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Synthesis of two isomers of 3-amino-7α-12α-dihydroxy-5β-cholanic acid

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Key words: *Cholic Acid, Stereoisomers, 3-amino-7α-12α-dihydroxy-5β-cholanic acid.*

Aim. With the aim of differences investigation in physical-chemical properties optimized synthesis of the two stereoisomers of 3-amino-7-12-dihydroxycholanic acid has been performed.

Methods and results. As the source compound natural cholic acid was used. 3α-amino-7α-12α-dihydroxy-5β-cholanic acid was obtained in high yield and low number of by-products. The yield of 3β-amino-7α-12α-dihydroxy-5β-cholanic acid was low because of steric interference and process needed additional treatment stages. Column chromatography was used for cleaning compounds. The structure was studied and confirmed by NMR method. Significant differences in signals of NMR spectra and physical-chemical properties 3β-amino-7α-12α-dihydroxy-5β-cholanic acid and 3α-amino-7α-12α-dihydroxy-5β-cholanic acid wasn't found or observed.

Синтез двох ізомерів 3-аміно-7α-12α-дигідрокси-5β-холанової кислоти

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З метою вивчення відмінностей у фізико-хімічних властивостях здійснили оптимізований синтез двох стереоізомерів 3-аміно-7-12-дигідроксихоланової кислоти. Як вихідну сполуку використали природну холеву кислоту. 3α-аміно-7α-12α-дигідрокси-5β-холанову кислоту отримали з високим виходом і малою кількістю побічних продуктів. Вихід 3β-аміно-7α-12α-дигідрокси-5β-холанової кислоти низький через стеричні перешкоди та потребував додаткових стадій очистки. Для очистки сполук використали колонкову хроматографію. Будову сполук вивчили методами ПМР. Встановили незначну різницю між сигналами ПМР спектрів і фізико-хімічними властивостями 3β-аміно-7α-12α-дигідрокси-5β-холанової кислоти та 3α-аміно-7α-12α-дигідрокси-5β-холанової кислоти.

Ключові слова: *холева кислота, стереоізомери, 3-аміно-7α-12α-дигідрокси-5β-холанова кислота.*

Актуальні питання фармацевтичної і медичної науки та практики. – 2015. – № 1 (17). – С. 22–26

Синтез двух изомеров 3-амино-7α-12α-дигидрокси-5β-холановой кислоты

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С целью изучения различий в физико-химических свойствах проведен оптимизированный синтез двух стереоизомеров 3-амино-7-12-дигидроксихоланової кислоты. В качестве исходного вещества использована природная холевая кислота. 3α-амино-7α-12α-дигидрокси-5β-холановая кислота получена с высоким выходом и низким количеством побочных продуктов. Выход 3β-амино-7α-12α-дигидрокси-5β-холановой кислоты был низким из-за стерических препятствий и требовал дополнительных стадий очистки. Для очистки соединений использована колоночная хроматография. Строение синтезированных соединений изучено методами ПМР. Установлена незначительная разница в сигналах ПМР спектров и физико-химических свойствах 3β-амино-7α-12α-дигидрокси-5β-холановой кислоты и 3α-амино-7α-12α-дигидрокси-5β-холановой кислоты.

Ключевые слова: *холевая кислота, стереоизомеры, 3-амино-7α-12α-дигидрокси-5β-холановая кислота.*

Актуальные вопросы фармацевтической и медицинской науки и практики. – 2015. – № 1 (17). – С. 22–26

At present analyzing literature we saw strong tendency in revealing methods of synthesis of stereo-compounds. First time researchers noticed feature of bile acids in 1937 when milder reduction with Pt successively converts dehydrocholic acid to 3α-hydroxy-7,12-diketo-, 3α,7α-dihydroxy-12-keto- and finally to 3α,7α,12α-trihydroxy-5β-cholanic acid. The acid obtained in this way is not identical with natural cholic acid, having twofold higher optical rotation and exhibiting different physiological activity [1]. It was first time when difference in physiology activity has been specified. Then searching for potential metabolites of bile acids, Chang et al. synthesized the 3β- and 12 β-epimers of cholic acid as well as 3 β,7α,12 β -trihydroxy-5 β-cholanic acid [2,3]. The 12β-hydroxy isomer has been obtained by reduction of the 12-keto derivative of cholic acid and its methyl ester with hydrogen in the presence of Raney-nickel.

