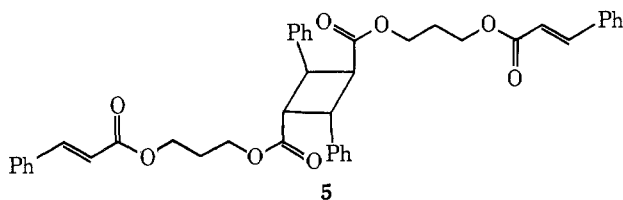


Table I. Relative Quantum Yields for Photoisomerization and Cyclization^a

Compd	Reaction	Solvent	Wave-length, nm	Catalyst	Rel quantum yield ^b
3 ^c	Cyclization	(C ₂ H ₅) ₂ O	300	None	0.05
1 ^c	Trans → cis	(C ₂ H ₅) ₂ O	300	None	0.90
1 ^c	Cyclization	(C ₂ H ₅) ₂ O	300	None	0.02
1 ^{c,d}	Cyclization	(C ₂ H ₅) ₂ O	300	CuCl	0.20
1 ^{c,d}	Cyclization	CHCl ₃	350	Ph ₂ CO	0.10
5 ^c	Trans → cis	(C ₂ H ₅) ₂ O	300	None	0.90

^a Standard: photoisomerization (trans → cis) of 1,3-propanediol-dicinnamate = 1.0 in diethyl ether at 300 nm. ^b Values are extrapolated to zero conversion. These values can be converted to absolute quantum yields by the use of 0.47 for the quantum yield of the standard.⁶ ^c Concentration 5×10^{-5} M. ^d Concentration 10^{-4} M.

which has the structure **5** was found not to undergo internal photocycloaddition under any of the experimental conditions that were tried. Reaction 1 is of interest from two points of view which follow.



(i) One concerns the factors which control the probability of a successful encounter between two cinnamate groups in a given molecule. The two important considerations according to the present investigation are the rate of a diffusive encounter between the chromophoric groups and the lifetime of the excited state of the molecule. Direct irradiation of **1** or **3** or **5** seems to lead to reaction from a singlet state as triplet sensitization gives quite different results. In this singlet state, trans → cis isomerization proceeds with about equal efficiency in all three instances (which is reasonable), but cyclization is twice as efficient in **3** ($s = 8$) as in **1** ($s = 17$), while **5** which has nearly the same separation between the chromophores as **3** does not cyclize at all to any detectable extent. The decrease in reactivity in going from **3** to **1** parallels the reported³ decrease in the quantum yields for the closure of bis anthroates with separations of 7 and 14 bonds and is attributable to decreased probability of an encounter between the ends of the chain with increasing chain length. The sharp contrast in behavior between **3** and **5** which have nearly the same separation suggest yet another consideration. Molecular models show that a [2 + 2] internal adduct of **5** would not suffer from angle strain whatever the stereochemistry of the addition may be, but severe limitations on its conformational mobility are placed by its *trans*-1,3-cyclobutane geometry. Therefore, separations between chromophores are comparable only when the geometries of the molecules are strictly similar.

For a given reactant molecule, the number of encounters between the ends is undoubtedly increased by going from a singlet to a triplet excited state. The marked effectiveness of triplet sensitizers on the photoreaction of various cinnamate esters (including polyvinyl cinnamate) is well documented in the literature.^{13,14} The data in Table I also bear this out. Cuprous chloride may also function by promoting the intersystem crossover through a heavy atom effect.¹⁵

(ii) A second point of interest is the stereochemistry of the addition in reaction 1. In the solid state it has been shown⁴ that a [2 + 2] photocycloaddition between cinnamate groups will give exclusively α -truxillic or β -truxinic acids or their derivatives. The conditions which govern the formation of one or the

other have been elegantly worked out. In contrast, in solution, as already mentioned, the δ -truxinic ester is a product of the photocyclization of **3** and is the only product from **4**. The present work suggests that the latter mode persists with even longer molecular separation between cinnamate groups. It may be noted that all three dicarboxylic acids are derived from *trans*-cinnamic acids. The stereochemistry that prevails in the photo cross linking of polyvinyl cinnamate which is usually irradiated as an amorphous film is an interesting question. In our earlier work,¹⁶ we had looked for only α -truxillic and β -truxinic acids among the hydrolysis products of the photolyzed materials. After establishing the stereochemistry in **2**, the earlier data were reexamined to see if we could have overlooked the presence of δ -truxinic acid. It was confirmed that an amorphous film of polyvinyl cinnamate gives only α -truxillic acid as we determined before. This indicates that the behavior of the cinnamate groups in the film is similar to those in a crystal rather than a fluid solution. The structure of the film deserves further examination.

