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# Bacteriochlorophylls modified at position C-3: long-range intramolecular interaction with position C-13²

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[3-Vinyl]-bacteriochlorophyll *a* and related pigments modified at C-3 and/or C-13² have been synthesized from bacteriochlorophyll *a*. The reactivity at C-3 is strongly influenced by the C-13² substituent, and vice versa. Spectroscopical data and comparison among derivatives modified at the isocyclic ring indicate that this interaction is related to formation of an intermediate enol(ate) structure. The possible role of enol(ate) formation in (bacterio)chlorophylls in nature is discussed.

## Introduction

The chemistry of plant chlorophylls (Chl *a*, *b*) is a well-studied part of porphyrin chemistry. A large amount of data has been accumulated on the chemical reactivity of side-groups and their physical and spectroscopic properties [1–3]. Much of that work is motivated by the importance of these chlorophylls in oxygenic photosynthesis. While most of these data originate from in vitro work in organic or micellar solution, much less is known on the structural and functional details in their native protein environment. In the case of bacteriochlorophylls, among which BChl *a* and *b* are most prominent, the situation is reversed. Due to the possibility to isolate bacterial light-harvesting complexes [4] and purple bacterial reaction centers [5] for more than 15 years, and to crystallize some of them, high precision structural data in their native protein environment are available [6–10]. However, their chemistry is relatively seldom investigated. In connection with the recently introduced methods for exchanging modified (bacterio)chlorophylls and -pheophytins into bacterial reaction centers [11–14] (no detailed reports have been given there on the pigment synthesis) and antennas [15–17], we have started to investigate the reactivity of side-groups in BChl *a*.

To understand the structure-function relationships of bacteriochlorophylls (and chlorophylls) in more detail, it is important to obtain structural links between the different naturally occurring (bacterio)chlorophyll structures. One such link is [3-acetyl]-chlorophyll *a* [18], bearing the 3-acetyl group characteristic of BChl *a* and *b*, and the macrocycle characteristic of the green plant Chl *a* and *b*. The complementary link is [3-vinyl]-Bchl *a*, which differs vice versa from Chl *a* by the macrocycle, and from BChl *a* by the presence of a C-3 vinyl instead of an acetyl group. Here, we wish to report a procedure to synthesize [3-vinyl]-BChl *a* and some related pigments, and discuss some physical properties of these pigments in vitro. Attention is given to a pronounced, and hitherto unreported, long-range interaction between substituents at the positions 3 and 13², which was observed during these studies. The reactivity at C-3 is strongly influenced by the nature of the C-13² substituent, and vice versa. The data suggest, that this 'connection' is related to formation of enol(ate) structures at the isocyclic ring. There has been considerable interest before in the enolisation and the epimerisation at C-13², and their possible involvement in photosynthesis [19–23]. The results are discussed in this context.

## Material and Methods

### *General conditions*

All chemicals and solvents used were reagent grade.

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Abbreviations: Chl, chlorophyll; Phe, pheophytin; BChl, bacteriochlorophyll; BPhe, bacteriopheophytin.

*Pigments*

The isolation of BChl *a* (**1**) and the syntheses and spectral characterizations of all derivatives are given in the Appendix.

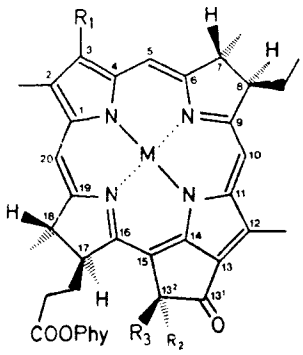
*Spectra*

Absorption spectra were recorded on a Lambda 2 photometer (Perkin Elmer). $^1$H-NMR spectra are recorded on an AX 360 MHz machine (Bruker model) in pyridine-$d_5$, if not stated otherwise. FAB mass spectra were obtained with the model CH 7a/SS 100 mass spectrometer (Varian MAT, Bremen). Extinction coefficients were determined by dissolving the pigments in ether to $A_{NIR}$ around 0.8. A precise absorption spectrum was recorded. The sample was then dried with a stream of nitrogen, and the Mg-contents determined by atomic-absorption spectroscopy. The reported values and error limits are avarages from three to six determinations.

