species, which may be present as impurities in Mn(III) preparations, can also give rise to EPR features at low field. Since $S = 2$ Mn(III) signals are quite weak if present at all, a small amount of an Mn(II) impurity can easily interfere with the identification of an Mn(III) signal. The two species can be distinguished, however, because the $S = {}^5/_2$ system will in general produce a resonance near $g = 2$ in addition to any other features at low field. In preliminary preparations of the monomeric Mn(III) complexes described above, features near $g = 2$ were observed in addition to the low-field signals. Upon further purification, the amplitudes of the $g = 2$ signals decreased markedly relative to the low-field features. This result, together with the consistency of the field positions and line shapes of the low-field features with an $S = 2$ spin Hamiltonian, provides additional confirmation of the assignment of the signals to Mn(III). In the case of the binuclear complex, a small amount of an Mn(III,IV) impurity produced a multiline signal centered near $g = 2$ that also decreased in amplitude relative to the low-field features upon further purification. In light of these considerations, we call into question the validity of recent reports of the assignment of $g = 2$ EPR signals to trivalent manganese species.[14]

In conclusion, we have observed and interpreted X-band EPR spectra of complexes of trivalent manganese. This work provides examples of spectral features observable in the $S = 2$ state of Mn(III) complexes and demonstrates the utility of X-band EPR for studying these systems. Studies of a similar nature on biological manganese centers and related inorganic model complexes are in progress.

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Ptilomycalin A: A Novel Polycyclic Guanidine Alkaloid of Marine Origin

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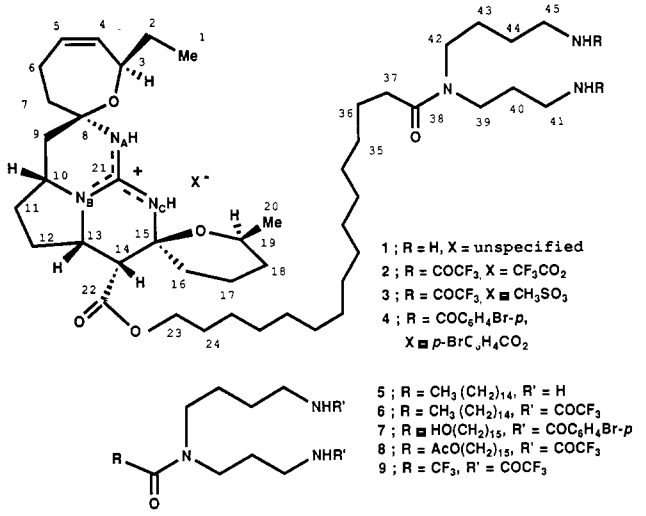
In the course of screening for novel bioactive agents from marine sponges, we have isolated from the Caribbean sponge *Ptilocaulis spiculifer*[1] and from a red *Hemimycale* sp. of the Red Sea the same antitumor, antiviral, and antifungal compound designated ptilomycalin A (**1**),[2] which possesses a unique polycyclic guanidine structure.

The molecular formula, $C_{49}H_{78}N_6O_7F_6$, of the bis(trifluoroacetyl) derivative **2**, an oil, $[\alpha]_D$ −15.8° (*c* 0.68, $CHCl_3$), was determined by HR-FAB mass spectrum (MH^+, *m/z* 977.5915 for $C_{49}H_{79}N_6O_7F_6$, Δ 1.0 mmu).

Right Half. The ^1H and ^{13}C NMR spectra (Bruker AM-500, $CDCl_3$)[3] of **2** suggested the presence of a spermidine moiety[4] as well as an aliphatic long chain (δ 1.25). A clue for the whole structure of the right half was obtained when **5** was isolated from both sponges as the bis(trifluoroacetyl) derivative **6**. Eventually, the structure of the right half (from position 23 to position 45) was established by reduction (excess $LiAlH_4$ in THF; 0 °C, 50 min) or methanolysis (2% MeONa in MeOH; reflux for 3 h) of **2** and by methanolysis (0.1% MeONa in MeOH, room temperature, 7 days) of the bis(*p*-bromobenzamide) **4**, which resulted in the isolation of the fragments **8** (after acetylation) and **7**, respectively.[5]



