# Microbial 7-OH Epimerisation of Bile Acids

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The microbial 7-OH epimerisation of cholic and chenodeoxycholic acids with *Xanthomonas maltophilia* CBS 827.97 to ursocholic and ursodeoxycholic acids with scarsity of oxygen is described. With normal pressure of oxygen the 7-ketocholic and the 7-ketochenodeoxycholic acids are obtained. No biotransformation is achieved in anaerobic conditions.

Bile acids are natural products, fundamental constituents of bile. Chenodeoxycholic acid and the 7-OH epimer ursodeoxycholic acid have important pharmaceutical applications related to their ability to solubilize cholesterol gallstones.[1] In recent years ursodeoxycholic acid has replaced chenodeoxycholic acid for the treatment of cholestatic liver diseases because of its similar efficacy and the complete lack of side effects.[2]

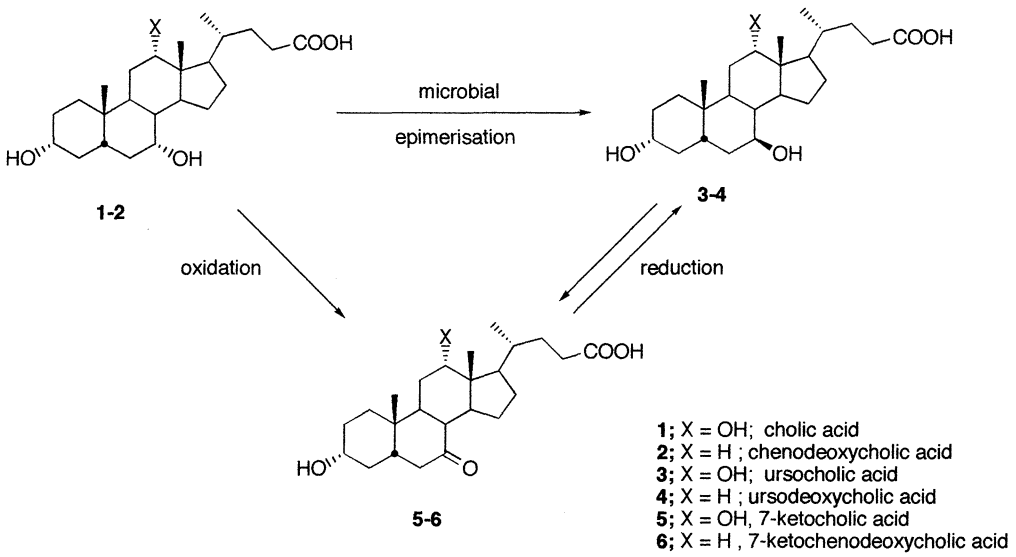
Both chenodeoxycholic and ursodeoxycholic acids are prepared on a large scale from raw, low cost materials with a high bile acid content. In particular bovine bile is the most commonly employed biological material. Its major component is cholic acid which is subsequently used as the starting reagent for the synthesis of chenodeoxycholic acid.[3,4] On the other hand ursodeoxycholic acid is industrially prepared by a sequence of chemical reactions, the last two steps of which involve the selective α/β inversion of the 7-OH carbon center.[5] During the last decade, different approaches have been proposed to overcome the well recognized drawbacks of the chemical sequences, i.e. the occurence of many by-products lowering the yield and the partial stereochemical control of the α/β inversion. In particular, biotransformations, mainly with anaerobic bacteria, have been successfully used to enhance the selectivity and to reduce the number of steps to obtain chenodeoxycholic or ursodeoxycholic acid.[6]

Excellent results have been obtained in this field using NAD(P)-dependent hydroxysteroid dehydrogenases[7] and various microrganisms (most of them anaerobic)[6] that regiospecifically oxidize the cholic acid. In a previous work we have isolated and classified several microrganisms from 50 enviromental samples withdrawn from ICE industry,[8] that extracts and purifies bile acids from raw materials (ox and pig bile), and screened them in oxidation reactions.[9] The same bacteria are tested in epimerisation screening of selected bile acids.

In this paper we describe the 7-OH epimerisation of cholic and chenodeoxycholic acids to ursocholic and ursodeoxycholic acids (Scheme), respectively, with *Xanthomonas maltophilia*.[10]

To a slowly stirred (pressure of oxygen about 50%) *Xanthomonas maltophilia* culture (1 L),[11] grown for 24 h in the presence of small amounts of the bile acid (**1-2**) sodium salt (0.25 g), is added the proper sodium salt (10 g) adjusting the pH to 8 with 10% NaOH and the incubation is continued for a further 4 h at 30°C without stirring (pressure of oxygen about 1-2%). The crude reaction products are analyzed by GLC.[9] The suspension is removed by centrifugation, the mixture is acidified with 5% HCl and extracted with ethyl acetate. Andrification and chromatography (silica, ethyl acetate/ acetic acid 50:1) afforded the pure products.

In these conditions the cholic acid **1** (7α-OH) afforded ursocholic acid **3** (7β-OH) in 75% yield while the chenodeoxycholic acid **2** (7α-OH) gave the ursodeoxycholic acid **4** (7β -OH, 27%) together with 23% of the 7-keto derivative **6**. On the other hand, prolonged incubation of chenodeoxycholic acid up to 24 h increased the amount of the 7-keto derivative (44%) and decreased the percentage of the 7β-OH ursodeoxycholic acid (10%). Both substrates, if the growing of the culture and the further incubation is achieved under vigorous stirring (normal pressure of oxygen), gave only the 7-keto derivatives **5-6** in about 80% yield. The scarsity of oxygen,



due to the slowly stirring during the growing of the microrganism and the quasi-lack of oxygen during the biotrasformation favour the epimerisation products **3-4** to disadvantage of the oxidation ones **5-6**. The biotransformation carried out in anaerobic condition has not afforded products. Partial purification of the enzyme responsible of the 7-OH inversion confirmed that it is an oxido-reductase β-stereospecific. The obtained acetone powder, in fact, is able to oxidize the 7-OH of the cholic acid **1** and subsequently reduces the 7-keto derivative **5** to 7β-OH. On the other hand the ursocholic acid **3** is oxidized in the same conditions to the ketone **6**. We can also point out that the normal pressure of oxygen is efficient to inactivate the reduction. Improvements of the epimerisation are under investigations.

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10 The strain has been isolated in ICE industry (Italian Patent MI97A 001745 (1997)) and deposited as CBS 927.97 (Centraalbureau voor Schimmelcultures, The Netherlands).

11 A culture medium containing, for 1 L of water, glucose (15 g), yeast exctract (5 g), soy peptone (3 g) and Nutriferm L 90 (15 g) and adjusted to pH 6.5 with 10% $H_2SO_4$, is inoculated with 10 mL of spore suspension and grown at 30 °C.