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On the Metabolism of 3α, 7α, 12α-Trihydroxycoprostanic Acid in the Rat *

Bile Acids and Steroids 44

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Tritium labelled D- and L-3α,7α,12α-trihydroxycoprostanic acids have been prepared. They have been found to be rapidly degraded to cholic acid in the rat liver, only a minor amount appearing in the bile as the taurine conjugate of the injected acid.

Bile acids with 27 carbon atoms have been found in various animals. (For a review see Haslewood [1]) 3α, 7α, 12α-25-L-trihydroxycoprostanic acid (II) has been isolated from the bile of *Alligator mississippiensis* [2] and also from the bile of *Rana nigromaculata nigromaculata* [3]. From the bile of this latter species the isomeric 3α, 7α, 12α-25-D-trihydroxy-coprostanic acid has also been isolated [3]. Both isomers have also been isolated previously from the bile of *Rana catesbiana* [4,5].

Tetrahydroxybinorsterocholanic acid (27 carbon atoms) has also been isolated from the bile of the domestic chicken [6].

Both the 25-D- and the 25-L-3α, 7α, 12α-trihydroxycoprostanic acids have been prepared by partial synthesis [7]. Haslewood has put forward the theory that the C-27 bile acids represent a primitive stage in the evolutionary chain leading up to the C-24 acids of many animals.

As the bile acids of mammals are end products of cholesterol metabolism one could expect a hydroxylated coprostanic acid to be an intermediate in the

* A preliminary report of this work was read at the Meeting of the Norwegian Biochemical Society in June, 1956 [18].

conversion of cholesterol into bile acids. This would also be in accord with the view that hydroxylations in the steroid nucleus might precede the total degradation of the side chain [8].

EXPERIMENTAL

L- and D-Trihydroxycoprostanic acids were prepared by anodic coupling of cholic acid with D- and L-methylhydrogen-a-methyl glutarate, respectively, followed by degradation of the resulting acids by the Barbier-Wieland procedure.

The sodium salts of the acids were tritiated by a platinum-catalyzed exchange with tritium water at 120° for four days [6]. The acids were then purified by chromatography with phase system F (see below) and crystallized. The L-trihydroxycoprostanic acid melted at 194—196° and had a specific activity of 2.2×10^5 c.p.m. when counted in an "infinitely thin" layer in a Tracerlab gas-flow counter. The D-form melted at 185° and had a specific activity of 5×10^5 c.p.m.

Bile fistulas were made on white rats weighing about 200 g. The bile was collected in 50 % ethanol. The conjugated bile acids were extracted with n-butanol after acidification to pH 1. Saponifications were carried out in sealed glass tubes in 2 N sodium hydroxide at 120° for 12 h, and the free acids extracted with ether after acidification.

The reversed-phase chromatographic technique worked out by Bergström, Sjövall and Norman [10-12] was used for separation of the bile acids. The following solvent systems were used.

System	Moving phase	Stationary phase
C (Ref.[12])	methanol / water 50/50	chloroform / "*iso*octanol" 50/50
D (Ref.[12])	water	butanol
F (Ref.[11])	methanol / water 55/45	chloroform / heptane 90/10

Each fraction from the chromatogram was titrated with 0.02 N sodium hydroxide, and an aliquot plated in an "infinitely thin" layer on an aluminium or copper planchet. These were then counted in a Tracerlab gas-flow counter. The specific activity of the isolated cholic acid was determined after combustion and conversion into tritiobutane by the methods of Glascock [13].

RESULTS

Excretion and conjugation. L-Trihydroxycoprostanic acid (1—3 mg) was neutralized with sodium hydroxide and injected intraperitoneally into a rat with a bile fistula. About 70 % of the administered activity was excreted within 24 h. The bile acids were extracted and chromatographed with phase system D. The resulting chromatogram is seen in Fig. 1. The first activity peak coincides with the titration peak caused by taurocholic acid at 10—20 ml. The second activity peak at 25—35 ml appears at the position of taurochenodeoxy acid. In this system, however, taurine-conjugated trihydroxycoprostanic acid moves as a taurine-conjugated dihydroxycholanic acid. Any free trihydroxycoprostanic acid that might occur would have remained in the stationary phase. The column was eluted and the material rerun with phase system F. No sign of free trihydroxycoprostanic acid was found. The second peak in Fig. 1 was saponified and rerun with unlabelled trihydroxycoprostanic acid with phase system F. The titration and activity peaks coincided.