Left Half. From the HOHAHA spectrum,[6] the protons could be classified into three spin-relaying groups: (i) CH_3-1 to CH_2-7, (ii) CH_2-9 to CH-14, and (iii) CH_2-16 to CH_3-20. Besides these proton signals, two D_2O-exchangeable signals appear at δ 10.22 and 9.87, which are ascribable to those of an ammonium or iminium group.[7] The fact that **2** is actually a salt was verified by the presence of one set of carbon signals ($CF_3CO_2^-$) [δ 116.8 (q, J = 292 Hz) and 162.7 (q, J = 34 Hz)] and also by the finding that shaking a $CDCl_3$ solution of **2** with an aqueous solution of sodium methanesulfonate resulted in the incorporation of 1 molar equiv of the methanesulfonate anion (**3**; detected by ^1H NMR spectrum; δ_{Me} 2.78) in the organic layer. Also, washing a $CDCl_3$ solution of **2** with 0.1 N NaOH brought about disappearance of the low-field NH signals, which reappeared slowly on standing. These properties suggested the presence of a strongly basic moiety in **2**.[8] When the ^{13}C NMR spectra ($CDCl_3$) were taken in the presence of 3 molar equiv of CD_3OD, the carbon signal at δ 149.09, which showed no correlation with protons in the COLOC[9] or

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(2) The freeze-dried animal (*P. spiculifer*) (15 g) was extracted with $CHCl_3$–MeOH (9:1), to give a brown oil (1.5 g), which was separated by chromatography (3×) on an NS-gel column (Nippon Seimitsu Kagaku 10503) eluted with MeOH–H_2O, affording **1** (30 mg), $[\alpha]_D$ −2.5° (*c* 0.7, $CHCl_3$).

(3) The NMR spectra were measured also in pyridine-d_5 by using 2–65 mg of **2**. The shifts ($CDCl_3$) described herein are those obtained for a 60 mM solution of **2**.

(4) Compound **2** exists in a mixture (3:1) of rotational isomers (at the amide linkage of C-38). This was confirmed by preparing **9**, which indicated that it also exists in a 3:1 mixture of isomers. The NMR properties of **9** parallel those of **2** with respect to the spermidine moiety.

(5) Many attempts to isolate the left half fragment were made in the reactions described herein. However, no trace of such a compound was obtained.

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(8) It is well-known that a solution of a strongly basic organic compound absorbs carbon dioxide from air, and the compound exists as a carbonate. (See, e.g.: Stecher, P. G., Ed. *The Merck Index*, 8th ed.; Merck & Co., Inc.: Rahway, NJ, 1968; p 510.)

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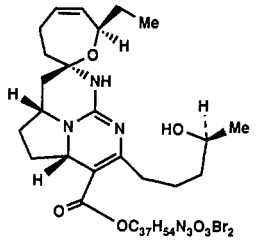
HMBC[10] spectra, was split into three peaks (δ 149.09, 149.02, and 148.94) due to an isotopic effect.[11] This observation means that the carbon is adjacent to the two exchangeable protons, and consideration of its chemical shift together with the fact that three nitrogens exist in the left half led to the conclusion that a guanidine moiety was present in **2**.[12] The isotopic shift experiment also caused considerable upfield shifts in the signals of two quaternary carbons at δ 83.9 (C-8) and 80.8 (C-15) (both Δδ = 0.08). The former was correlated (COLOC, HMBC) with H-6, H-3, and H-9 and the latter with H-14, H-16, and H-17. The N_AH signal at δ 10.22 showed a correlation peak with C-9, and the N_CH signal at δ 9.87 exhibited the peaks with C-15 and C-14. These data allowed us to propose the partial structure (plain) for the left half as depicted in **2** except for C-22.

