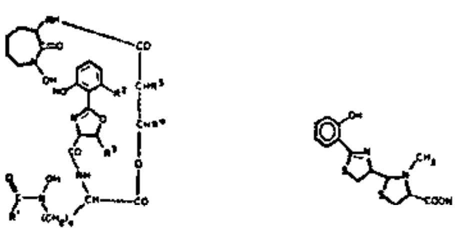
Rhodotorulic Acid Iron Complex

Fig. 1. Examples of hydroxamate siderophores. For the structural variations found in the ferrichromes see reference 1. For the well-known siderophore ferrioxamine B, R = H and R' = CH_3. The iron complex of rhodotorulic acid is a dimer, with an Fe^{3+} to ligand ratio of 2:3 (designated by the $Fe^{3+}/3$ in the structural formula).



Enterobactin

Pseudobactin



Mycobactin

$R_1 = 17$ $R_2 = R_5 = CH_3$

$R_3 = H$ $R_4 = C_2H_5$

Pyochelin

Fig. 2. The structures of representative phenolate siderophores.

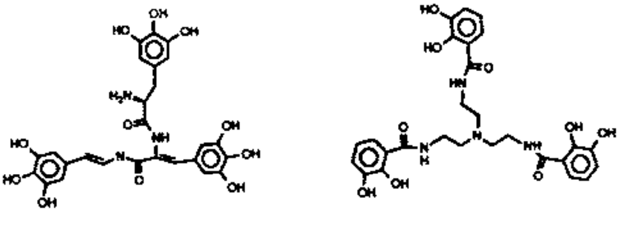
interaction. Such studies require a precise knowledge of the solution conformations and geometries of these iron-complexing agents made possible by the preparation of exchange inert metal complexes, which is reviewed elsewhere (ref. 1).

The intracellular release of Fe(III) from siderophore complexes, with formation constants in the range of 10^{25} to 10^{50}, requires ligand exchange or reduction and is a key part of the iron uptake process. Since many cellular constituents (such as amino acids, proteins, nucleic acids, or phosphate) are potential nonspecific iron-chelating agents, the manner by which the cell handles trace quantities of internal iron in the presence of many competing chelating agents remains one of the most challenging aspects of iron metabolism. Fundamental data on solution thermodynamics, kinetics, and electrochemistry are necessary in the examination of iron release mechanisms. Furthermore, the application of spectroscopic methods (such as Mössbauer spectroscopy) *in vivo* allows one to study the initial metabolic steps of iron assimilation via siderophores.

In an excellent recent review "The Genetics and Molecular Biology of Siderophore-Mediated Iron Transport in Bacteria," Jorge Crosa wrote "It is clear ... that siderophore mediated utilization by microorganisms of the host vertebrate iron, which is mostly bound by high-affinity iron binding proteins, becomes an important virulence factor in the establishment of an infection ..." (ref. 5). And later "These remarkable similarities between the procaryote and eucaryote iron transport systems underscore the importance of these findings with respect to the host-bacteria interactions leading to disease. An increased knowledge of the molecular mechanisms of microbial pathogenicity mediated by iron and host resistance will undoubtedly result from the studies of these systems. However, it is likely that bacteria will still have wonderful surprises awaiting us" (ref. 5). Although our concern has been primarily with the more coordination chemistry aspects of this research, we heartily concur.

In another classic review "Iron Withholding: A Defense Against Infection and Neoplasia" Eugene Weinberg described, for a very large number of diseases, the strong connection between pathogenicity of a microorganism in its animal host (particularly developed in the use of human diseases) and iron availability (ref. 7). He ends his article with "... research and clinical observations during the past 50 years have revealed the vigorous competition for growth-essential iron between bacterial, fungal, protozoan, and neoplastic invaders and their vertebrate hosts. The latter posses an array of mechanisms to withhold iron; the ability of invaders to overcome these mechanisms is an important component of virulence. Iron-withholding defense is compromised in a diversity of conditions, many of which involve aspects of iron overload in specific tissues. A number of possible methods and agents for strengthening the iron-withholding defense are now apparent. Research is needed to determine which of these might be safe, efficacious, and practical" (ref. 7). Although it is six years later and much progress has been made, this statement is still accurate. There have been major advances in our understanding of the genetic regulation of siderophore production and iron transport in microorganisms (refs. 5, 8). However, since the subject of this paper is biomimetic encapsulation, including the coordination chemistry and molecular aspects of siderophore mediated iron transport, no further background on the regulation aspect of this topic will be presented here.

The ability of catechol ligands strongly to complex higher oxidation state metal ions, and hence to alter the potentials for metal ion redox pairs, plays a role in at least one other area of chemistry than the siderophores. For a number of years it has been known that the tunicates (primitive marine organisms in the phylum *chordata*, commonly known as sea squirts) can selectively accumulate elements such as V, Fe, Mo and Nb (refs. 22-25). The black tunicate *Ascidia nigra* irreversibly absorbs vanadium as vanadate, concentrates the element by up to 10^7 relative to the marine concentration (ref. 26) and stores the vanadium in a reduced form. There has been controversy about whether the reduced form is predominantly V(IV) (refs. 27, 28) or V(III) (refs. 29, 30). Kustin et al. (refs. 31-33) have suggested that low-molecular weight pigments known as tunichromes serve as complexing agents for the accumulation and storage of intracellular vanadium in the tunicates. The tris(trihydroxyphenyl) compound tunichrome B (also known as compound An-1 (ref. 29), Figure 3) has been isolated from the blood of *Ascidia nigra* and suggested as such a complexing agent.



