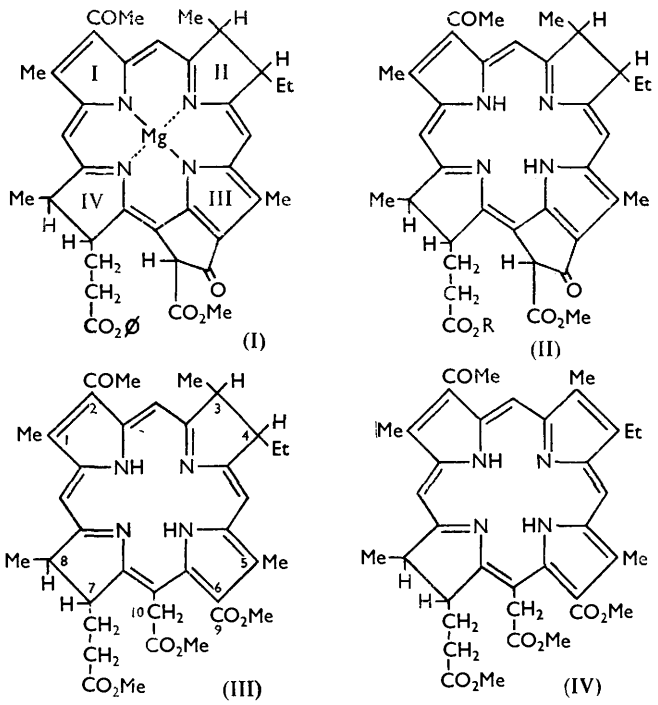
355. *Chlorophyll and Related Compounds. Part VII.* The Structure of Bacteriochlorophyll.*

By J. H. Golden, R. P. Linstead, and G. H. Whitham.

Hans Fischer's structure for bateriochlorophyll (I) has been strengthened and some aspects of its stereochemistry have been elucidated. Stepwise dehydrogenation of a bacteriochlorophyll derivative to the corresponding chlorin and porphin is described, together with some observations on the light absorption of the pigments.

The red and the purple photosynthetic bacteria contain, in addition to carotenoids, one chlorophyll component, bacteriochlorophyll, which appears to be associated in the cell with different proteins. The chlorophyllic component of the green sulphur bacteria appears to be a different substance.



The chemistry of bacteriochlorophyll has been extensively investigated by Fischer and his co-workers. Their structure [1] (I) is supported by evidence which may be summarised as follows: Conversion into bacteriochlorin e_6 trimethyl ester (III) *via* bacteriomethylphæophorbid (II; R = Me), analogous to similar transformations of chlorophyll-*a*, was followed by dehydrogenation [2] to the chlorophyll derivative (IV). Partial synthesis of this compound (IV) from chlorophyll [3] demonstrated that bacteriochlorophyll is a partially hydrogenated chlorophyll-*a* bearing an acetyl group at position 2 in place of a vinyl group.

* Part VI, *J.*, 1957, 733.

[1] Fischer-Orth, " Die Chemie des Pyrrols," Akademische Verlagsges., Leipzig, 1940, Vol. II, Part 2, p. 305.

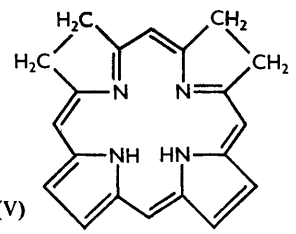
[2] Fischer, Lambrecht, and Mittenzwei, *Z. physiol. Chem.*, 1938, **253**, 1.

[3] Fischer, Lautsch, and Lin, *Annalen*, 1938, **534**, 1.

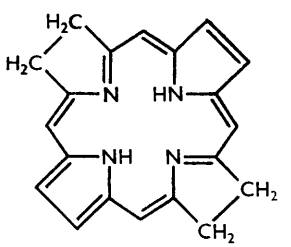
The precise level of hydrogenation of bacteriochlorophyll was not determined although it was assumed to be the tetrahydroporphin (dihydrochlorin), shown in the formulæ.

