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# Bacteriochlorophyll <latex>$e$</latex>: Structure and Stereochemistry of a New Type of Chlorophyll from Chlorobiaceae

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# Bacteriochlorophyll *e*: structure and stereochemistry of a new type of chlorophyll from Chlorobiaceae

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A so far unknown chlorophyll has been isolated from several strains of brown-coloured chlorobacteria and has been designated bacteriochlorophyll *e*. Comparison of the physical and chemical properties of the new chlorophyll with those of bacteriochlorophylls *c* and *d* (*Chlorobium* chlorophylls) allows one to deduce its structural formula.

The stereochemistry of the hydroxyethyl side chain of bacteriochlorophylls *c*, *d* and *e* can be determined by a modified Horeau analysis of these pigments, and the results thus obtained are in agreement with those derived from oxidative degradation experiments. The latter method allows one furthermore to prove the structure of bacteriochlorophylls *c* and *d* proposed by Holt, and to establish the absolute configuration at carbon atoms 7 and 8. The presence of a 8-methylsubstituent in bacteriochlorophylls *c* and *e* is unambiguously demonstrated by nuclear magnetic resonance.

## Introduction

Phototrophic bacteria are living in the anaerobic zones of many waters, in shallows, pools, ocean bays and also in slowly running rivers. Already at the end of the last century these organisms attracted the attention of biologists and biochemists because they are capable of a simple type of photosynthesis which can be regarded as an evolutionary precursor of the photosynthesis in higher plants.

While the sulphur-containing and the sulphur-free purple bacteria (Thiorhodaceae and Athiorhodaceae) posses bacteriochlorophyll *a* (Bchl *a*) or in some cases bacteriochlorophyll *b* (Bchl *b*) as photosynthetically active pigments, both magnesium complexes of tetrahydroporphyrin derivatives, the green sulphur bacteria (Chlorobiaceae) contain as major photopigments chlorophylls with a dihydroporphyrin skeleton together with only minor amounts of Bchl *a* which is apparently located in the reaction centres.

Differences in the absorption spectra between extracts from chlorobacteria and green plants were reported as early as 1897 (Ewart) and later confirmed by Metzner (1922), who reviewed the earlier literature and proposed the name bacterioviridin for the photopigment of green bacteria. From Metzner's experimental results Fischer, Lambrecht & Mittenzwei (1938) concluded that bacterioviridin might be 2-acetyl-2-desvinylchlorophyll *a* but neither the absorption spectra nor the solubility properties of bacterioviridin which was renamed later chlorobium chlorophyll by Larsen (1953) agreed with this hypothesis (Katz & Wassink 1939; Larsen 1953).

Differences in the absorption maxima reported by Goodwin (1955) and by Kaplan & Siberman (1959) as well as their own investigations on chlorobium chlorophylls led Stanier & Smith (1960; Stanier 1960) to the discovery that two types of chlorobium chlorophyll may occur in Chlorobiaceae. They were designated chlorobium chlorophyll-650 and chlorobium chlorophyll-660 according to their long wavelength absorption maxima in ether solution.

Our present knowledge of the structure of these chlorophylls is due mainly to the excellent analytical work of Holt and co-workers at Ottawa who found that both types consist of a mixture of homologues which can be separated under certain conditions (Holt & Morley 1960*a*, *b*; Holt & Hughes 1961; Hughes & Holt 1962; Holt, Hughes, Kende & Purdie 1962, 1963; Holt, Purdie & Wasley 1966; Morley & Holt 1961; Purdie & Holt 1965). These investigations have been reviewed elsewhere (Holt 1965, 1966). Together with the results of other groups (Rapoport & Hamlow 1961; Archibald, MacDonald & Shaw 1963; Archibald *et al.* 1966) the structures given in figure 1 could be derived for chlorobium chlorophylls-650 and 660.

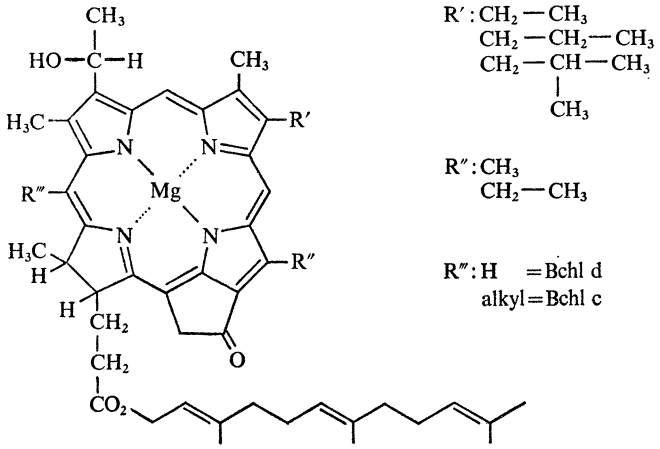



FIGURE 1. Structural formulas of the chlorobium chlorophylls-650 (Bchl *d*) and chlorobium chlorophylls-660 (Bchl *c*) according to Holt (1966). Alkyl may be $CH_3$ or $C_2H_5$.