Tunichrome b-1

TRENCAMH$_6$

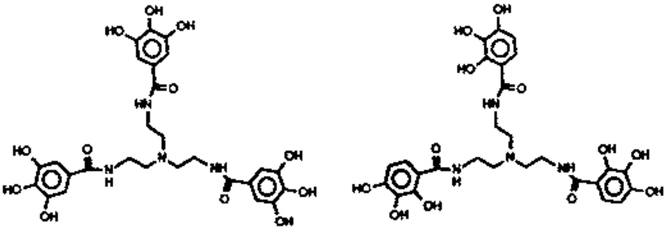


Fig. 3. Structures of tunichrome b-1 and the synthetic analogs described herein.

In the earlier introduction of siderophores the two major functional groups found in these ligands, hydroxamate (Figure 4, (a)) and catecholate (Figure 4, (c)), were described. Less common functional groups found in siderophores are hydroxypyridinone (Figure 4, (d)) (refs. 34, 35) and thiohydroxamic acid (in N-methylthioformohydroxamic acid, or thioformin) (Figure 4, (b)) (refs. 36, 37). The hydroxypyridinethione ligand structure (Figure 4, (e)) is not known in nature but can be regarded as either an aromatic thiohydroxamic acid derivative of (b) or as a thio derivative of the hydroxypyridonate (d).

With the goal of producing new iron sequestering agents of potential therapeutic utility in treating human iron overload, we have taken a biomimetic approach to the synthesis of ferric ion

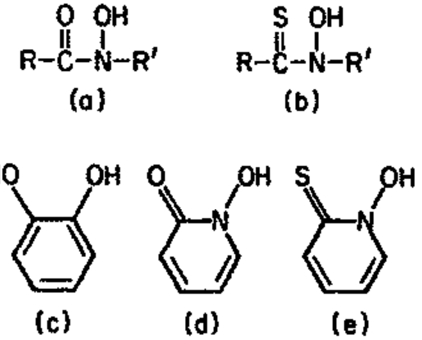


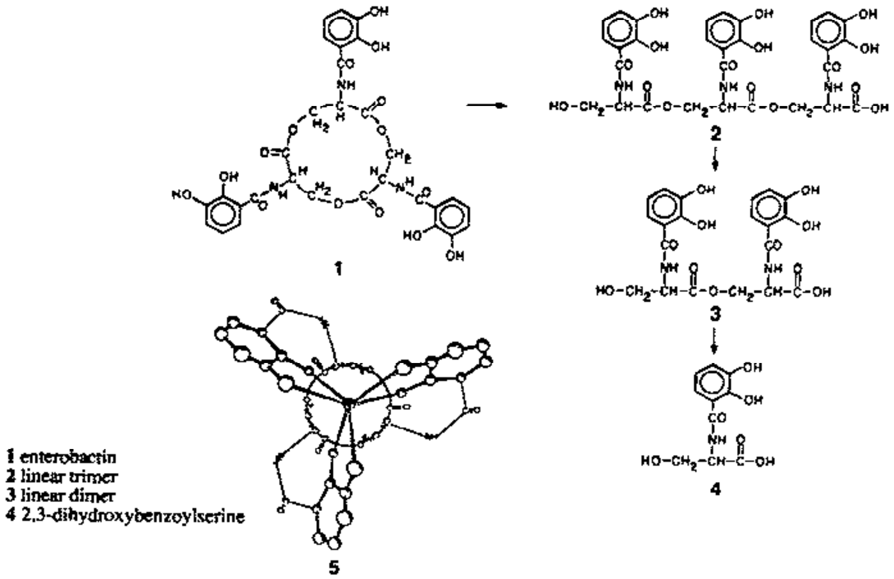
Fig. 4. Some bidentate chelating ligands commonly found in iron(III) sequestering agents.

specific sequestering agents based on the siderophores (ref. 38). Although enterobactin is the strongest iron(III) chelator known, it and close synthetic analogs are potentially dangerous since they are growth factors for microorganisms. The hexadentate catechol ligands also must lose up to 6 protons in sequestering Fe^{3} and hence are relatively ineffective in complexing Fe^{3+} below neutral pH. Hydroxypyridinones and hydroxypyridinethiones are stronger acids than catechol and hydroxamic acids; and, since they are monoprotic acids, hexadentate ligands formed from three of these ligand functional groups need to lose at most three protons to sequester Fe^{3+}. Indeed, the simple bidentate hydroxypyridinones 1,2-Hopo; 3,2-Hopo; and 3,4-Hopo (where x,y-Hopo is x-hydroxy-y(1H)-pyridinone) sequester iron more effectively than either catechol or acetohydroxamic acid at pH 7 (ref. 39). In addition, there is evidence that hydroxypyridonates may be orally active as human iron removal agents (ref. 40). Desferrioxamine B (Figure 1), the trihydroxamic acid siderophore which is the current clinical choice for human iron decorporation therapy, has several undesirable side effects and is orally inactive (refs. 41-43). The hydroxypyridonate and -pyridinethione ligand units therefore seem promising for the development of new iron sequestering ligands, as well as providing synthetic analogs for comparison with native siderophores.

II. ENTEROBACTIN AND ANALOGS

A. Stability and structure

Enterobactin (Figure 1) is hydrolytically unstable and this instability is linked to its iron transport and release mechanisms. The intermediate hydrolysis products have been isolated following each of the three stepwise hydrolysis reactions of enterobactin shown in Figure 5: to form a linear trimer, then dimer and finally the monomeric 2,3-dihydroxybenzoylserine amide. The protonation and conditional ferric complex formation constants of the linear trimer and linear dimer have been determined, as have the enthalpies of formation of the linear trimer complex and ferric enterobactin. Enterobactin is a better iron chelator than either its hydrolysis product (the linear trimer) or the synthetic mimic MECAM because it is able to complex iron with a minimum of added ring strain energy (an enthalpic effect) and because the conformational mobility of iron-free enterobactin is small due to extensive hydrogen bonding (an entropic effect). The similarity of the iron-free conformation to the conformation of ferric enterobactin may also be important from a kinetic standpoint. These features of enterobactin need to be kept in mind in attempts to synthesize similar ligands with very high affinities for metal ions.



