1.1 STRUCTURE AND OCCURRENCE OF CHLOROPHYLLS

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I. INTRODUCTION

The chlorophylls are a group of tetrapyrrolic pigments with common structural elements and functions. In chemical terms, they are cyclic tetrapyrroles of the porphyrin, chlorin, or bacteriochlorin oxidation state (Scheme 1), which are characterized by a fifth, isocyclic ring that is biosynthetically derived from the C-13 propionic acid side chain of protoporphyrin.* In chemical terms, chlorophylls are conventionally also characterized by a central magnesium atom. In a biological context, the definition is somewhat shifted. Only those among the pigments defined above should be regarded as true chlorophylls which function in photosynthesis. This biological definition excludes precursors or degradation products which conform to the chemical definition. It includes, on the other hand, the pheophytins, e.g., the derivatives which lack the central magnesium, because they are active in photosynthetic electron transport.

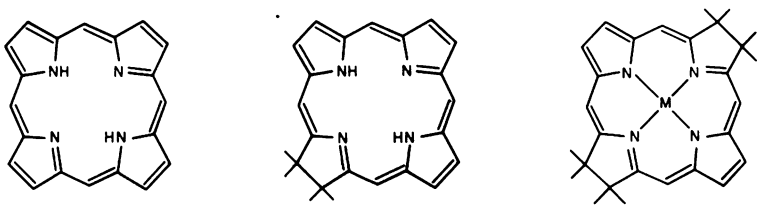
The additional structural elements of chlorophylls vary widely. The number of known structures isolated from photosynthetic organisms and active in photosynthesis has increased from 3 in 1960 to now well over 50 (Figure 1). As evidenced by the chlorophylls **c** (which should more correctly be termed chlorophyllides **c**), and which are typical and abundant in many algae,[2-4] neither the hydroporphyrin structure (e.g., reduced rings D, as in Chl **a**, or rings B and D as in BChl **a**) nor the terpenoid alcohol esterifying the C-17 propionic acid side chain are characteristic for the chlorophylls. Conversely, hydroporphyrins[5,6] functionally not related to chlorophylls, but containing related macrocyclic conjugation systems, have been found in several oxidoreductases,[7-16] as a pigment in a marine sponge,[16a] in a marine tunicate,[16b] and (as sex-determinant?) in the marine echiuroid, *Bonella viridis*.[17,18] In all structures containing an isocyclic ring V or remnants thereof, it is tacitly assumed that they are originally chlorophyll derived and then processed by the plant, the animal, or both.

II. CHLOROPHYLL a

Chl **a** is present in all organisms capable of oxygenic photosynthesis, where it occurs in both reaction centers (RC) and in all light-harvesting complexes (LHC) with the exception of the phycobiliproteins (Table 1; see also Table 2 for functions, occurrence, and spectra of chlorophylls). It functions as the primary donor in the RC of PS II, and Chl **a** or a closely related pigment (see below) is also the primary donor of photosystem I (PS I). Both reaction centers contain additional Chl **a** molecules, whose function is currently still unclear. In photosystem II (PS II), monomeric Chl **a** is believed to be located between the primary donor and the pheophytin **a** acceptor, based on the similarity of PS II to purple bacterial RC (see Chapters 3.5 and 5.3). A Chl **a**-type pigment is also discussed as the first electron acceptor (A_0) in PS I-RC. The intense absorptivity in the visible region is an important factor in light-harvesting by Chl **a**, and it is the major pigment in all chlorophyllous antenna complexes of oxygenic organisms. However, the intense bands of Chl **a** (like of all other chlorophylls) are quite narrow, and there is only moderate absorptivity in the green spectral region. In antennas, it is therefore almost always supplemented by additional light-harvesting pigments (see Chapters 3.2 and 3.3).

The molecular structure of chlorophyll **a** (Chl **a**) (Figure 2) has been established by total synthesis of the tetrapyrrole moiety[19] and the C-20 terpenoid alcohol, phytol.[20] The stereochemistry of the tetrapyrrole at C-17 and C-18 has been determined by relation to (−)α-santonin and dimethylpentan, that at C-13[2] and of the phytol by a combination of synthetic

* The semi-systematic IUPAC nomenclature[1] has been used throughout. See Figure 2 for a comparison with the still also common Fischer nomenclature system. Also used is the "bracket-system" for indicating substitutions, e.g., [3-acetyl]-Chl **a** is identical with 3-deethyl-3-acetyl-Chl **a**, an oxidation product of BChl **a**; and [7-formyl]-Chl **a** would be another name for Chl **b**. Chlorophyll nomenclature is detailed in Chapter 1.7 by Hynninen. See also the abbreviations list.



SCHEME 1: Oxidation states of the tetrapyrrole macrocycle present in the chlorophylls. From left: porphyrin, 17,18-dihydroporphyrin = chlorin, 7,8,17,18-tetrahydroporphyrin = bacteriochlorin.

and spectroscopic techniques (see Reference 21). Microcrystalline Chl has been known for a long time,[22] but crystals suitable for X-ray analysis have only been obtained from methylpheophorbide **a**[23] and methyl-[24] and ethylchlorophyllide **a**,[25] which confirmed the structure of the macrocyclic portion of the molecule. No crystal structure of the phytylated pigment has been obtained to date. The solution structure, including chlorophyll interactions and side-chain conformations, has mainly been determined by magnetic resonance methods (see References 26 and 27 and Chapter 4.4 by Abraham and Rowan), circular dichroism,[21,28,29] and vibrational spectroscopy (see Reference 30 and Chapter 4.6 by Lutz and Mäntele). All these studies indicate that the macrocycle and in particular the reduced ring D show a marked flexibility upon changes in substitution or of the central metal.

Chl **a** has been used as a reference compound in structure elucidation of many other chlorophylls and related pigments. It is readily available, e.g., from cyanobacteria (blue-green algae) which do not contain Chl **b**. Chl **a** provides a chiral and substituent pool from which a variety of reactions allow extensive modifications and correlations among the chlorophylls (see Chapters 1.7 by Hynninen and Chapter 1.6 by Smith). A key to many such modifications is improved methods for insertion of magnesium[31] because the demetalated pheophorbides are much more stable than the chlorophylls proper and hence often better suitable for chemical handling.

III. CHLOROPHYLL b

Chlorophyll **b** (Chl **b**) (Figure 3) is distinguished from Chl **a** by a 7-formyl instead of the 7-methyl-substitutent. Its structure has been established by chemical correlation with Chl **a**; the stereochemistry and esterifying alcohol of both pigments are identical. The X-ray structure of ethylchlorophyllide **b**[32] also shows a very similar conformation of the macrocycle and the substituents. Aggregation involving nucleophilic groups is markedly different, however, because of the presence of the additional carbonyl substituent at C-7 (see Reference 32 and Chapters 1.8 and 1.9.). Due to the electron-withdrawing effects of this substituent, the basicity of the central nitrogen is decreased,[22,57] and the spectroscopic properties are markedly changed. (See Section 4.) The 7-formyl-group is also a suitable substituent for chemical modifications *in vitro* (Chapter 1.7) and *in situ*.[175] As an example, it has been used by Davis et al.[176] to introduce a point-charge at a defined position at the chlorophyll periphery.

In the "green" series of oxygenic photosynthetic organisms (Table 1), Chl **b** accompanies Chl **a** and is generally present as a light-harvesting pigment in about a 1:3 ratio. This includes the prochlorophytes, green algae, and green plants. Whereas Chl **a** is complemented in most oxygenic organisms by either Chl **b** ("green line") or Chls **c** ("brown line") or phycobiliproteins ("blue and red line"; see chapter by Hiller *et al.*), Chl **b** has recently been identified together with c-type chlorophylls (see below) in a few chromophytes like, e.g., *Mantionella squamata,* which is of evolutionary significance (see Chapter 3.2 and Reference 161).

