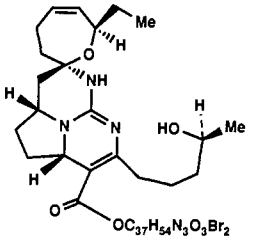
HMBC[10] spectra, was split into three peaks (δ 149.09, 149.02, and 148.94) due to an isotopic effect.[11] This observation means that the carbon is adjacent to the two exchangeable protons, and consideration of its chemical shift together with the fact that three nitrogens exist in the left half led 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 $\Delta\delta = 0.08$). 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 ($\Delta\delta$ = 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.

Acknowledgment. This work was supported financially by HBOI, Ft. Pierce, FL (Y.K.). We are grateful to Drs. S. Cross, P. McCarthy, and N. Burres, HBOI, for the antiviral, antimicrobial, and antitumor tests, respectively. We thank Drs. T. Kinoshita and N. Nakayama for FAB-MS measurements and Dr. M. R. Wälchli for taking the HMBC spectrum.

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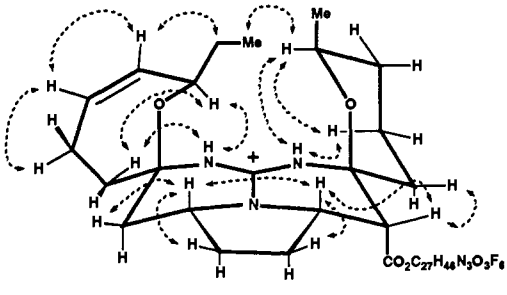
(14) NOEs indicated with arrows in structure **2a** (relative stereochemistry) were observed.



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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 16 000 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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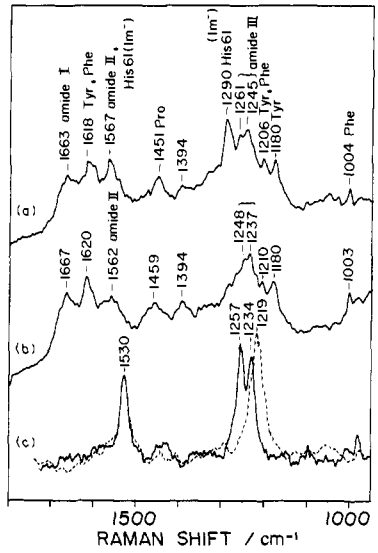

Figure 1. Ultraviolet resonance Raman spectra of 0.3 mM bovine erythrocyte superoxide dismutase (SOD) (a) and the metal-depleted apo-SOD (b) dissolved in 10 mM phosphate buffer (pH 7), and 50 mM amino acid histidine (solid line) and histidine-C(ϵ_1)d (broken line) in 3 M KOH (c) with 240-nm excitation (1 mW). Accumulation time, 20 min; slit width, 8 cm^{-1}.

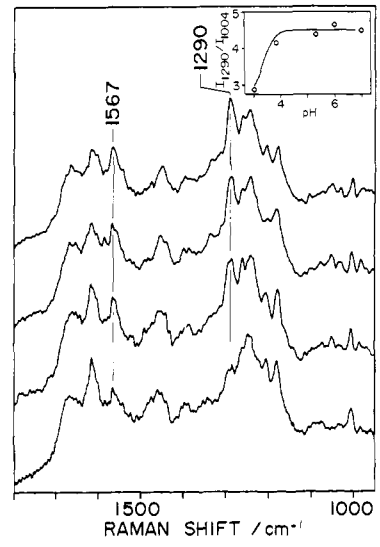

Figure 2. Raman pH titration of SOD (240-nm excitation). SOD was dissolved in water, and the pH was checked before and after each spectral measurement. The inset plots the relative intensity (I_{1290}/I_{1004}) against pH.

H_2 Raman-shifted quadrupled Nd:YAG laser. A quartz cylindrical cell containing the sample solution (0.3 mM) was spun to prevent decomposition by laser-light irradiation (average power, 1 mW at 30 Hz). Details of the UV Raman system will be reported elsewhere. Figure 1 shows the 240-nm-excited resonance Raman spectra of Cu,Zn-SOD (a) and apo-SOD (b). For comparison, Raman spectra of histidine and C(ϵ_1)-deuteriated histidine (His-C(ϵ_1)d) in very basic solution are shown in Figure 1c.

Assignments of most of the Raman bands of apo-SOD are straightforward. Two peaks in the amide III region at 1248 and 1237 cm^{-1} are due to irregular and β-sheeted parts of the molecule, respectively. The amide I band at 1667 cm^{-1} is an overlap of the bands of the irregular and β-sheeted parts. The amide II band is known to gain intensity with UV excitation[6] and is observed at 1562 cm^{-1}. Bands arising from the aromatic groups are at 1620 (Tyr and Phe), 1210 (Tyr and Phe), 1180 (Tyr), and 1003 cm^{-1} (Phe). The protein contains six Pro residues, and a relatively broad band around 1459 cm^{-1} is assigned to the imide stretching vibration.[7,8] Assignment of the 1394 cm^{-1} band to the overtone of amide V[9] cannot be ascertained in the present case since a similar band is observed in D_2O solution.

