The Structure and Properties of Phycocyanobilin and Related Bilatrienes*

W. J. Cole, D. J. Chapman, and H. W. Siegelman

ABSTRACT: Phycocyanobilin, the chromophore of the biliprotein C-phycocyanin, was isolated from trichloracetic acid denatured protein by boiling under reflux in methanol. The algal pigment was purified and crystallized as the dimethyl ester, and its ultraviolet–visible absorption spectra were compared with those of known bilatriene pigments. Alkaline potassium permanganate oxidation of phycocyanobilin revealed that the two central pyrrole rings contained the same β substituents as the IXα bile pigments. The dimethyl esters of phycocyanobilin (mol wt 614), mesobiliverdin (mol wt 614), and biliverdin (mol wt 610) had similar mass spectral fragmentation patterns. The structural differences between phycocyanobilin and mesobiliverdin were proven and confirmed by nuclear magnetic resonance comparisons and decoupling experiments. The facile isomerization of phycocyanobilin in alkaline media to a product identical with mesobiliverdin validated the structure proposed for phycocyanobilin. Within experimental error the optical activity of phycocyanobilin isolated from several sources was identical, $[\alpha]_D^{CHCl_3}$ +660°. It is proposed that regardless of the source, phycocyanobilin probably has the same structure and stereochemical configuration. There is a marked difference between the optical rotatory dispersion of phycocyanobilin and that of phycocyanin.

The photosynthetically active blue protein, C-phycocyanin, is the principal biliprotein of blue-green algae and certain red algae. It contains a bile pigment chromophore, phycocyanobilin (PCB).[1] Characterization of this pigment (Lemberg, 1928, 1930; Lemberg and Bader, 1933; Ó hEocha, 1963) has proved difficult because of poor yields and instability in the acidic media used. Fujita and Hattori (1962) obtained a blue pigment from algal cells by short-term extraction with hot methanol. We have isolated PCB from C-phycocyanin by a modification of their procedure. The complete characterization and properties of PCB and its relationships to the known bile pigments, mesobiliverdin and biliverdin, obtained by modern analytical techniques, are presented here. A preliminary report of these studies has appeared (Cole *et al.*, 1967).

Materials and Methods

Crystalline bilirubin was obtained from the Nutritional Biochemical Corp. 4-Methyl-3-carboxyethylpyrrole-2,5-dicarboxylic acid (I), 4-methyl-2-ethylpyrrole-2,5-dicarboxylic acid (II), and 4-methylpyrrole-2,3,5-tricarboxylic acid (III) were gifts from Dr. Z. Petryka, Northwestern Hospital, Minneapolis, Minn.

Chromatography. Thin-layer chromatography plates were prepared from silica gel (Absorbosil 5; Applied Science Laboratories, State College, Pa.) with a Desaga spreader. Samples were applied to the plates with a Radkin-Pellick streaker (Applied Science Laboratories). Solvent systems used (ascending) were: (A) carbon tetrachloride–methyl acetate (10:5, v/v), (B) benzene–ethanol (10:1, v/v), (C) ethanol–ammonia (*d* 0.88)–water (10:0.5:0.5, v/v), (D) carbon tetrachloride–glacial acetic acid (10:3.5, v/v), and (E) 1,2-dichloroethane–glacial acetic acid (5:1, v/v).

The blue-green algae *Plectonema boryanum*, *Phormidium luridum*, and *Calothrix membranacea* were grown in the synthetic D medium of Kratz and Meyer (1955) by the mass culture technique of Lyman and Siegelman (1967). The algal cells (1200 g wet weight) were frozen, thawed, and ground in a Waring Blendor together with solid carbon dioxide. The biliproteins were extracted with 0.1 M potassium phosphate buffer (pH 7). The cells were removed by centrifugation and reextracted (*ca.* four times) for additional biliprotein. The extracts were pooled, and the biliproteins were precipitated by the addition of ammonium sulfate to 50% saturation. The precipitated protein was made into a slurry with Celite 545 (Johns Manville Co.), poured into a column (15 × 30 cm), and washed with 55% saturated ammonium sulfate solution until the eluent was colorless. C-Phycocyanin was isolated free of allophycocyanin by ammonium sulfate elution chromatography with a linear gradient of 55% saturated ammonium sulfate solution (5 l.) and of 5% saturated ammonium sulfate solution (5.5 l.). The C-phycocyanin solution was made 1% with respect to trichloracetic acid, and the resulting precipitate was collected by centrifugation. The denatured protein (blue) was washed in the centrifuge with water (two 250-ml portions) to remove salts and with absolute methanol (four 250-ml portions) to remove yellow impurities. Ammonium sul-

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[1] Abbreviation used that is not listed in *Biochemistry 5*, 1445 (1966), is: PCB, phycocyanobilin.

