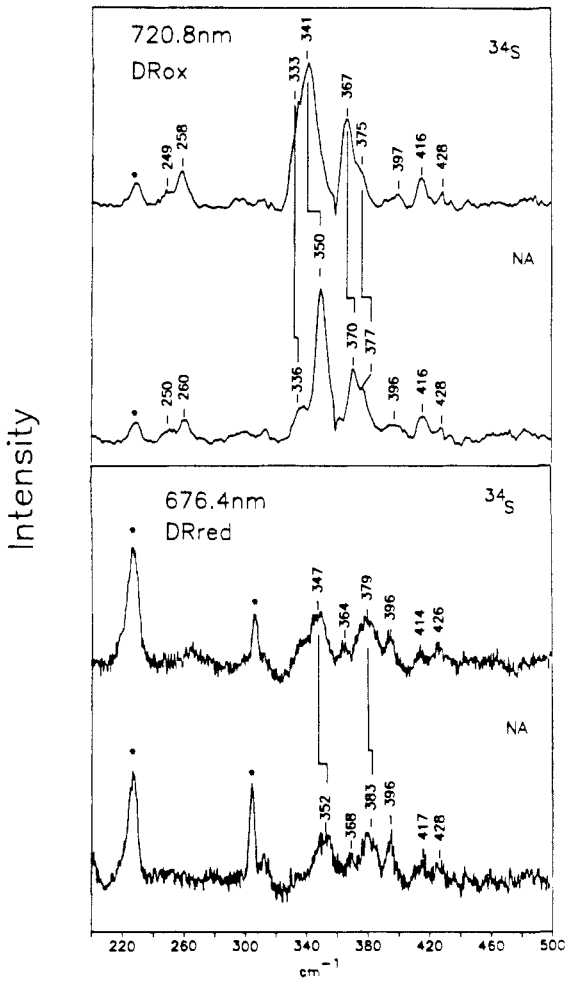

Figure 3. Low-frequency RR spectra, showing ^{34}S shifts of bands having Mo-S stretching character in oxidized (top) and reduced (bottom) DR. Excitation at 720.8 nm produces stronger enhancement for DRox, although the same features can be seen with 676.4-nm excitation. The spectrum of DRox was obtained by 180° backscattering from a frozen solution mounted on a cold finger cooled to 77 K. The 720.8-nm excitation line was provided by an Ar^+ pumped dye laser (Pyridine 2). The scattered light was collected with a Spex Triplemate equipped with a CCD (Photometrics). Conditions: 100-mW output laser power. The spectrum of DRred was obtained as described in Figure 2. Peaks marked with an asterisk are due to ice (228 cm^{-1}) or laser plasma lines (304 cm^{-1}). The ^{34}S-containing protein was obtained from *Rb. sphaeroides* grown on ^{34}S. ^{34}S, 90 atom %, obtained from Isotec, Inc., as elemental S was converted to $(NH_4)_2{}^{34}SO_4$ by boiling in a 3:1 mixture of HCl and HNO_3 in an oil bath at 170 °C until no more yellow fumes were seen. The solution was neutralized with NH_4OH and dried. Cultures of *Rb. sphaeroides* were grown for three generations on sulfate-free medium to deplete endogenous sulfur. The culture was then transferred to $^{34}SO_4$-containing medium for growth and isolation of ^{34}S-enriched DR.

428-cm^{-1} bands of DRred, but improved spectra are needed to confirm these shifts. (For DRox stronger enhancement was obtained at 720.8 nm, directly in resonance with the 720-nm absorption band.) The two Mo-S bonds of a dithiolene chelate should give rise to two stretching modes and can account for the two main ^{34}S-sensitive bands. The frequencies are nearly the same in the two oxidation states, but are slightly lower for DRox than for DRred, consistent with the slightly higher C=C frequency. These frequencies are expected to correlate negatively on electronic grounds.[12] Mo^{IV}-S frequencies have been reported in the 350-390-cm^{-1} range for various dithiolene complexes.[11,13] The weaker candidate ^{34}S-sensitive bands might arise from Mo-S bonds if the Mo is bound to the protein via cysteine ligands. EXAFS spectra of other molybdopterin proteins have indicated Mo-S bonds[14-17] in excess of two. Alternatively, the additional bands may arise from ligand deformation coordinates, e.g., SCC bending, coupled to the Mo-S stretches.

