was passed through a 1 × 25 cm column of silica gel eluting with hexane to give a trace of the azo compound followed by 2.39 g (94.1%) of pale orange solid 2,6,2′,6′-tetramethylazoxybenzene, mp 89.5–90.5 °C (lit.[6] mp 89.5–90 °C): IR ($CDCl_3$) 1430, 1320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.32 (s, 6 H), 2.47 (s, 6 H), 7.10–7.20 (m, 6 H).

2,6,2′,6′-Tetramethylazobenzene. A. From the Azo Dioxide. Hexachlorodisilane (6.5 g, 0.024 mol) was added dropwise to a solution of 2,6,2′,6′-tetramethylazobenzene *N,N′*-dioxide (2.7 g, 0.010 mol) in 50 mL of dry chloroform under a nitrogen atmosphere. The solution was heated at reflux for 7 days, monitoring progress by NMR. The solution was worked up in the same way as above to afford 2.3 g (96.5%) of deep red solid azo compound, mp 47–47.5 °C (lit.[6] mp 46–47 °C): IR (CCl_4) 1460 cm^{-1}; ^1H NMR as published.[2]

B. From the Azoxy Compound. Hexachlorodisilane (1.1 g, 0.0042 mol) was added to a solution of 1.0 g (0.0039 mol) of 2,6,2′,6′-tetramethylazoxybenzene in 30 mL of dry chloroform under a nitrogen atmosphere. The solution was heated at reflux for 7 days and worked up as above to afford 0.92 g (98%) of pure azo compound.

Registry No. 2,6-Dimethylaniline, 87-62-7; 2,2′,6,6′-tetramethylazobenzene *N,N*-dioxide, 101225-69-8; 2,2′6,6′-tetramethylazoxybenzene, 80101-88-8; 2,2′,6,6′-tetramethylazobenzene, 29418-31-3.

X-ray Structure Determination of the Naturally Occurring Isomer of Cyanobacterin

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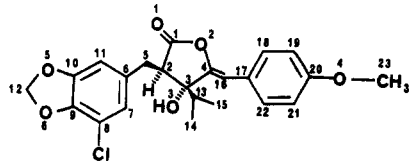
Cyanobacterin, **1** is an antibiotic which was isolated from the freshwater cyanobacterium (blue-green alga) *Scytonema hofmanni*, UTEX 2349. It was shown to be highly toxic to both cyanobacteria and other algae.[1] Further



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studies indicate that cyanobacterin acts by inhibiting photosynthetic electron transport in the oxygen-evolving system of photosynthesis (photosystem II).[2] Cyanobacterin contains two asymmetric centers at positions 2 and 3. From our previous spectral studies, we determined that the hydrogen and hydroxyl at carbons 2 and 3, respectively, are cis.[3] A recent report describes the synthesis and structure of racemic cyanobacterin.[4] Using our bioassay, we find that the synthetic material (kindly provided by P. Williard, Brown University) was approximately half as effective in inhibiting photosynthetic electron transport as the natural product.[2] Thus it appears that only the naturally occurring isomer has significant biological activity. We report here the absolute configuration determination of the naturally occurring isomer of cyanobacterin.

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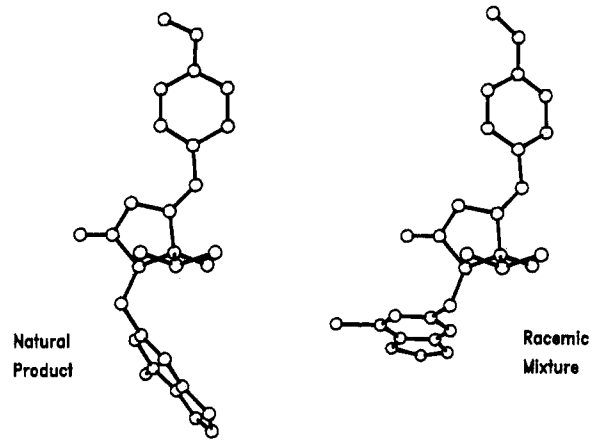

Figure 1. Cyanobacterin conformational differences in the solid state.