We became interested in chlorobium chlorophyll chemistry primarily because a co-worker of Professor Pfennig and myself found that brown coloured strains of chlorobacteria, isolated only recently in pure culture (Pfennig 1968), contain a third type of chlorobium chlorophyll, hitherto unknown. Following the nomenclature for bacteriochlorophylls proposed by Jensen, Aasmundrud & Eimhjellen (1964), according to which the chlorobium chlorophylls-660 and -650 are designated bacteriochlorophyll *c* and *d*, respectively, Gloe, Pfennig, Brockmann & Trowitzsch (1975) named this new chlorobium chlorophyll bacteriochlorophyll *e* (Bchl *e*).

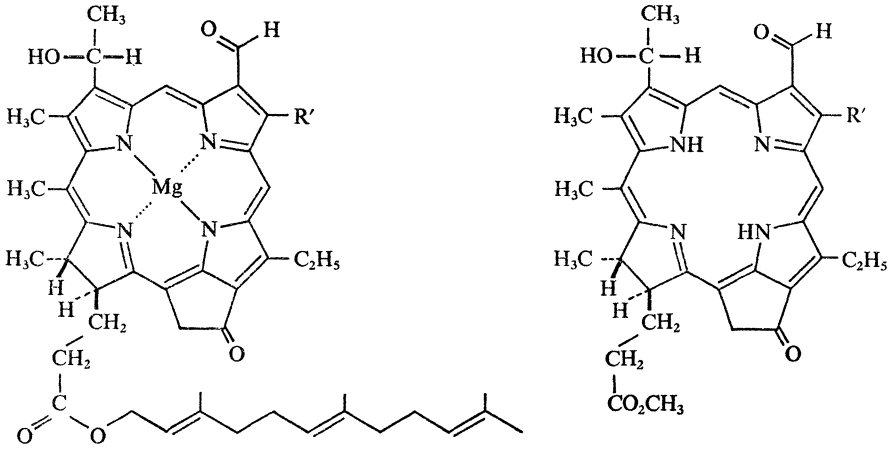
## STRUCTURE OF BACTERIOCHLOROPHYLL *e*

Bacteriochlorophyll *e* can be extracted with acetone or methanol from wet cells of *Chlorobium phaeobacteroides* and *Chlorobium phaeovibrioides*. It differs in absorption spectra and chromatographic behaviour on silica gel from all other known chlorophylls and has been purified by t.l.c. (Gloe *et al.* 1975). Treatment of Bchl *e* with dilute mineral acid removes the central magnesium which has been identified with Titan Yellow. The bacteriophaeophytine *e* (**2**) thus obtained can be transesterified to the corresponding bacteriomethylphaeophorbide *e* (**3**) by standard procedures.

Mainly from the results of spectroscopical investigations of **2** and **3** we were able to deduce structure **1** for bacteriochlorophyll *e* (Brockmann, Gloe, Risch & Trowitzsch 1975). Mass spectra of **2** and **3** revealed that Bchl *e* like Bchl *c* and Bchl *d* is a mixture of at least three homologues. The difference in molecular masses between **2** and **3** was the first indication that farnesol

was the esterifying alcohol. This was then obtained by alkaline hydrolysis of **2** and was identified with an authentic sample of *E,E*-farnesol by $^{1}$H-n.m.r. and mass spectrometry.

The most remarkable structural feature of Bchl *e* is probably the presence of a 3-formyl group. In the spectra of **3** this gives rise to an i.r. absorption at 1655 cm$^{-1}$, a $^{13}$C-n.m.r. signal at 188.0 parts/10$^{6}$, and an aldehyde proton magnetic resonance at 11.02 parts/10$^{6}$. Moreover the visible absorption spectrum of **3** is very similar to that of methylphaeophorbide *b*, small




