intense dipyrrolic and monopyrrolic fragment ions. The empirical formula and the fragmentation pattern are compatible with a mesobilirubinoid tetrapyrrole. Structures that are closely related to mesobiliverdin may be eliminated on the basis both of empirical formulas and fragmentation pattern. The base peak in the mass spectrum of dimethylmesobiliverdin was the parent ion at *m/e* 614, and this compound gave only low intensity dipyrrolic fragment ions.[11] In contrast, the base peak in the spectrum of phycocyanobilin corresponded to a dipyrrolic fragment ion *m/e* 288.1478; $C_{16}H_{20}N_2O_3$ requires 288.1474.

The results of nuclear magnetic resonance analysis are summarized in Table I. The ethylidene group deduced from the nmr data has very recently been reported by Rüdiger to occur in the bile pigment aplysioviolin extracted from the Mediterranean sea hare.[12]

Table I. Nmr[a] Assignments for Phycocyanobilin Based on Chemical Shifts in 0.1 *M* Pyridine-d_5 (Relative to Internal Hexamethylsiloxane (HMS)) and 0.1 *M* Trifluoroacetic acid (TFA) (Relative to External HMS)

Chemical shift, ppm			Relative area,	
Pyridine	TFA	$\|J\|$, cps (±0.1)	nearest integral value	Assignment
1.11	0.99	7.5, triplet	4[b]	CH_3CH_2[c]
1.34	1.37	7.5, doublet	3	CH_3CH[d]
1.58	1.84	7.1, doublet	3	CH_3CH=[e]
1.89	2.02			
1.95	2.06	Singlets	9	CH_3-ring
2.01	2.06			
2.34	2.32	7.6, quartet	2	CH_3CH_2[c]
2.70	2.65	Multiplets	4	-CH_2CH_2COOH
2.97	3.04		4	
3.2	3.43	Multiplet	1	CH_3CH[d]
3.17	...	Singlets	1	CH_3O
3.20	...			
5.71	5.97	Singlet	1	CH=
5.92	6.42	Singlet	1	CH=
6.17	6.62	7.2, 2.1, quartet of doublets	1	CH_3CH=[e]
7.09	7.37	Singlet	1	CH=
11.9[f]	...	Singlet	5	NH and COOH

[a] Nmr spectra determined with a Varian HA-100 spectrometer. [b] High value believed to be due to impurity. [c–e] The spin–spin interactions in these pairs of groups are confirmed by decoupling experiments. [f] Concentration dependent.

A structure consistent with all of our data is shown in Figure 1. The oxidation state assignment is based entirely on the mass spectrometric results and is made on the assumption that no thermally induced internal oxidation–reduction reactions occur in the phycocyanobilin in the mass spectrometer source. The ready exchange of one of the methine protons requires a mobile equilibrium between the keto (bilirubinoid) and enol forms of this bile pigment. The visible and nmr spectra indicate that the enol form predominates in solution. The distribution of side chains is arbitrary and is by analogy with the known bile pigments. It has not been proved that the substance isolated by our procedure actually occurs as such in nature, and the mode of attachment of the chromophore to the protein is still obscure.

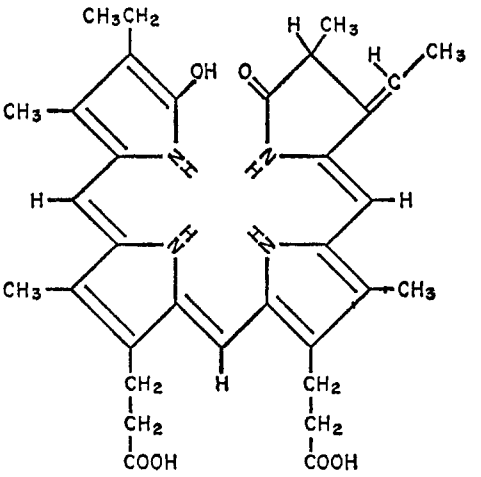

Figure 1. Proposed structure for phycocyanobilin.

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The Structure of Phycocyanobilin[1]

Sir:

C-Phycocyanin, a photosynthetically active algal protein, has a tetrapyrrolic chromophore, phycocyanobilin.[2] Evidence presented here shows the phycocyanobilin is 2-desethyl-2-ethylidene-1-protiomesobiliverdin, an isomer of mesobiliverdin (Figure 1).