In summary, RR spectra, in resonance with the red absorption bands of DMSO reductase, reveal the presence of C=C stretching and Mo-S stretching vibrational bands, thereby confirming the presence of a dithiolene chelate structure in the molybdenum cofactor.

Acknowledgment. We are grateful to Dr. Timothy Harris at Bell Laboratories, Murray Hill, NJ, for his help in obtaining the 720.8-nm excited spectrum of RDox. This work was supported by NIH Grants GM 13548 (to T.G.S.) and GM 00091 (to K. V.R.) and NIH Postdoctoral Research Grant ES07031-12 (to N.R.B.).

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Microviridin: A Novel Tricyclic Depsipeptide from the Toxic Cyanobacterium *Microcystis viridis*

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Cyanobacteria of *Microcystis* species have been extensively studied from the environmental, toxicological, biological, and chemical points of view because they are responsible for water blooms that frequently produce potent hepatotoxins. These toxins are classified as microcystins and are considered to be harmful to the health of humans, cattle, and wild animals.[1] During our work on the toxins,[2] we found that an HPLC fraction contained a peptide, which was different from microcystins, and exhibited tyrosinase inhibitory activity. We herein describe elucidation of its unusual structure.

An axenical clonal strain of *M. viridis* (NIES-102),[3] isolated from a bloom on Kasumigaura Lake, was cultivated in MA medium, and the dried cells (100 g) were extracted with methanol. The methanol extract was subjected to HPLC [Ultron C_{18}, CH_3CN/H_2O (25:75)], and a peak preceding the one containing microcystins LR + YR was collected and further purified by using a TSK gel DEAE-2SW column [20 mM phosphate buffer (pH 7.0) containing 0.3 M NH_4OAc] to afford a colorless solid, microviridin (87 mg).

Microviridin (**1**) [(Chart I); $[\alpha]_D$ +21.7° (*c* 0.95, MeOH); λ_{max} 220 (ϵ 57 000), 278 nm (ϵ 8800); $C_{85}H_{100}N_{16}O_{24}$ [HRFABMS *m/z* 1729.7220 $(M + 1)^+$]; negative in a ninhydrin test] produced 14 amino acids, Asp, Thr, Ser, 2 × Glu, 2 × Gly, 3 × Tyr, Phe, Lys, Trp, and Pro, on hydrolysis [TsOH/3-(2-aminoethyl)-indole/H_2O, 110 °C, 22 h].[4] The GCMS analysis using a chiral

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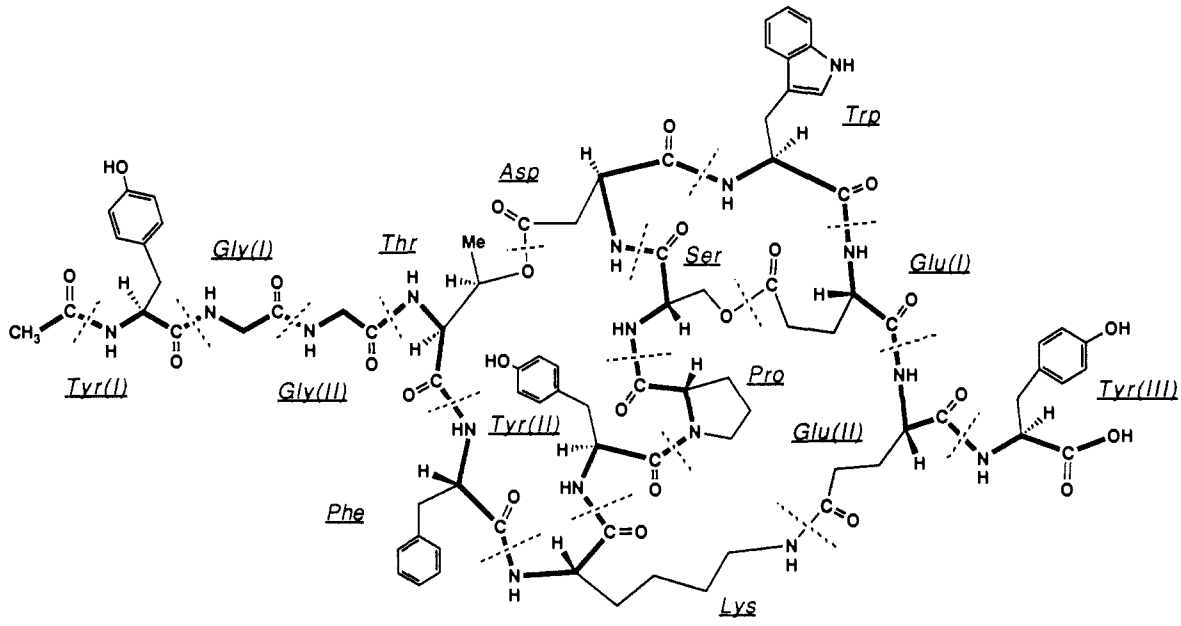
Chart I



