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Review Article

Biosynthesis and Biotransformation of Bile Acids

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SUMMARY

Introduction: Bile acids are steroidal compounds, which contain 24 carbon atoms. They can be classified into two major groups: primary and secondary.The most abundant bile acids: The primary bile acids include cholic acid and chenodeoxycholic acid, while the major secondary bile acids are deoxycholic acid and litocholic acid. Bile acids are important physiological agents for intestinal absorption of nutrients and are used for biliary lipid secretion, toxic metabolites and xenobiotics. The aim of this paper is to analyze biosynthesis and biotransformation of bile acids, as preparation for practical usage in laboratory and clinical conditions.

Topic: *Biosynthesis and biotransformation of bile acids*: The biosynthesis of bile acids is the dominant metabolic pathway for catabolism of cholesterol in humans. The classical route of biosynthesis of bile acids is embarking on the conversion of cholesterol into 7α–hydroxycholesterol using enzyme 7α–cholesterol hydroxylase (CYP7A1). This enzyme is one of the microsomal cytochrome P450 enzyme is localized exclusively in the liver. Classical road is the main road in the biosynthesis of bile acids, and its total contribution amounts to 90% for people, and 75% in mice. CYP 7A1 enzyme is considered to be sensitive to the inhibition of carbon monoxide, and the condition for the effect of NADPH, the oxygen, lecithin, and the NADPH-cytochrome P450 reductase. Bile acids are important signaling molecules and metabolic controls which activate the nuclear receptor and the G protein-coupled receptors (GPCR), a signaling lipid regulation of the liver, glucose and energy homeostasis. Also, bile acids maintain metabolic homeostasis. *Biotransformation of bile acids*: The conversion of cholesterol into bile acids just important for maintenance of cholesterol homeostasis, but also to prevent the accumulation of cholesterol, triglycerides and toxic metabolites as well as violations of the liver and other organs. Enterohepatic circulation of bile acids from the liver to the intestine and back to the liver occupies the most important role in the processes of absorption and distribution, as well as in metabolic regulation and homeostasis.

Conclusions: This physiological process is complicated and regulates the membrane transport system in the liver and intestine by means of nuclear receptors. It is very dangerous fact that toxic bile acids may be causes of inflammation, apoptosis and cell death. On the other hand activated GPCR signaling and nuclear bile acid protects against inflammation of the liver, intestine and macrophages. Bile acid metabolism disorders cause cholestatic liver disease, dyslipidemia, fatty liver disease, cardiovascular disease and diabetes.

Keywords: bile acids, biosynthesis, biotransformation

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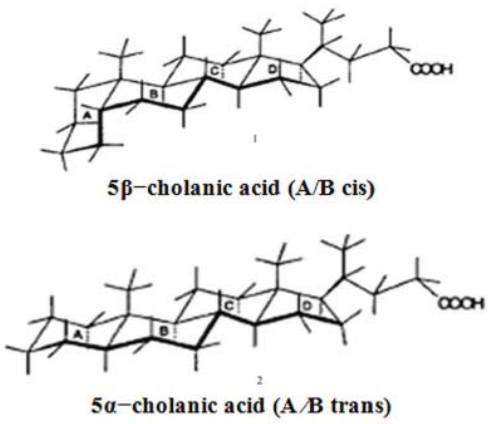
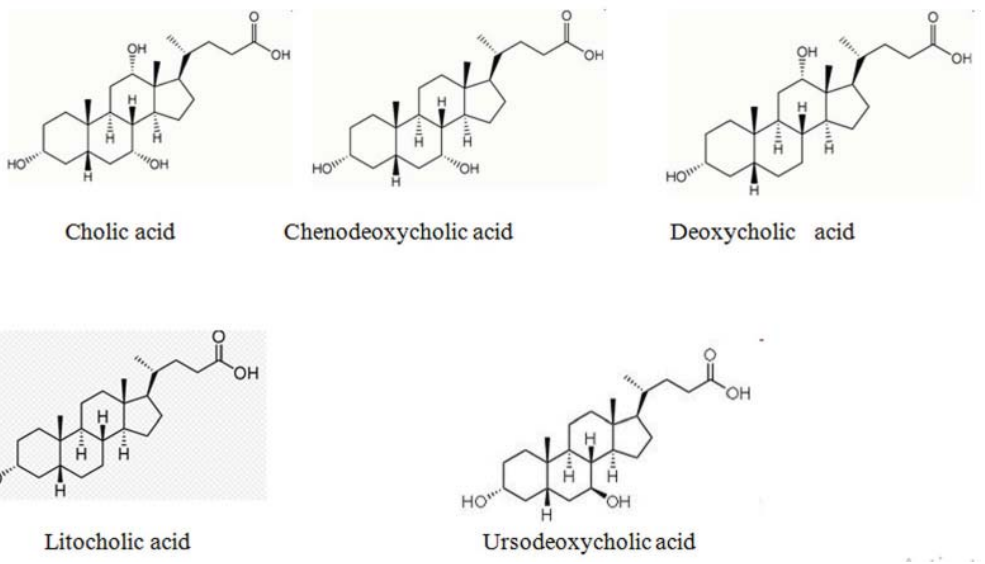
INTRODUCTION

All of the bile acids are characterized by the appearance of cyclopentanoperhydrophenanthrene ring. The primary bile acids are synthesized in the liver. Further, they can be conjugated with glycine and taurine, before secretion into the bile ducts and the digestive tract. The conjugation of reducing the hydrophobicity of a bile acid. Also, in this manner increases the amphiphilic nature of the bile acids and makes them less toxic. These conjugates are membrane permeable and have the ability to transform a series of lamellar lipid into the mixed micelles. The secondary bile acids (deoxycholic acid and litocholic acid) occur 7-α dehydroxylation and deconjugation of cholic acid and deoxycholic acid , wherein is then resorbed, conjugated and excreted from the bile. The modification of the primary bile acids in the secondary is carried out by means of anaerobic bacteria in the colon. The required amount of bile acids in the body are maintained by a recirculation phenomenon enterohepatic [1]. Bile acids have an important role in pharmaceutical terms. It is reflected in the emulsification of fats and cholesterol in the bile and intestines and reabsorption of the fat and fat-soluble vitamins in the intestinal lumen. In this case, the bile acids, used as replacement therapy in conditions when their synthesis is insufficient. On the other hand, it is important to note that chenodeoxycholic and ursodeoxycholic acids used in the treatment of breaking stones in the bile [2]. They may also have a significant antibacterial effect, with better results related to the cholic acid derivatives, but derivatives of deoxycholic acid. Since the antibacterial effect, are used for the production of new antibiotics, which are very efficient and have low toxicity content [3]. The fluorescent derivatives of bile acids, affect the characterization of the liver and transport through the intestine, as well as to itself during the transportation of biotransformation. In this case, the bile acid containing fluorophores attached to the side chain. Fluorophores have a significant impact, since the bound bile acids, affect on the transport of hepatocytes and enterocytes [4]. Pass through the cell membrane and affect on the biotransformation of hepatocytes during transport [5]. In aqueous solution the bile acid aggregate and form aggregates polymolecularity, rod clusters (so-called. Micelles). They may be incorporated into cholesterol, and phospholipids. Bile acids have an important role and in agriculture [1]. They are used as a food supplement in animals. Some of them due to increased growth, or at

Scheme 1. Conformational display of cholanic acid [7]



Scheme 2. The structures of the most widespread of bile acids [6]



prebiotic treatment may be contributory factors of increased secretion of cholesterol and bile stones creation. The aim of this paper is to analyze biosynthesis and biotransformation of bile acids.

TOPIC

The most abundant bile acids

Bile acids are an integral part of the bile of animals and humans. All the bile acids of higher vertebrates can be considered derivatives of cholanic acids, which is the steroid system to 24 carbon atoms. The diversity of a bile acid is a result of the presence of a variable number of hydroxyl groups in their molecules. In nature, the most widespread are cholic and deoxycholic acids. There are two isomeric of cholanic acid [2,6].