Plectonema boryanum (Indiana University 594) cells (1200 g wet weight) were ground in a Waring blender with solid carbon dioxide, and the biliproteins after extractions with 0.1 *M* potassium phosphate buffer (pH 6) were separated from cell debris by centrifugation and precipitated with ammonium sulfate. C-Phycocyanin was chromatographed free of allophycocyanin on a Celite 545 column by ammonium sulfate gradient elution. The solution containing C-phycocyanin was made 1% with respect to trichloroacetic acid; the denatured protein (blue) was removed by centrifugation and washed successively with water (two 250-ml portions) and absolute methanol (four 250-ml portions). The denatured C-phycocyanin was boiled under reflux with absolute methanol (2.5 l.) with stirring for 16 hr, and the phycocyanobilin solution (blue) was filtered free of protein residue (38 g) and concentrated. Boron trifluoride in methanol (14%; 2 ml) was added to the methanolic pigment solution (2 ml), and the mixture was boiled under reflux. After 3 min the solution was cooled, and chloroform (10 ml) and water (100 ml)

(10) A. H. Jackson, G. W. Kenner, H. Budzikiewicz, C. Djerassi, and J. A. Wilson, *Tetrahedron*, 23, 632 (1967).
(11) R. C. Dougherty, to be published. We are indebted to Drs. H. W. Siegelman and John Cole for a sample of dimethylmesobiliverdin.
(12) W. Rüdiger, *Z. Physiol. Chem.*, 348, 129 (1967).

(1) This work was performed at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.
(2) C. Ó hEocha, *Biochemistry*, 2, 375 (1963).

Table I. Nuclear Magnetic Resonance Assignments of (A) Phycocyanobilin Dimethyl Ester and (B) Mesobiliverdin Dimethyl Ester (~0.1 *M* in Pyridine-d_5)

A			B		
Chemical shifts[a]	Relative intensity	Assignment	Chemical shifts	Relative intensity	Assignment
1.10, triplet	3	Methyl of ethyl group	1.05, 1.07 two overlapping triplets	6	2 methyls of ethyl groups
1.33, doublet	3	Methyl of saturated carbon atom C_1			
1.59, doublet	3	Methyl of ethylidene group			
1.84, 1.96 singlets	9	3 β-methyl groups	1.80, 1.86, 1.88 singlets	12	4 β-methyl groups
2.34, quartet	2	Methylene of ethyl group	2.26, quartet	4	2 methylenes of ethyl groups
2.51, 2.54 two overlapping triplets	4	2 α-methylenes of propionic ester groups	2.51, triplet	4	2 α-methylenes of propionic ester groups
2.83, 2.91 two overlapping triplets	4	2 β-methylenes of propionic ester groups	2.84, triplet	4	2 β-methylenes of propionic ester groups
3.19, quartet	1	Proton of saturated carbon atom C_1			
3.46, 3.48 singlets	6	2 methoxyl groups	3.48, singlet	6	2 methoxyl groups
5.70, singlet	1	Methine proton	5.71, singlet	1	Methine proton
5.90, singlet	1	Methine proton	5.76, singlet	1	Methine proton
6.18, quartet of doublets	1	Methine proton of ethylidene group			
6.84, singlet	1	Methine proton	6.93, singlet	1	Methine proton
8.67, broad singlet[b]	3	3 NH protons	7.80, broad singlet[b]	3	3 NH protons

[a] Nmr spectra were determined with a Varian HA-100 nmr spectrometer (facilities kindly provided by Dr. J. J. Katz, Argonne National Laboratory, Argonne, Ill.) Chemical shifts are in parts per million from internal hexamethylsiloxane (HMS) (δ). [b] Chemical shifts (determined using a Varian A-60 nmr spectrometer) of NH protons in parts per million from internal TMS (δ) (pigment concentration ~0.1 *M* in $CDCl_3$); the NH resonances were barely perceptible in the 100-Mc experiment.

were added successively. The pigment extracting into the chloroform was washed with water (two 100-ml portions), filtered, and concentrated (3 ml), and the pigment was purified by thin layer chromatography

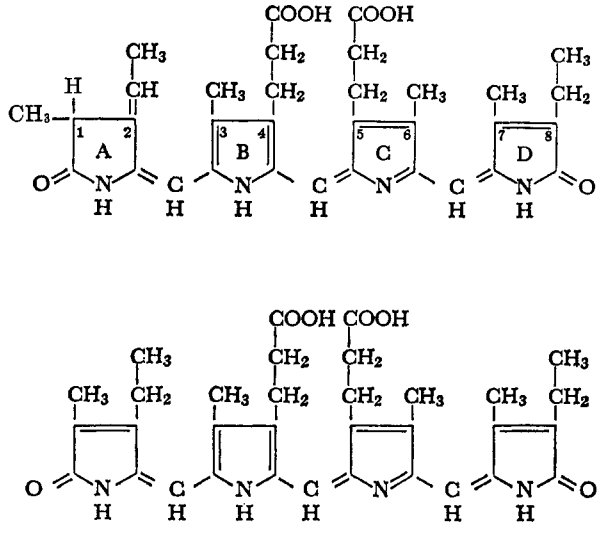

Figure 1. Structures of (top) phycocyanobilin and (bottom) mesobiliverdin.

