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MONO, DI AND TRIKETO DERIVATIVES OF CHOLIC ACID AND THEIR HYPOGLYCEMIC EFFECTS

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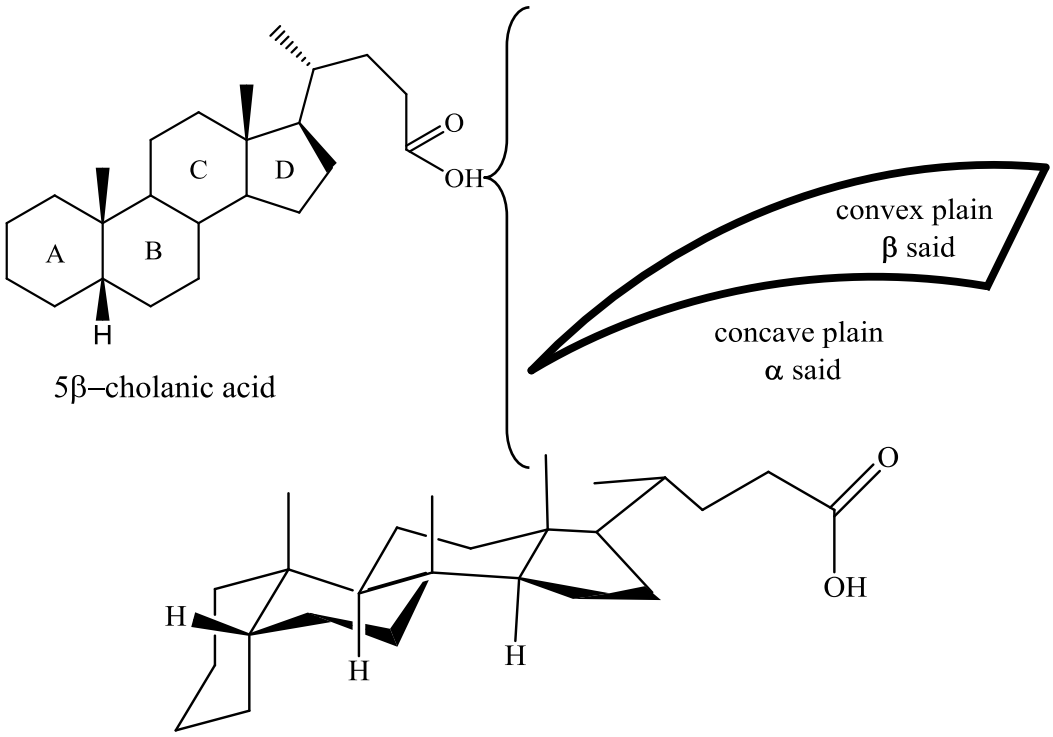
ABSTRACT

Bile acids are amphiphilic molecules, which consist of a hydrophobic and a rigid steroid nucleus to which they are attached a hydrophilic hydroxyl groups, as well as flexible aliphatic acid side chain. The number, position and orientation of hydroxyl groups in the bile acid molecules may vary. The steroidal core of bile acids constitutes a saturated cyclopentanoperhydrophenanthrene hydrocarbon skeleton, consisting of three six-member (A, B, C) and one five-membered ring (D). Natural bile acids are derivatives of 5β-cholanic acid, wherein A and B rings are cis-linked. The planar structure of amphiphilic bile acid, or the existence of a hydrophilic and hydrophobic surface molecule affects its physico-chemical characteristics and the ability to self-aggregation conditions. Monoketo derivatives of bile acids facilitate the permeability of the membrane. It has been shown that the 3α,7α−dihydroxy−12−keto−5β−cholanic acid exhibits hypoglycemic effect in diabetes type 1. Better effects are noted if mentioned 3α,7α−dihydroxy−12−keto−5β−cholanic acid is used in combination with hypoglycemic gliclazide or preparate of stevioside. The best effect in glycemic control was achieved when rats with type 1 diabetes were pretreated with probiotics, and then simultaneously with the use of a derivative of 3α,7α−dihydroxy−12−keto−5β−cholanic acid and gliclazide. The latest studies of diabetes in rodents in experimental models have shown that synthetic derivatives such as sodium 3α,7 −dihydroxy−12−keto−5β−cholanate results in the reduction of blood glucose concentration, which is the concentration reached 54% of that obtained following subcutaneous administration of the same dose of insulin. The aim of this paper is to explain the synthesis of various mono, di and tri keto derivatives of cholic acid, as well as to analyze the discovery of the association between bile acids (the most of 3α,7α−dihydroxy−12−keto−5β−cholanic acid) and glucose regulation, which gives us a new perspective in the design of hypoglycemic drugs in the treatment of diabetes.

KEYWORDS: Bile acids, keto derivatives of cholic acid, hypoglycemic drugs.