In the native SOD, the amide I peak is downshifted (1663 cm^{-1}) and the amide III peaks are upshifted (1261 and 1245 cm^{-1}) from those of apo-SOD. These frequencies are unusual for irregular and β forms, suggesting that the metal ion binding causes the tightening of the structure composed of eight antiparallel β strands and three external irregular loops.[2] Changes in the Raman bands of Tyr and Phe are negligible except for that of the 1619-cm^{-1} band. The Pro band at 1451 cm^{-1} is sharper than that in apo-SOD, indicating a more unified trans structure. A significant feature of Figure 1a as compared with Figure 1b is the emergence of a pair of strong bands at 1290 and 1567 cm^{-1}, the latter being overlapped with the amide II band. The peaks do not shift in D_2O solution.

Cu,Zn-SOD has an absorption at 256 nm, which has been assigned to a HisH $\rightarrow$ Cu^{2+} charge-transfer (CT) transition.[10] Accordingly, it might be considered that the two bands are enhanced in resonance with this transition. However, the intensity of the unoverlapped 1290-cm^{-1} band does not track the CT absorption but increases monotonically and steeply (via 253 and 246 nm) toward 240 nm. Since noncoordinated HisH has a $\pi \rightarrow \pi^*$ absorption around 210 nm and such an absorption is also expected for His$^-$, it is possible that the $\pi \rightarrow \pi^*$ transition is red-shifted upon coordination and the ring vibrations of coordinated HisH or His$^-$ are enhanced in resonance with the transition. A UV Raman study on $Cu(ImH)_4^{2+}$ has shown that the metal coordination does not change the intensity pattern of the spectrum very much except for significant enhancement of a band at 954 cm^{-1} with 240-nm excitation.[11] Since the simple two-peak pattern of SOD is much different from the resonance Raman pattern of HisH[6,12] and no band corresponding to the 954-cm^{-1} band of $Cu(ImH)_4^{2+}$ is observed, the two bands cannot be assigned to the coordinated HisH residue. On the other hand, amino acid histidine in basic solution (His$^-$) gives UV resonance Raman bands at two frequency regions as shown in Figure 1c, the enhancement being larger than in HisH but smaller than in SOD. The band at 1530 cm^{-1} does not shift on deuteriation at C(ϵ_1) (dotted line) and is assigned to the C(γ)C(δ_2) stretch. The doublet at 1257 and 1234 cm^{-1} becomes a singlet at 1218 cm^{-1} on C(ϵ_1) deuteriation and is assigned to the N(δ_1)C(ϵ_1)N(ϵ_2) symmetric stretch. (The doublet may arise from Fermi resonance.) These bands are expected to show large frequency shifts upon coordination to metal ion because the corresponding bands at 1459 and 1250 cm^{-1} of Im$^-$, which are also enhanced with 240-nm excitation, are reported to upshift to 1489 and 1273 cm^{-1} in $Co(Im^-)_2$.[13]

The SOD spectrum changes reversibly with pH (Figure 2). Concomitantly with decrease in pH, the 1290 and 1567 cm^{-1} bands decrease in intensity and finally disappear around pH 3.0. In the inset is plotted the intensity of the 1290-cm^{-1} band relative to that of the 1004-cm^{-1} Phe band against pH, and the pK_a is determined to be about 3.5. It has been reported that SOD undergoes a structural change below pH 4 due to release of the Zn ion from the protein.[14,15] On the basis of these and the referred findings, the two bands are assigned to the Im$^-$ ring of His61 coordinated by the Cu^{2+} and Zn^{2+} ions, and the disappearance of the bands is ascribable to protonation of His61, which occurs with the release of the Zn ion. The present observation gives direct evidence for the presence of the bridging His$^-$ in SOD in solution. Those His$^-$ bands will prove useful in studying the catalytic role of His61, which is still controversial.[16-24]

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Total Synthesis of (±)-Crassin by Titanium-Induced Pinacol Coupling