Biosynthesis and biotransformation of bile ACIDS

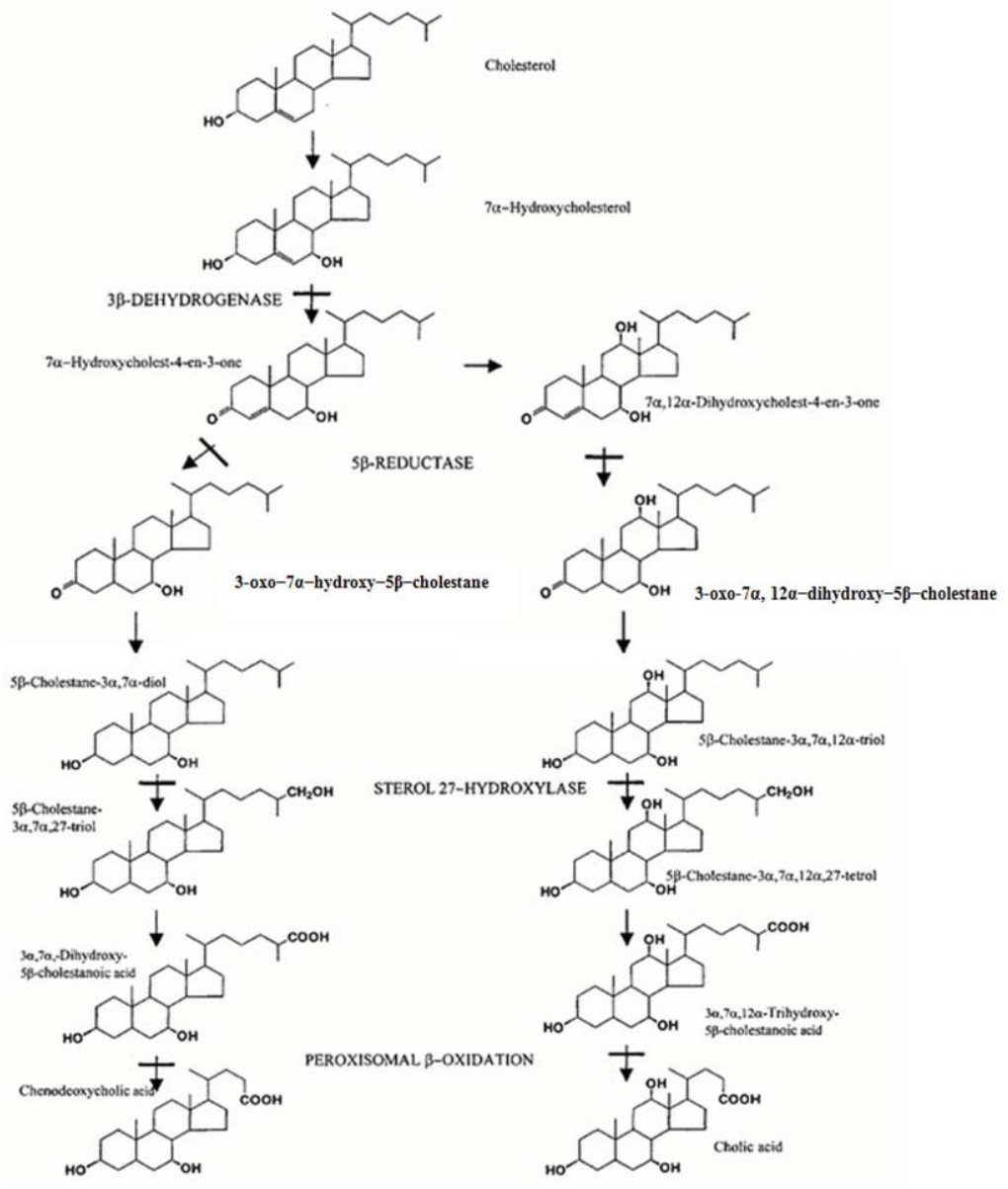
The biosynthesis of bile acids is the dominant metabolic pathway for catabolism of cholesterol in humans. It is important to note that the transformation of cholesterol into a bile acids occurs in complex biochemical pathways, which include the effect of different enzymes [7]. Many of these enzymes are predominantly expressed in the liver, and are localized in a number of different sub-cellular compartments. It is most often in the human liver in the adult is converted to about 500 mg of cholesterol to bile acids [8,9]. When it comes to the biosynthesis of bile acids, it involves modification of the ring of cholesterol, then oxidation, shortening the side chain and eventually carried bile acid conjugation with amino acids. Consider the classical route of biosynthesis of bile acids, which is embarking on the conversion of cholesterol into 7α– hydroxycholesterol using enzyme 7α–cholesterol hydroxylase (CYP7A1). This enzyme is one of the microsomal cytochrome P450 enzyme is localized exclusively in the liver [10].

Biosynthesis of bill acids

Classical road is the main road in the biosynthesis of bile acids, and its total contribution amounts to 90% for men, and 75% in mice. CYP 7A1 enzyme is considered to be sensitive to the inhibition of carbon monoxide, and the condition for the effect of NADPH, the oxygen, lecithin, and the NADPH-cytochrome P450 reductase. It is believed that this enzyme is very important for the reason, which suppresses the backflow of hydrophobic bile acids returning to the liver via the portal circulation [11]. By the enzyme cholesterol 7α–hydroxylase, cholesterol is converted to a 7α–hydroxycholesterol, which is in turn under the action of the enzyme 3β–hydroxy Δ^5–C_{27}–steroid oxidoreductases converted to 4-cholesten-7α-ol-3-one [11]. The hydroxyl group of cholesterol is in the C_3 β–orientation and must be epimerize to the α-orientation during the synthesis. The epimerization is catalysed by HSD3B7 (3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase) [11]. That the reaction is essential for the biosynthesis and functions of bile acids, it is proved by the presence of the hidden mutations at HSD3B7. Children who have these hidden mutations develop progressive liver disease, which is characterized as cholestatic jaundice. Further in the biosynthetic pathway of enzymes 12α sterol-hydroxylase ($CYP8B_1$) on 7α–hydroxy–4–cholesten–3-one obtained 7α, 12α–dihydroxy–4–cholesten–3-one [12]. This compound is then subjected to a reduction of the keto group to the hydroxyl group and simultaneous epimerization reaction of the double bond. Enzymes that are responsible for this process are AKR1C4 (3α–hydroxysteroid dehydrogenase) and AKR1D1 (Δ^4–3–oxosteroid 5β–reductase) influence on the process of forming 5β–cholestan–3α,7α,12α–triol [13]. Then lead to the oxidation of the side chain by the enzyme sterol 27–hydroxylase (CYP $27A_1$) to form 3α,7α,12α–trihydroxy–5β–cholestanoic acid, from which it is formed at the end of the most important primary bile acid–cholic acid [12,14]. At the same time of 7α–hydroxy–4–cholesten–3–one by the action of AKR1D1 and AKR1C4 formed 5β–cholestan–3α,7α–diol, followed by oxidation of the side chain occurs 3α,7α–dihydroxy–5β–cholestanoic acid. From it, then gets chenodeoxycholic acid (CDCA) [11]. The biosynthesis of bile acids via the acid pathway run to the mitochondrial sterol 27-hydroxylase (CYP $27A_1$) which converts cholesterol into 27-hydroxycholesterol and later in 3β–hydroxy–5–cholestenoic acid. Both compounds, a 27–hydroxycholesterol and beta-hydroxy-5-cholestenoic acid are 7α–hydroxylated in human liver mitochondria to the 3β,7α–dihydroxy–5–cholestenoic acid [12,15]. Sterol 7α–hydroxylated activity unlike of cholesterol 7α–hydroxylase convert 27–hydroxycholesterol in a 7α,

27–dihydroxycholesterol in liver microsomes in hamsters. Recently, the cytochrome P450 enzyme called oxysterol 7α–hydroxylase or $CYP7B_1$ originally cloned from mouse hippocampus and shown the possibility catalyzed 7α–hydroxylation of 25–hydroxycholesterol and 27–hydroxycholesterol, which are transported in 293 kidney cells [14,15].

Microsomal 3β–hydroxysteroid oxidoreductase/isomerase is then converted 3β, 7α–dihydroxy–5–cholestenoic acid in 7α–hydroxy–3–oxo–4–cholestenoic acid, which is a precursor for the synthesis of chenodeoxycholic acid [12]. However, little is known about the rest of the enzymes, which is involved in the conversion of metabolites in chenodeoxycholic acid [14]. The recent observation that can be formed a cholic acid and chenodeoxycholic acid when cholesterol 7α–-hydroxylase is inhibited in rat hepatocytes, provides suggestive evidence that hydroxylated intermediates in 12α–position products cholic acid via the acid pathway [14]. Has been shown to modify the bile acid signaling pathways in the cell, including calcium mobilization, cyclic AMP synthesis and protein kinase C activation [5]. Bile acids are activated protein kinase C/Janus N-terminal kinase pathway. Stimulate secretion of pro-inflammatory cytokines, tumor necrosis factor (TNF) and interleukin 1 (IL-1) from Kupffer cells (macrophages resistant hepatocytes), that are acti-