INTRODUCTION

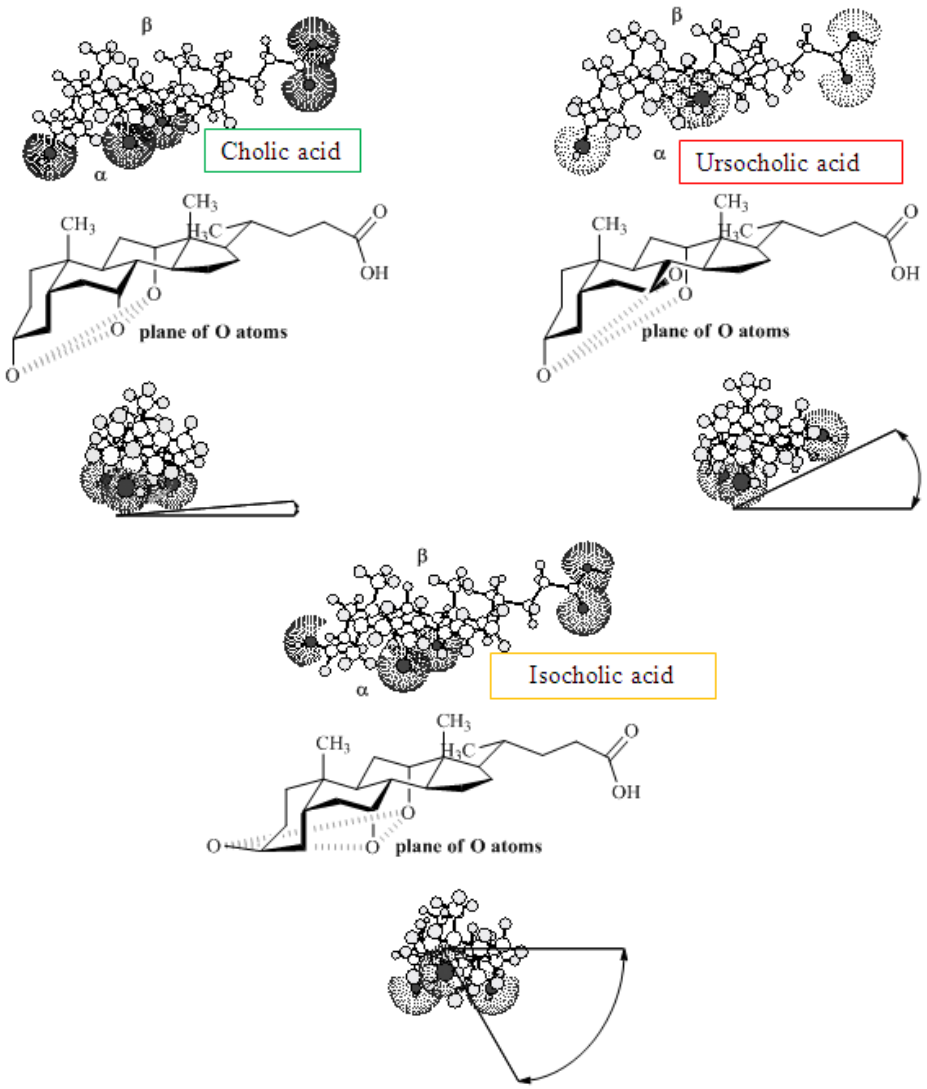
Bile acids are steroidal compounds consisting of a cyclopentanoperhydrophenanthrene ring and contain 24 carbon atoms. Bile acids, which are enzymatically produced in the liver of humans and other mammals are primary bile acids (cholic acid and chenodeoxycholic acid) from which, under the influence of anaerobic bacteria in the colon are obtained secondary bile acids (deoxycholic acid and lithocholic acid) by 7α−dehydroxylation and deconjugation reactions. Primary and secondary bile acids are hydroxy derivatives of 5β−cholanic acid.[1] Some lizards contain the bile acids, which represent the hydroxyl derivatives of 5α−cholanic acid. There is a difference in the geometry of interconnection of the rings A and B of the steroid skeleton in 5α−cholanic acid and 5β−cholanic acid. In 5β-cholanic acid, A and B rings are cis linked, while in the case of allo diastereoisomers are trans linked. In a large extent, the geometry of molecule of 5β−cholanic acid determines the properties of bile acids. Convex β and concave α surfaces are singled out on the steroidal skeleton of molecule of 5β−cholanic acid (Scheme 1).[1]



Scheme 1: Viewing of the concave and convex surface of 5β−cholanic acid and its configuration and conformational structure.[1]

The convex surface of the steroidal skeleton of bile acids is larger than the concave surface. It can be very important in examining the influence of the orientation of the hydroxyl and oxo groups on the hydrophobicity of the molecule. Hydroxyl groups of bile acids are most often oriented according to α side of the steroid skeleton.[2] Angular axial methyl groups on C_{10} and C_{13} have β orientation. By the X−ray diffraction it has revealed that the carboxyl group due to the conformational flexibility of the side chain can be found from the α side of the steroid ring system. The concave surface (α) of the steroidal skeleton of bile acids is polar-hydrophilic, while the convex surface (β) is nonpolar-hydrophobic. The presence of a hydrophobic and hydrophilic region in the bile acid molecule is referred to amphiphilicity. Bile acids in biological systems are classified into the ionic amphiphiles, since the carboxyl group is ionized under physiological conditions.[2] The cholic acid with two α axial (a) OH groups on C_7 and C_{12} carbon atoms and once an α equatorial (e) OH group (on C_3 carbon) belongs to a special group of amphiphilic compounds, ie. cholic acid is classified into a group of biplanar amphiphiles. At this molecule in space, oxygen atoms from the alpha OH groups are in the same plane, so-called polar plane (hydrophilic plane from the concave side of the steroid core).[2,3] The most complete separation of hydrophobic surface (β side of steroid skeleton) and hydrophilic surface (α side of the steroid skeleton) achieved in cholic acid, which is designated as biplanar. Ursocholic acid is an C_7 epimer of cholic acid, which means that OH group of this bile acid at C_7 carbon atom of the steroidal skeleton has the beta equatorial (e) OH group.

The change in the steric orientation of the C_7 OH group of ursocholic acid in regard to the orientation of the same OH group of cholic acid results in the movement of the polar plane to the convex (β) side of the steroidal system of rings. This results in a change of the ratio of the hydrophobic and hydrophilic surfaces of the molecules, i.e. leads to a decrease of biplanariness of ursocholic acid.[2,3] At the C_3 epimers of cholic acid, i.e. at isocholic acid, the C_3 OH group is a β axial orientation. It means that for this bile acid hypothetical plane of polarity (formed by oxygen from the groups: C_7 α (a)−OH, C_{12} α(a)−OH and C_3 β (a)−OH) cuts the steroid skeleton, because at isocholic acid, there is no real biplanariness (Scheme 2). For dihydroxy bile acids, such as chenodeoxycholic acid and deoxycholic acid, can not talk about real biplanariness, so the term is usually used: "molecules with hydrophilic edges"[2,3]