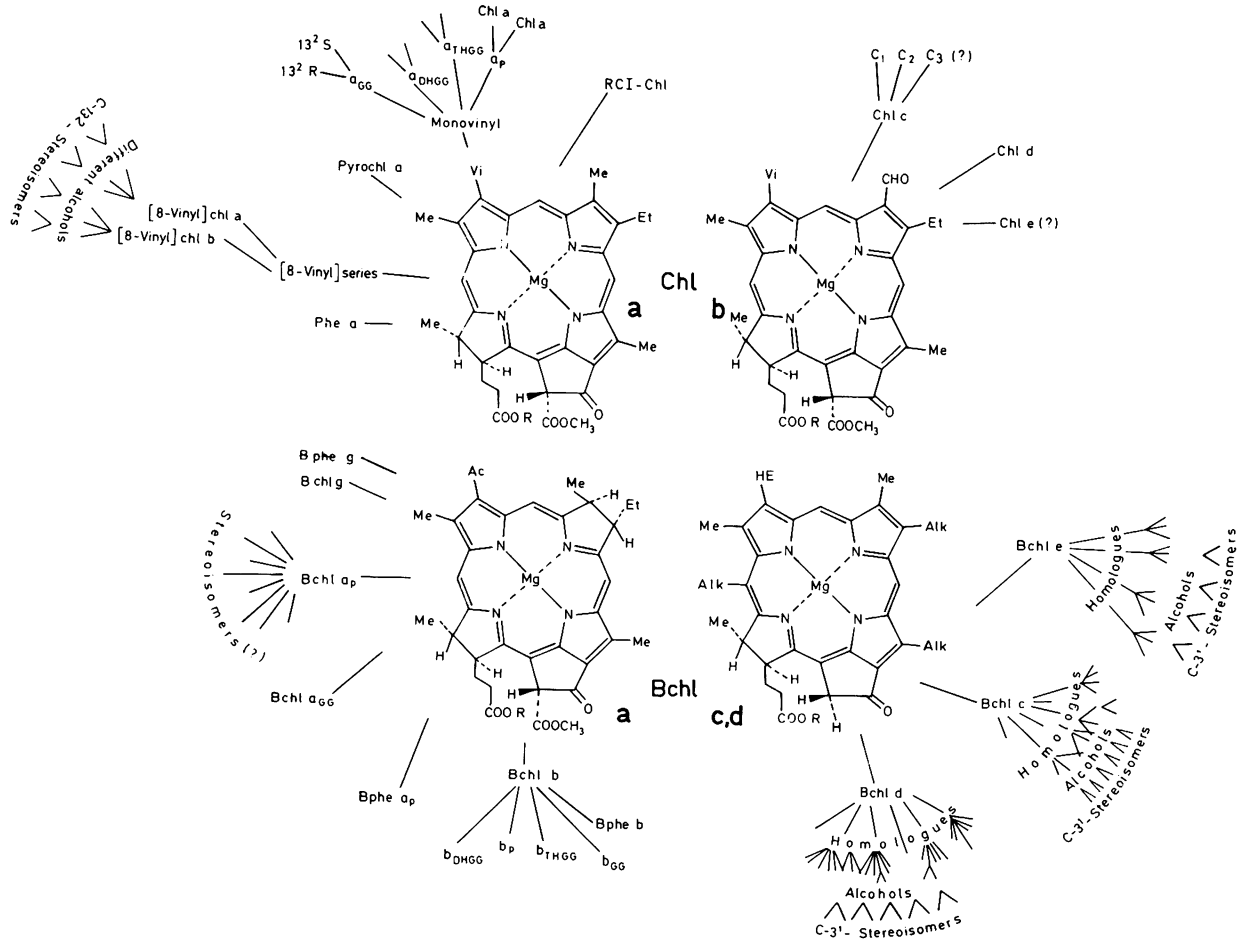


FIGURE 1. The chlorophyll explosion. The three established structures in 1960 were those of chlorophyll **a**, chlorophyll **b**, and bacteriochlorophyll **a** shown in the center. Subscripts refer to the esterifying alcohols discussed in the concluding section and shown in Figures.[13,14]

TABLE 1

	Chlorophyll				Bacteriochlorophyll					
	a	**b**	**c**[a]	**d**	**a**	**b**	**c**[b]	**d**[b]	**e**[b]	**g**
Purple bacteria[c]	−	−	−	−	+	+	−	−	−	−
Green bacteria[d]	−	−	−	−	+	−	+	+	−	−
Brown bacteria[d]	−	−	−	−	+	−	−	−	+	−
Erythrobacter	−	−	−	−	+	−	−	−	−	−
Protaminobacter[c]	−	−	−	−	+	−	−	−	−	−
Heliobacteria	?	−	−	−	−	−	?	−	−	+
Prochlorophytes[f]	+	+	−	−	−	−	−	−	−	−
Cyanobacteria	+	−	−	−	−	−	−	−	−	−
Rhodophytes	+	−	−	?	−	−	−	−	−	−
Cryptophytes	+	−	+	−	−	−	−	−	−	−
Chlorophytes	+	+	−	?	−	−	−	−	−	−
Micromonadophytes	+	+	+	−	−	−	−	−	−	−
Prymnesiophytes	+	−	+	−	−	−	−	−	−	−
Chrysophytes	+	−	+	−	−	−	−	−	−	−
Pyrrophytes	+	−	−	−	−	−	−	−	−	−
Diatomes	+	−	+	−	−	−	−	−	−	−
Dinoflagellates	+	−	+	−	−	−	−	−	−	−
Pheophytes	+	−	+	−	−	−	−	−	−	−
Green plants	+	+	−	−	−	−	−	−	−	−

[a] Chlorophyll **c** is a type name which covers an increasing number of Mg-pheoporphyrins (see text).

[b] Bchl **c**, **d**, and **e** are type-names for sets of homologous pigments differing in stereochemistry and degree of methylation (see text).

[c] Very little is known yet on this species.

[d] The green and the related brown bacteria investigated hitherto contain BChl **c** and/or BChl **d**, or they contain BChl **e** (see Table 3).

[e] Purple bacteria contain either BChl **a** or BChl **b**.

[f] [8-vinyl]-Chl **a** and [8-vinyl]-Chl **b** have been identified in deep water marine prochlorophytes.[62,178]

The majority of Chl **b** is found in the antenna complexes of PS II; in the LHC II-complex(es) it amounts to nearly 50% of the chlorophylls. Since the red absorption band of Chl **b** is at shorter wavelength, the Soret band at longer wavelength than the respective absorptions of Chl **a**, it extends the absorption of light from either side into the visible spectral region (see Chapter 4.1). Chl **b** is less abundant in the antenna of PS I, where its occurrence was debated in the past but has recently been confirmed. Both reaction centers lack Chl **b**, and it is probably also absent in the core antenna complex(es) of both photosystems (see Chapter 3.3).

IV. STRUCTURES RELATED TO CHLOROPHYLL a

The complexity of the photosynthetic apparatus, the large spectrum of organisms, and the variety of functions performed by Chl **a**, have stimulated a search for other chlorophylls. Several closely related pigments have indeed been isolated from plant material and suggested to be functional in photosynthesis. They generally occur in small amounts only. Moreover, with one exception (e.g., the [8-vinyl]-chlorophylls; see below), all these derivatives can principally be formed readily from chlorophylls by nonenzymatic reactions. This had led to considerable skepticism with regard to their involvement in photosynthesis, and an example for the pitfalls shall be given below. However, at least one such pigment, e.g., Phe **a** is present and functional *in situ*, in reaction centers of photosystem II.

TABLE 2
Functions, Occurrence, and Spectra of Chlorophylls

Pigment	Esterifying alcohol[a]	Occurrence	Function[b]	Chlorophyll	Pheophytin
Chlorophyll **a**[p] R_1 = H, R_2 = C_2H_5, R_3 = $COOCH_3$, R_4 = H $C_{55}H_{72}N_4O_5Mg$ MW = 892	Δ2[c]	All oxygenic photosynthetic organisms[p]	A + RC[d]	662, 430[l]	667, 535, 505, 408[l]
Chlorophyll **b**[p] $C_{55}H_{70}N_4O_6Mg$ MW = 906	Δ2	Green plants Algae[e] Prochlorophytes	A	644, 430	655, 525, 412
Chlorophylls $\mathbf{c}_1$, $\mathbf{c}_2$, $\mathbf{c}_3$ + others $C_{35}H_{30}N_4O_5Mg$ MW = 610 ($\mathbf{c}_1$)	H	Pheophyta Cryptophyta Pyrrophyta Chrysophyta Bacillariophyta Prasynophyta	A[f]	626, 576, 444[g] (627, 578, 448)	650, 592, 579, 532, 433[h]
Chlorophyll **d** $C_{54}H_{70}N_4O_6Mg$ MW = 894	Δ2	Rhodophyta Chlorella (?)	A[i]	688, 447	692, 547, 516, 421
Protochlorophyllide[j] (R′ = C_2H_5 or C_2H_3) $C_{35}H_{32}N_4O_5Mg$ MW = 612	H	Oxygenic photosynthetic organisms	P	623, 432[k]	638, 586, 564, 525, 417
[8-Vinyl]-protochlorophyllide (R′ = C_2H_3)	H	Photosynthetic bacteria	P		
Bacteriochlorophyll **a** $C_{55}H_{74}N_4O_6Mg$ MW = 910 (R = Δ2)	Δ2;Δ2,6,10,14	Photosynthetic bacteria	A + RC	773, 577, 358	749, 525, 385, 357
Bacteriochlorophyll **b** (R_1 = $COCH_3$) $C_{55}H_{72}N_4O_6Mg$ MW = 908 (R = Δ2)	Δ2; Δ2,10	Few species of photosynthetic bacteria[m]	A + RC	794, 580, 368	776, 528, 398, 368

Bacteriochlorophyll **g** (R_1 = C_2H_3) $C_{55}H_{72}N_4O_6Mg$ MW = 886	Δ2,6,10,14	*Heliobacterium chlorum*	A + RC	763, 575, 470, 418, 408	753, 518, 396, 388
Bacteriochlorophylls **c**[n]	Mainly farnesol, many others	Chlorobiaceae, Chloroflexaceae	A[o]	660, 432	664, 547, 515, 408
Bacteriochlorophylls **d**[n]		Chlorobiaceae, Chloroflexaceae	A	646, 458	658, 548, 505, 406
Bacteriochlorophylls **e**[n] (isomer mixture)		Chlorobiaceae, Chloroflexaceae (?)	A	654, 424	654, 534, 439

[a] See Figures 13 and 14 for the alcohol.

