

**Pigment of *Bacillus violaceus*.  
II. Pyrrolic Nucleus of Violacein.\***

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A process for the preparation of violacein, the violet pigment of *Bacillus violaceus* (*Chromobacterium violaceum*) has been described.<sup>1, 2</sup> This pigment is of interest since several workers have attributed a respiratory function to it.<sup>3-6</sup> The present paper summarizes the results of a preliminary chemical examination of amorphous violacein.

**Ultimate analysis.** Semi-micro combustions made in duplicate on 4 to 11 mg. samples dried to constant weight in vacuum at 100°C. gave the following percentage values: C 69.08, 69.47, Av. 69.28; H 6.94, 6.63, Av. 6.79; O (by difference) 16.34, 16.59, Av. 16.45; N 4.87, 4.79, Av. 4.83; Ash 2.77, 2.52, Av. 2.65. The ash contained a considerable amount of iron. Due to the low solubility of violacein in all common solvents including melted camphor, no satisfactory value could be obtained for the molecular weight. Other workers<sup>7, 8, 9</sup> have reported higher nitrogen and have overlooked the presence of iron, although in one case ash was reported.<sup>9</sup> There is but little agreement between the values reported by different investigators for percentile composition and molecular weight. Accordingly the constitution of the pigment is still in doubt.

**Solubility.** Although violacein gave intensely violet solutions with many solvents, its solubility was generally very low. Its solubilities as mg. per 100 cc. of saturated solution at 23-25° were: Methyl alcohol 86, 95% ethyl alcohol 99, acetone 76, tertiary butyl

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<sup>1</sup> Tobie, W. C., Ph.D. Thesis, Massachusetts Institute of Technology, 1933 (unpublished), upon which the present paper is partly based.

<sup>2</sup> Tobie, W. C., *J. Bact.*, 1935, **29**, 223.

<sup>3</sup> Ewart, A. J., *J. Linn. Soc. Botan.*, 1897, **33**, 123.

<sup>4</sup> Shibata, K., *Jahrb. wiss. Botan.*, 1912, **51**, 179.

<sup>5</sup> Friedheim, E. A.-H., *Compt. rend. soc. biol.*, 1932, **110**, 353.

<sup>6</sup> Friedheim, E. A.-H., *Arch. sci. phys. nat.*, 1932, **14**, 125.

<sup>7</sup> Reilly, J., and Pyne, G., *Biochem. J.*, 1927, **21**, 1059.

<sup>8</sup> Kögl, F., 1932, *Handbuch der Pflanzenanalyse* (Gustav Klein Ed.), III/2 *Spezielle Analyse* II, 1410.

<sup>9</sup> Wrede, F., and Rothhaas, A., *Z. physiol. Chem.*, 1934, **223**, 113.

alcohol 107, pyridine 389. Exposure of these solutions to strong sunlight caused the discharge of the violet color within a few hours.

*The pyrrolic nucleus of violacein.* An 8 mm. Pyrex tube was drawn to a capillary 12 cm. long, bearing a small bulb with an open end. A plug of asbestos was inserted next to the capillary, followed by a layer of zinc dust and a mixture of 45 to 270 mg. of violacein with 1 gm. of zinc dust. The free end of the tube was then fire-sealed. First the zinc dust and then the zinc dust-violacein mixture was heated with a burner. Water at first collected in the capillary, but at a dull red heat yellow-brown droplets of an oil condensed in the asbestos. These were driven into the capillary and bulb by further heating. The capillary was then sealed off and the oil treated with anhydrous sodium sulphate to absorb the water. The oil darkened rapidly in contact with the air. It was soluble in ethyl ether and petroleum ether, but insoluble in 95% ethyl alcohol. Attempted boiling-point determinations by the Sewaliboff method were inconclusive, due to the rapid carbonization of the oil when heated in contact with the air. A reddish deposit appeared on the thermometer where vapors of the oil came in contact with sulphuric acid fumes from the bath, suggesting the formation of pyrrol red. For convenience in running color-tests, portions of the oil were dissolved in petroleum ether which was allowed to evaporate spontaneously in test tubes. The residue in the tubes gave strong positive tests for a pyrrol as follows: Ehrlich test (p-dimethylamino-benzaldehyde and HCl) vivid pink, vanillin and HCl intense yellow or orange, Hopkins and Cole reaction (glyoxylic acid reagent and sulphuric acid) pink. No picrate or picramate could be obtained from the oil. Similar pyrrolic reduction-products were also obtained by reduction with hydriodic acid, or with zinc dust or sodium hydro-sulphite in NaOH solution. These products also gave strongly positive pyrrolic color-reactions. They showed very little volatility with steam.

*Other properties of violacein.* Reduction of a saturated solution of violacein in glacial acetic acid by warming with coppered granular zinc gave no pyrrol but yielded a pale straw-colored solution. On carefully neutralizing the acid and allowing the mixture to stand overnight, the original violet color reappeared. Apparently mild reduction converts violacein into a leuco base from which it is readily regenerated by the action of atmospheric oxygen. Violacein was stable in buffered alkali up to pH 11, but above that point it dissolved to a pink solution which later became brown. Strong alkali such as NaOH solution decomposed it with liberation of am-

monia. HCl exerted a destructive action on violacein in alcoholic solution. In an attempt to prepare an ash-free preparation, 172 mg. of the pigment was dissolved in 300 cc. of 95% ethyl alcohol and HCl gas was passed in until the violet color had changed to green. On addition of 500 cc. of water, a dark violet precipitate separated from a brown supernatant liquid. After filtration, washing, and drying, the weight of the precipitate was only 97 mg. indicating that over 40% had been destroyed by the HCl. Although the ash was reduced, the percent of nitrogen dropped to 3.43 indicating a partial decomposition of the molecule. By passing an excess of HCl into an alcoholic solution, the violet color was completely destroyed and could not be restored by neutralization.

The production of pyrrolic products by several different methods of reduction indicates that violacein contains one or more pyrrol rings in its molecule. The fact that the pyrrolic products exhibit little volatility with steam and are soluble in ethyl and petroleum ethers but insoluble in ethyl alcohol, suggests that the pyrrolic ring or rings may possess hydrocarbon side-chains. Probably violacein is chemically similar to prodigiosin, the red pigment of *Bacillus prodigiosus* which has been shown to be a tripyrrylmethane derivative.<sup>10</sup> The presence of iron is of possible significance in view of the supposed respiratory function of the pigment, and may indicate an analogy to hemoglobin, chlorophyll, and other metal-pyrrol compounds.

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### Influence of Certain Liver Poisons on Action of Parathyroid Extract.

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In 1932 when practically nothing was known of the mechanism of the action of the parathyroid hormone in raising the blood calcium level, this investigation was undertaken to test the hypothesis that some other tissue, particularly the liver, might be involved as an intermediary in the action of the hormone. Since then Selye,<sup>1</sup>

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<sup>10</sup> Wrede, F., and Rothhaas, A., *Z. physiol. Chem.*, 1933, **222**, 203.

<sup>1</sup> Selye, H., *Arch. Path.*, 1932, **14**, 60; *Endocrinology*, 1932, **10**, 547.