1 enterobactin
2 linear trimer
3 linear dimer
4 2,3-dihydroxybenzoylserine

Fig. 5. Hydrolysis products of enterobactin.

The iron complex of the hydrolysis product of enterobactin is sufficiently less stable than that of enterobactin so that hydrolysis of one of the ester bonds is a plausible effective prerequisite to iron removal from enterobactin by microbes. In contrast, the stability of the linear trimer complex with iron is comparable with the complexes of the highest affinity synthetic iron chelators (such as MECAMS). Hence the ability of microbes to remove iron from MECAM and other synthetic ligands lacking ester bonds should not be taken as evidence against the requirement of ester hydrolysis before iron removal from enterobactin. It is found that the greater stability of the enterobactin complex, compared with tri-catechol ligand analogs, is about 1/3 enthalpic and 2/3 entropic in origin.

The preferred Δ chirality of the iron center of enterobactin, imposed by the ligand chirality, is maintained when the triserine ring is hydrolyzed to form linear trimer. Assays of the mediation of iron uptake in *E. coli* RW193 show that both the linear dimer and linear trimer are nearly as effective as enterobactin in mediating iron uptake in this organism (ref. 44).

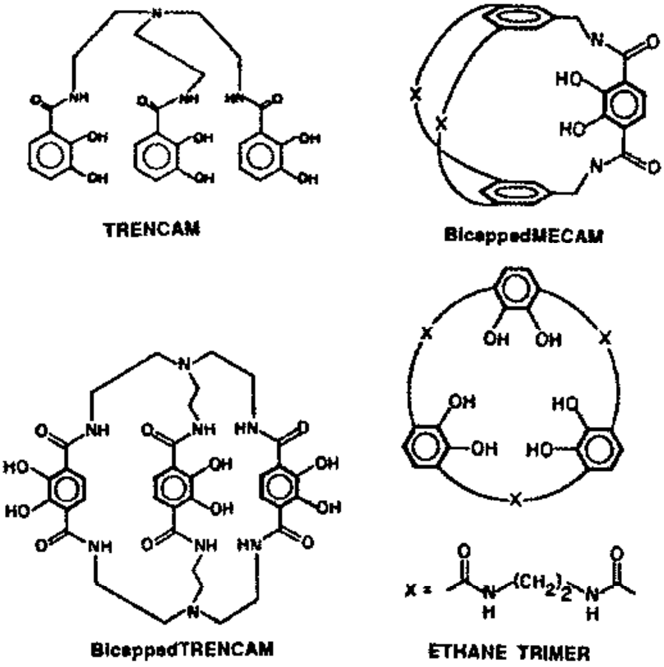
The significance of essential metal ions, particularly iron, to plants has been known for the last hundred years. However it is only relatively recently that the existence of transport proteins in the plasma membrane of plant roots has been established. Low-molecular weight complexing agents, sometimes called phytosiderophores in parallel to the bacterial iron transport agents, are now known for a large number of plants. Mechanisms which allow some plants to more efficiently take up iron have been widely studied (refs. 9, 10). Relatively recently siderophores have been found to act as iron uptake agents, or at least to promote plant growth (ref. 11). Two examples of such siderophores are pseudobactin (ref. 12) and pseudobactin A (ref. 13). Specific strains of *Pseudomonas fluorescens-putida* have been shown to promote growth and increase yields. These rhizobacteria rapidly colonize plant roots of the potato, sugar beet, and radish, giving significant yield increases (refs. 14, 15).

Many studies have examined the use of synthetic iron chelating agents used in agriculture. One example of this is the extensive addition of ferric EDTA to various fertilizers. There is evidence for a non-specific plasma membrane reductase which releases iron from synthetic chelates, but not from microbial siderophores (refs. 16, 17). The reductase activity appears not to be directly coupled to iron transport but rather serves to utilize iron from chelates with a low affinity for iron. Therefore the system functions by transferring electrons from plant material to extracellular chelated iron. Upon reduction, the unstable ferrous complex dissociates, migrates away from the chelating agent, is oxidized back to ferric iron, which then precipitates in the plant root (ref. 18).

The compounds for which the term phytosiderophore was originally applied (refs. 19, 20) are multidentate amino carboxylate ligands such as mugineic acid (ref. 20). However these compounds are also good complexing agents for several other ions, particularly copper, that are also essential for plants. It is known that plants promote processes to chemically modify the soil close to the their roots in ways that increase or decrease the absorption of crucial metal ions (required or toxic, respectively) (ref. 21).

In attempting to make very powerful iron(III) sequestering agents modelled on the siderophores, one approach is to impose a steric constraint on the ligand that predisposes it for metal complexation. Recently, Vögtle (refs. 45, 46), Martell (ref. 27), and we (refs. 48-50) have produced macrobicycles such as bicapped MECAM and bicapped TRENCAM and macrocycles such as ethane trimer (Figure 6) based not on the 2,3-dihydroxybenzamide moiety A found in enterobactin but on the 2,3-dihydroxyterephthalamide B as the binding subunit. Entropic considerations have been the dominant rationale behind this work. Margerum (ref. 51) and others (refs. 52-54) have used the term "macrocyclic effect" to describe the increased stability gained by a chelating agent when all binding subunits are incorporated into a macrocycle or macrobicycle. However, in this strategy toward a more effective chelating agent, the enthalpic properties of the new binding subunit have not been considered. The thermodynamic studies on the new sequestering agents have not shown a macrocyclic effect. The formation constant for the ethane trimer is 5 log units less than that for TRENCAM, a tripodal ligand with three 2,3-dihydroxybenzamide binding subunits, although this is offset by a correspondingly greater acidity (ref. 48). Vögtle and coworkers initially reported a formation constant of 10^{59} for **12**, a value 7 orders of magnitude higher than that for enterobactin (ref. 46), but recent studies show the 10^{59} estimate is too large by about 10 orders of magnitude. In all of these new macrocyclic ligands both the ligand topology and the binding subunit have been varied.

