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A New Bacteriochlorophyll from Brown-Colored Chlorobiaceae*

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Abstract. A new bacteriochlorophyll has been isolated by thin layer chromatography from all strains of the brown-colored Chlorobiaceae *Chlorobium phaeobacteroides* and *Chlorobium phaeovibrioides*. The new bacteriochlorophyll e —like the bacteriochlorophylls c and d—represents the major amount of bacteriochlorophyll in the cells in addition to small amounts of bacteriochlorophyll a. Bacteriochlorophyll e can be differentiated from the bacteriochlorophylls c and d by its absorption maxima in aceton and its different R_f-value in the thin layer chromatogram. The structure of the new bacteriochlorophyll e has been elucidated on the basis of mass spectra, ^{1}H- and ^{13}C-NMR-spectra, the UV/VIS-spectrum as well as IR-, ORD-, and CD-spectra. The new bacteriochlorophyll has the same relationship to bacteriochlorophyll c as chlorophyll b from green plants to chlorophyll a; therefore, bacteriochlorophyll e represents the first formyl-substituted chlorophyll from bacteria. Similar to the bacteriochlorophylls c and d, the new bacteriochlorophyll e consists of a mixture of at least three homologues which differ from each other by different substituents on the pyrrol rings II and III.

Key words: Bacteriochlorophyll e — Bacteriopheophytin e — Absorption Spectra — Bacteriochlorophylls c, d, e — Chlorophylls a, b — Structure — Homologues — *C. phaeobacteroides* — *C. phaeovibrioides*.

Until now the four different bacteriochlorophylls a, b, c, and d have been isolated from phototrophic bacteria (Jensen *et al.*, 1964). The present paper reports on a new bacteriochlorophyll which was isolated from all strains of the brown-colored phototrophic Chlorobiaceae. These strains belong to the two species *Chlorobium phaeobacteroides* and *Chlorobium phaeovibrioides* which were first described by Pfennig (1968). At that time, the bacteriochlorophylls of these new species have not been isolated and were characterized only by the absorption spectra of the living cells. In the course of studies on the nature of the bacteriochlorophylls a of all type strains of the Chlorobiaceae (Gloe and Pfennig, 1974) it was observed that the bacteriopheophytins of the major bacteriochlorophyll components of the brown-colored *Chlorobium* strains differed markedly in their chromatographic properties from the bacteriopheophytins c and d of the green *Chlorobium* strains (Stanier and Smith, 1960). This observation gave rise to the present study in which the new bacteriochlorophyll e will be characterized in detail.

Abbreviations Used. DSM = Deutsche Sammlung von Mikroorganismen, Göttingen; Bchl. = bacteriochlorophyll; Bph. = bacteriopheophytin; P = phytol; Gg = geranylgeraniol; F = farnesol; C = Chlorobium.

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Materials and Methods

Organisms. The following strains of phototrophic bacteria from the Deutsche Sammlung von Mikroorganismen, Göttingen, were used:

Chlorobium phaeobacteroides
Strains: 2430, 2431, 9230
DSM-No.: 266, 267, 268

Chlorobium phaeovibrioides
Strains: 2631, 2531, B 1 (Zenitani)
DSM-No.: 269, 270, ...

Rhodopseudomonas sphaeroides
Strain 17023 DSM-No.: 158 Bchl.: a

Chlorobium vibrioforme
Strain 6030 DSM-No.: 260 Bchl.: d

Chlorobium limicola forma thiosulfatophilum
Strain 6230 DSM-No.: 249 Bchl.: c

Chlorobium vibrioforme forma thiosulfatophilum
Strain 1930 DSM-No.: 265 Bchl.: d

Pelodictyon luteolum
Strain 2532 DSM-No.: 274 Bchl.: d

Culture Medium. With the exception of *Rhodopseudomonas sphaeroides*, all strains were grown in the following culture medium. The amounts are given for a total of 20 l medium: part I: $CaCl_2 \cdot 2\,H_2O$ 3 g, distilled water 200 ml; part II: $NaHCO_3$ 27 g, distilled water 4 l; part III: $Na_2S \cdot 9\,H_2O$ 18 g, distilled water 200 ml; part IV: trace element solution SL 4 (Pfennig and Lippert, 1966) 180 ml, NH_4Cl 6 g, $MgSO_4 \cdot 7\,H_2O$ 9 g, KCl 6 g, KH_2PO_4 6 g, Na-acetate 20 g, $Na_2S_2O_3$ 20 g, vit. B_{12}-solution (2 mg/100 ml) 36 ml, distilled water 600 ml; part V: distilled water 15 l.