Microviridin (1)

column (Chirasil-Val III capillary column) indicated that all the amino acids are in the L form. Analysis of H,H- and H,C-COSY and HOHAHA spectra[5] of **1** (CD_3OD or DMSO-d_6) led to the assignment of all the proton signals and the carbon signals except for those of quaternary carbons (Table I[5]).

Determination of the amino acid sequence was based on HMBC[5] and phase-sensitive NOESY spectra[5,6] of **1** measured in CD_3OH. For example, the carbonyl signal at δ_C 174.0 exhibits two HMBC correlation peaks to the acetyl protons (δ_H 2.01) and NH of Tyr (δ_H 8.33) which, in turn, shows an NOE cross peak to the acetyl protons. This established that the N-terminus of **1** was Tyr(I), the amino group of which was acetylated. The C=O at δ_C 174.8 shows J cross peaks to α-protons (δ_H 4.53) and β-protons (δ_H 2.97 and 3.15) of Tyr(I). Thus, this signal was assignable to Tyr-CO. This carbonyl also shows J cross peaks to NH (δ_H 8.55) and α-protons (δ_H 3.90, 4.04) of Gly(I). Appearance of the NOE cross peaks from Gly(I)-NH to Tyr(I)-NH as well as to α-protons of Tyr(I) confirmed the sequence of Ac-Tyr(I)-Gly(I). These procedures were repeated [summarized in Table II[5] (NOE) and Figure 1[5] (HMBC)] to lead to the entire amino acid sequence: Ac-Tyr(I)-Gly(I)-Gly(II)-Thr-Phe-Lys-Tyr(II)-Pro-Ser-Asp-Trp-Glu(I)-Glu(II)-Tyr(III)-OH.

The linkage between ϵ-NH of Lys and δ-CO of Glu(II) was confirmed by the HMBC cross peak from Glu(II)-δ-CO (δ_C 174.6) to Lys-ϵ-NH (δ_H 7.37) and NOESY peaks from Lys-ϵ-NH to Glu(II)-γ-H_2 (δ_H 2.12 and 2.47). In the ^1H NMR spectrum the signals of β-H of Thr and two β-H's of Ser appeared downfield at δ 5.56 and δ 4.84, 3.71, respectively, suggesting that Thr and Ser are esterified. Reduction of **1** ($NaBH_4$ in MeOH[7]) followed by hydrolysis afforded a product, the amino acid analysis of which revealed that Asp and 1 mol of Glu had disappeared, and instead, homoserine and 5-hydroxy-2-aminopentanoic acid[8] were detected. These findings implied that γ- and δ-carboxylic moieties of Asp and Glu, respectively, are involved in the ester linkages with Thr and Ser. The HMBC peak of Asp-γ-CO (δ_C 171.5) to Thr-β-H (δ_H 5.56) confirmed ester formation between Asp-γ-CO_2H and Thr-OH, and also the peak between Glu(I)-δ-CO (δ_C 173.3) and one (δ_H 3.71) of Ser-β-H_2 verified the ester bond of Glu(I)-δ-COO-Ser.

