SYNTHESIS OF TWO ISOMERS OF 3-AMINO-7A-12A-DIHYDROXY-5B-CHOLANIC ACID

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ABSTRACT

This paper presents an optimized synthesis of two stereoisomers of 3-amino-7-12-dyhydroxycholanic acid. It was possible to obtain α-isomer in sufficient quantity. Unfortunately we could not receive β-isomer with the best yield currently.

Keywords: *cholic acid, stereoisomers, 3-amino-7α-12α-dihydroxy-5β-cholanic acid*

INTRODUCTION

Analyzing literature at present we saw a strong tendency in revealing methods of synthesis of stereo-compounds. For the first time researchers noticed the feature of bile acids in 1937 when in a milder reduction with Pt they successively converted 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 a twofold higher optical rotation and exhibiting different physiological activity (1). It was the first time when difference in physiology activity was specified. Later, when searching for potential metabolites of bile acids, Chang et al. synthesized the 3β- and 12 β-epimers of cholic acid as well as 3β,7α,12 β -trihydroxy-5 β-cholanic acid (2,3). The 12β-hydroxy isomer was 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). In all these applications, there are significant advantages to the replacement of hydroxyl by amino functionality. 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 characterization 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, another 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-

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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 be 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 it was decided to synthesize an α/β-epimers of 3-amino-7,12-dihydroxy-5β-cholanic acid from raw natural cholic acid isolated from chicken gallbladders. They were interesting due to their as different as possible stereo position differences in pharmacological effects, so we took the task to create such stereoisomers at the 3rd position of the Carbon atom in cholic acid and to design the method of synthesis of such epimers, because literature has showed us an absence of a quick, suitable and convenient method of synthesis of the compounds.

METHODS

Synthesis of compound 2 (methylcholate). 30g (73.3 mmol) of cholic acid and 0.5 g (2.9 mmol) of p-toluolsulfoacid was 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 was dissolved in chilled ice water in 200 ml of methylene chloride and then 18.6ml of (0.134 mol) triethylamine was added to a solution. Then, to the reaction mixture, by stirring and cooling a 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 for another 1 h. Then it 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 was 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 $P(Ph)_3$ was 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 extracted with CH_2Cl_2; organic extracts were combined, evaporated under reduced pressure. The achievement of complete drying is not needed, because the resulting azide may be subject to decay, and the remainder of the solvent (tetrahydrofuran) does not 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 by stirring in a mixture of 250 ml tetrahydrofuran and 15-20 ml of water. The reaction mixture was mixed for 48 hours at room temperature, then 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 a 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

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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). A mixture, consisting of 95% $CHCl_3$ and 5% isopropyl alcohol, was used as eluent.

RESULTS AND DISCUSSION

During the literature analysis we found an optimal way to perform selective substitution of the hydroxyl-group by an amino-group (10-12), so we carried it out in the following way. Initially, we synthesized metylcholate by reacting cholic acid with methanol in acidic medium (compound 2). The 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 in sequential synthesis these reagents could complicate the interactions through the formation of undesirable compounds. The 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 the resulting substance was semiliquid. We came to a decision to using it in a half raw state. Subsequently, it has not affected the further results. Later on, through consistent interaction with sodium azide (compound 4) and recovery by triphenylphosphine, we succeeded in synthesizing 3α-amino-7,12-dihydroxycholanate methyl ester (compound 5).

