Bacteriochlorophyll b. Determination of its Configuration by Nuclear Overhauser Effect Difference Spectroscopy

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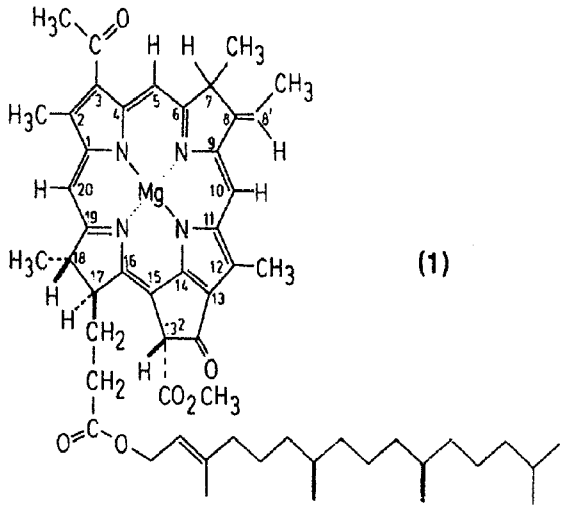
Bacteriochlorophyll b (Bchl b) (1) has been examined by Brockmann[1] and Scheer,[2] and its main structural features have been determined. However, the full stereochemistry of this natural product as yet remains unknown.



(1)

Bchl b (1) was first isolated by Eimhjellen[3] from *Rhodopseudomonas viridis*. Of all the photosynthetic pigments, it absorbs at the longest wavelength (λ_{max} = 1020 nm, *in vivo*). It is extremely sensitive towards light, oxygen, most solvents, and nearly all chromatographic materials.‡ Controlled chemical reactions which keep ring *B* intact, or even an oxidative degradation that converts this part of the molecule into a substituted succinimide, seem to be impossible.§ Consequently, no evidence was available about the absolute configuration at C-7, and the configuration of the exocyclic double bond at C-8.

In the course of our investigation of the n.m.r. spectra of chlorophyll derivatives we used nuclear Overhauser effect (n.O.e.) difference spectroscopy[4] for the assignment of the ^1H resonances. This led us to try the method for the determination of the configuration of the 8,8′-double bond in (1). Bchl b (1) was isolated from *Rhodopseudomonas viridis* by extraction with pyridine and purified by low-pressure chromatography[5] on cellulose columns. Irradiation at the resonance of the 8′-methyl group under n.O.e conditions did not lead to a significant change in signal intensity of the proton at C-10, while irradiation at the resonance of the 8′-proton produced a clear and reproducible enhancement of the intensity of the 10-proton signal (Figure). These data verify the postulated *E*-configuration for the 8,8′-double bond of Bchl b (1). This result is compatible with the stereochemistry of the ethylidene group in the structurally related phycocyanobilin.[6,7]

Still unsolved is the absolute configuration at C-7.¶ By analogy with the stereochemistry of phycocyanobilin and bacteriochlorophyll a[9,10] one might expect the *R*-configuration.

Experimental

Bacteria (5 g; deep frozen) were extracted by known methods with pyridine. The extract was chromatographed on a cellulose column with pyridine–water (8 : 1). The eluate was evaporated to near dryness under reduced pressure, taken up in 3 ml of [$^2H_5$]pyridine, once more reduced to near dryness, and then dissolved in degassed and oxygen-free [$^2H_5$]-pyridine for n.m.r. measurements with a Bruker WP 80 spectrometer (computer/BNC 28). All n.O.e. measurements were carried out in the gated homonuclear decoupling mode.

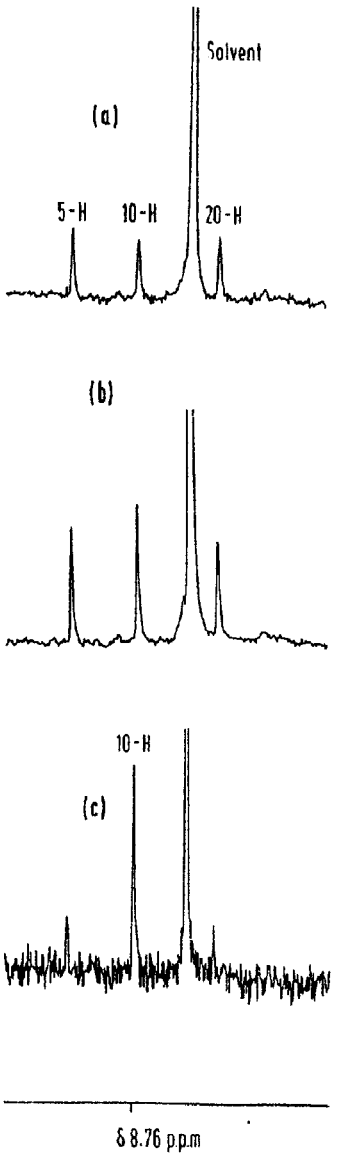
All solvents were distilled in a nitrogen stream and degassed. For purification Merck chromatographic material was used. During the work-up and after the n.m.r. investigations the u.v.–visible spectra of all solutions were recorded (Beckmann Acta M4) to check for possible decomposition. As far as possible all experiments were done in the dark.

†This is a Short Paper as defined in the Instructions for Authors [*J. Chem. Research (S)*, 1977, Issue 2, p. iv]; there is therefore no corresponding material in *J. Chem. Research (M)*.

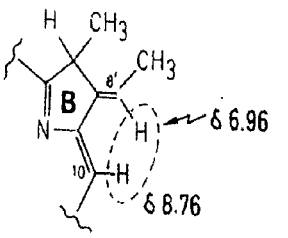
‡Bchl b is unstable, even on 'reversed phase material' (RP-8, RP-18).

§The demetallation to bacteriophaeophytine b with 0.1M-HCl can be carried out in reasonable yield.

¶The postulated absolute configuration at C-13[2] is based on the observation that o.r.d. spectra of debacteriophaeophorbide b methyl ester and (3-acetyl)phaeophorbide a methyl ester are identical; *cf.* ref. 8.



(a) n.O.e. control (irradiation at 20 p.p.m.)



(b) n.O.e. spectrum (irradiation at 6.96 p.p.m.)

(c) n.O.e. difference spectrum

Figure N.O.e. difference ^{1}H n.m.r. spectra of Bchl b (1) (low-field part) in [$^2H_5$]pyridine

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