Table I. Differences in Bond Lengths in the Solid State between the Natural Product and Synthetic Cyanobacterin

bond	natural product, Å	synthetic, Å
C1–O1	1.173 (6)	1.211 (12)
C5–C6	1.547 (6)	1.509 (12)
C7–C8	1.476 (7)	1.374 (14)
C8–Cl	1.703 (5)	1.750 (8)
C9–C8	1.398 (7)	1.361 (12)
C10–C9	1.293 (7)	1.398 (13)
O5–C12	1.224 (8)	1.421 (13)

Results and Discussion

The absolute configuration of the naturally occurring cyanobacterin was determined to be *R* at both asymmetric carbons from the difference in *R* factor based on the anomalous scattering of the Cl atom by using Friedel's pairs as suggested by Rogers.[5] The final *R* factor for the configuration in which both C2 and C3 are *R* was 0.069 (R_w = 0.095). For the *S* configuration the corresponding value was 0.076 (R_w = 0.105).

The solid state conformation of both the natural product and synthetic cyanobacterin are shown in Figure 1. A comparison of selected bond lengths for the two molecules is given in Table I. The C1–C2–C5–C6 torsion angle is 155° in the natural product and 84° in the synthetic compound. Therefore, in the natural cyanobacterin the two fused rings are folded back toward the rest of the molecule instead of being extended as they are in the synthetic material. Crystal packing for the naturally occurring cyanobacterin is influenced by the presence of a weak hydrogen bond (Cl···O3 at 3.3 Å) which essentially links the molecules together into pairs. However, since crystal packing energies are generally of the order of a few kcal,[6] both conformations would presumably be present in solution in equilibrium with perhaps even other arrangements. Mechanistic studies of enzyme–substrate interactions suggest that only one conformation will bind to a protein receptor. Biochemical analyses of the activity of cyanobacterin have not resolved which conformation is bound but do indicate that the unnatural *S* isomer obtained by chemical synthesis[4] is not active.

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Experimental Section

Isolation and Crystallization of Cyanobacterin. Batch cultures of *Scytonema hofmanni* (University of Texas Collection, 2349) were grown as described[1] and harvested after 4–5 weeks. Cyanobacterin was prepared by extraction of sonicated cells with *tert*-butyl methyl ether. Purification was completed by preparative TLC and HPLC. Presence of highly purified cyanobacterin was confirmed from NMR spectra. Details of purification and spectral data can be found in the previous publication.[3] Biological activity of the final product was determined by monitoring the inhibition of oxygenic photosynthetic electron transport in the cyanobacterium *Synechococcus* sp.[2] Highly purified cyanobacterin was crystallized by dissolving the compound in ethyl ether and adding a layer of hexane. The mixture was placed in a loosely stoppered vial and crystals grew in the hexane layer as the ether evaporated.

X-ray analysis: $C_{23}H_{23}O_6Cl$, crystal size (0.25 × 0.25 × 0.30 mm); orthorhombic; space group $P2_12_12_1$; a = 7.707 (1) Å, b = 15.616 (3) Å, c = 17.420 (3) Å, z = 4, d_{calcd} = 1.37 gm cm^{-3}, μ = 19.5 cm^{-1}. Measurements were obtained with a Nicolet R3M automatic diffractometer using Cu Kα radiation (λ = 1.54178 Å) with a graphite monochromator in the incident beam, 3393 reflections were measured (including Friedel equivalents for absolute configuration calculations) at room temperature using the θ–2θ scan technique with a variable scan rate out to a $2\theta_{max}$ = 116°. Data were corrected for Lorentz, polarization, and absorption effects (minimum and maximum transmission factors were 0.60 and 0.64, respectively). The structure was solved by direct methods.[7] Refinement was by full matrix least squares (non-H atoms anisotropic, H atoms coordinates only) using the 2837 reflections for which $|F_o| > 3\sigma|F_o|$ to a final R factor of 0.069, R_w = 0.095. The function minimized was $\sum\omega(|F_o| - |F_c|)^2$ where $\omega = 1/[\sigma^2(|F_o|) + g(F_o)^2]$ and g = 0.0003. The goodness of fit parameter was 4.7 which was acceptable when considering the marginal quality of the crystals available. Hamilton's test indicates that the probability that the configuration assignment is incorrect is less than 0.005.[8] The final difference map, except for diffraction ripples in the vicinity of the C1 atoms, was featureless. All calculations were carried out with the Shelxtl system of programs.[9]