on silica gel using the solvent system carbon tetrachloride–methyl acetate (10:5, v/v). The main band (deep blue) was scraped from the plate, and the pigment eluted with ethanol which, on concentration, gave phycocyanobilin dimethyl ester (long fibrous hairs). The pigment was recrystallized twice from chloroform–methanol (1:3, v/v) and migrated as a single component on chromatography in benzene–ethanol (10:1, v/v); over-all yield of the dimethyl ester 73 mg; mp 205–206° (uncor). *Anal.*[3] Calcd for $C_{33}H_{42}N_4O_6$: C, 68.38; H, 6.89; N, 9.12. Found: C, 67.94; H, 6.76; N, 9.14.

Mesobiliverdin was prepared by the ferric chloride oxidation of mesobilirubin.[4] The pigment was methylated, purified, and crystallized by the same procedure as that used for phycocyanobilin dimethyl ester; mp 231–231.5° (uncor), lit. mp 232–234° (cor). Phycocyanobilin was similar to but not identical with mesobiliverdin chromatographically and in electronic spectra (λ_{max} 374 (ϵ 47.9 × 10^3), 690 (ϵ 37.9 × 10^3) and 359, 685 mμ, respectively, in 5% HCl–MeOH, w/v).

The pigments were compared by nmr (Table I). The presence of one ethyl group in phycocyanobilin was confirmed when, irradiating at 2.34 ppm, the methyl triplet ($|J|$ = 7.5 cps) at 1.10 ppm collapsed to a singlet. When decoupled by irradiating at the frequency of the methane proton (3.19 ppm), the doublet ($|J|$ = 7.6 cps) at 1.33 ppm collapsed to a singlet, which indicated a methyl group attached to a saturated carbon atom. Irradiation at the frequency of the low-field quartet of doublets (6.18 ppm) caused the high-field doublet ($|J|$ = 7.4 cps) at 1.59 ppm to collapse to a singlet, which indicated a methyl group attached to an unsaturated carbon atom. Furthermore, irradiating at 1.59 ppm, the quartet of doublets at 6.18 ppm collapsed to a doublet, while irradiating at 3.19 ppm, the 6.18 ppm resonance collapsed to a quartet. This indicated that the carbon atom at C_2 contained an ethylidene group in which the methyl group split the methine proton into a quartet ($|J|$ = 7.5 cps) and each

(3) By Schwarzkopf Microanalytical Laboratory.
(4) H. Fischer, H. Baumgartner, and R. Hess, *Z. Physiol. Chem.*, 206, 201 (1932).

quartet peak was split into a doublet ($|J|$ = 2 cps), caused by long-range coupling of the proton at C_1 through the exocyclic double bond. Assignment of the other resonances obtained for phycocyanobilin followed directly from those of mesobiliverdin.

Mass spectral analyses[5] showed both phycocyanobilin and mesobiliverdin dimethyl ester had a strong molecular ion which required the empirical formula $C_{35}H_{42}N_4O_6$ as its base peak at *m/e* 614. The stabilities of the fully conjugated pigment systems were analogous to that of alkylated dipyrrylmethenes[6] in which fragmentation occurred primarily at the side-chain positions rather than at the methine bridge. In the case of the tetrapyrrole pigments most of the peaks with a high *m/e* ratio could be accounted for by the successive fragmentation of the propionic ester side chains. They did, however, show appreciable cleavage at the central methine bridge. Phycocyanobilin differed from mesobiliverdin in that it had an intense peak (97% of base) at *m/e* 599, which would be consistent with cleavage of the methyl group at the saturated carbon atom C_1. The results presented above are in agreement with the structure[7] proposed for phycocyanobilin (Figure 1, top), or with the β substituents of rings A and D interchanged. A tetrapyrrolic structure with the same ring A substituents has recently been proposed for aplysioviolin.[8] The structure for phycocyanobilin is further validated by its simple isomerization, when boiled under reflux with 1 *N* potassium hydroxide in methanol for 15 min, to a product identical, chromatographically, and in melting point, ultraviolet–visible, infrared, and nmr spectra, with mesobiliverdin.

