Steroids 107 (2016) 10–19

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

# New cholic acid derivatives: Biocatalytic synthesis and molecular docking study

M. Antonela Zígolo, Guadalupe García Liñares, Alicia Baldessari *

*Laboratorio de Biocatálisis, Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, piso 3, C1428EGA Buenos Aires, Argentina*

ARTICLE INFO

*Article history:*
Received 24 September 2015
Received in revised form 7 December 2015
Accepted 19 December 2015
Available online 21 December 2015

*Keywords:*
Cholic acid
Lipase-catalyzed
Molecular docking

ABSTRACT

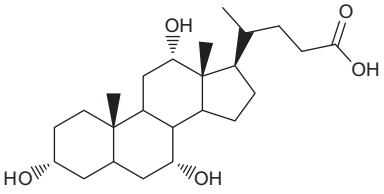
A series of cholic acid derivatives was synthesized by enzyme catalysis. Eleven acetyl and ester derivatives of cholic acid, eight of them new compounds, were obtained through regioselective lipase-catalyzed reactions in very good to excellent yield. The influence of various reaction parameters in the enzymatic esterification, acetylation and alcoholysis reactions, such as enzyme source, alcohol or acylating agent: substrate ratio, enzyme: substrate ratio, solvent and temperature, was studied. Moreover, in order to shed light to cholic acid behavior in the enzymatic reactions, molecular docking of the lipase with cholic acid and some derivatives was carried out.



## 1. Introduction

Enzymes are highly active and selective biocatalysts that turned out to be highly suitable for the synthesis of many complex organic compounds [1,2]. The application of enzymes for synthesizing specific compounds and the development of chemoenzymatic multi-step processes are key research topics nowadays. Among the enzymes exploited for synthetic purposes, lipases are very attractive due to their special properties: stability, recycling, broad substrate tolerance and non-aqueous media activity. Due to their stereo-. chemo- and regioselective nature, lipases have been employed for the preparation of compounds, which are not easily obtainable by chemical methods. Steroids have been suitable substrates for enzymatic transformations. In particular, the regioselective synthesis of steroid derivatives containing multiple hydroxyl groups has been undertaken through lipase-catalyzed acylation or deacylation procedures [3]. These mild and selective interconversions of functional groups are more accessible than the standard chemical approaches, because they avoid the tedious protection and deprotection steps [4].

Bile acids, widely distributed in nature as oxygenated metabolites of steroids, have been the subject of numerous pharmacological studies [5,6]. Their amphiphilic character generally increases cell membrane permeability [3]. For this reason their analogues are widely used in drug formulations as excipients (intestinal absorption enhancers, promoters, etc.) that can influence gastrointestinal solubility, and absorption of drugs [7].




1

Cholic acid (CA, **1**) is one of the most important human bile acids and has attracted significant attention primarily due to its wide availability and relatively low cost. Synthetic analogues and derivatives of cholic acid have been widely explored in different scientific areas such as combinatorial and supramolecular chemistry [8], syntheses of various receptors [9], as antimalarials and antiproliferatives, etc. [10].

As other bile acids, CA exhibits two different faces: a hydrophobic face (β) containing angular methyl groups and a hydrophilic face (α) with hydroxyl groups. This structure is responsible for their amphipathic nature and, consequently, in water self-associates to form multimers above a critical concentration, where hydrogen bonding plays a key role. Because of its amphipathic properties and ability to form micelles, cholic acid has been used

* Corresponding author.
*E-mail address:* alib@qo.fcen.uba.ar (A. Baldessari).

http://dx.doi.org/10.1016/j.steroids.2015.12.014
0039-128X/

as a building block in supramolecular chemistry to transport ion and polar molecules across the membrane [11].

Unlike other bile acids, CA is the only one that has three hydroxyl groups at the C3, C7 and C12 positions facing the concave side of the molecule. This feature gives higher water solubility and lower detergent power than the other bile acids [12]. Sometimes, the potential use of bile acid is difficult because of their low solubility in both hydrophilic and hydrophobic media. In order to find an increase in lipophilicity and enhance permeability features, we have recently reported the synthesis of derivatives of chenodeoxycholic (CDA), deoxycholic (DA) and lithocholic (LA) acids [13].

In the present work we describe an enzymatic strategy for the synthesis of a series of new derivatives of cholic acid, obtained by lipase-catalyzed esterification, acetylation and alcoholysis reactions. Due to its singular structure containing three hydroxyl groups, in the enzymatic reactions CA showed a different behavior from that observed for the three bile acids previously studied, containing one (LA) or two hydroxyl groups (CDA and DA). In fact, we set about examining the particular reaction conditions to obtain CA derivatives. This paper reports the results of our investigation in detail. Moreover, with the aim to rationalize the selective behavior of lipases with CA as substrate, molecular modeling studies were applied.