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Registry No. 1, 80902-00-7.

Supplementary Material Available: Tables of X-ray data for natural cyanobacterin: atomic coordinates and temperature factors, bond lengths, bond angles, anisotropic temperature factors, and hydrogen coordinates (5 pages). Ordering information is given on any current masthead page.

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An Unusual Cleavage of 2,5-Difluorobenzophenone

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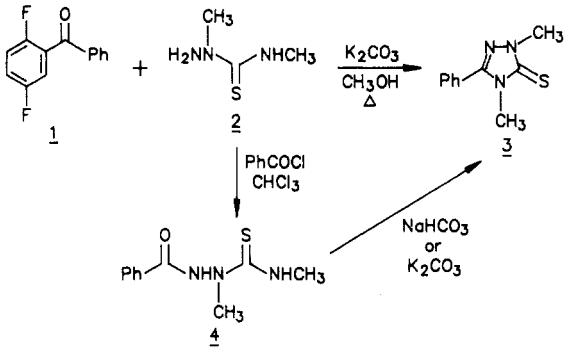
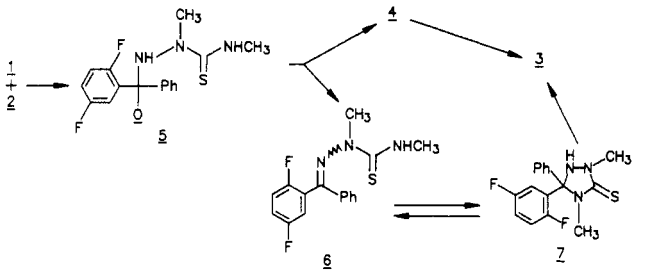
The alkaline cleavage of nonenolizable ketones has been the focus of considerable mechanistic[1-5] and synthetic[6-11] study. We have recently investigated the reactions of halo ketones and substituted thiosemicarbazides as routes to potential therapeutic agents.[12,13] During the course of our studies we observed an unusual reaction which resulted in both the facile cleavage of a diaryl ketone and the formation of a derivative of the 1,2,4-triazole ring system.

When 2,5-difluorobenzophenone (**1**),[13] 2,4-dimethylthiosemicarbazide (**2**),[14] and potassium carbonate were refluxed in methanol for 72 h, 2,4-dimethyl-5-phenyl-3*H*-1,2,4-triazole-3-thione (**3**)[15] was isolated in 73% yield. The



structure of **3** was initially based upon its ^{1}H NMR spectrum and was later confirmed by an alternate synthesis[15] via 1-benzoyl-2,4-dimethylthiosemicarbazide (**4**). In order to form **3**, it is apparent that at some time during the course of the reaction, the difluorophenyl moiety must be cleaved. The relatively mild conditions under which this cleavage occurred and the good yield of **3** which was isolated prompted us to investigate this reaction more closely.

Mechanistically, formation of **3** might be rationalized in two ways.[16] Condensation of **1** and **2** presumably involves the formation of a tetrahedral intermediate **5**. Cleavage of the aryl group at this stage is reminiscent of the classical Haller–Bauer[10] reaction and would be expected to yield benzylthiosemicarbazide **4**. Cyclization



of **4** under the alkaline reaction conditions should then

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