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- (11) All new compounds gave satisfactory elemental and mass spectral analyses.
- (12) We thank Professor I. Waltcher of the City University of New York for authentic samples of δ -truxinic acid and its ester and Professor D. G. Whitten of the University of North Carolina for NMR spectra of the octadecyl esters of δ -truxinic acid.
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Sirohydrochlorin. Prosthetic Group of a Sulfite Reductase Enzyme and Its Role in the Biosynthesis of Vitamin B₁₂

Sir:

Recent work in these laboratories^{1,2} and independently at Cambridge³ and Stuttgart⁴ has confirmed the role of uro'gen III (**1**) in the biosynthesis of vitamin B₁₂ (**2**). It has also been shown⁵ that, during the bioconversion of both uro'gen III (**1**) and the "ring C heptacarboxylic acid" (**4**) to cobrynic acid (**3**), formaldehyde can be trapped from the δ -meso (C-20) carbon of **1** and **4**. The relatively low but intact conversion of **4** to **3** (ca.

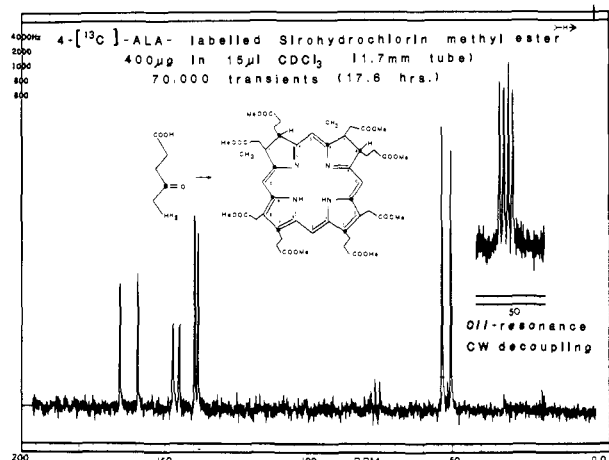


Figure 1. The ^{13}C FT spectrum of [4- ^{13}C]Ala-labelled sirohydrochlorin octamethyl ester with proton noise decoupling (main spectrum) and with off-resonance CW decoupling (inset) recorded on a Varian FT-80 instrument.

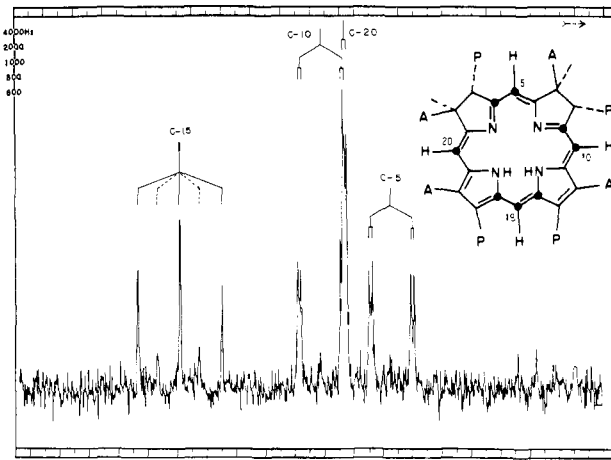
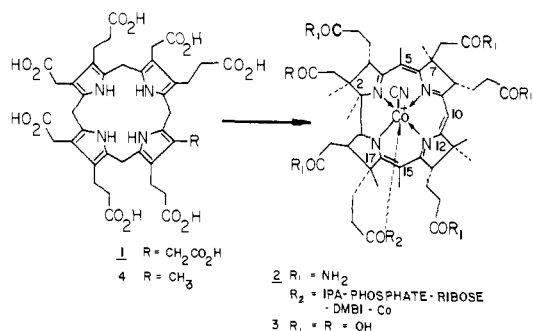


Figure 2. The meso carbon resonances of the proton noise-decoupled ^{13}C FT spectrum of [5- ^{13}C]Ala-labelled sirohydrochlorin octamethyl ester in CDCl_3 .