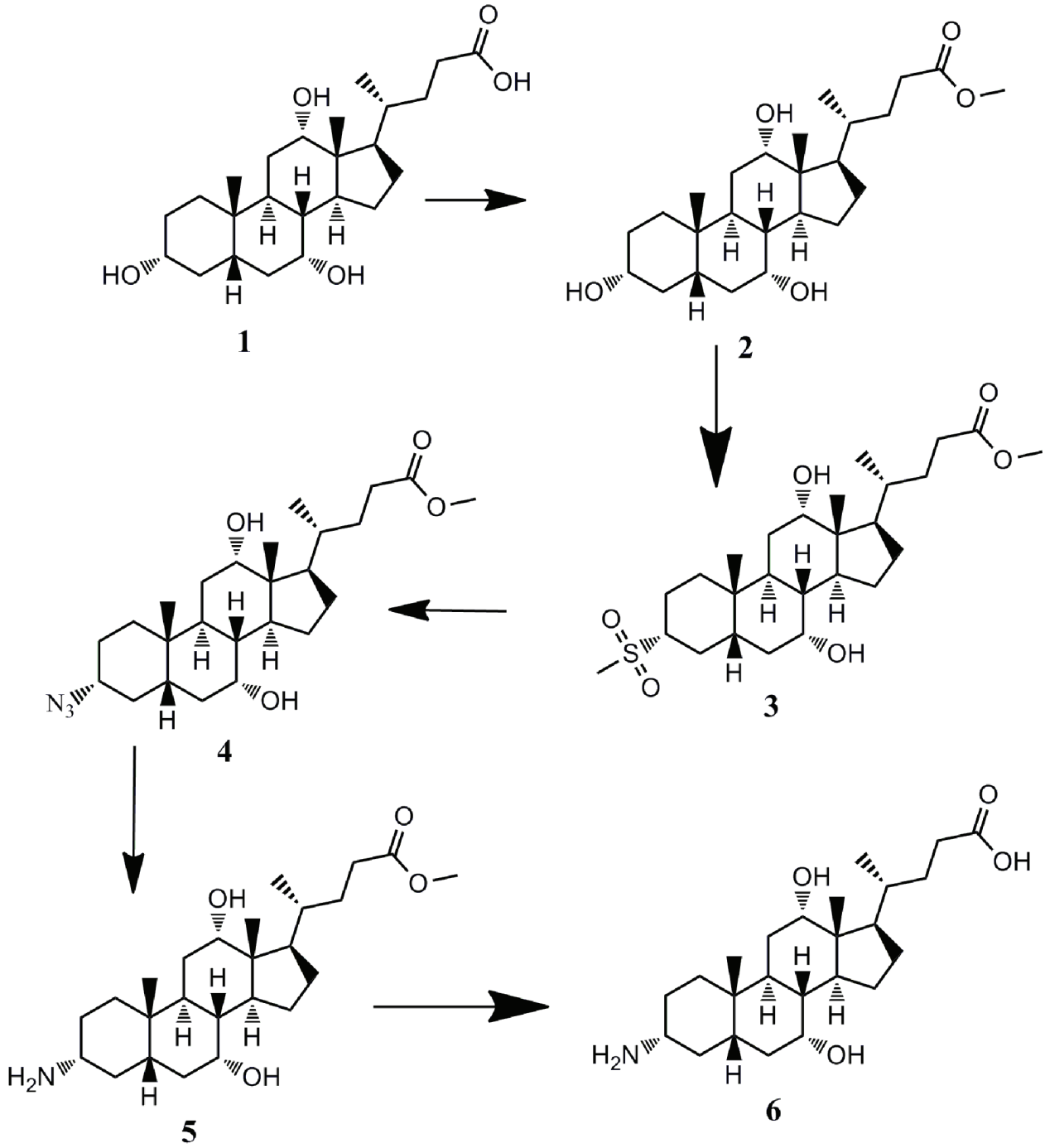
Hydrolysis was performed using the standard method by using sodium hydroxide in water/dioxane mixture to synthesize 3α-amino-7,12-dihydroxycholanic acid (compound 6). ^{1}H NMR spectra data of derived substances, yields and the melting points are shown in Table 1. They were measured at 200MHz in DMSO.

Table 1

№ 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 ^{0}C
methyl 3α-mesyl-7,12-dihydroxycholanate (3)	85,8%	2OH – 3,95; 3,85.	100 ^{O}***C***
methyl 3α-azide-7,12-dihydroxycholanate (4)	92,8%	2OH – 3,95; 3,75.	105 ^{O}***C***
methyl 3α-amino-7,12-dihydrocholanate (5)	90,1%	2OH – 4,0; 3,9. NH_2 – 3,75.	150-155^{0}C
3α-amino-7,12-dihydroxycholanic acid (6)	90,1%	2OH – 4,0; 3,9. NH_2 – 3,75. carboxylic group – 12.1;	150-155^{0}C

The reactions for the synthesis of β isomer differed by the abscence of mesylate preparation step and took place in four stages. Instead of a sequential mesylation, a Mitsunobu's reaction was used, and according to literature data concerning the reactivity of groups in bile acids the reaction was to be held at the third position of the Carbon atom. The source material for the synthesis of the corresponding methyl ester of 3β-amino-7,12-dihydroxy-5β-cholanic acid by the Mitsunobu's reaction was methyl cholate. The 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 an azido group with the change of the optical configuration



Schema 1

of the carbon atom, to which this functional group is bound. Then the azido group can be recovered to an amino in the way shown in Schema 3.

The method displayed at Schema 3 is used in the 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 the amino group. Figure 1 shows the spatial structure of the β stereoiso-

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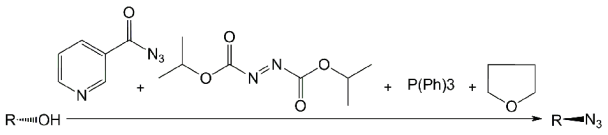

Schema 2

mer, 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 200MHz in DMSO.

In further investigations the synthesized compounds 6 and 9 have demonstrated a 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. The acids were used in further pharmacological experiments, the yield of 3β-amino compound is not very significant, and we need to find and develop other methods of synthesis, perhaps by using microwave waves.

