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ON THE PARTIAL SYNTHESIS OF OPTICALLY PURE BACTERIOPHEOPHORBIDES -C AND -D

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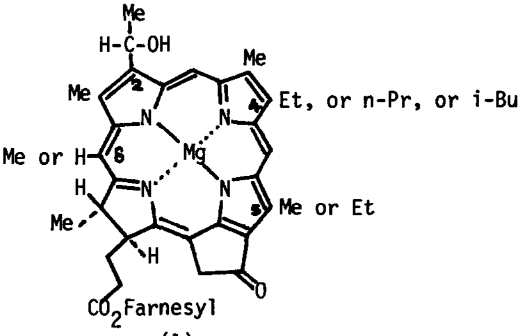
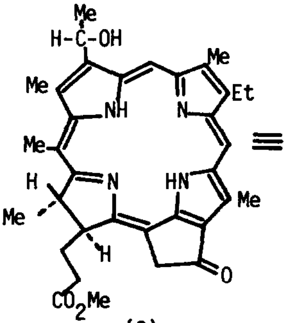
Abstract: Methyl bacteriopheophorbides-c or -d (obtained by partial synthesis, or by Markownikoff hydration of methyl pyropheophorbide-a, respectively) are obtained as a mixture of the (R,S) diastereomers at the 2(1-hydroxyethyl) group; these can be efficiently and completely separated using reverse phase high performance liquid chromatography (HPLC). Purity of the resolved mixture is demonstrated by 360 MHz NMR spectra.

The bacteriochlorophylls -c and -d are homologous mixtures of pigments isolated from certain green sulfur bacteria. Their general structures are exemplified in the structure (1), where the δ-meso-methyl is present in the bacteriochlorophylls-c, and a δ-meso-hydrogen, reminiscent of the plant chlorophylls, is present in the bacteriochlorophyll-d series.[1]

Brockmann Jr. et al.[2] recently communicated a partial synthesis of the 2-(R,S)-bacteriopheophorbide-c (2) using chlorin-e$_6$ trimethyl ester as their starting material. In a subsequent communication,[3] Risch and Reich described a procedure in which the readily available methyl 2-(R,S)-pyropheophorbide-a (3) could be enriched to an extent of 70% in the 2-(R) component by chromatography on a silicagel column coated with the chiral electron acceptor, (S)-(+)-2-(2,4,5,-7-tetranitro-9-fluorenylideneaminooxy)propionic acid.[4] It was anticipated[3] that refinement of the procedure might eventually produce complete separation of the diastereomers; HPLC on silicagel had been completely unsuccessful for the separation of (3) into its components.



(1)



(2)

Pheophorbide from "Band 6"[1] of the bacteriochlorophylls-c (Chlorobium chlorophylls, 660 Series)

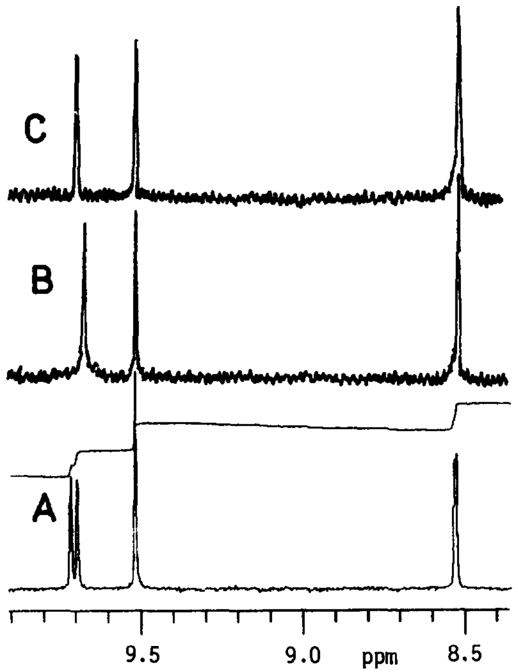


FIGURE 1: 360 MHz NMR spectrum (meso protons only) of A, the (R,S) mixture (3); B, pure (R) form; C, pure (S) form.

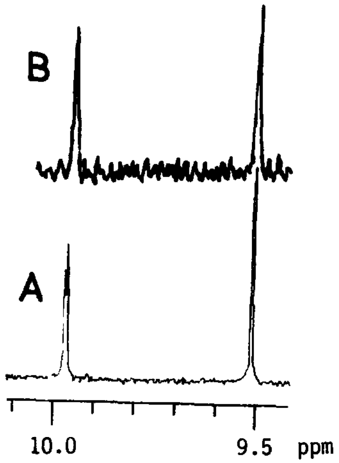


FIGURE 3: 360 MHz NMR spectrum of A, the (R,S) mixture (2); B, pure (R) form.

