ACTA CHEMICA SCANDINAVICA 11 (1957) 836—838

On the Conversion of 3α,7α,12α-Trihydroxycoprostanic Acid to Cholic Acid in Rat Liver Homogenates

Bile Acids and Steroids 46

S. BERGSTRÖM, R. J. BRIDGWATER and U. GLOOR

Department of Physiological Chemistry, University of Lund, Lund, Sweden

With tritium labelled 3α,7α,12α-trihydroxycoprostanic acid it has been found that the main reactions in a rat liver homogenate are the degradation to cholic acid followed by the conjugation with taurine. A minor part of the C_{27} acid was directly conjugated with taurine.

Recently Haslewood [1] isolated 3α,7α,12α-trihydroxycoprostanic acid (»coprocholic acid») from various members of the *Crocodylidæ* and it has also been found in certain species of *Amphibia* (see Ref.[1]). The two coprocholic acids stereoisomeric at C—25 have been prepared by Bridgwater [11]. These C_{27}-bile acids with the intact cholesterol carbon skeleton might be intermediates in the conversion of cholesterol into C_{24} bile acids in higher animals.

This would be in agreement with the suggestion that hydroxylation of the steroid nucleus might precede the β-oxydation of the side chain [2,3]. In connection with work done at this institute on the *in vivo* metabolism of 3α,7α,12α-trihydroxycoprostanic acid [4] we have also studied the reactions of this substance in rat liver homogenates.

EXPERIMENTAL

Tritium labelled L-3α,7α,12α-trihydroxycoprostanic acid was material prepared by Bridgwater and Lindstedt [4]. The radioactivity was 2.2×10^6 counts per min per mg counted in infinite thin layer in a windowless gas flow counter. Directly before use, the substance was purified twice by reversed phase partition chromatography in a system with chloroform/heptane 9:1 as stationary phase and 50 % methanol as moving phase [5-7]. The peak of the titration curve was at 65—70 ml effluent on the standard 4.5 g column at 23°.

Rat liver homogenates were prepared in 4 parts of Bucher medium [8] per part liver fresh weight as described earlier [9]. Unbroken cells and cell debris were spun down at 800 × g for 15 min at 0° and the supernatant used as total homogenate. In a typical experiment we used the following conditions: 38 ml 20 % rat liver homogenate and 2 ml buffer containing 40 mg adenosine triphosphate (crystalline, disodium salt, 3 H_2O,

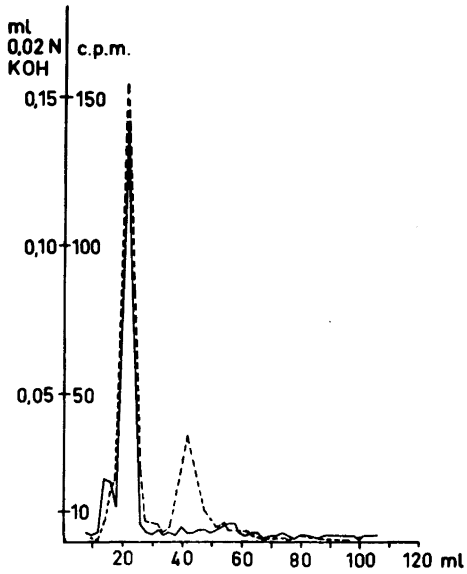

Fig. 1. Chromatography of the hydrolyzed taurine conjugates after incubating [T]-coprocholic acid with rat liver homogenates. Phase system F. Solid line: Titration values. Broken line: c.p.m. Absc. = ml effluent.

Sigma Chemical Corp., St. Louis, U.S.A.). 500 μg trihydroxycoprostanic acid sodium salt = 1.1×10^6 c.p.m. The incubation mixture was shaken in air for 2 h at 37°, pH 7.4 and at the end of this time 5 volumes ethanol were added to stop the reactions. The mixture was heated for 10 min in boiling water, cooled, the precipitate centrifuged off and the supernatant liquid evaporated under reduced pressure. The residue was partitioned between 1 N HCl and butanol and the combined butanol extracts again evaporated under reduced pressure. This gave the starting material for reversed phase column partition chromatography as worked out by Bergström, Sjövall and Norman [5-7]. The following solvent systems were used (Table 1).

Table 1. Solvent systems.

System	Stationary phase	Moving phase
C (Ref.[7])	chloroform / *iso*octanol 1:1	50 % methanol / water
D (Ref.[7])	butanol	water
F (Ref.[6])	chloroform / heptane 9:1	55 % methanol / water

Determinations of the radioactivity were done in "infinitely thin" layers on frosted aluminum or copper planchets in a windowless gas flow counter (Tracerlab).

After each experiment the extracts were first chromatographed with phase system D to separate the taurine conjugated acids from all distinctly less polar products, which stay in the stationary phase. The latter were then fractionated with phase system F. The taurine conjugated acids were saponified with 1 N NaOH for 8 h in sealed glass tubes at 120 °C, the acidified reaction mixture extracted with ether, washed, evaporated and chromatographed in phase system C together with unlabelled cholic acid.

RESULTS

In a typical experiment about 83 % of the activity was recovered in the taurine band (Phase system D). These fractions were combined and saponified and the isolated acids were rerun in phase system F together with unlabelled cholic acid. About 70 % were recovered in the cholic acid band near the front followed by a second band at 40—45 ml containing about 20 % *cf.* Fig. 1. When the material in the first band was rerun in system C the activity curve coincided with the titration curve of cholic acid. The identity of the labelled product with cholic acid was further strengthened by dilution with 100 mg unlabelled cholic acid and recrystallization four times from different solvents (aqueous ethanol, acetone, ethyl acetate) when the specific activity remained constant throughout the procedure.

The second band appeared at the same place as the coprocholic acid used. No unlabelled acid was available at that time for isotope dilution.

In vitro as *in vivo* the coprocholic acid was thus rapidly degraded to cholic acid that was conjugated with taurine. A minor part of the coprocholic acid was directly conjugated with taurine and thus protected from the degradation as has also been found *in vivo* [10].

The eluate of the butanol column, *i. e.* the acids that stayed on the column in this system, representing 17 % of the total activity, was rerun in phase system F when three distinct bands were obtained, with roughly equal isotope content, appearing at (a) 15, (b) 35, and (c) 75 ml. These minor peaks have not been identified with certainty but (b) follows coprocholic acid also in phase system C and might thus be unchanged coprocholic acid that has escaped conjugation, (a) is eluted before cholic acid in system C (glycocholic acid?). The structure of (c) is unknown. These compounds were not observed in the bile when labelled coprocholic acid had been injected intraperitoneally into bile fistula rats [4].

The investigation of the location of the various enzyme systems involved in the different fractions of the liver homogenate is being continued.

This work is part of investigations supported by *Statens medicinska forskningsråd* and *Knut och Alice Wallenbergs Stiftelse.*

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Received February 11, 1957.