Stereochemistry of the Bacteriochlorophyll-*e* Homologues

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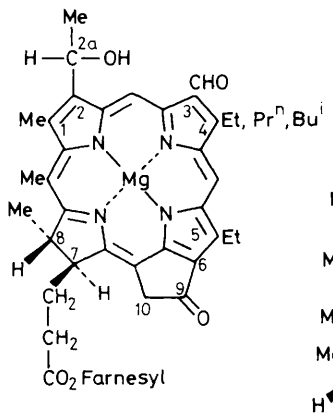
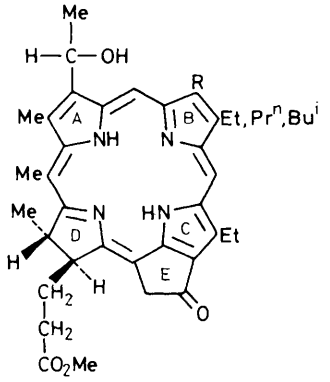
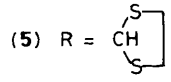
Using proton n.m.r. spectroscopy, reversed-phase h.p.l.c., and synthetic interconversion into methyl bacteriopheophorbides-*c* (**3**), the chirality of the 2-(1-hydroxyethyl) in methyl bacteriopheophorbides-*e* (and hence the bacteriochlorophylls-*e*) from *Chlorobium phaeobacteroides* is shown to be 95% (*R*) and 5% (*S*) for the [4-Et,5-Et] homologue, 40% (*R*) and 60% (*S*) for the [Pr^n,Et] compound, and approximately 1% (*R*) and 99% (*S*) for the [Bu^i,Et] homologue.

Green and brown photosynthetic sulphur bacteria (Chlorobiaceae) produce a large number of bacteriochlorophyll (BChl) pigments which are primarily used as light-harvesting antennae.[1] Green bacteria produce two different series of BChl, designated the BChl-*c* and -*d*. In the case of the BChl-*e* (**1**), Brockmann and coworkers have isolated these from the brown bacterium *Chlorobium phaebacteroides* (or *Cb. phaeovibrioides*).[2,3] The absolute stereochemistry in ring D of the BChls-*c*, -*d*, and -*e* has been established by Brockmann[2] by way of chromic acid degradation to maleimides. In addition, both the BChl-*c* and the BChl-*d* have been fully characterized with regard to both their peripheral substituents, and also the



(1)



(2) R = CHO
(3) R = Me
(4) R = CH(OMe)$_2$
(5) R = 

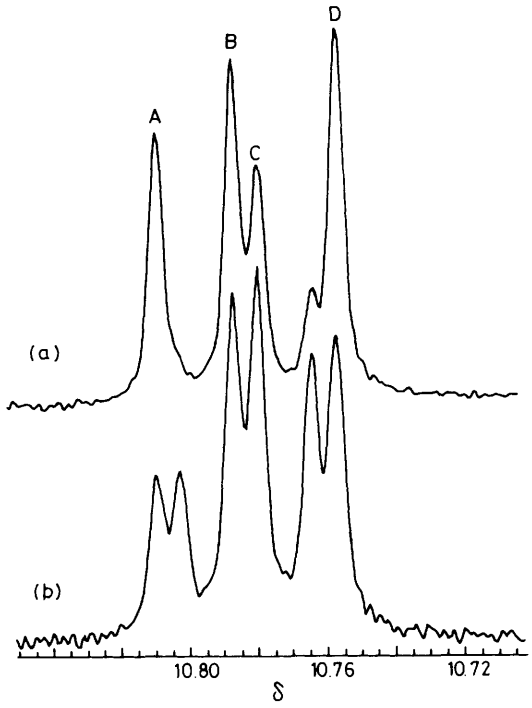


Figure 1. 360 MHz proton n.m.r. spectra (α-*meso*-proton region only, in $CDCl_3$ solution) of (a), the Bmph-*e* (**2**); (b) the Bmph-*e* after racemization of the 2-(1-hydroxyethyl) with aqueous trifluoroacetic/sulphuric acids. Assignments: A: (*S*)-[Bu^i,Et]; B: (*S*)-[Pr^n,Et]; C: (*R*)-[Pr^n,Et]; D: (*R*)-[Et,Et].

chirality of the 2-(1-hydroxyethyl) substituent. The chirality at C-2a shifts from (*R*) to (*S*) as the size of the alkyl moiety at C-4 increases. Thus BChl-*c* [Et,Et]† is exclusively (*R*), BChl-*c* [Pr^n,Et] is an (*R*,*S*) mixture, while BChl-*c* [Bu^i,Et] is completely (*S*).[4] Likewise, the chirality of BChl-*d* [Et,Me], [Et,Et], [Pr^n,Me], and [Pr^n,Et] is (*R*), whereas the [Bu^i,Me], [Bu^i,Et], [neopentyl,Me], and [neopentyl,Et] compounds all have the (*S*) absolute stereochemistry in the 2-side chain.[5] With the close structural relationships between the BChl-*c*, -*d*, and -*e*, we felt that there might be some (*S*) diastereoisomers present in the last of these. In the present communication we show that the BChl-*e* (**1**) also exist as both (*R*) and (*S*) diastereoisomers at the 2-position, and that the methyl bacteriopheophorbides-*e* [Bmph-*e*, (**2**)] can be transformed into the Bmph-*c* (**3**) without racemization of the 2-(1-hydroxyethyl) group.

