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CHEMICAL SYNTHESIS OF BILE ACIDS AND THEIR PHYSICO-CHEMICAL PROPERTIES

Tanja Šarenac* and Momir Mikov

University of Novi Sad, Faculty of Medicine, Department of Pharmacology, Toxicology and Clinical Pharmacology, 21000 Novi Sad, Hajduk Veljkova 3, Republic of Serbia.

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Correspondence to Author:
Tanja Šarenac

Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Republic of Serbia.

E-mail: tanjica.tanjci@gmail.com

ABSTRACT: The main roles of bile acids as amphiphilic molecules are the micellar solubilization of the lipids, in the course of the welding process and the regulation of cholesterol homeostasis, which is essential as a precursor for the synthesis of bile acids. From the hydrophobic and hydrophilic surfaces of bile acids directly depends the level of their attachment to the receptors, ion channels and their effectiveness in the solubilization of lipid (hydrophobic drugs). The toxicity of membrane of bile acids is mainly determined by their hydrophobicity. We have synthesized the methyl ester of 7α-hydroxy-3-oxo-5β-cholanoic acid. We have reviewed and discussed the effects of the structure of bile acids to their hydrophobicity, which is expressed by the retention parameters by reverse phase chromatography, and hydrophobic properties, which is represented by partition coefficient 1-octanol / water. It also discusses the role of the lipophilicity of bile acids in their interactions with biological systems.

INTRODUCTION: Bile acids belong to the group of water-soluble steroidal compounds, formed during the catabolism of cholesterol in the liver hepatocytes. The primary bile acids include cholic acid and chenodeoxycholic acid. These are conjugated with glycine or taurine in the hepatocytes of the liver. The primary bile acids include cholic acid and chenodeoxycholic acid. Bile acid synthesis represents the major route for elimination of cholesterol from the body. There are two parallel metabolic pathways; the classical (neutral) and the alternative (acidic) pathways. The microsomal cholesterol 7α-hydroxylase (CYP7A1) catalyzes the first and rate-controlling step in the classical pathway yielding 7α-hydroxycholesterol,

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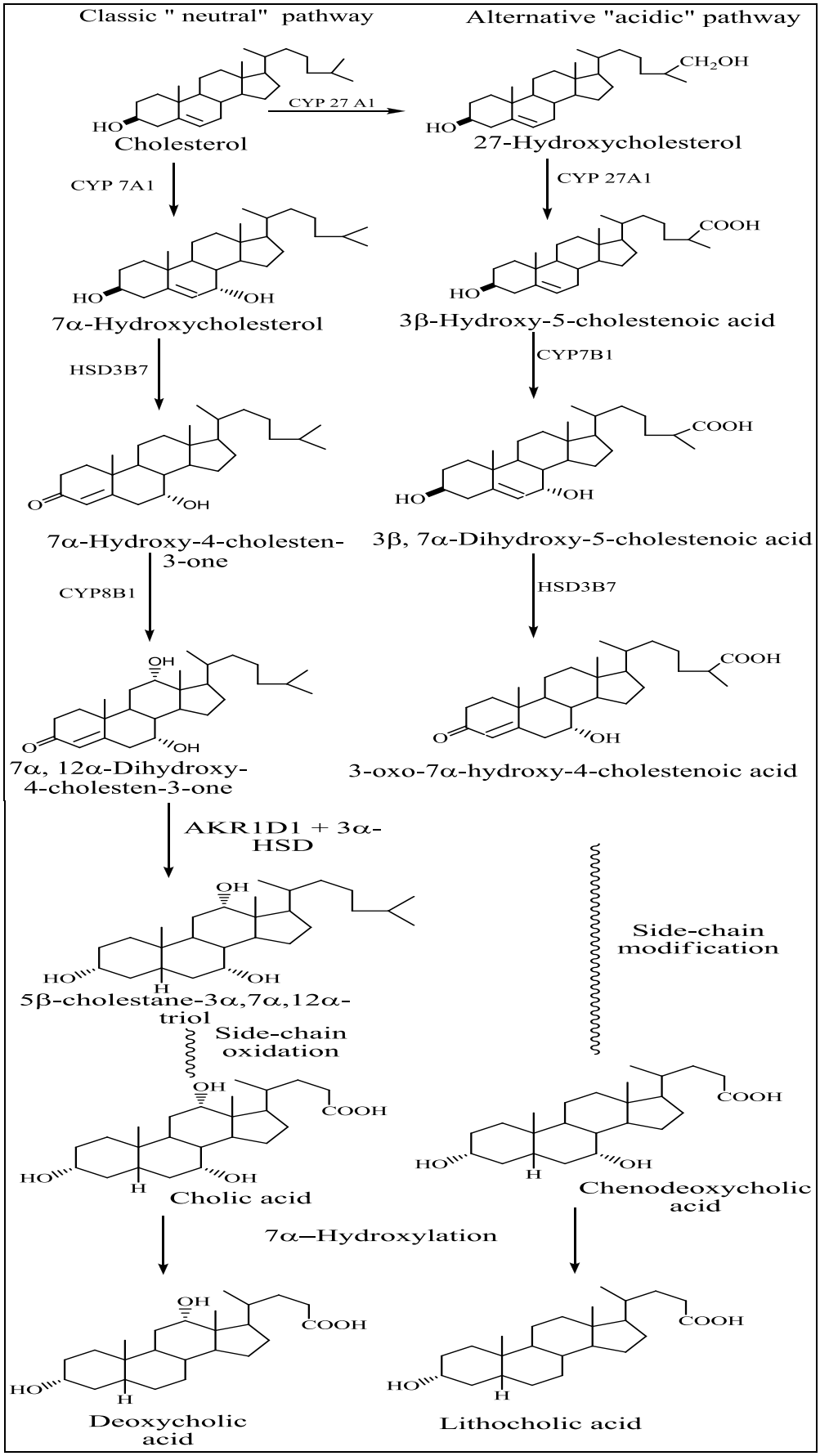
Whereas sterol 27-hydroxylase (CYP27A1) initiates the alternative pathway forming 27α-hydroxycholesterol. Cholic acid (CA) and chenodeoxycholic acid (CDCA) represent the two main end products of these pathways. Conjugation with glycine and taurine reduces the possibility of passive absorption through the biliary system and the proximal part of the small intestine. At the same time enabled active transport through the terminal ileum, and thus increases the solubility of bile acids.

At the same time enabled active transport through the terminal ileum, and thus increases the solubility of bile acids. First activation is carried out with ATP and CoA (CoA derivatives may be conjugated). Conjugation of primary bile acids with glycine enables the formation of glycocholic acid and glycochenodeoxycholic acid. In rodents, the majority of CDCA converted to a highly hydrophilic bile acids (α-MCA), and then to the β-MCA in the liver.



SCHEME 1: SIMPLIFED SCHEME OF BILE ACID SYNTHESIS *VIA* THE CLASSIC "NEUTRAL" AND ALTERNATIVE "ACIDIC" PATHWAYS

Before their excretion, it is important to note that almost all the bile acids conjugated in position C_{24} in peroxisomes of liver, thereby increasing their solubility. Usually, the bile acids are conjugated with glycine in humans, while in rodents mainly conjugated bile acids with taurine. Bile acids are present in the form of sodium salts, at physiological pH, which increases their solubility and amphipathic make them.

Secretion of bile acids in the bile ducts contributes expelling cholesterol and phospholipids in the gallbladder until the sodium ions and water monitored by diffusion across the narrow layer between the hepatocytes. Bile acids are stored with cholesterol and phospholipids in the gallbladder, and also concentrating the absorption of water and electrolytes.

At the apical membrane of cholangiocytes are localized the transporters, which transfer small amounts of bile acids from the bile duct back into circulation. Over peribiliary plexus are recycled back into the liver, but again secreted in bile. In response to food intake, it is important to note that enteroendocrine cells of the small intestine releases cholecystokinin (CCK), which stimulates contraction of the gallbladder, which releases bile into the upper part of the duodenum [1, 2].

The aim of this paper is to analyze a chemical synthesis of bile acids and their physico-chemical properties.

2. Chemical Synthesis of Bile Acids: The classical (neutral) pathway begins with the conversion of cholesterol into a 7α-Hydroxycholesterol by the enzyme of 7α-hydroxylase (CYP 7A1). In the second step, the obtained a 7α-hydroxycholesterol by the enzyme HSD3B7 (3β-hydroxy-Δ^5 C_{27} steroid dehydrogenase) is converted into a 7α-hydroxy-4-cholesten-3-one [3]. The 3β-HSD enzymatic system plays a crucial role in the biosynthesis of all classes of hormonal steroids. This compound is further converted into the 7α, 12α-dihydroxy-4-cholesten-3-one by the enzyme 12α-hydroxylase (CYP 8B1).

