THE CHEMISTRY OF THE LIPIDS OF TUBERCLE BACILLI

XXXV. THE CONSTITUTION OF PHTHIOCOL, THE PIGMENT ISOLATED FROM THE HUMAN TUBERCLE BACILLUS*

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INTRODUCTION

In Paper XXXIV of this series (1) we described the isolation of a pigment from the ether-soluble constituents obtained on saponification of the acetone-soluble fat. The present report deals with the determination of the chemical constitution of the pigment. In order to indicate its origin, we propose to designate the pigment by the name phthiocol.

Phthiocol crystallizes in yellow prisms which melt at 173–174°. It is volatile with steam and soluble in all common organic solvents except petroleum ether. It forms deep red, water-soluble salts with alkalies and other bases. In fact, a red color develops so easily in the presence of alkali that the heated portions of the melting point tubes become red as do also filter papers after filtering solutions of phthiocol.

The elementary composition of phthiocol agrees with the formula C_{11}H_{3}O_{3}. On direct acetylation a light yellow monoacetate is obtained and this compound, on reductive acetylation by refluxing with zinc dust and acetic anhydride, is converted into a colorless triacetate. This indicates a quinone linkage in the original substance and thus the 3 atoms of oxygen are accounted for, I

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as a hydroxyl group, the other 2 as a quinone. The yellow color of phthiocol and its volatility with steam point to an α-quinone rather than to a β-quinone. On oxidation in alkaline solution with hydrogen peroxide phthalic acid was obtained.

We conclude from the above data that phthiocol is 2-methyl-3-hydroxy-1,4-naphthoquinone.

\[ \text{OCH}_2 \text{OH} \]

This formula has been confirmed by synthesis. The synthesis will be reported in a separate publication.

The compound, 2-methyl-3-hydroxy-1,4-naphthoquinone, a metabolic product of the human tubercle bacillus, has not been described heretofore in the chemical literature and apparently has never been isolated previously from a natural product or prepared by synthetic methods. Phthiocol is an analogue of lapachol (2) and an isomer of plumbagin (3).

EXPERIMENTAL

The yellow crystalline pigment, obtained as described in Paper XXXIV (1), weighed 170 mg. It was necessary therefore to carry out our experiments on small quantities of material and to employ micromethods of analysis.

Preparation of Monoacetyl Derivative—The pigment, 34 mg., was dissolved in 4 cc. of pyridine, giving a deep red-colored solution to which, after cooling in ice water, was added 1 cc. of acetic anhydride. The solution became nearly colorless on adding the acetic anhydride, but after standing for a short time a light red color developed. After the solution had stood for 20 hours at room temperature, it was poured into 100 cc. of dilute hydrochloric acid. The mixture turned faintly cloudy, and after a few minutes light yellow-colored needles began to separate. After cooling, the

1 We are indebted to Professor H. T. Clarke of Columbia University for the microanalyses.
crystals were filtered off, washed thoroughly with cold water, and dried in a vacuum desiccator. The yield was 30 mg. The filtrate, which was slightly yellow in color, was extracted with ether. The ethereal solution was washed with water, filtered, and evaporated to dryness; a small amount of a yellow crystalline residue was obtained. This residue was combined with the crystals mentioned above and dissolved in 3 cc. of methyl alcohol. The yellow-colored solution was treated with norit, filtered, and the filter paper was washed with hot methyl alcohol. The norit apparently did not remove any of the coloring matter. The solution was concentrated to a volume of 3 cc. and diluted with 3 cc. of water, whereupon lemon-yellow delicate needles separated. After the mixture had been cooled in ice water for some time, the crystals were collected on a small Buchner funnel, washed with cold 50 per cent methyl alcohol, and dried in vacuo. The crystals weighed 25 mg. and under the microscope they appeared to be homogeneous.

The crystals showed no acid reaction on moist litmus paper, but when dissolved in methyl alcohol, the solution was faintly acid to litmus. An alcoholic solution of the substance gave a bright red color on the addition of potassium hydroxide.