The two " extra " hydrogen atoms in chlorophyll have been proved [4] to be in ring (IV). As to the location of the assumed second pair of " extra " hydrogen atoms in bacteriochlorophyll direct evidence is scanty and interpretation conflicting. Mittenzwei [5] suggested ring II on the grounds that oxidative degradation of a bacteriochlorophyll derivative yielded small amounts of an oil which was believed to be ethylmethylsuccinic anhydride, which was not obtained on oxidation of chlorophyll-*a* derivatives. Seely [6] criticised this deduction and made the improbable suggestion that the ethylmethylsuccinic anhydride arose from ring IV, by decarboxylation of the propionic acid side-chain. Seely proposed that the " extra " hydrogen atoms are on ring I or III, probably the latter. Barnard and Jackman [7] recently obtained important theoretical evidence which bears on this problem. Their molecular-orbital calculations on the position of the longest-wavelength band in the absorption spectra of the two possible types of tetrahydroporphins (V) and (VI) supported a structure of type (VI) for bacteriochlorophyll. Eisner's recent work [8] on the hydrogenation product of octaethylporphin is also relevant.

The present work, based on techniques already successful in the chlorin and the chlorophyll field, is divided into two main parts; (i) stepwise dehydrogenation of bacteriochlorin e_6 trimethyl ester (III) to provide unequivocal information as to the precise level of hydrogenation, and (ii) re-investigation of the degradative oxidation of bacteriochlorophyll derivatives with the object of identifying fragments derived from ring II. The light absorption of some of the pigments will also be briefly discussed.



(V)



(VI)

Dehydrogenations.—Although the ester (III) had been dehydrogenated to the chlorin (IV) by Fischer and his co-workers [2] their method was not attractive for quantitative purposes. Dehydrogenation by quinones of high redox potential by the general methods of Braude, Jackman, and Linstead,[9] as applied to hydroporphins by Eisner and Linstead,[10] was therefore used. The bacteriochlorin ester (III) in dry benzene solution at 20° with one mol. of 2 : 3-dichloro-5 : 6-dicyanobenzoquinone afforded an almost quantitative yield of the dihydro-ester (IV), whose absorption spectrum (see Table) agreed satisfactorily with that quoted by Stern and Pruckner.[11] Quantitative experiments showed unambiguously that the bacteriochlorophyll series contains two more hydrogen atoms than the chlorophyll series.

It is of interest that only one dehydro-compound is formed since *a priori* it might be expected that either the pair of hydrogen atoms characteristic of bacteriochlorophyll (in ring II ?) or those in ring IV would be abstracted by the quinone. Indeed it could not quite be taken for granted that the hydrogen would come off in 1 : 2-pairs and not in some other way. Consideration of the model for ester (III) shows that in the transition state

[4] Ficken, Johns, and Linstead, *J.*, 1956, 2272.
[5] Mittenzwei, *Z. physiol. Chem.*, 1942, **275**, 93.
[6] Seely, U.S. Atomic Energy Commn., 1953, U.C.R.L., 2417.
[7] Barnard and Jackman, *J.*, 1956, 1172.
[8] Eisner, *J.*, 1957, 3461.
[9] Braude, Jackman, and Linstead, *J.*, 1954, 3548.
[10] Eisner and Linstead, *J.*, 1955, 3749.
[11] Stern and Pruckner, *Z. phys. Chem.*, 1939, **185**, *A*, 140.

for dehydrogenation of ring IV the methylene group attached to position 7 must become coplanar with the macrocyclic ring, resulting in considerable steric compression between this methylene group and $C_{(10)}$ (also coplanar). Such compression is not involved in the transition state for dehydrogenation of ring II which is not flanked by a *meso*-substituent.

Further dehydrogenation of the acetylchlorin (IV) by the dichlorodicyanoquinone was slow, even at raised temperatures. However, this provided a convenient preparation of the corresponding porphin, oxochloroporphyrin e_6 trimethyl ester,[12] when excess of quinone was used. A quantitative study was impracticable owing to the fairly rapid rate of destruction of the quinone under these conditions.