[b] A = antenna or light-harvesting pigments; RC = reaction center pigments; P = biosynthetic precursor.

[c] Accompanied by Δ2,6,10,14; Δ2,10,14; and Δ2,14 as biosynthetic precursors. Further precursors contain a second vinyl group at C-8 (R_2 = C_2H_3).

[d] The reaction center of photosystem II contains pheophytin **a** (no central Mg) as intermediary electron acceptor. The 13^2-epimer of chlorophyll **a**, e.g., chlorophyll **a**′ has been correlated with photosystem I reaction centers.

[e] Not in xanthophytes, rhodophytes, cryptophytes, or cyanobacteria.

[f] Marine algae contain up to 50% of the chlorophylls **c**.

[g] $\mathbf{c}_1$, values for $\mathbf{c}_2$ in brackets. See text for $\mathbf{c}_3$ and others.

[h] Mixture of $\mathbf{c}_1$ and $\mathbf{c}_2$ in CH_2Cl_2.

[i] Possibly an artifact. However, some species are reported to contain up to 33% of the chlorophylls as chlorophyll **d**.

[j] Protochlorophyll occurs, in part, in the esterified form, e.g., with Δ2-phytaenol (= phytol) and its precursors. [8-vinyl]-protochlorophyllide is also termed bacterioprotochlorophyllide in the literature.

[k] The spectra are solvent dependent.[57]

[l] Monovinyl; the divinyl derivatives have a pronounced redshift (7 nm) in the Soret, and only a small redshift in the long-wavelength region.

[m] *Rhodopseudomonas viridis, Rp. sulfoviridis, Thiocapsa pfennigii, Ectothiorhodospira halochloris,* and *Et. abdelmalekii* contain bacteriochlorophyll **b**, and *Heliobacterium chlorum,* bacteriochlorophyll **g**.

[n] Very variable structure; see Table 3.

[o] Bacteriopheophytin **c** (or a similar pigment) has recently been reported to occur in reaction centers of green bacteria. See text.

[p] [8-vinyl]-Chl **a** and **b** have been found in greening tissues, in a *Zea mays* mutant, and in marine deep-water prochlorophytes (see text).

Adapted from Scheer, H., Chlorophylls, in *Handbook of Chromatography: Plant Pigments,* Vol. 1, *Fat-Soluble Pigments,* Köst, H.-P., Ed., CRC Press, Boca Raton, FL, 1988, 235.

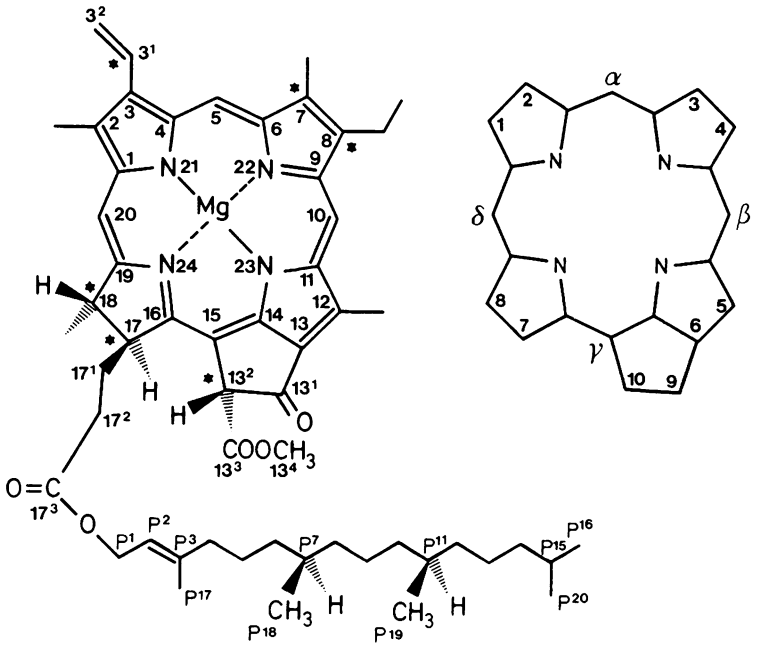


FIGURE 2. Structure of chlorophyll **a**, the most widely distributed chlorophyll; left the IUPAC[1] numbering system of the carbon skeleton, which is used with only few exceptions in this book; and right, the older but still frequently used Fischer numbering system. Carbon atoms which are asymetrically substituted in this or any of the other chlorophylls are marked by asterisks. See also footnote (p), Table 2, and text.

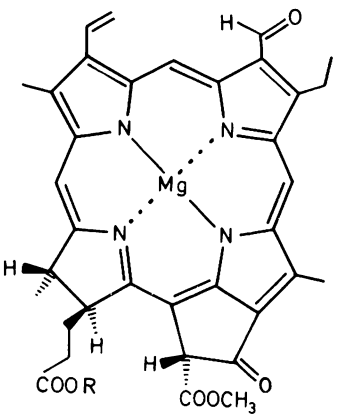


FIGURE 3. Chlorophyll **b**.

A. CHLOROPHYLL a′

Chl **a**′ (*"a-prime"*) was recognized early[33] as a contaminant of chlorophyll extracts and is reversibly interconvertible to Chl **a**. It is the 13^2S-epimer of Chl **a**[34,35] (Figure 4). The interconversion and procedures to isolate it in pure state have only recently been studied in more detail.[36-37] The key to this were chromatographic techniques which prevent the epimerization of the two isomers during and after separation, which prevent degradations, and which allow a ready analysis. The isolation procedure of Watanabe et al.[36] involves the grinding of washed leaves with anhydrous Na_2SO_4, sonication, and extraction with chloroform containing 0.8% ethanol as stabilizer, concentration, and HPLC at low temperatures

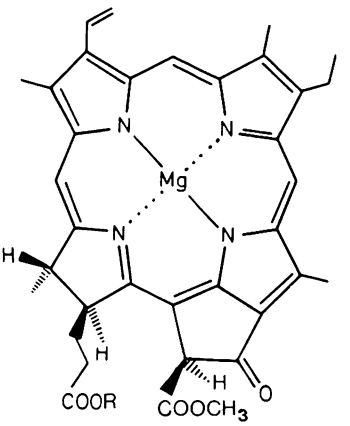


FIGURE 4. Chlorophyll **a**′.

on silica. The whole procedure takes only between 30 and 60 min. Microcrystallin Chl **a**′ has been prepared on a preparative scale by Hynninen and Lötjönen.[37]

Chl **a**′ is slightly less stable than Chl **a**. For the two epimers, $\Delta G^0 = 3.6$ kJ/mol has been determined, and an activation energy of $\Delta H^{\ddagger} = 46.5$ kJ/mol in the absence of base in most solvents.[38] This is sufficient to store and handle it in pure form under appropriate conditions, in particular in the absence of bases, which promote isomerization by abstraction of the 13^2-proton. The absorption spectra of the two epimers are very similar but they can be distinguished by NMR and CD spectroscopy,[21,26-28,35] by their polarities (and hence chromatographic mobilities; see Chapter 1.3 by Shioi). Differences have also been observed for their aggregation[39,40] (see also Chapter 1.8 by Katz et al. and 1.9 by Scherz et al.), and their reactivities.[41-43]

Epimerization at C-13^2 changes the shape of the molecule by steric interaction between the 13^2-$COOCH_3$ and the 17-propionic-acid side chain.[28] Many of the differences can therefore be rationalized in steric terms. However, the changed reactivity indicates that the electronic structure is different as well. An example is the electrophilic substitution at C-20, which proceeds more readily with Phe **a**′ than with Phe **a**.[41] Both steric and electronic structures may then be responsible for the different aggregation of the two chlorophyll epimers.

