METAL BINDING BY THE TRISOXAZOLE PORTION OF THE MARINE NATURAL PRODUCT DIHYDROHALICHONDRAMIDE#

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Abstract-Metal binding to the oxazole portion of dihydrohalichondramide (**1**), trisoxazole (**2**), and diphenyloxazole (**3**) are studied by fluorescence quenching and nmr techniques. The trisoxazole conformation is modeled by semiempirical computations. **1-3** are found to be relatively weak binders which do not exhibit a significant chelate effect. The biological action of **1** is not related to metal binding.

Dihydrohalichondramide (**1**) is a trisoxazole-containing metabolite of the sponge *Halichondria* sp. that is concentrated by the nudibranch *Hexabranchus sanguineus* for use as a chemical deterrent against predation.[1] The relative stereochemistry of **1** and related compounds has not been determined. Molecular models of the macrocyclic ring system suggest that metal ions might bind to the oxazole units of the macrocyclic ring. In this vein, we have surveyed the metal binding properties of **1**, the trisoxazole (**2**),[2] and 2,5-diphenyloxazole (**3**).

Heterocycles, such as oligopyridines,[3] -imidazoles,[4] and -thiazoles[4,5] are common accessories to the field of metal-coordination chemistry. Few oxazole metal complexes have been synthesized[6] and studied,[7] however, and little structural or stability constant data are available.

1,**2**, and **3**,[8] show strong fluorescence emissions around 340-360 nm. In the presence of metals such as Ag^+, Cu^{++}, Fe^{++}, Hg^{++}, or Pb^{++} this fluorescence is quenched. Following the quenching of fluorescence as a function of ligand and metal concentrations yields plots that fit well to a 1:1 metal-ligand binding isotherm.[9] From these data, stability constants for **1**, **2**, and **3** binding to various metals are derived (Table 1).[10]

Addition of silver triflate to a solution of **1** in methanol (2.5×10^{-2} M) manifests shifts in the region of the ^1H nmr spectrum assigned to the oxazole and olefinic protons. The shifts are most noticeable for the oxazole protons and the olefinic protons nearest the trisoxazole portion of the molecule (Figure 1). This suggests that the site of binding is at the trisoxazole subunit. Although the arm of **1** is conformationally mobile and can reach over the trisoxazole region of the molecule, there is no evidence from the nmr data to indicate that the arm participates in binding. Rough titration data give a binding constant, for the 1:1 binding of **1** and Ag^+, of the same order of magnitude as that derived from fluorescence measurements.

Dedicated to Ted Taylor on the occasion of his 70th birthday.

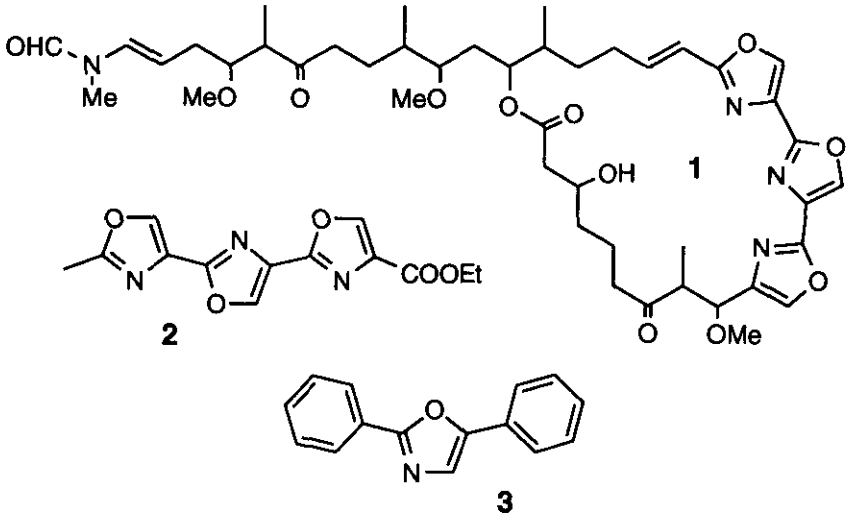


Table 1. Binding Constant Data for **1-3** with Various Metals.

Metal Salt	-pK$_b$[a]		
	1	**2**	**3**
$AgCF_3SO_3$	4.8	3.8	3.0
$Cu(CH_3COO)_2$	3.5	3.7	3.3
$FeSO_4$	3.3	3.3	3.5
$Hg(CH_3COO)_2$	3.8	2.8	3.7
$Pb(CH_3COO)_2$	2.0	2.3	3.7

a) average over several runs in methanol; metal salt added to the oxazole (0.05 mM).