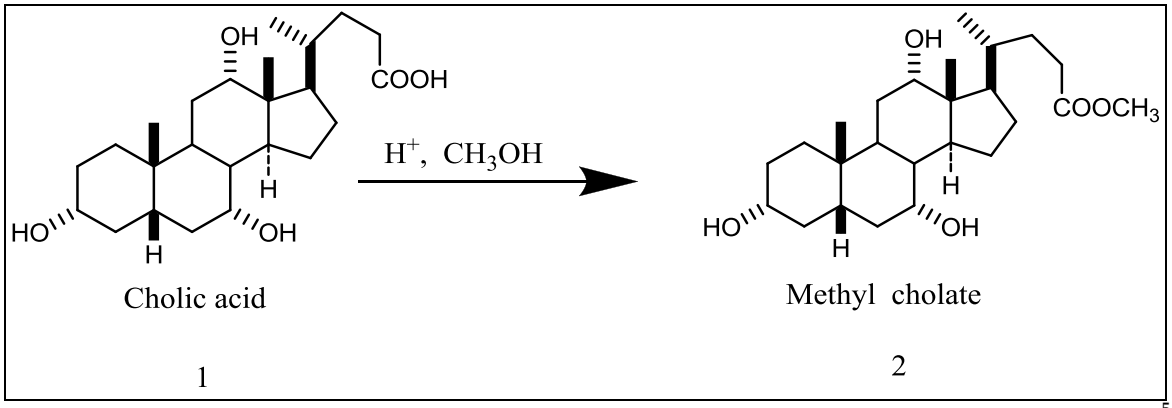
The 7α, 12α- dihydroxy- 4- cholesten- 3- one is subjected to the action of two important enzymes AKR1D1 (Δ^4−3β−oxosteroid 5β−reductase and AKR1C4 (3α−hydroxysteroid dehydrogenase), because it is formed a 5β−cholestane 3α, 7α, 12α-triol, which by its modification, *i.e.* shortening and oxidation of the side chain gets a cholic acid. Therefrom, with reaction of 7α-dehydroxylation and deconjugation under the action of bacterial enzymes on the cholic acid in the colon is formed secondary bile acid-deoxycholic acid **Scheme 1** [4].

Cholesterol in the alternative (acidic) pathway under the influence of the enzyme sterol 27-hydroxylase (CYP 27A1) is converted to 27-Hydroxycholesterol. Under the influence of the same enzyme, 27-Hydroxycholesterol is converted into the 3β-hydroxy-5-cholestenoic acid. Then it will be under the influence of CYP 7B1 (oxysterol 7α- hydroxylase) formed 3β, 7α- dihydroxy- 5-cholestenoic acid. 3β, 7α-dihydroxy-5-cholestenoic acid is converted by an enzyme HSD3B7 (3β-hydroxy-Δ^5 C_{27} steroid dehydrogenase) into the 3-oxo-7α-hydroxy-4-cholestenoic acid [3].

Enzyme is performed by the epimerization of the double bond and oxidation of the OH group in the position C3 to the keto group. The modification of the side chain from the 3-oxo-7α-hydroxy-4-cholestenoic acid occurs a chenodeoxycholic acid (CDCA). There from, with reaction of 7α-dehydroxylation and deconjugation under the action of bacterial enzymes on the chenode-oxycholic acid in the colon is formed secondary bile acid-lithocholic acid **Scheme 1** [4, 14].

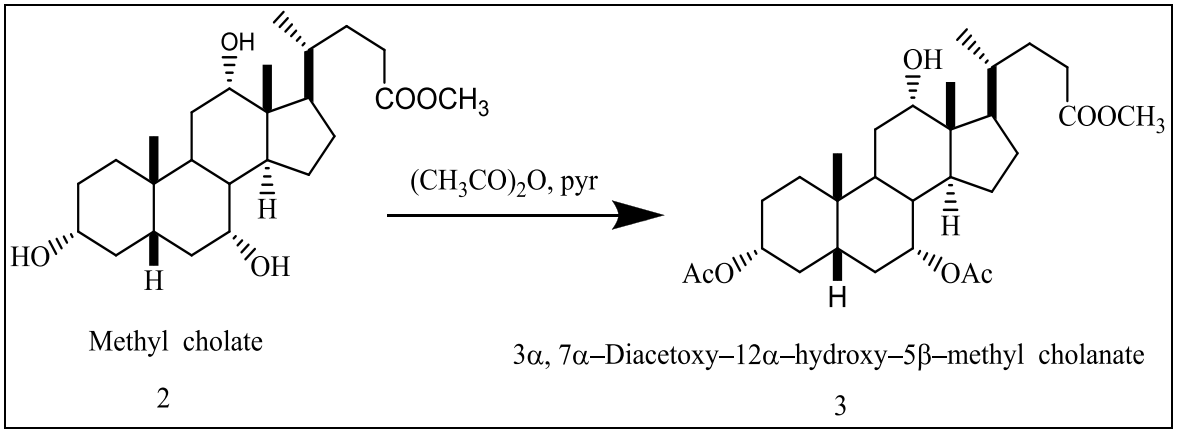
The enzyme AKR1D1 encoded by this gene is responsible for the catalysis of the 5 β-reduction of bile acid intermediates and steroid hormones carrying a Δ^4-3-one structure. Deficiency of this enzyme may contribute to hepatic dysfunction. 3α−hydroxysteroid dehydrogenase (3α-HSD), also known as aldo-keto reductase family 1 member C4, is an enzyme that in humans is encoded by the AKR1C4 gene [3, 14].

2.1. Synthesis and Significance of Ursodeoxycholic Acid: Ursodeoxycholic acid can be isolated from the bile of bears. The synthesis of ursodeoxycholic acid from cholic acid was described in a more of synthetic steps. In the first synthetic step is performed a methylation of the carboxyl group of cholic acid (1) to the methyl cholate (2), as shown in **Scheme 2** [1].



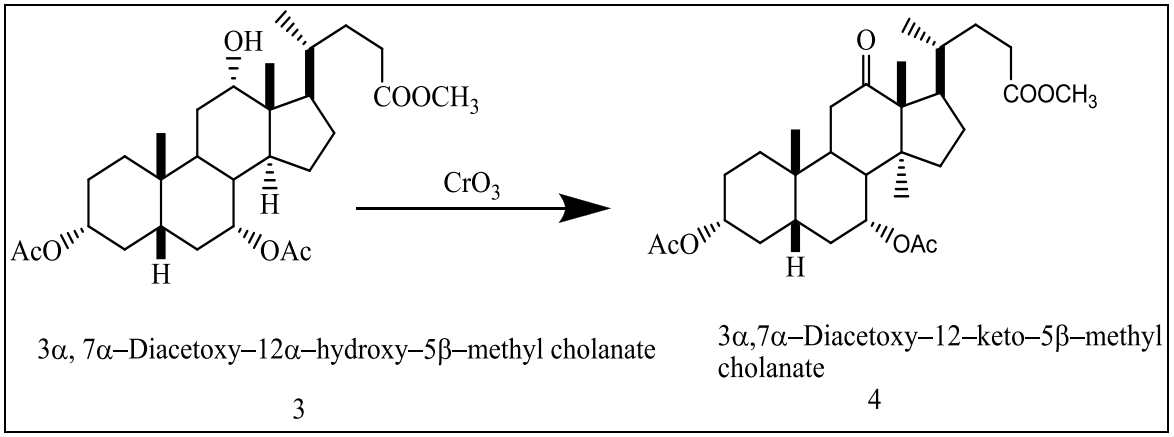
SCHEME 2: REACTION OF METHYLATION OF CHOLIC ACID TO THE METHYL CHOLATE [5]

The second synthetic step involves the acetylation of the hydroxyl groups in positions 3α- and 7α- of methyl cholate (2) addition of acetic anhydride in pyridine, wherein occurs the 3α,7α-diacetoxy-12α-hydroxy-5β-methyl cholanate (3), as shown in **Scheme 3** [5].



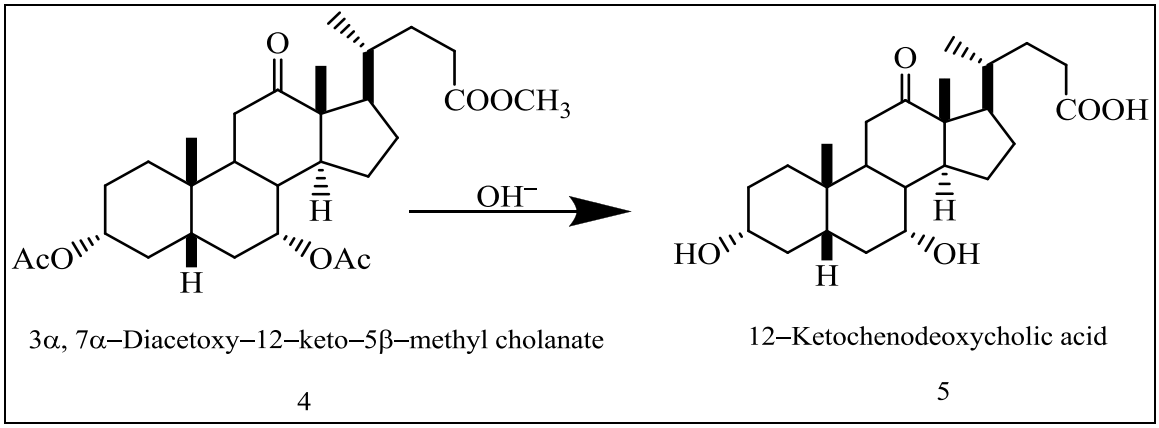
SCHEME 3: REACTION OF ACETYLATION OF METHYL CHOLATE TO THE 3α, 7α-DIACETOXY-12α-HYDROXY-5β-METHYL CHOLANATE

The third synthesis step involves the oxidation of OH group in position C_{12} of 3α, 7α-diacetoxy-12α-hydroxy-5β-methyl cholanate (3) addition of CrO_3 oxide of H_2CrO_4 acid, to give the 3α, 7α-diacetoxy-12-keto-5β-methyl cholanate (4), as shown in **Scheme 4** [5].