Cholic acid and its deoxy analogues have been variously exploited for the construction of synthetic receptors, novel amphiphiles, and scaffolds for the assembly of combinatorial libraries [4-6]. Amino groups can be derivatized rapidly and quantitatively, retain H-bond donor capabilities after acylation, and are highly hydrophilic when protonated. Herein Broderick et al. reported the first synthesis of 3α,7α,12α-triamino-5β-cholanic acid, a tris-deoxa-tris-aza analogue of methyl cholate, and the preliminary characterisation of its solution properties at neutral and acidic pH [7]. In particular they highlighted its potential as a "facial amphiphile", with enforced hydrophobic and hydrophilic surfaces which might confer useful recognition and transport properties in biphasic media. In 1993 other group of scientists have found an antibiotic that has been isolated from shark tissue [8] this compound exhibited antimicrobial activity towards Gram-negative and Gram-positive bacteria and it was designated as squalamine (3β-N-1-{N[3-(4-aminobutyl)]-1,3-diaminopropane-7α,

24S-dihydroxy-5β-cholestane-24-sulphate. It's structure was similar to 3α,7α,12α-triamino-5β-cholanic acid. 3α,7α,12α-triamino-5β-cholanic acid with its amphiphilic structure may be applicable to the transport of drugs, particularly those of the anionic type [7]. The three amino groups, directly bound to the steroid skeleton with its functionalized side chain, may allow the synthesis of receptors as well as being useful in combinatorial chemistry. New cationic steroid antibiotics have been prepared by binding tripeptides to a triamino analogue of cholic acid. These compounds were synthesized in the solid phase in an indexed library that was screened for antibacterial activity against Gram-negative and Gram-positive bacteria [9].

Based on the above analyzed literature we decided to synthesize an α/β-epimers of 3-amino-7,12-dihydroxy-5β-cholanic acid from raw natural cholic acid isolated from chickens gallbladders. They were interesting due to different stereo position as possible differences in pharmacological effects, so it has been tasked to create such stereoisomers at 3 position of Carbon atom in cholic acid and to design the method of synthesis of such epimers. Literature has showed us absence of short suitable and convenient method of synthesis of compounds.

Materials and methods

Synthesis of compound 2 (methylcholate). 30g (73.3 mmol) of cholic acid and 0.5 g (2.9 mmol) of p-toluolsulfoacid dissolved by heating in 300 ml of methanol. The reaction mixture was mixed at t = 50-60^{0}C for 48 hours, then was cooled, diluted with 400-500 ml of water and extracted with three portions of (300 ml) methylene chloride. Portions were united and evaporated under reduced pressure.

Synthesis of methyl 3-mesyl-7,12-dihydroxycholanate 3. 24.8 g (58.6 mmol) of compound 2 dissolved in chilled ice water in 200 ml of methylene chloride and then 18.6ml of (0.134 mol) triethylamine was added to solution. Then to the reaction mixture with stirring and cooling solution of 6.57 ml (85.2 mmol) mezylchloride in 100 ml methylene chloride was added dropwise; by adding the total volume over 40 min. The reaction mixture was mixed another 1 h. Then was washed with water and aqueous sodium bicarbonate solution, the organic layer was separated and evaporated under reduced pressure.

Synthesis of methyl 3-azido-7,12-dihydroxycholanate 4. 25.2 g (50.3 mmol) of compound 3 dissolved in 150 ml of dimethylformamide. Then 11.5 g (0.177 mol) of sodium azide was added to the solution, and the reaction mixture was mixed at t = 85-95 ^{0}C for 12 hours. Then the reaction mixture was diluted with 300 ml of water, extracted with three portions of 200 ml of methylene chloride; organic extracts were combined, washed by 5 portions of 150-200 ml water (to get rid of residual dimethylformamide) and evaporated under reduced pressure.

