ZEITSCHRIFT FÜR NATURFORSCHUNG

SECTION C

BIOSCIENCES

EDITED IN COLLABORATION
WITH THE INSTITUTES OF THE MAX-PLANCK-GESELLSCHAFT

VOLUME 36c 1981

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG
TÜBINGEN

Anschrift des Verlages: Postfach 2645, D-74 Tübingen
Satz und Druck: Konrad Triltsch, Würzburg

Section a
Physics, Physical Chemistry, Cosmic Physics

Section b
Inorganic and Organic Chemistry

Contents

Contents of Number 1/2

Original Communications

Contents of Number 3/4

Contents of Number 5/6

Original Communications

Contents of Number 7/8

Original Communications

Contents of Number 9/10

Original Communications

Contents of Number 11/12

Δ2,10-Phytadienol as Esterifying Alcohol of Bacteriochlorophyll b from *Ectothiorhodospira halochloris*

R. Steiner*, W. Schäfer**, I. Blos*, H. Wieschhoff*, and H. Scheer*

* Botanisches Institut der Universität München, Menzinger Str. 67, D-8000 München 19
** Max-Planck-Institut für Biochemie, Am Klopferspitz, D-8036 Martinsried

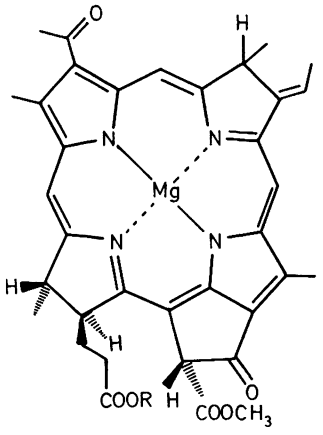
Z. Naturforsch. **36 c**, 417–420 (1981); received February 26, 1981

Photosynthesis, Photosynthetic Bacteria, Halophilic Bacteria, Bacteriochlorophylls, Diterpenoid Alcohols

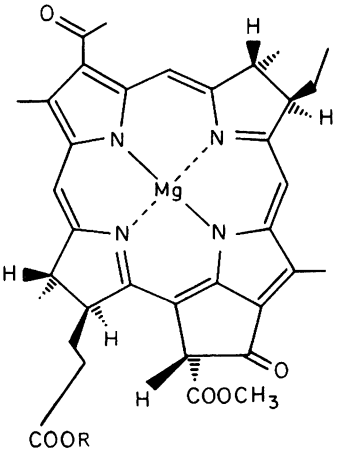
Bacteriochlorophyll b (bchl b) has been isolated from the halophilic photosynthetic bacterium, *Ectothiorhodospira halochloris*. The pigment and a series of derivatives thereof are different from Bchl b from *Rhodopseudomonas viridis* by HPLC analysis, but similar by uv-vis spectroscopy.

The chromatographic difference originates in different esterifying alcohols in the two pigments. The one from *Rp. viridis* (Bchl b_p) is esterified with Δ2-phytaenol (phytol), that from *E. halochloris* (Bchl $b_{\Delta 2,10}$) with Δ2,10-phytadienol. The structure of the latter has been established by isolation of the alcohol from the purified pigment, followed by (i) gaschromatography-mass spectroscopy and (ii) ozonolysis and dinitrophenylhydrazon-formation of the cleavage products, which were identified by gaschromatography-mass spectroscopy as 6-methyl-heptan-2-one, and 4-methyl-nona-1,8-dione.