The structure **1** was further reinforced by the following chemical reactions. **1** was treated with DPPA (diphenylphosphoryl azide) in DMF and then MeOH,[9] and the resulting urethane was hydrolyzed. Amino acid analysis revealed that 1 mol of Tyr had disappeared, which gave unambiguous evidence that the C-terminus is Tyr. Also **1** was esterified under mild conditions in anhydrous MeOH/HCl (room temperature, 24 h) to give a monoester (^1H NMR) [Tyr(III)-OMe in **1**]. Reduction of this ester with $LiBH_4$[10] and hydrolysis of the product resulted in disappearance of Asp, 1 mol of Glu, and 1 mol of Tyr and formation of homoserine and 5-hydroxy-2-aminopentanoic acid.

Interestingly, microviridin (**1**) was not detected (HPLC) in the "field" samples of *M. viridis* collected from lakes in Japan. This peptide seems to be rapidly decomposed by other bacteria, because **1** did not survive for 6 h when cells of the axenical strain of *M. viridis* containing **1** were incubated with unsterilized waters of the lakes.[11]

Microviridin (**1**) strongly inhibited tyrosinase activity to form melanin from tyrosine[12] at 3.3×10^{-4} M.

In conclusion, we have demonstrated that microviridin (**1**), a tetradecapeptide from *M. viridis* (cyanobacterium), possesses a novel tricyclic structure containing two ester linkages with the OH groups of Ser and Thr and an amide bond with ϵ-NH_2 of Lys. To the best of our knowledge, microviridin is the first example of a tricyclic depsipeptide from natural sources.

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Supplementary Material Available: Tables of NMR and NOE data and figures containing the long-range J_{CH} observed in the HMBC spectrum and ^1H NMR, ^{13}C NMR, H,H COSY, HOHAHA, H,C COSY, HMBC, and NOESY spectra for **1** (10 pages). Ordering information is given on any current masthead page.

Vacant Coordination Sites within the Tunnels of a Microporous, Neutral Framework Molybdenum Phosphate with 35 Vol % Void Space: Structure of $Mo_8O_{12}(PO_4)_4(HPO_4)_2 \cdot 13H_2O$

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We are investigating the synthesis and sorption properties of a new class of open framework solids with the goal of being able to carry out shape-selective and size-exclusionary chemical reactions within the pores of a microporous solid. If one could prepare a solid-state material that would have the accessible internal micropore volume, framework rigidity, and thermal stability of a zeolite, combined with a potentially catalytically active transition metal as an integral, covalently bonded part of the framework, then the possibility of performing both catalysis and separations simultaneously in one material may be achievable. In addition to a large number of solid-state alkali-metal Mo phosphates and molecular Mo phosphates with metal–metal bonds,[1] we have reported one previous example of a microporous molybdenum phosphate, $(Me_4N)_{1.3}(H_3O)_{0.7}[Mo_4O_8(PO_4)_2]\cdot 2H_2O$,[2] and there are three other examples of molybdenum phosphates that can be rendered microporous, namely, $(CH_3)_2NH_2[Mo_2P_3O_{12}(OH)_2]$[3] containing Mo^{5+}, $(NH_4)_3Mo_4P_3O_{16}$,[4] containing $Mo^{3.5+}$, and $(NH_4)Mo_2P_2O_{10}\cdot H_2O$[5] with both Mo^{4+} and Mo^{5+}. Here we discuss the synthesis, structural characterization, and sorption properties of $Mo_8O_{12}(PO_4)_4(HPO_4)_2\cdot 13H_2O$ (**1**). Phosphate **1** contains unusual Mo_4 units, with the MoO_6 octahedra bonded together via both edge- and corner-sharing modes, which together with the phosphate groups form a neutral framework that differs from the anionic frameworks found in all of our other molybdenum phosphates[1,2] as well most other zeolitic solids.[6] Some of the Mo atoms in **1** contain water ligands that can be removed while the crystallinity of the framework is maintained, Mo atoms thereby being generated with vacant coordination sites within the micropores of **1**. At least 35% of the internal volume of crystals of dehydrated **1** is empty space according to water absorption isotherms.