The 2,3-dihydroxyterephthalamide ligands form surprisingly stable complexes for such relatively simple bidentate ligands. Their greater acidity relative to the 2,3-dihydroxybenzamide unit (found in enterobactin and other naturally occurring ferric ion chelating agents, Figure 7) may be expected from the more extended π network available to the anionic charge produced upon deprotonation. However the deprotonated form of the terephthalamides is also stabilized by hydrogen bonding to the amide hydrogen, forming the stable six-membered rings seen in Figure 8. Recent X-ray studies have shown the presence of these hydrogen-bonded forms in a ferric complex of a tris(terephthalamide) ligand (ref. 50).



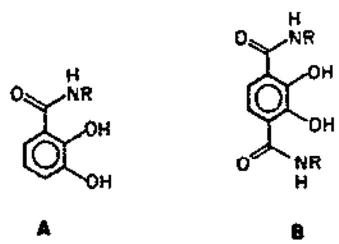
TRENCAM

BicappedMECAM

BicappedTRENCAM

ETHANE TRIMER

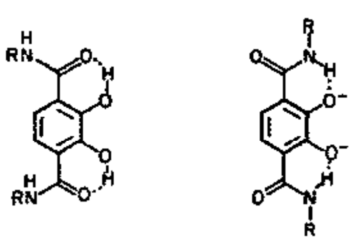
Fig. 6. Recently synthesized catechoylamide Fe(III) sequestering agents.



A

B

Fig. 7. Bidentate binding subunits used in catechoylamide (A) and 2,3-dihydroxytherephthalamide (B) Fe(III) sequestering agents.



acid form base form

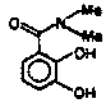
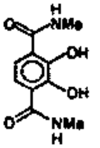
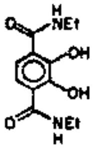
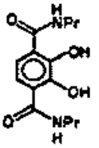
Fig. 8. Proposed acid and base forms of the 2,3-dihydroxyterephthalamide ligand.

The novel charge delocalization of these ligands slows the usual decrease in stepwise formation constants and strongly promotes the formation of the fully formed FeL_3 complex. This results in the highest iron binding affinity ever seen in a bidentate ligand. These binding subunits are therefore excellent iron chelators, even before incorporation into a macrocyclic or macrobicyclic structure.

The stability constant data and pM values shown in Table 1 demonstrate the dramatic increase in Fe(III) binding affinity that results from the substitution of a second amide group on the catechol ring. However this extra stability largely depends on the strong amide-proton to catechol-oxygen hydrogen bonding. Therefore terephthalamide catecholate groups formed from primary amines are particularly attractive constituents of multidentate ligands designed for high metal ion affinity.

TABLE 1

Protonation and formation constants of the 2,3-dihydroxyterephthalaides.[a]

		 1	 2	 3
$\log K_{110}$	17.8	16.4 (1)	16.3 (1)	16.0[b]
$\log K_{120}$	13.9	14.5 (1)	14.4 (1)	15.2 (1)
$\log K_{130}$	8.5	10.9 (1)	11.5 (1)	11.9 (1)
$\log \beta_{130}$	40.2	41.8	42.2 (1)	43.1 (1)
pM	15.0	21.1	21.6	22.7
$\log K_{011}$	12.1[b]	11.1 (1)	11.1 (1)	11.0 (1)
$\log K_{012}$	8.42	6.1 (1)	6.0 (1)	6.0 (1)

[a] pM = $-\log [Fe]$ at pH 7.4; $[L]_T = 10^{-5}$ M; $[Fe]_T = 10^{-6}$ M. [b] Estimate.

III. TUNICHROME AND ANALOGOUS VANADIUM COMPLEXES

As described in the introduction, the isolation of tunichrome (Figure 3), a tricatechol ligand, represented a substantive change in the biochemistry of the tunicates, where it has long been known that vanadium is present in extraordinarily high concentrations in certain species. The chemistry of catechol with vanadium has a long, and often confused, history. Some time ago we investigated the aqueous vanadium chemistry of simple catechol ligands (refs. 55). The principal finding was the first characterization of a stable aqueous V(IV) complex that did not contain the vanadyl ion but instead a pseudo-octahedral tris(catecholato)vanadate(IV) species (ref. 55). The structure of salts of $[V(cat)_3]^{2-/3-}$ (cat = catecholate dianion) compared the V(IV) and V(III) complexes, respectively. Also structurally characterized was a salt of the vanadyl complex $[VO(cat)_2]^{2-}$. Simple octahedral coordination, without the vanadyl (V = O) functionality, was a previously unobserved phenomenon attributed to the exceptional chelating ability of catechol, which is able in aqueous medium to displace the very stable vanadyl oxygen and achieve octahedral coordination at the metal center.

Isolation of the vanadyl and octahedral vanadium(IV) complexes made it possible to reconcile the controversy in the literature regarding the nature of vanadium(IV) catechol complexes -- both octahedral tris and square-pyramidal bis complexes exist. The failure of the numerous potentiometric investigations to find evidence for this complex formation was apparently because none of the investigators recognized that, for the equilibrium involved:

$$VO(cat)_2^{2-} + catH_2 = V(cat)_3^{2-} + H_2O$$

no protons are consumed or liberated, and thus tris complex formation would not be apparent in a potentiometric experiment. It *would*, however, be apparent in a spectrophotometric experiment, which accounts for the discrepancy in the literature (ref. 55) between the two sets of results. In basic solution the equilibrium

$$catH_2 + OH^- = catH^- + H_2O$$

becomes significant (pK_1 for catechol is 9.20, pK_2 is 11.93) which leads to the overall reaction:

$$VO(cat)_2^{2-} + catH^- = V(cat)_3^{2-} + OH^-$$

Thus, in contrast to the usual case in catechol complex formation, at high pH mass action tends to force the equilibrium to *lower* catechol/vanadium ratios (to the left in the above equation), which explains the facile synthesis of vanadyl bis(catecholate) in strongly basic solution.