Chl **a**′ has recently gained considerable interest by the finding of a constant ratio with the reaction center of PS I. After an initial report of 2 Chl **a**′/P700,[44] an improved HPLC system revealed a constant ratio of 1 Chl **a**′/P700.[45] It is therefore possible that Chl **a**′ has a functional role in the charge separation process in PS I. A recent report[184] using PS I-minus mutants indicated, however, that only about 50% of Chl **a**′ are related to this reaction center. In PS I, not only the primary donor P700, but also the primary acceptor $A_0$1 is believed to be a chlorophyll[46] (see also Chapter 5.3 by Parson). A test of this hypothesis has been attempted by a reconstitution experiment.[47] Treatment of inactive PS I particles with Chl **a**′ led to a light-induced difference spectrum which was similar to the light-induced P700 difference spectrum, but slightly blue-shifted. No such reaction was observed when the pigment was added to serum albumin. The bleaching was irreversible, however. In view of the ready formation of chlorophyll aggregates, it then remains to be demonstrated that the observed spectrum is not due to the photooxidation of such a complex, rather than of a reconstitution product (see Chapters 1.8, 1.9, and 2.5). It should be mentioned that no prime-pigments have been found in the crystal structure of bacterial reaction centers (see Chapter 3.5), although they can be incorporated *in vitro*.[140,141]. However, the latter are homologous to reaction centers of PS II, and one has to wait for results on PS I, or on bacterial reaction centers of type I, e.g., from *Chlorobium* or *Heliobacterium*.

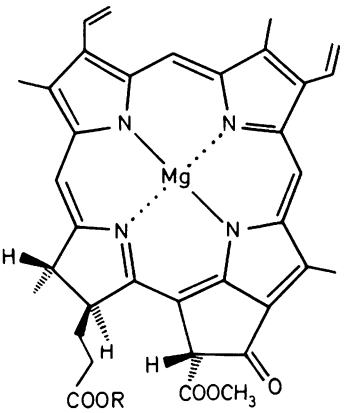


FIGURE 5. [8-vinyl]- or "Divinyl"-chlorophyll **a**.

B. [8-VINYL]-CHLOROPHYLL a AND OTHER [8-VINYL]-PIGMENTS

[8-Vinyl]-Chl **a**, which is often referred to as divinyl-Chl **a** (Figure 5), was first characterized in greening cucumber seedlings.[48] Subsequently, it has been detected in many different tissues. Its structure was first suggested from a red-shift in its fluorescence spectrum,[49] and was subsequently confirmed by NMR and mass spectroscopy[50-52] and chemical correlations.[53] [8-vinyl]-Chl **b** has been identified subsequently in *Zea mays* seedlings by similar techniques.[54] The diversity is reminiscent of the situation with Chl $\mathbf{c}_1$ and $\mathbf{c}_2$ which also differ by having a C-8 ethyl and vinyl substituent, respectively (see below).

Low-temperature fluorimetry[48,52,56] and reverse-phase HPLC (see References 52 and 57 and also the Chapter 1.3 by Shioi) allow the ready analysis of Chl **a**/**b** and their [8-vinyl]-analogues. The latter are slightly more polar than their respective parent compounds.

The discovery of these chlorophylls led to a reinvestigation of the pigment composition in greening tissue, from which a multiply-branched pathway has been proposed for chlorophyll biosynthesis which involves chlorophylls (= phytol esters), chlorophyllides (= free acids), and additional fatty-acid esterified chlorophylls, each of which can occur in the "normal" 3-vinyl-8-ethyl- and in the new "divinyl"-substitution form (see Reference 58 and Chapters 2.3 by Leeper and 2.4 by Griffiths). The hydrogenation of the 8-vinyl-group can proceed at different stages of biosynthesis, and the enzymes of chlorophyll biosynthesis can use both the 8-ethyl- and 8-vinyl-substituted precursors (see Chapters 2.3, 2.4, and 2.5). This complex pathway poses considerable analytical problems, and not all of the suggested intermediates and enzymes have been fully characterized.

The involvement of the multitude of chlorophylls in photosynthesis is to date only poorly explored. Most of the experiments cited have been carried out with greening systems. A number of mature tissues have been shown to contain certain amounts of [8-vinyl]-Chl **a**, and [8-vinyl]-Chl **b** "appear(s) to occur in small amounts in some green plant species".[56] Other reports employing high-resolution chromatography show little to none of them, however (see, e.g., Chapter 1.3 and the references in Reference 57). Very large amounts of [8-vinyl]-Chl **a** and **b** have been found in a mutant of *Zea mays*.[59] Although this mutant is impaired in photosynthesis due to the lack of stomata, the primary reactions involving chlorophylls are functional,[60] which suggests that [8-vinyl]-Chl **a** can replace at least partly Chl **a**. There is evidence, however, that marine phytoplankton,[61,178] including the recently discovered free-living prochlorophytes,[62] contain large amounts of [8-vinyl]-Chl **a**. A functional role of any of the other pigments in photosynthesis is presently unknown. Rebeiz et al.[63] have recently suggested a correlation of the chlorophyll biosynthesis pathway with the sensitivity to photodynamic damage. Light sensitivity due to overproduction of tetrapyrroles

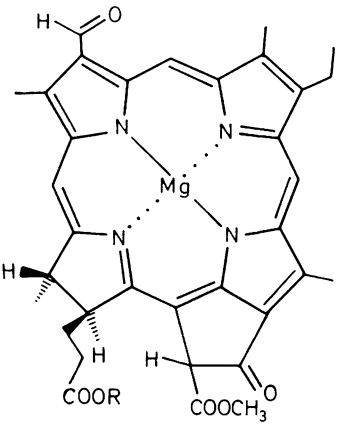


FIGURE 6. Chlorophyll **d**.

can be induced in plants by treatment with δ-amino-levulinic acid or bipyridyls, and their use as light-activated herbicides is currently being explored.[63]

C. PHEOPHYTIN a

Pheophytin **a** (Phe **a**) is a demetalated pigment and thus not a chlorophyll by the chemical, albeit by the biological definition. Demetalation of chlorophylls is promoted by acid and can be accelerated by amphiphiles (see Chapter 1.7 by Hynninen); demetalating enzymic activities have also been described in plants (see References 64 and 65 and Chapter 2.5 by Hendry and Brown). Chlorophyll preparations are thus often contaminated by pheophytins. The specific search for native pheophytin was triggered, and a strategy outlined, by the finding of BPhe **a** or **b** as a constituent of purple bacterial reaction centers, where it functions as an early electron acceptor (see below and Chapter 5.3 by Parson). It is transiently reduced under normal conditions, but can be trapped, e.g., by irradiation in the presence of an excess of electron donors. By applying similar techniques, a Phe **a**-type pigment was identified in plants first by difference absorption, ESR, and ENDOR spectroscopy[66] (see also Chapter 4.7 by Lubitz). The signals were interpreted as to arise from anion radical formation of pheophytin. Extraction under carefully controlled conditions yielded two molecules of Phe **a** (identified by its spectrum and chromatographic mobility) per PS II.[45] Recently the analysis of a highly enriched PS II preparation yielded a ratio of 2 Phe **a**/3 Chl **a**.[67] With preparations of this kind, the function of Phe **a** in electron transport has been fully substantiated (see Chapter 5.3).

D. CHLOROPHYLL d

Chl **d** differs from Chl **a** by the presence of a 3-formyl group (Figure 6).[68] It has been found together with isochlorophyll **d** (of unknown structure) in extracts from rhodophytes,[69,70] and there is a report on the spectrofluorometric indication in *Chlorella*.[71] Chl **d** can be formed artifactually from Chl **a**.[72-74] The status of Chl **d** and the arguments for its being a native pigment have been summarized by Holt[73] and Jackson,[74] and the subject does not appear to have been taken up in the meantime. No Chl **d**-containing pigment-protein complex has been isolated to the author's knowledge.