SCHEME 1

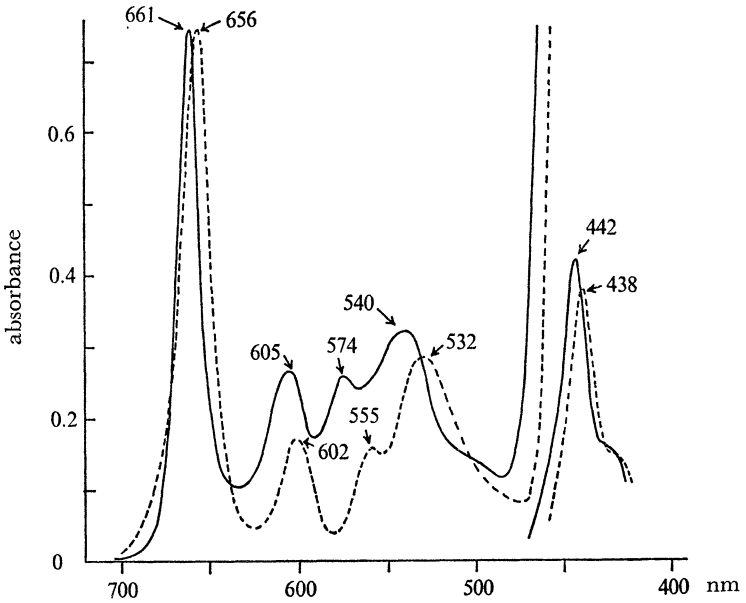
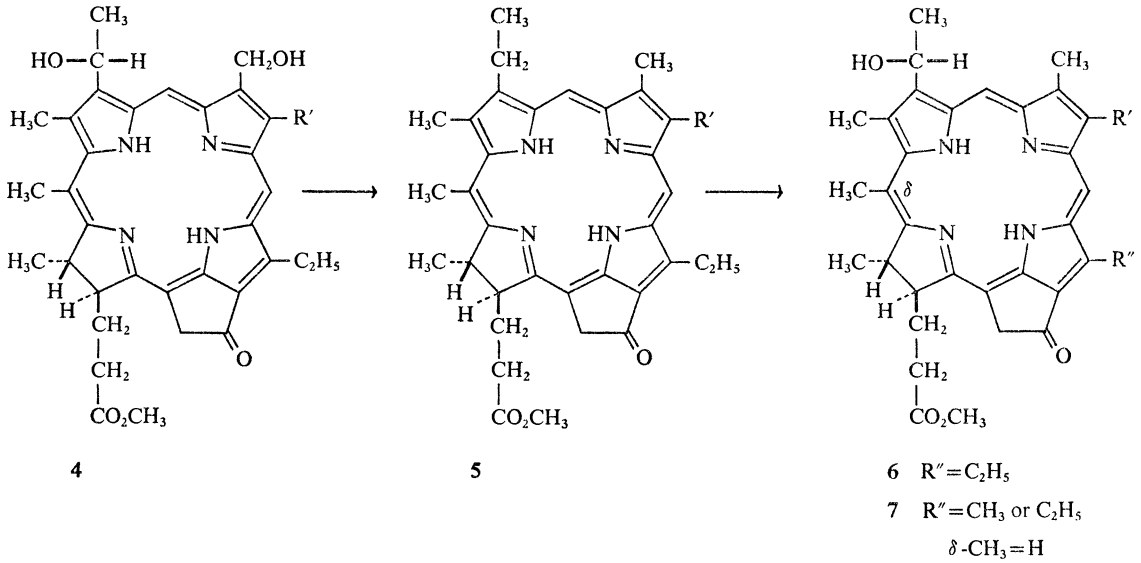



FIGURE 2. Visible absorption spectra of bacteriomethylphaeophorbide *e* (——) and methylphaeophorbide *b* (- - - -) in dioxane.

differences are obviously due to the 2-hydroxyethyl instead of a vinyl side chain and the additional presence of a δ-methyl substituent. The presence of only two unsubstituted methin bridges, of two aromatic methyl groups, an α-hydroxyethyl side chain, an ethyl group, the unsubstituted cyclopentanone ring, and of the reduced ring IV with methyl and propionic acid substituents could be derived immediately from the $^{1}H$- and $^{13}C$-n.m.r. spectra of **3**. The remaining signals of comparatively lower intensity in the $^{1}H$- and in the high field part of the $^{13}C$-n.m.r. spectra could be assigned in connection with the mass spectrum of **3** to the alkyl substituents which are different in the three components of **3**, namely an ethyl, *n*-propyl, and isobutyl group, respectively.

That the arrangement of these substituents is in the same order as usually found in chlorophylls can also be deduced from the n.m.r. spectra and is proved unambiguously by the following experiments.