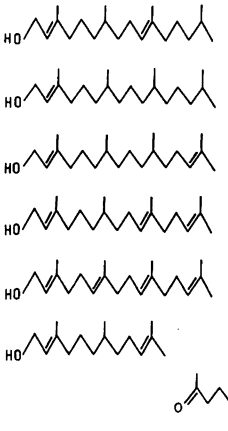
Bacteriochlorophyll b (**1 a**) is the photosynthetic pigment of only a few species of photosynthetic bacteria [1–4]. It replaces the common bacteriochlorophyll a (**2**) in the antenna, and at least in *Rhodopseudomonas viridis* – also in the reaction centers [5–7], and enables these organisms to use efficiently light down to 1020 nm. Recently, a new species from an extremely haline biotope, *Ectothiorhodospira halochloris*, has been found to contain

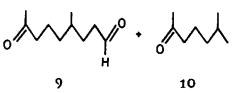


1 a: R = 3–OH
1 b: R = 4–OH



2 a: R = 4–OH
2 b: R = 7–OH





9 10

Abbreviations: Bchl, Bacteriochlorophyll; Bphe, Bacteriopheophytin; gc, Gas chromatography; ms, Mass spectrometry; hplc, High-performance liquid chromatography; DNP, Dinitrophenyl hydrazone; MW, Molecular weight.

Reprint requests to Prof. Dr. H. Scheer.

0341-0382/81/0500-0417 $ 01.00/0

Bchl b [8]. We wish to report this pigment to contain Δ2,10-phytadienol (**3**) instead of Δ2-phytaenol (phytol (**4**)) as a new kind of esterifying alcohol.

Materials and Methods

Ectothiorhodospira halochloris was grown anaerobically in the medium of Imhoff and Trüper [8] at 35 °C with white light from incandescent lamps (1500 lux). The cells were harvested after 10–14 days, washed twice with 0.01 M tris-buffer, pH 7.5 and stored frozen. *Rhodopseudomonas viridis* (DSM No. 133) was grown in a modified Hutner medium [9]. The chlorophylls were extracted by the method of Strain and Svec [10] and chromatographed twice on powdered sucrose containing 5% starch. The entire procedure was carried out with minimum exposure to light. Bacteriopheophytins b (Bphe b) were obtained by demetalation in methanol with 1% methanolic H_2SO_4 under nitrogen [11].

The isolation of the esterifying alcohols followed essentially the procedure of Schoch *et al.* [12]. The purified pigments were hydrolyzed with methanolic KOH, and the free alcohols chromatographed twice on silica. Small sections of the developed tlc plates were sprayed with $KMnO_4$ to spot the bands, which were scraped off from the remaining sections. The alcohols were eluted with acetone and dried in a stream of nitrogen.

All solvents were reagent grade or distilled prior to use. Sodium ascorbate (Merck, Darmstadt) and 2,4-dinitrophenylhydrazin (EGA) were reagent grade.

Chromatography

HPLC of the pigments was performed by a variation of the method reported earlier [13] on a RP 8 column (Knauer, Oberursel) in which mixtures of methanol and an aqueous solution of sodium ascorbate (1% w/v) were used as eluents. Capillary gc (25 m OV-1) was used for the analysis of the free alcohols and their trimethylsilylethers. The same type of column was used for the gc-ms experiments. Ozonolysis [14] of the alcohols, gc-ms identification of the cleavage products and ms-structure elucidation of their 2,4-dinitro phenylhydrazones was done as described earlier [15].

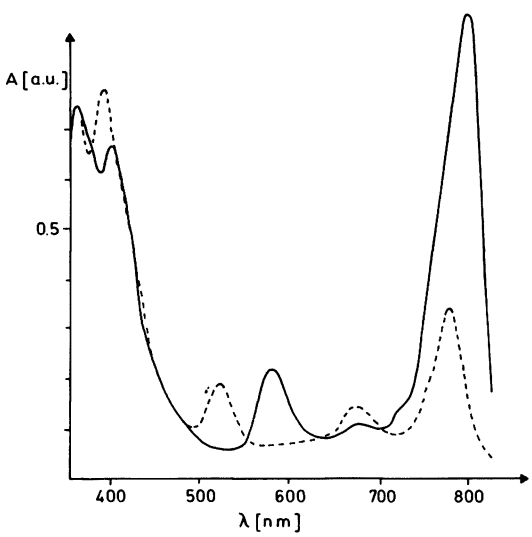


Fig. 1. Absorption spectrum of Bchl b (——) and Bphe b (---) from *Ectothiorhodospira halochloris*. The absorptions have been adjusted to equal intersities of the near-uv band of Bchl b and Bphe b, respectively. Bphe b is contaminated with demetalation by-products ($\lambda_{max} = 680$ nm) arising from oxidative isomerization. ε_{680} of these products is approximately one-third of $\varepsilon_{790}^{Bchl\,b}$.