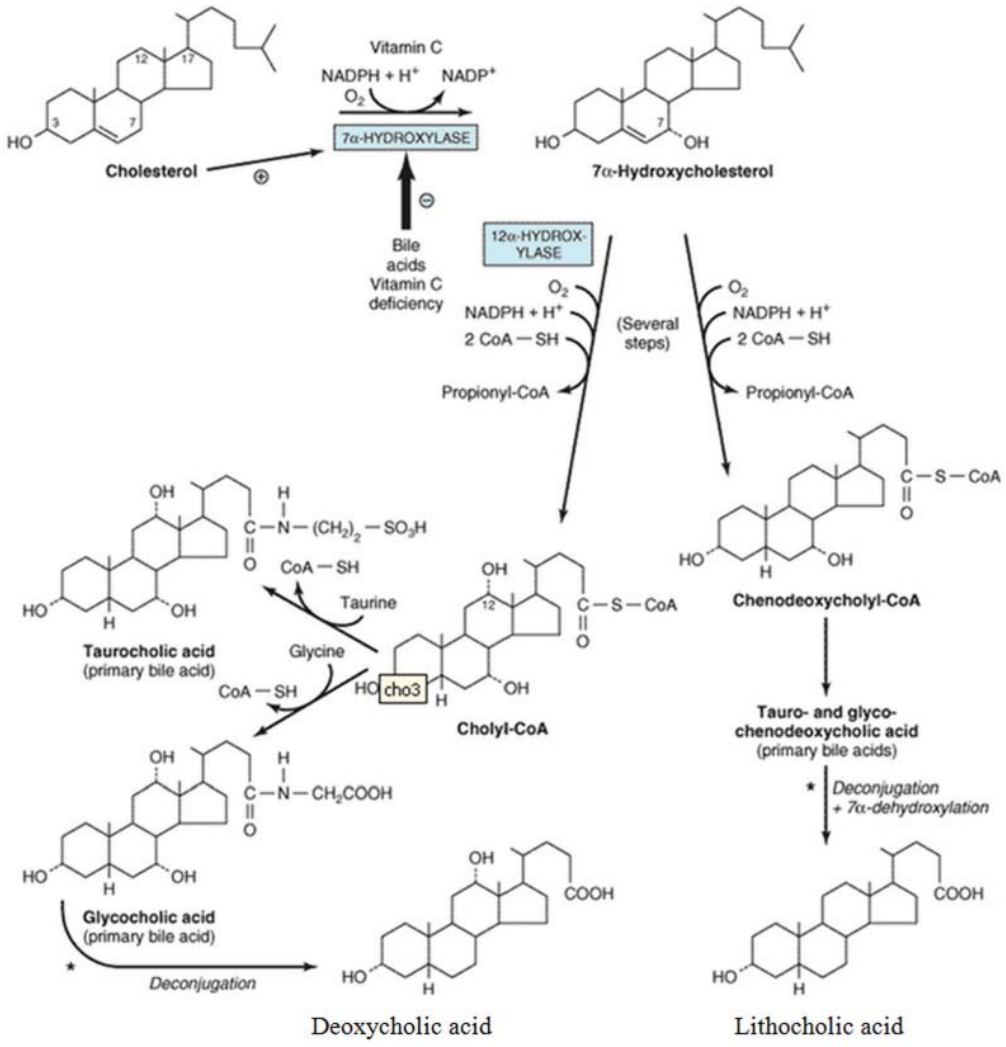
Scheme 3. Schematic view of primary bile acids biosynthesis pathway [17,18]

vated TNF receptor signaling protein and mitogen-activated kinase (MAPK)/JNK [16,17].

Scheme 3. gives a schematic view of the classical and alternative pathway in the biosynthesis of bile acids.

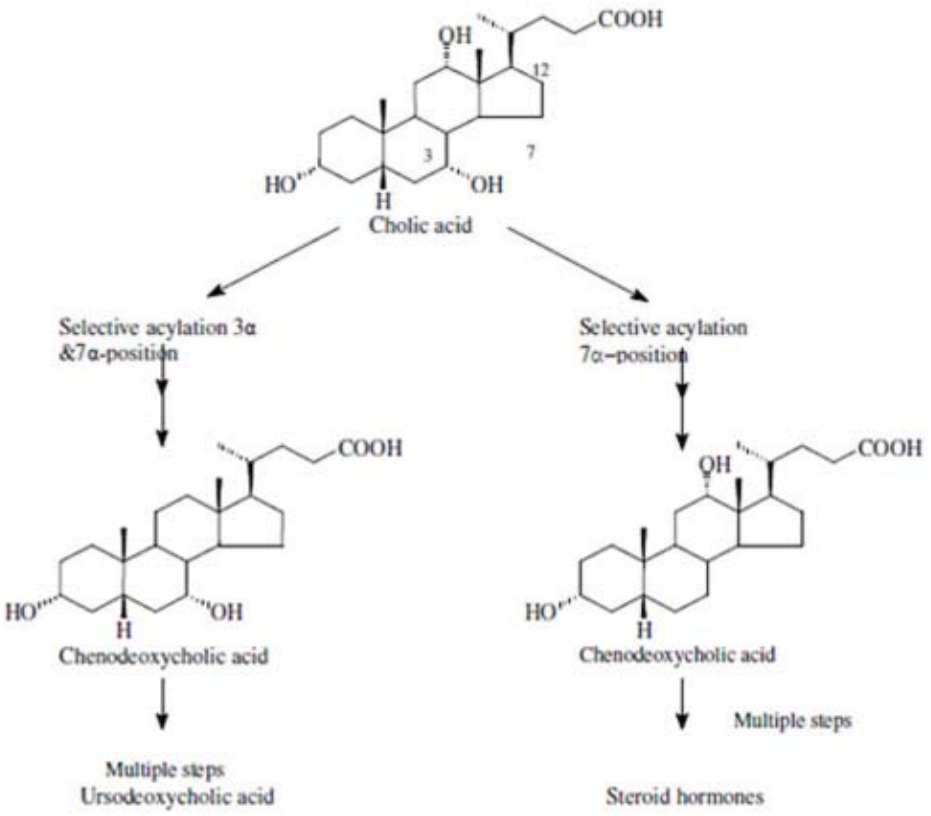
Conjugated bile acids produce of mitochondrial reactive oxidative species, which activated epidermal growth factor receptor and RAF-1/MEK/EPC signaling pathway. Free and conjugated bile acids have the ability to bind to the ligand binding domain of FXR, which consist of a heterodimer with retinoid X receptor and are linked to the inversely repetitive AGGTCA similar sequences with one nucleotide spacing (IR1) which is located in the promoters of target genes FXR, to stimulate the transcription of a gene. FXR plays a central role in the regulation of bile acid synthesis, extraction and transport of lipids, glucose and energy metabolism [17,18].

Conjugation increases the solubility of bile acids. Activation is carried out with ATP and CoA (CoA–derivatives, may be conjugated). Glycine forms glycocholic acid and glycotaurocholic acid (pK 4). In the bile at pH 6 they are significantly ionized and are good detergents. Taurine formes taurocholic acid and taurodeoxycholic acid (pK 2). They are almost completely ionized, and represent the best detergents in the bile. The ratio (glycine: taurine) =3:1 to 4:1. Under the action of bacterial enzymes, primary bile acids passing through the small intestine and colon are: deconjugation and dehydroxilation. In addition formed secondary bile acids: deoxycholic acid and lithocholic acid. Deoxycholic acid is reabsorbed and conjugated, and litocholic acid is partially reabsorbed, while the remainder is excreted in the feces [14,18,19].



Scheme 4. Conjugation of primary bile acids and synthesis of the secondary bile acids [8,9]

Scheme 5. Chemical transformation of cholic acid into ursodeoxycholic acid and steroid hormones [20]



Biotransfoirmations of bile acids

Early experiments involving chemical transformation of bile acids, which are carried out in order to determine their structure. In the last 50 years, carried out chemical transformations of bile acids are mainly aimed at interconversion of bile acids, the synthesis of steroid hormones and the synthesis of certain vitamins. Cholic acid is an important starting material for the synthesis of corticosteroids. It is also a necessary precursor of chenodeoxycholic acid, which is widely applied in the treatment of gallstones (Scheme 5) [20].

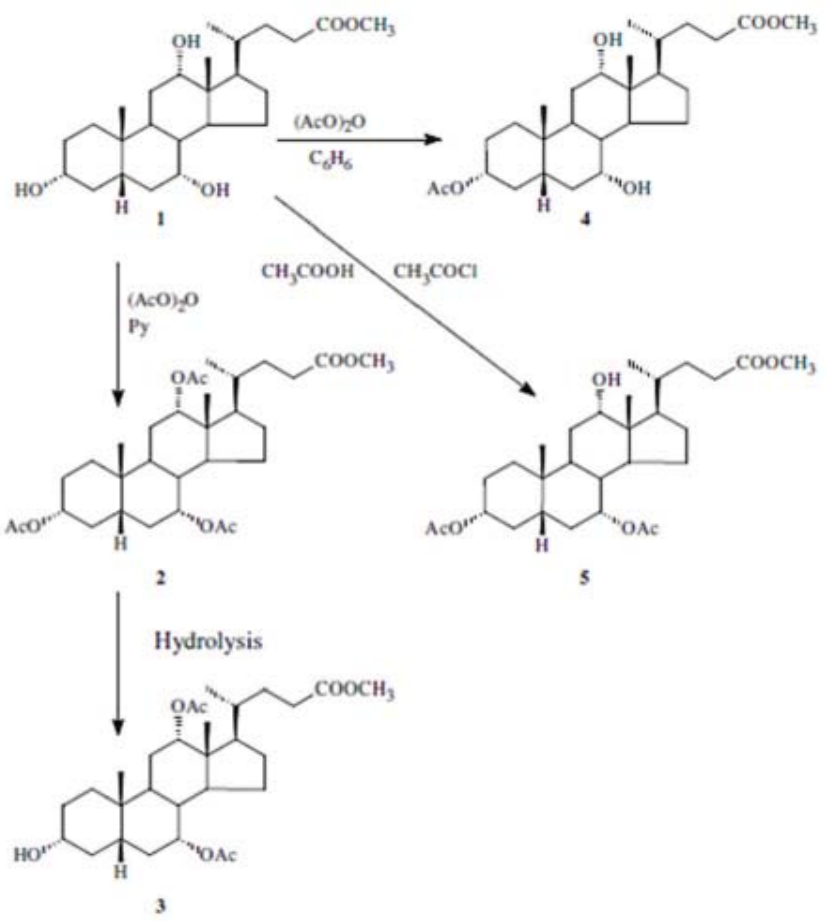
Regardless of whether the C_7 and C_{12} hydroxyl groups replaced by hydrogen cholic acid, certain artificial problems are inevitable. They include the following steps:

- the need for a selective protection of the two hydroxyl groups (usually by acylation), and
- selection of the reagent (mainly, ie. most often an oxidation) for the selective transformation of the remaining hydroxyl groups [20].

