ACTA CHEMICA SCANDINAVICA 16 (1962) 1765–1769

On the Extent of the Conversion of Scymnol to Cholic Acid in the Rat

Bile Acids and Steroids 120

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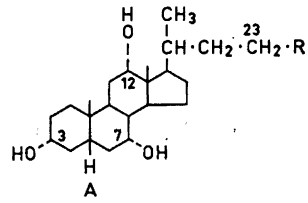
After intraperiotoneal administration of tritium labeled scymnol (VII) to bile fistula rats about 90 % of the radioactivity was excreted in bile during the first 24 h. Scymnol and two compounds which upon alkaline hydrolysis yielded material with chromatographic properties similar to those of anhydroscymnol, were the main metabolites. Cholic acid (I) accounted for only 0.5 % of the radioactivity excreted in bile. The bearing of these results on the theory that the mechanism of conversion of cholesterol to cholic acid in mammals represents a recapitulation of the evolution of bile salts is discussed.

The elucidation of the structure of bile alcohols and bile acids in different species has revealed that the composition of bile salts in the bile of an animal can often be correlated with its position in the evolutionary series [1,2]. Thus, bile alcohols and acids with 27 carbon atoms are found in more primitive animals, *e.g.* sharks, amphibians and certain reptiles, while acids with 24 carbon atoms are typical for the "modern" vertebrates, such as most bony fishes, snakes, birds and mammals. Cholesterol is the precursor of C_{27} as well as C_{24} bile salts, and it has been proposed that the mechanism of the conversion of cholesterol to C_{24} bile acids in mammals would entail the intermediary formation of C_{27} steroids similar to or the same as those found in the bile of lower species.

Studies on the mechanism of formation of cholic acid (I) in the rat have shown that the steroid nucleus of cholesterol is modified prior to the final oxidation of the side-chain and that 3α,7α,12α-trihydroxycoprostane (II) and the corresponding coprostanic acid (III), an acid found in the bile of amphi-



(I) A, R = $-{}^{24}COOH$

(II) A, R = $-{}^{24}CH_2\cdot{}^{25}CH(^{26}CH_3)(^{27}CH_3)$

(III) A, R = $-CH_2\cdot CH(COOH)(CH_3)$

(IV) A, R = $-CH_2\cdot CH(CH_2OH)(CH_3)$

(V) A, R = $-CH(O-CH_2)\cdot CH\cdot CH_2OH$

(VI) A, R = $-CHOH\cdot CH(CH_2O\cdot SO_3^-)(CH_2OH)$

(VII) A, R = $-{}^{24}CHOH\cdot CH(CH_2OH)(CH_2OH)$

bians and crocodiles, are rapidly degraded to cholic acid [3]. The conversion of cholesterol to these two compounds has, however, not yet been demonstrated in the rat [3]. The formation of trihydroxycoprostanic acid from trihydroxycoprostane has been shown to occur in rat and mouse liver *in vitro* [4] and the reaction involves the introduction of a hydroxyl group at C_{26} to yield the tetrol (IV) which is subsequently oxidized to the acid (III). It has been suggested that these changes represent the initial steps in the oxidation of the C_{27} side-chain. The later steps in the transformation of trihydroxycoprostanic to cholic acid have not been fully elucidated, but it appears likely that a β-oxidation occurs, splitting the side-chain between C_{24} and C_{25} yielding cholyl-CoA and propionyl-CoA [3–6].

In 1930, Windaus *et al.*[7] suggested that "scymnol", a C_{27} steroid alcohol obtained from the bile of sharks and carps and originally isolated by Hammarsten [8] in 1898, could be an intermediate in the conversion of cholesterol to cholic acid in mammals. However, as shown by Thannhauser [9], administration of a sample of Windaus' "scymnol" did not cause an increased formation of cholic acid in the bile fistula dog.

Structure (V) for "scymnol" was finally established [10], and it was further shown that this substance is an artefact, being formed during alkaline hydrolysis from the sulphate (VI) present in biles [11]. Because of this, it has been agreed [10,11] to rename the substance (V) "anhydroscymnol" and to transfer the name "scymnol" to the native bile alcohol (VII), which is the object of study now reported. Scymnol (VII) has been prepared from shark bile salts and also made by partial synthesis from cholic acid [11].