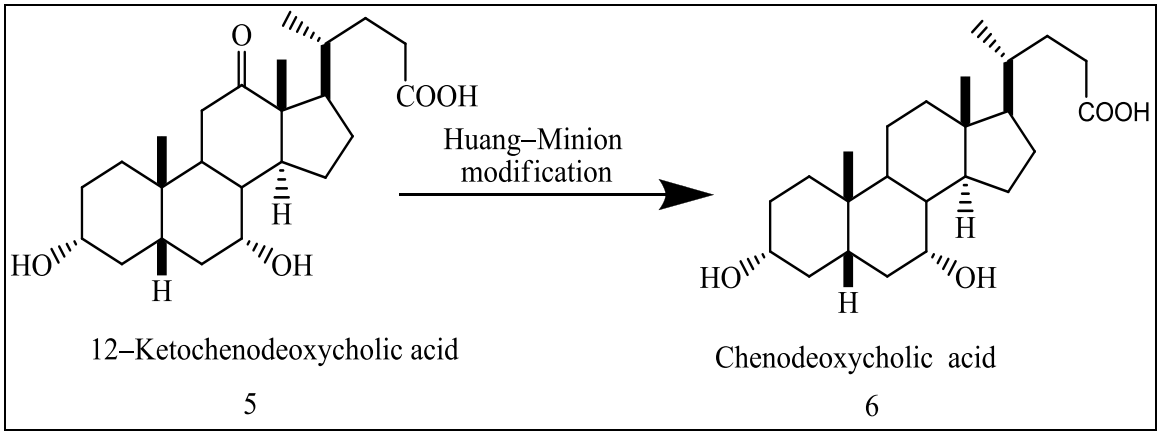
SCHEME 4: REACTION OF OXIDATION OF 3α, 7α–DIACETOXY-12α-HYDROXY-5β-METHYL CHOLANATE TO THE 3α, 7α-DIACETOXY-12-KETO-5β-METHYL CHOLANATE [5]

The fourth step is to saponify 3α, 7α-diacetoxy-12-keto-5β-methyl cholanate (4) to obtain a 12-ketochenodeoxycholic acid (5), as shown in **Scheme 5**.



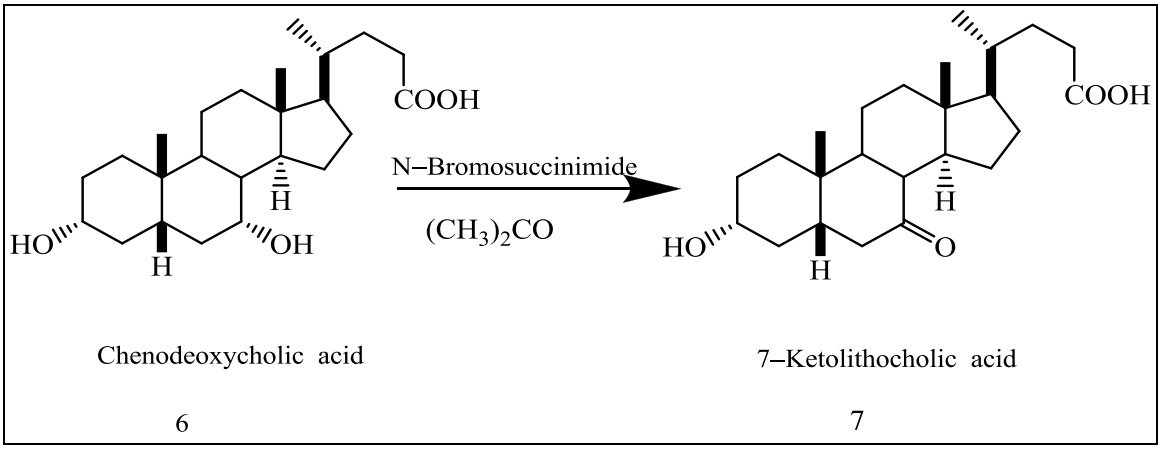
SCHEME 5: REACTION OF SAPONIFY OF 3*α*, 7*α*-DIACETOXY-12-KETO-5β-METHYL CHOLANATE TO OBTAIN A 12-KETOCHENODEOXYCHOLIC ACID [5]

The fifth step is to reduce the carbonyl group located at the 12 position of 12-ketochenodeoxycholic acid (5). In order to obtain the chenodeoxycholic acid (6) was used the Huang-Minion modification, as shown in **Scheme 6**.



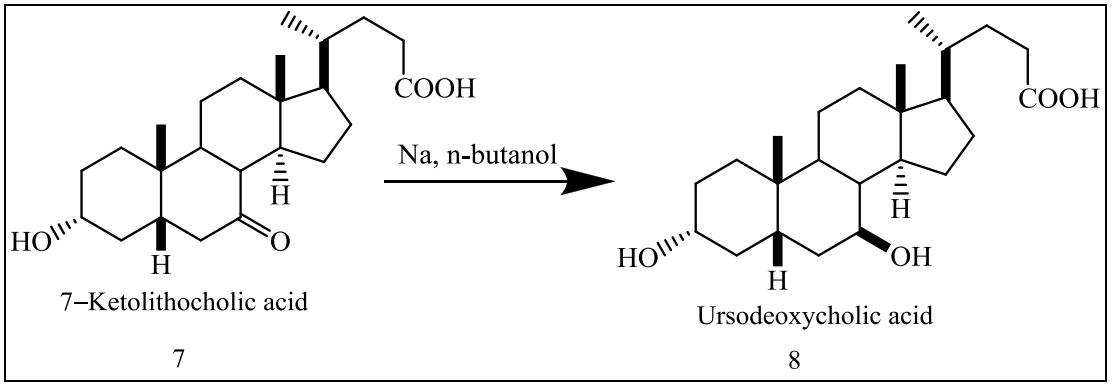
SCHEME 6: HUANG-MINION MODIFICATION OF 12-KETOCHENODEOXYCHOLIC ACID TO OBTAIN A CHENODEOXYCHOLIC ACID [5]

The sixth step is to oxidize the hydroxyl group located at the 7α position of chenodeoxycholic acid (6) by adding a N-bromosuccinimide in acetone, thus obtaining a 7-ketolithocholic acid (7) **Scheme 7**.



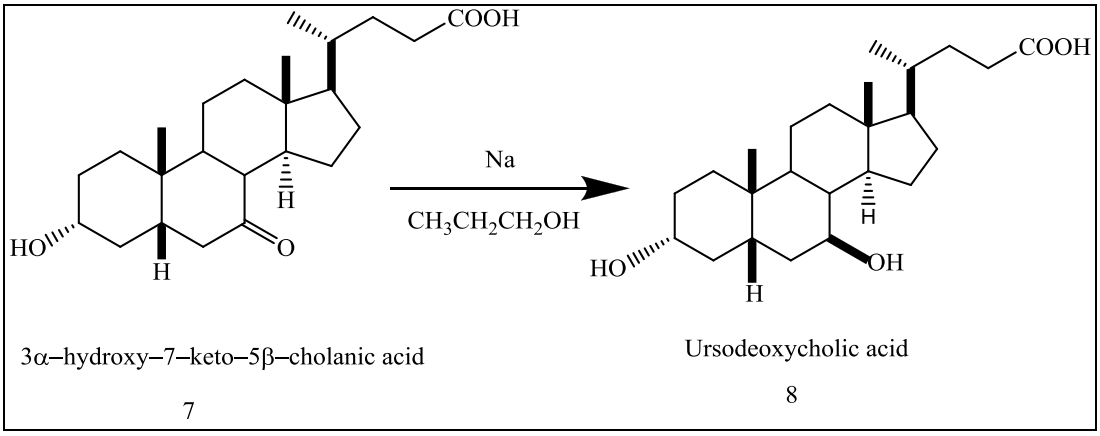
SCHEME 7: REACTION OF OXIDATION OF CHENODEOXYCHOLIC ACID TO THE 7-KETOLITHOCHOLIC ACID [5]

The seventh and final step is to reduce 7-ketolithocholic acid (7) by catalytic reduction carried out with sodium in n-butanol, thus obtaining the final product, ursodeoxycholic acid (8) **Scheme 8** [5].