Part II was flushed with CO_2 for about 1 hr (CO_2-saturation) and then mixed with parts I, IV and V and sterile filtered with CO_2-pressure through Seitz filters EKS. Part III was autoclaved and aseptically added to the rest of the medium. The pH of the medium was adjusted to 6.8. Strains with NaCl requirement (DSM-No. 260, 265, 269, 270 and B1) obtained 1% NaCl from a sterile 25% NaCl-solution. *Rhodopseudomonas sphaeroides* was grown in a culture medium for Rhodospirillaceae (Pfennig and Lippert, 1966).

Growth Conditions. All strains of the Chlorobiaceae were cultivated in bottles of 100 ml, 500 ml, 10 and 20 l of medium. The 100 and 500 ml cultures were incubated for 2 to 3 weeks at 50 foot candles light intensity in order to obtain a decent cell concentration, partially neutralized $Na_2S \cdot 9\ H_2O$ solution was added several times. The 10 and 20 l cultures were illuminated at first with 100 foot candles; when growth had started the light intensity was increased to 600–800 foot candles. Incubation temperature was 28–30° C in all cases.

Bacteriochlorophylls. For the extraction of bacteriochlorophyll, 1 g of wet bacteria was treated with about 100 ml of acetone, magnetically stirred in the dark for about 30 min and then centrifuged. This procedure was repeated using a mixture of 135 parts of acetone and 25 parts of carbontetrachloride. Both extracts were then combined.

Bacteriopheophytins. The bacteriochlorophyll extracts were two times treated with about 100 ml 0.5 N hydrochloric acid to obtain the magnesium–free bacteriopheophytins. During this procedure an acidic acetone-water phase and a carbontetrachloride phase containing all the photopigments are formed. After separation of the phases, the carbontetrachloride phase was washed several times with distilled water to remove all acid. After addition of anhydrous Na_2SO_4 to bind the remaining water, the carbontetrachloride extract was used for the separation of the photopigments by thin layer chromatography (see also Gloe, 1973).

Bacteriomethylpheophorbids. The purified bacteriopheophytins were converted to the corresponding bacteriomethylpheophorbids by standing for 2 hrs at room temperature with methanol containing 4% of concentrated sulfuric acid. By heating this mixture additional methylation of the 2-hydroxy-ethyl-group occurred.

Chromatography. Bacteriopheophytin was isolated from the accompanying pigments by chromatography on silica gel plates (Merck number 5553):

1. Solvent system: carbontetrachloride:acetone = 92:8 was used to remove bacteriopheophytin a and the fast moving non–bacteriochlorophyll photopigments (mostly carotenoids).

2. Solvent system: carbontetrachloride:acetone = 85:15 was used to remove the slow moving photopigments. In this system bacteriopheophytin e has an R_f-value different from that of bacteriopheophytin c and d. The R_f- and R_B-values were determined by using a solvent system carbontetrachloride:acetone = 90:10; bacteriopheophytin a_P from *Rhodopseudomonas sphaeroides* was used as a marker for the calculation of the R_B-values. The bacteriochlorophylls were separated from the accompanying photopigments by repeated chromatography on silica gel plates using solvent system 2. This is possible because bacteriochlorophyll c, d, and e do not undergo allomerization.

Absorption Spectra. Absorption spectra of living cells were determined with a Zeiss spectrophotometer DMR 21 with remission attachement. All other absorption spectra were measured without remission attachement using acetone as the solvent and 1 cm cuvettes. The short wavelength range between 300 and 350 nm was measured with an UV Spectrophotometer Leitz Unicam SP 800. Details about the methods used for the elucidation of the chemical structure of the new bacteriochlorophyll will be published elsewhere (Brockmann, Trowitzsch, and Gloe, in preparation).

