(1 H, dd), 1.9–1.6 (2 H, m), 1.65 (3 H, br s), 0.97 (3 H, d); IR ($CHCl_3$) 1725 cm^{-1}; MS, m/e calcd for $C_{10}H_{16}O_2$ 168.1151, found 168.1156.

Methyl *trans*-2,4-Dimethyl-3-cyclopentene-1-acetic Acid. Methyl ester from acid 3c (Nu = CH_3): ^1H NMR δ 5.15 (1 H, m), 3.67 (3 H, s), 2.46 (2 H, m), 2.33 (2 H, m), 2.16 (1 H, m), 1.97 (1 H, m), 1.68 (3 H, br s), 1.00 (3 H, d); IR ($CHCl_3$) 1730 cm^{-1}. Anal. Calcd for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59. Found: C, 71.37; H, 9.30.

Methyl 4,4-Dimethyl-2-cyclopentene-1-acetic Acid. Methyl ester of acid 2c (Nu = CH_3): ^1H NMR δ 5.51 (2 H, m), 3.67 (3 H, s), 2.38 (1 H, m), 2.43 (1 H, dd, J = 7 Hz, 15 Hz), 2.31 (1 H, dd, J = 7 Hz, 15 Hz), 1.96 (1 H, dd, J = 8 Hz, 13 Hz), 1.27 (1 H, dd, J = 7 Hz, 13 Hz), 1.09 (3 H, s), 1.03 (3 H, s); IR ($CHCl_3$) 1730 cm^{-1}. Anal. Calcd for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59. Found: C, 71.20; H, 9.34.

Acknowledgment. We thank the National Institutes of Health (GM 33372) for funding this work. D.P.C. also thanks the Dreyfus Foundation, the Alfred P. Sloan Foundation, and Eli Lilly for Fellowships.

Registry No. **1a**, 38110-76-8; **1b**, 100948-63-8; **1c**, 100948-64-9; **2a** (Nu = Me), 100948-65-0; **2a** (Nu = $(CH_2)_2CH{=}CH_2$), 100948-66-1; **2b** (Nu = Me), 100948-67-2; **2b** (Nu = Me, methyl ester), 100948-75-2; **2b** (Nu = $THPOCH_2C(CH_3)_2CH_2$), 94957-76-3; **2b** (Nu = 2-ethyl-1,3-dioxane), 100948-68-3; **2c** (Nu = Me), 100948-69-4; **2** (Nu = Me, methyl ester), 100948-77-4; **2c** (Nu = 2-ethyl-1,3-dioxane), 100948-70-7; **3a** (Nu = Me), 100948-71-8; **3a** (Nu = Me, methyl ester), 100948-74-1; **3a** (Nu = $(CH_2)_2CH{=}CH_2$), 100948-72-9; **3c** (Nu = Me), 100948-73-0; **3c** (Nu = Me, methyl ester), 100948-76-3; **i**, 100948-78-5.

Synthesis of 2,6,2′,6′-Tetramethylazobenzene and the Azodioxy and Azoxy Compounds

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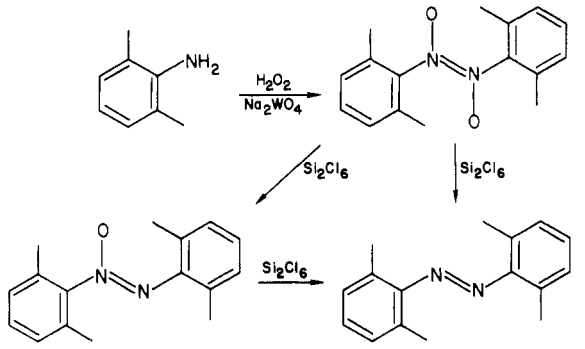
Received November 19, 1985

The method of choice for detection of traces of blood involves the use of 2,6,2′,6′-tetramethylbenzidine which gives a blue color with hydrogen peroxide in the presence of blood peroxidase.[1] This benzidine is made in low overall yield from 2,6-dimethylaniline.[2] The yield loss is in the first step, the oxidation of the aniline to the title azo compound. The ancient[3] oxidation with potassium ferricyanide gives only a 14.7% yield.[1,2] The use of silver(II) oxide[4] or silver carbonate[5] gave 33% and 35% yields. The azo compound has also been prepared by lithium aluminum hydride reduction of 2,6-dimethylnitrobenzene, but again the yield was only 13%.[6]