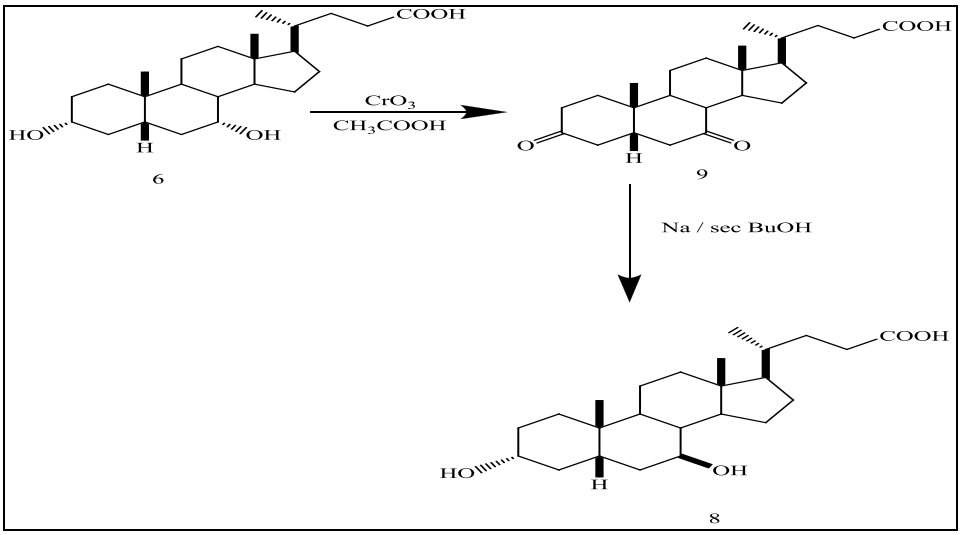
SCHEME 8: REACTION OF REDUCTION OF 7-KETOLITHOCHOLIC ACID TO THE URSODEOXYCHOLIC ACID [5]

Ursodeoxycholic acid is very important because of its ability to dissolve cholesterol-gallstones. Previously, the synthesis were based on the reduction of 3α-hydroxy-7-keto-5β-cholanoic (7) acid with sodium in alcohols, and selected by catalytic hydrogenation in acidic medium. The ursodeoxycholic acid (8) was obtained in high yield in a patented process, including sodium in propanol **Scheme 9** [6].



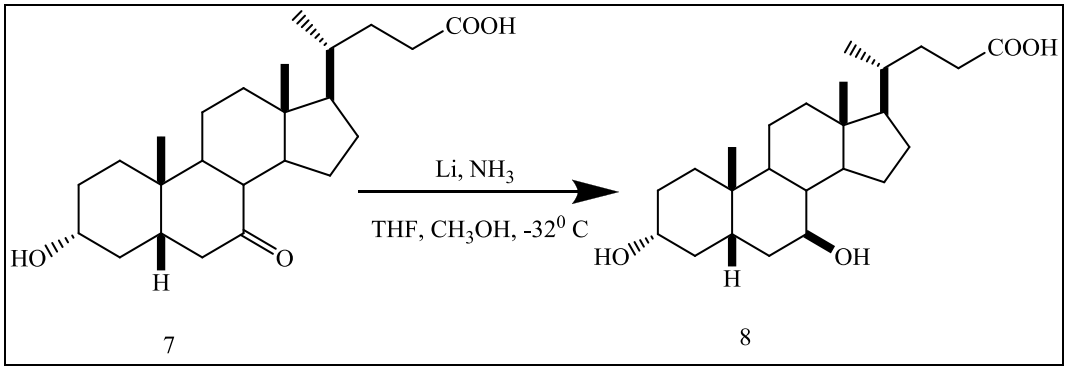
SCHEME 9: THE SYNTHESIS OF URSODEOXYCHOLIC ACID FROM 3α-HYDROXY-7-KETO-5β-CHOLANIC ACID [6]

Samuelsson was also synthesized ursodeoxycholic acid in high yield, using the same reactions. Kitakaro and Sato are carried out the oxidation of chenodeoxycholic acid (6) using CrO_3 in glacial acetic acid. In this way, they got a 3.7-diketo-5β-cholanic acid (9). Further reduction of this compound with sodium in the secondary butanol was obtained a ursodeoxycholic acid (8) **Scheme 10**[6].



SCHEME 10: THE TRANSFORMATION OF CHENODEOXYCHOLIC TO URSODEOXYCHOLIC ACID [6]

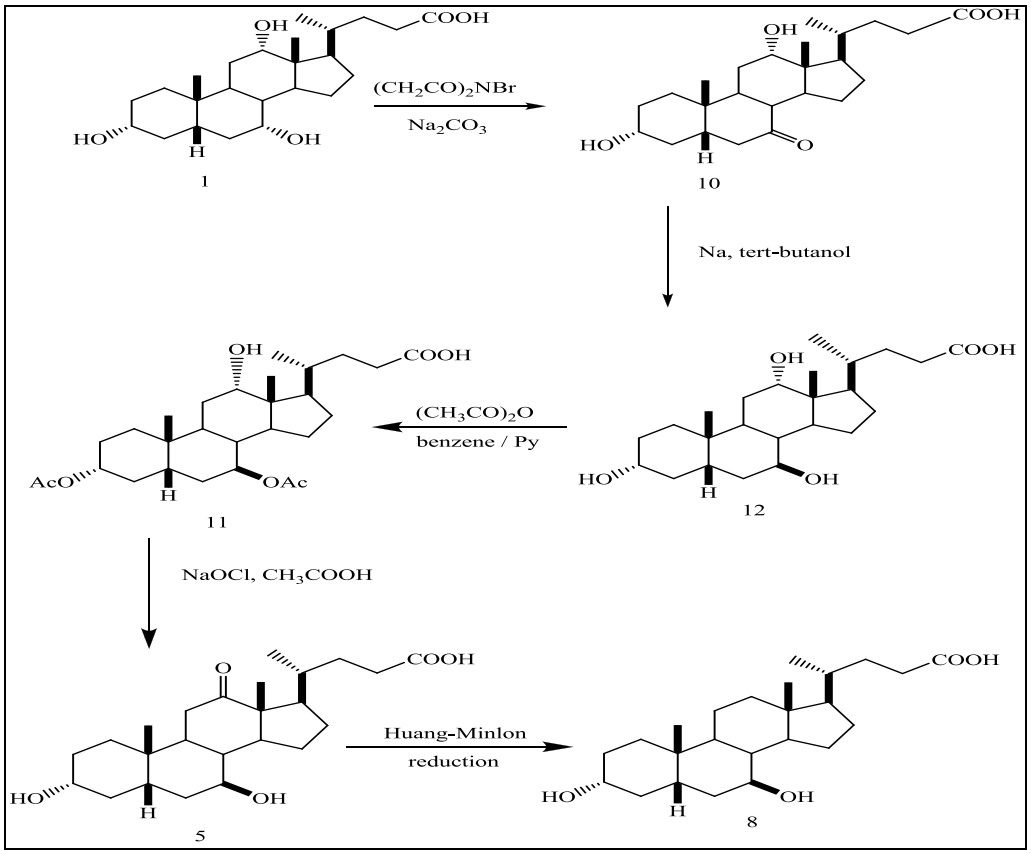
The ursodeoxycholic acid is obtained by Birch's reduction using the Li in liquid ammonia and using tetrahydrofuran or ethanol as solvents at a temperature of -32 °C to give the 3α-hydroxy-7-keto-5β-cholanoic acid (7) **Scheme 11** [6].



SCHEME 11: BIRCH REDUCTION OF 3α-HYDROXY-7-KETO-5β−CHOLANIC ACID TO URSODEOXYCHOLIC ACID[6]

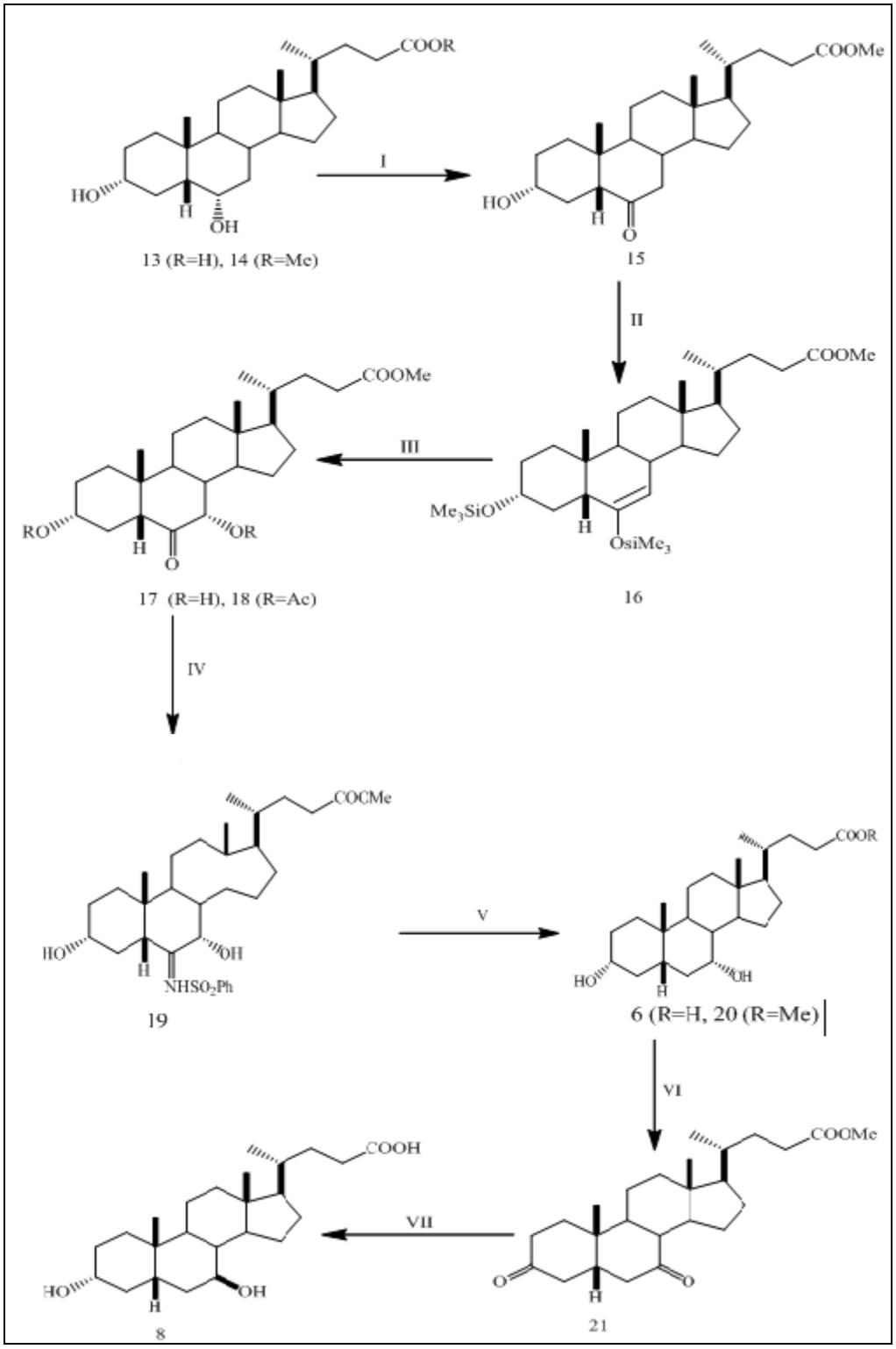
Synthesis of ursodeoxycholic acid from cholic acid is shown in **Scheme 12**. Selective oxidation of cholic acid (1) in an aqueous solution of sodium carbonate with N-bromosuccinimide resulting in the formation of 3α, 12α-dihydroxy-7-keto-5β-cholanoic acid (10).