The air oxidation of many of these catechol vanadium complexes is slow, and in the case of $[V(cat)_3]^{2-}$ yields initially a blue product ($[V(cat)_3]^-$), which is very similar in appearance to the blue-violet starting material. For this reason the air sensitivity of this system was not always appreciated, and hence many of the results in the older literature must be viewed with suspicion.

The structure determination of the $[Et_3NH]_2[V(cat)_3 \cdot CH_3CN$ established the existence of the tris(catecholato)vanadate(IV) complex in the solid state and this complex retains its structure in CH_3CN solution, as substantiated by ESR and electrochemical measurements at room temperature. However, an EPR spectrum was not observed for aqueous solutions of this complex, although the reversible redox behavior was consistent with the complex remaining intact. Since the triethylammonium cations apparently form a neutral tight ion pair with the $[V(cat)_3]^{2-}$ complex in CH_3CN (ref. 55) (through hydrogen bonding to the octahedral oxygens), ion dissociation in aqueous solution could lead to significant differences in the behavior of the aqueous complex. Recently this problem has been reexamined in an attempt to reach a more definitive conclusion about the formation and nature of the tris(catecholato)vanadate(IV) species in aqueous solution (ref. 56). The results confirmed that complexes with 1:1, 2:1, and 3:1 ligand-to-metal ratios are formed in the $V^{IV}O$-catechol system in aqueous solution. The first two species involve the coordination of one or two bidentate catecholates, respectively, to vanadium, which achieves the square-pyramidal geometry typical of the $V^{IV}O$ complexes. All the spectroscopic evidence points to the conclusion that the 3:1 species is a V(IV) complex, consistent with the previous interpretation (ref. 55). The conversion from a vanadyl to pseudo-octahedral complex results in a profound change in the EPR spectra, as shown in Figure 9 (low temperature data summarized in Table 2). In aqueous solution, where hydrogen bonding of triethylammonium ions to the phenolate oxygens is unimportant, the species retains the same geometry as in the solid state. Whatever the solvent (water, CH_3CN, or DMSO) or the counterion (Na^+, Tl^+, or Et_3NH^+), the tris(catecholato) species gives the same ESR spectra, which supports a d_{z^2} ground state. In all the known cases of d_{z^2} ground state for V(IV), the coordination geometry is distorted toward a trigonal-prismatic geometry (ref. 57). The catecholate ligand is notable in stabilizing distorted-trigonal-prismatic geometries in the trischelated complexes, the extreme example being a recently detected perfectly trigonal-prismatic ferric complex

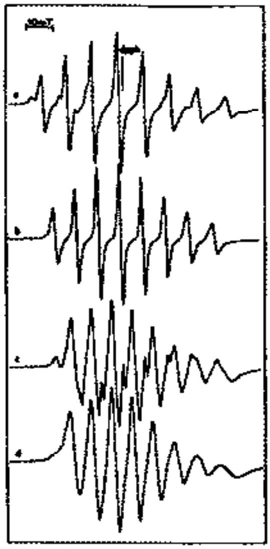


Fig. 9. Room temperature ESR spectra of aqueous solutions of VO^{2+} and catechol as a function of total metal concentration, ligand-to-metal molar ratio (L:M) and pH: 4 x 10^{2+}, L:M = 1, pH 4.0 (a) 4 x 10^{-3} M VO^{2+}, L:M = 2, pH 7.5 (b); 4 x 10^{-3} M VO^{2+}, L:M = 100, pH 7.0 (c); 2.4 x 10^{-2} M VO^{2+}, L:M = 100, pH 7.2 (d), from reference 56.

with a macrocyclic catechol ligand (refs. 49, 50). This property is the result of constraints imposed by the short ligand bite (ratio of the O-O ring distance to the M-O distance). The metal geometry is severely trigonally distorted in $[Et_3NH]_2[V(cat)_3]\cdot CH_3CN$ (refs. 49, 50), the twist angle between the triangular faces of the coordination polyhedron being ≈ 39°. This angle is expected to be 60° for an ideal octahedron and 0° for a trigonal prism. Crystal field diagrams, calculated by the method of Companion and Komarynsky (ref. 58), show that for the 39° twist angle the d_{z^2} orbital is lowest by 0.67 Dq. Hence, the change of the ground state from d_{xy} in mono- and bis(catecholate)oxovanadium(IV) complexes to largely d_{z^2} in tris(catecholato)vanadate(IV), as substantiated by the ESR data, is fully consistent with the solid-state structure. The trigonal-prismatic distortion imposed by the tris chelation has a dramatic effect on the ordering of the vanadium electronic levels, producing a change of the ground state from d_{xy} (typical of $V^{IV}O$ square-pyramidal complexes) to d_{z^2}.

TABLE 2

ESR parameters of vanadium catechol complexes.[a]

complex	g_0	A_0	g_z	A_z	$g_{x,y}$	$A_{x,y}$
[VO(cat)]	1.968	96	1.943	170	1.976	63
$[VO(cat)_2]^{2-}$	1.975	82	1.947	154	1.981	50
$[V(cat)_3]^{2-}$	1.955	76	1.991	14	1.937	107

[a]ESR measurements at ambient temperature and at 100 K; A values in 10^{-4}, from ref. 56.

While the binding of tunichrome b-1 to vanadium has not been structurally characterized, the presence of three pyragallol moieties (Figure 3) suggests the possibility of a tris-"catechol" mode of bonding. This type of bonding has been suggested in a recent communication that describes the synthesis of vanadium complexes of N,N',N"-tris(2,3,4-trihydroxybenzoyl)-1,5,10-triazadecane (ref. 59). However, the first structure of a vanadium-pyrogallol complex determined by X-ray diffraction shows it to be a pyrogallol-bridged dimer (ref. 60).