## Results and Discussion

*Structures*

Ultraviolet-Vis-NIR absorption, $^1$H-NMR and mass spectra of all products are consistent with the given structures. The 3-acetyl group was converted to the vinyl group by $NaBH_4$ reduction to yield [3$\alpha$-hydroxyethyl]-BChl *a*, followed by dehydration in refluxing toluene. The 3-$\alpha$-hydroxyethyl-derivative (**4**) has the absorption maxima and $^1$H-NMR shifts as described in Ref. [26]. The epimer mixture at C-3$^1$ gives rise to the splitting of some signals, as in the BChl *c*, *d*, *e* series [30,31]. Reduction of the 13$^1$-C=O group, which occurs readily in chlorophylls [32,33], has (quite surprisingly)




| | R1 | R2 | R3 | M |
|---|---|---|---|---|
| 1 | $COCH_3$ | $COOCH_3$ | H | Mg |
| 1′ | $COCH_3$ | H | $COOCH_3$ | Mg |
| 2 | $COCH_3$ | H | H | Mg |
| 3 | $COCH_3$ | $OH/COOCH_3$ * | | Mg |
| 4 | $HCOHCH_3$ * | $COOCH_3$ | H | Mg |
| 5 | $HCOHCH_3$ * | H | H | Mg |
| 6 | $HCOHCH_3$ * | $OH/COOCH_3$ * | | Mg |
| 7 | $CHCH_2$ | $COOCH_3$ | H | Mg |
| 8 | $CHCH_2$ | H | H | Mg |
| 9 | $CHCH_2$ | $OH/COOCH_3$ * | | Mg |
| 10 | $COCH_3$ | $COOCH_3$ | H | $H_2$ |
| 11 | $COCH_3$ | H | H | $H_2$ |
| 12 | $COCH_3$ | $OH/COOCH_3$ * | | $H_2$ |
| 13 | $HCOHCH_3$ * | $COOCH_3$ | H | $H_2$ |
| 14 | $HCOHCH_3$ * | H | H | $H_2$ |
| 15 | $HCOHCH_3$ * | $OH/COOCH_3$ * | | $H_2$ |
| 16 | CHCH2 | $COOCH_3$ | H | $H_2$ |
| 17 | $CHCH_2$ | H | H | $H_2$ |
| 18 | $CHCH_2$ | $OH/COOCH_3$ * | | $H_2$ |

Phy = phytyl ($C_{20}H_{39}$)

* Epimeric mixture.

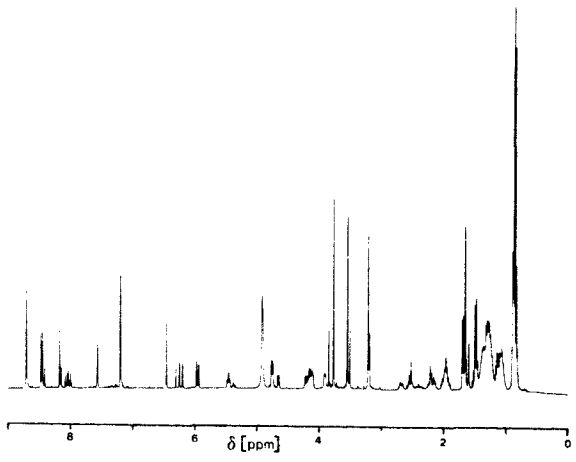



Fig. 1. $^1$H NMR spectrum of [3-vinyl]-BChl *a* (**7**) (13$^2$-epimer mixture) in pyridine-$d_5$. Solvent signals at 7.2, 7.56 and 8.69 ppm (pyridine) and at 4.88 ppm (water).

never been observed under these conditions, not even at greatly prolonged times.

The $^1$H-NMR spectrum of [3-vinyl]-BChl *a* (**7**) in pyridin (Fig. 1) shows the typical AB pattern of a vinyl group at $\delta = 8.02$, 6.20 and 5.93 ppm, the high-field shift of all signals with respect to chlorophyll is due to the reduced ring-current of bacteriochlorins [34]. The splitting of the methin proton signals at 8.46/8.45, 8.44/8.41 and 8.16/8.14 and the 13$^2$ H-signal at 6.44/6.29 ppm indicates a 13$^2$ epimeric mixture of about 70% 13$^2$S-(**7**) and 30% 13$^2$R-(**7**). All other expected proton signals are present. After pyrolysis of the mixture, the resulting (**8**) shows no split signals in the $^1$H-NMR-spectrum.

The best way to produce 13$^2$-hydroxylated BChl's is prolonged standing in methanol under aerobic conditions. The desired oxygenation (13$^2$-OH) and the undesired methoxylation (13$^2$-$OCH_3$) at this position are competing processes which occur with different yields (see Ref. 25). To obtain 13$^2$-hydroxylated BChl's the product mixture was subsequently separated by chromatography on DEAE-cellulose column [27]. Although

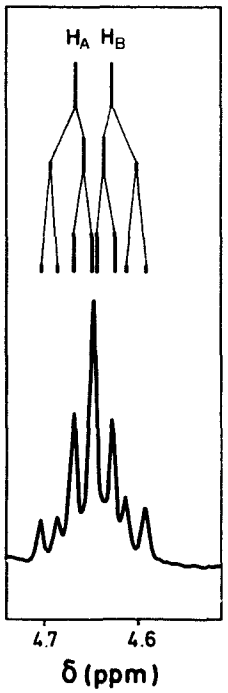



Fig. 2. $^1$H NMR spectrum of $13^2$-hydroxy-BChl *a* (**2**) ($13^2$-epimer mixture) in pyridine-$d_5$. The splitting of the $P_1$-$CH_2$ signal (centered at 4.65 ppm) is characteristic for $13^2$ hydroxylated bacteriochlorophylls in pyridine.