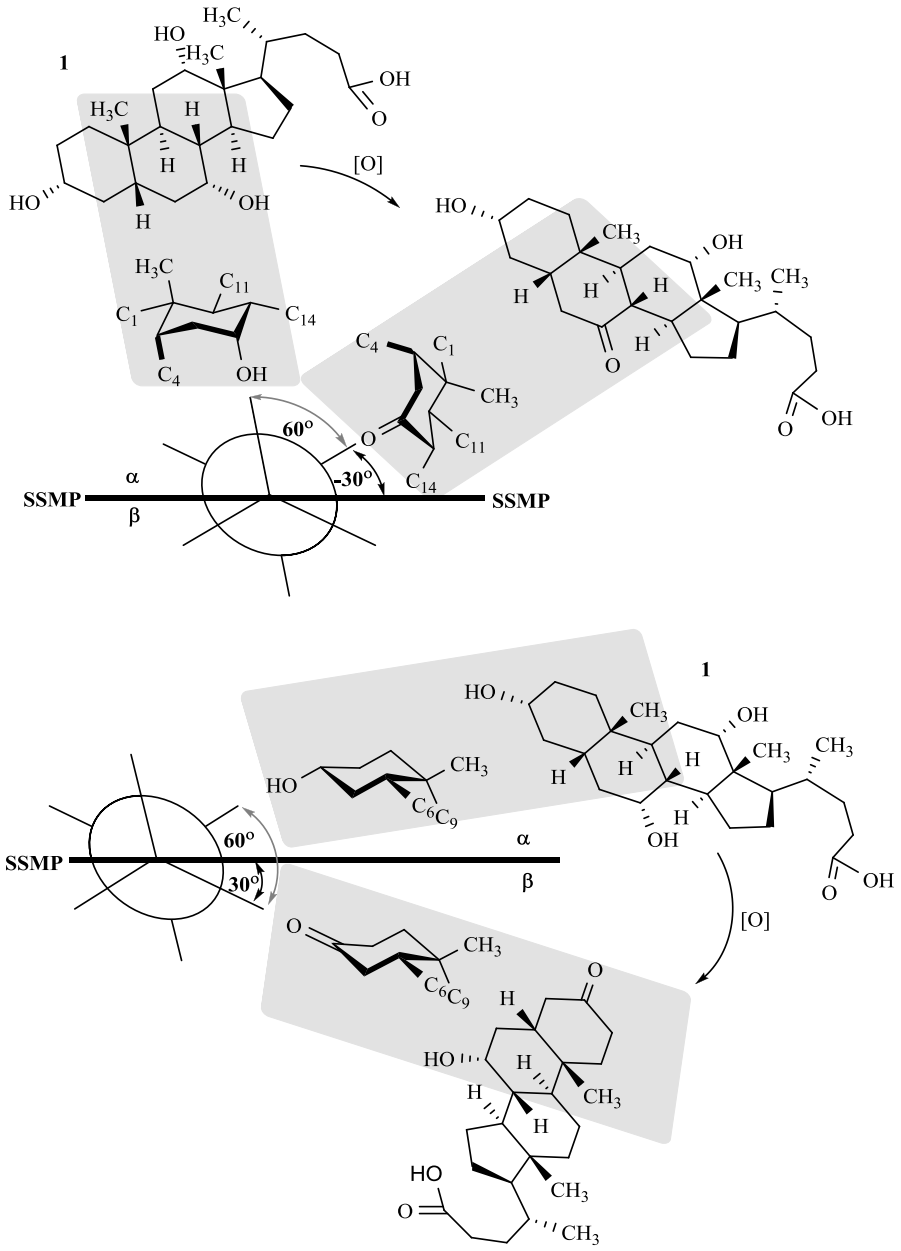


Scheme 2: Review of spatial oxygen atoms from α OH groups of cholic, ursocholic and isocholic acids, located in the same plane, so-called polar plane.[2,3]

Reactions of obtaining a keto derivatives of cholic acid

Keto derivatives of bile acids in humans can be found in traces in the feces and the results are microbiological transformations of primary and secondary bile acids in the intestinal flora. Some lizards, feathered animals, monkeys, rats and sea pigs can also be found in the bile in about 1% of other bile acids. As the keto derivatives in nature are not represented, they are obtained synthetically in the laboratory.[1]

In the oxidation of steroidal hydroxyl groups in the bile acids, using conformational analysis, the following stereochemical observations are made: In oxidation of any α axial (a) OH group of cholic acid (OH groups at C_7 and C_{12} methylene groups of steroidal skeletons), are formed the oxo groups, whose oxygen atoms have α equatorial (e) positions. Oxygen atoms from C_7 and C_{12} oxo groups are shifted by 60^0 relative to the position of the α(a)−OH group (Newman's projection formula), ie. with the median plane of the steroidal ring system (SSMP) made an angle of -30^0. The α equatorial (e) OH group (OH group at C_3) is oxidized into the keto group, whose oxygen atom has a β (s) orientation, that is forming an angle from 30 ° with the middle plane of the steroidal system of rings. When the OH groups of the cholic acid are converted into the keto groups, it leads to the forming of derivatives in which the oxygen atom is moved to the β side of the steroid skeleton, which then reflects on the change in the polar surface of the resulting of keto derivatives. Regioselective oxidations of OH groups from the steroidal skeleton are most commonly used to produce keto derivatives of bile acids (Scheme 3).[1,2,3]

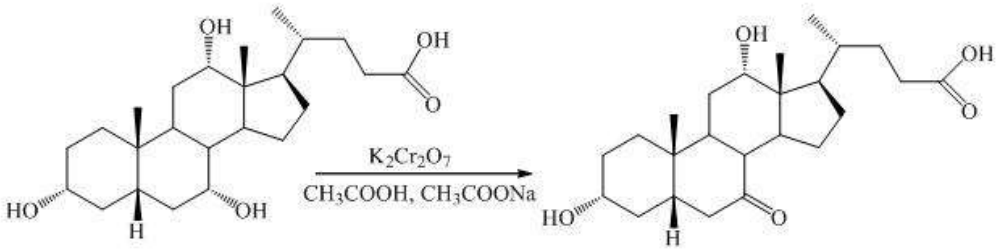


Scheme 3: Representation of regioselective oxidation of OH groups at C_7 and C_{12} methylene groups of steroidal skeletons to the keto groups, whose oxygen atoms are shifted by 60^0 relative to the position of the α(a)−OH group and made an angle of -30^0 with the median plane of steroidal ring system and the regioselective oxidation of α equatorial (e) OH group (OH group at C_3), which is oxidized into the keto group, whose oxygen atom has a β (s) orientation, that is forming an angle from 30 ° with the middle plane of the steroidal system of rings.[1]

It is observed a various reactivity of the three OH groups of cholic acid in the oxidation reactions. The order of reactivity is determined: C_7> C_{12}> C_3. The same rules do not apply for the catalytic oxidation, nor for oxidation by Oppenauer. In these cases, due to the changed mechanisms, the C_3 OH group is first oxidized. The oxidation of axial OH groups in the relation to the equatorial OH groups of the solid cyclohexane derivatives is faster, which can be explained in the following ways:

- The exposure of OH group by steric interactions during the reaction.
- The great reduction of repulsive interactions in oxidation of axial alcohols.
- Easier access of the base from the equatorial direction for the discontinuation of the C−H connection in the phase determining the rate of oxidation reaction.[1,4]

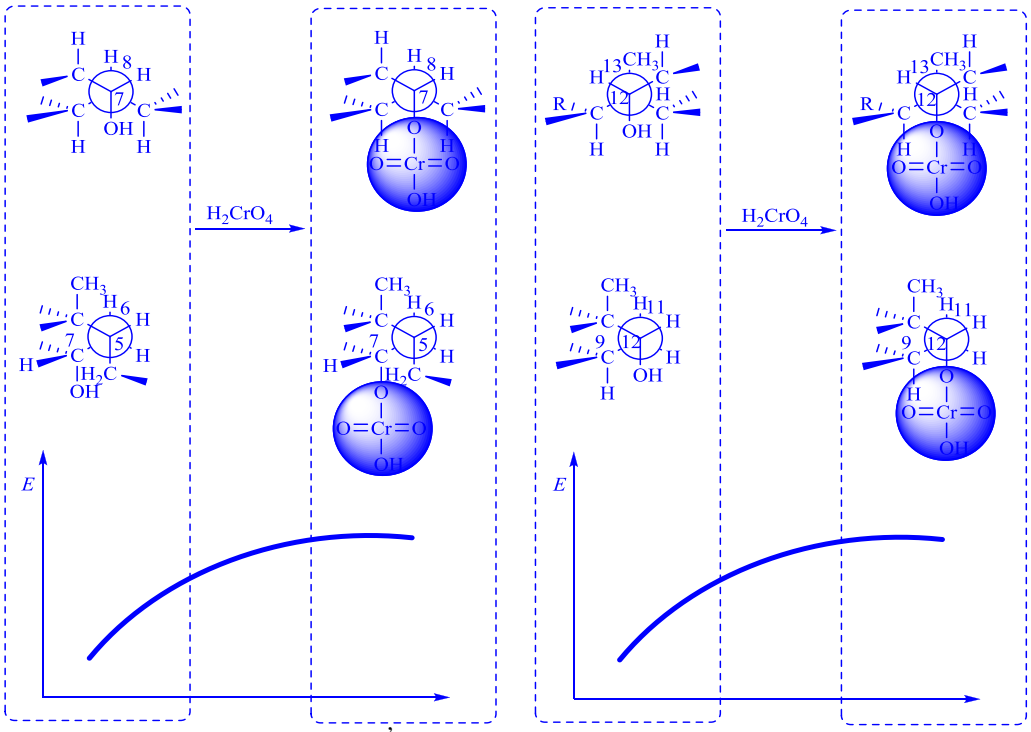
Haslewood is first explained the regioselective oxidation of hydroxyl group at C_7 of cholic acid to the 3α,12α−dihydroxy−7−keto−5β−cholanic acid. The regioselective oxidation is carried out with an aqueous solution of potassium dichromate in acetic acid in the presence of sodium acetate (Scheme 4).[1]



