

Unusual chelates isolated from Ascidiacea

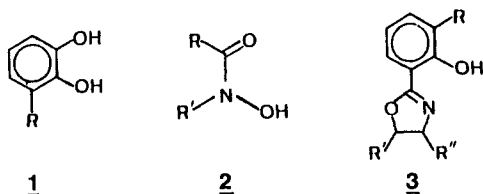
Clifford J. Hawkins

Department of Chemistry, University of Queensland, St Lucia,
Australia 4067

Abstract - Molecules with donor groups capable of strong metal binding have been extracted from a range of marine organisms including the Ascidiacea. These range from small molecular weight bidentate and multidentate chelates to proteins containing the catechol side groups. The range of structures is reviewed and some properties of the metal complexes formed by ascidian compounds are presented.

INTRODUCTION

Nature is an extremely imaginative coordination chemist, synthesizing small molecular weight chelates with most interesting combinations of donor groups, as well as large biopolymers with intricate structures that contort to produce an array of donors tailored to encapsulate specific metals. The siderophores are some of the best known families of strong small chelates. They have been designed by Nature to sequester iron(III) for microorganisms (bacteria, fungi, and microalgae) and utilize chelators such as catechols (1) (e.g. enterobactin), hydroxamic acids (2) (e.g. ferrichrome), and 2-hydroxyphenyl-2'-oxazoline (3) (e.g. mycobactin).¹

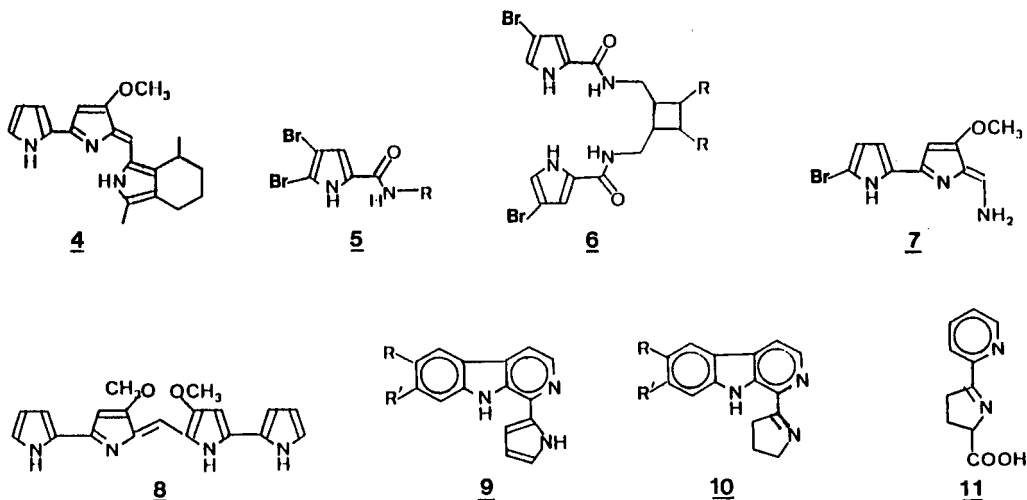


In the marine kingdom, a broad range of ligating groups are found in compounds from most phyla, including the phylum Chordata, to which the Ascidiacea belong. These latter organisms are filter feeding benthic marine invertebrates that have the distinction that some species concentrate vanadium in their blood cells to levels in excess of 1% dry weight. In relating structures of isolated chelates to phylogeny, some caution is needed because algal symbionts are frequently found in association with marine organisms, and the compounds of interest may originate from the algal cell rather than the host. Indeed for some aplousobranch ascidians, it has been shown in our laboratory that the predominant chelates extracted have come from the Prochloron algal symbiont, but interestingly the structures of these compounds have been dependent on the host.

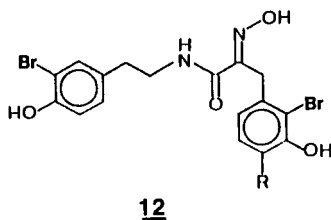
CHELATES FROM MARINE ORGANISMS

The pyrrole group and related nitrogen heterocycles are frequently found, sometimes in association with the peptide group: for example, prodigiosin (4) from the marine bacteria, Alteromonas rubra and Beneckea gazogenes.²