The copper derivative of the acetylchlorin (IV) proved to be more readily dehydrogenated and was amenable to quantitative study, which showed that two hydrogen atoms are abstracted in conversion to the porphin level. It is believed that this represents the first quantitative dehydrogenation of a natural chlorophyll derivative, although it has already been realised with the simple chlorins.[10]

Oxidative Degradation.—The ester[13] (III) was chosen as the most suitable bacteriochlorophyll derivative for degradation as it could be fairly readily obtained in a chromatographically pure, crystalline state. If structure (III) is correct then by analogy with similar oxidations of macrocyclic pigments[4,14] it would be expected that oxidative degradation of ester (III) should lead to ethylmethylsuccinimide from ring II, and dihydrohæmatinimide from ring IV. Rings I and III would be expected to undergo more extensive degradation.

Oxidation of ester (III) by chromium trioxide in sulphuric acid under the conditions used by Ficken, Johns, and Linstead[4] was followed by separation of the product into neutral and acidic fractions. The neutral fraction was examined by paper chromatography, the spots being detected by an imide spray:[15] an intense doublet was formed, corresponding closely to ethylmethylsuccinimide plus ethylmethylmaleimide, in addition to a faint spot corresponding to dihydrohæmatinimide. The ethylmethylmaleimide could arise by dehydrogenation of ring II before degradation. An infrared spectrum of the crude neutral fraction (in $CHCl_3$) exhibited strong imide bands but none due to an anhydride, showing that under our conditions no detectable amounts of ethylmethylsuccinic anhydride were formed (cf. ref. 5). The imide mixture was hydrolysed to the corresponding acids by concentrated hydrochloric acid (which had been shown[16] not to cause inversion of configuration at the carbon atoms α to the carboxyl groups). The crude hydrolysate was treated with cold neutral potassium permanganate to destroy ethylmethylmaleic acid (ethylmethylsuccinic acid and dihydrohæmatinic acid were shown to be unaffected under these conditions). Paper chromatography of the residue afforded spots probably corresponding to dihydrohæmatinic and ethylmethylsuccinic acid. Further, conversion of the mixed acids into their di-*p*-bromophenacyl esters followed by chromatography on neutralised alumina gave a crystalline *p*-bromophenacyl ester, the infrared spectrum (KBr disc) of which was identical with that of synthetic di-*p*-bromophenacyl *transoid*-ethylmethylsuccinate[16,17] and characteristically different from that of the *cisoid*-ester. This *p*-bromophenacyl ester was optically active, showing that racemisation had not occurred during the manipulations and therefore (as in the case of chlorophyll and its degradation products) the *transoid*-configuration of the acid represents the configuration in the ester (III), *i.e.*, the hydrogen atoms in ring II are *trans*-orientated.

Paper chromatography of the acid fraction from the oxidation gave a spot corresponding to dihydrohæmatinimide. Hydrolysis of the imide mixture and permanganate oxidation as before was then followed by paper chromatography; a strong spot corresponding to

[12] Fischer and Riedmair, *Annalen*, 1933, **505**, 87.
[13] Fischer and Hasenkamp, *ibid.*, 1935, **515**, 148.
[14] Muir and Neuberger, *Biochem. J.*, 1949, **45**, 163; 1950, **47**, 97.
[15] Reindel and Hopper, *Chem. Ber.*, 1954, **87**, 1103; Rydon and Smith, *Nature*, 1952, **169**, 922.
[16] Golden and Linstead, following paper.
[17] See Ficken, Johns, and Linstead, *J.*, 1956, 2280, for nomenclature.

dihydrohæmatinic acid was obtained, besides other spots probably due to oxalic and succinic acid.