On treatment with sodium borohydride in wet tetrahydrofuran only the formyl group of **3** is reduced. The diol (**4**) thus obtained can be transformed with Raney nickel in methanol to a mixture of compounds (**5**) which is identical in chromatographic behaviour and in all spectroscopic properties, including o.r.d. and c.d. spectra, with the mixture of compounds obtained by Raney nickel reduction of bacteriomethylphaeophorbide *c* (**6**) from *Chlorobium limicola*, strain Tassajara no. 6230 (Collection Professor Pfennig, Göttingen).




**4** **5** **6** $R''=C_2H_5$; **7** $R''=CH_3$ or $C_2H_5$, $\delta$-$CH_3$=H

SCHEME 2

The diol (**4**) turned out to be the best derivative of Bchl *e* from chromatographic separation of the homologues. Probably because of the vicinity of the different alkyl substituents at C4 to the polar 3-hydroxymethyl group, which is strongly responsible for the affinity to the adsorbent, **4** is separated into three distinct bands on silica gel with $CCl_4$/acetone (5 : 1) as eluent. These bands contain in the order of increasing $R_f$ values pure compounds with ethyl, propyl and isobutyl groups, respectively, as was shown by $^{1}H$-n.m.r. and mass spectrometry.

To avoid misunderstandings in future we propose the following nomenclature for bacteriochlorophyll *e* and its derivatives, which can be extended to the bacteriochlorophylls *c* and *d* as

well. If a pure component (fraction) is described the abbreviations of the substituents at C4 and C5 should be added in brackets behind the name of the compound. Accordingly the three diols (**4**) have to be designated 3-hydroxymethyl-3-desformyl-bacteriomethylphaeophorbide *e* [Et-Et], 3-hydroxymethyl-3-desformyl-bacteriomethylphaeophorbide *e* [*n*-Pr-Et], and 3-hydroxymethyl-3-desformyl-bacteriomethylphaeophorbide *e* [*i*-Bu-Et].

## Stereochemistry of bacteriochlorophyll *e*

7(*S*), 8(*S*) configuration of Bchl *e* and its derivatives follows from the identity of the o.r.d. and c.d. spectra of **5** obtained either from **3** or from **6** as the absolute stereochemistry of **6** is proved by oxidative degradation (next section). The chirality of the third centre of asymmetry at the hydroxyethyl side chain we have deduced by application of a modified Horeau analysis (Brockmann & Risch 1974). In the reaction with an excess of racemic α-phenylbutyroylimidazolide **3** forms preferentially the ester with (*R*)-α-phenylbutyric acid, hence the alcohol, i.e. the hydroxyethyl group must be (*R*) configurated (Risch 1975).

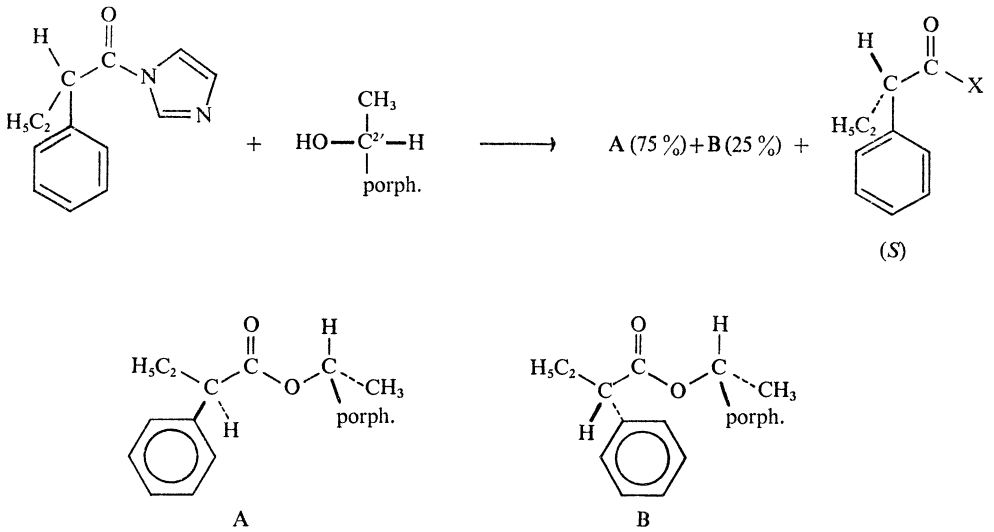



Figure 3. Determination of the chirality at C2′ of bacteriomethylphaeophorbide *c*, *d*, and *e* by a modified Horeau analysis. Porph. is used as abbreviation for the macrocycle, X is either imidazolyl or OH.