Results and Discussion

The absorption spectrum of the crude extract of *E. halochloris* is typical of Bchl b ($\lambda_{max} = 794$, 578, 408, 368 nm) [16] and indicates only a small amount of carotenoids (shoulder at $\lambda \sim 450$ nm). The latter are removed during the chromatographic purification, along with products formed by oxidative isomerization ($\lambda_{max} = 680$ nm) [17] of Bchl b. The isolated pigment and its pheophytin are uv-vis spectroscopically identical with the respective pigments from *Rp. viridis* (Fig. 1). They are different, however, chromatographically, as are the products formed from Bchl b of the two organisms by oxidative photoisomerization in acetone [17]. A constant factor of 1.3 has been found for the retention times (r') of each pair of the corresponding pigments from both organisms [19]. Hydrolysis of Bchl b (1.45 mg) from *E. halochloris* yields only a single $KMnO_4$-positive band on tlc. The gc analysis reveals one major component (0.35 mg) which elutes in the region of diterpenoid alcohols, but is different from each of the four alcohols **4**–**7** arising from sequential hydrogenation of Δ2,6,10,14-phytatetraenol (geranylgeraniol (**7**)) in greening plants [12]. It is accompanied by two minor peaks, one of which is identical in the gc with **7**. The same chromatographic pattern has been found for the respective trimethylsilylethers.

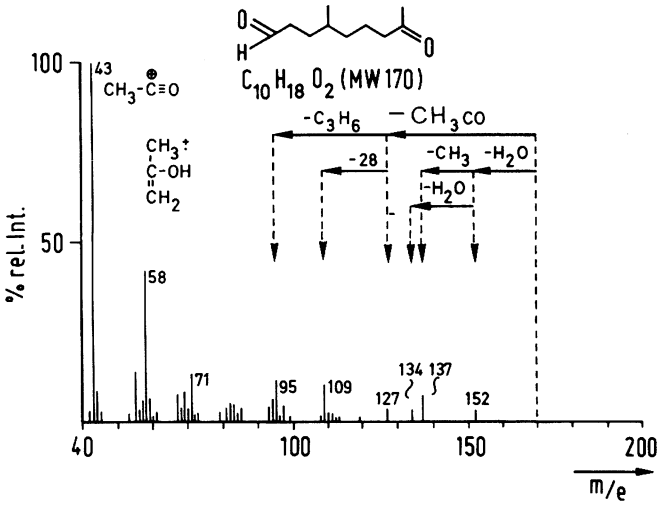


Fig. 2. Mass spectrum and fragmentation scheme of the ketoaldehyde **9**.

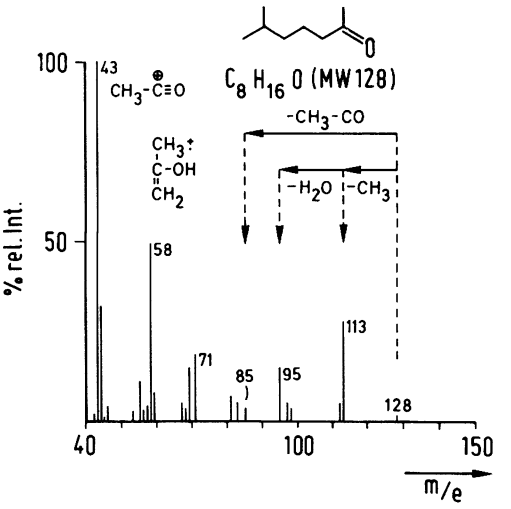


Fig. 3. Mass spectrum and fragmentation scheme of the aldehyde **10**.