The synthesis of two isomeric tris(trihydroxyphenyl) derivatives containing the β,β',β"-triaminotriethylamine (TREN) backbone (Figure 3) has recently been reported (ref. 28). The vanadium(IV) and (III) complexes of these ligand complexes provide models for the possible hexadentate binding of tunichrome b-1 to vanadium in the polymeric solid complex. [Following the convention of the original paper (ref. 28), when the degree of ligand protonation needs to be explicit, it will be denoted by putting the number of protons at the end of the ligand name. Thus, the TREN catechol derivative, with six dissociable protons, as the neutral molecule is $TRENCAMH_6$. For the pyrogallol derivative, with nine dissociable protons, the neutral ligand is $TRENPAMH_9$, and the tris(catechol)vanadium(IV) complex is $[V(TRENPAMH_3)]^{2-}$.]

The visible spectra of the vanadium(IV) complexes $[V(TRENCAM)]^{2-}$ and $[V(2,3,4\text{-}TRENPAM)]^{2-}$ in H_2O both contain two overlapping absorbances between 550 and 700 nm with $\varepsilon = 7000$, as well as a higher energy absorption near 440 nm with $\varepsilon \sim 5000$ (ref. 26).

Consistent with the assignments for the $[V(cat)_3]^{2-}$ (cat = catechol dianion) complex (ref. 55) these absorptions were assigned as ligand-to-metal charge-transfer bands (ref. 28). The VIS-UV spectrum of the vanadium(III) complex $[V(TRENCAM)]^{3-}$ lacks these intense ligand-to-metal charge-transfer bands, which is ascribed to the relatively low reduction potential of the V(III) metal center. It is instead dominated by the ligand-based π-π* absorption at 330 nm and d-d absorptions between 550 and 700 nm. Similarly, there is a dramatic difference between the two vanadium(IV) complexes $V(cat)_3{}^{2-}$ and $VO(cat)_2{}^{2-}$, since the latter shows only relatively weak d-d transitions (ref. 55). In the vanadium-tunichrome preparations of Oltz et al. (ref. 61), the EPR spectrum at 77 K was reported to have A = 162.9, A = 59.7 (10^{-4} cm^{-1}), and g = 1.990. The VIS-UV spectrum showed a maximum at 647 nm. Although not noted in that paper, these results are very similar to the vanadyl-bis(catecholate) complexes (for several catechol ligands) $VO(cat)_2{}^{2-}$, for which $A_{||} \approx 160$, $A_{\perp} \approx 50$ (10^{-4} cm^{-1}), $g_{||} \approx 1.95$, $g_{\perp} \approx 1.99$, and $\lambda_{max} = 656$ (ref. 55). The parameters for the $V(cat)_3{}^{2-}$ complexes are very different, with (in CH_3CN) $A_{|} \approx 15$, $A_{\perp} \approx 100$ (10^{-4} cm^{-1}), $g_{||} \approx 1.99$, $g_{\perp} \approx 1.94$; and $\lambda_{max} \approx 552$ ($\varepsilon = 9200$) (Table 2). This strongly indicates the predominance of a vanadyl-bis(catecholate) mode of coordination of the vanadium tunichrome (ref. 61) *for the conditions used in that preparation*, although the strong background absorbance in the VIS-UV must be from another chromophore. To summarize, the bis(catechol)vanadium(IV) complexes are very strongly absorbing in EPR spectra and weakly absorbing in the VIS-UV. For the tris complexes just the opposite is true.

A view of the trianion $[V(TRENCAM)]^{3-}$ is shown in Figure 10. The complex lies on a three-fold axis in space group P2,3. As can been seen from the figure, the six catechol oxygens form a

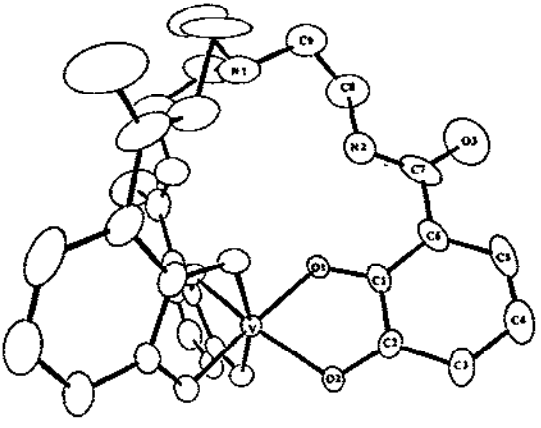


Fig. 10. Molecular structure of the complex anion in $K_3[V(TRENCAM)]\cdot 4CH_3OH\cdot 2H_2O$ as observed down the 3-fold axis.

distorted octahedral environment around vanadium. The twist angle of the top trigonal face relative to the bottom is 40.8 (6)° versus 60° for an octahedron. The approximately 5° decrease in the twist angle of $[V(TRENCAM)]^{3-}$ relative to $[V(cat)_3]^{3-}$ is significant and is consistent with steric constraints imposed on the catechol rings by hydrogen bonding between the catechol oxygen and the amine hydrogen of the TREN backbone. This is illustrated by the complex ferric bicapped-TRENCAM (ref. 50), in which the two TREN ligands occupy two capping positions above and below the catechol rings and induce a twist angle of zero.