the resulting $13^2$ epimers can be separated on analytical TLC or HPLC, no attempts were made for a preparative separation. A remarkable feature in the NMR spectra of $13^2$-hydroxylated epimers concerns the signal at 4.63 ppm, which is assigned to the phytyl P1-$CH_2$-group. It does not appear, as it usually does, as a doublet, but rather as a multiplet with seven resolved lines, which is the AB-part of an ABM system with $J_{AB} = 12.7$ Hz and $J_{AM} = J_{BM} = 7.2$ Hz (Fig. 2).

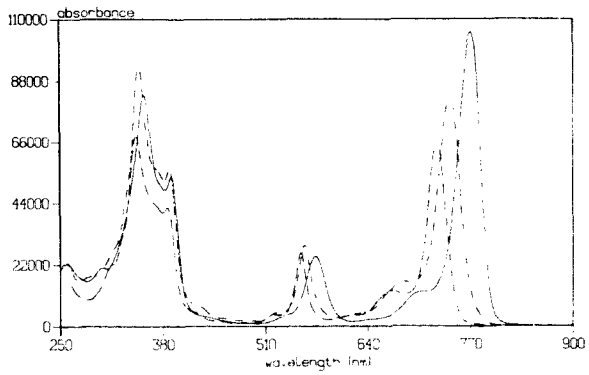



Fig. 3. Quantitative absorption spectra of bacteriochlorophylls, modified at position 3 in diethylether: BChl *a* (**1**), (———); [3-α-hydroxy-ethyl]-BChl *a* (**4**), (— — —) and [3-vinyl]-BChl *a* (**7**), (· - · - ·).

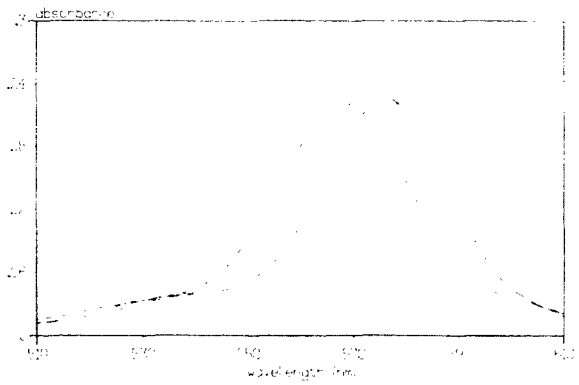


Fig. 4. $Q_X$-absorption band of bacteriochlorophylls modified at position $13^2$: BChl *a* (**1**), (———); $13^2$-demethoxycarbonyl-BChl *a* (**2**) (— — —); and $13^2$-hydroxy-BChl *a* (**3**), (· - · - ·).

This effect is typical for all $13^2$-hydroxylated pigments investigated. Such a pattern is probably the result of an increased anisochronicity, which results from interaction between the $13^2$-OH and the phytol residue in the (natural) $13^2$S-configuration.

*Absorption spectra and extinction coefficients*

Fig. 3 shows the quantitative absorption spectra in diethylether of BChl *a* and the derivatives (**4**) and (**7**), modified only at position 3. Compared with BChl *a* (**1**), there is, for both modifications (as expected [35]), a characteristic short-wavelength shift of the $Q_Y$ and the $Q_X$ bands. For $3^1$-OH-BChl *a*, the $Q_Y$ band is shifted from 771 nm to 728 nm, and the $Q_X$ band from 573 nm to 555 nm. The $Q_Y$ band of [3-vinyl]-BChl *a* shifts from 771 nm to 745 nm, and the $Q_X$ band from 573 nm to 560 nm. It is worth noting the lowered extinction coefficient of the $Q_Y$ band for both modifications. It decreases from $\epsilon_{771} = 105 \cdot 10^3$ for BChl *a* over $\epsilon_{745} = 83 \cdot 10^3$ for [3-vinyl]-BChl *a* to $\epsilon_{728} = 66 \cdot 10^3$ $M^{-1}$ $cm^{-1}$ for [3-α-hydroxyethyl]-BChl *a* in diethylether.