Synthesis of methyl ester of 3β-azido-7,12-dihydroxycholanic acid 7. 26.4 g (67 mmol) of methyl ester of cholic acid 2 and 26.4g (100.8 mmol) of $P(Ph)_3$ dissolved in 300 ml of tetrahydrofuran cooling by an ice bath. After complete dissolution 19,9 ml (100.5 mmol)of diisopropyl ether azodicarbonic acid (DEAD) was added dropwise to the mixture; after 15 min. 12.9 g (87.2 mmol) of azide of nicotinic acid was added to the mixture. The mixture was mixed at room temperature until disappearance of the starting material on TLC (eluent - 3% isopropyl alcohol in $CHCl_3$). The reaction took about half an hour. Then the reaction mixture was diluted with water and extraced with CH_2Cl_2; organic extracts were combined, evaporated under reduced pressure. The achievement of complete drying in not needed, because the resulting azide may be subject to decay, and the remainder of the solvent (tetrahydrofuran) don't interfere with the implementation of the next stage.

Synthesis of 3α and 3β-amino-7,12-dihydroxycholanate methyl esters 5 and 8. 21.4 g corresponding azide (4 or 7) (46.7 mmol) and 18.4 g of triphenylphosphine (70.2 mmol) was dissolved with stirring in a mixture of 250 ml tetrahydrofuran and 15-20 ml of water. The reaction mixture was mixed 48 hours at room temperature, then was diluted with 350-400 ml of water, extracted with three portions of 200 ml of methylene chloride, the organic extracts were combined and evaporated under reduced pressure.

Synthesis of acids from methyl 3α and 3β-amino-7,12-dihydroxycholanates 6, 9 (method of saponification of esters to acids). 0.70 mmol of corresponding ester (5 or 8) and 0.03 g (0.75 mmol) of sodium hydroxide dissolved in a mixture of 10 to 15 ml of water and 30 ml of dioxane. The reaction mixture was heated at t = 75 85 ^{0}C for 2-3 hours, then diluted with 50 ml of 5% solution of HCl, extracted with two portions of 30 ml of methylene chloride, the organic extracts were consolidated and evaporated under reduced pressure. The reaction was monitored by the method of thin layer chromatography (TLC). As eluent was used mixture consisting of 95% $CHCl_3$ and 5% isopropyl alcohol.