In the second phase, the 3α, 12α-dihydroxy-7-keto-5β-cholanoic acid (10) under the influence of the sodium in tert-buthanol is converted into the 3α, 7β, 12α-trihydroxy-5β-cholanoic acid (12). Then in the third phase the 3α, 7β, 12α-trihydroxy-5β-cholanoic acid (12) was obtained a 3α, 7β-diacetoxy-12α-hydroxy-5β-cholanoic acid (11) by the selective acetilation with acetic anhydride and pyridine in benzene, which in fourth phase, after oxidation using the sodium hypochlorite in acetic acid was obtained a 3α, 7β-dihydroxy12-keto-5β-cholanic acid (5) and finally in fifth phase, this compound by the Huang-Minlon reduction gave ursodeoxycholic acid (8) **Scheme 12** [6].



SCHEME 12: THE SYNTHESIS OF URSODEOXYCHOLIC ACID FROM CHOLIC ACID [6]

Derivatives of hyodeoxycholic acid (13) are also used for the synthesis of ursodeoxycholic acid. Methyl hyodeoxycholat (14) subjected to a selective oxidation reaction with pyridinium dichromate (PDC), led to the formation of the methyl ester derivative of 3α-hydroxy-6-oxo-5β-cholanoic acid (15), which was treated with trimethylsilyl chloride in the lithodeoxycholic acid, yields a 3α, 6-enol sylil ether (16) **Scheme 13** [6].



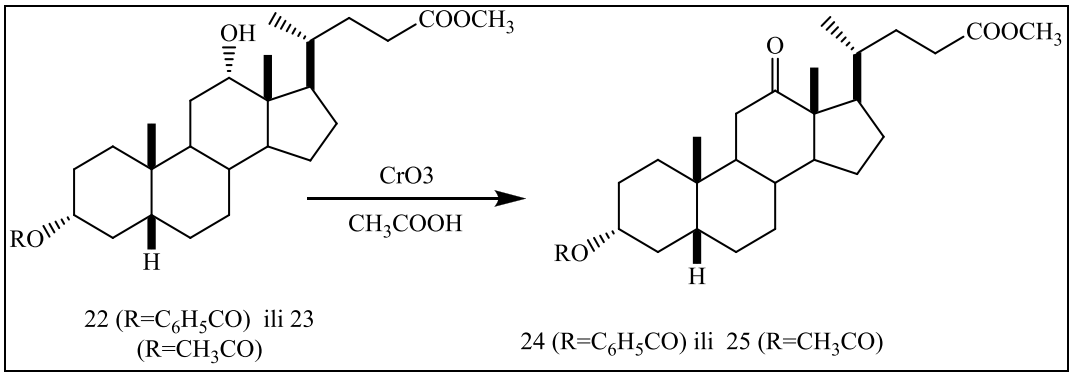
SCHEME 13: SYNTHESIS OF URSODEOXYCHOLIC ACID FROM METHYL HYODEOXYCHOLATE

Reagents I- PDC (pyridinium dichromate) – CH_2Cl_2; II- LDA (lithodeoxycholic acid), II- TMSCl (trimethylsilylchloride), THF; III- m-CPBA (meta-chloroperoxybenzoic acid), CH_2Cl_2 (methylene chloride) ili O_3, Py – CH_2Cl_2; IV- Benzene sulfonyl hydrazide ($C_6H_5SO_2NHNH_2$), 0.5% HCl – MeOH; V- $NaBH_4$, HOAc; VI- Jones reagent; VII. Li - NH_3 [6].

This 3α, 6-silyl enol ether (16) was oxidised with m-chloroperoxybenzoic acid to provide a methyl-3α, 7α- dihydroxy- 6- keto- 5β- cholanate (17). Certain hydrazide (19) was prepared by reaction of the methyl ester of 3α, 7α- diacetoxy- 6-keto-5β-cholanoic acid (18) with a hydrazide benzene-sulfonic acid, which was reduced with sodium borohydride ($NaBH_4$) giving chenodeoxycholic acid (6). Methyl chenodeoxycholate (20) was oxidized with Jones reagent to a corresponding diketo derivative (21) which are subject to a reduction in liquid ammonia to give the desired ursodeoxycholic acid (8) **Scheme 13** [6].

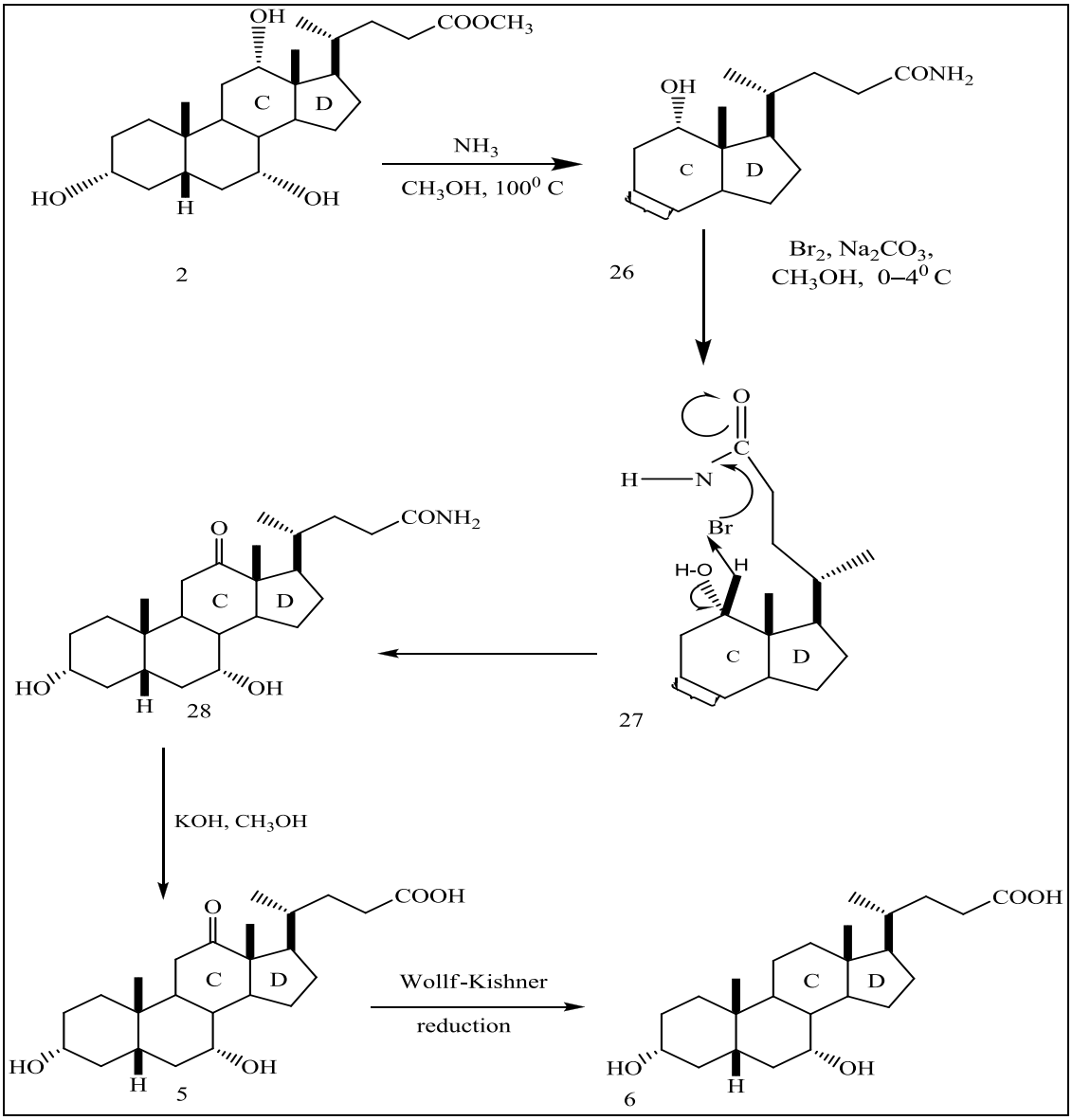
Ursodeoxycholic acid and its taurine and glycine derivatives are hydrophilic bile acids able to prevent apoptosis in cells induced by hydrophobic bile acids. Ursodeoxycholic acid and chenodeoxycholic acid showed a significant dose-dependent cytotoxicity in cancer cell lines of human breast and were able to inhibit cell proliferation and induce apoptosis in cancer cells of human breast [7]. Ursodeoxycholic acid and chenodeoxycholic acid displayed the poor effects on the sustainability of cancerous cells of human prostate. Natural bile acids such as ursodeoxycholic acid and chenodeoxycholic acid were found to be effective in causing the dissolution of cholesterol gallstones in humans. Bile acids reduce the amount of cholesterol, which is excreted from bile [7]. Ursodeoxycholic acid, increases the amount of cholesterol in the liver, converting it into bile acids, which can contribute to reducing the amount of cholesterol absorbed from the small intestine [7].