The mass spectrum of the major *E. halochloris* alcohol (MW 294) and its TMS-ether (MW 366) is typical for a phytadienol.

For the localization of the two double bonds, 0.2 mg of the hydrolysate purified by tlc were subjected to microozonolysis [14]. Gc/ms analysis of the cleavage products revealed, besides by-products, two peaks which have been asigned to structures **9** and **10**. The fragmentation of the ketoaldehyde **9** (Fig. 2) is analogous to the fragmentation of 4,8-dimethyl-1,12-dioxotridecan [15]. The second smaller peak with shorter retention time is assigned to keton **10** (Fig. 3).

In addition, the products from ozonolysis were converted to their dinitrophenylhydrazones, separated by tlc and their mass spectra recorded as described earlier [15]. The ms spectra confirm the structures assigned above. Characteristic fragments for DNP-**9** are *m/e* 530 (M^+), 512 ($M-H_2O$), 495 ($M-[OH+H_2O]$), 333 ($M-[(NO_2)_2-C_6H_5-NH-NH]$) and 331 ($M-[(NO_2)_2-C_6H_3-NH-NH_2+H]$). For DNP-**10** (MW 308) signals at *m/e* 293 ($M-CH_3$) 275 ($M-[CH_3+H_2O]$), 258 ($M-[CH_3+H_2O+OH]$) and also 273 ($M-[OH+H_2O]$) are typical [20]. Because **9** and **10** derive from a phytadienol, the structure of the alcohol is 3,7,11,15-tetramethyl-Δ2,10 hexadecadienol (2,10-phytadienol (**3**)).

The identification of **3** indicates a rather high degree of variability in the esterifying alcohols of bacteriochlorophylls. Bchl a commonly contains Δ2-phytaenol (phytol (**4**)) with the exception of Bchl a from *Rhodospirillum rubrum* esterified with Δ2,6,10,14-phytatetraenol, (**7**) [21, 22]. **4** is also the esterifying alcohol of Bchl b from *Rp. viridis* [16, 18], which is to our knowledge the only other Bchl b-containing organism from which **1** has been analyzed hitherto. Bchls c, d and e each contain farnesol (**8**) as the major esterifying alcohol [23–25], but a minor fraction of them is esterified by a wide variety of alcohols including nonterpenoid ones [26, 27]. The function(s) of these alcohols are still unknown. They are generally believed to serve as a hydrophobic anchor of the pigment. An interesting observation is the presence of **7** in Bchl a of *R. rubrum* reaction centers, but not in the Bphe a of the same complex which contains **4** instead [28]. The latter points to a more intricate interaction of these alcohols with their native invironment. In plant chlorophylls, the Δ2-phytaenol (**4**) generally [12] present is thought to stabilize the photosynthetic membrane [29, 30]. The occurence of **3** in *E. halochloris* Bchl b may then be an adaptation to its extremely haline environment.

The presence of **3** in this pigment is also interesting from a biosynthetic point of view. In greening plants, the hydrogenation of **7** to **4** has been shown to proceed regioselectively via **6** and **5** [12]. *E. halochloris* must then contain an enzyme with a different specificity, which may be true for other bacteria as well.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. We thank Dr. Imhoff and Prof. Trüper for providing to us the *E. halochloris* culture.