Scheme 4: Regioselective oxidation of hydroxyl group at C_7 of cholic acid to the 3α,12α−dihydroxy−7−keto−5β−cholanic acid.[1]

All of three hydroxyl groups of cholic acid can be oxidize by the chromic acid, but hydroxyl group at C_7 is first oxidized, than the hydroxyl group at C_{12} and finally is oxidize the hydroxyl group at C_3. The order of reactivity of hydroxyl groups is the same in oxidation with bromine in the base environment.[1]

The mechanism is confirmed by the isotope effect. It has been proven that the termination of C−H bond determines the rate of oxidation reaction. The change of the tetragonal C atom (sp^3) of the alcohol group in the trigonal C atom (sp^2) of the keto group, leads to the elimination of the repulsive interactions, which destabilize the intermediate chromatic ester. The oxidation rate will depend from the size of the reduction of repulsive interactions. In Newmann's projection formulas, the C_9 and C_{14} methine groups, or C_4 methylene groups are in synclinal positions in relative to the C_7 axial of chromatic ester functional group. Due to the close steric arrangement of the chromatic ester functional group at C_7 and the axial hydrogen associated with the above synclinal groups, there is a repulsive force (Van der Waals Reflective Force). (Scheme 5).[1]



Scheme 5: The representation of Newmann's projection formulas during the oxidation of all three hydroxyl groups of cholic acid, using a strong oxidation agent-chromic acid (H_2CrO_4) in which C_9 and C_{14} methyne groups or C_4 methylene group are in synclinal positions relative to the C_7 axial chromatic ester functional group.[1]

The chromester group at C_{12} is also in the synclinal position relative to the methine groups C_9, C_{14} and C_{17}. Between the chromester group at C_{12} and the axial hydrogens, which are bound to the above C atoms, appear the Van der Waalls's reflective interactions. Both of functional groups of esters of chromic acid at C_7 and C_{12} are in the synclinal position with three carbons, so the same reactivity would be expected. The reactivity of hydroxyl group at C_{12} in the oxidation reaction is reduced due to the greater sheltering of the equatorial hydrogen at C_{12} (which needs to be eliminated in the second phase) by the side chain at C_{17} (Figure 1).[1]

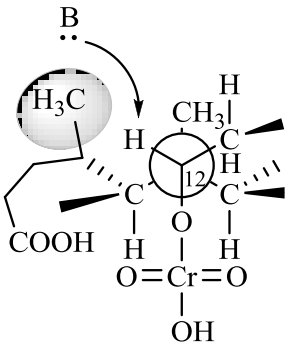


Figure 1: Review of chromatic ester functional group of Newmann's projection formulas, which is in synclinal position relative to the three carbons and the attack of voluminous base which abstracts the axial proton.[1]

Hydroxyl group at C_3 is in synclinal position in relative to the hydrogens of adjacent methylene groups. The introduction of a more voluminous chromatic ester functional group at C_3 does not lead to a significant increase of repulsive interactions and doesn't increase the reactivity.[1]

Due to the voluminousity of the base, which abstracts the axial proton, increases the 1,3−diaxial interaction and leads to increasing the activation energy, that is slowing down the oxidation reaction, if the oxidation of the equatorial OH group at C_3 is carried out in the second oxidation stage, which determining the reaction rate (Figure 2).[1]

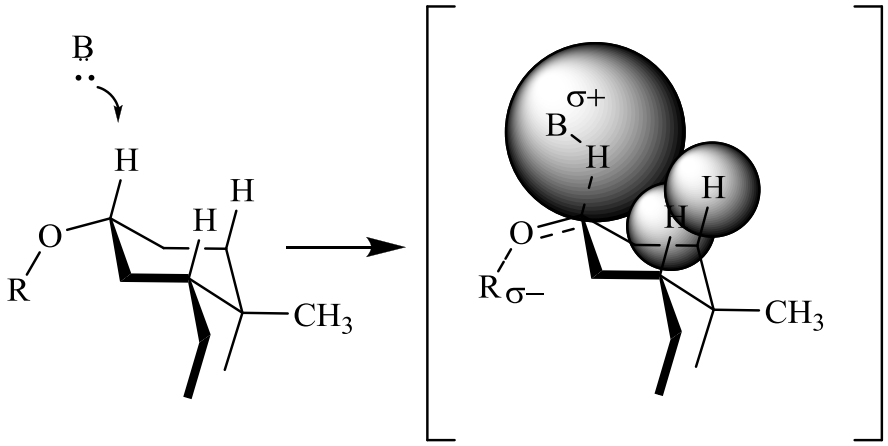
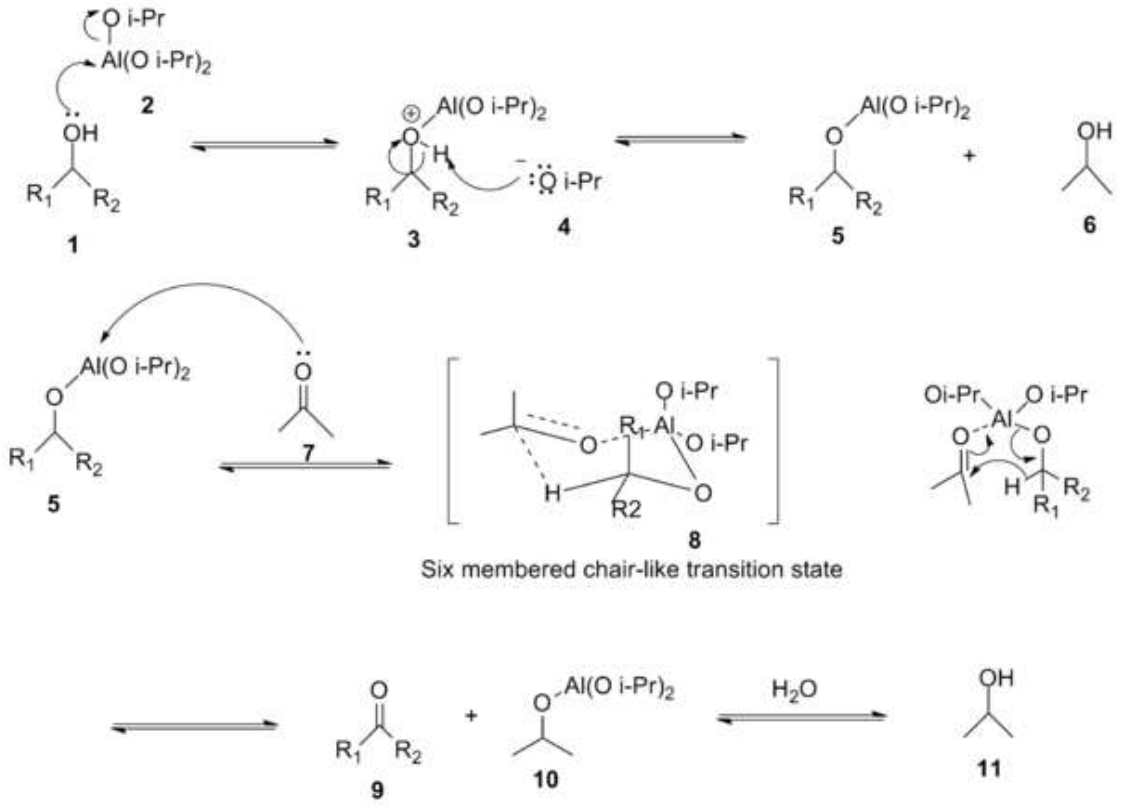


