STEROIDS 73 (2008) 1385–1390

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7α- and 12α-Hydroxysteroid dehydrogenases from *Acinetobacter calcoaceticus lwoffii*: a new integrated chemo-enzymatic route to ursodeoxycholic acid

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ARTICLE INFO

Article history:
Received 2 April 2008
Received in revised form
13 June 2008
Accepted 26 June 2008
Published on line 12 July 2008

Keywords:
12α-Hydroxysteroid dehydrogenase
7α-Hydroxysteroid dehydrogenase
Oxidation
Cholic acid
Ursodeoxycholic acid

ABSTRACT

We report the very efficient biotransformation of cholic acid to 7-keto- and 7,12-diketocholic acids with *Acinetobacter calcoaceticus lwoffii*. The enzymes responsible of the biotransformation (i.e. 7α- and 12α-hydroxysteroid dehydrogenases) are partially purified and employed in a new chemo-enzymatic synthesis of ursodeoxycholic acid starting from cholic acid. The first step is the 12α-HSDH-mediated total oxidation of sodium cholate followed by the Wolf–Kishner reduction of the carbonyl group to chenodeoxycholic acid. This acid is then quantitatively oxidized with 7α-HSDH to 7-ketochenodeoxycholic acid, that was chemically reduced to ursodeoxycholic acid (70% overall yield).

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1. Introduction

Bile acids (BAs), their conjugates and salts are natural products and fundamental constituents of bile [1]. Among them chenodeoxycholic and 7-OH epimer ursodeoxycholic acid (UDCA) have important pharmaceutical applications related to their ability to solubilize cholesterol gallstones [2,3]. Both these acids are prepared on a large scale from raw, low cost materials with high bile acid content as bovine bile. Its major component is the relatively inexpensive cholic acid that is therefore used as starting materials for the synthesis of chenodeoxycholic acid [4,5] and ursodeoxycholic acid [6,7] through different synthetic pathways [7–9]. The first step of the chemical procedure that affords these valuable drugs is the alkaline hydrolysis of the conjugates (i.e. taurinates and glycinates) obtained from bovine bile followed by a sequence of classic chemical reactions [1]. Among them, two steps are particularly crucial: the regioselective oxidation of the 12α-OH group [4] and the selective α/β inversion of the 7-OH carbon center [6]. Chemically the regioselective oxidation of 12α-OH can be obtained after the problematic protection of the 3- and 7-OH groups, lowering the oxidation yields, while the α/β inversion can be achieved through the regioselective oxidation of the 7α-OH function and the subsequent radicalic reduction with Na in ethanol [7] or with Li in liquid NH_3 [10].

Hoffmann [5] has described a seven-step sequence that represents the pure chemical approach to this problem. More recently, Sawada et al. [11,12] reported the preparation of

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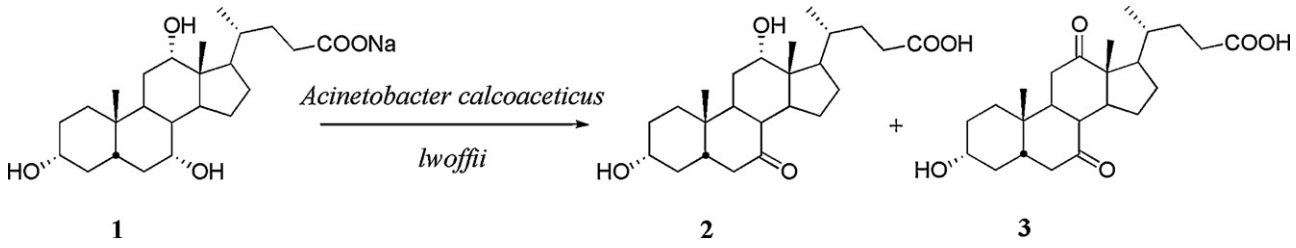
doi:10.1016/j.steroids.2008.06.013



Scheme 1

chenodeoxycholic acid by bioconversion of dehydrocholic acid mediated by bacteria. Additionally, in an our previous work this problem was resolved chemically by selective reduction of dehydrocholic acid [13]. On the other hand, various NAD-dependent 12α-hydroxysteroid dehydrogenases were purified from *Eubacterium lentum* [14] and *Clostridium* group P, strain C48–50 [15,16] and have been efficiently utilized in the synthesis of 12-ketochenodeoxycholic acid [17,18] and 12-ketoursodeoxycholic [19,20].

In this paper we report the oxidation of C_7–OH and C_{12}–OH groups of sodium cholate **1** (Scheme 1) by *Acinetobacter calcoaceticus lwoffii*, a strain isolated from a soil sample of ICE industry [21], and further partial purification of 7α-HSDH and 12α-HSDH. These enzymes are used in a new integrated chemo-enzymatic synthesis of ursodeoxycholic acid **7** starting from sodium cholate **1** (Scheme 2).