Decarboxylation and hydroxylation at C-$13^2$ only have a small effect on the absorption spectra, as in the chlorophyll series. There is, however, a small but distinct difference in the $Q_X$ region (Figs. 4 and 5). The $13^2$-demethoxycarbonyl( = pyro) compounds (**2**) and (**8**) shift always slightly to longer wavelengths, as compared to the respective (**1**) and (**7**), bearing a $13^2$-carbomethoxy substituent. The shift is most obvious in the short-wavelength flange. The $13^2$-hydroxylated products (**3**) and (**9**), in contrast, always shift to slightly shorter wavelengths, as compared to the $13^2$ H compounds (**1**) and (**7**). This effect, irrespective of the modifications at C-3 (see Materials and Methods section for the [3-α-hydroxyethyl]-bacteriochlorophylls (**4**), (**5**) and (**6**)), is also present in the $Q_X$ band of the corresponding metal-free compounds, e.g., the bacteriopheophytins (see Materials and Methods section

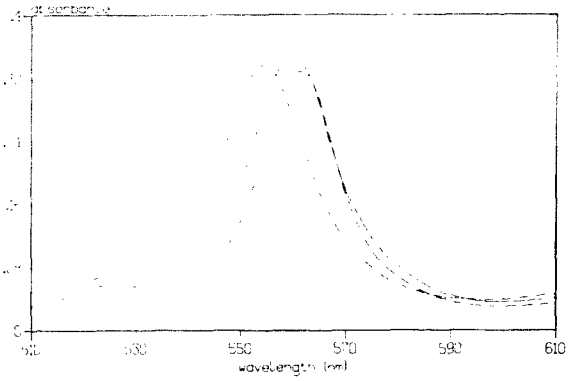



Fig. 5. $Q_X$-absorption band of [3-vinyl]-bacteriochlorophylls modified at position $13^2$: [3-vinyl]-BChl *a* (**7**) (———), [3-vinyl]-$13^2$-demethoxycarbonyl-BChl *a* (**8**) (— — —) and [3-vinyl]-$13^2$-hydroxy-BChl *a* (**9**), (· – · – ·).

and Ref. 29). $Q_X$ band shifts are also induced by a different modification, e.g., by the ligand number of the central Mg in chlorophylls and bacteriochlorophylls [36]. In the latter case, the change in ligation corresponds to a change in the macrocycle geometry (but see Ref. 37), which is less planar when the central Mg has only five ligands (e.g., one axial ligand). The underlying effect for the $13^2$-substituent shifts is also possibly steric changes of the macrocycle, because the $13^2$ substitution pattern can influence the 'puckering' of the macrocycle [38].

*Water elimination and reciprocal interaction between positions C-3 and C-$13^2$*

The elimination of water at the C-3 substituent of [3-α-hydroxyethyl]-BChl *a* (**4**) proceeds smoothly in refluxing toluene under anhydrous conditions to yield [3-vinyl]-BChl *a* (**7**). It is remarkable that this elimination reaction at position 3 is strongly dependent on the $13^2$ substituent. The $3^1$, $13^2$-dihydroxylated pigment (**6**) does not yield the respective [3-vinyl]-pigment (**9**). Rather it remains stable even after several hours of refluxing. The $^1$H-NMR spectra of $13^2$ hydroxylated BChl's consistently showed that they are very hygroscopic, which could be a cause for the changed reactivity. This was ruled out, however, by extensive drying. Repeated evaporation of dry toluene from the pigments under a stream of nitrogen, before attempting the elimination, did not promote the dehydration at the C-3 substituent. Moreover a similar influence of the $13^2$-substituents was found with the $13^2$-demethoxycarbonyl(= pyro) BChl *a* (**5**). This pigment is not as hygroscopic as are $13^2$-hydroxy-BChl's, but again the elimination reaction was negative. Even after 6 h of refluxing in toluene, (**5**) is stable and does not yield the expected (**8**). These experimental data confirm a distinct influence of the $13^2$ substitution pattern on the reactivity of C-$3^1$. One way to rationalize the interaction over such a distance would be a bimolecular mechanism in which one pigment catalyses the reaction of the other, e.g., in a 'head-to-tail' aggregate [39–42]. To test this idea, the reaction was followed at lowered concentrations of pigments in toluene, and also in pyridin, which is known to prevent aggregations. In neither case was there a marked change in the water elimination kinetics. Taken together these experimental results exclude intermolecular interactions as the origin for the changed reactivities, and leave as alternative an intramolecular long-range interaction of the C-$13^2$ on the C-3 sites.

Interestingly, there is also evidence for the reverse influence of the $3^1$-substituent on reactions of the isocyclic ring. The hydroxylation rate of [3-vinyl]-BChl *a* (**7**) at C-$13^2$ in methanol is much lower than that of BChl *a* (**1**). Further results come from reactions in alkaline methanol [43,44]. Under conditions where (**1**) is converted to (Rhodo)bacteriochlorin-$e_7$-methylester, the isocyclic ring V remains intact in (**7**) [29].