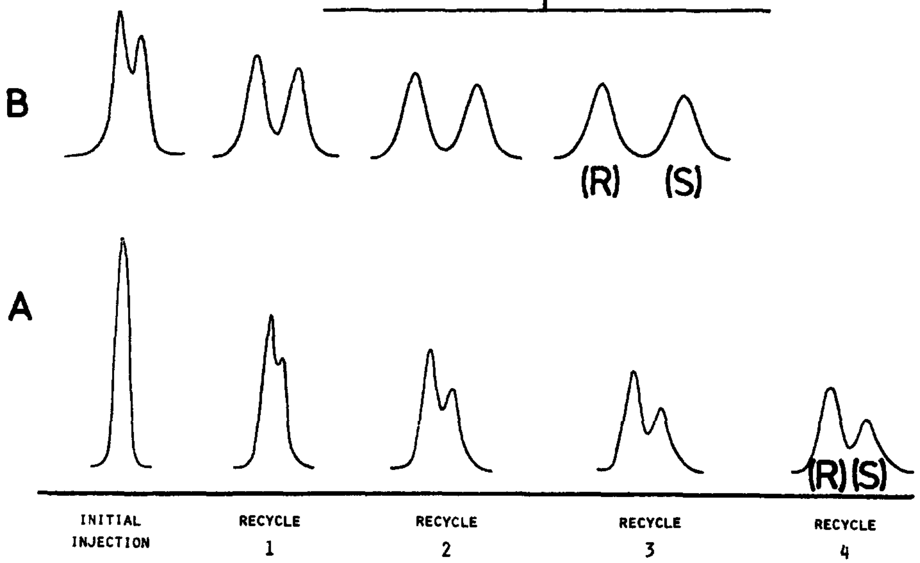
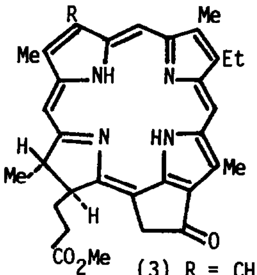


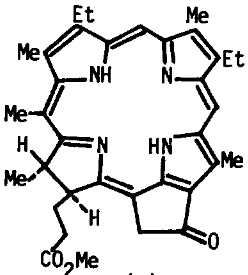
FIGURE 2: HPLC chromatogram, with recycling, of A, 2-(R,S)(1-hydroxyethyl) mixture (3); B, 2-(R,S)(1-hydroxyethyl) mixture (2). A Waters Associates ALC/GPC-201 Instrument with 405 nm UV detector was used, with two μ-Bondapak C18 columns (7.8 mm ID x 30 cm). Solvent was 15% H_2O in MeOH, and the flow rate was 3 mL/min, giving a pressure of approx. 2000 psi. Brockmann Jr., *et al.* (*Annalen*, 419 (1979)) have shown the 2-(R) compound to be the natural diastereomer.

These communications[2,3] have prompted us to report our recent work in this area. We have completed partial syntheses of the methyl mesobacteriopheophorbide (4)[5] and of the methyl pyropheophorbides (3) and bacteriophorbides (2)[6] using routes similar to those of Brockmann Jr. et al.[2] In the present Letter we show that the synthetic materials (2) and (3) can be readily separated into their optically pure components using reverse phase HPLC.

Methyl pyropheophorbide-a (5) was treated with HBr/HOAc[7] to give the corresponding 2-(1-hydroxyethyl) diastereomeric mixture (3). NMR spectroscopy indicated (Figure 1A) that the presence of the diastereomeric mixture could be detected. HPLC separation (Figure 2A) on reverse phase, even at the first pass, showed a mixture to be present and after five recycles the mixture was completely separated. The NMR spectra in Figure 1B and 1C demonstrate the purity of the two components. When the NMR spectrum of the synthetic methyl bacteriopheophorbide-c (2) was measured (Figure 3A) a mixture was again indicated, though the separation between the β-meso protons for each diastereomer was less obvious. However, HPLC separation (Figure 2B) was more readily achieved, and complete separation on even a preparative scale was achieved after only three recycles. One notable feature in Figures 2A and 2B is that the diastereomeric mixture is not 1:1 for the (R,S) forms; integration of the peak areas (assuming equal extinction coefficients for the two diastereomers) gives one a ratio closer to 3:2. Finally, the fact that the faster running (major) component is the 2-(R) form (i.e. the natural, optically pure component) for compound (2), was confirmed by the HPLC chromatogram shown in Figure 4. Here the natural mixture of methyl bacteriopheophorbides from *Chloropseudomonas ethylicum* is shown, and above it the same mixture doped with optically pure synthetic material. The arrow shows the retention volume for the synthetic 2-(S) material.



(3) R = CH(OH)Me
(5) R = $CH=CH_2$



(4)

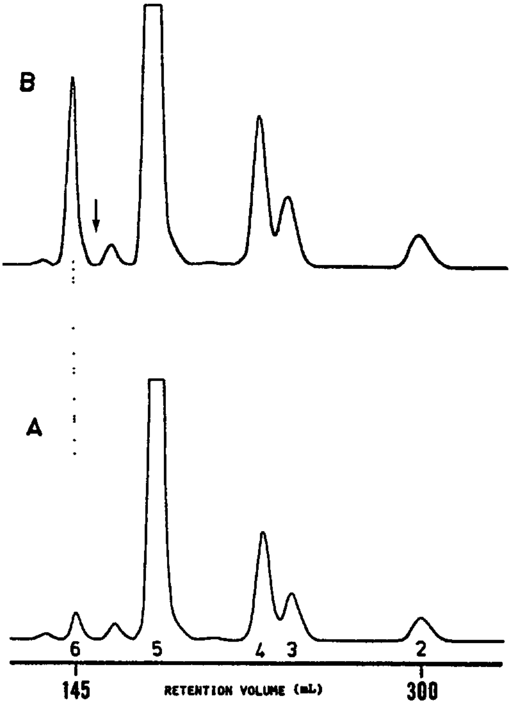


FIGURE 4: HPLC chromatograms, on reverse phase, of A, the natural mixture of methyl bacteriopheophorbides-c from *Chloropseudomonas ethylicum*; and B, the same mixture, but doped with synthetic 2-(R)(1-hydroxyethyl)-pheophorbide. The arrow shows the retention volume for the 2-(S) diastereomer. Numbers in chromatogram A refer to the bands using Holt's nomenclature.[1]

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