## Structure and stereochemistry of bacteriochlorophyll *c* and *d*

As mentioned above, the bacteriomethylphaeophorbide *e* (**3**) has been converted to a mixture of compounds (**5**) which could also be obtained from bacteriochlorophyll *c* derivatives. This, however, can only be a proof for the constitutional formula of Bchl *e* and its absolute configuration at C7 and C8 if the structure of Bchl *c* and its absolute configuration is known.

Whereas the constitutional formulas of the bacteriochlorophylls *d* proposed by Holt (Purdie & Holt 1965) seem to be generally accepted, there is some disagreement in the literature with respect to the nature and the position of the *meso*-alkyl group of bacteriochlorophyll *c* (Mathewson, Richards & Rapoport 1963*a*, *b*; Katz, Dougherty & Boucher 1966). Furthermore some of the phylloporphyrins prepared by a series of reactions in low yield from pure Bmph *c* components could not be identified unambigously by comparison with synthetic samples (Cox *et al.* 1967;

Cox, Jackson & Kenner 1971). Finally, nothing was known about the stereochemistry of Bchl *c* and *d*.

We have isolated Bchl *c* and Bchl *d* from *Chlorobium limicola*, strain Tassajara no. 6230, and from *C. limicola*, strain Lascelles no. 8327,† in order to study the stereochemistry in these pigments and to clarify open questions concerning their structure. The bacteriomethylphaeophorbides *c* and *d* (Bmph *c*, and Bmph *d*) prepared in the usual way gave $^{1}$H- and $^{13}$C-n.m.r. spectra very similar to each other with the exception that in the Bmph *c* spectra the signals for an additional *meso*-methyl group are present. As can be seen from figure 4 chemical shift differences are only observed for signals of protons and carbon atoms near to or at the δ-position. Hence the additional *meso*-methyl group must be bound to Cδ (Risch 1975; Tacke 1975). This is in full agreement with results of reductive degradation experiments by R. A. Chapman *et al.* (1971). It should be noted that the $^{13}$C chemical shift changes of the 1- and 8-methyl group caused by a δ-methyl substituent are very similar to those (−6.31 and +2.27 parts/$10^6$) caused by a δ-bromine (Lincoln, Wray, Brockmann & Trowitzsch 1974).

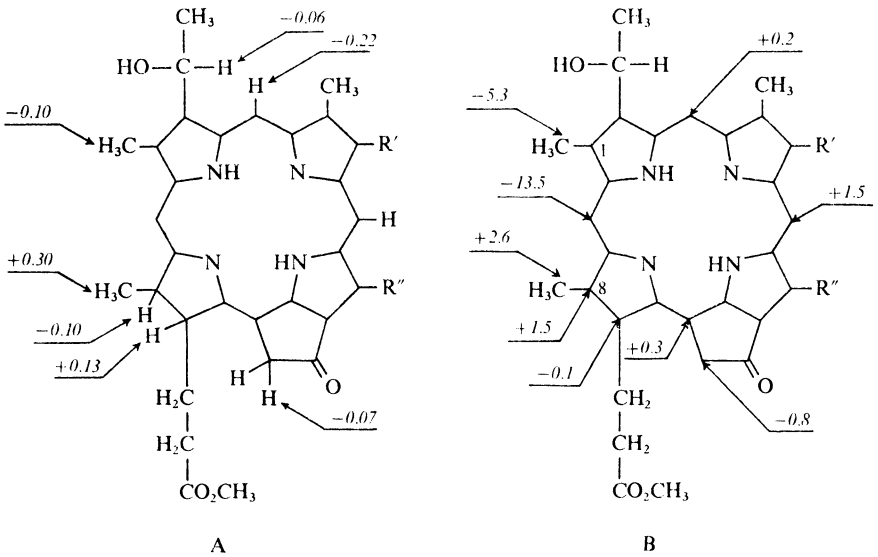



FIGURE 4. Chemical shift changes in the $^{1}$H-(A) and $^{13}$C-n.m.r. spectra (B) of Bmph *c* and Bmph *d* caused by the additional *meso*-methyl group in Bmph *c*. Values in parts/$10^6$.

