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A practical chemoenzymatic approach to the synthesis of 3-hydroxy metabolites of tibolone

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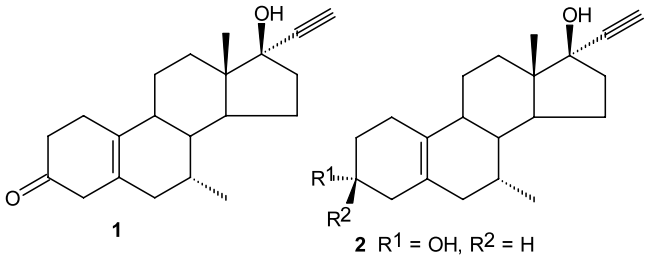
Abstract—Stereoselective preparation of the 3-hydroxy metabolites of tibolone is easily accomplished by diastereoselective *Candida antarctica* lipase B-catalyzed transformations of 3α- and 3β-alcohols or acetates mixtures in organic solvents, in suitable amounts for biological studies.

1. Introduction

Tibolone **1** is a synthetic steroid with oestrogenic, progestenic and androgenic properties, used in the treatment of menopausal complaints.[1] It is quickly metabolized into its main active metabolites, the 3α- and 3β-hydroxy derivatives **2** and **3** and into the Δ^4-isomer.



1

2 R^1 = OH, R^2 = H

3 R^1 = H, R^2 = OH

As a result of their affinity for the oestrogenic receptor and the inhibition of enzymes involved in the biosynthesis of estradiol, the 3-hydroxy metabolites may provide new possibilities in the treatment of oestrogen-dependent breast tumors.[2,3] Thus, their preparation in diastereomerically pure form is highly desirable.

However, as reported in the case of norethynodrel, a tibolone analogue lacking the 7α-methyl group, the reduction of **1** affords a mixture of isomeric 3-hydroxy derivatives, with the product ratio depending on the reducing agent employed.[4] Recently,[5] through NMR studies and quantum-chemical investigations, we could unambiguously assign the C-3 configurations of **2** and **3** and determine that, the 3α-diol **2** is, in any case, the predominant isomer in the reduction. Due to the presence of the 5–10 double bond, the A ring is in a 'quasi-chair' conformation and the 3α-hydroxy group is equatorial. This diastereoisomer can be obtained in pure form by lithium tri-*tert*-butoxyaluminum hydride reduction of **1**,[†] whereas the preparation of the 3β-alcohol **3** needs additional synthetic work: diisobutylaluminum hydride (DIBAL) reduction of **1** affords a 7/3 mixture of alcohols **2** and **3**. The β-isomer, even after repeated chromatography and recrystallization, is always isolated in the presence of the α-isomer (5–10%); better results with respect to isomer purity (but low yields) are achieved by inversion of the configuration at C-3 of the 3α-isomer **2** by Mitsunobu reaction,[6] followed by hydrolysis of the thus obtained ester.[5]

The well known capabilities of hydrolytic enzymes, such as lipases, in regio- and stereoselective transformations of polyhydroxylated steroids[7,8] prompted us to study a chemoenzymatic approach to the preparation of pure 3α- and 3β-diols **2** and **3**. Moreover, the mild conditions of lipase-catalyzed reactions in organic solvents leave the 5–10 double bond and 17α-ethynyl group present in these tibolone metabolites unreacted.

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† In this case a 96/4 ratio between the 3α- and 3β-diol is observed and the predominant isomer is isolated by crystallization.

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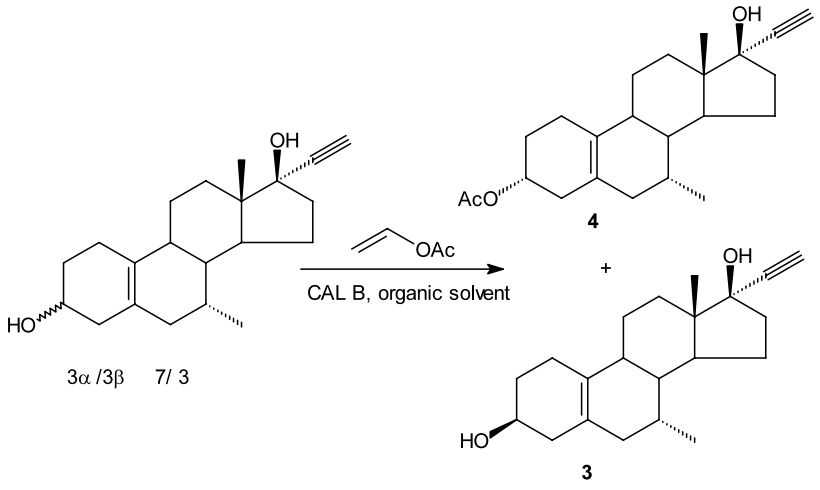
2. Results and discussion