Results and Discussion

All six different pure culture strains at present available of the two species of brown-colored Chlorobiaceae, *Chlorobium phaeobacteroides* and *Chlorobium phaeovibrioides* were found to possess as a major photopigment a new bacteriochlorophyll which proved to be different in all properties from the bacteriochlorophylls c and d hitherto known to occur in the green-colored Chlorobiaceae. In the following, the new bacteriochlorophyll will be designated bacteriochlorophyll e. In addition to the new bacteriochlorophyll e, all strains were shown to contain as a minor component bacteriochlorophyll a_P which has been identified chromatographically by the method described by Gloe and Pfennig (1974) as well as by mass spectra of the bacteriopheophytins of the type strains of the two species.

Absorption Spectra of Living Cells

Typical absorption spectra of living cell suspensions of three different *Chlorobium* species containing the three different bacteriochlorophylls c, d and e of the Chlorobiaceae are given in Fig. 1. The high absorption maximum in the range of 510–525 nm is due to the absorption of the special carotenoids isorenieratene and β-iso-

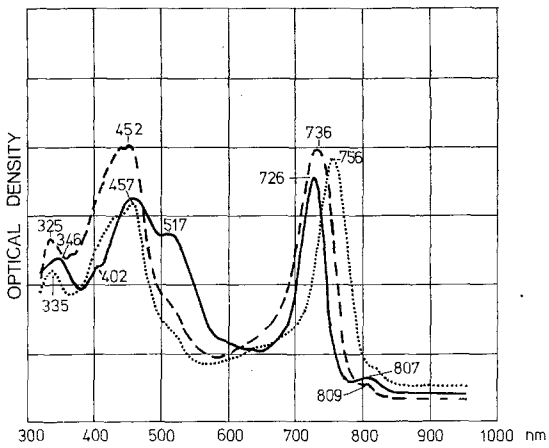

Fig. 1. Absorption spectra of living cells of three different species of the Chlorobiaceae containing three different bacteriochlorophylls. *Chlorobium limicola forma thiosulfatophilum* strain 6230 ····· (Bchl. c); *C. vibrioforme* strain 6030 ---- (Bchl. d); *C. phaeovibrioides* strain 2631 —— (Bchl. e)

renieratene (Liaaen Jensen, 1965) occurring in the brown colored, bacteriochlorophyll e containing *Chlorobium*-species. As a preliminary and rough indication of the kind of bacteriochlorophyll present in a phototrophic bacterium, the position of the long wavelength absorption maximum in the spectrum of living cells may be used. On the basis of absorption spectra of more than 40 *Chlorobium* strains with different bacteriochlorophylls, the absorption ranges given in Table 1 have been summarized. As can be seen from this table as well as Table 2, the position of the long wavelength absorption maximum of different strains having the same kind of bacteriochlorophyll is not identical but spread over a certain wavelength range. These differences in the position of the absorption maxima of different strains must be due to differences in the kind of bacteriochlorophyll to protein binding in the photosynthetic apparatus of the different strains.

The absorption maxima of living cells of all strains of the Chlorobiaceae studied in the present investigation are summarized in Table 2 in order to show the degree of variation in the position of the absorption maxima, particularly for the bacteriochlorophyll e containing brown *Chlorobium* strains. It can be seen that in particular the absorption maximum in the shortest measured wavelength range is situated at higher wavelengths (between 345 and 352 nm) and that the main long wavelength absorption maximum is situated at lower wavelengths (between 715 and 724 nm) in the bacteriochlorophyll e containing *Chlorobium* strains as compared to the bacteriochlorophyll c or d containing *Chlorobium* strains. From Tables 1 and 2 it is apparent, that the ranges of the position of the long wavelength absorption maxima for the three different bacteriochlorophylls c, d, and e are not well separated from each other but are just overlapping. This means that in some cases (see Table 2, strains 1930 and 2532 in comparison to strains 2631 and 2531) the position of this absorption maximum in the spectrum of living cells does not provide an unequivocal characteristic for the kind of bacteriochlorophyll present. Therefore, these data must be confirmed by the chromatographic identification of the extracted bacteriochlorophylls and their bacteriopheophytins.