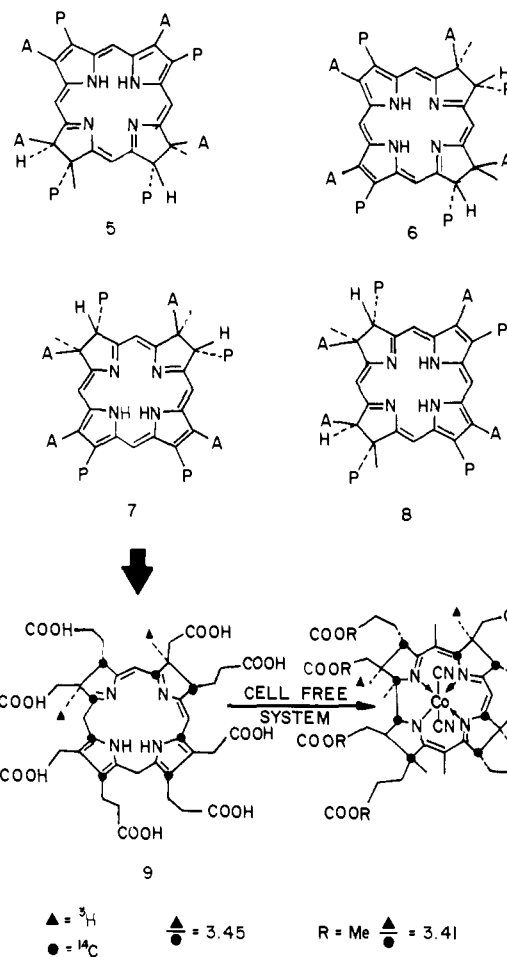
an order of magnitude less than the incorporation of **1** into **3**) suggests reassessment of the location of the heptaacid (**4**) on the metabolic grid of the porphyrinogen-corrin pathway. In this paper we describe the characterization of a new isolate from a modified version of the *P. shermanii* cell-free system⁶ which has considerable bearing on the porphyrinogen-corrin connection.

A novel heme-like prosthetic group in a rather widespread class of enzymes which catalyze the six-electron reduction of sulfite to sulfide in *E. coli* (E.C. 1.8.1.1) was first characterized in 1973⁷ and named siroheme. Removal of the iron from this species afforded an orange fluorescent compound—sirohydrochlorin. By modifying our cell-free technique it has been possible to isolate from *P. shermanii* almost 1 mg of an orange fluorescent substance.

Inspection of the UV, CD, mass and ^1H NMR spectral data for the methyl ester of the *P. shermanii* metabolite and of sirohydrochlorin ester from *E. coli* NADPH-sulfite reductase leaves no doubt that the substances, which also show complete correspondence in TLC R_f values, are identical in every respect. The molecular constitution was also confirmed by high resolution mass determination of the molecular ion at m/e 974 which revealed the composition of $\text{C}_{50}\text{H}_{62}\text{N}_4\text{O}_{16}$ (974.4160).

As pointed out by Siegel et al.^{7,8} the UV spectrum of sirohydrochlorin is diagnostic of the isobacteriochlorin class requiring the two reduced rings to be adjacent (see structures **5-8**).

In the ^1H NMR spectrum recorded at 270 MHz the 3 H singlet resonances at δ 1.87 and 1.93 are consistent with the known chemical shifts of methyl groups on the reduced ring of a chlorin.⁹⁻¹¹ Similarly a 2 H multiplet at δ 4.1 also falls within the range of shifts expected for methine hydrogens in such systems.⁹⁻¹² Decoupling experiments showed that the methylene hydrogens of the acetate moieties adjacent to the



reduced ring (δ 2.72–2.75) were not coupled to the δ 4.1 multiplet (or any other resonance). Structures **5** and **8** are, therefore, eliminated as they would require coupling between these two resonances. Furthermore, incubation of 60 mg of [4- ^{13}C]aminolevulinic acid (90% enriched) with the *P. shermanii* cell-free homogenate yielded a purified sample of sirohydrochlorin (400 μg) whose proton-decoupled and off-resonance CW decoupled spectra are in Figure 1. It is clear that only structures **6** and **7** are compatible with the observation of two doublets (C-3 and C-8) (see insert, Figure 1), since structures **5** and **8** would exhibit only one such enriched sp^3 carbon bearing hydrogen. Finally, incubation with [5- ^{13}C]aminolevulinic acid enabled us to show that sirohydrochlorin has

structure **7**. The expected labeling pattern is as shown in Figure 2. In the proton-decoupled spectrum, the C-15 resonance is a triplet ($J = 72$ Hz) due to 1,2 coupling with two adjacent enriched sites. (A lower intensity doublet is also present owing to those molecules having only one adjacent enriched site.) The C-5 and C-10 resonances both occur as doublet of doublets due to 1,2 and 1,4 couplings while C-20 shows only a 1,4 interaction. The four-meso-hydrogen ^1H NMR resonances occurred as doublets ($J = 183$ Hz) at δ 8.54, 7.46, 7.36, and 6.78. As discussed by Bonnett et al.,^{12a} the upfield meso-hydrogen resonance of an isobacteriochlorin may be assigned to that between the reduced (methylated) rings and the downfield resonance to that between the nonreduced rings. Thus the δ 8.54 hydrogen would be coupled to C-15 in structure **7** and to C-20 in structure **6**. The former case was confirmed by a selective heteronuclear decoupling experiment. Additionally, the δ 6.78 meso hydrogen was shown to be coupled to the upfield meso carbon which is therefore C-5.