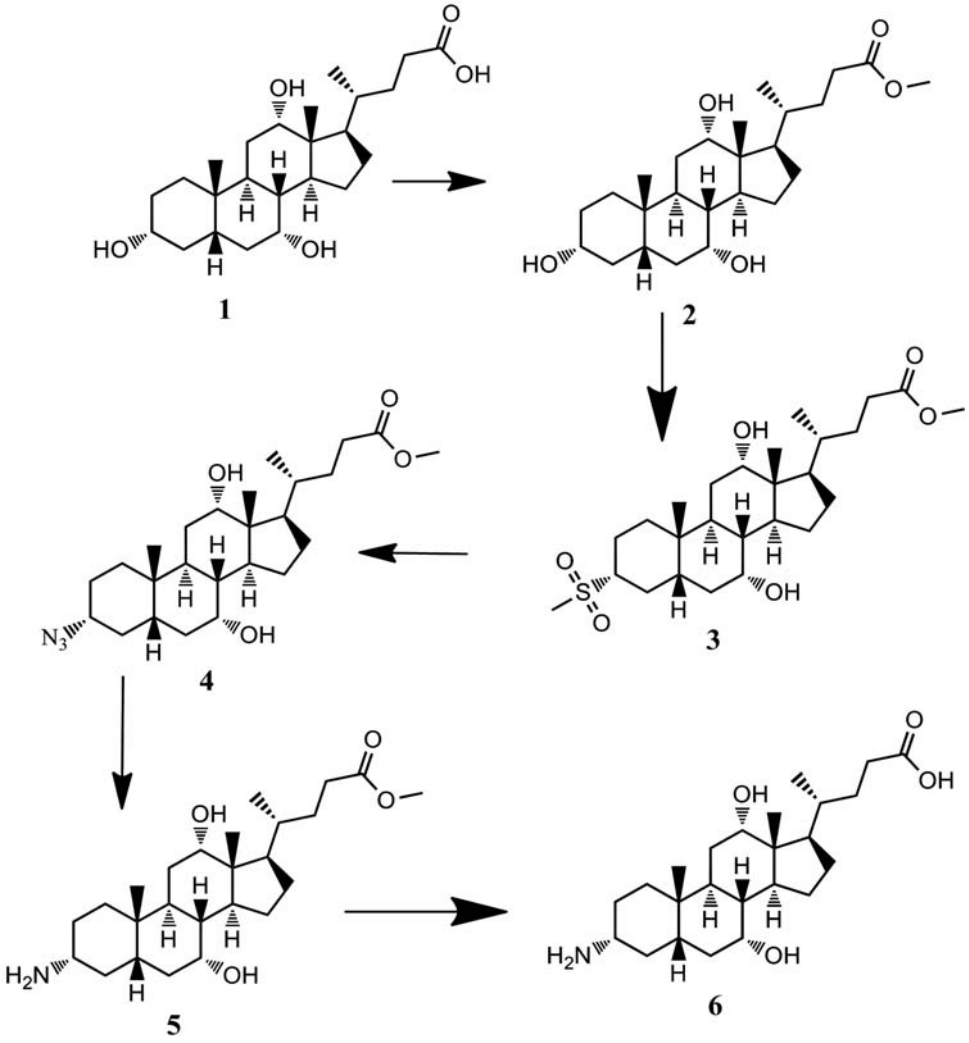
Results and discussion

During the literature analysis we found an optimal way to perform selective substitution of hydroxyl-group by amino-group [10-12], so we carried out it in a way showed below. Initially, we synthesized metylcholate by reacting cholic acid with methanol in acidic medium (compound 2). General steps are depicted in *schema 1*.

As it was predicted, carboxylic group has undergone this reaction very easily and its purpose was to protect it. This procedure is required because these reagents in sequential synthesis could complicate interactions through the formation of undesirable compounds. First stage is also common for the synthesis of 3β stereoisomer. During regioselective mesylating of compound 2 we obtained methyl 3-mesyl-7,12-dihydroxycholanate (compound 3). The main problem of using mesylchloride was complicated evaporation as it was drying hardly and tiring and resulting substance was semiliquid. We came to decision of using it in a half raw state. Subsequently it hasn't affected on further results. Later on through consistent interaction with sodium azide (compound 4) and recovery by triphenylphosphine we gain succeed in synthesis of 3α-amino-7,12-dihydroxycholanate methyl ester

Schema 1

Synthesis steps of 3α-amino-7,12-dihydroxycholanic acid



(compound 5). Hydrolysis was performed using standard method by using sodium hydroxide in water/dioxane mixture to synthesize 3α-amino-7,12-dihydroxycholanic acid(compound 6). ^{1}H NMR spectra data of derived substances, yields and the melting points are shown in *table 1*. They were measured at 200MHz in DMSO.

Table 1

Physico-chemical properties and NMR Spetra data of substances in scheme 1

№ of compound (name)	Yield	The signals of protons (or characteristic protons) 1H NMR of compounds	The melting point, °C
Methyl cholate (2)	86,1%	3OH–3,9; 3,8;3,6	150–155
Methyl 3α-mesyl-7,12-dihydroxycholanate (3)	85,8%	2OH–3,95; 3,85	100
Methyl 3α-azide-7,12-dihydroxycholanate (4)	92,8%	2OH–3,95; 3,75	105
Methyl 3α-amino-7,12-dihydrocholanate(5)	90,1%	2OH–4,0; 3,9 NH2–3,75	150–155
3α-amino-7,12-dihydroxycholanic acid (6)	90,1%	2OH–4,0; 3,9 NH2–3,75 carboxylic group – 12.1	150–155