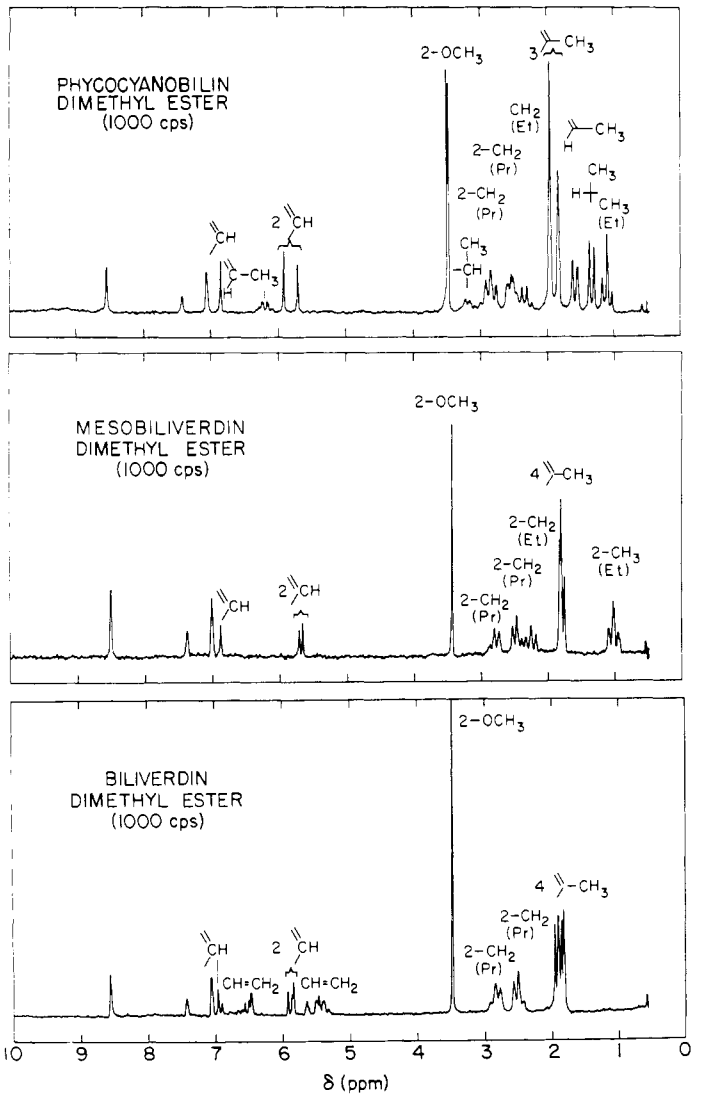


FIGURE 1: Nuclear magnetic resonance spectra of bilatrienes (approximately 0.1 M in pyridine-d_5). Chemical shifts are in parts per million (δ) from internal hexamethylsiloxane.

fate solutions of C-phycocyanin obtained by column chromatography were also used to prepare native crystalline protein. After precipitation with excess ammonium sulfate, the biliprotein was collected by centrifugation and redissolved in water, and the solution was brought to 15% saturation by the slow addition of saturated ammonium sulfate solution. Crystalline C-phycocyanin was obtained from the cooled solution (4°) after 24 hr.

Isolation of PCB Dimethyl Ester. PCB was split from C-phycocyanin by boiling the denatured protein (isolated from 1200 g of wet cells) under reflux with absolute methanol (2.5 l.), with stirring, for 16 hr. The methanol solution (blue) was filtered free of protein residue (green, 38 g) and concentrated. The pigment was converted into the dimethyl ester by boiling under reflux with 7% boron trifluoride in methanol (5 ml) for 3 min. Chloroform (10 ml) and water (100 ml) were added successively to the cooled solution, and the pigment was extracted into chloroform. The chloroform solution was washed with water (two 250-ml portions), filtered through chloroform-moistened filter paper, and concentrated (3 ml). The pigment was purified by thin-layer chromatography with solvent system A. The main band (deep blue) was scraped from the plate (approximately 18 minor bands detected) and eluted from the silica with chloroform and ethanol. The pigment crystallized as long fibrous hairs on concentration. Two recrystallizations from chloroform–methanol (1:3, v/v) gave small blue needles of PCB dimethyl ester; yield 73 mg.