E. OTHER PIGMENTS

A variety of pigments derived from loss of the 13^2-$COOCH_3$ group or the long-chain terpenoid alcohol, or demetalation, transmetalation, chlorination at C-20, pyrolysis from oxidative reactions at the isocyclic ring V, or a combination thereof, have been isolated

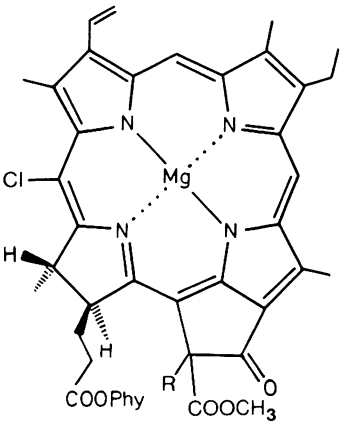


FIGURE 7. Chlorophyll-RCI (epimer mixture at C-13², R = OH)[78,79] and 20-Chloro-chlorophyll **a** (R = H).

from various plant sources. These pigments are generally believed to be degradation or biosynthesis leakage products of chlorophylls, which are formed enzymatically or nonenzymatically (see Reference 75 and Chapter 2.5). Since many studies have been done with decaying biological material, or processed plants, it is currently uncertain which of the products are members of the natural turnover. For none of the pigments a function in photosynthesis has been demonstrated.

The difficulties and pitfalls involved in the study of function of these pigments shall be exemplified with Chl-RC I. This pigment was first identified by absorption difference spectroscopy in an extract from the photosystem II-less mutant C6E of the green alga, *Scenedesmus obliquus*.[76] Its name was chosen because it was isolated from various tissues and subchloroplast preparations at a constant ratio to P700, the primary donor of PS I, and because it had, like P700, a red-shifted spectrum (7 to 15 nm, solvent-dependent, for the Q_Y1- and 2 to 4 nm for the Soret band). From spectral similarities with BChl **c**, a substitution at a *meso*-position was suggested.[77] The structure analysis, carried out with pigments derived from *Scenedesmus*[78] and from the cyanobacterium, *Spirulina geitleri*,[79] showed that chlorophyll-RC I is 13²-hydroxy-20-chloro-Chl **a** (Figure 7). Although chlorinated compounds are known from many plants (see references in References 78 and 79), this was the first chlorinated chlorophyll to be isolated from plant material.

Since both substituents are known artifacts,[41,80-87] proof of the occurrence of Chl-RC I *in situ* was important. Whereas Dörnemann and Senger[78] found a good 1:1 correlation with P700, Watanabe et al.[45] could not detect this pigment at all, but rather varying amounts of a spectroscopically similar one which was later shown to be 20-chloro-Chl **a**.[88] Katoh and Yasuda[89] found only minor amounts of chlorinated chlorophylls after radioactive chlorine labeling. Critical considerations of function[79,84] and spectroscopic properties[90] of either structure were inconclusive in confirming or dismissing the identity of Chl-RC I with P700. From its redox properties, it would rather have been compatible with the primary acceptor A_0,[79] which also appears to be a chlorophyllous pigment.[46]

The artifactual introduction of the 13²-OH substitutent was suggested by the isolation of two epimers (13²*R* and 13²*S*, Figure 5) in variable proportions.[78,79] Since the epimerization of Chl-RC I at C-13² is no longer possible via enolization of the β-ketoester system as in Chl **a**, this indicated a nonenzymatic step during its introduction. Gentle work-up conditions of fresh plant material gave no indication for the presence of chlorinated chlorophylls, but 20-chloro-Chl **a** occurs in aged plant material and can be formed during extraction.[88] Hydroxylation on silica plates (one of the original purification steps) was demonstrated sub-

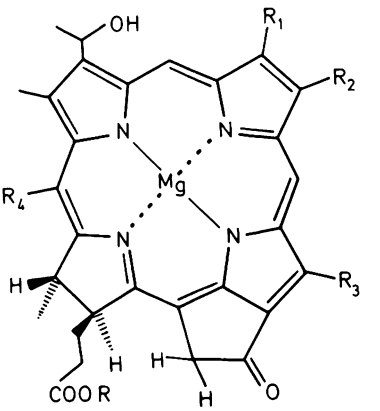


FIGURE 8. Structure of bacteriochlorophylls **c**, **d**, and **e**. See Table 3 for substituents R, R_1, R_2, and R_3, and text for stereochemistry.

sequently.[91] Combined with the conflicting analytical results, this showed that Chl-RC I has *no* relation to photosystem I.[92]

It is a different question, however if 20-chloro-Chl **a** is always an artifactual pigment. Fresh tissue seems to contain very little if any of the pigment,[45,89] but decaying tissue can, on the other hand, accumulate amounts in excess of 1%.[45] This may indicate that the pigment could be involved in chlorophyll breakdown. Chemical evidence to this comes from the finding that 20-Chl-Chl **a** (like C-20 methylated pheophorbides) readily forms bile-pigment(s) after irradiation with visible light.[93,177] Introduction of a substituent at the C-20 position then seems to facilitate the ring opening reaction, possibly due to buckling introduced into the macrocycle by steric hindrance. It is likely that the biodegradation of chlorophylls (like that of hemes[94]) proceeds via bile pigments.* However, none of the tetrapyrrolic products found in aging or degreening organisms (see Reference 75 and Chapter 2.5) offer any obvious advantage with respect to conversion to bile pigments. Chlorination (or any other substitution at C-20) could then be a preparatory step for breakdown, and at the same time reduce the risk of photodynamic damage by the cyclic tetrapyrroles (see Chapter 5.4).

V. BACTERIOCHLOROPHYLLS c, d, AND e

This complex group of pigments (Figure 8 and Table 2) is present in green[73] and brown or red[96] sulfur bacteria (Chlorobiaceae) and in Chloroflexaceae.[97] BChl **c** and **d** were originally named chlorobium chlorophylls 660 and 650,[73] respectively; they are now classified biologically as bacteriochlorophylls. Chemically, they are chlorins with only one reduced pyrrole ring (D), whereas the classical bacteriochlorins have two such rings (B, D). Accordingly, their absorption spectra in solution are similar to those of the green plant Chl **a** and **b**. *In situ*, their absorptions (710 to 740 nm, Q_Y-band) are intermediate between the plant chlorophylls and the BChl **a**, **b**, or **g**.

The BCHl **c** and **d** were each recognized early[73] as a complex mixture of pigments with a number of common structural features: they are chlorins, they lack the 13^2-carbomethoxy group, and they bear an α-hydroxyethyl substituent at position C-3. The pigments of the BChl **c** series differ from the **d**-series by the presence of a C-20 methyl substituent, which is responsible for the red-shifted absorption spectrum. The more recently detected BChl **e** group has this substituent, too, but also carries a 7-CHO group, as does Chl **b**.[96] The name BChl **f** has been reserved for the (yet to be positively identified nature[174]) pigments bearing this 7-CHO group as well, but lacking the 20-CH_3 group.[98] Since the 20-CH_3-substituent in

* Biosynthesis of the chlorophyll-derived bioluminescent bile pigments[95] is unknown.

BChl **c** is introduced by methylation of BChl **d**,[99,100] BChl **f** should likewise be an intermediate for BChl **e**. Pigments of this substitution type have recently been synthesized to aid their search (see References 98, 174, and Chapter 1.6 by Smith).

The variable parts of the structures of each series of these bacterial chlorophylls are the substituents at C-8 and C-10, the stereochemistry at C-3^1, and the long-chain esterifying alcohol at C-17^3 (Table 2). By combining these structural elements, a rather impressive number of different pigments can be written down. Not all of them have been found (yet?), but the number is large enough that their identification has been linked closely with the advance of high resolution chromatographic techniques[57,101,110] (and see Chapter 1.3) and has required a considerable synthetic effort (see, e.g., References 99, 102, 103, 174 and Chapters 1.6 and 1.7). Most of the results summarized here have been taken from a few more recent publications.[100,102,174] (See also Chapter 1.6), which should be consulted for more details and further references.