Table 1. Wavelength ranges for the long wavelength absorption maxima of living cell suspensions of *Chlorobium* strains with different bacteriochlorophylls

Bacteriochlorophyll	Range of long wavelength absorption
c	745–760
d	725–745
e (new)	715–725

Chromatography

Most important for the identification of the bacteriochlorophylls from Chlorobiaceae is the differentiation between bacteriochlorophyll d and e. From Table 3

Table 2. Maxima of the absorption spectra of living cells of all tested strains

Strain	DSM-No.	Bchl.	λ_{max} in nm; relative intensities in brackets				
6230	249	c	335 (57) –	457 (83)	515 (sh)	756 (100)	812 (18)
6030	260	d	325 (55) –	452 (97)	493 (sh)	736 (100)	809 (7)
1930	265	d	– 405 (90)	449 (100)	515 (sh)	727 (95)	800 (7)
2532	274	d	323 (50) 404 (95)	445 (100)	508 (sh)	725 (96)	805 (4)
2431	266	e	347 (81) 403 (89)	460 (100)	511 (sh)	721 (89)	808 (5)
2430	267	e	348 (83) 397 (78)	454 (100)	517 (sh)	720 (87)	808 (sh)
9230	268	e	345 (77) 403 (95)	452 (100)	525 (74)	719 (91)	805 (8)
2631	269	e	346 (65) 402 (59)	458 (96)	517 (80)	726 (100)	807 (12)
2531	270	e	351 (62) 402 (62)	460 (81)	521 (73)	724 (100)	805 (6)
B 1	...	e	346 (79) 404 (67)	454 (100)	518 (90)	715 (100)	806 (5)

Table 3. R_f- and R_B-values and colors of different derivatives of bacteriochlorophylls d and e on silica gel plates

Derivatives	isolated from	R_f-values ×100	R_B-values ×100	colors
Bacteriomethylpheophorbid e	2631	5.57	8.43	darkgreen
Bacteriomethylpheophorbid d	6030	11.0	15.8	blue-violet
Bacteriopheophytin e	2631	18.1	26.85	darkgreen
Bacteriopheophytin d	6030	37.6	55.9	blue-violet
Bacteriopheophytin a	2631 6030 17023	67.0	reference	red-violet

it can be seen that the derivatives bacteriopheophytin and bacteriomethylpheophorbid of the new bacteriochlorophyll e differ remarkably from the corresponding derivatives of bacteriochlorophyll d not only in their color but also in the R_f-values and the more reliable R_B-values which are calculated on the basis of bacteriopheophytin a as a reference compound. The derivatives of bacteriochlorophyll c show similar characteristics as the derivatives of bacteriochlorophyll d.

Absorption Spectra of Purified Bacteriochlorophylls c, d and e

The absorption spectra of chromatographically purified bacteriochlorophyll d and e in acetone from *Chlorobium vibrioforme* strain 6030 (d) and *Chlorobium phaeovibrioides* strain 2631 (e) are shown in Fig. 2. Table 4 presents in addition the positions of the characteristic absorption maxima in acetone of all purified bacteriochlorophylls e from the six different brown colored *Chlorobium* strains at present available in pure culture; for comparison the corresponding absorption maxima of the bacteriochlorophylls c and d are given. It is apparent that the isolated bacteriochlorophylls e in acetone show similar overall differences in the position of the absorption maxima as the spectra of whole cells: the long wavelength absorption maximum (646—648 nm) is situated at shorter wavelengths than that of the bacteriochlorophylls c and d while the Soret maximum (456—459 nm) is situated 30 nm or more at longer wavelengths. This difference in the Soret region is to some extent also expressed in the position of the minor absorption maximum of bacteriochlorophyll e in the range 336—340 nm as compared, to 330 nm for bacteriochlorophyll d. This absorption maximum which is characteristically found also in the absorption spectra of whole cells of all strains of the Chlorobiaceae has hitherto not been described in the literature. In the course of the study of the bacteriochlorophylls e extracted by acetone or methanol from *Chlorobium* cell material, it was observed that in some cases the extracts contained up to 50% bacteriopheophytin e. Reliable absorption spectra of bacteriochlorophyll e can therefore only be obtained after thin layer chromatography of the cell extracts and isolation of the purified bacteriochlorophyll e.