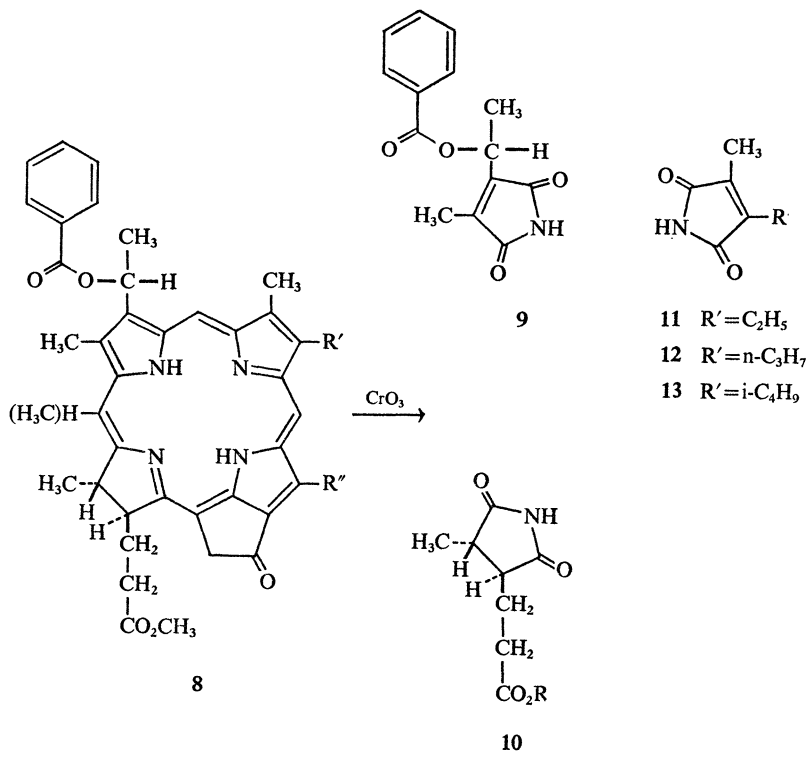
The stereochemistry at the hydroxyethyl side chain of Bmph *c* (**6**) and *d* (**7**) was first determined by us by use of the modified Horeau analysis (Brockmann & Risch 1974) as described in the previous section (see figure 3). It was found to be *R* as in bacteriochlorophyll *e* (Risch 1975). This finding has been proved by the following classical method (Tacke 1975).

If **6** or **7** are benzoylated at the 2′-hydroxy group prior to the chromic acid oxidation five imides are obtained by oxidative degradation of **8**. The three known dialkyl maleinimides (**11–13**) were identified by thin-layer and vapour phase chromatography as well as by $^{1}$H-n.m.r. and mass spectra with authentic samples. The dihydrohaematinimide (**10**, R = H) was converted to its methyl ester (**10**, R = $CH_3$), which gave o.r.d. and c.d. spectra identical to those of 2(*S*), 3(*S*)-dihydrohaematinimide methylester obtained from chlorophyll *a* (Brockmann

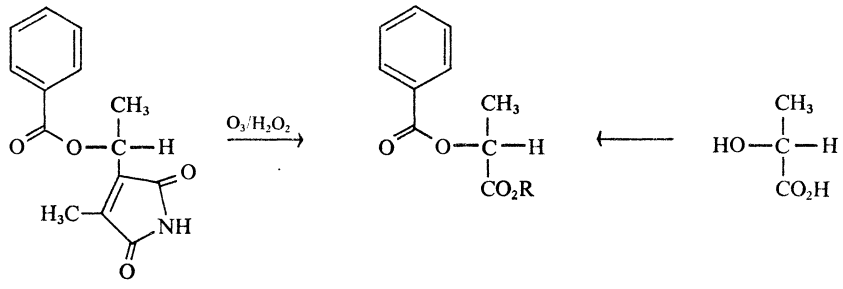
† We are grateful to Professor Pfennig, Göttingen, for the *C. limicola* strains and for valuable advice concerning their cultivation.

1971). Finally, the fifth imide was found to be optically active and its structure (**9**) was deduced from spectroscopic data. It has been further degraded with ozone and hydrogen peroxide to (*R*)-benzoyllactic acid (**14**, R = H), which was identified as its methylester (**14**, R = $CH_3$) by comparison with an authentic sample prepared from (−)-lactic acid (**15**). Thus the chirality of all three asymmetric carbon atoms of Bchl *c*, Bchl *d* and with the exception of C2′ of Bchl *e* is correlated chemically with the known configuration of simple compounds.