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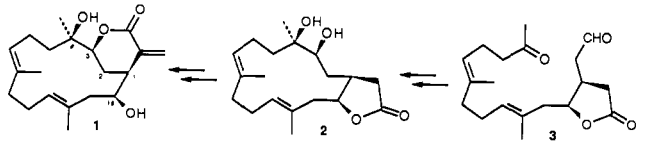
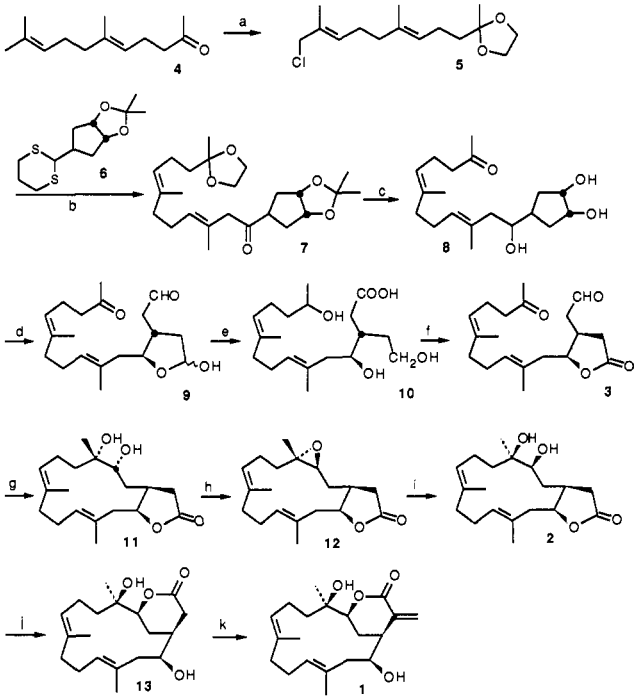
Crassin (**1**), a diterpenoid cembrane isolated in 1960 from the Caribbean gorgonian *Pseudoplexaura porosa*, has a remarkable range of biological activities. Its acetate has mild analgesic,[1] antibiotic,[2] and antineoplastic[3] properties and shows in vitro activity against human epidermoid carcinoma of the nasopharynx (KB cell line) at concentrations of 2 μg/mL, while crassin itself is about 2 times as potent.[3] Both compounds are also active against the PS cell line,[4] and cinnamoyl esters of crassin show significant in vitro antileukemic activity.[3] This bioactivity, together with the difficulties posed by large-ring synthesis, have prompted synthetic efforts by several groups in the last decade.[5-7] We now report the first total synthesis of crassin by a route that significantly extends the range of the titanium-induced carbonyl-coupling reaction.[8]

Our idea was to take advantage of the fact that crassin is regenerated after base-induced hydrolysis followed by acid-catalyzed relactonization.[9,10] It therefore follows that the isomeric butyrolactone **2** might also be convertible into norcrassin (**13**) by translactonization. Compound **2**, in turn, is a cyclic 1,2-diol that might be accessible by titanium-induced pinacol coupling of the corresponding keto aldehyde **3** according to our recently published procedure.[11]



There are two evident drawbacks to this plan. One is that we have no obvious method of stereocontrol over the coupling reaction; four stereoisomeric diols might result. A second, more crucial problem is that titanium-induced carbonyl-coupling reactions have thus far been limited almost exclusively to the synthesis of hydrocarbons.[8] Low-valent titanium is a powerful oxophile capable of reducing all kinds of carbonyl groups, and it is not clear that a lactone grouping in the molecule can survive the coupling conditions. Nevertheless, the simplicity of the overall scheme, the relative ease of hydroxyl inversion if the wrong stereoisomer predominates, and the desire to extend the titanium-induced carbonyl-coupling reaction for the synthesis of complex, oxygenated macrocycles led us to attempt the synthesis.

Scheme I. Total Synthesis of Crassin (**1**)[a]



[a] (a) SeO_2, *t*-BuOOH, 76%; then $HOCH_2CH_2OH$, H^+; then NCS, PPh_3, 85%. (b) BuLi; then **6**; then $AgNO_3$, NCS, H_2O, 70%. (c) $NaBH_4$, then H_3O^+. (d) $NaIO_4$, THF, 73% from **7**. (e) $AgNO_3$, NaOH; then $NaBH_4$. (f) *p*-TSA, CH_2Cl_2; then $(COCl_2)_2$, DMSO, 56% from **9**. (g) $TiCl_3(DME)_{1.5}$, Zn-Cu, DME, 20%. (h) MsCl, $PhCH_2NMe_3OH$, 68%. (i) H_3O^+, 77%. (j) NaOH, H_2O, then H_3O^+, 100%. (k) LDA, then CH_2O; then MsCl, DBU, 53%.

Keto aldehyde **3** was prepared by the route shown in Scheme I.[12] Starting with geranylacetone (**4**), selective allylic oxidation with selenium dioxide,[13] acetalization, and treatment with *N*-chlorosuccinimide and triphenylphosphine[14] gave the chloride **5**. Alkylation with the anion of dithiane **6**[15] followed by removal of the thioacetal group[16] gave **7**, and reduction with $NaBH_4$ followed by acid-catalyzed hydrolysis gave keto triol **8**. Cleavage of **8** by treatment with periodic acid then gave an intermediate dialdehyde, which underwent stereoselective cyclization to yield exclusively the trans-disubstituted hemiacetal **9**, thereby differentiating be-

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