[1] K. E. Eimhjellen, O. Aasmundrud, and A. Jensen, Biochem. Biophys. Res. Comm. **10,** 232 (1963).
[2] G. Drews and P. Giesbrecht, Arch. Mikrobiol. **53,** 255 (1966).
[3] K. E. Eimhjellen, H. Steensland, and J. Traetteberg, Arch. Microbiol. **59,** 82 (1967).
[4] K. E. Eimhjellen, Arch. Microbiol. **73,** 193 (1970).
[5] N. L. Puchey, N. L. Kerber, and A. F. Garcia, Arch. Microbiol. **109,** 301 (1976).
[6] T. L. Trosper, D. L. Benson, and J. P. Thornber, Biochim. Biophys. Acta **460,** 318 (1977).
[7] B. J. Clayton and R. K. Clayton, Biochim. Biophys. Acta, **501,** 470 (1978).
[8] J. F. Imhoff and H. G. Trüper, Arch. Microbiol. **114,** 115 (1977).
[9] G. Cohen-Bazire, W. R. Sistrom, and R. Y. Stanier, J. Cell. Comp. Physiol. **49,** 25 (1957).
[10] H. H. Strain and W. A. Svec, in: The Chlorophylls, (L. P. Vernon and G. R. Seely, eds.), p. 21, Academic Press, New York 1966.
[11] M. S. Davis, A. Forman, L. K. Hanson, J. P. Thornber, and J. Fajer, J. Phys. Chem. **83,** 3325 (1979).
[12] S. Schoch, U. Lempert, and W. Rüdiger, Z. Pflanzenphysiol. **83,** 427 (1977).
[13] S. Schoch, U. Lempert, H. Wieschhoff, and H. Scheer, J. Chromatogr. **157,** 357 (1978).
[14] M. Beroza and B. A. Bierl, Analyt. Chem. **39,** 1131 (1967).
[15] S. Schoch and W. Schäfer, Z. Naturforsch. **33 c,** 408 (1978).
[16] H. Scheer, W. A. Svec, B. T. Cope, M. H. Studier, R. G. Scott, and J. J. Katz, J. Am. Chem. Soc. **96,** 3714 (1974).
[17] The reaction yields at least four pigments of the chlorophyll a type, of which only 3-acetyl-3-devinylchlorophyll a [18] and its 8(1-hydroxyethyl)-derivative [16] have been identified. They all arise from the isomerization of the labile exocyclic $\Delta 8,8^1$-double bond present in **1** [16] to an endocyclic $\Delta 7$-bond.
[18] H. Brockmann, jr. and I. Kleber, Tetrahedron Lett. **1970,** p. 2195.
[19] Details of the HPLC results are to be published separately (H. Scheer, J. Benz, H. Wieschhoff, S. Schoch, R. Steiner, and W. Rüdiger, in preparation).
[20] J. Seibl, Org. Mass Spectrom. **3,** 1089 (1970).
[21] J. J. Katz, H. H. Strain, A. L. Harkness, M. H. Studier, W. A. Svec, T. R. Janson, and B. T. Cope, J. Am. Chem. Soc. **94,** 7938 (1972).
[22] H. Brockmann, jr. and G. Knobloch, Arch. Microbiol. **85,** 123 (1972).
[23] A. S. Holt, D. W. Hughes, H. J. Kende, and J. W. Purdie, Plant Cell. Physiol. **4,** 49 (1963).
[24] H. Rapoport and H. F. Hamlow, Biochem. Biophys. Res. Comm. **6,** 134 (1961).
[25] A. Gloe, N. Pfennig, H. Brockmann jr., and W. Trowitzsch, Arch. Microbiol. **102,** 103 (1975).
[26] M. Caple, H. Chow, R. M. Burns, and C. E. Strouse, in: Chlorophyll-Proteins, Reaction Centers and Photosynthetic Membranes, (J. M. Olson and G. Hind, eds.), p. 56, Brookhaven Symp. Biol. **28,** New York 1977.
[27] A. Gloe, Dissertation, Universität Göttingen 1977.
[28] E. Walter, J. Schreiber, E. Zass, and A. Eschenmoser, Helv. Chim. Acta **62,** 899 (1979).
[29] A. Rosenberg, Science **157,** 1191 (1967).
[30] S. Patton, Science **159,** 221 (1968).