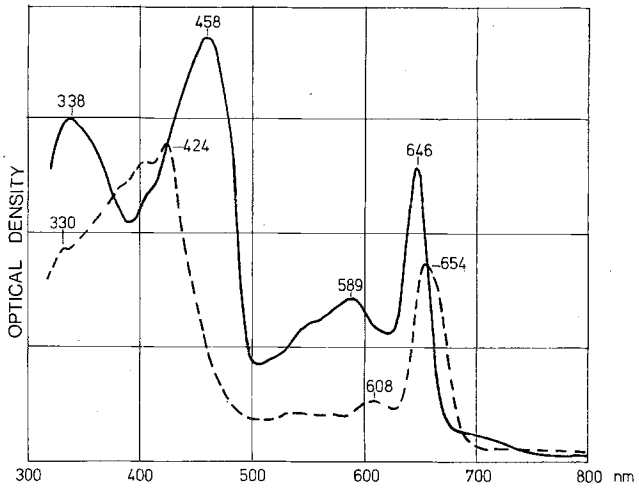

Fig. 2. Absorption spectra of purified bacteriochlorophylls in acetone. *Chlorobium vibrioforme* strain 6030 ---- (Bchl. d); *C. phaeovibrioides* strain 2631 —— (Bchl. e)

Absorption Spectra of Purified Bacteriopheophytins c, d and e

Characteristic changes in the absorption properties occur, when the bacteriochlorophylls are converted into their magnesium—free derivatives, the bacteriopheophytins. Absorption spectra of chromatographically purified bacteriopheophytins d and e in acetone are given in Fig. 3. Table 5 summarizes the positions of the characteristic absorption maxima of all bacteriopheophytins studied in correspondence to the bacteriochlorophylls presented in Table 4. While the difference in the position of the long wavelength absorption maxima of bacteriopheophytin d and e is very small, the Soret maxima of the bacteriopheophytins e are—as in the case of the bacteriochlorophylls (Table 4)—situated about 30 nm at longer wavelengths than those of bac-

Table 4. Maxima of the absorption spectra of the purified bacteriochlorophylls of all tested strains

Strain	DSM-No.	Bchl.	λ_{max} in nm; relative intensities in brackets					
6230	249	c	338 (40)	428 (100)	490 (39)	—	622 (29)	660 (63)
6030	260	d	330 (45)	424 (100)	—	—	608 (17)	654 (61)
2431	266	e	336 (42)	458 (100)	—	548 (sh)	593 (12)	647 (32)
2430	267	e	338 (34)	456 (100)	—	545 (sh)	594 (10)	647 (24)
9230	268	e	336 (38)	459 (100)	—	544 (sh)	592 (17)	646 (32)
2631	269	e	338 (77)	458 (100)	—	550 (sh)	589 (36)	646 (66)
2531	270	e	336 (68)	458 (100)	—	547 (sh)	594 (sh)	646 (28)
B 1	...	e	340 (32)	458 (100)	—	544 (sh)	589 (sh)	648 (24)