## 2. Experimental

### 2.1. General

Chemicals and solvents were purchased from Merck Argentina and Sigma–Aldrich de Argentina and used without further purification. Lipase from *Candida rugosa* (CRL) (905 U/mg solid) and type II crude form porcine pancreas lipase ((PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *Candida antarctica* lipase B (CAL B): Novozym 435 (7400 PLU/g) and Lipozyme RM 1 M (LIP) (7800 U/g) were generous gifts of Novozymes Spain and *Carica papaya* lipase (CPL) is the remaining solid fraction of papaya latex, after wash off of proteases using distilled water was a generous gift of Dr. Georgina Sandoval (CIATEJ). All enzymes were used "straight from the bottle". Enzymatic reactions were carried out on Innova 4000 digital incubator shaker, New Brunswick Scientific Co. at the corresponding temperature and 200 rpm. E/S: enzyme amount in mg/substrate amount in mg. To monitor the reaction progress aliquots were withdrawn and analyzed by TLC performed on commercial 0.2 mm aluminum-coated silica gel plates (F254) and visualized by 254 nm UV or immersion in an aqueous solution of $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.04 M), $Ce(SO_4)_2$ (0.003 M) in concentrated $H_2SO_4$ (10%).% Conversion was determined by analytical reverse-phase HPLC employing a Phenomenex Phenogel column 5 μM 10E5A, 300 × 7.8 mm and eluting with MeOH:$H_2O$ 80:20 at 1.00 mL/min. Melting points were measured in a Fisher Johns apparatus and are uncorrected. Optical rotation values were measured in a $CHCl_3$ solution with a Perkin Elmer-343 automatic digital polarimeter at 25 °C. $^1$H NMR and $^{13}$C NMR spectra were recorded at room temperature in $CDCl_3$ as solvent using a Bruker AM-500 NMR instrument operating at 500.14 MHz and 125.76 MHz for $^1$H and $^{13}$C respectively. The $^1$H NMR spectra are referenced with respect to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at $\delta$ = 7.26 ppm. Coupling constants are reported in Hertz (Hz). $^{13}$C NMR spectra were fully decoupled and are referenced to the middle peak of the solvent $CDCl_3$ at $\delta$ = 77.0 ppm. Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quadruplet; qn, quintet; dd, double doublet, etc. IR spectra were recorded with a Nicolet Magna 550 spectrometer. High Resolution Mass Spectrometry was recorded with Thermo Scientific EM/DSQ II – DIP. The results were within ± 0.02% of the theoretical values.

### 2.2. Enzymatic esterification. General procedure

Lipozyme (1 g) was added to a solution of cholic acid (100 mg) in DIPE (10 mL) and the corresponding alcohol (20 eq). The suspension was shaken (200 rpm) at 40 °C and the progress of the reaction monitored by TLC/HPLC. Once the reaction was finished, the enzyme was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography on silicagel employing mixtures hexane:ethyl acetate as eluent (6:4–2:8). Reuse experiments: the filtered and washed enzyme was used in the next enzymatic esterification under the same reaction conditions.

### 2.3. Enzymatic acetylation. General procedure

CAL B (1 g) was added to a solution of ethyl cholate (100 mg) in hexane (10 mL) and ethyl acetate (0.25 mL). The suspension was shaken (200 rpm) at 40 °C and the progress of the reaction monitored by TLC/HPLC. Once the reaction was finished, the enzyme was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography on silicagel employing mixtures hexane:ethyl acetate as eluent (7:3–1:1). Reuse experiments: the filtered and washed enzyme was used in the next enzymatic acetylation under the same reaction conditions.

### 2.4. Enzymatic alcoholysis. General procedure

To a solution of the peracetylated cholic acid (100 mg) in 10 ml of the indicated solvent containing 5 mol equiv of ethanol, CAL B (1 g) was added. The suspension was shaken (200 rpm) at 40 °C and the progress of the reaction was monitored by TLC. After indicated time, the enzyme was filtered off, the solvent was evaporated, and the crude residue purified by column chromatography on silica gel employing mixtures hexane:ethyl acetate as eluent (8:2–1:1).

### 2.5. Chemical peracetylation

In a typical procedure, a mixture of cholic acid (400 mg, 1 mmol), acetic anhydride (2 mL, 20 mmol), pyridine (2 mL) and DMAP (10 mg) was stirred at room temperature for 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was partitioned between saturated solution ammonium chloride (20 mL) and methylene chloride (20 mL). The aqueous phase was extracted with methylene chloride (3 × 10 mL). The combined organic layers were washed with saturated solution of sodium chloride (5 × 20 mL), dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography (silica gel) employing mixtures of hexane/EtOAc as eluent (8:2–1:1).

#### 2.5.1. Ethyl 3α,7α,12α-trihydroxy-5β-cholanate (2a)

Yield 85%; white solid, mp 160–161 °C; $[\alpha]_D^{25}$ + 22.2° (*c* 1.0, $CHCl_3$). $^1$H NMR ($CDCl_3$, 500 MHz) δ4.12 (2H, q, *J* = 6.7 Hz, –OC*H*$_2$-$CH_3$), 3.96 (1H, t, *J* = 2.9 Hz, H-12), 3.83 (1H, dd, *J* = 2.5, 3.0 Hz, H-7), 3.43 (1H, tt, *J* = 11.2, 4.1 Hz, H-3), 2.35 (1H, m, H-23b), 2.22 (1H, m, H-23a), 1.24 (3H, t, *J* = 6.8 Hz, –OCH$_2$C*H*$_3$), 0.98 (3H, d, *J* = 6.4 Hz, H-21), 0.88 (3H, s, H-19), 0.67 (3H, s, H-18); $^{13}$C NMR ($CDCl_3$, 500 MHz) δ 174.4 (C-24), 73.1 (C-12), 71.9 (C-7), 68.4 (C-3), 60.2 (–O*C*H$_2$CH$_3$), 30.9 (C-23), 22.4 (C-19), 17.3 (C-21), 14.2 (–OCH$_2$C*H*$_3$), 12.5 (C-18). HRMS: $[M+Na]^+$ Calcd. $C_{26}H_{44}NaO_5$ 459.3086 Found: $C_{26}H_{44}NaO_5$ 459.3090.