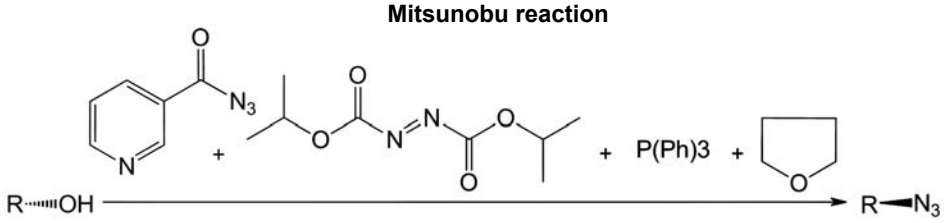
Reactions for the synthesis of β isomer differed by absence of mesylate preparation step and took place in four stages. Instead of a sequential mesylation it was used a Mitsunobu's reaction, and according to literature data concerning the reactivity of groups in bile acids the reaction was to be held at third position of Carbon atom. Source material for synthesis of corresponding methyl ester of 3β-amino-7,12-dihydroxy-5β-cholanic acid by the Mitsunobu's reaction was methyl cholate. And reaction of obtaining β-azide passed in one stage (*schema 2*). It should be noticed that other stages were similar to 3α-amino–epimer synthesis.

The essence of Mitsunobu's reaction is the transformation of the hydroxyl group into azido group with the change of the optical configuration of the carbon atom, of which this functional group is binded. Then azido group can be recovered to amino through the way shown in *schema 3*.

Method displayed at *schema 3* is used in synthesis of methyl ester of 3β-amino-7,12-dihydroxycholanic acid (compound 13) an analog of methyl ester of 3α-amino-7,12-dihydroxy-5β-cholanic acid with the opposite configuration of third carbon atom that carries amino group. *Figure 1*