Figure 2: The representation of voluminous base which abstracts axial proton.[1]

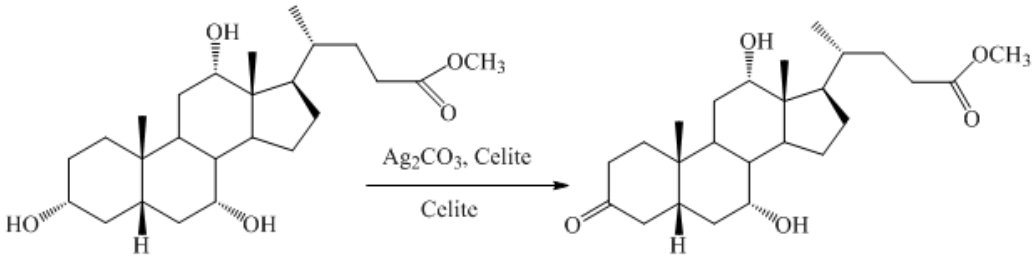
Regioselective oxidation of C_7 OH group of cholic acid with potassium dichromate even at 0 ºC is very small.[1] A significantly better regioselectivity is achived by oxidation of cholic acid to the 3α, 12α−dihydroxy−7−oxo−5β−cholanic acid with bromine in 1:1 mixtures of methanol and water in the presence of $NaHCO_3$.[1,2] It is formed the intermediate hypobromous ester, so the explanation of regioselectivity is similar to the oxidation with dichromate. Selective oxidation of equatorial OH group in the presence of axial OH group at Oppenauer oxidation can be explained by considering the transient state of transfer of hydride anion from the bile acid to the carbonyl carbon of acetone.[4] The equatorial oxygen is bound by coordinate covalent bond for the central ion of aluminium. In this case there is not a 1,3−diaxial interaction with the reagent (aluminium complex). If the axial OH group is bound by the coordinate covalent bond, then due to the volumizing of the aluminium complex, a 1,3−diaxial interaction is formed, which increases the energy of bound bile acids. In addition the 1,3−diaxial interaction increases the transition state of the hydride ion transfer for the same transient state, when the bile acid is bound via the equatorial oxygen.[4]

In the first step of the mechanism of Oppenauer oxidation, the oxygen from hydroxyl group of alcohol (1) is bonded by its free electronic pair, to the aluminium from aluminium isopropoxide (2), thereby forming the complex (3), which is then deprotonated with the alkoxide ion in the second step, thereby forming an alkoxide intermediate (5). In the third step both the oxidant acetone (7) and the substrate alcohol are bound to the aluminium from the alkoxide intermediate.[4] The acetone is coordinated to the aluminium which activates it for the hydride transfer from the alkoxide. The aluminium catalyzed hydride shift from the α carbon of the alcohol to the carbonyl carbon of aceton proceeds over a six-membered transition state (8). The desired ketone (9) is formed after the hydride transfer (Scheme 6).[4]



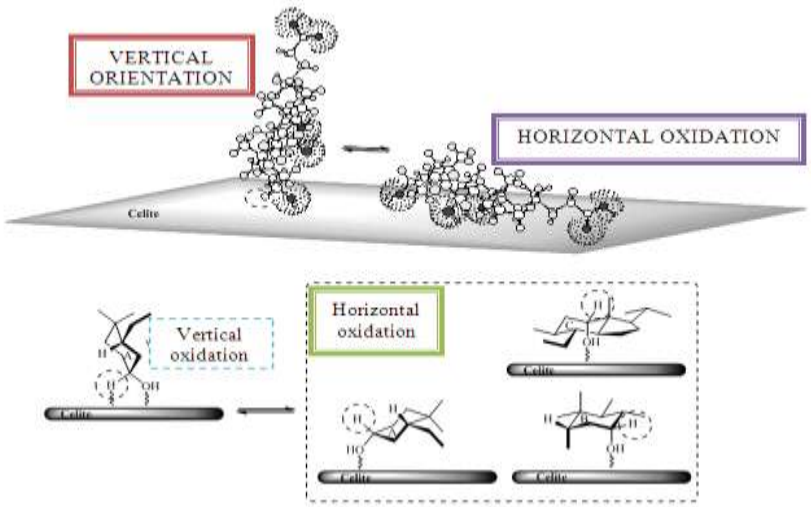
Scheme 6: Display of the Oppenauer oxidation mechanism.[4]

Danielss described that in oxidation of bile acids by Oppenauer mechanism is formed a complex mixture of products and the yields of the 3−monoketo derivatives are small. Tsreng improved the selective oxidation of the hydroxyl group at C_3 carbon of the bile acids using the celite, which is impregnated with Ag_2CO_3. Tsreng oxidized the methyl ester of bile acids in boiling toluene and he determinated that the methyl ester of lithocholic acid is most oxidized on the celite, while the introduction of hydroxyl group reduces the oxidation rate (Scheme 7).[1]