2.2. Synthesis of 12-Oxo Derivative of the Selected Bile Acids: Kaziro and Shimada were first explored the selective oxidation of deoxycholic acid using the chromic acid. They translated deoxycholic acid to 3α-hydroxy-12-keto-5β-cholanoic acid. Selective oxidation of the 12α-hydroxyl group of deoxycholic acid is the crucial step in the synthesis of lithocholic acid. The first step is acylation of the 3α-hydroxyl group to followed by oxidation of the 12α-hydroxyl group. In the **Scheme 14** is shown the critical step in the synthesis of lithocholic acid involving oxidation of the 12α-hydroxy group of deoxycholic acid esters by the influence of chromium VI oxide in acetic acid **Scheme 14** [8].



SCHEME 14: THE CRITICAL STEP IN THE SYNTHESIS OF LITHOCHOLIC ACID INVOLVING OXIDATION OF THE 12α-HYDROXYL GROUP OF DEOXYCHOLIC ACID ESTERS [8]

In the first step of the synthesis, the cholic acid is treated with ammonia in methanol, whereby it is formed a amide of cholic acid. Then, in the second phase the amide of cholic acid may be selectively oxidized into the corresponding 12-keto derivative using an equivalent amount of bromine in alkaline methanol in the presence of sodium carbonate via formation of an N-bromoamide in the side chain. The amide of 3α, 7α- dihydroxy- 12-keto- 5β-cholanoic acid was treated with potassium hydroxide and methanol and it is formed a 3α, 7α-dihydroxy-12-keto-5β-cholanoic acid [13] **Scheme 15**. 3α, 7α-dihydroxy-12-keto-5β-cholanoic acid is detected in human feces. This acid and its esters, are the main intermediates in the synthesis of chenodeoxycholic acid and cholic acid. This keto acid could be prepared by oxidation of the methyl ester of 3α, 7α- diacetoxy- 12α- hydroxy- 5β-cholanoic acid with CrO_3. Its subsequent reductive Wolf-Kishner's method, is obtained chenode-oxycholic acid **Scheme 15** [8, 13].



SCHEME 15: CHOLIC ACID OXIDATION TO THE 12-KETO DERIVATIVE *VIA* AN N-BROMOAMIDE AND SUBSEQUENT FORMATION OF CHENODEOXYCHOLIC ACID

The effect of keto derivatives of cholic acid (sodium salt and methyl ester) and it was found that sodium 3α, 7α- dihydroxy- 12- keto- 5α- cholanat promoted absorption of quinine, enhanced the analgesic effects of morphine and prolonged the sleep time induced by pentobarbital. Methyl 3α, 7α-dihydroxy-12-keto-5α-cholanat also improved the effect of morphine and increased the time of sleeping induced by pentobarbital, but inhibited quinine uptake [7, 8].

2.3. Physico-chemical Properties of Bile Acids: In water, when is dissolved amphiphilic molecules (bile acids), occurs a process of interrupting hydrogen bonds between the water molecules. Then comes to the formation of hydration layer around the solute (bile acids). Amphiphilic solution is divided into two subsystems: a hydrophobic and a hydrophilic part. At the both subsystem is built a cage around the solvatacion amphiphilic molecules of bile acids. Between the water molecules, formed the same number of hydrogen bonds. The change in enthalpy for each subsystem, during the formation of hydration shell, is zero [9, 10].

$$\Delta H(a) \approx \Delta H(b) \approx 0 \text{ .....(Eq.1)}$$

During the process of forming layers of hydrated water molecules decreases entropy (translational and rotational).

$$\Delta S(a) \approx \Delta S(b) < 0 \text{ .....(Eq. 2)}$$

Molecules of water with a hydrophilic side of bile acids (amphiphilic molecules) are stabilized, while the molecules with the hydrophobic side of bile acids unstabilized. The ratio of hydrophilic and hydrophobic surface bile acids determines the overall change in Gibbs free energy of formation of hydration cage (ΔG). At molecules of bile acids, hydrophobicity is expressed through the logP value [9, 10]. It can be examined by using chromatographic parameters and over the partition coefficient. For hydrophilic subsystem there is an additional negative enthalpy ΔH < 0, which is distributed in the environment (interior solution), thereby giving a positive entropy change (environment) [11].

In general hydrophobicity of the bile acids can be expressed in terms of the logarithm of the partition coefficient (log P) between 1-octanol and water. As for the tests bile acids, through chromatographic techniques generally applied reverse high performance liquid chromatography (HPLC) and reverse phase thin layer chromatography, high-resolution (HPTLC) [9, 10].

If the examination by reversed phase liquid chromatography, it is clear that there is a balance between the molecules of bile acids from the polar mobile phases (BA aq) and bile acids (BAs), which are adsorbed on a hydrophobic stationary phase [11]. This equilibrium is characterized by a constant balance K_{ad} = [BA(s)]/[BA(aq)], which according to the Jones connected to the retention factor [11].

$$K=(Kad * Vsp)/Vpp = Kad*\varphi \text{..... (Eq. 3)}$$

Vsp is the volume of the stationary phase of hydrocarbons, and V_{pp} is the volume of polar phase. Their relationship is defined as the characteristics of the column. Retention factor (capacity factor) is represented as:

$$K= t_{ba}-t_0/t_0$$

*t_0 elution time of the mobile phase, t_{ab} - elution time of bile acids

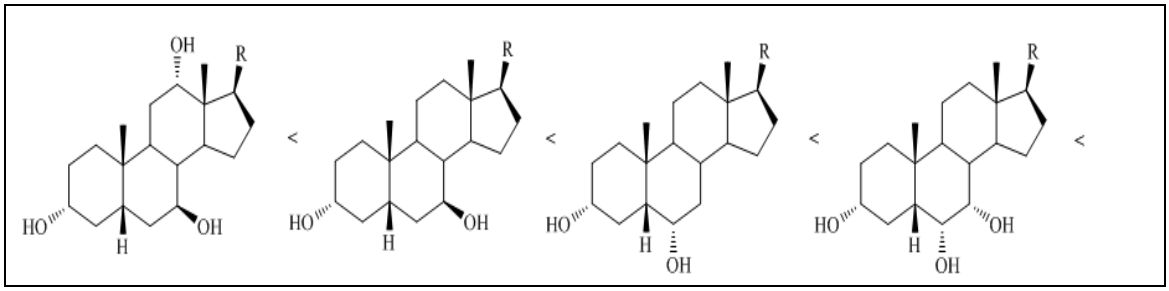
The standard change of Gibbs energy for the reaction can be represented as:

$$\Delta G°ad = -RT \ln k_{ad} = -RT \ln k + RT \ln\varphi \text{.....(Eq. 5)}$$

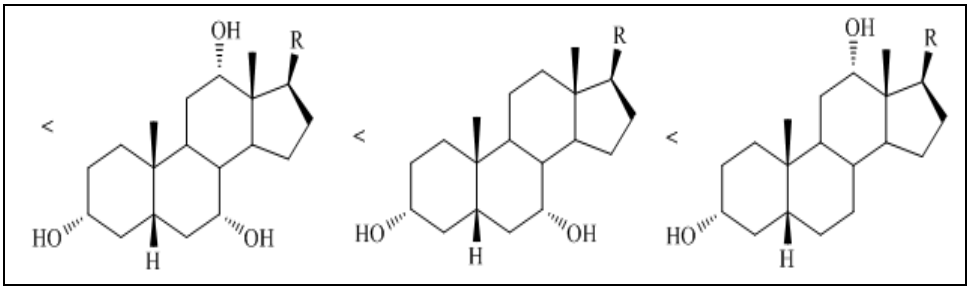
Both chromatographic methods (reverse phase HPLC and RPTLC), for adsorption of bile acids on a hydrophobic stationary phase, contribute that the molecules of the hydrophilic solvent to leave a mobile phase, giving a positive contribution to the entropy. Unsolvated hydrophobic surface of bile acids, bind to the surface of the adsorbent hydrophobic interactions, such as London's dispersion forces, induced dipole. On the basis of this leads to the establishment of a negative enthalpy of contribution to the process of adsorption. Negative enthalpy is wasted in a solution of mobile phase (environment), thus giving a positive change in the entropy of the environment [10].