In unhindered aryl cases the N–N bond formation may occur at several levels of oxidation, but the 2,6-dimethyl groups lessen these possibilities.[7] The exception is at the nitroso level where ortho substituents interfere with coplanarity and conjugation in the monomer, favoring the azodioxy compound. In solution nitrosobenzene is essentially all monomer under conditions where nitrosomesitylene and 1,3-dimethyl-2-nitrosobenzene are mostly dimer.[8]

Scheme I



We have found that the title azo compound can be prepared in 92% overall yield by first oxidizing the aniline to the azodioxide and then reducing it to the azo level (Scheme I). Sodium tungstate catalyzed[9] oxidation of 2,6-dimethylaniline with hydrogen peroxide gives the crystalline, colorless azo dioxide in 98% yield. Heating this with 2.5 mol/mol of hexachlorodisilane in chloroform gives the dark red azo compound in 97% yield. With 1.1 mol/mol of hexachlorodisilane, the azoxy compound may be made in 94% yield.

Earlier workers[10] employed Caro's acid (potassium persulfate in sulfuric acid) to oxidize 2,6-dimethylaniline to the azo dioxide in 20% to 60% yield. The azoxy compound was made previously by oxidation of the azo compound.[6]

Hexachlorodisilane has been used to deoxygenate alkyl amine oxides[11] nitrones,[12] and a few cyclic cis azo dioxides[13,14] and azoxy compounds.[15]

Experimental Section

The NMR spectra were run on a Varian EM-360 spectrometer with tetramethylsilane as an internal standard.

2,6,2′,6′-Tetramethylazobenzene *N,N′*-Dioxide. 2,6-Dimethylaniline (47.2 g, 0.390 mol), 60 mL of water, 8.0 g of sodium tungstate, and 100 mL of ether were placed in a 500-mL Erlenmeyer flask and cooled in an ice bath. Hydrogen peroxide (132 mL of 30%, 1.17 mol) was added dropwise to the mixture with stirring over a 40-min period. The resulting mixture was left at room temperature overnight. The pale yellow product was separated by filtration and washed with 50 mL of ice-cold ether to afford 51.8 g (97%) of white crystalline solid, mp 134.5–135 °C (lit.[8] mp 133.5–134 °C): IR (mineral oil) 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 12 H), 7.12 (s, 6 H).

2,6,2′,6′-Tetramethylazoxybenzene. Hexachlorodisilane (3.0 g, 0.011 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. After the initial exothermic reaction, the solution was heated at reflux overnight. Cold 1 M aqueous sodium hydroxide was added until the water layer was basic. The chloroform layer was separated and the water layer was extracted with three 50-mL portions of ether. The combined chloroform and ether solution was dried with sodium sulfate and rotary evaporated to leave an orange-red solid. This

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0022-3263/86/1951-1614$01.50/0

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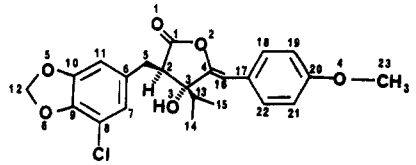
Cyanobacterin, **1** is an antibiotic which was isolated from the freshwater cyanobacterium (blue-green alga) *Scytonema hofmanni*, UTEX 2349. It was shown to be highly toxic to both cyanobacteria and other algae.[1] Further



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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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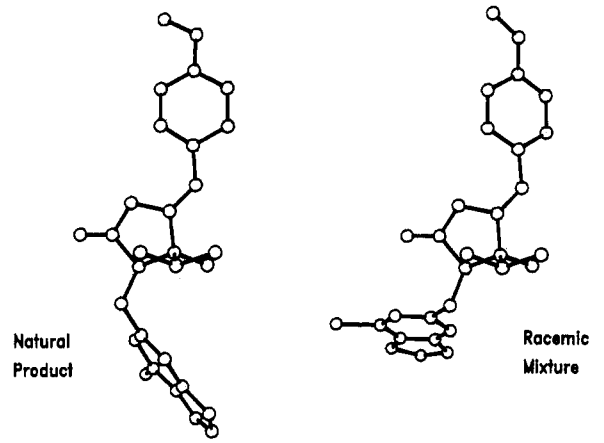

Figure 1. Cyanobacterin conformational differences in the solid state.

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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