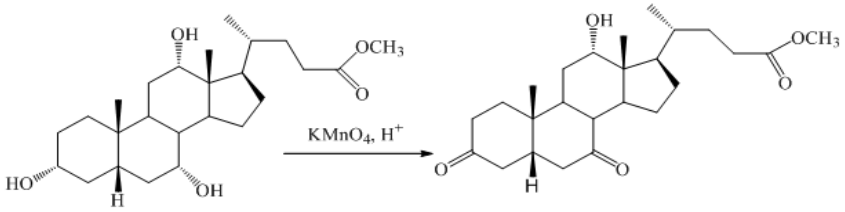
Scheme 7: Oxidation of methyl ester of cholic acid with silver carbonate on celite in boiling toluene to give a methyl 7*α*,12*α*−dihydroxy−3−keto−5β−cholanate.[1]

Kakis and his associates explained the different oxidation rate of the hydroxyl group at C_3 of the bile acids on the celite assuming two types orientation of bile acids on the adsorbent surface: vertical and horizontal, which are in balance. In the vertical orientation, the C_3 OH group and C_3 hydrogen are bound to the surface of the celite, which is necessary for oxidation, while the others hydroxyl groups and hydrogens are free, which explains the selectivity. In the horizontal orientation, all hydroxyl groups and carboxyl group are bound to the surface of adsorbents. Any hydrogen atoms at carbon atoms, which have an hydroxyl group, don't bind to the surface of the adsorbent. Therefore this orientation is inactive in the oxidation reaction. If bile acid has a lot of hydroxyl groups, then the balance is moved to the horizontal orientation and oxidation slowed down. Thus, the methyl ester of lithocholic acid is oxidized for one hour. The balance is moved to the active form, i.e. to the vertical orientation. For oxidation of the OH group at C_3 carbon of methyl ester of cholic acid, it takes seven hours (Scheme 8).[1]



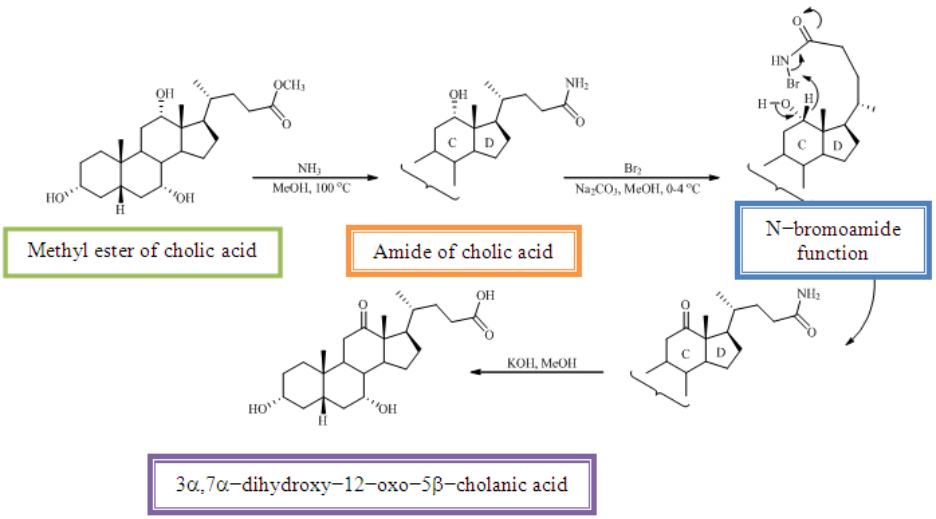
Scheme 8: Vertical and horizontal orientation at bile acids.[1]

Kuwada and associates noted that the methyl ester ofcholic acid in the acidic environment and at room temperature with potassium permanganate is selective oxidized to methyl ester of 12α−hydroxy−3,7−diketo−5β−cholanic acid (Scheme 9).[1]



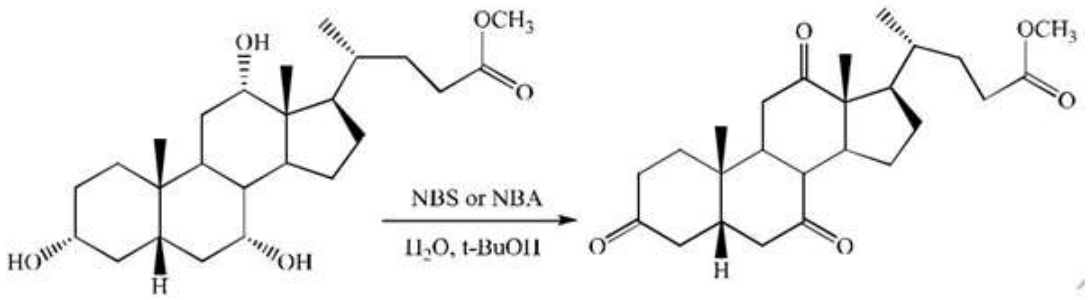
Scheme 9: Selective oxidation of methyl ester of cholic acid to methyl ester of 12α−hydroxy−3,7−diketo−5β−cholanic acid with potassium permanganate in the acidic environment.[1]

Miljkovic et al. explained the method for the regioselective oxidation of the C_{12} OH group of methyl ester of cholic acid. The methyl ester of cholic acid is converted into the amide of cholic acid, which is then oxidized with equivalent amount of bromine in alkaline methanol. The regioselectivity of the reaction is determined by the N−bromoamide function from the side chain. In the reaction obtaines the 3α, 7α−dihydroxy−12−keto−5β−cholic acid (Scheme 10).[1]



Scheme 10: Regioselective oxidation of C_{12} OH group of the methyl ester of cholic acid to obtaines the 3α,7α−dihydroxy−12−keto−5β−cholanic acid.[1]

Fiser and Rajagoplan have come to the conclusion that N−bromsuccinimide (NBS) or N−bromacetamide are the strongest oxidation agents in aqueous tert-butanol. Under the mentioned conditions, in the case of methyl ester of cholic acid, all OH groups are oxidized to give the methyl ester of 3,7,12−triketo−5β−cholanic acid (Scheme 11).[1]



Scheme 11: Oxidation of methyl ester of cholic acid to the methyl ester of 3,7,12−triketo−5β−cholanic acid.[1]

Hypoglycemic effects of bile acids

Bile acids are important endocrine molecules, which initiating signalling by the nuclear farnesoid X receptor (FXR) and G−protein coupled receptor (TGR_5). By the signalling of bile acids is carried out the different influence on glucose, lipid and energy homeostasis, which functions as additional to the classic road of bile acids in lipid solubilisation and absorption in the intestine. It has been proven that bile acids (BA) exert variety of metabolic effects besides the known role in cholesterol homeostasis.[5]