The structural variations are summarized in Table 3. The somewhat conservative BChl **c** from *Chloroflexus aurantiacus* has a C_2-substituent (ethyl, Et) at C-8, as is commonly encountered in tetrapyrroles.[97] *N*-propyl (Pr) and *iso*-butyl substituents (^{i}Bu) have been identified in BChl **c, d,** and **e** from different *Chlorobium* species; they all arise from methylation of the terminal C-8^2 carbon originating from methionine.[99,100,104] In BChl **d** from *Cb. vibrioforme* and BChl **e** from *Cb. phaeovibrioides* and *Cb. phaeobacterioides,* there even occurs a neopentyl substituent (^{n}Pent) as the last member of the series.[103] 12-Methyl (Me) and 12-Et-substituents are encountered in BChl **c** and **d** from different *Chlorobium* species, whereas BChl **c** from *Cf. aurantiacus* contains only the common 12-Me-,[97] and BChl **e** from *Cb. phaeovibrioides* only the unusual 12-Et-substituent.[105] Together with the presence of a 20-Me substituent in BChl **c** and **e**, it thus appears that extensive, but regiospecific methylation reactions occur in the *Chlorobium* species. Remarkably, the sites of methylation (terminal methyl of the 8-Et substituent, benzylic position at C-12, aromatic C-20) have no obvious chemical or biosynthetic features in common. This indicates the presence of several methylating enzymes, or the methylation of precursors in which these positions are chemically more similar. It has recently been suggested that methylation offers an ecological advantage by introducing a redshift in the antennas of the Chlorobiaceae.[100]

The absolute configuration at C-3^1 (Reference 21) has been determined by chemical degradation correlation with lactic acid[105] and other chlorophylls,[102,103] Horeau analysis,[97,107] and X-ray crystal structure analysis.[108] It is now readily accessible by HPLC analysis.[103] Increasing methylation at C-8^2 is accompanied by a gradual change in stereochemical preference for the asymmetric C-3^1. It is *(R)* configured in the lesser methylated homologues (up to 8-Pr) of BChl **d** from *Cb. vibrioforme* and *S* configured in the higher methylated ones.[103] *(R,S)* mixtures are also found in BChl **e** from *Cb. phaeobacteroides,* again with an increasing proportion of *(S)* with increasing degree of methylation.[109]

The major esterifying alcohol at C-17^3 is farnesol in the Chlorobiaceae[73,105] and stearol in *Cf. aurantiacus*[97] (but see Reference 189 for the latter). Significant amounts of other alcohols are present in either case (see Table 2). The most extensive study[110] has been carried out with BChl **c** from *Chlorobium limicola* forma *Thiosulfatophilum* 2 K. It has been resolved without modification into 12 components containing 6 different alcohols. Among them there are familiar (farnesol, geranyl-geraniol, phytol) and a rare isoprenoid alcohol (Δ2,6), but also a well known (cis-hexadecenol) and a hitherto unknown fatty alcohol (undecylfuran-methanol; see also section on esterifying alcohols).

Besides their well-established antenna function, there are several reports indicating that a BChl **c**-like pigment could be an early electron acceptor in type I bacterial reaction centers, e.g., in the Chlorobiaceae[111] and the Heliobacteria.[112] (See References 185 and 186 for reviews.) The difference maximum of this component (≈ 670 nm) is close to the absorption maximum of BChl **c**, which is present in *Pc. aestuarii.* The pigment has not been identified

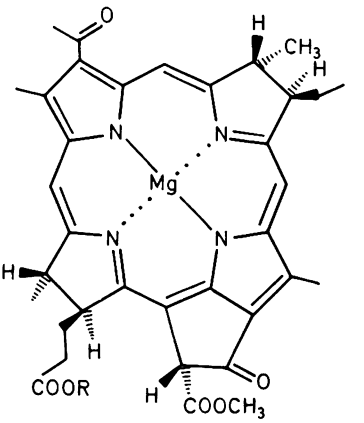


FIGURE 9. Bacteriochlorophyll **a**. See text for esterifying alcohol R.

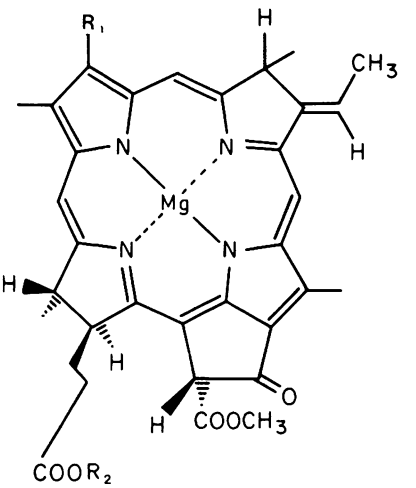


FIGURE 10. Bacteriochlorophyll **b** (R_1 = $COCH_3$) and bacteriochlorophyll **g** (R_1 = C_2H_3). See text for esterifying alcohol R_2.

yet in *Heliobacterium chlorum*. Indeed, considering environmental effects the difference peak can be due to almost any chlorin-type pigment, e.g., Chl **a, d,** BChl **c, d,** or their pheophytins (Table 3). It is not even necessary that the same pigment be present in both types of bacteria, and the presence of Chl **a** has been speculated on chemical reason in *Hb. chlorum*.[113] This situation is reminiscent of photosystem I, in which (a pigment related to) Chl **a** has been suggested as an early acceptor.[46]

VI. BChl a-RELATED STRUCTURES

A. BACTERIOCHLOROPHYLLS a AND b

BChl **a** (Figure 9) is the most widely distributed bacteriochlorin pigment.[114] It occurs in most photosynthetic bacteria (Table 1), and is the only bacteriochlorophyll in most *Rhodospirillales*. In several species, it is replaced by BChl **b**, (Figure 10, R = $COCH_3$), which was first isolated from *Rhodopseudomonas viridis*,[115] but has subsequently been

identified in *Rp. sulfoviridis*,[116] in several *Ectothiorhodospira* species,[117,118] in *Thiocapsa pfennigii*,[119] and in some other purple nonsulfur-bacteria.[120] Much interest has recently focused on this pigment and the species, *Rp. viridis*, due to the crystal structure analysis of its reaction center (see Chapter 3.5).

BChl **b** differs from BChl **a** by the presence of a C-8 ethylidene group[121,122] which is responsible for its chemical lability.[122,123] The stereochemistry of BChl **a** and **b** at the reduced ring D and the isocyclic ring is identical to that of Chl **a** (17*R*, 18*R*, 13^2R[21,122]). The common asymmetric C-7 at ring B is *R*-configured in BChl **a**;[21] that of BChl **b** is still unknown, but may be expected to be the same. C-8 in BChl **a** is *R*-configured as well.[121] The 8-ethylidene group of BChl **b** has *E*-configuration.[124] The crystal structure of a BChl **a** derivative[155] and several BChl proteins[130,134-136] has confirmed this stereochemistry.

Reaction centers of photosynthetic bacteria (with the exception of *Heliobacteria*) contain either BChl **a** or BChl **b** as the primary donor, P870 and P960, respectively (see Chapters 3.5 and 5.3). In purple bacteria, the two monomeric BChl **a** or **b**, B800 or B830, respectively, have identical pigments, whereas in the green bacterium, *Cf. aurantiacus*, the one on the inactive branch (termed M or B) is replaced by BPhe **a**[125] (see also below). BChl **a** or BChl **b** is also the only antenna chlorophyll in purple bacteria. BChl **a** has also been found in some bacteria which are not classified among the common photosynthetic bacteria.[126-129,179,180] At least two of them, *Erythrobacter spec.* and *Protaminobacter ruber* have been shown to synthesize BChl **a** and photophosphorylate at rather high oxygen tension, contrary to the "classical" photosynthetic bacteria.[114] A recent classification can be found in Reference 180.

There are also several antenna fraction(s) containing BChl **a** in the green bacteria (see Chapter 3.1 by Hawthornethwaite and Cogdell). One of them, a water-soluble fraction from *Chlorobium limicola* forma *thiosulfatophilum* 2 K, was the first chlorophyll-protein for which a high-resolution crystal structure had been determined[130] (and see Chapter 3.5). It is thought to be a link in energy transfer from chlorosomes to the core antenna surrounding the reaction centers, which contains BChl **a** as well. A somewhat similar picture has also been arrived at for *Cf. aurantiacus*.[131,132]

B. BACTERIOPHEOPHYTINS a AND b

BPhe **a** was the first chlorophyll "alteration" product identified as a native constituent of photosynthetic complexes. Both BPhe **a** and **b** occur in reaction centers from photosynthetic bacteria, where they accompany their respective parent chlorophylls (see Chapters 3.5 and 5.3). They have distinct absorptions at the short-wavelength side of the Q_y band system, and in particular the Q_x band around 530 nm is well separated from the BChl Q_y band around 600 nm. Interactions with the surrounding protein and other pigments require pigment extraction for quantitation. A ratio of 2 BPhe to 4 BChl has been determined analytically for *Rs. rubrum* (see below for the difference in esterifying alcohols),[133] and this ratio has been verified by the recent X-ray results on reaction centers of *Rp. viridis* and *Rb. sphaeroides*[134-137] (and see Chapter 3.5). In view of the very similar absorption spectra of other reaction centers to either one or the other of these two species, the 2:4 ratio is now generally accepted for purple photosynthetic bacteria.