The V-O1 and V-O2 distances of 1.985(5) and 2.007(5)Å, respectively, are not significantly different from the V-O distances found in $K_3[V(cat)_3]$ (ref. 55). The vanadium in $[V(TRENCAM)]^{3-}$ is slightly displaced from the plane of the catechol ring; the dihedral angle between the catechol plane and the plane of the vanadium and the two ligating oxygens is 14°. As with the only other tris(catecholamide) metal complex structurally characterized, ferric bicapped-TRENCAM, the tertiary nitrogen of the TREN backbone adopts the "in" conformation. The aqueous redox behavior of $[V(TRENCAM)]^{2-}$ has a reduction wave at E_f = -0.37 V vs. NHE that is quasi-reversible, with a peak separation of 125 mV (20-200 mV s^{-1}) and a ratio of cathodic to anodic peak currents of 1.2 (ref. 55). These and other data were consistent with a quasi-reversible reduction process. Cyclic voltammetric behavior $V(TRENCAM)^{2-/3-}$ in DMF at a platinum disk electrode showed quasi-reversible waves at E_f = -0.544 and +0.531 V vs. NHE, corresponding to the V(IV/III) and V(V/IV) couples, respectively (ref. 28).

For $[V(2,3,4\text{-}TRENPAM)]^{2-}$ (7) in aqueous solution a wave at E_f = -0.67 V vs. NHE (20-200 mV s^{-1}) was found with a peak separation of 65 mV and a ratio of peak cathodic to anodic currents of nearly 1.0, again consistent with a one-electron, Nernstian process (ref. 28). Above pH 7.0 the cyclic voltammograms of $[V(TRENPAM)]^{2-}$ exhibit a formal potential that becomes more negative with an increasing basicity of the solution. This was attributed to the increasing negative charge that develops on the complex (Figure 3) as the hydroxyl groups in the 4-position becomes fully deprotonated, thereby making reduction of $[V(2,3,4\text{-}TRENPAMH_3)]^{2-}$ more difficult. This behavior is in contrast to that observed for $[V(TRENPAM)]^{2-}$, for which E_f shows negligible pH dependence from pH 5 to 12. For the $[V(2,3,4\text{-}TRENPAMH_3)]^{2-}$ complex, the first acid dissociation constant complex is determined as $K_1 = 3.3 \times 10^{-8}$ M (ref. 28).

These results indicate that tunichrome b-1 complexes of vanadium(III/IV) would show similar variation in their redox couples at high pH. However, the relevant pH values for the biochemistry of vanadium tunichrome range from neutral to very acidic. At neutral pH in the presence of excess pyrogallol groups, vanadium(IV) can be expected to form the intensely colored tris(catechol) species. However, comparison of the EPR properties reported for vanadium-tunichrome preparations (ref. 61) with model vanadium(IV)-catechol complexes would indicate predominantly bis(catechol)vanadyl coordination. In any case, the vanadium(III) complexes must remain very highly reducing. The standard potential of pyrogallol is +0.79 V (ref. 62) and decreases 60 mV per pH unit (up to about pH 9) so that at pH 7 the potential is ~ 0.37 V. The potentials of the vanadium(IV/III) couples reported (ref. 28) for the tunichrome analogs are ~ -0.4 V. It is concluded that excess pyrogallol ligand is barely able to reduce the vanadium(V)-pyrogallol complex at neutral pH (and it becomes progressively more difficult as the pH decreases) and is certainly incapable of reducing the vanadium(IV) complex, although this has been suggested (refs. 31, 32). The highly reducing vanadium(III) complex of tunichrome must be generated in some other way. If the role of the vanadium in the tunichromes is to act as a catalyst in the cross-linking of the animals' tunic, then the oxygen sensitivity of the vanadium(III)-catechol complexes may be the essential feature of these compounds.

IV. NON-CATECHOL LIGANDS IN SIDEROPHORES OR ANALOGS

The anion of hydroxypyridonate (Figure 4) is isoelectronic with the catecholate dianion. For the isomer shown (1,2-Hopo) one hydroxyl is bound to the nitrogen. In the other two isomers (3,2-Hopo and 3,4-Hopo) the nitrogen is one or two carbon atoms away, respectively, from the nearest phenolate oxygen. An antibiotic siderophore isolated from bacteria in the *Pseudomonas* family is a 1,2-Hopo, 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone (refs. 63, 64). *In vitro*, this compound and its copper and ferric complexes are reported to show moderate activity against Gram-positive bacteria, fungi and *Trichomonas vaginali* (ref. 63). Topical application protected guinea pigs against infection with *Microsporum canis*. However, some systemic toxicity in mice was observed. Other examples of hydroxypyridonate siderophores are also shown (refs. 34, 35, 65).

The effectiveness at low pH of tricatechol enterobactin analogs is limited by their weak acidity and loss of six protons on bonding iron(III). The hydroxypyridonates and hydroxamates are stronger, monoprotic acids. These ligands need only lose three protons to form six-coordinate bidentate complexes, which makes them competitive for iron at pH 7 with similar catecholate ligands, despite the much lower formation constants of the iron complexes. Indeed, the simple bidentate hydroxypyridinones 1,2-Hopo, 3,2-Hopo, and 3,4-Hopo (where "x,y-Hopo" is x-hydroxy-y(1H)-pyridinone) bind iron more completely than either catechol or acetohydroxamic acid under standard conditions at pH 7 (ref. 39). Although hydroxypyridinones have been shown to bind effectively to divalent transition metals, the selectivity of bidentate hydroxypyridinones toward iron(III) over that toward divalent metals such as copper and zinc is comparable to that of catechol and better than that of hydroxamates.

Bidentate hydroxypyridinones are promising agents for therapeutic removal of iron(III). The kinetic ability of 1,2-Hopo and 3,4-Hopo derivatives to remove iron from transferrin has been reported by Kontoghiorghes (refs. 68, 69). We have presented quantitative kinetic data which show that mimosine (a natural product amino acid derivative of 3,4-Hopo) and 3,4-Hopo remove iron *in vitro* from human iron transferrin at rates similar to those for tricatecholate ligands at concentrations as low as 2×10^{-4} M (ref. 70). Some new synthetic hydroxypyridonate ligands show promising *in vivo* removal of plutonium in test animals (refs. 71, 72).