These results are in complete agreement with structure (III) for bacteriochlorin e_6 trimethyl ester and provide the further information that the hydrogen atoms in ring II are *trans*-orientated. That the hydrogen atoms in ring IV are also *trans* to one another follows from the conversion of ester (III) into the chlorophyll-*a* derivative (IV) and the earlier proof by Ficken, Johns, and Linstead [4] that in chlorophyll-*a* the hydrogen atoms in ring IV are *trans*-orientated. It is not possible to assign the relative configuration of the hydrogen atoms in ring II to those in ring IV on the known evidence: this would require a knowledge of the absolute configuration of the two rings. The only remaining stereochemical point is the configuration of the 10-methoxycarbonyl group in bacteriochlorophyll. Since the hydrogen atom at position 10 is very powerfully activated by three electron-attracting groups the 10-methoxycarbonyl group might be expected to be in the thermodynamically more stable configuration, *viz.*, *trans* to the propionic acid side-chain in ring IV.

Absorption spectra of the pigments prepared during this study are given in the annexed Table. Previous data on bacteriochlorophyll derivatives have been recorded by Pruckner *et al.*[11,18] and by Weigl.[19] The band at *ca.* 750 mμ appears to be characteristic of bacteriochlorophyll derivatives; it is shifted bathochromically to 770 mμ by the introduction of magnesium, *i.e.*, in bacteriochlorophyll itself, and hypsochromically to 727 mμ on reduction of the acetyl group (cf. VII). Although a quantitative spectrum of bacteriochlorophyll was not obtained it is apparent from results during the isolation (see p. 1730) that the ε value of the 770 mμ band in methanol must be considerably less than that reported for solutions in ether.[19,20]

Light absorption data (λ *in* mμ).

λ	ε	λ	ε	λ	ε	λ	ε	λ	ε
Bacteriophæophytin (II; R = phytyl) In dioxan		Bacteriomethylphæophorbid (II; R = Me) In dioxan		Bacteriochlorin e_6 (III) In dioxan		Me_3 ester In C_6H_6		2-Acetylchlorin e_6 Me_3 ester (IV) In C_6H_6	
297	39,800	298	18,200	358	112,000	307	46,800	411	105,000
359	107,000	360	112,000	385	79,400	359	138,000	507	10,600
387	57,500	385	57,500	456	3,470	387	112,000	542	8,080
496	6,200	496	6,030	493	5,250	457	3,470	626	4,360
530	29,500	530	27,500	522	29,500	493	5,010	682	50,400
627	3,500	625	3,710	627	2,190	525	27,500		
682	10,000	682	10,200	688	7,760	631	3,720		
751	70,800	752	72,400	750	93,300	691	7,240		
						754	93,300		
Cu deriv. of (IV) In C_6H_6		Oxochloroporphyrin e_6 Me_3 ester (X *) In dioxan		Cu deriv. of (X) In C_6H_6		Ester (VII) In C_6H_6		*b*-Octaethyltetrahydroporphin (VIII) In C_6H_6	
423	73,000	348	22,400	419	198,000	379	67,100	374	180,000
511	3,750	413	224,000	550	7,600	448	2,390	434	9,200
556	3,010	516	9,330	601	15,700	477	3,860	463	20,300
658	43,500	559	12,000			506	20,100	491	49,300
		587	7,760			613	1,930	604	2,500
		642	1,990			665	6,710	662	6,200
						691	6,820	685	14,100
						727	61,200	721	150,000

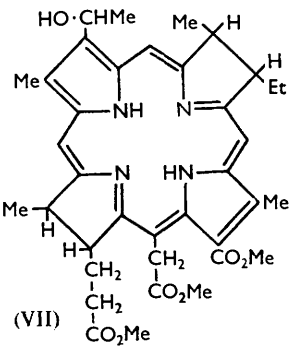
* = IV without the " extra " hydrogen atoms.

An additional characteristic of the bacterio-derivatives is the double Soret band at 360 and 385 mμ; the acetyl group appears to be necessary for this feature, as it is not shown by compounds (VII) or (VIII).

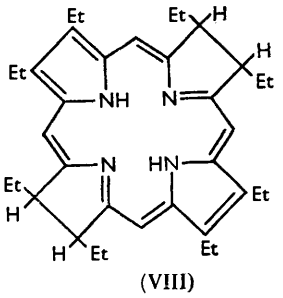
[18] Pruckner, *Z. phys. Chem.*, 1940, **187**, *A*, 257.
[19] Weigl, *J. Amer. Chem. Soc.*, 1953, **75**, 999.
[20] Holt and Jacobs, *Amer. J. Bot.*, 1954, **11**, 718.