In the L− cells in the intestinal epithelium, TGR_5 activation by bile acids stimulates the release of gut hormones such as glucagon−like peptide−1 (GLP−1), leads to the improved glucose homeostasis. In plasma, concentration of bile acids rapidly growing in response to glucose ingestion, which probably underpins many of the mechanisms, by which bile acids play a role in regulation of the body`s response to food intake.[5] In pancreatic β−cells, TGR_5 and FXR activation enhances insulin secretion, while in the liver, FXR activation may inhibit gluconeogenesis with increasing glycogen synthesis and improving hepatic insulin sensitivity, although a role of FXR in gluconeogenesis remains controversial. Glucose itself stimulates expression of cholesterol 7α−hydroxylase, which catalyses the rate−limiting step in bile acid production. Morever, the therapy by bile acid sequestrant and their keto derivatives improves the glycaemic control in type 2 diabetes and in addition affects on lipid−lowering action.[6]

Based on the latest studies of diabetes in rodents in experimental models, it has been shown that synthetic derivatives, such as sodium 12−monoketo cholate, don't only possess hypoglycemic activity, but also potentiate the effect of insulin. Glucose reduction is presented in rats after subcutaneous administration of insulin and after nasal administration of saline solution and insuline.[6] Nasal administration of the mixture containing insulin and sodium 3α,7α−dihydroxy−12−keto−5β−cholanate results in a decrease in blood glucose concentrations, which reached a 54% from the dose obtained after subcutaneous administration of the same insulin dose.[6,7] However, following nasal administration of bile salts, blood glucose levels reached 36% of the amount obtained after subcutaneous administration of insulin. The discovery of the link between bile acids and glucose regulation gives us a new perspective in design of hypoglycemic drugs in the treatment of diabetes.[7] The association of bile acids and glycemic regulation was observed in patients with type 2 diabetes when cholestyramine, a derivate of bile acids was used to regulate dyslipidemia, which apart from lowering total and LDL cholesterol, lowered glycemic risk by 13%.[6] According to Duran−Sandoval and associates, expression of FXR (farnezoid X receptor) was reduced in rats with streptozotocin-induced diabetes and hyperglycemia and normalized after insulin administration. According to research by Zhang and his associates, in starvation has increased the expression of FXR. Activation of FXR ligands or increased expression of constituent FXR reduces glucose levels in animal models due to obesity and diabetes and FXR deficient mice showed hyperglycemia. All of these studies sugest that bile acids involved in glucose metabolism via the FXR dependent pathway. Some of the other signaling pathways to which bile acids may affect on glucose metabolism (independent of FXR) include the membrane receptor of bile acids, TGR_5 and effect of bile acids on cell hydration. Due to the amphiphilicity of the molecules, bile acids have the role of transport promoters and stimulators of absorption of various of therapeutic agents.[7] Bile acids increase the permeability of the membrane for molecules of different molecular weights, including insulin and potentiate the effect of hypoglycemic and hypolipemic drugs.[6,7] The effect of bile acids in glucose homeostasis can be achieved by hypoglycemic effect (via FXR dependent and independent pathway) and by improving the absorption of drugs. Modification of the amount or composition of bile acids, manipulation with intestinal flora and manipulation with bile acid receptors (FXR and TGR5) are also interesting and promising ways of research in order to develop new anti−diabetic drugs.

The use of bile acid sequestrants (cholestyramine, cholestipol and cholesevelam) in hyper− and dyslipidemia has been previously accepted. They also lower glucose levels in patients with type 2 diabetes, most likely through FXR−dependent pathways. On the territory of United State of America, Food and Drug Administration in 2009, it allowed the use of a drug under the generic name COLESEVELAM to improve glycemic control in type 2 diabetes, in addition to changes of lifestyle habits. In addition to the positive regulation of LDL and HDL cholesterol, this preparation has led to a rise in triglycerides of 11−22%. Monoketo derivative of cholic acid (3α, 7α−dihydroxy−12−keto−5β−cholanic acid) is a stable low-toxic semi-synthetic bile acid. Bile acids and bile salts improve the permeability of biological membranes of the gastrointestinal wall, buccal and nasal mucosa, cornea and blood−brain barriers) for molecules of low molecular weight, but also for larger molecules, such as insulin. The properties that allow them (solubility, hydrophobicity, micelle formation, etc.) vary in depending from structure and configuration of the molecules.[6,7,12]

Alloxan is a pyrimidine derivative, whose structural formula is largely resembling a glucose molecule and acts in the body as an analogue of glucose. It is a very unstable chemical compound, so the half−life of alloxan is very short. Aloxan induces the production of ROS, which amoung other things, leads to the deterioration of β−cells of the pancreas, and at the same time presents a model for the study of diabetes.[8] Aloxan is a toxic glucose analogue, which is subject to cyclic redox reaction in β−cells of the pancreatic cells, the ultimate product of which is dialuric acid and ROS, which are ultimately responsible for the death of β-cells. The condition that arises is called "alloxan diabetes." In aqueous solution, it spontaneously transforms into anti−diabetic aloxic acid within a few minutes. Because of this, it must be quickly applied after preparation. It is actually a protoxin, which via the $GLUT_2$ receptor enters in the beta cells of pancreas, where it produces ROS and toxic dialuric acid (during <1h).[8]

After the use aloxan, it is distinguish four phases:

- First phase (transient hypoglycemic phase) begins with the use of aloxan and lasts about 30 minutes; and represents a short-term hypoglycemic response that occurs to the transient stimulation of insulin secretion.[8]
- Second phase (first hypoglycemic phase) usually lasts 2−4 hours and occurs as a result of a blockade of insulin secretion with accompanying hypoinsulinemia.[8]
- Third phase (second hypoglycemic phase) tipically occurs 4 to 8 hours after an alloxan injection and lasts for several hours. As a result of toxin-induced rupture of secretory granules and cell membrane of beta cells, comes to overflowing circulation with insuline, that leads to severe transient hypoglycemia.[8]

PXR and CAR receptors are induced in the regulation of glucose and lipid metabolism. Activation of both receptors results in a hypoglycemic effect, which primarily arises from the suppression gene for enzymes PEPCK and G_6Pase involved in the gluconeogenesis process.[6] On the other hand, it has been established in humans that exposure to certain pesticides and polychlorinated biphenyls (PCBs), which are PXR activators, leads to hyperglycemic effects.[6,7,8] It should be borne in mind that these are weak activators, as well as ligands for other receptors, so that the role of PXR in signaling pathways of importance for the development of diabetes should be further examined. The basic effects mediated by the TGR_5 receptor in the body are not related to maintenance of bile acid homeostasis, but primarily for energy metabolism and homeostasis of glucose, although they also affect on the function of the billiary tract, enteric nervous system and immune system. TGR_5 is considered a significant pharmacological target for the treatment of metabolic syndrome or hypoglycemia and obesity. It has been shown that TGR_5 activation leads to a hypoglycaemic effect by stimulating the synthesis and releases of GLP-1 (glucagon like peptide 1) from enteroendocrine L-cells to calcium-dependent mode. The incretin decreases glucagon secretion and increases insulin release, although the activation of TGR_5 on β−cells of pancreas results in a decrease in insulin release on c AMP and potassium-dependent route.[6,8] Bile acids via the TGR_5 receptor promote both energy metabolism by activating the iodotyronin deodoinase 2 (DIO_2) enzyme that converts the T_4 hormone thyroid gland into the T_3 active form, which stimulates beta oxidation of fatty acids in brown fat tissue in mice or in skeletal muscle in humans. In this way, the administration of bile acids can prevent, but also reduce the obesity, induced by the diet with high lipid content. Activation of TGR_5 also has an immunomodulatory effect. It has been shown that the TGR_5 is responsible for the suppression of macrophage activation by bile acids by inhibiting the signaling pathway of the nuclear factor kB (NF-kB).[8]