*Which role does keto-enol tautomerism play?*

A common aspect of the two compounds, (**5**) and (**6**) which do not eliminate water from the C-3 substituent, is that enolization at the isocyclic ring is greatly reduced (**5**) or even inhibited (**6**). (**5**) is no longer a β-ketoester, and enolization is no longer possible if the $13^2$-H of (**1**) or (**4**) is substituted by an OH-group as in (**6**). The readily enolizable β-ketoester is present in most (bacterio)chlorophylls, but the functional significance of this group is still unclear [19–23]. Enols can principally be formed by tautomeric shift of the $13^2$-H to either the $13^1$-carbonyl or the $13^3$-methoxycarbonyl oxygen, but all enolic structures reported so far are $13^1$-ene-$13^1$-ols [19–23,45]. Enolates can be formulated from all enols by proton dissociation. In most cases, the carbonyl tautomer is strongly favored and accounts, for example, for the absorption and vibrational spectra, and no significant amounts of chlorophyll enol-tautomers are detected in organic solvents by the latter [46]. There is, for example, no distictive spectroscopic difference between the readily enolizable (**1**), the more difficult enolizable (**2**), and the non-enolizable (**3**), whereas the known enols differ considerably in their absorption from the keto-compounds. However, the ready H-exchange [47] and epimerisation of the chiral center C-$13^2$ [48] are explained by this mechanism.

Vinylogous enolizations are possible as well, and, for example, account for the $^1$H/$^2$H-exchange at the 12-$CH_3$-group [49]. It is possible that the long-range effects described above are due to a more extensive vinylogous enolization. Enol(ates), which are substituted at the β-position by a suitable nucleophilic group, are prone to elimination forming αβ-unsaturated ke-

tones. A number of enzymatic ([50–52] and for a review see Ref. 53) and non-enzymatic [54–57] reactions of this type have been studied in detail. Via the tetrapyrrolic $\pi$-system, the water elimination from (**4**) can then also be rationalized by a vinylogous mechanism.

It is worth noting the long distance over which the supposed enol(ate) formation can influence the reactivity of side-groups. There is another long-range intramolecular reaction known in chlorophylls, e.g., the electrophilic chlorination of Phe *a* [58], which depends on the stereochemistry at C-13$^2$. But in this case, probably steric rather than electronic factors are important.

It has been speculated for a long time that enolizations are important in vivo [19–23], because all bacteriochlorophylls and bacteriopheophytins in reaction centers carry the enolizable $\beta$-ketoester system. The long-range effect observed here is a further aspect to these speculations. It was significant in this context, that exchange of the enolizable (**1**) in bacterial reaction centers at the sites $B_{A,B}$ by the non-enolizable (**3**), did not interfere with charge separation, stability and VIS/NIR spectra [11,29]. The same is true for an exchange of (**10**) by (**12**) (or (**18**)) in the $H_B$ binding site, while such an exchange was not readily possible at the $H_A$ binding site [29]. An enol formation of BPhe *a* in $H_A$ which has been suggested from Raman spectroscopy [59], would explain the latter exchange results. However, ENDOR [60] and more recent Raman data [61] seem to exclude any enolic character of BPhe *a* in $H_A$ in the ground or doublet states. In any event, in order to rationalize the possible role of enol(ate) formation of BPhe *a* in $H_A$ it will be necessary to obtain more experimental and theoretical results about the influence of enol(ate)s on excited states and charge transfer states. One good tool for this will be the exchange experiments [11–14,29] in combination with IR/Raman spectroscopy (in particular with a stabilized BPhe $a^{\cdot-}$).

While the previous discussion was mainly concerned with static aspects, transient enol(ate) formation is another one, especially in photoenolization (for a review see Ref. 62). The photochemistry of aryl carbonyl compounds, such as acetophenone and benzophenone, is characterized by very efficient hydrogen abstraction from solvent or other hydrogen donors to give ketyl radicals, which then either couple to give pinacols or abstract hydrogen to give alcohols. However, the presence of an ortho-substituent carrying benzylic hydrogens almost completely quenches this intermolecular reaction and often renders the aryl carbonyl compound photochemically inert [63,64]. The mechanism of this quenching has been recognized as involving intramolecular hydrogen abstraction from the ortho-substituent by the carbonyl in a Norrish type II reaction to give a biradical, which can collapse to ground state dienols. This is preferable for anti-conformers, the syn-conformer converts directly from the excited $S^1$ state to ground state dienols. The unstable di-enols rapidly 're-ketonize' to give back the starting material, so that the initial photochemical excitation energy is ultimately dissipated as heat. Their ability to direct the de-excitation pathway has led to the use of these compounds to stabilize or destabilize polymers towards light.

For all known bacteriochlorophylls there is such a stabilizing 'ortho' substituent, e.g., the 12-$CH_3$ group (in *syn*-conformation). This could play the same stabilizing role in quenching excited states as an ortho methyl substituent in aryl carbonyl compounds. It is clear that, under normal photosynthetic conditions, this is an undesirable process. On the other hand, this quenching could become important in an environment or under conditions where the absorbed energy cannot be used in regular energy transfer (e.g., during biosynthesis or high light intensity conditions) or when they are functional in electron transfer. The functionality of the 'vinylogous' 12-$CH_3$ group depends on the environment; by interaction with this group, the reactivity of the excited state could be modulated. One such modulation may occur by the interaction of Glu-L141 and 13$^1$ C=O of BPhe-$H_A$ in reaction centers.