Reaction centers from the green bacterium, *Cf. aurantiacus*, however, differ by the replacement of one BChl **a** by a BPhe **a**. Since the histidine residue binding the central magnesium of the monomeric B_B (alternatively termed B_M) on the inactive branch in purple bacterial reaction centers is replaced by isoleucine, it has been suggested that this is the site occupied by BPhe **a** in *Cf. aurantiacus*.[138,139] Replacement of BChl **a** by BPhe **a** was also achieved by a series of site-directed mutations with *Rb. capsulatus* and *Rb. sphaeroides* (see Chapter 3.7 by Bylina and Youvan and Reference 181). Transformation of BChl-binding histidines by isoleucines always leads to replacement of the respective BChl by a BPhe, and

vice versa. This indicates that either the character of this amino acid determines if a Mg-containing BChl or a Mg-free BPhe is incorporated, or that a missing histidine as a ligand to the central Mg stimulates the loss of the metal. The former argument of a selection from pigments offered is supported by two independent lines of evidence: in reaction centers from *Rs. rubrum,* the BChl is esterified with geranylgeraniol (Δ2,6,10,14), the BPhe with phytol (Δ2), which argues against a simple demetalation.[133] Recently, exchange experiments with reaction centers from *Rb. sphaeroides* have shown that, irrespective of a series of modifications at the periphery of the tetrapyrrole, all magnesium-complexes which did exchange did so with the monomeric BChl, and all free bases which could be introduced were incorporated at BPhe sites.[140,141,182] It has also been shown that BPhe is not bound by the B873 apoprotein of *Rs. rubrum.*[104]

One of the two BPhes is the first (or probably rather the second[142]) electron acceptor in the primary charge separation in bacterial photosynthesis (see Chapter 5.3). Based on the crystal structure of *Rp. viridis* reaction centers, this PPhe is identified as the one situated on the A or "active" branch of the superficially symmetric structure. The site is alternatively termed H_A or H_L (see Chapter 3.5). The function of the second BPhe (M or B) coordinated to the M-subunit is less understood. It may then only be a remnant which is no longer active, but other functions like structural,[141] tuning of the reaction center absorptions by interaction with the other pigments,[143] "safety valve" acting in cooperation with the neighboring BChl and carotenoid (see References 136 and 144 and Chapter 3.5) are possible.

C. STEREOISOMERS

BChl **a** and **b** have the same enolizable β-ketoester system as Chl **a** and can thus also form the "prime-pigments" BChl **a**′ and BChl **b**′, respectively. They are much less characterized, however, and to the author's knowledge only a partial structural and spectroscopic study has been carried out.[34,183] Under standard isolation conditions (methanol, acetone or mixtures thereof, and in particular in the presence of bases), epimerization is likely and extracts contain generally the epimers in varying ratios. The equilibrium mixture contains approximately 20% BChl **a**′[34] or **a**′[145], with the percentage varying with the solvent used[183] (see also Chapter 1.3). Reaction center crystals show only the presence of the thermodynamically more stable 13^2R-epimer[134-137] (and see Chapter 3.5). A series of minor pigment components has been suggested to represent stereoisomers of BChl **a** at the reduced C-7; -8; -17, and -18,[146] but a confirmation for this is still lacking.

D. BACTERIOCHLOROPHYLL g*

This pigment has been isolated from *Heliobacteria,* e.g., bacilliform, brownish-green, strictly anaerobic, nitrogen-fixing bacteria from soil whose phylogenetic relation to other photosynthetic bacteria is unclear.[147] It has the chromophore of BChl **b**, but carries a vinyl rather than an acetyl group at C-3* (Figure 10, R = C_2H_5),[148] and farnesol rather than phytol (Δ2) as the esterifying alcohol.[113] It is very labile and readily forms pigments of the Chl **a** spectral type bearing an oxidized C-8 side chain.[148] These are similar to the reaction products of BChl **b**,[123] with the exception of the C-3 substituent and the C-17^3-esterifying alcohol (Figure 11).

BChl **g** is present as antenna and reaction center chlorophyll of *Hb. chlorum* and other *Heliobacteria.* The reaction centers have not been isolated, and the ESR[149] and optical data[150] are presently inconclusive if the primary donor (P840) is dimeric, too. As in the Chlorobiaceae, a BChl **c**-like pigment has been suggested as electron acceptor (see above). The chromophore is isomeric to Chl **a** and could arise from the latter by isomerization of the

* The name BChl **f**[94] has been reserved to the (yet to be positively identified in nature) pigments(s) differing from BChl **e** by the absence of the C-20 methyl group, similar to the difference between BChl **d** and BChl **c** (see above).

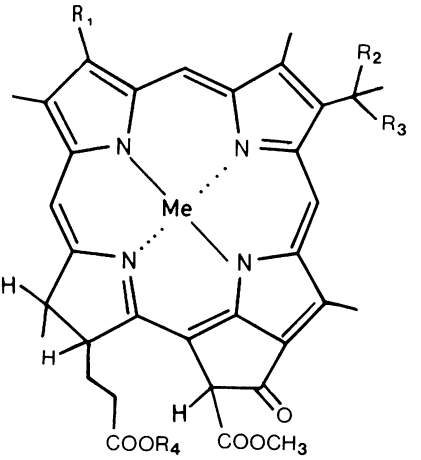


FIGURE 11. Type structure of the isomerization and/or oxidation products of BChl **b** and BChl **g**.

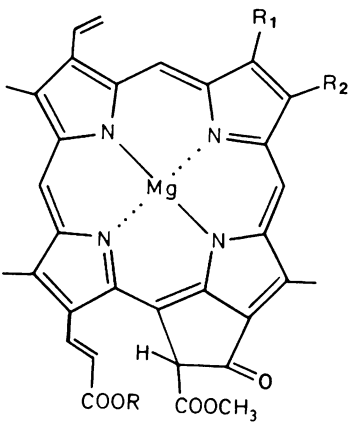


FIGURE 12. Chlorophylls of the **c**-type. Chl $\mathbf{c}_1$: $R_1 = CH_3$, $R_2 = C_2H_5$; Chl $\mathbf{c}_2$: $R_1 = CH_3$, $R_2 = C_2H_5$; Chl C_3: $R_1 = COOCH_3$, $R_2 = C_2H_3$.

endocyclic Δ7,8 into the exocyclic Δ8,8′ double bond. It has been suggested that this could be a key reaction in the biosynthesis of the BChl **a**, too.[113,151]

VII. CHLOROPHYLL c

Chl **c** or chlorophyllide (Chlid) **c** (see below) is the common name for what were originally considered two[152-154] and now are more than three[3,156-159] chlorophylls which are widely distributed and abundant in the chromophyte algae (Table 1) (see References 3, 4, and 158 and Chapter 3.2 by Hiller et al.). These pigments have the fully unsaturated porphyrin macrocycle. They generally (but see Reference 159) do not carry a long-chain esterifying alcohol at the C-17 acrylic acid side chain and should therefore be termed chlorophyllides (Chlid) rather than chlorophylls. To the author's knowledge, the stereochemistry at the only asymmetric C-13[2] is unexplored. Three Chl **c** structures are currently established (Figure 12). They all have an acrylic side chain at C-17 in common. Chl $\mathbf{c}_1$ and Chl $\mathbf{c}_2$ differ by the presence of an 8-ethyl- and 8-vinyl-substituent, respectively.[152-154] This situation is

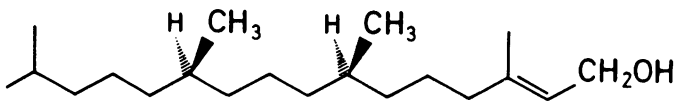


FIGURE 13. Phytol.

reminiscent of the [8-vinyl] precursors of Chl **a** and **b**, which are porphyrin-free acids, too (see above). Chl $\mathbf{c}_3$ has recently been shown to carry a $COOCH_3$- substituent at ring B, and the structure shown in Figure 12 has been suggested.[156] Interestingly, a Chl **c**-type pigment bearing an oxygenated substituent at this position had been postulated only a little while ago[160] based on geochemical evidence (see Chapter 1.13 by Callot).

It had generally been accepted that algae contain either Chl **b** *or* Chl **c**. However, the chlorophyte *Mantionella squamata* and the related *Micromonas pulsilla* contain both Chl **b** and Chl **c**, and light-harvesting complexes isolated from them gave evidence that both pigments function in photosynthesis.[161,162] These species also contain besides Chl **a** and Chl **b** a third chlorophyll species which had been assumed to be Mg-3,8-divinyl-pheoporphyrin $\mathbf{a}_5$ monomethylester a precursor of Chl **a**.[187] Recently this pigment was reinvestigated and was shown to be chromatographically and spectrally similar to Chl $\mathbf{c}_1$. The new Chl **c** types from both prasinophytes are rather labile and appear to have two vinyl-groups as do the chromatographically different Chl $\mathbf{c}_2$ and 3,8-divinyl-pheoporphyrin $\mathbf{a}_5$.[163] Last, but probably only for the moment, there has been a report on a **c**-type chlorophyll bearing a phytol residue at the propionate side chain.[159] A careful examination[161] will be required in all these cases to prove a participation in photosynthesis.

