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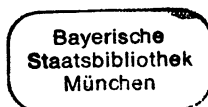
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$\Delta 2,10$ -Phytadienol as Esterifying Alcohol of Bacteriochlorophyll b from *Ectothiorhodospira halochloris*

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

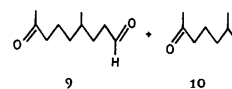
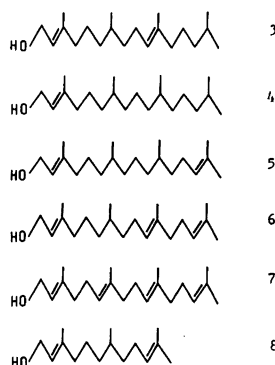
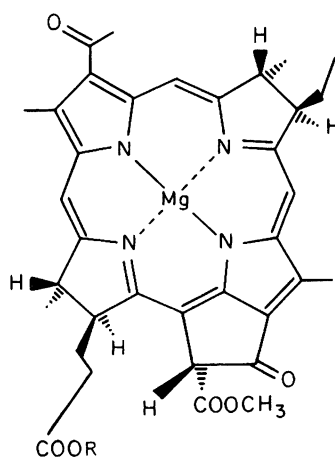
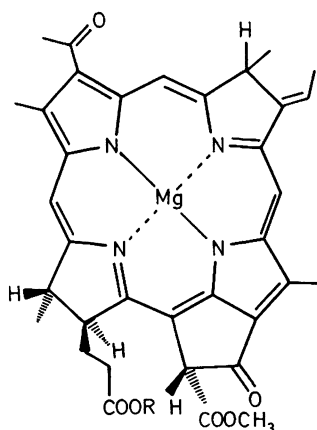
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Photosynthesis, Photosynthetic Bacteria, Halophilic Bacteria, Bacteriochlorophylls, Diterpenoid Alcohols

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 *Rhodospseudomonas 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 $\Delta 2$ -phytaenol (phytol), that from *E. halochloris* (Bchl b _{$\Delta 2,10$}) with $\Delta 2,10$ -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 (**1a**) 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 *Rhodospseudomonas 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



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

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Bchl b [8]. We wish to report this pigment to contain $\Delta^2,10$ -phytadienol (**3**) instead of Δ^2 -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. *Rhodospseudomonas 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 H₂SO₄ 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 KMnO₄ 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].

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 KMnO₄-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 $\Delta^2,6,10,14$ -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.

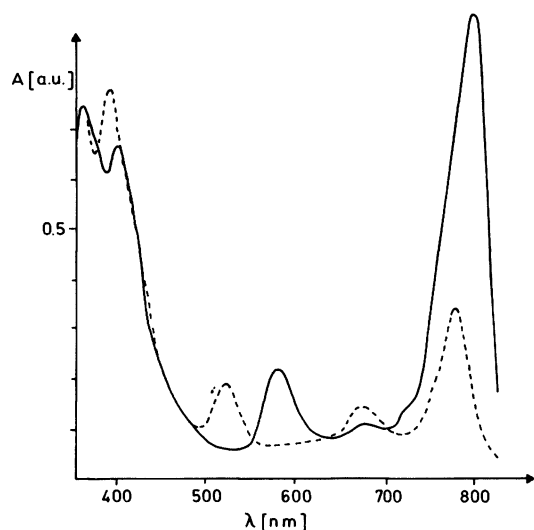


Fig. 1. Absorption spectrum of Bchl b (—) and Bphe b (---) from *Ectothiorhodospira halochloris*. The absorptions have been adjusted to equal intensities of the near-uv band of Bchl b and Bphe b, respectively. Bphe b is contaminated with demetalation by-products ($\lambda_{\max} = 680$ nm) arising from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

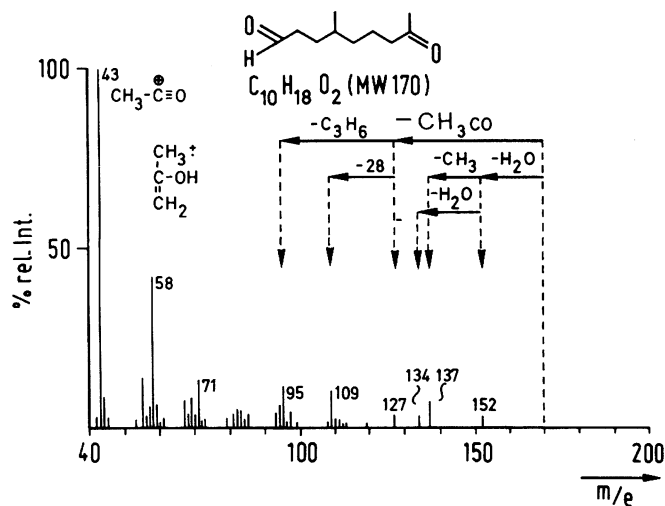


Fig. 2. Mass spectrum and fragmentation scheme of the ketoaldehyde **9**.

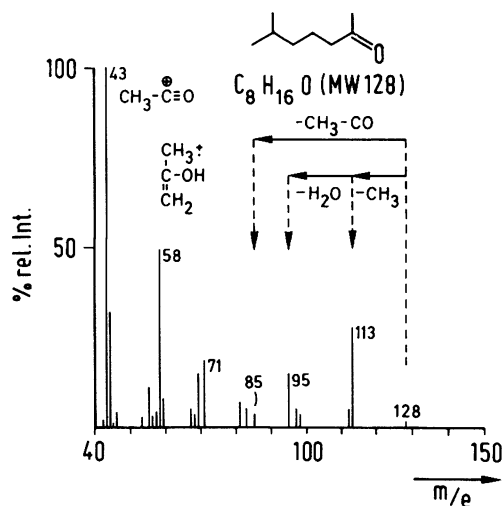


Fig. 3. Mass spectrum and fragmentation scheme of the aldehyde **10**.

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 assigned 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 ($M-[(NO_2)_2-C_6H_5-NH-NH_2+H]$). 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- Δ 2,10 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 Δ 2-

phytaenol (phytol (**4**)) with the exception of Bchl a from *Rhodospirillum rubrum* esterified with Δ 2,6,10,14-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 environment. In plant chlorophylls, the Δ 2-phytaenol (**4**) generally [12] present is thought to stabilize the photosynthetic membrane [29, 30]. The occurrence 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

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