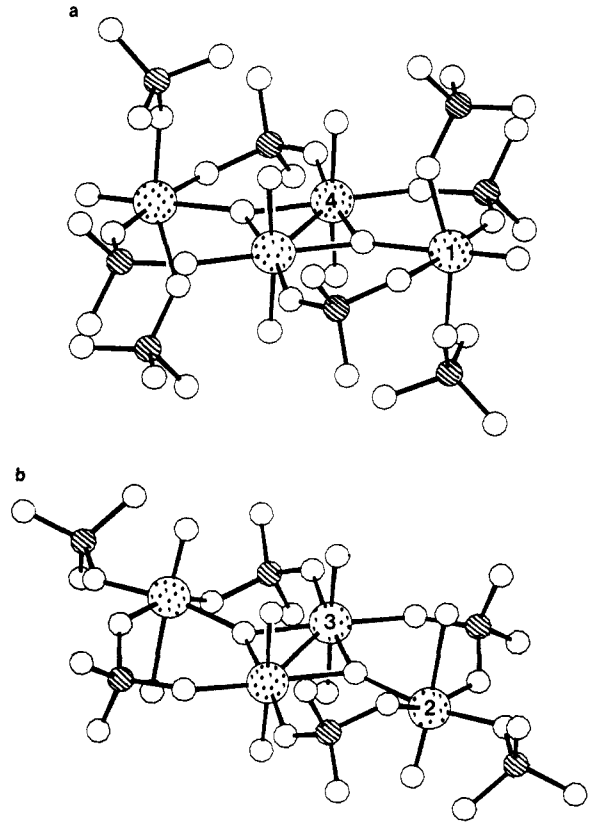

Figure 1. The two Mo_4 tetramers in **1**: (a) tetramer **2** containing Mo(1) and Mo(4) and (b) tetramer **3** containing Mo(2) and Mo(3). Stippled circles, Mo; striped circles, P; remaining circles, O.

The reaction of MoO_3, Mo, H_3PO_4, and H_2O in a mole ratio of 7:1:12:300 for 6 days at 200 °C gives a 40% yield of orange-brown crystalline $Mo_8(H_2O)_6P_6O_{34}(OH)_2\cdot 7H_2O$ as the only solid precipitating from solution. The product was shown to be single phase by comparison of the powder X-ray diffraction pattern to the powder pattern calculated from the coordinates obtained from the single-crystal structure determination.

Phosphate **1** has a complicated, low-symmetry ($P\bar{1}$) three-dimensional structure[7] with a framework composed of MoO_6 octahedra and PO_4 and $PO_3(OH)$ tetrahedra sharing edges and corners. The structure can be described in terms of the two crystallographically independent, centrosymmetric, nearly planar Mo_4 units present in the unit cell. These two tetramers, labeled **2** and **3** in Figure 1, are both composed of two edge-sharing MoO_6 octahedra, with Mo–Mo single bonds (2.659 (5) and 2.611 (6) Å), with the two O atoms involved in this shared edge also serving as a corner for two additional MoO_6 octahedra, making these particular oxygens each three coordinate to three different Mo atoms.

Tetramer **2** (Figure 1a), centered around the $\bar{1}$ site at ($^1/_2$,0,0), has two of the four Mo atoms (Mo(1)) in the 5+ oxidation state according to bond-strength bond-length calculations[8] and the characteristic geometry.[9] The molybdenyl groups on Mo(1) are trans to three-coordinate O(5). The two Mo(4) atoms in the central edge-sharing octahedral dimer are 2.611 (6) Å apart, consistent with a Mo–Mo single bond and an oxidation state of 5+. Refinement of the X-ray data shows two terminal O atoms on each Mo(4) which appear to be a molybdenyl O and a water O atom disordered over the two sites.[10] In tetramer **3** (Figure

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(7) Crystal data for **1**: triclinic, space group $P\bar{1}$ with a = 10.466 (5) Å, b = 12.341 (4) Å, c = 8.228 (7) Å, α = 94.75 (4)°, β = 111.46 (5)°, γ = 89.48 (3)°, V = 985 (2) Å^3, $R(R_w)$ = 0.067(0.072). Framework O atoms: isotropic refinement. All others: anisotropic for 182 variables and 1592 data with $I > 3\sigma(I)$.

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