BChl-*e* (**1**) were isolated from *Chlorobium phaeobacteroides* as described by Brockmann *et al.*[2,3] These workers have shown that only the 5-ethyl series of pigments is produced by this bacterial strain. Thus, in our h.p.l.c. traces of the Bmph-*e* (**2**) produced by treatment of the BChl-*e* with methanol–sulphuric acid, we expected to see only three peaks (*i.e.* [Et,Et], [Pr^n,Et], and [Bu^i,Et]). We were therefore surprised to see six clearly resolved bands in the h.p.l.c. trace. Further synthetic and analytical work showed that the three extra peaks were from the dimethyl acetals (**4**), produced by transformation of the 3-formyl group with the methanolic acid.

The reversed-phase h.p.l.c. of pure Bmph-*e* was investigated under several different solvent and column conditions but at no time was there any diastereoisomeric separation of the homologues. Lack of satisfactory resolution by the h.p.l.c. column was suspected because no separation was achieved even when the Bmph-*e* were previously treated under racemization conditions (aqueous trifluoroacetic acid, TFA).

N.m.r. spectroscopy was subsequently shown to be a better technique for identification of diastereoisomers. Unlike for the Bmph-*c* and -*d*, the *meso*-proton region in the 360 MHz proton n.m.r. spectrum of the homologous mixture of Bmph-*e* (**2**) was quite diagnostic for identification of homologues and diastereoisomers. The α-*meso*-protons (Figure 1a) adjacent to the stereocentre of interest are the protons most affected by the chiral 2-(1-hydroxyethyl) group. Using n.m.r. monitoring, it was possible to achieve complete racemization by treating Bmph-*e* with 80% TFA–water, spiked with a small amount of sulphuric acid. Racemization was evidenced by the doubling of the α-protons for all three homologues in the proton spectrum (Figure 1b). The results of the racemization experiments and n.m.r. spectra showed that at least two homologues ([Et,Et] and [Pr^n,Et]) exist‡ as mixtures of diastereoisomers.

This conclusion was confirmed by synthetic interconversion of the Bmph-*e* (**2**) to Bmph-*c* (**3**), the latter being known to give clear h.p.l.c. separations for individual diastereoisomers.[4] It has been reported[3] that selective acetal formation is possible with toluene-*p*-sulphonic acid, and ethanedithiol in the Bmph-*e* series, but no attempt was made to preserve the all important 2-(1-hydroxyethyl) group, and this was completely transformed into 2-ethyl. Thus, the Bmph-*e* mixture (**2**) was treated with 1 equiv. of ethanedithiol and 1.1 equiv. of boron trifluoride–ether adduct to give the dithioacetal (**5**)§ which,

† The [Et,Et] (*etc.*) nomenclature refers to the substituents at the 4- and 5-positions, respectively.

‡ We cannot eliminate the possibility that the 1% (*R*) diastereoisomer observed here has arisen through chemical scrambling of the 2-(1-hydroxyethyl) group. This does, however, establish the upper limit for racemization at this value.

§ The crude dithioacetal (**5**) was not characterized, but n.m.r. spectroscopy of the α-*meso*-proton resonances showed that the original stereochemistry of the hydroxyethyl group had been retained.

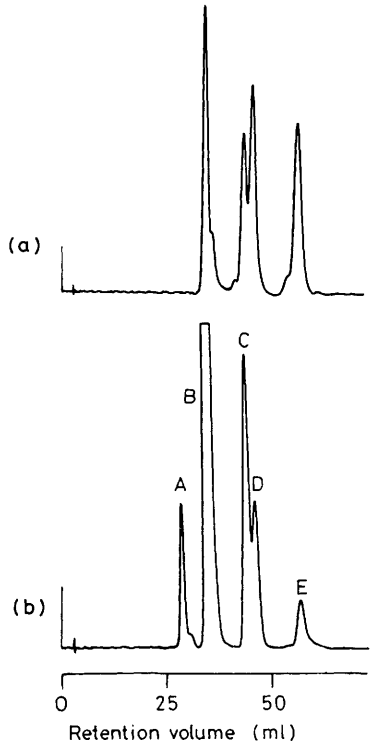


Figure 2. H.p.l.c. traces (Waters Associates RCM-100, 5 micron C-18 μBondapak column, 1.5 ml/min of 88 : 12 methanol : water, detector set at 670 nm) of (a) Bmph-*c* (**3**) obtained from Bmph-*e* (see text); (b) natural Bmph-*c* isolated from *Prosthecochloris aestuarii*.[4] Assignments: A: (*R*)-[Et,Me]; B: (*R*)-[Et,Et]; C: (*R*)-[Pr^n,Et]; D: (*S*)-[Pr^n,Et]; E: (*S*)-[Bu^i,Et].

after treatment with Raney nickel for 1 h at 40 °C gave desulphurized material which was characterized by spectrophotometry, n.m.r., and h.p.l.c. The 360 MHz n.m.r. spectrum confirmed the product as Bmph-*c*, but because of coincidental α-*meso*-proton chemical shifts, no stereochemistry could be inferred from the spectrum. However, the reversed-phase h.p.l.c. (Figure 2a) showed that the synthetic Bmph-*c* [Et,Et] is almost entirely (*R*) configuration at the 2-(1-hydroxyethyl), but with about 5% of the (*S*) configuration also present (h.p.l.c. recycling not shown). The synthetic [Pr^n,Et] homologue of Bmph-*e* is approximately 40% (*R*) and 60% (*S*), these values being reversed from those determined for the same homologues in the natural Bmph-*c* (Figure 2b). The [Bu^i,Et] homologue is greater than 98% (*S*) stereochemistry, as would be expected from the Bmph-*e* n.m.r. spectrum. Thus, by a combination of synthetic, chromatographic, and spectroscopic techniques, it was shown that the Bmph-*e* (and by analogy, the BChl-*e*) exist as three homologues, each of which occur as different mixtures of diastereoisomers, but following the general pattern outlined already for the stereochemistry of the Bmph-*c* and Bmph-*d*.

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