The Formation in Vitro of (±)-Stercobilin from Bilirubin*

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Stercobilin was first isolated from feces in 1932 (1), and it was soon observed to differ from the crystalline urobilin prepared from mesobilirubinogen (2, 3). This urobilin is optically inactive, hence designated as i-urobilin, in contrast to sterobilin, which was shown by Fischer, Halbach, and Stern, (4, 5) to be levorotatory. Fischer and Halbach also demonstrated a formal relationship of l-stercobilin to the IXα bile pigments (5) by virtue of its oxidation to glaucobilin, which in turn could be reduced to mesobilirubinogen. Although both the latter and i-urobilin yield methylethylmaleimide on CrO₃ oxidation, sterobilin does not (2, 3). Gray and Nicholson (6) have shown more recently that sterobilin, on CrO₃ oxidation, yields methylethylsuccinimide and hematinic acid; thus, Structure I (Fig. 1) is indicated for sterobilin hydrochloride. This structure may be contrasted with Structure II, for i-urobilin, as proven by Siedel and Meier's synthesis (7), shown here in the bislactam form.

Although when l-stercobilin is formed in the intestine by bacterial reduction of bilirubin, the addition of 6 moles of H₂ is required, Fischer and Niemann (8) showed that an alkaline solution of bilirubin, in the presence of colloidal palladium at room temperature, took up only 4 moles of hydrogen to give the leuko compound mesobilirubinogen or i-urobilinogen, which is readily oxidized to the pigment i-urobilin (II).

This report is in accord with recent findings (9) that 2-oxoΔ₂-pyrrolines are not readily hydrogenated under these conditions, and the present work was prompted by the observation (9) that these systems may be hydrogenated under more vigorous conditions.

EXPERIMENTAL PROCEDURE AND RESULTS

Hydrogenation of Bilirubin

Bilirubin (Nutritional Biochemicals Corporation) was recrystallized before being used by extraction from a thimble with hot chloroform, and was dried to constant weight under vacuum at 80°C.

Bilirubin, 100 mg, was suspended in 20 cc of glacial acetic acid and hydrogenated at 1 atmosphere of pressure and at 60°C in the presence of 100 mg of 10% palladium-charcoal catalyst. In three successive runs, the uptake of H₂, corrected to normal temperature and pressure, was 23.5, 22.4, and 23.1 cc (6.12, 5.85, and 6.02 moles), respectively. The colorless solution, after hydrogenation, was diluted with 20 cc of water; 12 cc of 5% aqueous CuSO₄ were added, and the solution was aerated for 2 hours. The catalyst was separated and washed with 20 cc of water and 15 cc of concentrated HCl. The yellow pigment was extracted into chloroform, which was then separated, washed with water, and dried by filtration. Evaporation of the chloroform to approximately 2 cc and addition of hot acetone caused the (±)-sterobilin hydrochloride to be precipitated as an amorphous powder, which was separated, washed with acetone, and dried (15 to 20 mg).

Identification of the Product

1. Analytical and Spectroscopic—The pigment was crystallized during displacement of boiling methanol by ethyl acetate, forming orange plates; slow decomposition >180°C. Elemental analysis upon drying of the crystals in a vacuum at 80°C yielded

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<th>Compound</th>
<th>Calculated</th>
<th>Found</th>
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<tr>
<td>Ca₅H₁₄N₄O₂HCl</td>
<td>C 62.8%, H 7.51%, N 8.88%</td>
<td>C 62.54%, H 7.67%, N 9.12%</td>
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In methanol-10% HCl (weight per volume), 801, the absorption maximum was at 492.2 μm, measured by a Zeiss grating spectrophotometer, and at 462.5 μm, measured by a Beckman DK spectrophotometer.

The amorphous powder precipitated from chloroform by acetone had an infrared spectrum, by the KBr micropellet method (10), identical with natural l-stercobilin hydrochloride. When the powder was crystallized from ethyl acetate-methanol and dried at 80°C in a vacuum, however, an extra band appeared in the carbonyl region (5.8 μ). This spectrum reverted to the previous one on reprecipitation from chloroform-acetone.

Oxidation in methanolic ferric chloride, by the spectroscopic method recently described, to differentiate between the urobilins (11, 12) showed the characteristic ratio and behavior of sterobilin, as contrasted with the i- and d-urobilins.

2. CrO₃ Oxidation—The synthetic material, 53.0 mg, was dissolved in 3 cc of 50% (volume for volume) H₂SO₄ and treated with 2.5 cc of 1.5 N CrO₃. The solution was stirred for 6 hours and then was allowed to stand at room temperature for a further 20 hours. Water, 10 cc, was then added, and the solution was extracted with chloroform, 3 × 5 cc, which was washed (2 × 5% aqueous K₂CO₃, 1 × water) and evaporated to dryness. The oily residue was then dissolved in 0.25 cc of methanol, and the solution was used to spot for chromatography in the system ethanol-water-concentrated ammonium hydroxide (80:16:4, by volume) (6) developed at 5°C for approximately 18 hours. The position of the imides was revealed by exposure to chlorine. The i-urobilin yield methylethylmaleimide, with k₅₀ values of 0.80 and 0.72, respectively, were used as markers. The neutral imide from the oxidative degradation had an k₅₀ of 0.71.

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SUMMARY

Bilirubin, in the presence of a large quantity of 10% palladium-charcoal catalyst in glacial acetic acid at 90° and 1 atmosphere of hydrogen, smoothly takes up 6 moles of hydrogen to give (±)-stercobilinogen. This compound was not isolated but was converted directly to (±)-stercobilin in 15 to 20% over-all yield by aerial oxidation in the presence of cupric sulfate as an oxidative catalyst.

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REFERENCES

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