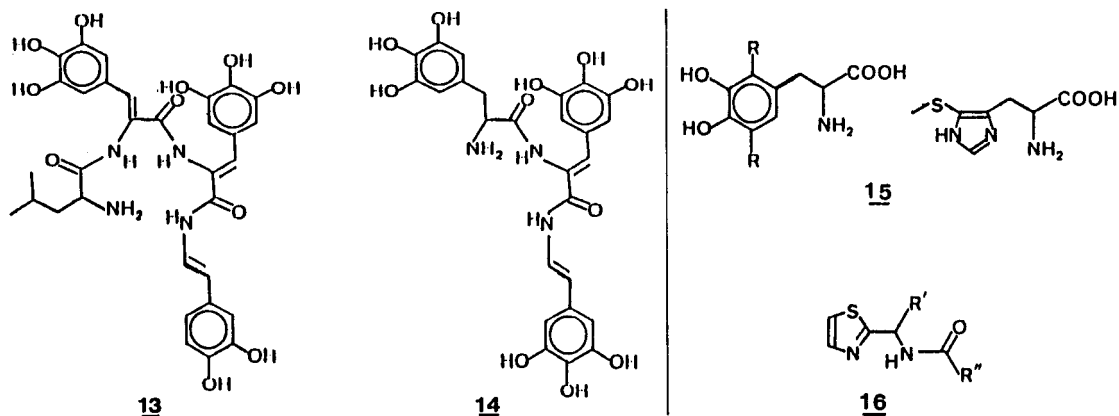
roidin (5) and sceptrin (6) from *Agelas* species of sponge;³ tambjamine B (7) from a *Tambje* nudibranch;⁴ a tetrapyrrole (8) from the aplousobranch ascidian, *Sigillina cyanea*;⁵ and the eudistomins such as structures (9) and (10) from the aplousobranch ascidian, *Eudistoma olivaceum*.⁶ The latter eudistomin has the same coordinating groups as the iron(II) chelator pyrimine(II) isolated from a *Pseudomonas* sp.⁷



The oxime group occurs in the bastadins (12) from the sponge, *Ianthella basta*.⁸

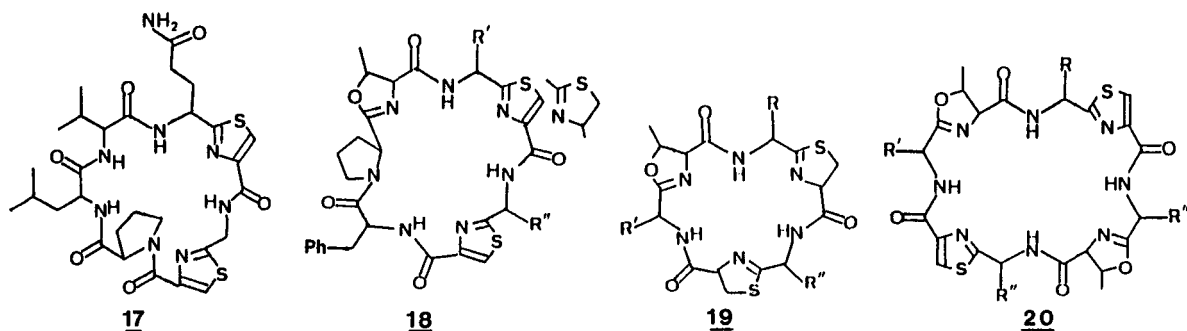


The peptides and 3,4,5-trihydroxyphenyl groups are both found in celenamide D (13) from the sponge, *Cliona celata*,⁹ and in tunichrome B1 (14) from the phlebobranch ascidian, *Ascidia nigra*.¹⁰ Celenamide D also has the catechol group.



The adenochromines such as compound A (15) from the cephalopod, *Octopus vulgaris*, has a particularly interesting array of donor groups with catechol, thioether, imidazole, and amino acid groupings.¹¹

The thiazole ring is found in the dysidenins (16) from the sponge, *Dysidea herbacea*,¹² and in a range of azacrown cyclic compounds (17-20). There is still some uncertainty about the structure of dolastatin-3 (17) isolated from the sea hare *Dolabella auricularia*,¹³ but its structure is closely related to those of other 21-azacrown-6 compounds, ulicyclamide and lissoclinamides (18), some of which have thiazoline rings, from the aplousobranch ascidian, *Lissoclinum patella*.¹⁴ The oxazoline ring in ulicyclamide and the lissoclinamides is also found with thiazole or thiazoline rings in 18-azacrown-6 and 24-azacrown-8 macrocycles, the bistratamides (19) and patellamides (20), from *Lissoclinum bistratum*¹⁵ and *L. patella*,^{14,16,17} respectively. Patellamide A has one oxazoline ring formed from serine rather than threonine.