Anal. Calcd for $C_{35}H_{42}N_4O_6$ (614.7): C, 68.38; H, 6.89; N, 9.12. Found: C, 67.94; H, 6.76; N, 9.14; mp 205–206° uncor.

Preparation of Mesobiliverdin Dimethyl Ester. Mesobilirubin was prepared by the hydrogenation of bilirubin (3 g) dissolved in 0.1 N sodium hydroxide (160 ml) in the presence of 10% palladium on charcoal (0.8 g) according to the method of Fischer (1914). The pigment was crystallized from glacial acetic acid and recrystallized from pyridine; yield 1 g; $\lambda_{max}^{CHCl_3}$ 433 nm. The mesobilirubin (375 mg) dissolved in glacial acetic acid (250 ml) was then oxidized with ferric chloride (0.5 g in glacial acetic acid, 8 ml) to mesobiliverdin (Fischer *et al.*, 1932). The pigment was converted into the dimethyl ester and purified by the same method as PCB dimethyl ester. The material separated into a faint blue leading band, a major deep blue band, and a minor blue trailing band. The major band was removed from the plate, and the pigment was eluted with ethanol and evaporated to dryness. Two recrystallizations from chloroform–methanol (1:5, v/v) gave mesobiliverdin dimethyl ester (230 mg), mp 231–234° cor.

Preparation of Biliverdin Dimethyl Ester. Bilirubin (200 mg) in absolute methanol (260 ml) was boiled under reflux with 20% solution of ferric chloride in 12 N hydrochloric acid (14 ml) for 1 hr. To the cooled solution, chloroform (50 ml) and water (500 ml) were successively added. The pigment extracting into the chloroform was washed with water (two 250-ml portions), filtered, concentrated, and purified by thin-layer chromatography in solvent system B. The material separated into two green bands, which were removed from the plate, and the pigments were eluted with ethanol. The major band was recrystallized twice from chloroform–methanol (1:2, v/v) and proved to be biliverdin dimethyl ester (60 mg), mp 205–206°, lit. (Siedel, 1960) mp 206–209°. The minor band was recrystallized with difficulty from chloroform–methanol, mp >300°.

Isomerization of PCB. PCB dimethyl ester (500 mg) was boiled under reflux in the presence of 1 N potassium hydroxide in methanol (20 ml) for 15 min. After neutralization of the solution with glacial acetic acid, the pigment was extracted into chloroform, converted into the dimethyl ester, and purified by the same method as the PCB dimethyl ester. The major pigment, recrystallized from chloroform–methanol (1:3 v/v), migrated as a single component on cochromatography with mesobiliverdin in solvent systems A and B. Both compounds had identical ultraviolet–visible, infrared, and nmr spectra.

Oxidation of PCB. PCB dimethyl ester (33 mg) was suspended in 2 N potassium carbonate (5 ml) and oxidized at room temperature by the gradual addition of a saturated aqueous solution of potassium permanganate. The α,α'-pyrrolecarboxylic acids obtained were extracted according to the method of Nicolaus (1960). The oxidation products were compared by thin-layer chromatography in solvent systems C, D, and E with the authentic pyrrolic acids I, II, and III. They were identified as violet to red-violet zones by successively spraying the chromatograms with diazotized sulfanilic acid and 1 N sodium hydroxide. PCB gave only one oxidation product, which was identical with the pyrrolic acid I.

Nuclear Magnetic Resonance Studies. The principal bilatriene spectra were determined with a Varian HA-100 nuclear magnetic resonance spectrometer. The chemical shifts (parts per million, δ) were internally referenced to hexamethylsiloxane (pigment concentration approximately 0.1 M in pyridine-d_5). The chemical shifts of the NH protons were determined with a Varian A-60 nuclear magnetic resonance spectrometer internally referenced to tetramethylsilane (pigment concentration approximately 0.1 M in deuteriochloroform).

Mass spectra were determined with an AEI MS-9 mass spectrometer using a "direct" inlet system heated to 200–250° and an ionizing voltage of 70 eV.

Optical rotatory dispersion curves were determined with a Cary 60 recording spectropolarimeter. Measurements were made on solutions in sealed quartz cells, 10-mm path length. The maximum absorption of the solutions in the 550–650-mm spectral region ranged from 1.1 to 1.6 absorbance units. The maximum rotations observed were in the range 0.01–0.03°. The base line was recorded by using the appropriate blank solution in the cell.

Absorption spectra were determined with a Cary 14 spectrophotometer.