For comparison with Eisner's synthetic *b*-octaethyltetrahydroporphin [8] (VIII) the ester (VII) was prepared by reduction of the bacteriochlorin (III) with borohydride. This compound could not be obtained crystalline, possibly because it was a mixture of epimers, and consequently only approximate ε values can be quoted.

Infrared Spectra.—The infrared spectra of macrocyclic pigments have not been studied



(VII)



(VIII)

in great detail. The most extensive work is that of Falk and Willis [21] on a series of porphyrins and hæms and of Thomas and Martell [22] on *mes o*-tetraphenylporphins. Apart from a few early results by Pruckner [18] of limited range, the only bacteriochlorophyll derivative studied is the parent pigment.[23]

For the pigments described in this paper, the N–H stretching band is a weak band at 3290—3240 cm.$^{-1}$, better observed in solution spectra (in $CHCl_3$); it is absent in the metallo-derivatives. The conjugated ester band (1720—1705 cm.$^{-1}$) attributable to the 6-methoxycarbonyl group in the esters (III) and (IV) is resolved from the normal ester band (1730 cm.$^{-1}$) in the KBr-disc spectra but not in solution spectra ($CHCl_3$). In derivatives (II; R = phytyl and Me) containing the keto-group in a five-membered ring the above conjugated-ester band is replaced by one at 1690 cm.$^{-1}$. A band at 1665—1655 cm.$^{-1}$ appears to be diagnostic of the conjugated 2-acetyl group; in the ester (VII) this band is absent. These band assignments indicate that the carbonyl groups at positions 2 and 6 in bacteriochlorophyll must be conjugated with a π-electron system. This is in agreement with the assignment of the two " extra " hydrogen atoms to positions 3 and 4.

A band at 1618—1616 cm.$^{-1}$ appears in all the chlorophyll and bacteriochlorophyll derivatives examined but is absent in the porphins. Bacteriochlorophyll derivatives appear to be characterised by a series of intense bands in the 1000—800 cm.$^{-1}$ region; the chlorophylls and porphyrins only showed relatively weak absorption in this region.

Experimental

Unless otherwise stated, alumina for chromatography is Peter Spence's Grade " H " alumina deactivated and neutralised by addition of 5 c.c. of 10% acetic acid per 100 g. of alumina. M. p. marked (K) were determined on a Kofler block. The solvent mixture used for paper chromatography was ethanol–water–ammonia (*d* 0·88) (80 : 15 : 5).

Infrared measurements were made by Mr. R. L. Erskine using a Grubb-Parsons double-beam instrument. Elementary analyses were carried out in the Micro-analytical Laboratory (Miss J. Cuckney) of Imperial College.

Extraction of Bacteriophæophytin (II; R = phytyl).—Bacteria (314 g.; *Chromatium* sp., El Agheila) were ground with methanol (500 c.c.) and then centrifuged. The colourless supernatant liquid was discarded; the deposit was ground with fresh methanol (500 c.c.) and re-centrifuged. The dark green supernatant liquid was collected and the deposit repeatedly re-extracted

[21] Falk and Willis, *Austral. J. Sci.*, 1951, **4**, *A*, 579.
[22] Thomas and Martell, *J. Amer. Chem. Soc.*, 1956, **78**, 1338.
[23] Weigl and Livingston, *ibid.*, 1953, **75**, 2173.

in this way until the extracts were pale yellow. The combined extracts were filtered through a fine sintered-glass filter to remove suspended particles, and gave *ca.* 2·5 l. of solution. Spectroscopic estimation of the bacteriochlorophyll content, using the absorption at 770 mμ and Holt and Jacobs's *E* value,[20] gave a value of 439 mg.