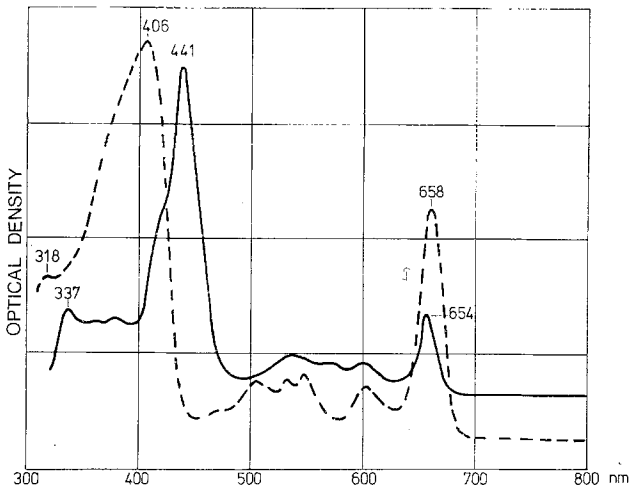

Fig. 3. Absorption spectra of purified bacteriopheophytins in acetone. *Chlorobium vibrioforme* strain 6030 ---- (Bph. d); *C. phaeovibrioides* strain 2631 —— (Bph. e)

teriopheophytin c and d. The Soret absorption maxima of the bacteriopheophytins c, d, and e as compared to those of the corresponding bacteriochlorophylls (Table 4) are shifted about 20 nm towards shorter wavelengths. On the other hand it appears to be typically for bacteriopheophytin e that the position of the minor absorption maximum at 332—337 nm is almost unchanged as compared to bacteriochlorophyll e while in the case of the bacteriopheophytins c and d for this absorption maximum a much larger shift towards shorter wavelengths is observed.

In conclusion, the comparison between the absorption spectra of the purified bacteriochlorophylls and bacteriopheophytins c, d, and e in acetone shows, that the differences in the absorption characteristics between the new bacteriochlorophyll e and the bacteriochlorophylls c and d are more pronounced than the differences between the bacteriochlorophylls c and d.

Mass Spectrometry

The derivatives bacteriopheophytin and bacteriomethylpheophorbid were prepared from the bacteriochlorophyll e of *Chlorobium phaeovibrioides* strain 2631. The mass spectra obtained revealed that the derivatives are mixtures of homologous compounds. Bacteriopheophytin e gives rise to at least three different molecular ions at m/e 798, 812, and 826. The mass spectrum of bacteriomethylpheophorbide e shows the corresponding ions at m/e 608, 622, and 636, however, with lower relative intensity. The fragment ions of bacteriomethylpheophorbide e which arise by loss of water show a higher relative intensity at m/e 590, 604, and 618. The presence of a mixture of homologous molecules has also been confirmed by a ^{1}H-NMR-spectrum. Furthermore the mass spectra allowed the identification of the alcohol, which is esterified with the propionic acid side chain (see position R_6 in Fig. 4 and Table 6); the difference in molecular weight between bacteriopheophytin e and bacteriomethylpheophorbide e is in agreement with farnesol. Furthermore, the mass spectrum of bacteriopheophytin e shows an ion at m/e 204 which is a derivative of farnesol formed by Mc Lafferty-rearrangement and ionization of the olefine part. After hydrolysis and isolation of the alcohol, the identity with farnesol was

Table 5. Maxima of the absorption spectra of the purified bacteriopheophytins of all tested strains

Strain	DSM-No.	Bph.	λ_{max} in nm; relative intensities in brackets										
6230	249	c	320 (25)	—	379 (sh)	—	408 (100)	481 (sh)	515 (17)	—	547 (22)	604 (15)	664 (65)
6030	260	d	318 (20)	—	390 (sh)	—	406 (100)	472 (sh)	505 (14)	533 (14)	548 (16)	604 (12)	658 (55)
2431	266	e	336 (18)	—	378 (17)	418 (sh)	438 (100)	—	—	534 (17)	567 (sh)	599 (14)	655 (37)
2430	267	e	332 (27)	358 (22)	376 (25)	418 (sh)	437 (100)	—	—	534 (11)	567 (sh)	596 (9)	654 (29)
9230	268	e	332 (14)	—	378 (14)	418 (sh)	438 (100)	—	—	533 (7)	569 (sh)	598 (6)	654 (16)
2631	269	e	337 (26)	360 (22)	380 (23)	418 (sh)	441 (100)	—	—	538 (12)	573 (10)	601 (10)	654 (25)
2531	270	e	334 (19)	354 (20)	380 (19)	420 (sh)	440 (100)	—	—	530 (9)	570 (sh)	598 (6)	655 (18)
B 1	...	e	333 (16)	—	378 (19)	418 (sh)	438 (100)	—	—	534 (8)	569 (6)	598 (6)	654 (19)