Table 2

№ 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 ^{0}C
methyl 3β-azide-7,12-dihydroxycholanate (7)	-	2OH – 3,95; 3,85.	110-130 ^{O}***C***
methyl 3β-amino-7,12-dihydrocholanate (8)	15,6 %	2OH – 4,0; 3,8. NH_2 – 3,7.	140-145^{0}C
3β-amino-7,12-dihydroxycholanic acid (9)	82,3%	2OH – 4,1 , 3,9 . carboxylic group – 12.0;	150-160^{0}C

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 we had to carry out purification using column chromatography monitoring end by TLC. This was perhaps due to the imperfect methods as well as the characteristic arrangement of atoms in the molecules shown in Fig. 1. Such problems with the synthesis of α amine did not crop up, and the yield was great.

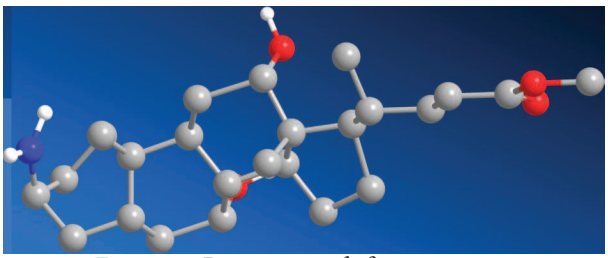

Figure 1. 3D structure of 3β-amino-7,12-dihydroxycholanic acid

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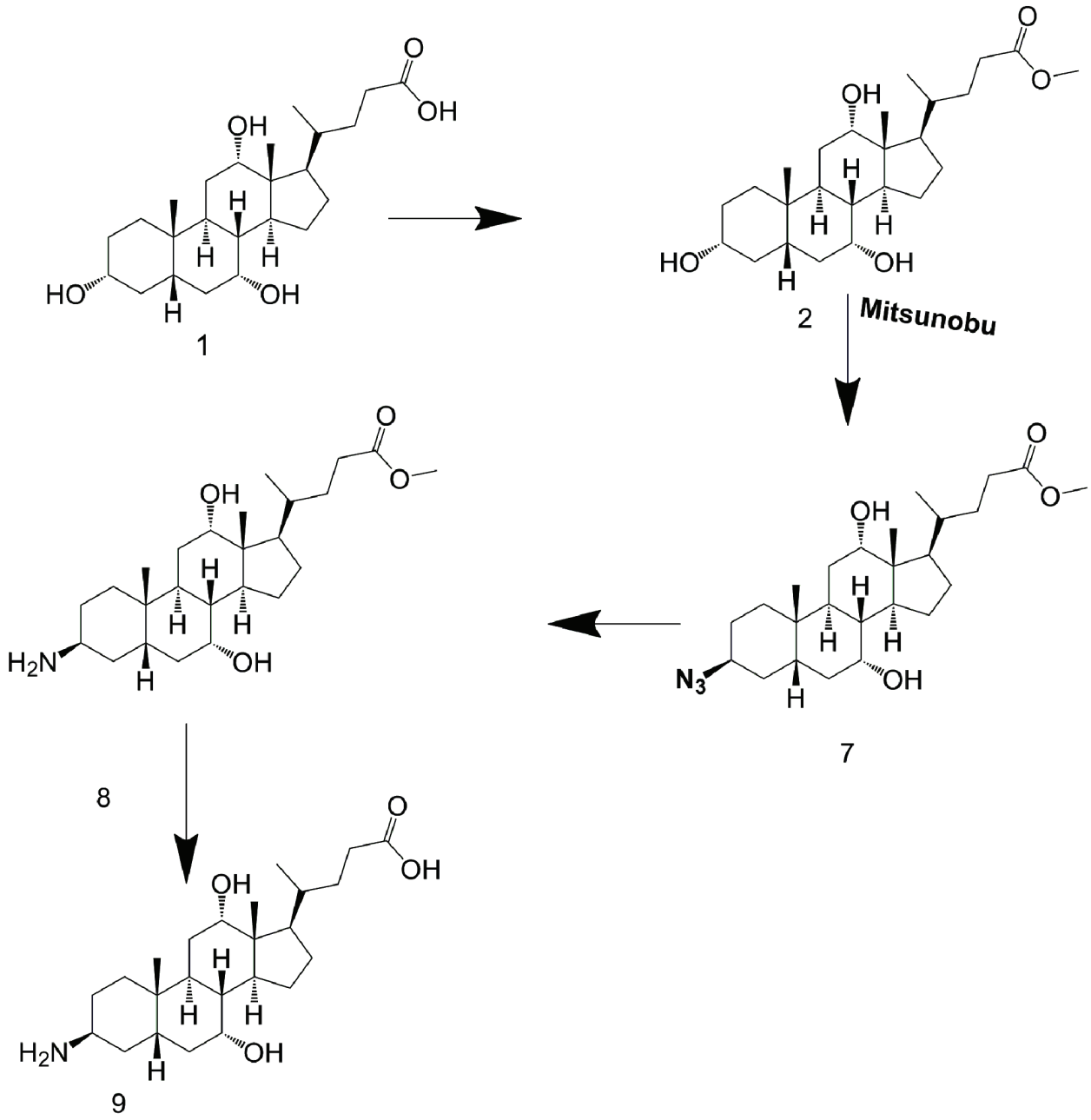

Schema 3

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