When heated in a capillary tube, the substance melted at 101–102° and on cooling the melt solidified at 86° to a yellow crystalline mass which remelted at 106–107°. The melting points were sharp.

**Analysis**—3.445 mg. substance: 1.36 mg. H₂O, 8.59 mg. CO₂

\[ \text{C}_{11}\text{H}_{18}\text{O}_4\cdot\text{CH}_4\text{CO} \] (230). Calculated. C 67.82, H 4.34

Found. " 68.00, " 4.42

**Molecular Weight**—0.227 mg. of substance in 2.420 mg. of camphor gave a depression of 16.3° of the melting point. Found, mol. wt. 230.

The values found on analysis as well as the molecular weight are in agreement with the calculated composition of a monoacetyl derivative and the results indicate that the pigment contains one hydroxyl group.

**Preparation of Triacetyl Derivative**—The monoacetyl derivative was prepared as just described; 50 mg. of the pigment yielded 60 mg. or 98 per cent of the lemon-yellow-colored crystals. The product was refluxed for 30 minutes with 10 cc. of acetic anhydride, 2 cc. of glacial acetic acid, 0.5 gm. of fused sodium acetate, and 1.5
gm. of zinc dust. The clear colorless solution was allowed to cool and was decanted from the undissolved zinc into 100 cc. of cold water. The zinc dust was rinsed twice with boiling glacial acetic acid and the washings were decanted into the aqueous solution. When the acetic anhydride was decomposed, a compact colorless substance separated and was filtered off, washed with water, and dried in vacuo. The substance weighed 65 mg. Extraction with chloroform removed from the filtrate an additional small quantity of the triacetyl derivative which was combined with the main lot and crystallized from 50 per cent methyl alcohol, yielding 73 mg. of colorless prismatic crystals, m. p. 148°.

The substance was recrystallized from methyl alcohol by adding water; 66 mg. of small colorless prisms were obtained. This preparation has a peculiar melting point, or rather two melting points. When heated in a capillary tube, the substance fuses quite sharply at 148° to an opaque mass which on continued heating becomes clear at 158–159°. The melted substance does not crystallize on cooling but remains as a transparent glassy mass.

Analysis—3.596 mg., 4.034 mg. substance: 1.65 mg., 1.88 mg. H₂O, 8.53 mg., 9.53 mg. CO₂, C₁₅H₂₀O₇(CH₃CO)₃ (316). Calculated. C 64.55, H 5.05

Found. " 64.69, " 5.13
" 64.77, " 5.21

Molecular Weight—0.214 mg. of substance in 2.754 mg. of camphor gave a depression of 10°. Found, mol. wt. 311.

The results of the analyses and the value for the molecular weight are in close agreement with the calculated composition of a triacetyl derivative.

Oxidation of Pigment. Formation of Phthalic Acid—For the oxidation, 50 mg. of the crystalline pigment were dissolved in 5 cc. of 0.1 N NaOH, giving a dark red solution to which were added 2.5 cc. of superoxol (Merck). On standing at room temperature, the color gradually disappeared and after 3 hours the solution was colorless. The solution was acidified with hydrochloric acid and extracted several times with ether. After the ether was distilled off, a nearly colorless crystalline residue was obtained which weighed 35 mg.

The substance was recrystallized from water, yielding colorless
long prismatic crystals. On moist litmus paper the crystals showed a strong acid reaction. The powdered substance when rapidly heated melted with effervescence at 210°. The melt solidified at 110° and remelted at 130-131°. There was no change in the melting points when some of the substance was mixed with phthalic acid. There separated in the cool portion of the melting point tube a colorless crystalline deposit of phthalic anhydride. There was not a sufficient quantity of the purified substance for analysis, but we believe that the melting points given above show that the oxidation product was phthalic acid.

SUMMARY

The yellow crystalline pigment isolated from the acetone-soluble fat of the human tubercle bacillus has been named phthiocol.

The reactions, properties, and derivatives of phthiocol indicate that it is 2-methyl-3-hydroxy-1,4-naphthoquinone.

BIBLIOGRAPHY


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