Cholic acid contains one C_3 equatorial OH group and two axial hydroxyl groups in the position of C_7 and C_{12}. A equatorial hydroxyl group in the position of C_3 is subject to rapid esterification than the axial hydroxyl groups in the positions of C_7 and C_{12}. It has confirmed the greater reactivity of the C_7 axial hydroxyl group compared to the axial hydroxyl group in the position of C_{12}. Greater reactivity of the C_7 can not be attributed only to steric effects. These characteristics reactivity cholic acid are experimentally confirmed in numerous synthetic studies [20].

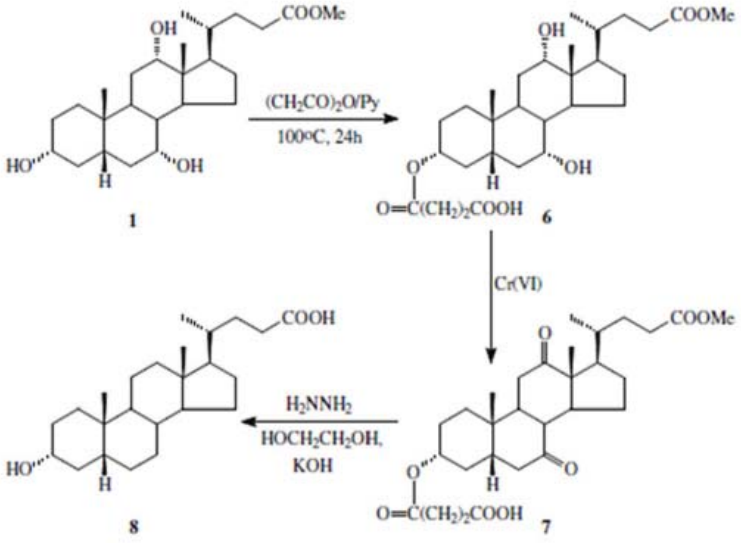
Borshe obtained a 3-carbethoxy derivative (cathylate) in a good yield, by the influence of ethyl chloroformate ($ClCO_2C_2H_5$) on methyl cholate in pyridine. When it is used the excess of acetyl chloride in acetic acid, the triacetyl derivative is obtained in a yield of 30-35%. Later, Fieser and associates obtained the 3-monocathylate in good yield, using piriding-dioxane as a solvent. Although, Wieland and Kapitel were synthesized the methyl 3α, 7α–diacetoxy–12α–hydroxy–5β–cholanate (compound 5) by reaction with acetic anhydride in pyridine, as well as by the influence of acetyl chloride in acetic acid on the methyl cholate (compound 1) [20].

In addition, they also synthesized a 3α, 7α–diacetyl derivatives, using a acetyl chloride or acetic anhydride and HCl. In the same study they were described a synthesis of 7α–acetyl derivative of cholic acid by partial acid saponification of 3α, 7α–diacetyl deriva-



Scheme 6. The synthesis of various acetyl derivatives from a methyl cholate [20]

tive. Plattner Heusser also synthesized a methyl triacetylcholate (compound 2), by the reaction of acetylation of methyl cholate (compound 1) with the acetic anhydride in pyridine, monitoring the selective hydrolysis of methyl of 3α, 7α, 12α–triacetoxy–5β–cholanate (compound 2) to give a methyl of 3α–hydroxy–7α, 12α–diacetoxy–5β–cholanate (compound 3) (1% HCl-CH_3OH, 20 hours). By the influence of acetyl chloride in acetic acid on the methyl cholate, comes to building a methyl ester of 3α, 7α–diacetoxy–12α–hydroxy–5β–cholanoic acid (compound 5). Also, the same group of authors synthesized the 3α–acetoxy–7α, 12α–dihydroxy–5β–cholanate (compound 4) (m.p. 149-1500 C from the system of acetone-



Scheme 7. The synthesis of lithocholic acid from methyl cholate [20]

diethyl ether $(CH_3)_2CO$-$(C_2H_5)_2O$). In a 50% yield by partially acetylated of methyl cholate (the molar ratio of acetic anhydride: cholic acid was (4:1) in a reflux of benzene for 2 hours (Scheme 6) [20].

Hauser and Wuthier are first selectively esterified a methyl cholate (compound 1) with succinic anhydride in dry pyridine, to give a methyl of 3α–succinoyloxy–7α, 12α–dihydroxy–5β–cholanate (compound 6). Without further purification this was oxidized to compound 7 in 90% yield, by the following reduction of diketone (methyl of 3α–succinoy loxy–7,12–diketo–5β–cholanate, (compound 7)) using by hydrazine in ethanediol and potassium hydroxide to give a lithocholic acid (compound 8) (Scheme 7) [20].

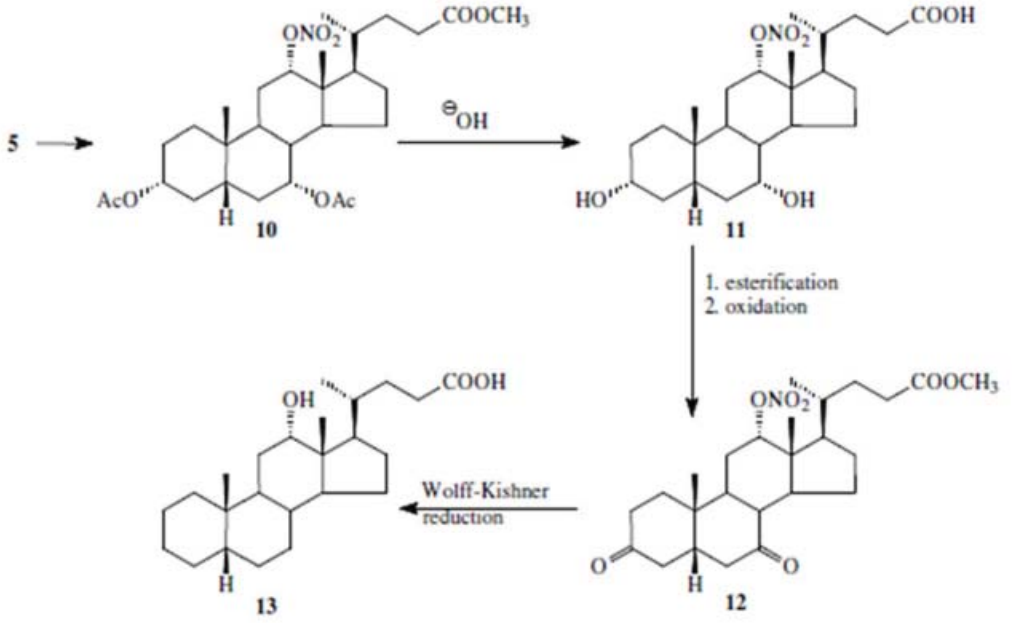
In order to receive a chenodeoxycholic acid, Fisher et al also synthesized of 3α,7α–diacetyl derivative by the reaction of acetylation of methyl cholate with acetic anhydride in pyridine and pyridine-dioxane at room temperature for about 20 hours. The yield was 70%. Different reactivity of the C_7 axial hydroxyl group of esters of carboxylic and mineral acids has been used in the synthesis of 12α–hydroxy–5β–cholanoic acid (compound 13) and deoxycholic acid.

Starting from the compound 5 (methyl of 3α,7α–diacetoxy–12α–hydroxy–5 β–cholanate) converted to the corresponding methyl of 3α,7α–diacetoxy–12α–nitro–5β–ch olanate (compound 10), which after mild alkaline hydrolysis led to the formation compound 11 (3α, 7α–dihydroxy–12α–nitro–5β–cholani c acid). Compound 11 was esterified, and then oxidized to methyl 3,7–diketo–12α–nitro–5 β–cholanate (compound 12), which are then converted to the 12α–hydroxy–5β–cholanoic acid (compound 13) by Wolff–Kishner reduction (Scheme 8) [20].