The structure of scymnol does not fit into the above formulated mechanism for the oxidation of the side-chain in the formation of C_{24} bile acids. Thus, if scymnol were a precursor of cholic acid in mammals, this would indicate the presence of an alternative and quite different pathway in the side-chain oxidation. The existence of such a pathway would be inconsistent with the hypothesis that the conversion of cholesterol to cholic acid in mam-

mals is a recapitulation of the evolution of bile salts, for selachians phylogenetically represent a development of an early side-branch of the path of evolution leading to mammals.

The partial synthesis of scymnol has made it possible to study accurately its metabolism. This communication is a report on the metabolism of randomly tritium labeled scymnol in the bile fistula rat.

EXPERIMENTAL

A sample of partially synthetic scymnol [11] was exposed to 2 C of tritium gas for 3 weeks according to the method of Wilzbach [12] in the apparatus described by Bergström and Lindstedt [13]. The sample was then treated with 5 % (w/v) KOH in methanol for 2 h at room temperature. The solution was acidified with HCl, extracted with water-saturated butanol and the butanol extract washed with water until neutral. After evaporation of the butanol the residue was chromatographed twice on a column of hydrophobic kieselguhr with solvent system C:3 [14]. The main radioactive peak was pooled, unlabeled scymnol added, and the mixture was again chromatographed with system C:3. The radioactive peak was eluted at the same place as the carrier added. The labeled scymnol obtained had a specific activity of ~8 μC/mg.

4–8 μC of tritium labeled scymnol was injected intraperitoneally as serum albumin stabilized emulsions [4] into bile fistula rats. Bile was collected in 24 h portions. The bile was acidified with HCl and extracted with water-saturated butanol. The butanol extracts were washed with water until neutral and evaporated. The residue was chromatographed with solvent system D[15]. Bile and chromatographically isolated fractions were hydrolyzed with 2 N NaOH at 120° in a sealed steel tube for 14 h. The saponification mixtures were acidified and extracted with butanol. The residues of the butanol extracts were chromatographed first with solvent system C:1 [14] and then with system C:3.

RESULTS AND DISCUSSION

After intraperitoneal administration of tritium labeled scymnol about 90 % of the radioactivity was excreted in bile in 24 h. No significant amounts of radioactivity were excreted in bile during the following days or in the urine collected during the first two days.

Chromatography with system C:1 of hydrolysed bile obtained during the first 24 h showed that only 5 % of the radioactivity was eluted within the titration peak of cholic acid, while most of the remaining radioactivity appeared considerably earlier, with elution volume similar to that of scymnol and anhydroscymnol, which are eluted close to each other with this solvent system. As scymnol sulphate upon vigorous alkaline hydrolysis is converted into anhydroscymnol, as mentioned earlier, it is obvious that chromatography of hydrolysed bile does not distinguish between free and conjugated scymnol excreted in bile.

In order to obtain information on this point, unhydrolysed bile, collected during the first 24 h after injection of labeled scymnol, was chromatographed with solvent system D (Fig. 1). About 50 % of the radioactivity put on the column was retained in the stationary phase. Rechromatography of this labeled material, together with unlabeled scymnol, in system C:3 showed that the peaks of radioactivity and added carrier coincided.

As shown in Fig. 1, the radioactivity in the effluent of the column was distributed in several peaks appearing in connection with the two main titra-

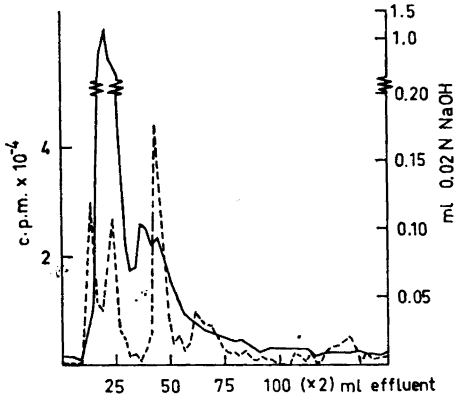


Fig. 1. Chromatography of the first 24 h portion of unhydrolysed bile after intraperitoneal administration of 8 μC of tritium labeled scymnol. Column: 9 g hydrophobic kieselguhr. Phase system D. Solid line: titration values. Broken line: radioactivity.