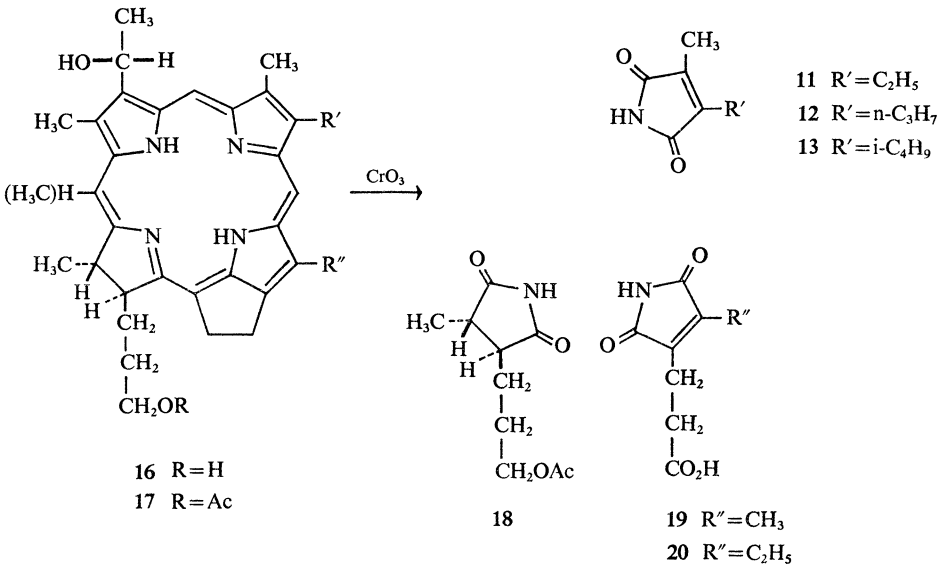
In order to obtain also ring III of phaeophorbides as an imide by chromic acid oxidation and thus to determine the nature of the C5 substituent Holt *et al.* (1966) developed a multistep procedure. However, because of the extremely low yields this method did not seem to be the appropriate way to check whether our bacteriochlorophylls *c*, *d* and *e* contain also components




SCHEME 3



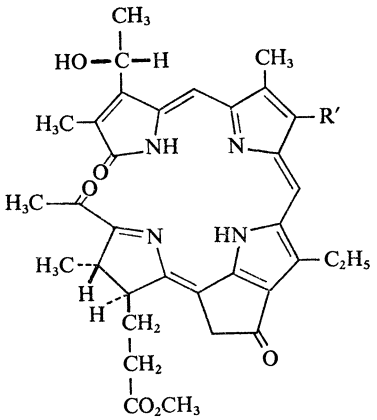

SCHEME 4




SCHEME 5

with a 5-methyl group. An alternative method is to convert the phaeophorbides under investigation to 9-deoxo-derivatives. This can be achieved in a one-step reaction with lithium aluminum hydride in the presence of zinc chloride (Bode 1971). However, under these conditions also the ester group is reduced and in order to prevent reoxidation in the following reaction **16** was acetylated. Chromic acid oxidation of the product (**17**) thus obtained transforms ring III of the macrocycle to haematinimide (**19**) or homohaematinimide (**20**) while the imide obtained from ring IV is **17**.

Applying this procedure to our bacteriomethylphaeophorbides *c* and *d* we found that only the Bmph *d* from *C. limicola*, strain Lascelles no. 8327, contained components with a 5-methyl group. Using isopropyl ether as eluent bacteriomethylphaeophorbides *d* [R-Et] and bacteriomethylphaeophorbides *d* [R-Me] are clearly separated on silica gel.



**21** R=$CH_3$ or $CH_2OH$
R′=$C_2H_5$, n-$C_3H_7$ or i-$C_4Hg$

SCHEME 6

After the discussion of chromic acid degradation as an analytical tool, another interesting reaction which causes the breakdown of the macrocylic system of δ-methyl substituted phaeophorbides should at least be mentioned. By photo-oxidation compounds **1**, **4** and **6** are converted to the linear tetrapyrroles (**21**) in excellent yield (Belter 1975).

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## References (Brockmann)


Archibald, J. L., MacDonald, S. F. & Shaw, K. B. 1963 *J. Am. chem. Soc.* **85**, 644.

Archibald, J. L., Walker, D. M., Shaw, K. B., Markovac, A. & MacDonald, S. F. 1966 *Can. J. Chem.* **44**, 345–362.

Belter, H. C. 1975 Ph.D. Thesis, Technische Universität Braunschweig.

Bode, J. 1971 Ph.D. Thesis, Technische Universität Braunschweig.

Brockmann, Jr, H. 1971 *Justus Liebigs Ann. Chem.* **754**, 139–148.

Brockmann, Jr, H., Gloe, A., Risch, N. & Trowitzsch, W. 1975 *Fustus Liebigs Ann. Chem.* (in the Press).

