JOURNAL OF BACTERIOLOGY, Dec., 1966

Vol. 92, No. 6
Printed in U.S.A.

# Pigment of *Nocardia corallina*

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Received for publication 18 July 1966

The pigment of *Nocardia corallina* is reported, on the basis of solubility and color of partially purified samples, to be a lipochrome (V. Reader, Biochem. J. **18**:1039, 1925) and probably a carotenoid (R. Webb, Ph.D. Thesis, University of Oklahoma, Norman, 1956). In this paper, the crystallization of the major pigment is reported, together with evidence not in agreement with a carotenoid structure.

*N. corallina* was grown for 1 month on nutrient agar plus 0.5% fructose. The resulting growth was dehydrated in methanol and extracted for 18 hr at 50 C with methanol containing 10% KOH and benzene (1:3, v/v) by shaking in an evacuated, foil-wrapped flask. The benzene phase was

TABLE 1. *Visible absorption maxima of the red pigment*

| Solvent | Absorption maximum |
|---|---|
| | mμ |
| Methanol | 465 |
| Absolute alcohol | 465 |
| Normal hexane | 470 |
| Chloroform | 472 |
| Carbon tetrachloride | 475 |
| Benzene | 476 |
| Carbon disulfide | 497 |

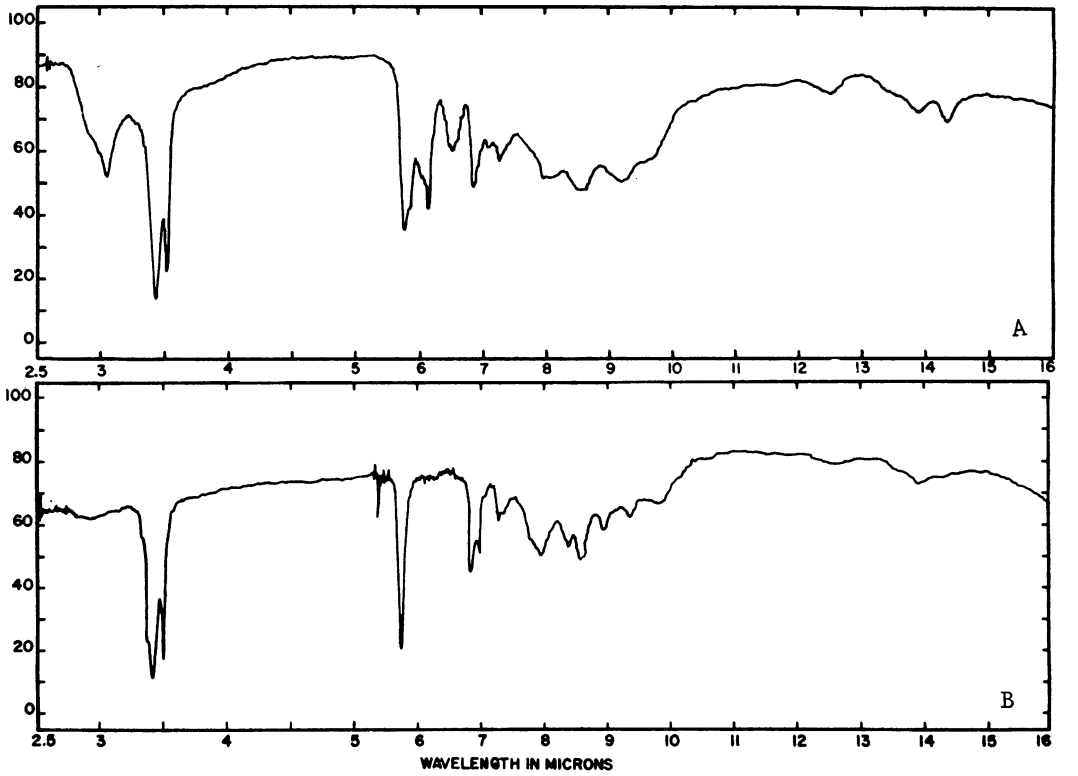


FIG. 1. *(A) Infrared spectrum of the red pigment crystallized from methanol and precipitated from water with 0.1 N HCl; (B) infrared spectrum of the yellow pigment. A was run as a KBr pellet; B, as a smear on NaCl discs.*

filtered, washed with deionized water, dried with anhydrous $Na_2SO_3$, and refiltered. After concentration in partial vacuum, the solution was added, dropwise, into cold acetone in a chilled Morton filter, and a residue was removed by filtration. The pigment was concentrated to dryness, dissolved in benzene, and added to a column of Sea Sorb 43 and Hyflo Super Cel (1:1, w/w). A red component was tightly adsorbed near the top of the column, and the effluent was concentrated to a yellow oil. The oil was rechromatographed as described above; the effluent was concentrated and then dissolved in methanol, and a white precipitate was removed. The red portion of the column was mechanically separated, extracted with methanol, and filtered. A red pigment crystallized from methanol solution.

The absorption maxima of the crystallized red pigment (Table 1) show a single peak in each solvent with a shift toward longer wavelengths in less polar solvents. The purified pigment was also soluble in water, producing a basic *p*H of 9.5 to 10. Upon acidification with 0.1 N HCl at 4 C, the pigment precipitated and was insoluble in distilled water and in 5% $NaHCO_3$, but was dissolved by heating with 10% KOH. The infrared spectrum of the red pigment is shown in Fig. 1A. The yellow oil had a single absorption maximum at 434 m$\mu$. Its infrared spectrum is shown in Fig. 1B.

The red pigment reacted slowly with 2,4-dinitrophenylhydrazine to form a yellow precipitate which was washed three times with 95% ethyl alcohol. This precipitate, presumably a phenylhydrazone, had a melting range of 122 to 124 C and a molecular weight of 264 (Rast), indicating oxidative cleavage of the pigment.

The red pigment was positive for phenolic group, and gave a positive Carr-Price test (P. Karrer and E. Jucker, *Carotenoids*, Elsevier, New York, 1950). No optical rotation was detected in benezene solution. The melting range was 186 to 189 C, and chemical analysis showed a molecular weight of 893: C, 62.6%; H, 9%; N, 5%; and C-methyl group, 3.7%.

The molecular weight, nitrogen content, water solubility, and infrared spectra (L. Bellamy, *The Infrared Spectra of Complex Molecules*, 2nd ed., John Wiley & Sons, New York, 1958) do not substantiate a carotenoid structure. The Carr-Price test may be misleading, since it is not absolutely specific for carotenoids (Karrer and Jucker, *Carotenoids*, Elsevier, New York, 1950). The infrared spectra and phenylhydrazone test give evidence for the carbonyl group, but there is no color change correlated with *p*H change; hence, the carbonyl is not conjugated with the chromophore (G. Turian, Helv. Chim. Acta **33:** 1303, 1950). The data available are consistent with a partial formula of $C_{46}H_{80}N_3$, with an undetermined amount of oxygen present.