Several authors have shown that Chl $\mathbf{c}_1$ and Chl $\mathbf{c}_2$ are protein-bound in the light-harvesting antenna of chromophyte algae (see Reference 188 and the chapter by Hiller *et al.*). The Chl **c** pigment isolated from the prasinophytes was also proved to function in light-harvesting[161,162] and therefore fits into the definition of a true Chl **c**. Recently, Wilhelm and Wiedemann (unpublished results) could demonstrate that Chl $\mathbf{c}_3$ functions together with Chl $\mathbf{c}_2$ in the light-harvesting antenna of a prymnesiophyte. A careful examination will be required in the other cases to prove a participation in photosynthesis.

VIII. ESTERIFYING ALCOHOLS

Phytol (Δ2-phytaen-1-ol, Δ2*, Figure 13) is the most common esterifying alcohol of chlorophylls. Small amounts of chlorophylls esterified with higher unsaturated phytanols are found in green plants and other oxygenic photosynthetic organisms.[164-166] They are very rare in green plants, and are believed to be biosynthetic precursors of Chl $\mathbf{a}_{\Delta 2}$ and Chl $\mathbf{b}_{\Delta 2}$ (see Chapters 1.3 by Shioi and 2.5 by Rüdiger and Schoch). In bacteria, bacteriochlorophylls carrying alcohols other than phytol are more frequent. As in green plants, these include the biosynthetic precursors of phytol (see Chapter 1.3), but in several species they are the main pigments which are functional in photosynthesis. BChl **a** from *Rhodospirillum rubrum* contains Δ2,6,10,14 (geranylgeraniol).[167] Interestingly, a small but significant pigment pool in this species, e.g., the BPhe **a** in the reaction center, is esterified with Δ2.[133] This indicates not only a specific binding site, but also a specific biosynthetic pathway and not just a demetalation reaction of BChl **a** for its formation. BChl **b** from *Ectothiorhodospira halochloris* and *Et. abdelmalekii* contains Δ2,10 as the major esterifying alcohol, besides smaller amounts of Δ2 and a trienol (probably Δ2,6,10).[168,169]

The C-15 isoprenoid, farnesol, is the major esterifying alcohol in the BChl **c, d, e** of *Chlorobiaceae*,[73,110] whereas BChl **c** from *Cf. aurantiacus* contains a mixture of fatty acid

* A short notation for alcohols with the phytan-1-ol skeleton is used by indicating the position of double-bond, e.g., phytol = Δ2, geranylgeraniol = Δ2,6,20,14 (see Figure 14).

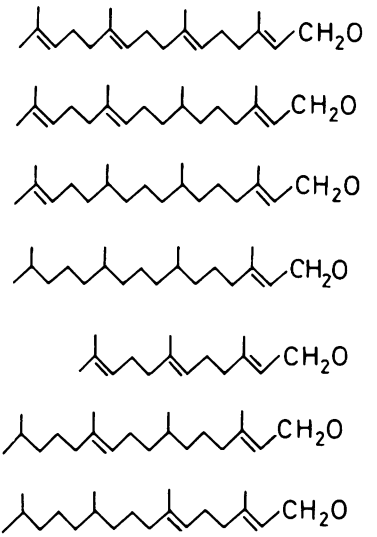


FIGURE 14. Other isoprenoid esterifying alcohols of chlorophylls and abbreviations used in the text. From top to bottom: geranylgeraniol (Δ2,6,10,14); Δ2,10,14-phytatrienol (Δ2,10,14); Δ2,14-phytadienol (Δ2,14); phytol (Δ2, see also Figure 13), farnesol, Δ2,10-phytadienol (Δ2,10); Δ2,6-phytadienol (Δ2,6).

TABLE 3
Well-Studied Bacteriochlorophylls c, d, e in Different Organisms

Organism	Type	R_1	R_2	R_3	3[a]	R[b]
Cf. aurantiacus	**c**	Me	Et	Me	R	Stearyl, Δ2, Δ2,6,10,14, others[c]
Cb. limicola forma *thiosulfatophilum*	**c**	Me	Et, ^{n}Pr, ^{i}Bu	Me, Et	R, S	Farn, others[d]
Pc. aestuarii						
Cp. ethylicum						
Cb. vibrioforme	**d**	Me	Et, ^{n}Pr, ^{i}Bu, ^{neo}Pent	Me, Et	R, S	Farn, others[d]
Cb. phaeovibrioides	**e**[c]	CHO	Et, ^{n}Pr, ^{i}Bu, ^{neo}Pent (?)	Et	R, S	Farn, others[d]
Cb. pheobacteroides						

Note: See Figure 8 for structures and locations of R—R_3.

[a] Absolute configuration at C-3[1]. The R:S ratio depends on the R_2-substituent and decreases with its increasing size.
[b] See Figure 14 for abbreviations.
[c] *Cb. phaeobacteroides* may also contain BChl **f**.[174]
[d] See Reference 110.
[e] See Reference 189.

(C-16, C-18, C-18:1) and isoprenoid alcohols (Δ2 and Δ2,6,10,14).[97,189] Farnesol is also present in BChl **g** from *Heliobacterium chlorum*.[113] The BChl **c, d,** and **e** from *Chlorobiaceae* contain also minor fractions esterified with a variety of alcohols, some of them being isoprenoids, but also the unbranched fatty alcohol, stearol[110] (see Table 3).

The alcohol portion constitutes approximately 30% by weight of the chlorophyll molecule. In moderately polar environment, it hardly affects its chemical and optical properties. In hydrophobic or aqueous environments or at polarity boundaries, however, the alcohols profoundly affect the properties of the pigment, as evidenced, e.g., by the large chromat-

ographic differences on reverse phases (see Chapter 1.3) or differences in their aggregation.[171] The natural environment of chlorophylls is rather hydrophobic. The aforementioned specific esterification of bacteriochlorophylls points undoubtedly to a biological significance in these variations, but this is still poorly investigated on the molecular level. The very well-defined electron densities for most of the alcohol atoms in crystals of the BChl **a** protein from *Chlorobium*[130] and the reaction centers of *Rp. viridis* and *Rb. sphaeroides*[134-137] contrast with the much less-defined ones of the carotenoids in the latter (if present), and indicate very specific interactions.

This is supported by the finding of alcohol-specific exchange reactions of the B800 BChl molecules ($B_{A,B}$) photosynthetic reaction centers.[170] Whereas BChl $\mathbf{a}_{\Delta 2,4,10,14}$ can be introduced into reaction centers from *Rb. sphaeroides*, BChl $\mathbf{a}_{\Delta 2}$ is much more difficult to exchange into reaction centers from *Rs. rubrum*, in which BChl $\mathbf{a}_{\Delta 2,6,10,14}$ is the naturally occurring pigment. BChl **a** esterified with different alcohols are accepted by the B873 apoprotein, but only the "native" BChl $\mathbf{a}_{\Delta 2,6,10,14}$ gave a complex with the correct cd-spectrum.[155]

Distinct differences among BChls esterified with different alcohols were also observed in very hydrophobic environments and at interfaces, where chlorophylls readily aggregate. BChl **a**-micelles, both in mixed organic-aqueous solvents and in micelles with the detergent Triton X-100, show a much more pronounced aggregation with the pigment esterified with Δ2,6,10,14 as compared to Δ2.[171]

The function(s) of the particularly large variety of alcohols in BChl **c, d,** and **e** is presently unclear. *Chlorobium* species contain large amounts of an active chlorophyllase,[172] so some of the pigments may be artifactual. They may, on the other hand, be important for the chlorosome organization. The interior of this organelle is very rich in BChl **c, d,** or **e** (see Chapter 3.1 by Hawthornethwaite and Cogdell and Chapter 5.1 by Sundström and van Grondelle), and there is one report indicating that it may be devoid of protein altogether.[173] In any event, it is likely that the chlorosome is not fully homogeneous. The variety of alcohols (and of peripheral substituents, see above) may be related to this, and by, e.g., determining their location within the superstructure. The esterifying alcohol may also have a function in adapting the BChl **b**-containing *Ectothiorhodospira* species to their very alkaline and high-salt biotope,[117] and in adapting *Chloroflexus aurantiacus* to high temperatures.[131,132]

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