(5) Determined with an AE1 MS-9 mass spectrometer by W. Milne, National Institutes of Health, Bethesda, Md.
(6) A. H. Jackson, G. W. Kenner, H. Budzikiewicz, C. Djerassi, and J. M. Wilson, *Tetrahedron*, **23**, 3642 (1967).
(7) This structure is similar to but not identical with that proposed by H. L. Crespi, L. J. Boucher, G. Norman, J. J. Katz, and R. C. Dougherty, *J. Am. Chem. Soc.*, **89**, 3642 (1967).
(8) W. Rüdiger, *Z. Physiol. Chem.*, **348**, 129 (1967).

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Tri(cyclopentadienylmanganese)tetranitrosyl. A Metal Cluster Compound with Doubly and Triply Bridging Nitrosyl Groups

Sir:

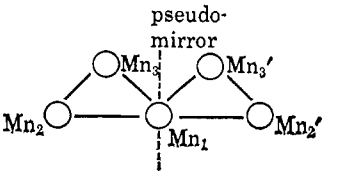
King and Bisnette[1] have reported a compound which they formulated as $(\pi\text{-}C_5H_5)_6Mn_6(NO)_8$, and for which they proposed a cyclohexane-like ring of Mn atoms with six doubly bridging and two triply bridging NO groups (symmetry D_{3d}; should have two NO bands in the infrared). Other hexanuclear structures have also been proposed;[2] none of the structures fits the observed infrared spectrum if the three NO stretching bands (1520, 1475, 1313 cm^{-1} for the solid) are correctly assigned.

(1) R. B. King and M. B. Bisnette, *Inorg. Chem.*, **3**, 791 (1964).
(2) *Cf.* B. F. G. Johnson and J. A. McCleverty, *Progr. Inorg. Chem.*, **7**, 346 (1966). These include a more compact D_{3d} (not S_6) structure and a cubic structure (proposed independently by F. A. Cotton and E. L. Muetterties, assuming a hexanuclear molecule; the cubic structure should have but one NO stretch in the infrared).

Because of the uncertainties regarding the structure of this unusual substance, further investigations were undertaken. A preliminary report is presented here.[3]

Single crystal X-ray diffraction studies have shown that this substance is, in fact, a trinuclear compound, $(\pi\text{-}C_5H_5)_3Mn_3(NO)_4$, containing a triangular array of manganese atoms in which the occurrence of bridging nitrosyl groups, of both the double and triple types, is substantiated for the first time by X-ray methods. Black, seemingly crystalline material, kindly supplied by Dr. R. B. King, was in the shape of hexagonal tablets, but examination with the precession camera showed it to be a microcrystalline mass. Recrystallization of this material yielded a small number of crystals which were twinned. A new batch of the compound was then prepared and recrystallized from a chloroform–pentane mixture. Most of these crystals were also twinned but three single crystals were obtained. The cell constants determined for the twinned crystals of King and the single crystals we prepared are the same, thus confirming the identity of our compound with that of the compound prepared by King. They are $a = 13.34 \pm 0.01$, $b = 7.95 \pm 0.03$, $c = 16.82 \pm 0.01$ A; $\beta = 107.8 \pm 0.1°$. The space group, determined uniquely from the systematic absences, is $P2_1/c$. From the density (1.88 ± 0.05 g cm^{-3}) the unit cell must contain 12 manganese atoms or one-half of the proposed hexanuclear molecule as the asymmetric unit.

Attempts to solve the structure by Patterson techniques showed a pseudo-centering translation in Patterson space and did not yield a useful model of the structure. Attempts to resolve the structure using superposition functions were equally fruitless. The heavy atom structure was finally determined by MAGIC,[4] a digital computer program using the Hauptman–Karle methods to determine the signs of reflections in centric structures. The structure is disordered by the presence of a pseudo-mirror plane perpendicular to the *a* axis at $x = 1/4$. One of the manganese atoms lies approximately in this plane and is reflected into itself appearing at full weight in the Fourier synthesis. The remaining two metal atoms are reflected through this plane to form a second triangle, thus



The last four manganese atoms all appear at about one-half the height of the first one. This solution of a disordered structure seems indicative of the wide usefulness of such methods.

Successive Fourier syntheses computed with signs fixed by the model allowed the resolution of all nonhydrogen atoms except two of the carbon atoms, which, from the ring geometry, can be presumed to superpose on Mn_2 and Mn_2' due to the disorder. Least-squares refinement of positional and temperature parameters

(3) Further refinement of the crystal structure is being carried out by R. C. Elder at the University of Chicago and will be reported later.
(4) E. B. Fleischer, A. L. Stone, and R. B. K. Dewar, "MAGIC—Multiphase Automatic Generation from Intensities in Centric Crystals," program for IBM 7094 computer, University of Chicago, March 1966.