At hydroxy bile acid derivatives (which are also the most studied in the literature) on the basis of the hydrophobicity of the retention time in reverse phase chromatography to grow in the following series of conjugated bile acid: R = CH (CH_3) CH_2 CH_2COOH) ursocholic acid (configuration of the OH group: 3α, 7β, 12α) < ursodeoxycholic acid (3α, 7β) < chiodeoxycholic acid (3α, 6α) < chiocholic acid (3α, 6α, 7α) < chenodeoxycholic acid (3α, 7α) < deoxycholic acid (3α, 12α).

The upper sequence is identical at unionized and ionized bile acids or in the case glyco-(R=CH (CH_3) $CH_2CH_2CONHCH_2COOH$) and tauro conjugated ($CH_2CH_2CONHCH_2CH_2SO_3H$) bile acids. This indicates the importance of the steroid nucleus of bile acids in adsorption on a hydrophobic stationary phase [11].



Ursocholic acid Ursodeoxycholic acid Chiodeoxycholic acid Chiocholic acid



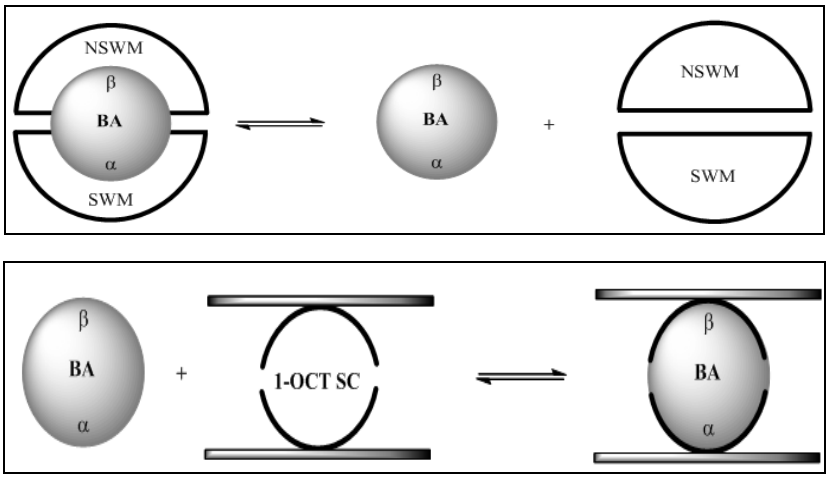
Cholic acid Chenodeoxycholic acid Deoxycholic acid

SCHEME 16: THE ORDERY OF HYDROPHOBICITY OF BILE ACIDS [11]

From the above order of hydrophobicity of bile acids can be concluded that the β side of steroidal skeleton at least hydrophobic bile acids in relation to the axial α (a) OH group with OH groups have shifted to the high levels of the steroid skeleton (SSMP). The water molecules from the hydrated layers are moved completely on the side β steroid skeleton. As the bile acid biplanar compound, a hydrophobic plane (stationary phase) is connected, the side of the steroid skeleton, which is largely hydrophobic [12]. Typically with natural bile acids and their oxo derivatives are β convex side of the steroid skeleton (as an exception to the enantiomer of cholic acid with β configuration with all three OH groups, where is α side of steroidal skeleton more hydrophobic than the β side of the steroid skeleton). Between CMC values of bile salts and value of retention parameters (RPHPLC) there is a good correlation, indicating the importance of hydrophobicity (particularly hydrophobicity of β side by steroid nucleus.) amphiphilic in their selfassociation [11, 12].

Dependence of a change of retention coefficients or hydrophobicity index with a pH value of the mobile phase with the unconjugated bile acids and glyco-conjugated bile acid is sigmoid curve (tauro-conjugates do not show pH dependency). For each bile acids non-ionized form is more hydrophobic than the ionized form. What is a bile acid hydrophobic to more retention ratios differ between the molecular and ionized forms. For each bile acid hydrophobicity there following order: tauro-conjugated < glyco-conjugated < unconjugated.

At the stage of unsolvation removal of bile acids from the hydration layer SWM did enthalpy unfavourable, since the break up additional hydrogen bond between the OH group of the steroid skeleton or water molecules, it is necessary to bring the heat ΔHε. Formally, the transition of molecules (ions) of bile acid from the aqueous solution in 1-octanol can be seen **Scheme 17** as the sum of the reaction (process) the complete dehydration of the molecule, where the water molecules from the solvated layer come back into the interior of the solution, and the reaction installation unsolvated bile acid into the solvated cage 1-octanol (OCT-1 SC) [10].

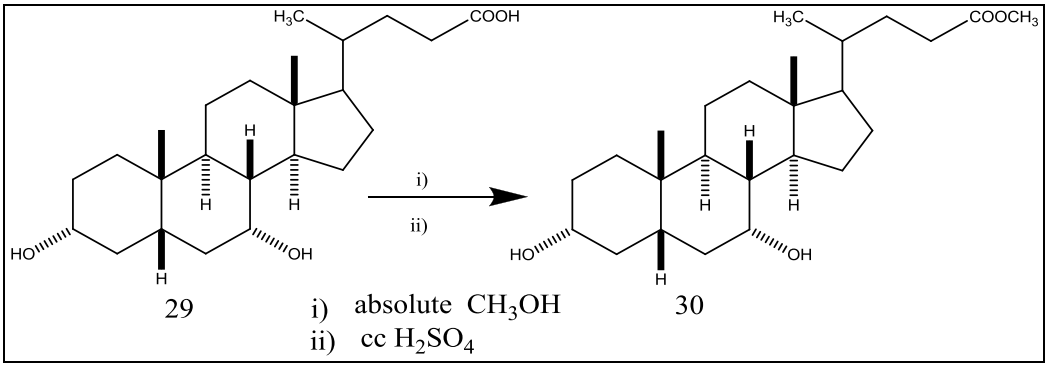


SCHEME 17: BA = BILE ACIDS, SWM = STABILISED WATER MOLECULES, NSWM = NONSTABILISED WATER MOLECULES, 1-OCT SC= 1-OCTANOL SOLVATATION CAGE [10]

3. MATERIAL AND METHODS: All chemicals used in the experimental work were purchased from the manufacturer Sigma-Aldrich and before use are not further purified. To elicit a TLC plate was used H_2SO_4 1:1 with heating in a hot plate. Chromatographic purification of the substances was performed by column "flash chromatography" making use of the manufacturer Merck silica gel (0.04 - 0.063 mm).

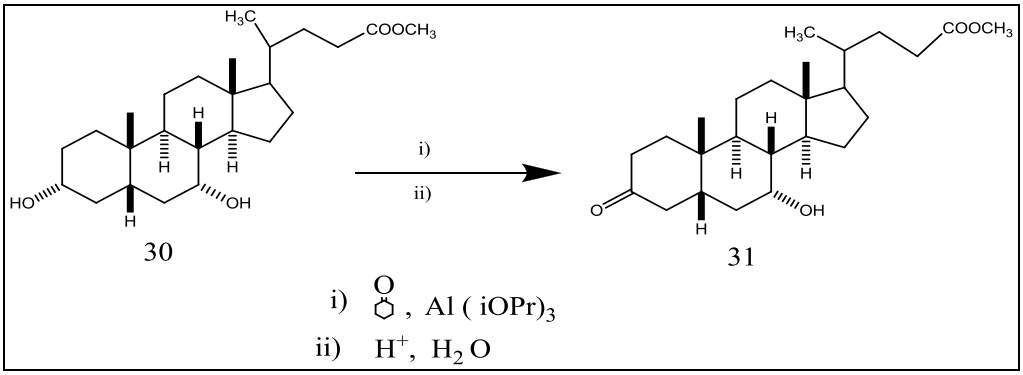
3.1. Synthesis of Methyl Ester of Chenodeoxycholic Acid: A solution of chenodeoxycholic acid (29), (5g, 1.28×10^{-2} mol) in absolute methanol (CH_3OH) (50 ml) was heated with stirring the reaction mixture as long as the substance does not dissolve. After that, the heating was interapted and added 4 drops of cc H_2SO_4. Heating was performed with reflux for 1 h and 30 min. After completion of the reaction, the volume of the reaction mixture was reduced by evaporation of CH_3OH on a rotavapor. Then the concentrated reaction mixture was transferred to 200 ml of H_2O. NaCl was added, because of salting out of the reaction mixture.

The crystals were separated by vacuum filtration and dried in the air, whereby were obtained a large white crystals of methyl ester of chenodeoxycholic acid (30) in a yield of 96.72%.