Brockmann, Jr, H. & Risch, N. 1974 *Angew. Chem.* **86**, 707–708; *Angew. Chem. int. Edit.* **13**, 664–665.

Chapman, R. A., Roomi, M. W., Norton, J. C., Krajcarski, D. T. & MacDonald, S. F. 1971 *Can. J. Chem.* **49**, 3544–3564.

Cox, M. T., Fletcher, R., Jackson, A. H., Kenner, G. W. & Smith, K. M. 1967 *Chem. Commun.*, pp. 1141–1142.

Cox, M. T., Jackson, A. H. & Kenner, G. W. 1971 *J. Chem. Soc.* (C), pp. 1974–1981.

Ewart, A. J. 1897 *Ann. Bot.* **11**, 486–487.

Fischer, H., Lambrecht, R. & Mittenzwei, H. 1938 *Hoppe Seyler's Z. Physiol. Chem.* **253**, 1–39.

Gloe, A., Pfennig, N., Brockmann, Jr., H. & Trowitzsch, W. 1975 *Archs Microbiol.* **102**, 103–109.

Goodwin, T. W. 1955 *Biochim. biophys. Acta* **18**, 309–310.

Holt, A. S. 1965 In *The chemistry and biochemistry of plant pigments* (ed. T. W. Goodwin), pp. 3–28. London: Academic Press.

Holt, A. S. 1966 In *The chlorophylls* (ed. L. P. Vernon & G. R. Seely), pp. 111–118. New York: Academic Press.

Holt, A. S. & Hughes, D. W. 1961 *J. Am. chem. Soc.* **83**, 499–500.

Holt, A. S., Hughes, D. W., Kende, H. J. & Purdie, J. W. 1962 *J. Am. chem. Soc.* **84**, 2835–2836.

Holt, A. S., Hughes, D. W., Kende, H. J. & Purdie, J. W. 1963 *Pl. Cell. Physiol.* **4**, 49–55.

Holt, A. S. & Morley, H. V. 1960*a* *J. Am. chem. Soc.* **82**, 500–501.

Holt, A. S. & Morley, H. V. 1960*b* In *Comparative biochemistry of photoreactive systems* (ed. M. B. Allen), pp. 169–179. New York: Academic Press.

Holt, A. S., Purdie, J. W. & Wasley, J. W. F. 1966 *Can. J. Chem.* **44**, 88–93.

Hughes, D. W. & Holt, A. S. 1962 *Can. J. Chem.* **40**, 171–176.

Jensen, A., Aasmundrud, O. & Eimhjellen, K. E. 1964 *Biochim. biophys. Acta* **88**, 466–487.

Kaplan, I. R. & Silberman, H. 1959 *Archs Biochem. Biophys.* **80**, 114–124.

Katz, E. & Wassink, E. C. 1939 *Enzymologia* **7**, 97–112.

Katz, J. J., Dougherty, R. C. & Boucher, L. J. 1966 In *The chlorophylls* (ed. L. P. Vernon & G. R. Seely), pp. 185–251. New York: Academic Press.

Larsen, H. 1953 *K. norske Vidensk. Selsk. Skr.* Nr. 1, 1–205.

Lincoln, D. N., Wray, V., Brockmann Jr., H. & Trowitzsch, W. 1974 *J. chem. Soc. Perkin II*, pp. 1920–1925.

Matthewson, J. W., Richards, W. R. & Rapoport, H. 1963*a* *J. Am. chem. Soc.* **85**, 364–365.

Mathewson, J. W., Richards, W. R. & Rapoport, H. 1963*b* *Biochem. biophys. Res. Commun.* **13**, 1–5.

Metzner, P. 1922 *Ber. dtsch. bot. Ges.* **40**, 125–129.

Morley, H. V. & Holt, A. S. 1961 *Can. J. Chem.* **39**, 755–760.

Pfennig, N. 1968 *Arch. Mikrobiol.* **63**, 224–226.

Purdie, J. W. & Holt, A. S. 1965 *Can. J. Chem.* **43**, 3347–3353.

Risch, N. 1975 Ph.D. Thesis, Technische Universität Braunschweig.

Rapoport, H. & Hamlow, H. P. 1961 *Biochem. biophys. Res. Commun.* **6**, 134–137.

Stanier, R. Y. 1960 In *Comparative biochemistry of photoreactive systems* (ed. M. B. Allen), pp. 69–72. New York: Academic Press.

Stanier, R. Y. & Smith J. H. C. 1960 *Biochim. biophys. Acta* **41**, 478–484.

Tacke, R. 1975 Ph.D. Thesis, Technische Universität Braunschweig.






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