The methanolic solution was divided into 500 c.c. portions. Each was diluted with ether (500 c.c.), water (1 l.) added with swirling, and the colourless aqueous layer discarded. The combined ethereal layers (*ca.* 1 l.) were shaken with 22% hydrochloric acid (75 c.c.) to remove magnesium: the solution changed from bright green to dark violet. The ether layer was thoroughly washed with water until free from acid; emulsions at this stage were broken by the cautious addition of acetone. The dried (Na_2SO_4) solution (1·6 l.) was filtered and the bacteriophæophytin content was estimated, from the absorption at 747 mμ, to be 657 mg. The solution was concentrated under reduced pressure to 10 c.c., cooled to 0°, and filtered. The bacteriophæophytin (containing some sulphur and carotenoids) was washed with a little ether and then with methanol and dried (983 mg.).

In several isolations by the above procedure the ratio of spectroscopically estimated bacteriophæophytin to spectroscopically estimated bacteriochlorophyll (Holt and Jacobs's *E*) was between 1·54 and 1·75 (average 1·65).

A small quantity of the above material was purified by precipitation from the minimum of acetone with excess of hot methanol. Three-fold repetition of this process yielded chromatographically homogeneous bacteriophæophytin, m. p. 203° (K) (Found: C, 73·9; H, 8·7. Calc. for $C_{55}H_{76}O_6N_4$: C, 74·3; H, 8·6%).

Bacteriomethylphæophorbid (II; R = Me).—This material, prepared by Fischer and Hasenkamp's method [13] from bacteriophæophytin by ester exchange with methanolic hydrogen chloride, crystallised when the reaction mixture was cooled; the average yield from crude bacteriophæophytin was 78%, of material of m. p. 225—230° (K). A portion was purified by chromatography on alumina and crystallised from acetone–methanol as dark blue rhombs, m. p. 233—235° (K) (Found: C, 69·1; H, 7·0, 7·1; N, 9·0. Calc. for $C_{36}H_{40}O_6N_4$: C, 69·2; H, 6·45; N, 9·0%).

Bacteriochlorin e_6 Trimethyl Ester (III).—This was prepared [13] by methanolysis of bacteriomethylphæophorbid with diazomethane–methanol in the presence of pyridine, and purified by chromatography on alumina and elution with benzene–ether (4 : 1). Crystallisation from acetone–methanol gave steely-black needles (70%), m. p. 208—210° (K) (Found: C, 68·0; H, 7·35, 7·4; N, 8·4. Calc. for $C_{37}H_{44}O_7N_4$: C, 67·7; H, 6·75; N, 8·5%).

2-Acetylchlorin e_6 Trimethyl Ester (IV).—(*a*) Nitrogen was bubbled through a solution of bacteriochlorin e_6 trimethyl ester (20 mg.) in dry benzene (10 c.c.), 2 : 3-dichloro-5 : 6-dicyanobenzoquinone (7·3 mg., 1·05 mol.) in dry benzene (10 c.c.) was added, and the whole set aside at 20° for 30 min. in the dark. The pigment was then absorbed on a column of alumina. Elution with benzene–ether (9 : 1) afforded 2-acetylchlorin e_6 as a narrow dark brown band, followed by a trace of oxochloroporphyrin e_6 trimethyl ester as a pale blue zone. Evaporation of the brown solution and crystallisation of the residue from acetone–methanol gave 2-acetylchlorin e_6 (19 mg., 95%) as dark brown crystals with a blue metallic lustre, m. p. 245—250° (K) (Found: C, 68·0; H, 6·7; N, 8·4. Calc. for $C_{37}H_{42}O_7N_4$: C, 67·9; H, 6·5; N, 8·6%).