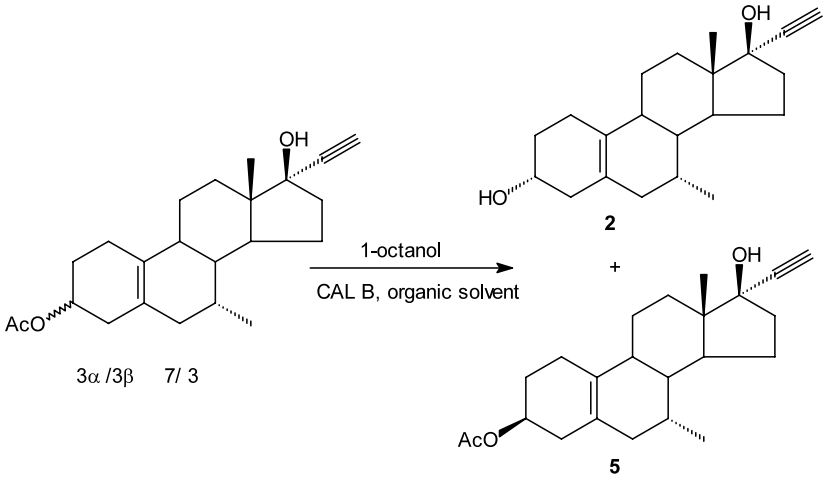
Lipases from *Pseudomonas cepacia* and *Candida rugosa* (PCL and CRL), which have been applied successfully in selective transformations of hydroxy steroids and their esters,[7,8] are not useful in the case of the diols we wish to investigate. In fact, under irreversible transesterification conditions, using vinyl acetate as the acyl donor,[9,10] PCL does not transform the 7/3 mixture of isomeric **2** and **3** and CRL shows some activity only in chloroform (60% of the 3α-isomer was transformed after 5 days), leaving the starting material completely unreacted in other solvents (acetonitrile, tetrahydrofuran, *tert*-butylmethyl ether and toluene). Significant results are obtained by means of *Candida antarctica* lipase (CAL-B, Novozym 435) with vinyl acetate in different solvents, the best diastereoisomeric separation being achieved in toluene (Scheme 1): the α-isomer is quantitatively transformed into the corresponding 3-acetate, after 1 h, while the 3β-alcohol is not affected by the enzymatic reaction, even after 24 h. For the CAL-B-catalyzed alcoholysis of the 7/3 mixture of acetates **4** and **5** (using octanol as acyl acceptor[11] in toluene) longer times are required, the enzymatic reaction occuring with the same preference for the 3α-isomer, leaving the 3β-acetate unreacted (Scheme 2).

The results from CAL-B-catalyzed reactions in the screened solvents are collected in Table 1. The study shows that better results are obtained under transesterification conditions than in alcoholysis, and the only unsuitable solvent for the separation of the diastereoisomeric pair of tibolone metabolites is chloroform.

When CAL-B is employed in regioselective transformations of polyhydroxylated steroids[12,13] it shows a preference for the alcoholic functions on the A ring: for substrates with the A/B ring fusion in the *trans* configuration or with a 5,6-double bond, the equatorial 3β-hydroxyl is acylated, whereas the equatorial 3α-hydroxy derivatives are transformed when the A/B ring fusion is in the *cis* configuration. The preference of CAL-B for acylation at the 3-equatorial hydroxyl group is con-



Scheme 1.



Scheme 2.

Table 1. CAL B-catalyzed transesterification of **2**/**3** and alcoholysis of **4**/**5**

Solvent	Irreversible transesterification			Alcoholysis		
	Reaction time (h)	Conversion of **2** (%)[a]	% Ratio of unreacted substrates **2**/**3**	Reaction time (h)	Conversion of **4** (%)[a]	% Ratio of unreacted substrates **4**/**5**
Acetonitrile	2.5	100	0/100	72	100	0/100
Tetrahydrofuran	3	100	0/100	72	93	11/89
tert-Butylmethylether	5	100	0/100	24	88	20/80
Toluene	1	100	0/100	24	100	0/100
Chloroform	27	0	7/3	48	33	55/45

[a] Determined by GLC.

firmed by our results: in fact in the case of the isomeric mixture of **2** and **3**, having a 'quasi-chair' A ring conformation, CAL-B is able to transform only the equatorial 3α- and not the axial 3β-isomer.

In conclusion, the good diastereoselectivity showed by CAL-B provides a facile method for preparation of both tibolone metabolites **2** and **3** in pure form, in the amounts required (gram-scale) for biological evaluation, avoiding careful and tedious chromatography and crystallization exercises.