Dayal et al describe the reaction of esterification, deacetylation and deformylation of bile acids executed under microwave conditions. They used a methanesulfonic acid or p-toluenesulfonic acid in methanol, as the catalyst instead of the commonly used strong mineral acids (The Schemes 9 and 10). Recently exposure to microwave radiation (MW) has become very popular in terms facilitating of fast esterification, hydrolysis and conjugation of bile acids. Reactions, which are carried out by standard procedures were compared , with a final reactions by the influence of microwaves, obtained in the same purity and in the same yield , but in a much shorter period of time [20].

In the first phase, (25R)–3α,7α,12α–trihydroxy–5β–cholestan–26-oic acid (compound 14) was treated with methanesulfonic acid or p-toluenesulfonic acid in methanol under microwave iradiation for a time period of 50 s, to give a (25R)–methyl 3α,7α,12α–trihyd roxy–5β–cholestan–26–oate (compound 15). In the second stage the (25R)–methyl 3α,7α, 12α–trihydroxy–5β–cholestan–26-oate (compound 15) is subjected to mild alkaline hydrolysis, whereby obtained the (23R)–3α,7α,

Scheme 8. The synthesis of 12α–hydroxy-5β–cholanic acid [20]

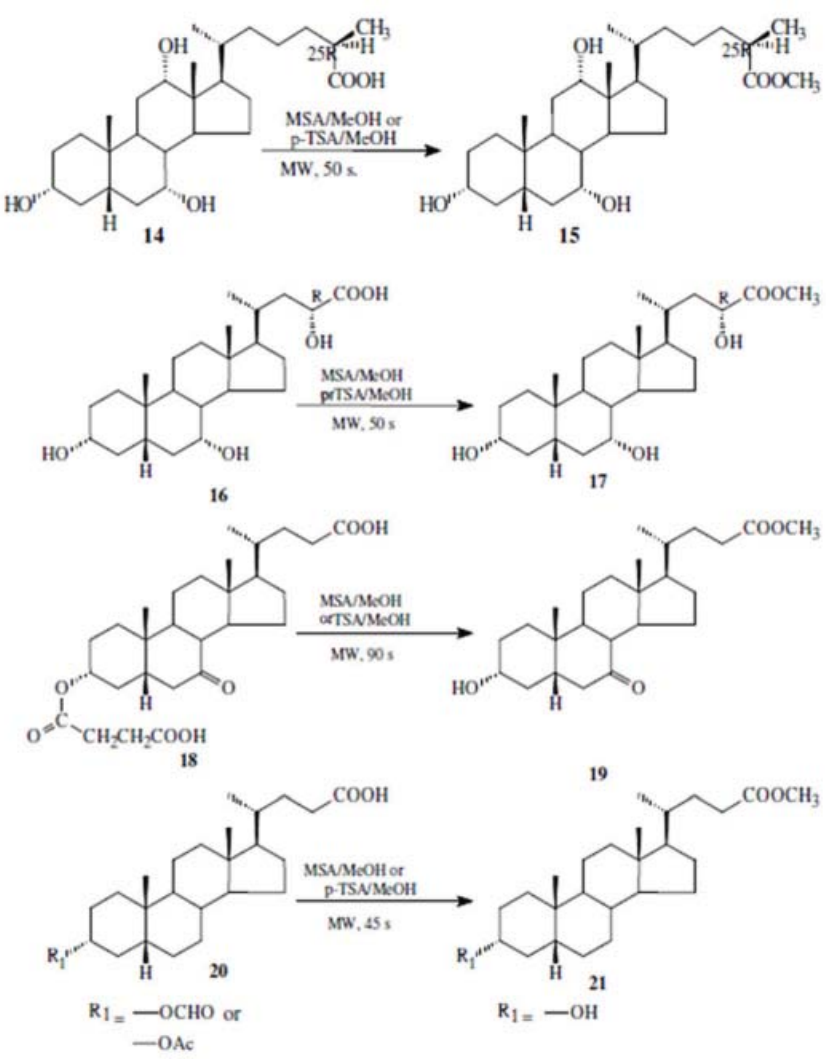


23–trihydroxy–5β–cholanoic acid (compound 16). In the third synthetic stage the (23R)–3α,7α, 23–trihydroxy–5β–cholanoic acid (compound 16) was treated with methanesulfonic acid in methanol or p-toluenesulfonic acid in methanol, to give a (23R)–methyl of 3α,7α,23-trihydroxy–5β–cholanate (compound 17). The reaction was conducted under the influence of microwaves in a period of 50s. In the fourth synthetic stage the (23R)–methyl of 3α, 7α, 23–trihydroxy–5β–cholanate (compound 17) was treated with succinic anhydride in dry pyridine, to give a 3α–succinoyloxy–7–keto–5β–cholanic acid (compound 18). In the fifth step, the 3α–succinoyloxy–7–keto–5β–cholanic acid (compound 18) with methanesulfonic acid in methanol or p–toluenesulfonic acid in methanol, to give a methyl–3α–hydroxy–7–keto–5β–cholanate (compound 19). The reaction was conducted under the influence of microwaves in a period of 90s [20].

In the sixth step the 3α–hydroxy–7-keto–5β–cholanate (compound 19) was subjected to the reaction of selective acetylation or formylation in the position of C3, as well as the Wolff-Kishner reduction of the keto group in position 7, to give a 3α–acetoxy (formyloxy)–5β–cholanic acid (compound 20) [20].

In the seventh step the 3α–acetoxy (formyloxy)–5β–cholanic acid (compound 20) was treated with methanesulfonic acid in methanol or p-toluenesulfonic acid in methanol, to give a methyl lithocholate (compound 21). The reaction was conducted under the influence of microwaves in a period of 45s (Scheme 9) [20].

Compound 22 (3α, 7β, 12α–trihydroxy–5β–cholanoic acid) was treated with 94% formic acid and perchloric acid, as a catalyst, to give the 3α, 7β, 12α–triformyloxy–5β–cholanic acid (com-

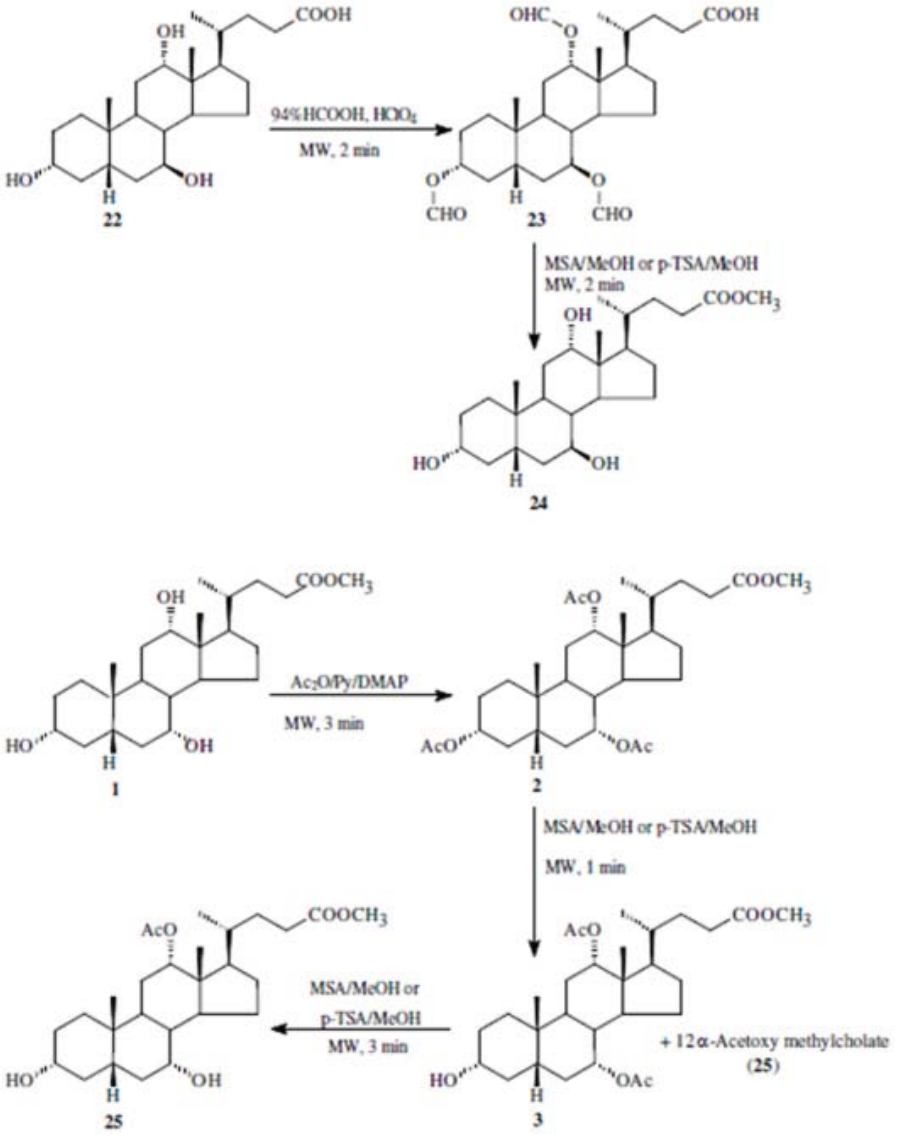


Scheme 9. Esterification, deacetylation, and deformylation reactions of bile acids carried out under microwave (MW) irradiation [20]