The synthetic procedure shown in Figure 11 provides a convenient route to these sometimes difficult to prepare compounds. The amidation of 2,5-dimethoxytetrahydrofuran-2-carboxylic acid methyl ester by primary alkylamines followed by acid-catalyzed rearrangements yields N-alkyl-3-hydroxy-2-pyridinones (ref. 73). The rearrangement reaction is promoted by the presence of metal ions, such as Fe^{3+}, Cu^{2+}, and Zn^{2+}, which have high affinities for the bidentate hydroxypyridinone binding group. The structures of N-butyl-3-hydroxy-2-pyridinone (HL') and its 3:1 complex with iron(III) ($FeL'_3 \cdot 3H_2O$) were determined by X-ray crystallography. Bond lengths involving the pyridine ring indicate that a catechol-like aromatic resonance form is significant particularly in the iron complex (ref. 73). The visible spectra for $[FeL'_n]^{(3-n)+}$ (n = 1 - 3) are virtually identical with those of previously characterized complexes of iron(III) and 3-hydroxy-2(1H)-pyridinone (3,2-Hopo, HL) (ref. 39). The log K_a (protonation constant) of HL' and log K_3 for formation of FeL'_3 (from $[FeL'_2]^+$ and $[L']^-$) are higher by 0.5 log unit than those of the unsubstituted HL and FeL_3 (ref. 73).

As noted in the introduction, while hydroxypyridinethione ligands (Figure 4, (e)) are closely related to either thiohydroxamate or hydroxypyridonate ligands (both of which are found in siderophores) there are no natural products that are hydroxypyridinethiones.

Recently hexadentate thiohydroxamate ligands derived from 2-mercaptopyridine-1-oxide-6-carboxylic acid and triamines have been prepared by reaction of the amines with the active amide produced from the carboxylic acid and 1,1'-carbonyldiimidazole in DMF (Figure 12) (ref. 74). The structure (Figure 13) of the complex N,N',N''-tris(1-oxo-2(1H)-pyridinethione-6-carboxyl)-2,2',2''-triaminotriethylamine iron(III) 0.5 $CHCl_3$ shows that the coordination polyhedron around ferric ion is intermediate between octahedral and trigonal prismatic geometries, and is similar to that of the ferric complex of 1-hydroxy-2(1H)-pyridinone (ref. 74). Although detailed thermodynamic data are not yet available, the complex appears to be extremely stable.

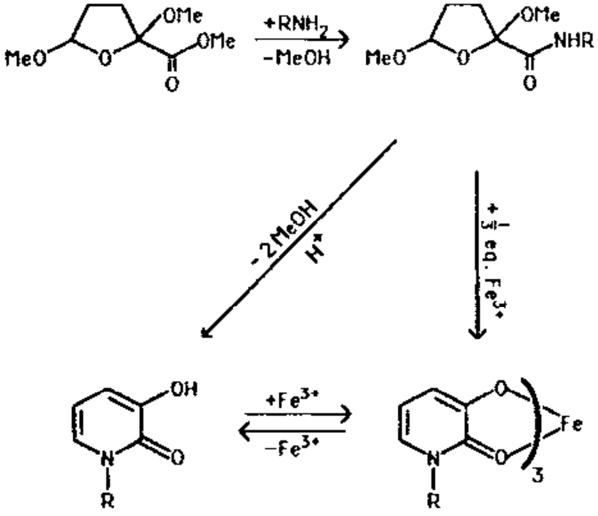


Fig. 11. A synthesis scheme showing the preparation of N-substituted 1,2-dihydroxypyridinone from 2,5-dimethoxytetrahydrofuran, from reference 73.

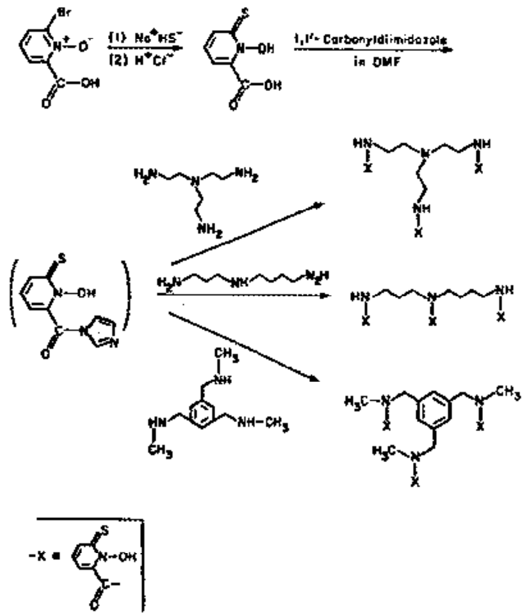


Fig. 12. A synthesis scheme showing the preparation of hexadentate hydroxypyridinethione ligands, from reference 74.

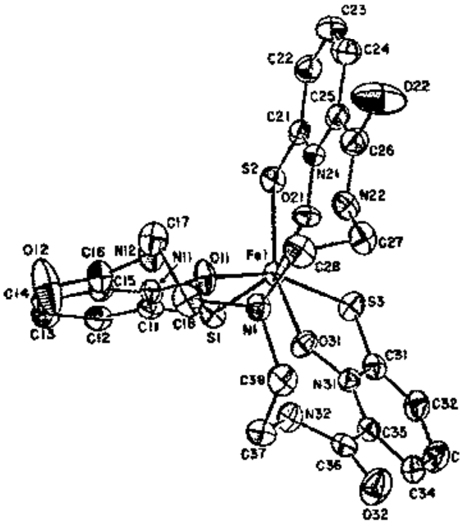


Fig. 13. A perspective view of the hydroxypyridinethione iron complex with the number scheme of the non-hydrogen atoms. The non-hydrogen atoms are drawn at 50% probability contours of the thermal motion. Hydrogen atoms are not included for simplicity.

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It is a pleasure to dedicate this paper to Professor Fred Basolo on the occasion of his 70th birthday. I am grateful that I was a Ph.D. student of his. I also thank my several past and present coworkers whose work is represented here (identified in the citations).

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