Identification of cholic acid. A bile sample from a rat that had received L-trihydroxycoprostanic acid was saponified and run on a chromatogram with phase system C. An activity peak coinciding with the titration peak caused

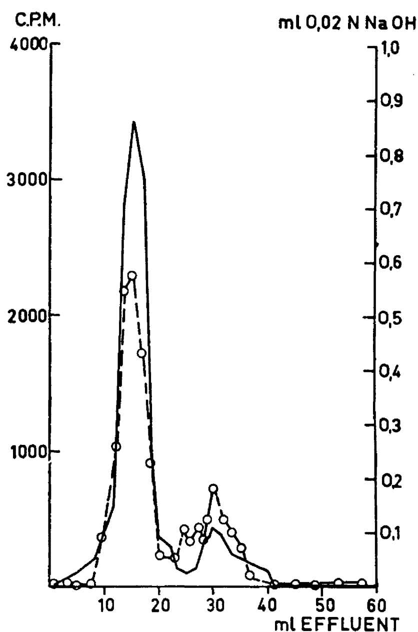


Fig. 1. Chromatogram of conjugated bile acids after administration of tritium-labelled L-trihydroxycoprostanic acid. Column: 4.5 g of hydrophobic kieselguhr. Phase system D. Solid line: titration values. Broken line: isotope determinations.