14-(25R)–3α,7α,12α–Trihydroxy–5ß–cholestan-26–oic acid;
15-(25R)–Methyl 3α,7α,12α–trihydroxy–5ß–cholestan–26–oate;
16-(23R)–3α,7α,23–Trihydroxy–5ß–cholanic acid;
17-(23R)–Methyl 3α,7α,23–trihydroxy–5ß–cholanate;
18-3α–Succinoyloxy–7–keto–5ß–cholanic acid;
19-Methyl 3α–hydroxy–7–keto–5ß–cholanate;
20-3α–Acetoxy–(or 3α–formyloxy)-5ß–cholanic acid;
21-Methyl-lithocholate;
MSA-methanesulphonic acid;
p–TSA-p–toluenesulphonic acid

Scheme 10. Esterification, deacetylation, and deformylation reactions of bile acids carried out under microwave (MW) irradiation [20]

22-3α,7Β,12α–Trihydroxy-5Β–cholanic acid;
23-3α,7Β,12α–Triformyloxy–5Β–cholanic acid;
24-Methyl of 3α,7Β,12α–trihydroxy–5Β–cholanate;
1-Methyl of 3α,7α,12α-trihydroxy-5Β-cholanate;
2-Methyl of 3α,7α,12α-triacetoxy-5Β-cholanate;
25-Methyl of 3α,7α-dihydroxy-12α–acetoxy–5Β–cholanate;
3-Methyl of 3α-hydroxy-7α,12α-diacetoxy-5Β-cholanate;
DMAP -(4-N,N"–dimethylamino antipyrine);
MSA-methanesulphonic acid;
p-TSA-p-toluenesulphonic acid



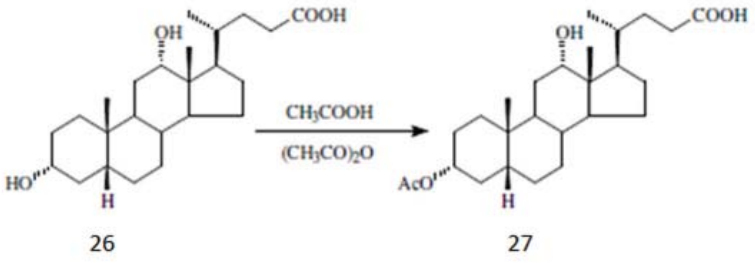
pound 23). The reaction was conducted under the influence of microwaves in a period of 2 min.

Then, the compound 23 (3α,7β, 12α–triformyloxy–5β–cholanic acid) reacted with methanesulfonic acid in methanol or p-toluenesulfonic acid in methanol, whereby formed the methyl of 3α, 7β, 12α–trihydroxy–5β–cholanate (compound 24) (Scheme 10) [20].

Compound (1) (methyl cholate) was treated with acetic anhydride in pyridine and 4–N,N"–dimethylaminoantipyrine and finally it was obtained the compound 2 (methyl of 3α, 7α, 12α–triacetoxy–5β–cholanate). The reaction was conducted under the influence of microwaves in a period of 3 min. [20]

Then the methyl of 3α, 7α, 12α–triacetoxy–5β–cholanate (compound 2) was treated with methanesulfonic acid in methanol or p-toluenesulfonic acid in methanol, whereby formed the methyl of 3α–hydroxy–7α,12α–diacetoxy–5β–cholanate (compound 3). Later by the influence of the same reaction it was obtained the methyl of 3α, 7α-dihydroxy–12α–acetoxy–5β–cholanate (compound 25) from the methyl of 3α–hydroxy–7α,12α–diacetoxy–5β–cholanate (compound 3). The reaction was conducted under the influence of microwaves in a period of 3 min [20].

In later studies, Wieland and coworkers described the synthesis of 3α–acetoxy–12α–hydroxy–5β–cholanoic acid. They explained a selective acylation of deoxycholic acid on C3,

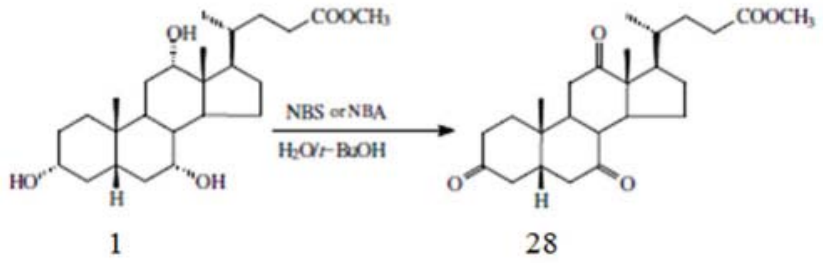


Scheme 11. Selective 3α–acetylation of deoxycholic acid [20]

which is performed in order to obtain a lithocholic acid [20].

Deoxycholic acid (26) is heated to the boiling point with acetic anhydride and glacial acetic acid for 3 hours. After that, compound 27 (3α–acetoxy–12α–hydroxy–5β–cholanic acid) was obtained in 25% yield by crystallization from diethyl ether. (Scheme 11). 3α-Acetoxy-12α-hydroxy-5β-cholanoic acid and 3α–propionyloxy–12α–hydroxy-5β-cholanic acid are obtained in good yields (59% and 67%) by reaction of deoxycholic acid with the corresponding acids, by using of perchloric acid as a catalyst [20].

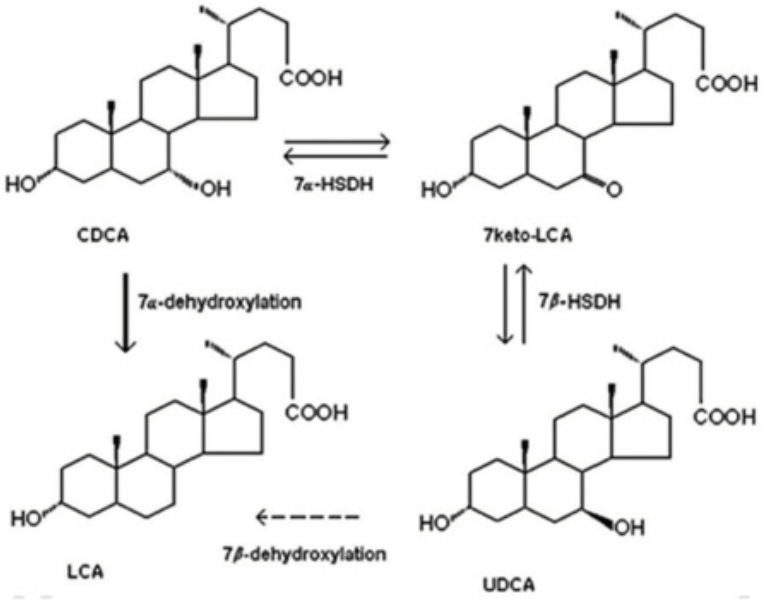
Fieser and Rajagopalan examined the selective oxidation of 7α–OH group of cholic acid and 6β–OH group in the cholestane–3β, 5α, 6β–triol with N-bromosuccinimide (NBS) or N-bromoacetamide (NBA) in various solvents. Their investigations revealed that



Scheme 12. Complete oxidation of methyl cholate to a methyl of 3,7,12–triketo–5ß–cholanate [20]

oxidation was most effective in aqueous tert-butanol, where all the 3-OH group of methyl cholate (compound 1) were oxidized a methyl of 3,7,12–triketo–5β–cholanate (compound 28) in a quick and efficient manner (Scheme 12) [20].

Biotransformation of bile acids, as shown in Scheme 13, takes place in the first synthetic step on the chenodeoxycholic acid by means of 7α–HSDH (hydroxysteroid dehydrogenase, EC 1.1.1. 159) with a synthetic name (7α– hydroxysteroid NAD+



Scheme 13. Biotransformation of bile acids [20]

7–oxidoreductase), which catalyzes the oxidation of 7α–hydroxy group of bile acids to the keto group in position 7, to give the 7–keto derivative of litocholic acid. Also enzymes Bacteroides fragilis and Clostridium Sordi can use $NADP^+$. 7–keto derivative of litocholic acid (3α–hydroxy–5β–cholan–24–oic), in the second synthetic step, under the action of the enzyme 7β–hydroxysteroid dehydrogenase is converted into the ursodeoxycholic acid (3α,7β–dihydroxy–5β-cholan–24–oic). Is carried out by reduction of the keto group at the C7 position to the 7β –OH group. In the third step, the synthetic ursodeoxycholic acid (7β–OH epimer of chenodeoxycholic acid) reacted by 7β-dehydroxylation, whereupon occurs the chenodeoxycholic acid (3α, 7β–dihyd roxy–5β–cholane–24–oic). In the final fourth step occurs a 7α–hydroxylation of chenodeoxycholic acid, thereby forming a lithocholic acid. (Scheme 13) [20].