(*b*) 1 c.c. portions of a benzene solution of bacteriochlorin e_6 trimethyl ester (341 mg./l.) were mixed severally with 0·40 c.c. and 0·80 c.c. (0·5 and 1·0 mol.) of a solution (162 mg./l.) of 2 : 3-dichloro-5 : 6-dicyanobenzoquinone, and the mixtures were made up to 10 c.c. After 20 min. at 20° the intensities of absorption in the 755 and the 682 mμ region were measured, after appropriate dilution, and the concentrations of starting material and product estimated:

Quinone added (mol.)	Bacteriochlorin unchanged (%)	Acetylchlorin formed (%)
0·5	4·9	48
1·0	0	93

Oxochloroporphyrin e_6 Trimethyl Ester (IV minus 2H).—2-Acetylchlorin e_6 trimethyl ester (20 mg.) in dry benzene (10 c.c.) was heated under reflux in nitrogen with 2 : 3-dichloro-5 : 6-dicyanobenzoquinone (35 mg., 5 mol.) during 30 min. Chromatography on alumina and elution with benzene–ether (9 : 1) gave a bright purple solution. Evaporation followed by crystallisation of the residue from chloroform–methanol gave blue prisms (16 mg., 80%), m. p. 270—272° (K) (Found: N, 8·5. Calc. for $C_{37}H_{40}O_7N_4$: N, 8·6%).

Copper Derivative of Pigment (IV).—2-Acetylchlorin e_6 trimethyl ester (100 mg.) was heated

in boiling benzene (30 c.c.) under nitrogen; cupric acetate (100 mg.) in hot methanol was added. After 2 min. the bright blue-green solution was cooled, washed with water, dried, and evaporated. The residue was chromatographed on alumina, elution with benzene–ether (8 : 2) giving the *copper derivative* of the pigment as a sharp green band. Crystallisation from chloroform–methanol afforded blue-green needles (108 mg., 98%) (Found: C, 61·9; H, 5·9; Cu, 9·0. $C_{37}H_{40}O_7N_4Cu$ requires C, 62·0; H, 5·6; Cu, 8·9%).

Copper Derivative of Pigment (X = IV minus 2H).—(*a*) The preceding copper derivative (50 mg.) in boiling dry benzene (20 c.c.) was heated under nitrogen with 2 : 3-dichloro-5 : 6-dicyanobenzoquinone (16 mg., 1 mol.) during 30 min. Purification was by chromatography on alumina; the pigment was eluted as a green band with benzene–chloroform (4 : 1). Solutions in benzene and chloroform are dark red in fairly concentrated and blue-green in dilute solution. Evaporation of the eluate followed by crystallisation from chloroform–methanol gave the *derivative* as dark-purple needles (45 mg., 90%) (Found: C, 61·9; H, 5·7; Cu, 9·0. $C_{37}H_{38}O_7N_4Cu$ requires C, 62·2; H, 5·4; Cu, 8·9%).

(*b*) 1 c.c. portions of a solution (491 mg./l.) of the copper derivative of (IV) were mixed severally with 0·23 and 0·46 c.c. (0·5 and 1·0 ml.) of a solution of 2 : 3-dichloro-5 : 6-dicyanobenzoquinone (325 mg./l.). The solutions were heated at 80° for 30 min., cooled, and diluted to 10 c.c. After appropriate dilution the intensities of absorption in the 658 mμ region (starting material) and the 550 and the 600 mμ region (product) were measured and the concentrations estimated:

Quinone added (mol.)	Copper deriv. of (IV)	Copper deriv. of (X) (%)	
		602 mμ	550 mμ
0·5	50	44	40
1·0	5	88	90

(*c*) Oxochloroporphyrin e_6 trimethyl ester (10 mg.) was heated under nitrogen in boiling benzene (10 c.c.), and cupric acetate (20 mg.) in hot methanol was added. After 2 min. the red-green solution was cooled, washed with water, dried, and evaporated. The residue was chromatographed on alumina; elution with benzene–chloroform (4 : 1) gave the copper derivative (8 mg.), spectroscopically identical with that obtained as in (*a*) above.

Oxidation of Bacteriochlorin e_6 Trimethyl Ester.—The deep blue solution of bacteriochlorin e_6 trimethyl ester (500 mg.) in 50% sulphuric acid (40 c.c.) was treated with ice (30 g.), cooled to −12°, treated with chromium trioxide (2 g.) in water (15 c.c.) with stirring during 1 hr., stirred for a further 4 hr. at −12°, allowed to reach room temperature, and then continuously extracted with ether for 48 hr. Evaporation of the extract afforded a yellow oil whose solution in water (10 c.c.) was adjusted to pH 10 (NaOH) and continuously extracted with ether for a further 48 hr., giving a neutral fraction (A). The aqueous layer was acidified to pH 3 with dilute hydrochloric acid and re-extracted with ether for 48 hr., giving an acid fraction (B).