It has been proven that the keto derivatives of cholic acid, exactly the most effective 3α, 7α−dihydroxy−12−keto−5β−cholanic acid, compared to diketo and triketo derivatives of cholic acid exhibits hypoglycemic effect in type 1 diabetes, but also to achieve better effects when applied concomitantly with hypoglycemic glyclazide or stevioside preparations, but when any of these substances are given individually. The best effects in glycemic control were achieved when rats with type 1 diabetes were pretreated with probiotics , and then simultaneously with the use of a derivative of 3α, 7α−dihydroxy−12−keto−5β−cholanic acid and gliclazide.[9] Al Salami and associates found that the transporter function was disturbed or suppressed in diabetes. It has been found that monoketo derivatives of cholic acid act as inhibitors of efficient transporter and transfer of substances in the direction from mucosa to serosa, by inhibition of Mpr_3 transporter. This

discrepancy with in vivo study results is believed to be due to in vivo biotransformation (metabolic transformation) of the monoketo derivative which in vivo promote the absorption of gliclazide in the ileum.[10] This is support by the fact that with intravenous administration (regardless of the change in glyclazide or probiotic pretreatment), the pharmacokinetic properties of monoketo derivatives of bile acids remain unchanged, but are significantly altered in the case of oral administration.[9,10,11]

Bile acid-FXR signaling promotes glycogen synthesis and inhibits gluconeogenesis. It is found decreased phosphoenolpyruvate carboxylase kinase and glucose−6−phosphatase gene in diabetic mice given GW4064 (a farnesoid X receptor agonist). What's more, it is found that increased concentration of bile acids inhibited the expression of gluconeogenesis related genes through bile acid− FXR-SHP pathway.[12]

The activation of FXR by GW4064 in insulin-resistant ob/ob mice reduced hyperinsulinemia and improved glucose tolerance. Glucose tolerance reduced by TGR5-deficiency and improved by TGR5 over expression in high-fat diet-fed mice via increased GLP1 and insulin secretion.

The key role of FXR in the control of insulin sensitivity, FXR expression has recently been reported to play a role in pancreas, where it regulates glucose-induced insulin secretion.[13,14]

Bile acids are involved in the regulation of hepatic glucose metabolism by FXR-mediated pathways. The expression of FXR itself is decreased in rat models of type 1 and T2D, an effect that could be reversed by the administration of insulin.[14]

Gliclazide is antidiabetic drug with antioxidative properties that are independent of any effect on glucose level. Gliclazide has favorable hemobiological properties and other extrapancreatic effects which make gliclazide potentially useful in type 1 diabetes mellitus. its scavenging effect and low affinity for binding to SUR receptors in brain gliclazide is a good candidate for the investigation as a protector of brain cells in diabetes.[10]

In patients with newly diagnosed type 2 diabetes mellitus in Hausler's study were used the medicaments to treat hypoglycemia, which could influence on insulin sensitization, while excluded participants who have already used hypoglycemic drugs.[14]

Taylor's study showed a positive correlation between secretion of total bile acid in urine and HbA1c in patients with diabetes. The association between insulin resistance and total bile acids in the diabetic population was not influenced by the Hb1Ac level, indicating that the change in the level of bile acids in the dysglycemic state is mainly due to insulin sensitivity instead of glucose levels. Taylor's studies have shown that may be a compensatory increase in bile acid signaling in order to maintain glucose homeostasis.[14]

Great progress in treatment of type 1 diabetes mellitus is the discovery of the new treatment, which avoids and even replaces the absolute requirement for injected insulin. The need for multiple treatment of comorbidity associated with T1DM, increases demand for the development of new therapeutic alternatives with new mechanisms of action.[5,14]

DISSCUSION

Diabetes mellitus (DM) is one of the most commonly occurring endocrine disorders, affecting 6% of the world's population. It is classified as Type 1 (T1D) and Type 2 (T2D) diabetes. Diabetes mellitus type 1 is a autoimmune disease characterized by significant inflammation and the distruction of β−cells of pancreas with partial or complete disruption of insulin production and the inability of the body to control glycemia. The spectrum of symptoms of hypoglycemia is varied from autonomic activation to behavioral changes to altered cognitive function to seizures of coma. The short and long−term complications include neurologic damage, trauma, cardiovascular disease and death.

In the treatment of type 2 diabetes mellitus, glyclazide used in combination with keto derivatives of bile acids (3α, 7α−dihydroxy−12−keto−5β−cholanic acid) shows a pleiotropic effect outside the β−cells of pancreas an extrapancreatic effect, such as antiinflammatory and cellular protective effects that could be useful in treating diabetes mellitus type 1.

The positive effects of glyclazide in diabetes mellitus type 1 are even more expressed when combined with other hypoglycaemic agents, such as probiotics and keto derivatives of bile acids. There is a clear synergistic effect between glyclazide, bile acids and probiotics, illustrated by reduction in blood glucose level and the improvement of diabetic complications. Bile acids may affect on the secretion of the increted hormone in particular of glucagon−like peptide 1 (GLP−1) and the glucose-dependent insulinotropic polypeptide.

CONCLUSION

It has been proven that treatment with colesevelam increased the level of GLP-1, as well as postprandial GLP-1 and glucose level of insulinotropic polypeptides. Bile acids perform a pleiotropic metabolic effects, while the physico−chemical properties of various bile acids affect on their function. In healthy people, insulin resistance is associated with increased 12α−hydroxylated bile acids (cholic acid, deoxycholic acid and their conjugated forms). Relationships of 12α−hydroxylated and 12α−non−hydroxylated bile acids were associated with key insulin resistance characteristics, including higher levels of insulin, proinsulin, glucose, glucagon,

triglycerides and lower level of HDL cholesterol. A higher ratio of 12α−hydroxylated and 12α−non−hydroxylated bile acid is associated with lower insulin sensitivity and higher triglycerides in plasma, which has been proven earlier by control of gender, age, BMI and glucose tolerance status. 12α−Hydroxylated bile acids and 12−keto derivatives of bile acids have a role of treating metabolic abnormalities of diabetes mellitus and increased the possibility of developing insulin sensitization therapy.

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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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