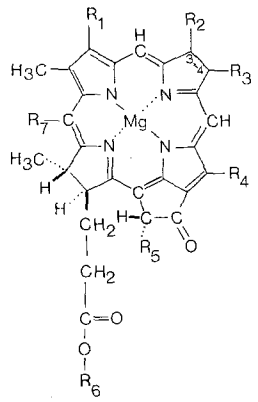

Fig. 4. Structure of all bacteriochlorophylls and the chlorophylls a and b. The different substituents present in the positions R_1 to R_7 are given in Table 6

confirmed by an ^{1}H-NMR-spectrum. A proton signal at 11.18 ppm and the lack of a methyl resonance at 3.30 ppm in the ^{1}H-NMR-spectrum of bacteriomethylpheophorbide e suggested the presence of a formyl group on pyrrol ring II. Similar characteristics are observed in the ^{1}H-NMR-spectrum of pyromethylpheophorbide b, a derivative of chlorophyll b from green plants. The presence of formyl groups on pyrrol rings II has been confirmed by IR- and UV/VIS-spectra of both derivatives. The structure of bacteriochlorophyll e given in Fig. 4 and Table 6 was further confirmed by ^{13}C-NMR-, ORD-, and CD-spectra as well as by elemental analysis. The details of the physicochemical characterization of the new bacteriochlorophyll e will be published in a separate paper (Brockmann, Trowitzsch, and Gloe, in preparation).

Table 6. Presentation of the different substituents of the positions R_1 to R_7 in Fig. 4 for all bacteriochlorophylls and the chlorophylls a and b

Pigment	R_1	R_2	R_3	R_4	R_5	R_6	R_7	References
Chlorophyll a	$-CH=CH_3$	$-CH_3$	$-CH_2-CH_3$	$-CH_3$	$-C(=O)-O-CH_3$	P	$-H$	Fischer and Wenderoth (1939) Brockmann, Jr. (1971)
Chlorophyll b	$-CH=CH_3$	$-C(=O)-H$	$-CH_2-CH_3$	$-CH_3$	$-C(=O)-O-CH_3$	P	$-H$	Fischer and Wenderoth (1939) Brockmann, Jr. (1971)
Bacteriochlorophyll a	$-C(=O)-CH_3$	$-CH_3$ [a]	$-CH_2-CH_3$ [a]	$-CH_3$	$-C(=O)-O-CH_3$	P/Gg	$-H$	Mittenzwei (1942) Brockmann, Jr., and Kleber (1969)
Bacteriochlorophyll b	$-C(=O)-CH_3$	$-CH_3$ [b]	$=C(H)-CH_3$ [b]	$-CH_3$	$-C(=O)-O-CH_3$	P	$-H$	Scheer *et al.* (1974)
Bacteriochlorophyll c	$-C(OH)(H)-CH_3$	$-CH_3$	$-C_2H_5$ / $-C_3H_7$ / $i-C_4H_9$	$-C_2H_5$ / $-CH_3$ (?)	$-H$	F	$-CH_3$	Holt *et al.* (1966)
Bacteriochlorophyll d	$-C(OH)(H)-CH_3$	$-CH_3$	$-C_2H_5$ / $-C_3H_7$ / $i-C_4H_9$	$-C_2H_5$ / $-CH_3$ (?)	$-H$	F	$-H$	Purdie and Holt (1965)
Bacteriochlorophyll e	$-C(OH)(H)-CH_3$	$-C(=O)-H$	$-C_2H_5$ / $-C_3H_7$ / $i-C_4H_9$	$-C_2H_5$	$-H$	F	$-CH_3$	–

[a] No doublebond between C-3 and C-4; additional H-atoms are in position C-3 and C-4.
[b] No doublebond between C-3 and C-4; an additional H-atom is in position C-3 (Scheer *et al.*, 1974).