Results

Nuclear Magnetic Resonance. The nuclear magnetic resonance spectra of the dimethyl esters of PCB, mesobiliverdin, and biliverdin are shown in Figure 1. The three peaks at lowest field are due to solvent impurity. The areas under the curves were calculated from integrated spectra by using the strong methoxyl (6 H) resonances (3.46–3.48 ppm) as the internal standard. The resonances of the two β-ethyl groups of mesobiliverdin were confirmed by irradiation at the frequency of the methylene quartet (4 H) at 2.26 ppm: the two overlapping methyl (6 H) triplets ($^1J_1$ = 7.6 cps) at 1.05 ppm collapsed to two singlets. When decoupled by irradiation at the frequency of the methylene quartet (2 H) (2.34 ppm) of PCB, the methyl triplet (3 H, $^1J_1$ = 7.5 cps) at 1.10 ppm collapsed to a singlet, which confirmed the presence of a β-ethyl group. Biliverdin gave no resonances at high field due to ethyl groups, but showed a series of multiplets at low field due to two vinyl groups ($CH{=}CH_2$ multiplets (4 H) range from 5.32 to 5.66 ppm; $CH{=}CH_2$ multiplets (2 H) range from 6.46 to 6.78 pp). The ABX patterns were not resolved. Phycoerythrobilin, an algal bile pigment having one vinyl group (Chapman *et al.*, 1967a), has one multiplet (2 H) at 5.33 ppm ($CH{=}CH_2$) and another (1 H) at 6.25 ppm ($CH{=}CH_2$) in deuteriochloroform, internally referenced to tetramethylsilane. The relationship between the two methyl groups (doublets at 1.33 and 1.59 ppm), the methane proton (quartet at 3.19 ppm) at high field, and the methine proton (quartet of doublets at 6.18 ppm) at low field of PCB was confirmed by decoupling experiments (Figure 2). The doublet (3 H, $^1J_1$ = 7.6 cps) at 1.33 ppm collapsed to a singlet when irradiated at 319 cps, and the doublet (3 H, $^1J_1$ = 7.4 cps) at 1.59 ppm collapsed to a singlet when irradiated at 6.8 cps. Furthermore, the quartet of doublets at 6.18 ppm collapsed to a doublet and to a quartet when irradiated at the frequencies of 159 and 319 cps, respectively. The resonances of the NH proton absent in the 100-Mc spectra were observed as broad singlets (3 H) at 8.67, 7.80, and 7.90 ppm (from internal tetramethysilane) for PCB, mesobiliverdin, and biliverdin, respectively, when measured in deuterichloroform with a 60-Mc spectrometer.

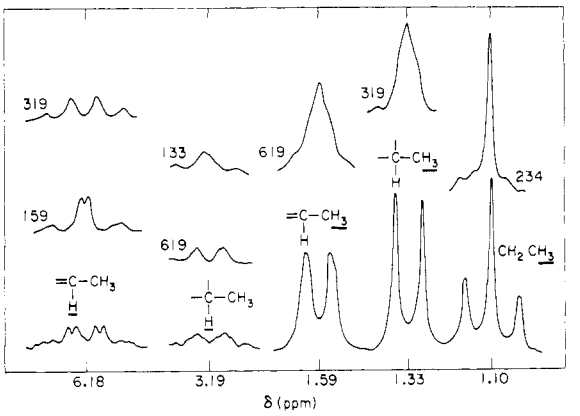


FIGURE 2: Partial nuclear magnetic resonance spectrum (bottom) of phycocyanobilin (in pyridine-d_5) together with decoupled resonances and irradiation frequencies (cycles per second).

Mass Spectrometry. The mass spectra of dimethyl esters of PCB, mesobiliverdin, and biliverdin are shown in Figures 3–5, respectively. The pigments exhibited a strong molecular ion as their base peak. The molecular ions at *m/e* 614 for PCB and mesobiliverdin required the empirical formula $C_{35}H_{42}N_4O_6$; the molecular ion at *m/e* 610 for biliverdin required the empirical formula $C_{35}H_{38}N_4O_6$. The probable fragmentations involved are given in Figure 6. Since the principal fragments of PCB and mesobiliverdin are the same, the possible structures only of those derived from mesobiliverdin and biliverdin are shown. The peaks with a high *m/e* ratio are accounted for by the successive fragmentation of the propionate side chains (CH_3, OCH_3, $COOCH_3$, CH_2COOCH_3, and $CH_2CH_2COOCH_3$). For mesobiliverdin the existence of metastable peaks, *m** 584 (614–599), calcd 584.4, and *m** (614–583), calcd 553.5, confirmed the order M − CH_3 − OCH_3. The ions *m/e* 454 and 450 for mesobiliverdin and biliverdin, respectively, may represent cleavage from both propionate groups