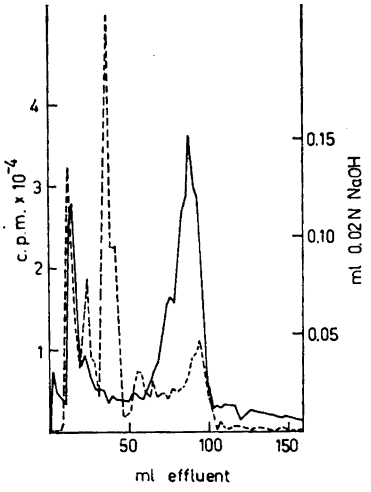


Fig. 2. Chromatography of hydrolysed taurocholic acid band, 13—30 ml effluent, Fig. 1. Column: 4.5 g hydrophobic kieselguhr. Phase system C:1. Solid line: titration values. Broken line: radioactivity.

tion peaks caused by taurocholic (13—30 ml effluent) and taurochenodeoxycholic acid (34—60 ml effluent). The taurocholic acid band was pooled, hydrolysed and chromatographed on phase system C:1 (Fig. 2). The main part of the radioactivity was eluted as three distinct peaks, more polar than cholic acid while about 25 %, which corresponds to 5 % of the total radioactivity excreted in the bile, appeared within the titration peak of cholic acid. The fractions containing the cholic acid were combined and after addition of unlabeled cholic acid crystallized repeatedly from ethyl acetate. The specific activity reached a constant value after 5 crystallizations. The radioactivity thus identified as cholic acid represents 0.5 % of the total radioactivity excreted in bile. This must be considered a minimum value, as it can be expected that tritium labeled scymnol on conversion to cholic acid loses a part of the tritium located in the side-chain. However, as the amount of radioactivity excreted in bile nearly equals that administered, this loss of tritium cannot have had a very pronounced influence on the order of magnitude found for the conversion of scymnol to cholic acid.

The isotope eluted between 30 and 50 ml effluent (Fig. 2) showed chromatographic properties similar to those of anhydroscymnol when rechromatographed with system C:3, but the identity of the radioactive compound with anhydroscymnol was not further established. The preceding two radioactive peaks were not identified.

Chromatography of the hydrolysed taurochenodeoxycholic acid fraction (*cf.* Fig. 1) demonstrated that about one third of the radioactivity in this

fraction had chromatographic behaviour similar to that of anhydroscymnol while the remainder consisted of two unknown compounds more polar than anhydroscymnol.

The results obtained thus show that the main part, about 70 %, of intraperitoneally administered scymnol was excreted in bile as scymnol and two compounds that yielded material chromatographically similar to anhydroscymnol upon alkaline hydrolysis. The original structure of the latter two compounds is not known but it appears likely that they represent conjugates of scymnol with, *e.g.*, sulfuric acid, as they are much more polar than scymnol. Cholic acid accounted for only 0.5 % of the radioactivity excreted in bile, while the remaining 30 % was distributed chiefly in two uknown compounds more polar than scymnol before as well as after hydrolysis.

The very poor conversion of scymnol to cholic acid by rat liver can be considered as evidence in favour of the hypothesis that the conversion of cholesterol to cholic acid in mammals follows a pathway, which does not include scymnol, and may be different from that leading to cholic acid in most teleostean fishes. This view accords with what seems obvious from the natural distribution of cholic acid: that this substance has been produced at least twice during evolution [11].

Acknowledgements. The skilful technical assistance of Miss C. Carlon is gratefully acknowledged.

This work is part of investigations supported by the *National Institutes of Health*, Bethesda, Md., USA (Research Grants H-2842 and A-4303 and Research Fellowship HF 7600).

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Received March 6, 1962