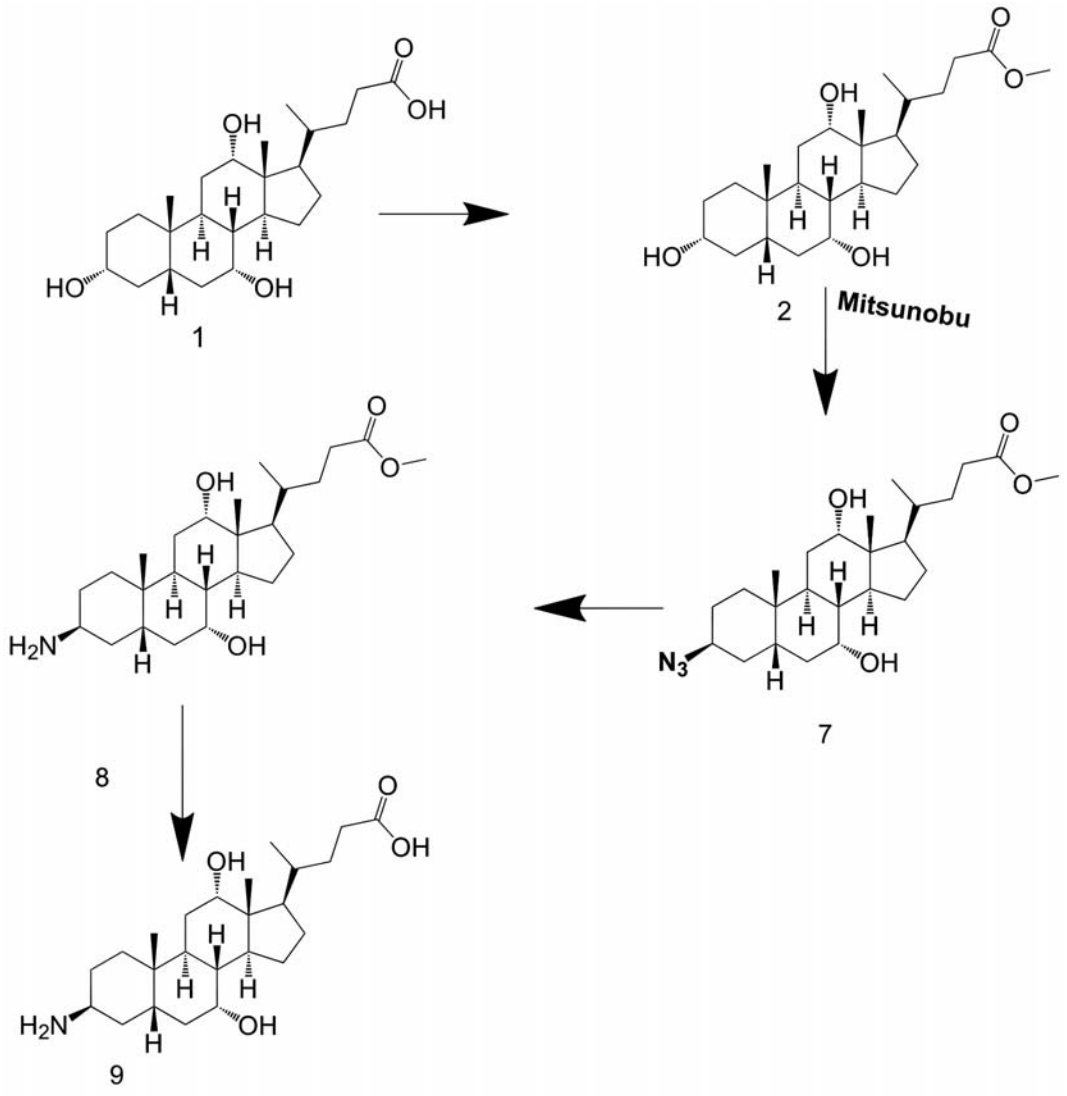
Schema 2

Mitsunobu reaction



Schema 3

Synthesis steps of 3β-amino-7,12-dihydroxycholanic acid



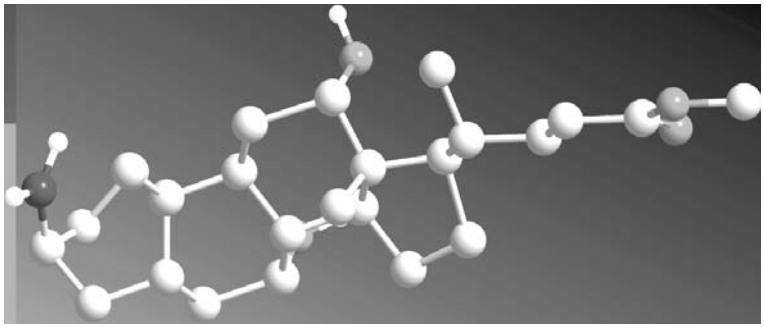


Fig. 1. 3D structure of 3β-amino-7,12-dihydroxycholanic acid.

Table 2

Physico-chemical properties and NMR Spetra data of substances in scheme 3

№ of compound (name)	Yield	The signals of protons (or characteristic protons) ^{1}H NMR of compounds	The melting point
Methyl cholate (2)	86,1%	3OH – 3,9; 3,8; 3,6	150–155°C
Methyl 3β-azide-7,12-dihydroxycholanate (7)	-	2OH – 3,95; 3,85	110–130***C***
Methyl 3β-amino-7,12-dihydrocholanate (8)	15,6 %	2OH – 4,0; 3,8. NH_2 – 3,7	140–145°C
3β-amino-7,12-dihydroxycholanic acid (9)	82,3%	2OH – 4,1 , 3,9 . carboxylic group – 12.0	150–160°C

shows the spatial structure of the β stereoisomer, it was built using «ChemBioOffice ChemBio3D Ultra 12.0».

^{1}H NMR spectra data of derived substances and yields and the melting points are shown in *table 2*. They were measured at 200 MHz in DMSO.

Methyl 3β-azide-7,12-dihydroxycholanate was not dried due to low stability and the potential opportunity to breakup, and then it was used for the synthesis of amine, so the yield of amine was recalculated towards methyl cholate. It should be noted that all reactions that involve β position passed harder and with the less yield, also it had to carry out purification using column chromatography monitoring end by TLC. Perhaps due to imperfect methods as well as through the characteristic arrangement of atoms in the molecules shown in *fig. 1*. Such problems with the synthesis of α amine didn't crop up, and the yield was great.

In further investigations synthesized compounds 6 and 9 have demonstrated wide spectrum of antibacterial properties towards cultures of aerobic bacteria and fungi. Other physiological activities are being studied now.

Conclusions

The synthesis of α/β 3-amino-7,12-dihydroxycholanic acids has been successfully carried out. We have successfully confirmed their structures and measured their physical-chemical properties. The comparison of properties was held and was observed the difference in properties. The yield of 3β-amino compound is not very significant, due to structural obstacles.

The acids were used in further pharmacological experiments, and it needs to find and to develop other methods of synthesis, perhaps by using microwave waves**.**

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