The structural proposal (**7**) for sirohydrochlorin was examined by two sets of biosynthetic experiments. Specimens of sirohydrochlorin labeled (in $\sim 10\%$ radiochemical yield) by separate incubations⁶ with δ -[4- ^{14}C]aminolevulinic acid and S-[$^3\text{H}_3\text{C}$]adenosylmethionine were purified chromatographically as the octamethyl esters, hydrolyzed, and reincubated as singly and doubly labeled species after sodium amalgam reduction (**7** \rightarrow **9**). The singly labeled species was incorporated by the "corrin synthetase"⁶ preparation into cobyrinic acid **3** (isolated as cobester) in 0.4–1.9% radiochemical yield. Intact incorporation of the reduced version (**9**) is clearly revealed by retention of the $^3\text{H}/^{14}\text{C}$ ratio (3.45) in the isolated cobester (3.41). When *unreduced* **7** was incubated with the corrin synthetase preparation, similar incorporations (0.3–2.8%) were observed. The absolute stereochemistry of **7** is therefore established via bioconversion to corrin.¹³

Other workers^{14,15} have recently isolated a metabolite from *P. shermanii* with similar UV and mass spectral characteristics to those of sirohydrochlorin. The Cambridge group postulated structure **7** for their metabolite after assuming that it was on the B₁₂ pathway and further that ring C was not methylated. This paper¹⁶ shows unequivocally that sirohydrochlorin is indeed identical with a *P. shermanii* metabolite, that it has structure **7**, and that it is an intermediate on the corrin pathway. The possibility that siroheme represents a prebiotic sulfate-reducing agent⁸ and, further, that both sirohydrochlorin and vitamin B₁₂ producing anaerobic organisms predate the evolution of heme-synthesizing aerobes¹⁷ suggest that the reductive methylation of reduced porphyrins may be a phenomenon of considerable antiquity (three billion years).

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Enhanced Chemiluminescence from the Silica Gel Catalyzed Decomposition of a 1,2-Dioxetane

Sir:

In 1969 Kopecky and Mumford first demonstrated that the thermolysis of 1,2-dioxetanes yields electronically excited carbonyl products.¹ It has subsequently been shown that simple isolable 1,2-dioxetanes afford predominantly triplet excited states upon spontaneous decomposition in solution.² Transition metals,³ electron-rich olefins,⁴ and amines⁴ catalyze the cleavage of 1,2-dioxetanes. However, these processes give ground-state products and therefore result in reduced light emission. We now report the first observation⁵ of *enhanced* chemiluminescence associated with the catalytic decomposition of a stable, crystalline 1,2-dioxetane.

2,3-Di(2-anthryl)-1,4-dioxene (**1**,⁶ 100 mg) was photo-oxygenated for 30 min at -78°C in 100 mL of CH_2Cl_2 using 1 g of polymer-bound Rose Bengal⁷ as sensitizer with two 500-W tungsten-halogen lamps and a UV-cutoff filter. The reaction mixture was filtered and concentrated at 10°C on a rotary evaporator to 50 mL. Addition of 1 mL of pentane and cooling to -25°C gave pure **2** as a pale yellow solid in 51% yield: UV (*o*-xylene) λ_{max} 327 nm ($\log \epsilon$ 3.68), 343 (3.83), 361 (3.93), 380 (3.82);⁸ ^1H NMR (100 MHz, CDCl_3 , 0°C) δ 4.51 (m), 4.99 (m), 6.5–8.6 (m, aromatic). Additional **2** could be obtained by concentrating the reaction solution further;⁹ however, this material also contained cleavage product **3**.

Thermolysis of the dianthryl-substituted 1,2-dioxetane **2** in *o*-xylene results in quantitative formation of the diester **3**¹⁰ and is accompanied by light emission. The rates of decomposition of **2**, the formation of **3**, and the emission of light are first order and identical ($k = 6.56 \times 10^{-3} \text{ s}^{-1}$ at 84.1°C). The