The methyl ester of 3α,12α–dihydro xy–7α–acetoxy–5β–cholanic acid (compound 29) was treated with a mixture of concentrated nitric acid and acetic anhydride, resulting in formation of the corresponding methyl of 3α,12α–dinitro–7α–acetoxy–5β–cholanate (compound 30).

By the influence of alkaline hydrolysis from the methyl of 3α,12α–dinitro–7α–ac etoxy–5β–cholanate (compound 30) was obtained the 3α,12α–dinitro–7α–hydroxy–5β–cholanic acid (compound 31).

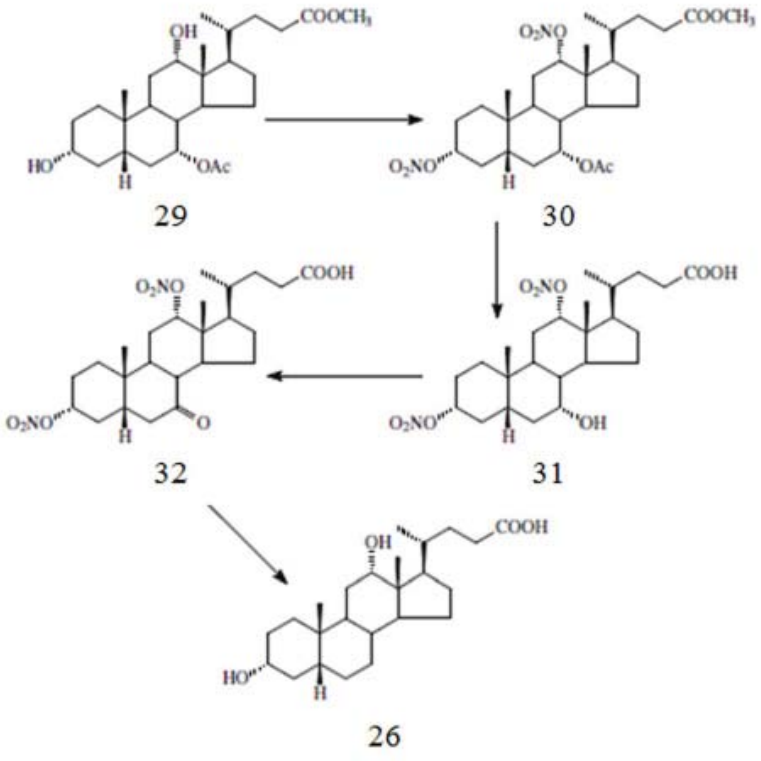
The oxidation of 3α,12α–dinitro–7α –hydroxy–5β–cholanic acid (compound 31) with chromium trioxide in glacial acetic acid gave the 3α, 12α–dinitro–7–keto–5β–cholanic acid (compound 32) which, by Wolff–Kishner reduction, yielded deoxycholic acid (compound 26) (Scheme 14) [20].

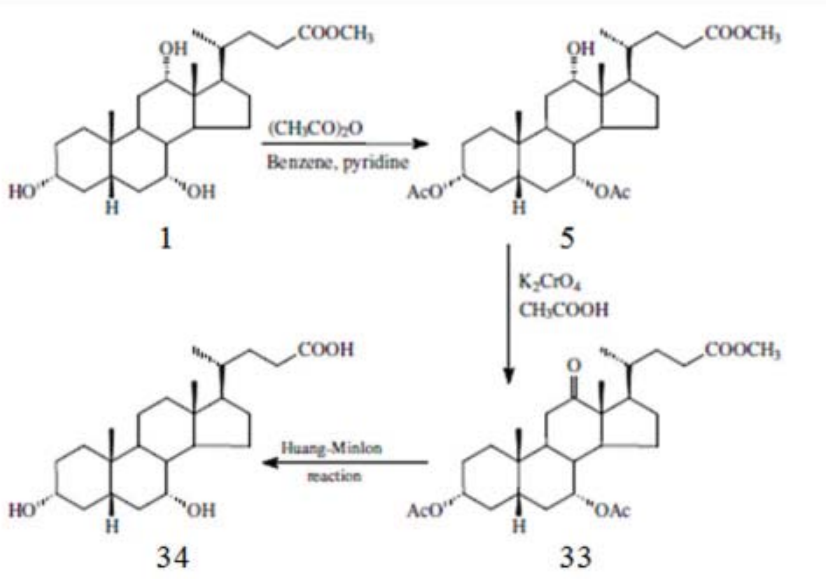
Fieser and Rajagopalan synthesized chenodeoxycholic acid (compound 34) starting from methyl cholate (compound 1). The reaction of selective acylation of the methyl cholate methyl (compound 1) in positions of C3 and C7 was carried out in benzene, using acetic anhydride in the presence of pyridine.

As a product is obtained methyl 3α, 7α–diacetoxy–12α–hydroxy–5β–cholanat e (compound 5), which is then oxidised with sodium chromate (Na_2CrO_4) in acetic acid to methyl of 3α,7α–diacetoxy–12–keto–5β–chol anate (compound 33). Reduction of this keto ester by the Huang-Minlon reaction results in the formation of chenodeoxycholic acid (compound 34) (Scheme 15) [20].

Hydrophobic bile acids such as CDCA (chenodeoxycholic acid) is the most

Scheme 14. The synthesis of deoxycholic acid via a 3α, 12α–dinitro–7–keto–5ß–cholanic acid [20]





Scheme 15. The synthesis of chenodeoxycholic acid from methyl cholate [20]

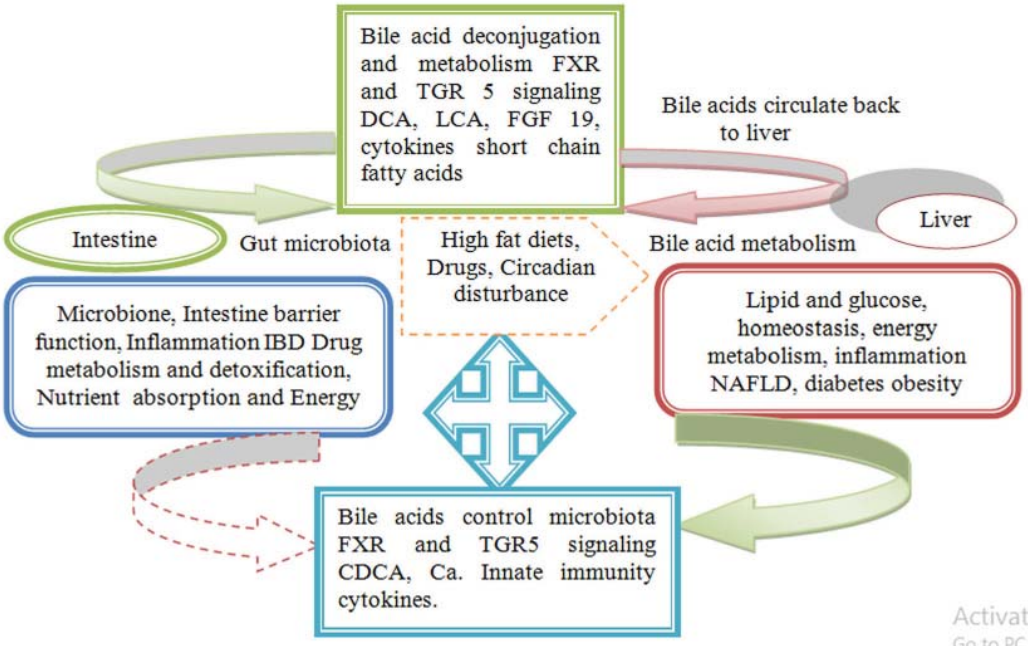
efficient endogenous FXR ligand, and a hydrophilic bile acid, such as for example ursodeoxycholic acid and muricholic acid do not activate FXR [18]. Bile acids bind and activate pregnane X receptor (PXR) and the vitamin D receptor (VDR) receptors. These two receptors have a very important roles in the detoxification of bile acids, drugs and xenobiotics [21]. Deoxycholic acid actives FAS receptor and JNK induction of acide sphingomyelinsized generated ceramide in primary rat hepatocytes [16]. Also, very important information is that the bile acids stimulate insulin receptor signaling. In the brown adipose tissue, bile acids have the ability to activate the receptor TGR5, $G\alpha_i$ protein-coupled receptor. TGR5 receptor stimulates cAMP production, which induces iodothiron diodinase (D2) and produces thyroid hormone T3, which contributes to the stimulation of energy metabolism and improvement of glucose tolerance and insulin sensitivity [10].