SCHEME 18: SYNTHESIS OF METHYL ESTER OF CHENODEOXYCHOLIC ACID FROM THE CHENODEOXYCHOLIC ACID

3.2. Synthesis of Methyl Ester of 7α-Hydroxy-3-Oxo-5β−Cholanoic ACID: In a solution of methyl ester of chenodeoxycholic acid (30) (1.15 g, 0.003 mol) in cyclohexanone (10 ml) was added aluminium isopropoxide Al $(iOPr)_3$ (0.41g, 0.002 mol). The reaction mixture was heated at 100 °C for 5 hours. After completion of the reaction, the reaction mixture was acidified with HCl 1: 1 and the excess cyclohexanone was removed by distillation with steam. The solid residue after distillation in the bubble was separated from the aqueous layer and dissolved in chloroform. The aqueous layer was extracted with chloroform in a separating funnel. The combined chloroform extracts and the chloroform solution of solid residue after distillation were evaporated after drying with Na_2SO_4. The residue after evaporation was purified on a silica gel column, whereby was used a dichloromethane-acetone (11:2). The yield of methyl ester of 7α-hydroxy-3-oxo-5β-cholanoic (31) is 60.53%.



SCHEME 19: SYNTHESIS OF METHYL ESTER OF 7α-HYDROXY-3-OXO-5β-CHOLANOIC ACID

4. RESULTS: NMR spectra were recorded on a Bruker AC - 250 with an operating frequency assigned to the protons of 250.13 MHz and for the carbon nucleus C_{13} 62.9 MHz. The spectra were recorded in deuterated chloroform.

4.1. ^{1}H NMR spectrum of 7α-hydroxy-3-oxo-5β-cholanoic acid

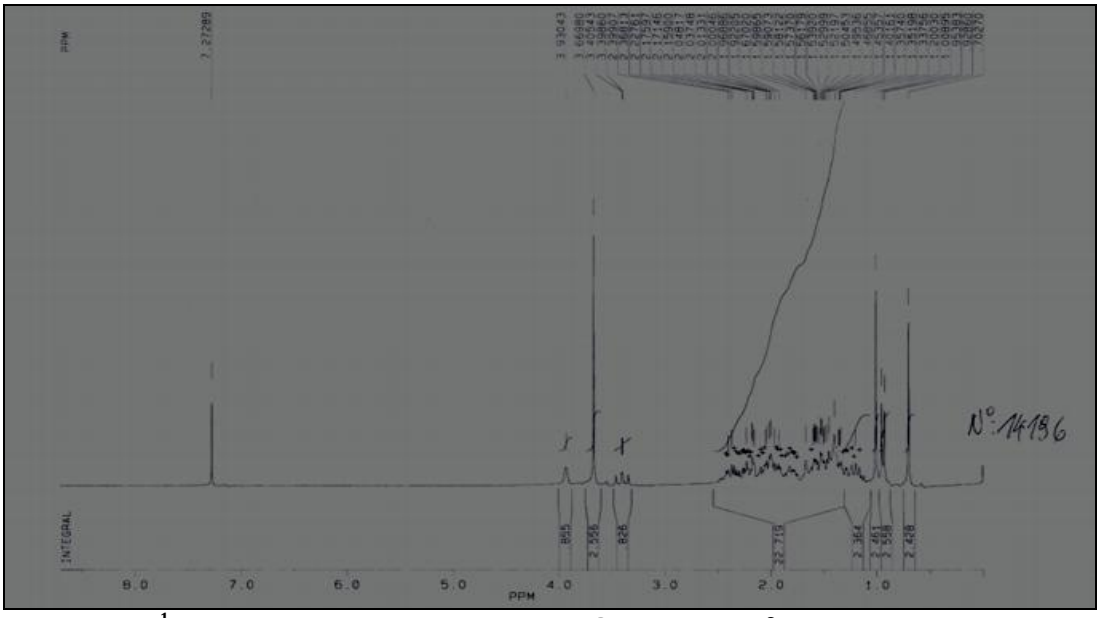


FIG. 1: ^{1}H NMR SPECTRUM OF 7α−HYDROXY-3-OXO-5β-CHOLANOIC ACID

^{1}H NMR ($CDCl_3$, ppm): 0.70 (s, 3H, CH_3-18); 0.93 (d, 3H, CH_3-21, J = 6.3 Hz); 1.01 (s, 3H, CH_3-19); 1.20-2.40 (steroidni skelet); 3.40 (dd, 1H, H_a (CH_2-2), J_{gem} = 14.9 Hz, $J_{vic.}$= 4.5 Hz); 3.66 (s, 3H, COOMe); 3.93 (s, 1H, CH-7).

4.2. ^{13}C NMR spectrum of 7α-hydroxy-3-oxo-5β-cholanoic acid

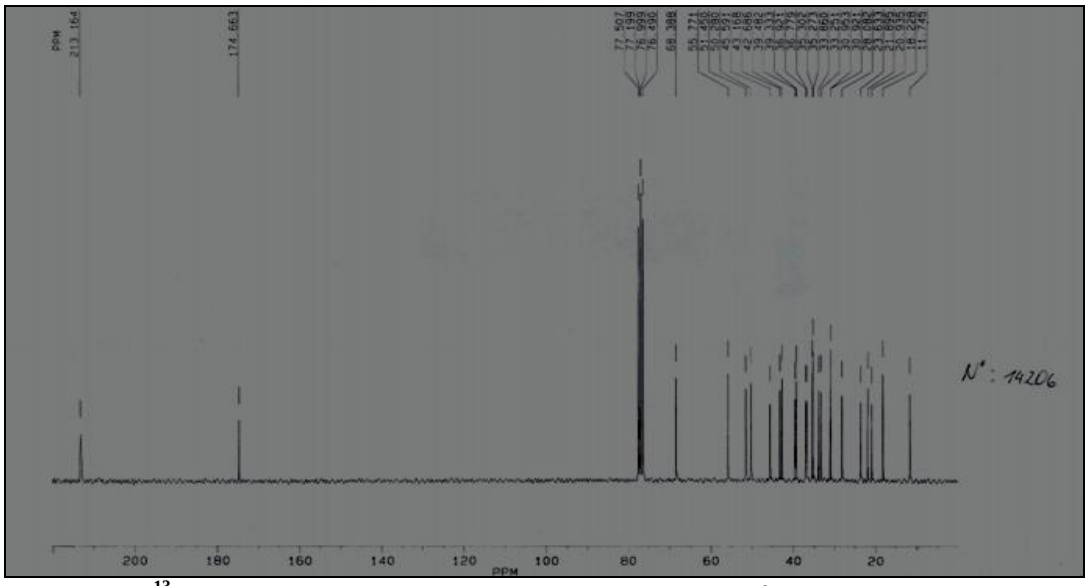


FIG. 2: ^{13}C NMR SPECTRUM OF 7α-HYDROXY-3-OXO-5β-CHOLANOIC ACID

^{13}C NMR ($CDCl_3$, ppm): 11.7 (C-18); 18.2 (C-21); 20.9 (CH_2); 21.9 (C-19); 23.6 (CH_2); 28.1 (CH_2); 30.92 (CH_2); 30.95 (CH_2); 33.25 (CH); 33.86 (CH_2); 35.27 (Cq); 35.30 (CH); 36.77 (CH_2); 36.92 (CH_2); 39.33 (CH); 39.48 (CH_2); 42.68 (Cq); 43.16 (CH); 45.59 (CH_2); 50.28 (CH); 51.45 (CH_3); 55.77 (CH) 68.38 (CH), 174.7 (C24); 213.2 (C3).

DISCUSSION: In this paper we analyzed the importance of ursodeoxycholic acid in the treatment of gallstones, primary sclerosing cholangitis, primary billary cirrhosis, choledo-cholithiasis and hepatolithiasis associated with Caroli syndrome. We discussed the syntheses of 12-keto derivatives of bile acids and of their sodium salts and methyl esters that have a role in promoting the absorption of quinine, followed by improving the analgesic effect of morphine and prolongation of time to sleep caused by pentobarbital.

The above-mentioned 12-keto derivatives of selected bile acids take part in improving the permeability of the blood-brain barrier. Special emphasis we put on our synthesis of 3-keto derivatives of selected bile acids such as a 3-oxo-7α-hydroxy-5β-cholanoic acid We determined a application of 3-keto derivatives of selected bile acids in medicine, which included the production of beneficial effects on sexually transmitted diseases, primary biliary cirrhosis, gall stones, cystic fibrosis, cancer, diabetes and leukemia.

The hydrophobic β side steroid skeleton by the hydrophobic stationary phase in 5β - bile acid derivatives is determined by the number and orientation of the OH and oxo groups, or their spacing of the steroid skeleton, and thus forms the steroid skeleton. Overall hydrophobic surface (lipophilicity) of the bile acid is generally determined by the number of OH and oxo group, and to a lesser extent their orientation. The lipophilicity of the bile acids is best determined by the logarithm of partition coefficient between 1-octanol and water. In biological systems, some phenomens are better described by the hydrophobicity β side steroid skeleton.

CONCLUSION: The amphiphilic molecules in the aqueous solutions form the aggregates - micelles. In the course of increasing the amount in the solution of the amphiphilic molecules, more water molecules participating in the hydration of the hydrophobic surface which is the entropic adversely. Between the critical micelle concentration value of bile acid salts and value retention parameters (RPHPLC) there is a good correlation, indicating the importance of hydrophobicity (particularly hydrophobicity of β side by steroid nucleus) amphiphile in their selfassociation. Self association of bile acid salts also best describes the hydrophobicity of the convex side of the steroid system of rings.

At the same time the presence of oxo and OH groups in the steroid nucleus of bile acids favor the formation of aggregates (aggregates, such as reverse micelles) bile acids and certain drugs (verapamil, lidocaine) in chloroform (model for depo effect in the cell membrane).

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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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