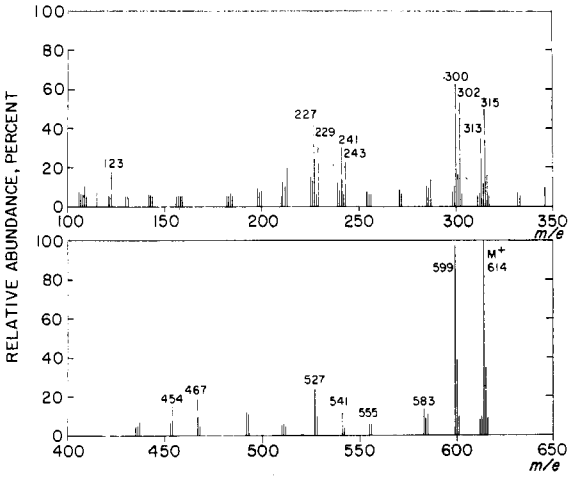


FIGURE 3: Mass spectrum of phycocyanobilin dimethyl ester (relative abundance >5%).

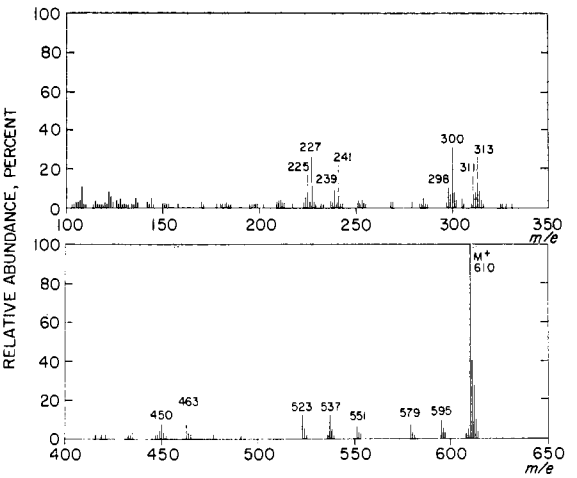


FIGURE 5: Mass spectrum of biliverdin dimethyl ester (relative abundance >2%).

($-CH_2COOCH_3$ + CH_2COOCH_3); however, the nature of the respective ions at *m/e* 467 and 463 is obscure. PCB differed from mesobiliverdin in having an intense ion (97% of base at *m/e* 599 (M − CH_3). The pigments show appreciable fragmentation by rupture at the central methine bridge carbon atom. Each dipyrrolic fragment shown in Figure 6 could arise from both halves of the tetrapyrrole. There is no indication from mass spectra which ion is favored, and only one has been indicated for convenience. Other dipyrrolic ions, at *m/e* 227, 229, 241, and 243 for PCB and mesobiliverdin and *m/e* 225, 227, 239, and 241 for biliverdin, may be derived by further fragmentation of the propionate side chains of the ions V, VI, VII, and VIII. No peaks are observed between *m/e* 350 and 400, which indicates the absence of ions representative of the central dipyrrylmethene unit. The ion at *m/e* 123 for mesobiliverdin, however, may be represented by the monopyrrole IX.

Optical Rotatory Dispersion. The optical rotatory dispersion curves of C-phycocyanin (750–215 nm) and PCB (750–250 nm) isolated from *Ph. luridum* are shown in Figure 7. The curve for C-phycocyanin, although similar to that reported by Boucher *et al.* (1966), is presented here for convenient comparisons. It shows a positive Cotton effect at long wavelengths and a negative Cotton effect at shorter wavelengths, associated with the red and blue absorption bands of the chromophore. The positive Cotton effect (amplitude 1025 ± 200°) has a peak at 635 nm and a trough at 535 nm with a crossover point at 610 nm (λ_{max} 620 nm in 0.01 M phosphate buffer, pH 7). The negative Cotton effect (amplitude 700 ± 200°) has a peak at 322 nm and a trough at 388 nm with a crossover point at 333 nm (subsidiary λ_{max} 360 nm). A large trough ($[\alpha]_D$ 3650 ± 200°) at 227 nm probably arises from the protein α-helix configuration and has a crossover point at 219 nm.

