THE PIGMENTS OF THE PHOTOSYNTHETIC BACTERIUM RHODOMICROBIUM VANNIELII

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Previous studies concerning the pigment complex of the photosynthetic bacteria belonging to the *Thiorhodaceae* and the *Athiorhodaceae* have indicated that this complex consists essentially of one green pigment called bacteriochlorophyll and a varying number of yellow to red carotenoid pigments (French, 1937; van Niel, 1944; van Neil *et al.*, 1935; Wassink *et al.*, 1939). The present investigation was undertaken to characterize further the recently described photosynthetic bacterium, *Rhodomicrobium vannielii* (Duchow and Douglas, 1949), with respect to its pigment complex.

Mass cultures of the organism were grown on the media used by Duchow and Douglas (1949) under constant illumination for 10 to 20 days at approximately 25 C. The pigments were extracted from the centrifuged organisms first by a shaking with methanol to extract the green component, followed by repeated shakings with chloroform to extract the carotenoid components.

The green component in methanol was chromatographed with powdered magnesium trisilicate¹ as an adsorbent. Only one band formed and an alcoholic solution of the pigment was found to be spectroscopically identical with previously described bacteriochlorophyll (Wassink *et al.*, 1939). In the intact cells absorption maxima resulting from the chlorophyll complex were found at 590, 805, and 880 m μ .

The extracted carotenoids were transferred to Skelly solve B and the solution chromatographed on a column consisting of 2 parts by weight magnesia² plus 1 part "hyper-flo celite." Six bands formed and were eluted and collected at the bottom of the column. These were examined spectroscopically and found to be either identical with or closely related to previously described bacterial pigments. (All spectroscopic measurements were made with a Beckman model DU quartz spectrophotometer using 1-cm cells.)

Pigment 1 (numbered in the order eluted from the chromatogram) was identified as *beta*-carotene both by spectroscopic similarities and by mixed chromatography on alumina with an authentic sample of purified *beta*-carotene. Pigments 2 and 5 had identical maxima in the visible range. However, pigment 5 had an additional maximum in the near ultraviolet at $362 \text{ m}\mu$, which was exactly 141 m μ from the longest absorption maxima of pigment 2. The same relationship held between pigments 3 and 6, with pigment 6 having the additional maximum 141 m μ from the longest maximum of pigment 3. For these reasons it was con-

¹ Merck and Company, Rahway, New Jersey.

² California Chemical Company, Newark, California, adsorptive powdered magnesia no. 2641.

sidered probable that pigments 5 and 6 were *cis* isomers of their respective *trans* forms, namely pigments 2 and 3 (Zechmeister and Polgar, 1943). Pigments 2 and 5 correspond spectroscopically to the previously described bacterial pigment rhodopin (Karrer and Solmessen, 1935), whereas pigments 3 and 6 correspond with rhodovibrin (Karrer and Solmessen, 1935). Pigment 4 was identified as either identical with or closely related to spirilloxanthin on the basis of absorption maxima (van Niel and Smith, 1935). Figure 1 shows the absorption spectra of these pigments in the solvent benzene.



Figure 1. Absorption spectra of the pigments of *Rhodomicrobium vannielii* in benzene Extinction values represent the extinction of the pigment solution extracted from 18 grams of moist cells and dissolved in 50 ml of solvent. They thus represent the relative contribution of each pigment to the pigment complex of the organism.

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