Mass spectra of the bacteriopheophytins a from *Chlorobium phaeobacteroides* strain 2430 and *Chlorobium phaeovibrioides* strain 2631 confirmed earlier results obtained by thin layer chromatography (Gloe and Pfennig, 1974) *i.e.* the esterifying alcohol of the bacteriochlorophyll a present in these organisms is phytol.

Fig. 4 and Table 6 present the structural details of all presently known bacteriochlorophylls and of the chlorophylls a and b of green plants.

It is apparent from Table 6 that R_2 of the new bacteriochlorophyll e is a formyl-group as in chlorophyll b. This formyl-group is the differentiating structural characteristic of bacteriochlorophyll e to bacteriochlorophyll c. The fact that bacteriochlorophyll d differs from bacteriochlorophyll c only with respect to R_7 suggests the possible existence of a further "bacteriochlorophyll f" which differs from bacteriochlorophyll e in the same way as bacteriochlorophyll c from bacteriochlorophyll d. Future studies will show whether this hypothetical bacteriochlorophyll f exists in nature.

References

Brockmann, H., Jr.: Zur absoluten Konfiguration der Chlorophylle. V. Die absolute Konfiguration der Chlorophylle a und b. Justus Liebigs Ann. Chem. **754**, 139–148 (1971)

Brockmann, H., Jr., Kleber, I.: Zur absoluten Konfiguration der Chlorophylle. 3. Mitteilung. Zur absoluten Konfiguration des Bacteriochlorophylls a. Angew. Chem. **81**, 626–627 (1969)

Fischer, H., Wenderoth, H.: Zur Kenntnis von Chlorophyll. Justus Liebigs Ann. Chem. **537**, 170–177 (1939)

Gloe, A.: Untersuchungen der Bacteriochlorophylle roter und grüner Schwefelbakterien auf das Vorhandensein von Phytol, Geranylgeraniol oder anderer Substituenten. Diplomarbeit, Universität Göttingen (1973)

Gloe, A., Pfennig, N.: Das Vorkommen von Phytol und Geranylgeraniol in den Bacteriochlorophyllen roter und grüner Schwefelbakterien. Arch. Microbiol. **96**, 93–101 (1974)

Holt, A.S., Purdie, J.W., Wasley, J.W.F.: Structures of Chlorobium Chlorophylls (660). Canad. J. Chem. **44**, 88–93 (1966)

Jensen, A., Aasmundrud, O., Eimhjellen, K.E.: Chlorophylls of photosynthetic bacteria. Biochim. biophys. Acta (Amst.) **88**, 466–479 (1964)

Liaaen Jensen, S.: Bacterial Carotenoids. XVIII. Arylcarotenoids from *Phaeobium*. Acta chem. scand. **19**, 1025–1030 (1965)

Mittenzwei, H.: Über Bakteriochlorophylle. Hoppe-Seylers Z. physiol. Chem. **275**, 93–121 (1942)

Pfennig, N.: *Chlorobium phaeobacteroides* nov. spec. and *Chlorobium phaeovibrioides* nov. spec., zwei neue Arten der grünen Schwefelbakterien. Arch. Mikrobiol. **63**, 224–226 (1968)

Pfennig, N., Lippert, K.D.: Über das Vitamin B_{12}-Bedürfnis phototropher Schwefelbakterien. Arch. Mikrobiol. **55**, 258–266 (1966)

Purdie, J.W., Holt, A.S.: Structures of *Chlorobium* chlorophylls (650). Canad. J. Chem. **43**, 3347–3353 (1965)

Scheer, H., Svec, W.A., Cope, B.T., Studier, M.H., Scott, R.G., Katz, J.J.: Structure of Bacteriochlorophyll b. J. Amer. chem. Soc. **96**, 3714–3716 (1974)

Stanier, R.Y., Smith, J.H.C.: The Chlorophylls of green bacteria. Biochim. biophys. Acta (Amst.) **41**, 478–484 (1960)

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