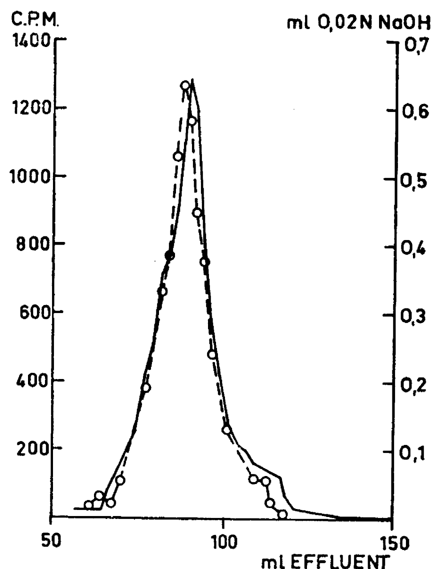


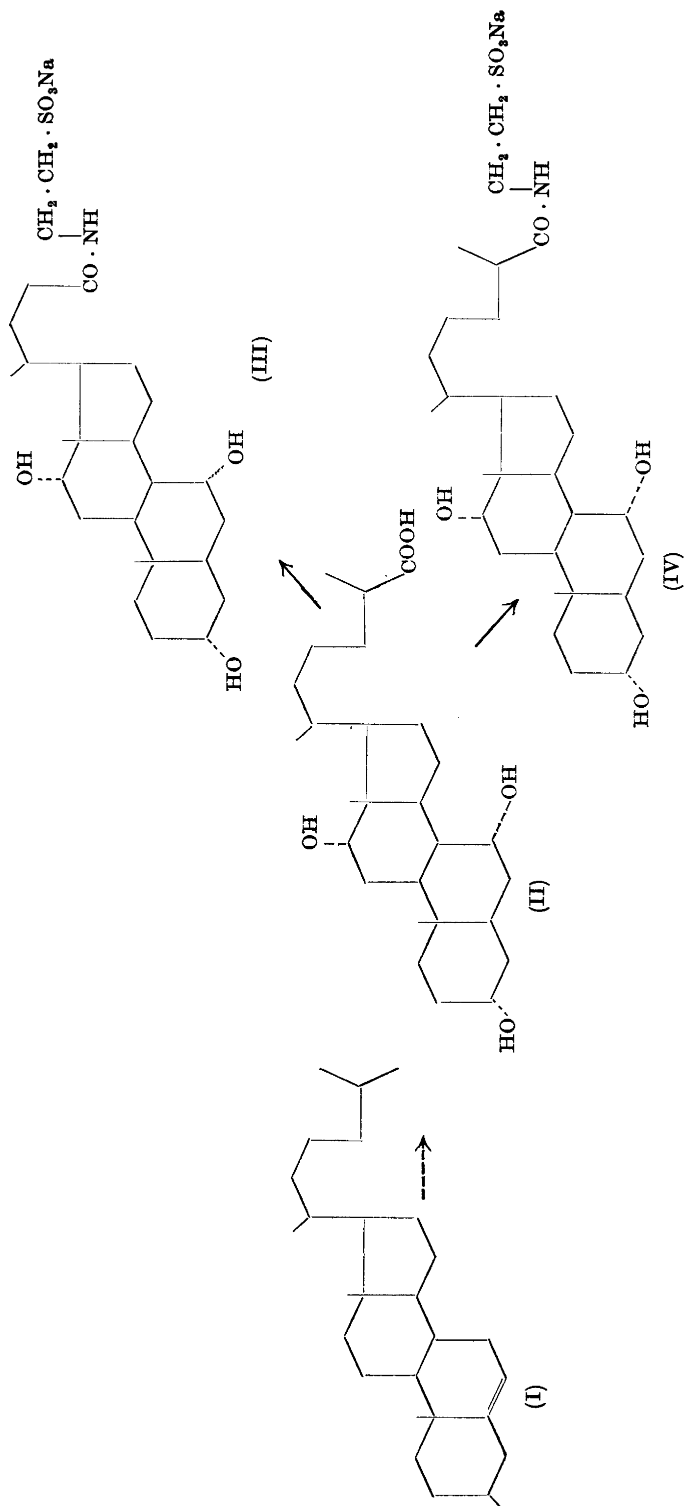
Fig. 2. Chromatogram of cholic acid after administration of tritium-labelled L-trihydroxycoprostanic acid. Column: 4.5 g of hydrophobic kieselguhr. Phase system C. Solid line: titration values. Broken line: isotope determinations.

by cholic acid was found (Fig. 2). The acid isolated from the combined fractions (60—120 ml effluent, *cf.* Fig. 2) was diluted with carrier cholic acid and the material recrystallized five times from different solvents (EtOH/aq, acetone/aq, EtOAc). Duplicate determinations of specific activity were carried out on material from the last four crystallizations. This specific activity was found to remain constant.

D-*Trihydroxycoprostanic acid.* When the D-form was given to a rat with a bile fistula and the bile acids fractionated, results identical with those described for the L-form were obtained.

DISCUSSION

As a result of earlier investigations it would appear probable that the first steps in the conversion of cholesterol into bile acids in mammals are hydroxylations in the steroid nucleus, which are then followed by the degradation of the side chain, the latter proceeding at a rapid rate. It has thus been shown that both 3α, 7α, 12α-trihydroxycoprostane, 3α,12α-dihydroxycoprostane and 3α,


(I)
(II)
(III)
(IV)
COOH
CO · NH
CH₂ · CH₂ · SO₃Na
OH
HO

7α-dihydroxycoprostane give rise to bile acids more rapidly than cholesterol itself[14–16].

It is assumed that the degradation of the side chain starts with a methyl-oxidation followed by a β-oxidation. This latter oxidation is known to be unhindered by the presence of a methyl group in the α-position (*cf.* Tryding and Westöö[17]). The present investigation clearly shows the rapid rate of the β-oxidation. It is also evident that the oxidation does not proceed beyond the cholic acid stage, the enzymatic reaction probably being blocked by the bulky steroid nucleus. It is of interest to note that the steric configuration at carbon atom 25 is of no importance, both stereoisomers being attacked to approximately the same extent.

Although no C-27 bile acids have been found in mammals they may appear as short-lived intermediates in the conversion of cholesterol(I) into bile acids.

The trihydroxycoprostanic acid (II) that was not degraded to cholic acid (III) appeared as a taurine-conjugate (IV), no trace of glycine-conjugate or free acid being found. Since both the conjugation[19,20] and the β-oxidation presumably proceed *via* a CoA-compound there is apparently a competition between the dehydrogenation and the conjugation resulting in a preponderance of the products of the former reaction.

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