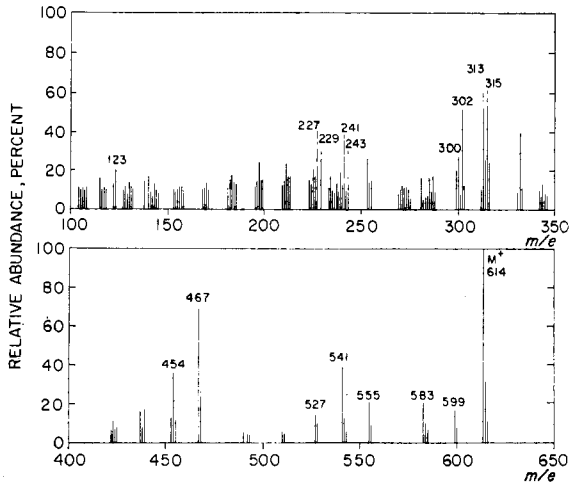
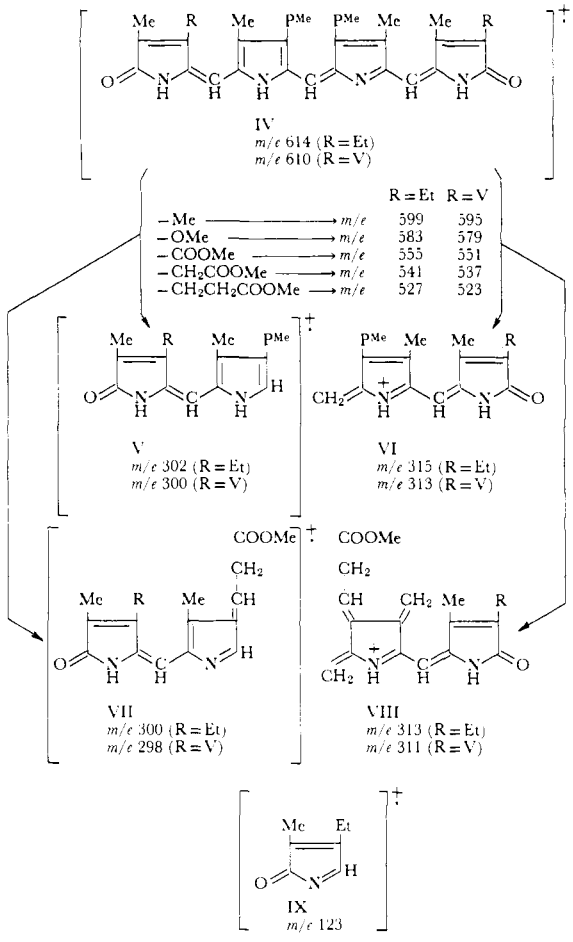


FIGURE 4: Mass spectrum of mesobiliverdin dimethyl ester (relative abundance >5% from *m/e* 650 to 250, and >10% from *m/e* 250 to 100).

The optical rotatory dispersion curves for PCB derived from *P. boryanum* and *C. membranacea* were identical in shape and magnitude, within experimental error, with those for PCB isolated from *P. luridum* and shown in Figure 7. The optical rotatory dispersion curve of PCB ($[\alpha]_D^{CHCl_3}$ +660°) is almost the mirror image of the protein curve. It has a negative Cotton effect at long wavelengths and a positive Cotton effect at short wavelengths. The negative Cotton effect (amplitude 1200 ± 200°) has a peak at 615 nm and a trough at 688 nm with a crossover point at 650 nm (λ_{max} 600 nm in $CHCl_3$). The positive Cotton effect (amplitude 1900 ± 200°) has a peak at 384 nm and a trough at 325 nm with a crossover point at 361 nm (λ_{max} 368 nm in $CHCl_3$).

Discussion

The absorption spectra of PCB in acid media strongly resemble those of the known bilatrienes, mesobiliverdin and biliverdin (Table I). In free-base form phycocyanobilin has spectral characteristics intermediate between those of a violin and a verdin. Comparison of the absorption maxima of the free base and the zinc complex, and of the fluorescence emission maxima of the zinc complexes (W. J. Cole, D. J. Chapman, and H. W. Siegelman, in preparation), indicates an increasing order



Me=CH_3; Et=CH_2CH_3; V=$CH{=}CH_2$; P^{Me}=$CH_2CH_2COOCH_3$.

FIGURE 6: Possible structures of the principal ions obtained from the fragmentation of mesobiliverdin (R = CH_2CH_3) and biliverdin (R = $CH{=}CH_2$).

of conjugation going from PCB to mesobiliverdin to biliverdin.

