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Comparative Studies of 'Bile Salts'

3. THE CONVERSION OF PYTHOCHOLIC ACID TO DEOXYCHOLIC ACID

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Pythocholic acid, $C_{24}H_{46}O_5$, from the bile of Boidae was shown, as reported in Part 2 of this series (Haslewood & Wootton, 1951), to be a cholanic acid having three secondary hydroxyl groups substituted probably at C-3, C-12 and C-15 or C-16 in the molecule. Such a substance might be convertible to ordinary deoxycholic acid (3 α :12 α -dihydroxy-cholanic acid), and the present report gives details of successful experiments leading to this end. Opportunity is taken to record an investigation of the bile salts of two more species of Boidae.

RESULTS AND DISCUSSION

The dibenzoate (I) of pythocholic lactone was readily obtained. When this was dissolved in warm dilute ethanolic potassium hydroxide, the lactone ring was opened. The resulting acid was precipitated at once and converted into its methyl ester. Without purification, this was oxidized with chromic oxide and a non-crystalline product, which must have consisted chiefly of (II), was isolated. Treatment of this by the Kishner-Wolff method gave deoxycholic acid (III) in a yield of 71%, calculated on the crystalline dibenzoate. Since cholic acid can be converted to deoxycholic acid without change of configuration and in good yield by essentially similar methods (Gallagher & Long, 1943; Haslewood, 1943; Schneider & Hoehn, 1943), the above experiments leave no reasonable doubt that pythocholic lactone is correctly formulated as a 3 α :12 α -dihydroxy compound; pythocholic acid is therefore almost certainly 3 α :12 α :16(or 15)-trihydroxy-cholanic acid.

Results of an examination of the bile salts of two more species of Boidae are given in Table 1. These figures continue to support the view that pythocholic acid is characteristic of the bile of Boidae.

Table 1. *Approximate amounts of bile acids in two species of Boidae*

(Details as described by Haslewood & Wootton, 1951.)

Species	Pythocholic acid (% of 'bile acids'; approx. minimal figures)	Cholic acid (% of 'bile acids'; approx. minimal figures)
Anaconda, <i>Eunicotes murinus</i>	25	8
Reticulated python, <i>Python reticulatus</i>	48	8

EXPERIMENTAL

General. All melting points are uncorrected. Al_2O_3 was obtained from Hopkin and Williams and neutralized as described by Shoppee (1949). Micro-analysis was done by Drs Weiler and Strauss, Oxford. Optical rotations were determined in a 1 dm. micro tube. 20% CrO_3 = a solution made by dissolving CrO_3 (20 g.) in the minimal amount of water and making to 100 ml. with acetic acid.

Conversion of pythocholic lactone to deoxycholic acid

Pythocholic lactone dibenzoate. The lactone (50 mg.) was dissolved in pyridine (0.5 ml.) with benzoyl chloride (0.2 ml.) and the solution heated at about 90° for 30 min. It was cooled and diluted with excess aqueous HCl. Methanol was now added to a concentration of about 60% (v/v) and the solid which gradually formed was collected, washed with approx. 50% (v/v) methanol-water and crystallized from ethanol. Yield, 45 mg., m.p. 198–202°. This substance was

recrystallized from ethanol, from which it appeared as short white needles.

Pythocholic lactone dibenzoate had m.p. 200–201°. (Found: C, 76.1; H, 8.0. $C_{38}H_{46}O_6$ requires C, 76.3; H, 7.7%.) The substance was recovered unchanged (m.p. 199–201°, not depressed by the starting material) after treatment in the cold with 20% CrO_3 in acetic acid.

bomb was opened it was found that the solvent had evaporated. The white residue was dissolved in water and the solution acidified with HCl. The precipitated acid was collected, washed, dissolved in ethanol and the solution filtered. Evaporation left a residue which with ether gave the deoxycholic acid-ether complex as white needles of m.p. 155–160°. Yield 0.155 g. (71%, calculated on the weight of

Conversion of the dibenzoate to a product containing (II). The dibenzoate (0.33 g.) was dissolved in ethanol (33 ml.) and treated at about 60° with KOH (3.3 ml. of an approx. 40% (w/v) aqueous solution). As soon as the alkali had been added, the flask was shaken and the mixture at once diluted with 2–3 vol. of cold water. The solution, which had remained clear, was acidified immediately with aqueous HCl. The flocculent white precipitate was collected as quickly as possible on a filter, washed with water and dissolved in ethanol. This was saturated with diazomethane, carried over in N_2 from decomposing *N*-nitrosomethylurea (1 g.). The solution was evaporated on the water pump. The gummy residue, which showed no tendency to crystallize with organic solvents, was dissolved in acetic acid (4 ml.) at 25° and treated with 20% CrO_3 (0.4 ml.). After 15 min. at 25° with occasional shaking, the solution was diluted with water, and when separation of a solid seemed complete, this was collected and washed with water. This product showed no tendency to crystallize. It was dried by evaporation with ethanol, dissolved in benzene and the solution run on to Al_2O_3 (3.5 g.) in a column. Benzene (140 ml.) eluted the whole of the material (0.33 g.).

Deoxycholic acid from (II). The above material (0.33 g.) was transferred with ether to a small metal bomb. After evaporation of the ether, the residue was dissolved in ethanol (5 ml.) in which was dissolved sodium (0.3 g.) and hydrazine hydrate (0.5 ml. of approx. 95%). The bomb was sealed and heated at 200–210° for 4 hr. When the cooled

dibenzoate used). From acetic acid, this substance gave the deoxycholic-acetic acid complex as typical white needles, m.p. 139–141°, not depressed by authentic material prepared from ox-bile. $[\alpha]_D^{19} = +45 \pm 1^\circ$ in ethanol (*c*, 1.0). The product (50 mg.) of m.p. 139–141° was oxidized in the usual way with CrO_3 to give dehydrodeoxycholic acid, crystallizing from dilute ethanol as typical glistening leaflets of m.p. 177–179°, not depressed by authentic material. $[\alpha]_D^{19} = 87^\circ \pm 2^\circ$ in ethanol (*c*, 0.7).

Methyl pythocholate, pythocholic lactone and cholic acid from Eunicotes murinus and Python reticulatus

These were prepared exactly as described in Part 2 (Haslewood & Wootton, 1951) for other species of Boidae. No unexpected difficulties were encountered.

SUMMARY

1. Pythocholic lactone dibenzoate was prepared. When this was dissolved in ethanolic alkali, the lactone ring was opened without significant hydrolysis of benzoyl groups. Re-lactonization was prevented by methylation of the carboxyl group, and the free hydroxyl group was then oxidized to

carbonyl by chromium trioxide. Kishner-Wolff reduction of the non-crystalline product gave deoxycholic acid in good yield. Pythocholic acid is therefore almost certainly 3 α :12 α :16(or 15)-tri-hydroxycholanic acid.

2. The bile salts of two more species of Boidae have been examined. Both contained pythocholic acid and smaller amounts of cholic acid.

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