2. Experimental

Sodium salt of cholic acid **1** and chenodeoxycholic acid **5a** have been supplied by ICE industry [21].

NAD^+, phenylmethyl sulphonyl fluoride (PMSF), β-mercaptoethanol, egg white lysozyme, DEAE–sepharose, and LDH from rabbit muscle were obtained from Sigma.

Melting points are uncorrected and were determined on a 510 Büki melting point instrument. ^1H and ^{13}C NMR spectra were obtained with a Varian Gemini 300 spectrometer operating at 300 MHz (^1H) and 75 MHz (^{13}C), with Me_4Si as internal standard.

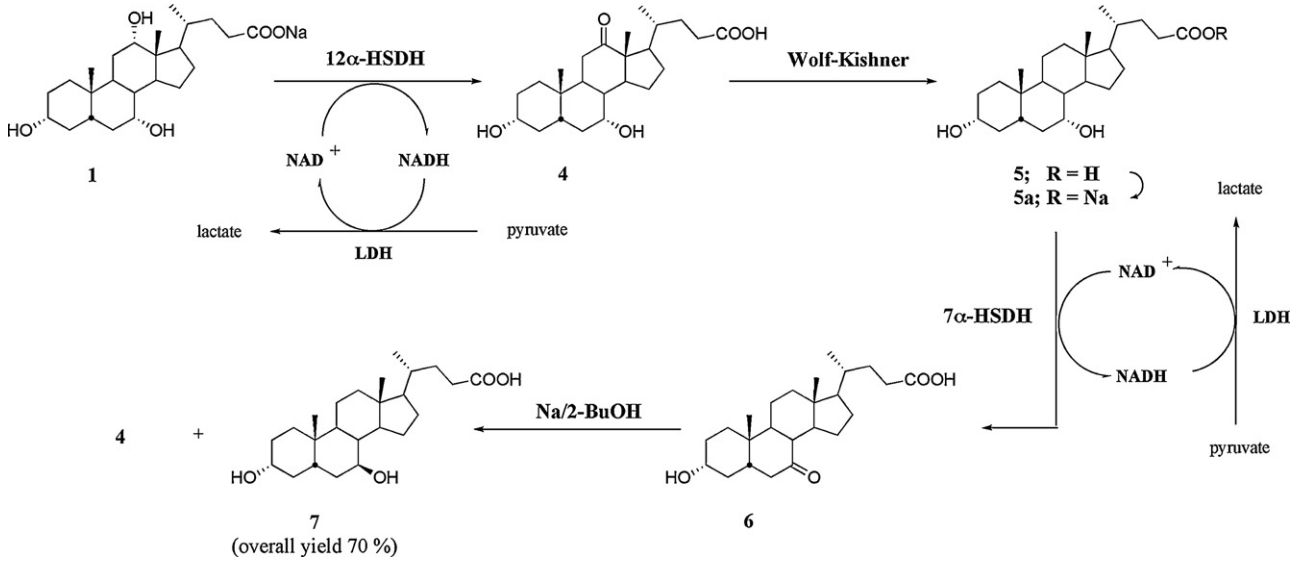
TLC were performed on precoated silica gel plates (thickness 0.25 mm, Merck) and silica gel (Fluka, Kiesegel 60, 70–230 mesh) was used for preparative column chromatography.

Gas chromatographic analyses were performed on a Carlo Erba HRGC 5160 Mega series chromatograph. The reaction products, previously derivatized with trifluoroacetic anhydride and hexafluoroisopropanol, were analyzed by GLC on fused capillary column SE52 (25 m × 0.32 mm) from Mega s.n.c.: helium as carrier gas (0.55 atm); temperature 250 °C for 5 min, 250–300 °C (5 °C/min) and then 300 °C for 3 min.

Retention times (in min) for the series of cholic and chenodeoxycholic acids were reported in a previous paper [22].

2.1. Biotransformation of sodium cholate 1 with A. calcoaceticus lwoffii

A loopful of *A. calcoaceticus lwoffii* from a culture on plate count agar was inoculated in plate count broth (100 mL) containing glucose (1 g/L), yeast extract (2.5 g/L) and tryptone (5 g/L). After 48 h at 30 °C and 100 rpm, sodium cholate **1** (0.1 g) was added and the culture was maintained in the same conditions for 48 h. The biomass was removed by centrifugation (10,000 rpm, 15 min) and 20% HCl was added to reach pH 2. The acidified solution was extracted with ethyl acetate (3× 60 mL), the



Scheme 2

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