Oxidation. The α,α'-pyrroledicarboxylic acids, I and II or I and III, can arise from the four pyrrole rings of mesoporphyrin and protoporphyrin, respectively, on oxidation with alkaline permanganate. Only the two central rings of linear tetrapyrroles, however, will give similar products on oxidation (Gray *et al.*, 1958). Therefore the isolation of 4-methyl-3-carboxyethylpyrrole-2,5-dicarboxylic acid (I) from the alkaline permanganate oxidation of PCB confirms that the β positions of its two central rings contain methyl and propionic acid substituents.

Mass Spectrometry. The mass spectra of the dimethyl esters of PCB, mesobiliverdin, and biliverdin, which have intense molecular ions as base peak, bear a strong resemblance to those of dipyrrylmethenes containing β-alkyl substituents (Jackson *et al.*, 1967). The dipyrrylmethenes are particularly stable toward cleavage at the linking methine-bridge carbon atom and show ions due principally to fragmentation of the β substituents. The presence of metastable peaks in the tetrapyrrole spectra confirmed that the ions with a high *m/e* were obtained by successive fragmentation of the β-propionic

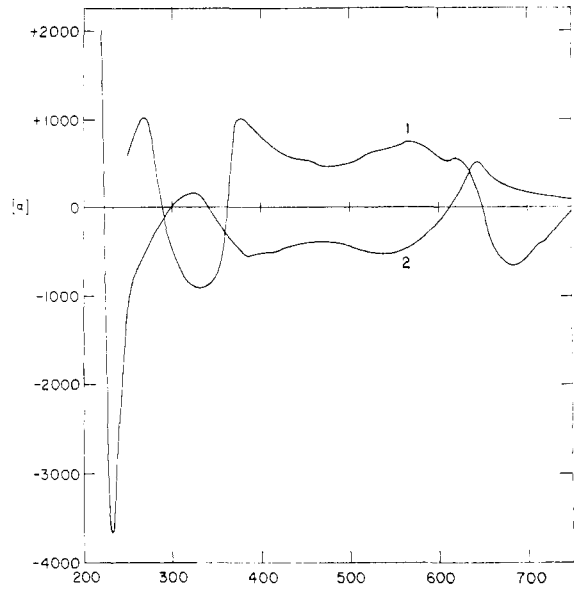


FIGURE 7: Optical rotatory dispersion curves of (1) phycocyanobilin (*ex P. luridum*) in chloroform (3.94 mg/100 ml) and (2) C-phycocyanin (*ex P. luridum*) in 0.01 M potassium phosphate buffer (pH 7) (14.7 mg/100 ml).

acid ester side chains. The intense peak (97% of base) at *m/e* 599 for PCB, however, could also represent cleavage of a nuclear methyl group from a saturated carbon atom. The bile pigments do show some cleavage at the central methine bridge as indicated by the ions V, VI, VII, and VIII (Figure 6), although these structures can result only from considerable hydrogen transfer. They undergo further fragmentation at the propionate side chains. Similar dipyrrolic structures have been proposed for the ions derived by cleavage at the relatively weak methylene bridge of bilirubin (Jackson *et al.*, 1967). Concomitant with the relative stability of the methine bridges of PCB, mesobiliverdin, and biliverdin is the absence of strong peaks arising from tripyrrolic fragments and dipyrrolic fragments representative of the two central rings. The structures of ion fragments presented in Figure 6 are in accord with the known structures XIa and b (Figure 8) for mesobiliverdin (Siedel, 1935) and biliverdin (Fischer and Plieninger, 1942), respectively, although they were originally formulated as bis-lactim compounds.

Nuclear Magnetic Resonance. The presence of a β-ethylidene group in PCB was confirmed by the decoupling experiments; the methyl doublet at 1.59 ppm collapsed to a singlet when irradiated at the frequency of the methine proton (quartet of doublets) at 6.18 ppm. The methyl group (doublet) at 1.33 ppm was shown to be attached to a saturated carbon atom by irradiation at the frequency of the methane quartet at 3.19 ppm. The results indicate that the ethylidene group and the methyl group (1.33 ppm) are situated on the same pyrrole ring, since the quartet of doublets at 6.18 ppm collapsed to a quartet and to a doublet when irradiated at 3.19 and 1.59 ppm, respectively. Thus the methyl of the ethylidene group splits the methine proton into a quartet ($^1J_1$ = 7.5 cps), and each quartet peak is split into a doublet ($^1J_1$ = 2 cps) by long-range coupling to the pro-

TABLE I: Absorption[a] and Fluorescence Maxima (nanometers) of Bilatrienes and Derivatives.

