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Synthesis of the Tritiated 25-Methyl Homologues of 3α,7α,12α-Trihydroxy Coprostane and Coprocholic Acid

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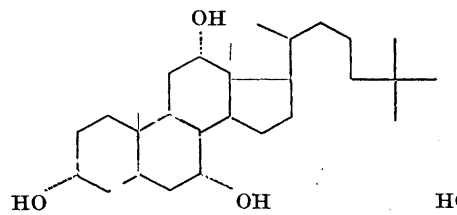
The compounds have been prepared through electrolysis of tritiated cholic acid together with *tert.* butyl acetic acid, respectively the monoester of 2,2-dimethylsuccinic acid.

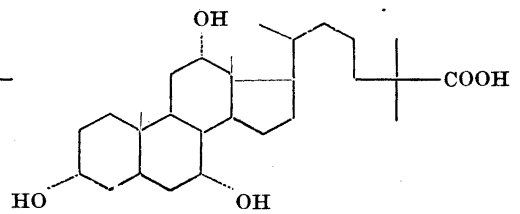
Previous work at this institute has shown that the hydroxylating enzyme systems involved in the transformation of cholesterol into bile acids have very exacting requirements as far as the structure of the side chain is concerned.

A 12α-hydroxyl is introduced resulting in the formation of cholic acid when labelled cholesterol (*cf.* Summary [1]), 3α,7α-dihydroxycoprostane [2] or 7α-hydroxycholesterol [3] is administered. If the side chain contains a carboxyl at the end of the C_5-cholanic or the C_8-"coprocholanic" side chain, however, the molecule is hydroxylated at other positions. Furthermore the 7α-hydroxylating enzyme system present in rat liver microsomes that transforms taurodeoxycholic acid into taurocholic acid does not work on free deoxycholic acid [1] *in vitro*.

As a step in the investigation of the influence of the side chain structure on the hydroxylating system and on the taurine conjugating system and also as a useful substrate in studying the ω- or methyloxidation we have prepared 3α,7α,12α-trihydroxy-25-methylcoprostane (I), *i.e.* a coprostane with the stable cholic acid structure in the ring system and a tertiary butyl group at the end of the side chain, thus blocking any β-oxidation after a possible ω-oxidation.



I



II

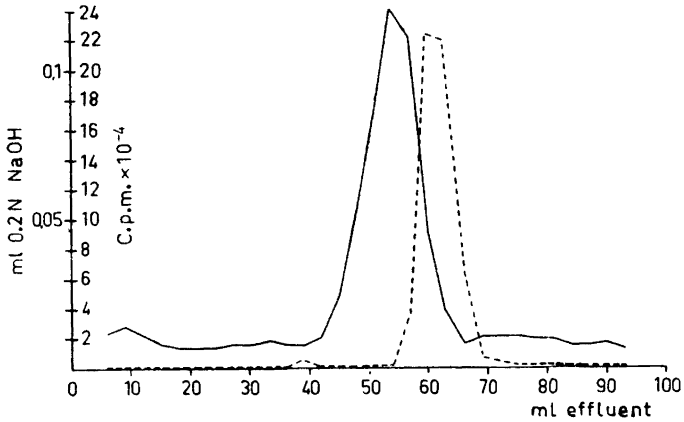


Fig. 1. Reversed phase partition chromatography of deoxycholic acid (solid line) and [T]-25-methyl-coprocholic acid (<0.1 mg) (broken line, cpm) in 55 % aqueous methanol/chloroform-heptane 9:1, *cf.* Ref.[1]

We have also prepared the corresponding possible metabolite that would be formed if one of the terminal methyl groups was oxidized to a carboxyl, *i.e.* 3α,7α,12α-trihydroxy-25-methylcoprocholanic acid (II).

The preparation of these two compounds (I and II) from tritium labelled cholic acid is described in this publication. Some data on the metabolism of these compounds are given in a separate communication.

EXPERIMENTAL

3,7,12-Trihydroxy-25-methylcoprostanic acid (25-methyl-coprocholic acid). Tritiated cholic acid (250.8 mg) was dissolved in methanol (50 ml) and 2,2-dimethyl-succinic acid mono-methyl ester (tertiary) (2.33 g) and sodium (35 mg in one ml of methanol) was added.

The solution was electrolyzed as described earlier [4] until a pH of about 7.5 had been reached. The solution was then taken to dryness *in vacuo* and the residue distributed between ether and water. The ether phase was washed neutral with carbonate and brought to dryness leaving a residue of 884 mg.

The crude product was dissolved in methylene chloride and chromatographed on a column of silicic acid (dried at 120° for 24 h) and Hyflo Supercel. The material eluted with 6 % methanol in methylene chloride. The centre of the material eluted with this solvent was saponified in 2 N potassium hydroxide for 4 h at 130°.

Of the free acid recovered (129 mg) 70 mg was subjected to reversed phase partition chromatography on hydrophobic Kieselguhr (18 g) with 60 % aqueous methanol as mobile phase and chloroform-heptane as stationary phase [5].

The well defined peak between 100 and 180 ml effluent was combined and crystallized from aqueous acetone. The product melted at 224—226°C. (Found: C 72.8; H 10.5. Calc. for $C_{28}H_{48}O_5$: C 72.4; H 10.4).

A sample of the acid was also run with deoxycholic acid in a system of 55 % aqueous methanol and chloroform-heptane 9:1.

As seen in Fig. 1 the peak of the labelled material appeared at 60—65 ml effluing slightly after the deoxycholic acid (55 ml) whereas coprocholic acid has been found to appear at 40—45 ml and cholic acid at 20—25.

The addition of three carbons to the side chain of cholic acid thus does not quite counterbalance the effect of one hydroxyl in this system, whereas the further addition of one methyl group makes the acid less polar than deoxycholic acid.

3,7,12-Trihydroxy-25-methylcoprostane. Tritiated cholic acid (50 mg) was dissolved in methanol (5 ml). Tertiary butylacetic acid (316 mg) was added together with sodium methylate (7 mg). The platinum electrodes were 5×7 mm at a distance of 2 mm from each other and the electrolysis was run as indicated above. The neutral product was chromatographed on silicic acid and the material eluted with methylene chloride containing 2 % of methanol, combined and recrystallized from aqueous acetone yielding 14 mg of a product melting at 203°. (Found: C 77.3; H 11.7. Calc. for $C_{28}H_{50}O_3$: C 77.4; H 11.6).

We are very grateful to Dr. S. Lindstedt for supplying us with cholic acid labelled with tritium through catalytic exchange essentially according to the method of Trenner *et al.*[6]

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