Irrespective of the mechanistic details the results show how the reactivity of bacteriochlorophylls could be influenced sensitively by substituents of the macrocycle, and probably also, by the environment of these substituents. This, then, may provide for switching or modulating the photophysical and photochemical properties of the pigment molecules in a reversible, environment-controlled, manner. It may be relevant to the different functions of bacteriochlorophylls in photosynthesis, and, in a dynamic manner, for the diode properties of reaction centers.

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**Appendix**

*Pigment isolation, synthesis and characterization*

*BChl a (1).* Isolated from *Rhodobacter sphaeroides* 2.4.1, as described before [12] as a C-13$^2$ epimer mix-

ture with R/S around 90/10. Spectra: absorption in ether, $\lambda_{max}$ [nm] (relative intensities): $S_A$ * 357 (0.78), $S_B$ * 391 (0.50), $Q_X$ 573 (0.23), $Q_Y$ 771 nm (1); ($\epsilon_{771}$ = $(105 \pm 5.0) \cdot 10^3 \cdot M^{-1} \cdot cm^{-1}$ in ether); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 9.52 (5-H); 8.64 (10-H); 8.50 (20-H); 6.56 ($13^2$R-H); 6.42 ($13^2$S-H); 3.45 (2-$CH_3$); 3.12 ($3^2$-$CH_3$); 1.70 (7-$CH_3$, d, $J$ = 7.2 Hz); 3.56 (12-$CH_3$); 1.53 (18-$CH_3$, d, $J$ = 7.1 Hz); 3.78 ($13^2$-$COOCH_3$); 4.75 ($P_1$); 5.45 ($P_2$); 1.65 ($P_3$).

*$13^2$-Demethoxycarbonyl-BChl a (= Pyro-BChl a) (2).* Synthesized as described in Ref. 24. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 35 (0.77), $S_B$ 390 (0.43), $Q_X$ 576 (0.22), $Q_Y$ 771 nm (1); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 9.49 (5-H); 8.72 (10-H); 8.61 (20-H); 5.29 ($13^2$R-H, d, $J$ = 19.5 Hz); 5.07 ($13^2$S-H, d, $J$ = 19.6 Hz); 3.52 (2-$CH_3$); 3.10 ($3^2$-$CH_3$); 1.7 (7-$CH_3$); 3.68 (12-$CH_3$); 1.65 (18-$CH_3$); 4.75 ($P_1$); 5.47 ($P_2$); 1.67 ($P_3$).

*$13^2$-Hydroxy-BChl a (3).* Synthesized as described in Ref. 25. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 357 (0.78), $S_B$ 391 (0.55), $Q_X$ 568 (0.22), $Q_Y$ 771 nm (1); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 9.65 (5-H); 8.71 (10-H); 8.60 (20-H); 3.48 (2-$CH_3$); 3.15 ($3^2$-$CH_3$); 1.74 (7-$CH_3$, d, $J$ = 7.1 Hz); 3.56 (12-$CH_3$); 1.39 (18-CH, d, $J$ = 7.1 Hz); 3.57 ($13^2$-$COOCH_3$); 9.66 ($13^2$-OH); 4.65 ($P_1$); 5.35 ($P_2$); 1.58 ($P_3$).

*[3-α-Hydroxyethyl]-BChl a (4) (modified after Ref. 26): 1 (3–5 mg)* was dissolved in 100 ml ethanol and stirred under nitrogen at 4°C. After 10 min, $NaBH_4$ (10 mg) was added and the reaction mixture kept stirred. The reaction is followed by Vis-NIR absorption spectroscopy (blue-shift of the $Q_Y$ band from 771 nm to 714 nm in the reaction mixture) and was generally complete after 30–60 min. The mixture was then separated between diethyl ether and water. The ether phase, which contains the pigments, was washed repeatedly with water and then dried over NaCl. The products were purified on a DEAE-cellulose column [27]; the four $3^1/13^2$ diastereomers were not separated by this procedure. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 346 (1.03), $S_B$ 387 (0.64), $Q_X$ 555 (0.39), $Q_Y$ 728 nm (1); $\epsilon_{728}$ ($66.1 \pm 3.2 \cdot 10$ $M^{-1}$ $cm^{-1}$ in ether); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 8.88 *, 8.84 *, 8.83 * (5-H); 8.37 *, 8.35 * (10-H); 8.02 *, 8.00 *, 7.99 * (20-H); 6.41 ($13^2$R-H); 6.26 *, 6.25 * ($13^2$S-H); 3.23 *, 3.18 * (2-$CH_3$); 1.63 (7-$CH_3$); 3.51 *, 3.48 * (12-$CH_3$); 1.46 (18-$CH_3$); 375 *, 3.83 *, 3.74 * ($13^2$-$COOCH_3$); 6.45 ($3^1$-H); 7.38 ($3^1$-OH); (* split signal due to presence of $3^1/13^2$ diastereomers).