TGR5 receptor is not expressed in hepatocytes, and is usually localized in the sinusoidal endothelial cells. In endocrine cells, TGR5 has a role in the stimulation of glucagon, which is similar to peptide 1 and has antidiabetic activity. FXR receptor produces FGF19, which is secreted from the intestine to blood circulation in response to postprandial efflux of bile acids to inhibit bile acid synthesis in the liver [10]. CDCA and GW4064 rapidly induce FGF19 mRNA expression, FGF19 protein secretion, and tyrosine phosphorylation of FGFR4, but inhibit CYP7A1 mRNA expression in primary human hepatocytes suggesting that liver-produced FGF19 is secreted from hepatocytes to activate FGFR4 signaling in hepatocytes by an autocrine or paracrine mechanism [9,13]. In the intestine, bacteria overgrowth damages intestine barrier function and causes IBD, diarrhea, and impaired drug metabolism, detoxification, and absorption. Bile acids control gut bacteria overgrowth and protect against inflammation. Gut microbiota also play a role in biotransformation of bile acids and affected bile acid composition and metabolism via FXR and TGR5 signaling in the liver [16,17]. In the liver, high levels of bile acids cause liver injury. Bile acids also have anti-inflammatory functions by activating FXR and TGR5 signaling in hepatocytes to protect against metabolic diseases such as NAFLD, diabetes, and obesity (Scheme 16) [18–21].

Most of the litocholic acid is excreted in the feces. A small amount of litocholic acid circulates in the liver, where is conjugated on the 3-hydroxy position sulfotransferase (SULT2A1) and rapidly secreted into a bile [5,22].

Bile acid and steroid hormones, inflammatory cytokines and growth factors preventing the transcription of the CYP7A1 through the 5'-upstream region of the promoter. The proximal promoters of CYP7A1 at rats are ligand-activated transcription factors, which play important roles in embryogenesis, development and metabolism. Human CYP7A1 promoter does not bind LXR and does not produce LXR, due to a change in the DR4 motifs in BARE-I sequences. It has been

Scheme 16. Bile acid and gut microbiota [5,22]



confirmed that transgenic mice, carrying the human CYP7A1, do not respond to the high levels of cholesterol in the diet, are not induced by the transgene and synthesis of bile acids is not stimulated by these mice. HNF_4 transactive a CYP7A1 promoter activated with coactivator peroxisome proliferator-activated γ–receptor coactivator 1α. Mutation certain DR1 sequences drastically reduces the basal activity of CYP7A1 promoter and his answers to the inhibition of bile acid. Insulin regulates transcription factors Fox O1 is associated with insulin sequence in response to the CYP7A1 promoter in rats and contributions CYP7A1 transcription in rats [4,22].

CONCLUSION

Bile acids are synthesized via the classic pathway initiated by cholesterol 7α–hydroxylase ($CYP7A_1$), and via alternate pathways, one of which is initiated by sterol 27-hydroxylase ($CYP27A_1$). These studies used mice lacking cholesterol 7α–hydroxylase ($Cyp7A_1$) to establish whether the loss of the classic pathway affected cholesterol homeostasis differently in males and females, and to determine if the rate of bile acid synthesis via alternate pathways was responsive to changes in the enterohepatic flux of cholesterol and bile acids. The acidic pathway may be quantitatively important in the synthesis of bile acids in patients with liver diseases and newborns. In mice, the majority of the bile acids is conjugated with taurin , to form taurine conjugates. Conjugation of bile acids contribute to increased ionization and solubility in physiological pH conditions, prevent precipitation of Ca^{2+}, then reduce the passive absorption and resistant to cleavage using pancreatic carboxypeptidase. In the distal intestine, conjugated bile acids are first deconjugated, and then bacterial 7α–dehydroxylase converted a cholic acid and chenodeoxycholic acid into a deoxycholic acid and litocholic acid.

Most litocholic acid is excreted via the feces, a small quantity is distributed in the liver and rapidly conjugated with sulfidation process, and then excreted from the bile. As the main method for detoxification of bile acids in humans servant sulphation. Bile acids such as CDCA and UDCA have been useful in the treatment of gallstones. UDCA is able to sequester cholesterol from the surface of gallstones, thereby making them smaller and easier to be excreted. The secondary bile acids, DCA and LCA present in the colon, have been shown to act as tumor promoters. The proposed mechanism may offer some insight into the benefits of a diet low in saturated fat and high in fibre. UDCA and CDCA derivatives have been shown to be antiproliferative and are capable of inducing apoptosis in carcinoma cells.

In mice chenodeoxycholic acid hydroxylated to α–muricholic acid and 6β–muricholic acid, which are classified in two main primary bile acids in mice.

7α–hydroxy group in the chenodeoxycholic acid can be epimerized to 7β–OH group of ursodeoxycholic acid. Hydroxylation at 6α/β or 7β–position leads to increase the solubility of bile acids, reducing their toxicity. Physiological importance of FXR receptor depends of pathways in the regulation of metabolism of bile acids. Bile acids activate FXR receptor, which plays a key role in maintaining metabolic homeostasis. The activated membrane G protein-coupled bile acid receptor (Gpbar-1) plays a role in the stimulation of energy metabolism. Then, it is important to emphasize that protects the liver and intestine from inflammation and steatosis (fatty liver) and improves insulin sensitivity. Activated GPCR, sphingosine-1-phosphate receptor 2 (S1P2) is important in lipid metabolism. Also recently discovered role of bile acids in the integrated regulation of lipids, glucose and energy metabolism. The serum of bile acids is higher in patients with prior gastric bypass than in overweight and severely obese patients without gastric bypass, and serum bile acids were positively correlated with serum glucagon-like peptide-1 (GLP-1). The mechanism underlying FXR/FGF19/FGFR4 signaling in inhibition of *CYP7A1* transcription and bile acid synthesis remains to be elucidated.

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CONFLICT OF INTEREST

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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Biosinteza i biotransformacija žučnih kiselina

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KRATAK SADRŽAJ

Uvod: Žučne kiseline su steroidna jedinjenja, koja sadrže 24 C atoma. One se mogu svrstati u dve velike grupe: primarne i sekundarne. Najzastupljenije žučne kiseline: Primarne žučne kiseline su holna kiselina i henodeoksiholna kiselina, dok su glavne sekundarne žučne kiseline deoksiholna i litoholna kiselina. Žučne kiseline su važni fiziološki agensi za crevnu apsorpciju hranljivih materija i koriste se za sekreciju lipida putem žuči, toksičnih metabolite i ksenobiotika. Cilj ovog rada je analiza biosinteze i biotransformacije žučnih kiselina, kao priprema prakticne primene u laboratorijiskim i kliničkim uslovima.

Tema: *Biosinteza i biotransformacija žučnih kiselina:* Biosinteza žučnih kiselina je dominantan metabolički put za katabolizam holesterola kod ljudi. Klasičan put biosinteze žučnih kiselina započinje konverzijom holesterola u 7α–hidroksiholesterol pomoću enzima 7α–holesterol hidroksilaze (CYP7A1). Ovaj enzim je jedan od citohrom P450 mikrozomalnih enzima, koji su lokalizovani isključivo u jetri. Klasičan put je glavni put u biosintezi žučnih kiselina, a njegov ukupan doprinos iznosi kod ljudi 90%, a 75% kod miševa. CYP7A1 enzim se smatra osetljivim na inhibiciju ugljenmonoksida (CO), a uslovom za efekat NADPH, kiseonika, lecitina i NADPH–citohroma P450 reduktaze. Žučne kiseline su važni signalni molekuli i metaboličke kontrole, koje aktiviraju nuklearni receptor i kuplovani G–protein (GPCR), signalnu regulaciju lipida u jetri, glukoze i energetske homeostaze. *Biotransformacija žučnih kiselina*: Konverzija holesterola u žučne kiseline je prvenstveno od značaja za održavanje homeostaze holesterola, ali i za sprečavanje nagomilavanja holesterola, triglicerida i toksičnih metabolita, kao i povrede jetre i drugih organa. Enterohepatska cirkulacija žučnih kiselina iz jetre u creva i nazad (recirkulacija) do jetre, zauzima najvažniju ulogu u procesima apsorpcije i distribucije, kao i u metaboličkoj regulaciji i homeostazi.

Zaključak: Ovaj fiziološki proces je komplikovan i reguliše transportni membranski sistem u jetri i crevima putem nuklearnih receptora. Veoma je opasna činjenica da toksične žučne kiseline mogu biti uzročnici inflamacije, apoptoze i ćelijske smrti. Sa druge strane, aktivira se GPCR signalizacija i nuklearne žučne kiseline štite jetru, creva i makrofage od upale. Poremećaji metabolizma žučnih kiselina izazivaju holestatske bolesti jetre, dislipidemije, bolest masne jetre, kardiovaskularne bolesti i dijabetes.

Ključne reči: žučne kiseline, biosinteza i biotransformacija

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