Material from fraction (A), a yellow oil (44 mg.), was examined by paper chromatography, the spots being detected by an imide spray: R_F 0·43 (faint) (dihydrohæmatinimide), 0·81—0·82 and 0·86—0·88 (ethylmethylsuccinimide and ethylmethylmaleimide). Authentic R_F values with this solvent system [16] are: *cis*-dihydrohæmatinimide, 0·41; *trans*-dihydrohæmatinimide, 0·45; *cis*-ethylmethylsuccinimide, 0·79; *trans*-ethylmethylsuccinimide, 0·81; ethylmethylmaleimide, 0·87.

The infrared spectrum of the crude neutral fraction (A) in $CHCl_3$ included strong imide bands but no anhydride bands.

Hydrolysis of fraction (A) by concentrated hydrochloric acid (1 c.c.) under reflux for 1 hr. was followed by removal of the acid under reduced pressure, dissolution of the residue in water, and adjustment of the pH to 7 with sodium hydrogen carbonate. The solution was treated with neutral permanganate (30 mg. in 5 c.c. of water) and kept at 25° for 1 hr. After decolorisation with sulphur dioxide the solution was extracted with ether for 48 hr., giving a colourless oil which was examined by paper chromatography. Development with ethanolic bromocresol-green revealed two main spots: R_F 0·14 (dihydrohæmatinic acid?) and 0·38 (ethylmethylsuccinic acid?). Authentic R_F values are: *cisoid*-dihydrohæmatinic acid, 0·12; *transoid*-dihydrohæmatinic acid, 0·11; *cisoid*-ethylmethylsuccinic acid, 0·43; *transoid*-ethylmethylsuccinic acid, 0·40.

The oil was converted into the *p*-bromophenacyl ester in the usual way, the product being isolated with chloroform and chromatographed on alumina (neutralised with ethyl acetate).

The fractions eluted with benzene–light petroleum (3 : 2) afforded di-*p*-bromophenacyl *transoid*-ethylmethylsuccinate (24 mg., 6%), m. p. 112—113° (after two crystallisations from aqueous methanol), $[\alpha]_D^{24}$ −37°. The infrared spectrum (KBr disc) was identical with that of authentic racemic material.[16]

The acid fraction (B), a red-brown oil (200 mg.), was hydrolysed with concentrated hydrochloric acid and oxidised with neutral permanganate, as for (A), giving an acidic oil (131 mg.). Paper chromatography showed the presence of material of R_F 0·14, corresponding to dihydrohæmatinic acid, as well as spots probably due to oxalic and succinic acids.

Borohydride Reduction of Bacteriochlorin e_6 Trimethyl Ester.—Bacteriochlorin e_6 trimethyl ester (20 mg.) in dioxan (5 c.c.) was treated with potassium borohydride (20 mg.) in 50% aqueous dioxan (50 c.c.) and set aside at 20° for 1 hr., the solution changing from olive-green with a violet tinge to bright green. Water was added and the pigment isolated with ether. Chromatography on alumina and elution with benzene–ether (9 : 1) gave a small quantity of starting material (4 mg.), followed by a bright green band which on evaporation gave a green uncrystallisable gum (13 mg.), whose infrared spectrum (in $CHCl_3$) indicated replacement of the acetyl group by hydroxyethyl, the ester groups being retained.

These investigations were made possible by the kindness and skilful collaboration of Dr. G. A. Miller and his colleagues of the Antibiotic Research Station, Clevedon, who made available the *Chromatium* bacteria used as a source of pigment; also by financial support from the Rockefeller Foundation whom we thank.

IMPERIAL COLLEGE, S. KENSINGTON, LONDON, S.W. 7. [*Received, November 27th*, 1957.]