*[3-α-Hydroxyethyl]-$13^2$-demethoxycarbonyl-BChl a (5).* Synthesized by chemical reduction of **(2)**, as described for the synthesis of **(4)** from **(1)**. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 346 (1.015), $S_B$ 385 (0.64), $Q_X$ 555 (0.358), $Q_Y$ 729 nm (1); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 8.76 *, 8.75 * (5-H); 8.47 (10-H); 8.13 (20-H); 5.20 ($13^2$-H); 6.26 ($13^2$-H); 3.30 *, 3.27 * (2-$CH_3$); 1.61 (7-$CH_3$); 3.63 (2-$CH_3$); 1.55 (18-$CH_3$); 6.43 ($3^1$H); 7.31 ($3^1$-OH); (* split signal due to mixture of $3^1$ epimers).

*[3-α-Hydroxyethyl]-$13^2$-hydroxy-BChl a (6). (4) (5 mg)* was dissolved in methanol (250 ml) and kept for 4 days at 4°C in the presence of air [25]. There were several products which were separated on a DEAE-cellulose column [27]. The main band contained a mixture of $3^1/13^2$ diastereomers of **(6)**, which were not separated. A subsequent repurification on RP-18 columns (Adsorbex, Merck) is sometimes necessary to remove by-products. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 346 (1.14), $S_B$ 386 (0.84), $Q_X$ 550 (0.40), $Q_Y$ 728 nm (1); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 9.00 *, 8.97 * (5-H); 8.46 (10-H); 8.12*, 8.11 * (20-H); 5.37 ($13^2$R-H); 3.26 *, 3.21 * (2-$CH_3$); 3.52 (2-$CH_3$); 3.54 ($13^2$-$COOCH_3$); 6.51 ($3^1$-H); (* split signal due to mixture of $3^1/13^2$ diastereomers).

*[3-Vinyl]BChl a (7).* Purified **(4)** (1 mg) was dried in vacuum over $CaCl_2$ for 12 h and then dissolved in toluene (50 ml), dried over molecular sieve (3 Å). The mixture was refluxed under argon for 1–2 h. The reaction was usually followed by absorption spectroscopy (red-shift of the $Q_Y$ band from 739 to 750 nm in the reaction mixture). After the reaction was completed, the solvent was removed by 35°C in vacuum. The final product, which was already rather pure, was purified on a DEAE-cellulose column [27]. The $13^2$ epimers were not separated. The high yield of the reaction was confirmed by HPLC analysis with a diode array absorption detector [14]. The silica gel system used [28], allows the separation of the $3^1$ and $13^2$ diastereomers of **(4)** and the $13^2$ epimers of **(7)**. There are no colored by-products detectable by HPLC analysis of the final product. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 351 (1.13), $S_B$ 389 (0.67), $Q_X$ 560 (0.35), $Q_Y$ 745 (1); $\epsilon_{745}$ ($82.5 \pm 2.5 \cdot 10^3$ $M^{-1}$ $cm^{-1}$ in ether); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 8.45 (5-H); 8.41 (10-H); 8.14 (20-H); 8.03 ($H_X$ d, $J$ = 11.6 Hz); 5.94 ($H_A$ d, $J$ = 11.6 Hz); 6.21 ($H_B$, d, $J$ = 17.9); 6.44 ($13^2$R-H); 6.29 ($13^2$S-H); 3.20 (2-$CH_3$); 1.68 (7-$CH_3$, d, $J$ = 7.2); 3.53 (12-$CH_3$); 1.47 (18-$CH_3$, d, $J$ = 7.2 Hz); 3.75 ($13^2$-$COOCH_3$); 4.74 ($P_1$); 5.44 ($P_2$); 1.64 ($P_3$); FAB-mass: 896 ($M$ + 2H, 38%); 895 ($M$ + H, 67%); 894 ($M^+$, 70%); 616 ($M$–phytol + H, 100%); 154 (30%); 136 m/z (24%).

*[3-Vinyl]-$13^2$-demethoxycarbonyl-BChl a (8).* Obtained from **(7)** according to Ref. 24, by refluxing in pyridine under argon for 18 h. Spectra: absorption in

* $S_{A,B}$ = main components of the Soret band system, $Q_{X,Y}$ = visible and near-IR absorption bands, $P_{1,2,3}$ = phytol hydrogen NMR signals C1($H_2$), C2(H) and C3($CH_3$), respectively.

ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 351 (1.07), $S_B$ 388 (0.65), $Q_X$ 562 (0.32), $Q_Y$ 747 nm (1); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 8.56 (5-H); 8.38 (10-H); 8.29 (20-H); 8.02 ($H_X$, $J_{AX}$ = 11 Hz, $J_B$ = 18 Hz); 5.90 ($H_A$, d, $J$ = 11.6 Hz); 6.19 ($H_B$ d, $J$ = 17.7 Hz); 5.20 ($13^2$R-H, d, $J$ = 19.5 Hz); 4.97 ($13^2$S-H, d, $J$ = 19.4 Hz); 3.27 (2-$CH_3$); 1.64 (7-$CH_3$, d, $J$ = 9.6 Hz); 3.65 (12-$CH_3$); 1.61 (18-$CH_3$ d, $J$ = 7.4 Hz); 4.74 ($P_1$); 5.45 ($P_2$); 1.66 ($P_3$).

*[3-Vinyl]-13²-hydroxy-BChl a (9), (7) (5 mg)* was dissolved in methanol (250 ml) and kept in the dark for 6 days at 4°C in the presence of air. There are several products which were separated on a DEAE-cellulose column [27]. The main fraction contains the $13^2$ isomeres of (**9**), which were not separated. A subsequent repurification on RP-18 columns (Adsorbex, Merck) was sometimes necessary. Spectra: absorption in ether $\lambda_{max}$ [nm] (relative intensities): $S_A$ 350 (1.09), $S_B$ 387 (0.79), $Q_X$ 555 (0.33), $Q_Y$ 744 nm (1); $^1$H-NMR in pyridine-$d_5$: $\delta$[ppm] (proton): 8.56 (5-H); 8.55 (10-H); 8.27 (20-H); 8.09 ($H_X$, $J_{AX}$ = 11 Hz, $J_B$ = 18 Hz); 5.96 ($H_A$, d, $J$ = 11.5 Hz); 6.24 ($H_B$, d, $J$ = 7.9 Hz); 3.23 (2-$CH_3$); 1.71 (7-$CH_3$, d, $J$ = 7.2 Hz); 3.54 (12-$CH_3$); 1.33 (18-$CH_3$ d, $J$ = 7.1 Hz); 3.54 ($13^2$-$COOCH_3$); 9.45 ($13^2$-OH); 4.63 ($P_1$); 5.34 ($P_2$); 1.57 ($P_3$); FAB-mass: 912 ($M$ + 2H, 26%); 911 ($M$ + H, 44%); 910 ($M^+$, 50%); 632 ($M$–Phytol + H, 20%); 155 (100%), 136 $m/z$ (100%).

*Modified bacteriopheophytins.* All metal-free products are obtained by demetalation in diethyl ether under nitrogen with 15% HCl at 4°C from the corresponding magnesium-containing pigments **1**–**9**. The final products were purified by preparative thin-layer chromatography on silica gel. The absorption spectra in diethyl ether $\lambda_{max}$ [nm] (relative intensities) are given below. Further spectroscopical data are given in Ref. 29.

*BPhe a (10)*: $S_A$ 357 (1.60), $S_B$ 384 (0.88), $Q_X$ 525 (0.40), $Q_Y$ 749 nm (1).

*13²-Demethoxycarbonyl-BPhe a (11)*: $S_A$ 357 (1.47), $S_B$ 383 (0.79), $Q_X$ 527 (0.36), $Q_Y$ 749 nm (1).

*13²-Hydroxy-BPhe a (12)*: $S_A$ 358 (1.54), $S_B$ 383 (0.94), $Q_X$ 521 (0.37), $Q_Y$ 750 nm (1).

*[3-α-Hydroxyethyl]-BPhe a (13)*: $S_A$ 351 (2.25), $S_B$ 378 (1.34), $Q_X$ 509 (0.76), $Q_Y$ 712 nm (1).

*[3-α-Hydroxyethyl]-13²-demethoxycarbonyl-BPhe a (14)*: $S_A$ 350 (2.22), $S_B$ 377 (1.233), $Q_X$ 511 (0.70), $Q_Y$ 713 nm (1).

*[3-α-Hydroxyethyl]-13²-hydroxy-BPhe a (15)*: $S_A$ 350 (2.20), $S_B$ 376 (1.46), $Q_X$ 506 (0.70), $Q_Y$ 713 nm (1).

*[3-Vinyl]-BPhe a (16)*: $S_A$ 353 (2.31), $S_B$ 380 (1.24), $Q_X$ 513 (0.70), $Q_Y$ 725 nm (1).

*[3-Vinyl]-13²-demethoxycarbonyl-BPhe a (17)*: $S_A$ 353 (2.37), $S_B$ 380 (1.31), $Q_X$ 514 (0.65), $Q_Y$ 725 nm (1).

*[3-Vinyl]-13²-hydroxy-BPhe a (18)*: $S_A$ 353 (2.17), $S_B$ 379 (1.34), $Q_X$ 510 (0.64), $Q_Y$ 726 nm (1).

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