Oxazole ligands(**1-3**)show relatively weak binding in comparison to their azo and thio cognates. This is consistent with proton affinity and ionization potential data. Relative to oxazole, the proton affinities of imidazole, pyridine, and thiazole are 60 kJ/mol, 54 kJ/mol, and 23 kJ/mol, respectively.[11] The first ionization potential of oxazole (10.2 eV) is greater than that of imidazole (8.78 eV) and thiazole (9.5 eV).[11] Both factors demonstrate the poor character of oxazole as a Lewis base.

Among **1-3**, there is a remarkable similarity in binding constants for a given metal. In general, binding constants range between 10^2 and 10^4 for all systems studied. This appears peculiar when compared to the "chelate effect"[12] seen in terpyridine complexes. A deeper look at the trisoxazole structure and conformational energies is illuminating.

Semiempirical calculations on **4** using the AM1 and PM3 Hamiltonians[13,14] predict four different minimum-energy conformers (Figure 2). **I,** with a bay region composed of C, N, and O, is the lowest-energy conformer. The chelating conformer (**IV**) is ca. 13 kJ/mol (AM1) or 18 kJ/mol (PM3) higher in energy. The chelate angle in **IV** is ca. 30° as compared to ca. 60° in terpyridine. Thus, the nitrogen lone-pair vectors converge further out in

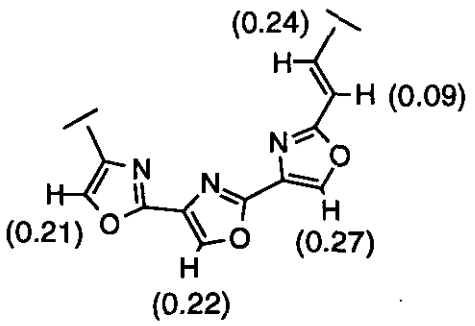
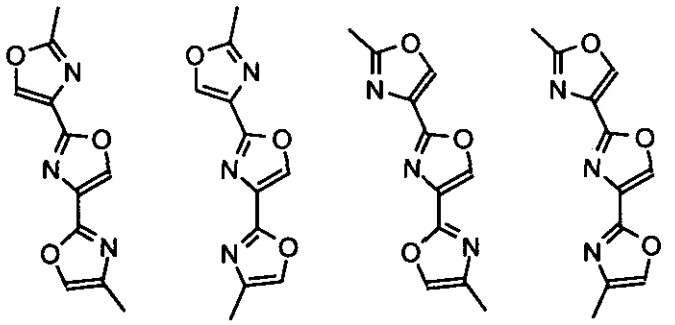


Figure 1. Nmr shift differences ($\Delta\delta$, ppm) after the addition of ca. 1 eq. of $AgCF_3SO_3$ to **1** in $CDCl_3$.



	I	II	III	IV
AM1	0.0 kJ/mol	4.9 kJ/mol	6.5 kJ/mol	12.8 kJ/mol
PM3	0.0 kJ/mol	7.4 kJ/mol	8.7 kJ/mol	18.2 kJ/mol

Figure 2: Relative conformational energies of **4** from semiempirical methods using AM1 and PM3.

IV than in terpyridine. Indeed, a metal atom sitting at a normal bond distance from the central nitrogen sits outside of bonding range from the other two nitrogens in **IV**.

The binding energies found for **3** are on the order of the energy cost required for **4** to adopt the chelating conformation **IV**. In addition, the poor chelate angle and electron-withdrawing substituent on **2** make it unlikely that the second and third nitrogens provide additional binding energies comparable to the first. These competing effects account for the relatively small difference in pK_b seen between **3** and **2** (*cf.* Table 1). Lack of a significant increase in binding to **1** may be the result of steric hindrance to the binding site by substituents on the ring or a non-chelating low energy conformation for the trisoxazole subunit.[15]

Investigations of the antifungal properties of **1**, **2**, and **3** have shown that only **1** is an active antifungal agent. Metal binding in **1** and **2** is similar, however. Therefore, we conclude that direct metal binding is not important in the mechanism of action of **1** as an antifungal.

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15 The present analysis assumes that the dominant interaction is with the nitrogen of the oxazole; other interpretations may arise if binding at oxygen is dominant. Calculations using both the PM3 and the AM1 Hamiltonian show that the HOMO for **4 I-IV** is mostly π in nature. These calculations predict that the first orbitals with appreciable "lone pair" character have energies ca. 1 eV (100 kJ/mol) below the HOMO. This aids in a rationalization of the lack binding affinity of **1-3**.

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