The IR spectrum ($CHCl_3$) of **1** exhibits an absorption at 1730 cm^{-1} assignable to an ester group. In the COLOC spectrum of **2**, the ester carbonyl carbon (δ 168.6) showed the cross peaks to H-13 (δ 4.29) and H-14 (δ 2.94). Comparison of the chemical shifts of H-13 and H-14 indicated the ester carbonyl group to be attached to C-14. This assumption was verified by the fact that **10** was obtained as the major methanolysis product of **4**. The ^1H NMR spectrum of **10** indicated that (i) the ester linkage O-C-H_2-23 still survived, (ii) H-14 was lost, (iii) the pattern of H-13 changed from dt (J = 10.5, 5 Hz) into dd (J = 10.5, 6 Hz), and (iv) H_2-16 were markedly shifted downfield (Δδ = 0.84 and 1.24). The UV maxima (MeOH) of **10** were observed at 237 and 342 nm, the former being due to the *p*-bromobenzoyl moieties and the latter due to a dihydropyrimidinecarboxylate chromophore.[13] The IR absorption ($CHCl_3$) at 1655 cm^{-1} is consistent with the moiety.[13]



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The COLOC spectrum of **2** suggested the correlation between the ester carbonyl carbon (C-22; δ 168.6) and H_2-23 (δ 4.08 and 4.05), and this gave a proof that the hydroxy terminal of the right half was connected to C-22 through an ester linkage.

The stereochemistry of **2**[14] was determined on the basis of NOESY and ROESY[15] experiments. The NOE between H-19 and H_3-1 was essential to determine the stereochemistry at C-15.

Compound **1** shows cytotoxicity against P388 (IC_{50} = 0.1 μg/mL) and antimicrobial and antifungal activity against *Candida albicans* (MIC = 0.8 μg/mL) as well as very good antiviral activity (HSV) at a concentration of 0.2 μg/mL.

In conclusion, we have elucidated the structure of ptilomycalin A (**1**), a new class of polycyclic guanidine alkaloids, which are linked through an ω-hydroxy acid to spermidine.

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Supplementary Material Available: Tables I (NMR properties of **2**) and II (deuterium exchange studies on the ^{13}C NMR spectrum of **2** in the presence of 3 molar equiv of CD_3OD), ^1H NMR spectra of **1**, **2**, **8**, and **9**, ^{13}C NMR spectrum of **9**, HOHAHA spectrum of **2**, and CHCOSY spectrum of **2** (11 pages). Ordering information is given on any current masthead page.

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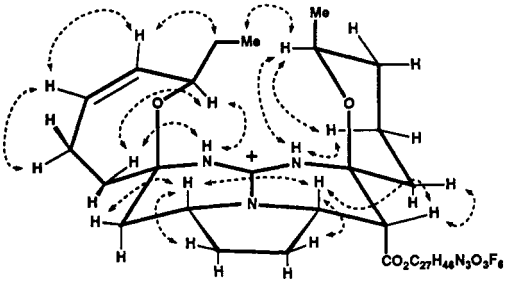
(14) NOEs indicated with arrows in structure **2a** (relative stereochemistry) were observed.



2a

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Ultraviolet Resonance Raman Spectra of Cu,Zn-Superoxide Dismutase: Detection of an Imidazolate Bridge between the Metal Ions in Solution

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Cu,Zn-superoxide dismutase (SOD) from bovine erythrocyte catalyzes the dismutation of superoxide to H_2O_2 and O_2.[1] The enzyme is a dimer with a subunit molecular weight of about 16000 containing one catalytic Cu^{2+} and one Zn^{2+} per subunit. In crystal, the Cu ion is coordinated by four histidine residues and the Zn ion is coordinated by three histidine residues and one aspartic acid residue.[2] Among the coordinated histidines, His61 takes a unique structure (His^-) by binding to Cu^{2+} and Zn^{2+} at $N(\epsilon_2)$ and $N(\delta_1)$, respectively, to form an imidazolate (Im^-) bridge between the two metal ions, while each of the other histidines (HisH) binds to one of the ions.[2] Although ESR studies have detected the magnetic interaction between metals through the bridging ligands,[3,4] the Im^- ring itself in solution has not been detected yet. Recently, UV resonance Raman spectroscopy is becoming a useful tool in studying the structure and microenvironments of proteins under physiological conditions.[5,6] We report, for the first time, UV resonance Raman spectra of SOD and the metal-depleted apo-SOD in solution. Raman bands arising from a single Im^- ring of His61 are clearly observed in resonance with its $\pi \rightarrow \pi^*$ transition, and the effects of coordination on the main-chain structure are noticed.

SOD was purchased from Boehringer Mannheim (3000 units/mg). The excitation light at 240 nm was obtained from an

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