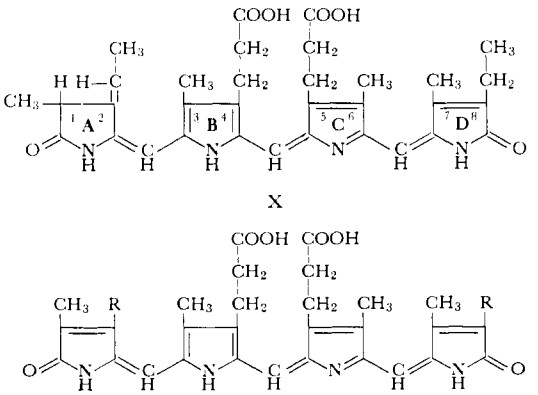
Bilatriene Dimethyl Ester	5% Pyridine in $CHCl_3$	E_2/E_1[b]	Acid[c] $CHCl_3$	E_2/E_1	5% HCl in MeOH (w/v)	E_2/E_1	Zn Complex in EtOH	E_2/E_1	Zn Complex Fluorescence
Phycocyanobilin	*367*, 590	2.72	*375*, 662	0.94	*374*, 690	1.26	*375*, 664	1.01	695
Mesobiliverdin	*368*, 635	3.30	*362*, 655	1.22	*359*, 655	1.73	*367*, 685	1.31	713
Biliverdin	*377*, 660	3.40	*380*, 668	1.33	*375*, 695	2.01	*383*, 705	1.30	733

[a] Principal wavelength maximum in italics. [b] E_2/E_1 is the calculated ratio of the two absorption maxima. [c] Chloroform solution shaken with 3 N hydrochloric acid.

ton attached to the saturated carbon atom. Such a configuration is given in ring A of structure X for PCB (Figure 8). The decoupling experiments indicate that the proton at C_1 and the proton of the ethylidene group are in a *cis* configuration relative to one another. The ethyl group confirmed by decoupling is assigned position 8 in ring D; however, it is possible that the β substituents of rings A and D may be interchanged. Resonances (3 H) due to the NH protons obtained from the 60-Mc nuclear magnetic resonance spectra indicate that the three pigments contain one pyrrolenine nitrogen atom. The assignments of the other three nuclear methyl groups (1.84 and 1.96 ppm), the methylenes of the propionate groups ($CH_2CH_2COOCH_3$ (4 H) at 2.51 and 2.54 ppm; $CH_2CH_2COOCH_3$ (4 H) at 2.83 and 2.91 ppm), and the bridge methine protons (5.70, 5.90, and 6.84 ppm) of PCB are by analogy similar to those of mesobiliverdin and biliverdin.

Substitution of the β substituents of ring A of PCB into the corresponding ion structures proposed for the fragmentation of mesobiliverdin (Figure 6) satisfies the fragmentation pattern of PCB. The resonances due to the three methine protons confirm that PCB is a bilatriene, and its proposed structure, X, is further validated by its facile isomerization, in the presence of base, to mesobiliverdin. The pigment PCB obtained by the methanol reflux of C-phycocyanin is identical with that obtained by enzymic cleavage (Siegelman *et al.*, 1967) and is considered the native chromophore. Crespi *et al.* (1967) have proposed a similar but not identical structure for the algal pigment. Oxidative degradation of native C-phycocyanin and characterization of the resulting imides led Rüdiger *et al.* (1967) to propose a structure identical with X for PCB. With an asymmetric carbon atom at position C_1, the possibility of PCB being optically active was confirmed by optical rotatory dispersion studies. The pigment is the first bilatriene from either natural or synthetic sources known to possess optical activity ($[\alpha]_D^{CHCl_3}$ +660°). The crossover point at 650 nm of the negative Cotton effect is unusually distant from the corresponding absorption maximum (600 nm). It is clear that the configuration of the native protein and the environment it imposes on the chromophore drastically affect the rotation of the pigment, the negative Cotton effect of the isolated chromophore being completely reversed to a positive Cotton effect. These results partially explain the reduction of optical activity in the long-wavelength region of urea-denatured C-phycocyanin (Boucher *et al.*, 1966).

C-Phycocyanin from a variety of blue-green algae (Chapman *et al.*, 1968), allophycocyanin, and R-phycocyanin (Chapman *et al.*, 1967b) all possess the same chromophore, PCB. Since the optical rotatory dispersion curves of PCB isolated from three sources of C-phycocyanin were identical, it is probable that the algal pigment has the same structure and stereochemical configuration, regardless of its source.



X

XI a, R = $-CH_2CH_3$; b, R = $-CH{=}CH_2$

FIGURE 8: Structures of phycocyanobilin (X), mesobiliverdin (XIa), and biliverdin (XIb).

Acknowledgments

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