COPPER (II) PATELLAMIDE

For most of the above chelates their roles and the importance of metals in these roles are not known. The coordination of the patellamides and bistratamides is being studied in our laboratory.¹⁸ Although molecular models suggest two metals can be bound concurrently by two sets of four donor groups within the 24-azacrown-8 macrocycle, the evidence to date supports the coordination of only one metal ion. For example, when copper(II) is added to a methanolic solution of patellamide D ($R, R'' = \text{CH}_2\text{CH}_2$; $R', R''' = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$; $R'' = \text{CH}_3$)¹⁸ the circular dichroism (CD) parameter ϵ (based on the concentration of patellamide D) increased until 1.5 equivalents of copper (II) have been added and then remains unaltered.¹⁸ The CD spectrum has a negative Cotton effect at 687nm with a negative shoulder at 580nm, whereas patellamide E (also called ascidiacyclamide: $R, R'' = \text{CH}(\text{CH}_3)_2$; $R', R''' = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$)¹⁶ gives a positive band at 675nm. In assessing the CD spectra it is important to recall that the thiazole amino acids have the D configuration while the oxazoline amino acids are L. For copper(II) complexes of tripeptides with a side group in the amino terminal position, the L-phenylalanyl peptide has a positive Cotton effect at about 560nm, opposite in sign to other peptides studied with L-amino acid residues including Gly-L-Phe-Gly and Gly-Gly-L-Phe except for L-Pro-Gly-Gly which also gives a positive Cotton effect.¹⁹ Molecular models suggest that the most likely quadridentate chelation involves the two peptide nitrogens adjacent to one of the thiazole rings, that thiazole's nitrogen, and the other peptide nitrogen that lies between an oxazoline and a thiazole ring. In this planar coordination, the second thiazole lies above the plane near one of the apical coordination sites.

PROTEINS

The Ascidiacea have three sub-orders that differ in their metal chemistry.²⁰ The phlebobranch ascidians other than those from the morphologically advanced Corellidea family concentrate vanadium(III) in their blood cells in very large amounts with less than 5% of the vanadium present as vanadium(IV). In the sub-order, Aplousobranchia, the morphologically more primitive genera have high concentrations of vanadium in their blood cells with a significant percentage in oxidation state IV. The vanadium(III) complex involves a prosthetic group and a protein.²¹ In other genera, vanadium is either not found in every species, or is absent in all species. In the third sub-order, the Stolidobranchia, no vanadium is stored in the blood cells. Throughout all these sub-orders iron is present in the cells.

An iron protein has been extracted from the blood cells of the Stolidobranch, Pyura stolonifera. Its apo-protein, called ferreascidin, has been characterized to have a molecular weight of 10000da with by far the highest percentage of aromatic amino acids that has been found in a natural protein: 42% tyrosine, 17% 3,4-dihydroxyphenylalanine, 8.5% phenylalanine, and 7.5% histidine.²² The iron(III) complex of ferreascidin has not been fully characterized. With one equivalent of iron it has an absorption spectrum with a maximum at 540nm. With two equivalents, maxima occur at 570nm and 388nm, cf bis(catecholato)iron(III) 570nm and 382nm.²³ The visible charge-transfer band moves with pH: for FaFe below pH4.5 it occurs at about 650nm; between pH4.5 and 6.5 at 570nm; and above about pH6.5 at 540nm. For FaFe₂ the changes occur at higher pH. The first two steps approximate those for [Fe(cat)]⁺ and [Fe(cat)₂]⁻.²³ The change at pH6.5 - 7.0 possibly results from the deprotonation and coordination of a histidyl imidazole.

The blue FaFe₂ has a room temperature zero-field Mössbauer spectrum with a quadrupole doublet with δ 0.38 ± 0.01 and ΔE_Q 0.675 ± 0.01 mm s⁻¹ (v's Fe)²⁴. This is typical of iron(III)-phenolate complexes such as [FeEHPG]- δ 0.37, ΔE_Q 0.77 mm s⁻¹ (EHPG, ethylenebis[(o-hydroxyphenyl)glycine]), and Fe₂Tr δ 0.38, ΔE_Q 0.75 mm s⁻¹ (Tr, transferrin).²⁵

The blood cells that contain the ferreascidin also contain a small molecular weight pigment with a strong UV absorption at 273nm. In the presence of this pigment the FaFe and FaFe₂ complexes absorb at 502nm²⁴ close to the maximum for [Fe(cat)₃]³⁻ which occurs at 490nm.²³

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