3. Experimental

All solvents and reagents were purchased from Sigma-Aldrich. All reactions were monitored by TLC on silica gel 60 F_{254} plates (Merck) with detection by spraying with 10% phosphomolybdic acid in ethanol solution and heating at 110°C. Column chromatography was performed on silica gel 60 (0.063–0.200 mm) (Merck). Differential scanning calorimetry (DSC) were performed on a Perkin Elmer DSC-7 instrument. GLC analysis were performed on a Hewlett Packard HP5890 instrument at 260°C oven temperature, with an HP5-WB capillary column (25 m×0.32 mm i.d., 0.52 μm film thickness). Optical rotations were determined on a Perkin Elmer model 241 polarimeter in a 1 dm cell at 25°C. All NMR spectra were recorded in $CDCl_3$ solutions with a Bruker AM-500 spectrometer. Chemicals shifts are reported on the δ (ppm) scale and are relative to TMS as internal reference.

3.1. 17α-Ethynyl-7α-methyl-5(10)-estren-3ξ,17β-diols, 2 and 3

To a solution of **1** (1 g, 3.2 mmol) in toluene (60 mL) at 0°C, was added DIBAL (1 M in hexane, 6.9 mL). After stirring at 0°C for 3 h, the reaction mixture was treated with a saturated aqueous NH_4Cl solution (20 mL) and filtered through a Celite pad; the organic phase was separated and the aqueous phase extracted with ethyl acetate (3×20 mL). The collected organic phases were dried over Na_2SO_4 and evaporated at reduced pressure. The residue (1 g) was purified on column chromatography (silica gel 1/20) by elution with hexane/ethyl acetate (7/3), a mixture of **2** and **3** (0.8 g, 80%) was obtained. GLC: 3α-diol T_R 12.75 min, 3β-diol T_R 12.56 min. In the ^1H NMR spectrum two multiplets at 3.94 and 4.16 ppm were present, in a 7/3 ratio, due to the H-3 of 3α- and 3β-diol, respectively.[5]

3.2. General procedure for CAL-B-catalyzed irreversible transesterification

In a typical experiment the 7/3 mixture of **2** and **3** (1 g, 3.18 mmol) was dissolved in the required solvent (200 mL). Vinyl acetate (1.25 mL, 13.5 mmol) and CAL-B (Novozym 435, 3.5 g) were added; the suspension was kept at 30°C under stirring for the required time (see Table 1). The reaction progress was monitored by GLC. The enzyme was removed by filtration and the residue obtained after evaporation at reduced pressure was purified by silica gel column chromatography; elution with hexane/ethyl acetate (8/2) afforded pure 3α-acetate **4** (0.85 g); endothermic peak fusion (DSC) at 70°C (acetone/water); $[\alpha]_D$ +118 (*c* 1, chloroform); ^1H NMR δ 0.74 (d, 3H, 7α-CH_3), 0.84 (s, 3H, CH_3-18), 2.02 (s, 3H, CH_3CO), 2.55 (s, 1H, CH-21), 4.83 (m, 1H, CH-O). Pure 3β-diol **3** (0.24 g) was recovered with hexane/ethyl acetate (7/3) as eluant; its chemico-physical data are in agreement with those reported in Ref. 5.

3.3. 17α-Ethynyl-7α-methyl-5(10)-estren-3α,17β-diol, 2

Acetate **4** (0.8 g, 2.2 mmol) in methanol (28 mL) was treated with a solution of K_2CO_3 (1.1 g, 8.0 mmol) in water (3 mL) at room temperature (3 h). After addition of water (15 mL) the precipitate was recovered by suction affording pure 3α-diol **2** (0.65 g, 95%); its chemico-physical data are in agreement with those reported in Ref. 5.

3.4. 17α-Ethynyl-7α-methyl-5(10)-estren-3ξ,17β-diol, 3-acetates, 4 and 5

The 7/3 mixture of **2** and **3** (0.312 g, 1 mmol) was treated with acetic anydride (0.5 mL, 5.3 mmol) in pyridine (1 mL) at 0°C for 3 h. The reaction mixture was poured into water (3 mL) and extracted with dichloromethane (3×3 mL). The collected organic phases were washed with water (2×4 mL), and dried over sodium sulfate. Evaporation of the solvents at reduced pressure afforded a mixture of acetates **4** and **5** (0.32 g, 90%). GLC: 3β-acetate T_R 15.73 min, 3α-acetate T_R 16.95 min. In the ^1H NMR spectrum two

multiplets at 4.81 and 5.03 ppm were present, in a 7/3 ratio, due to the H-3 of 3α- and 3β-acetate, respectively.

3.5. General procedure for CAL-B-catalyzed alcoholysis

In a typical experiment a 7/3 mixture of acetates **4** and **5** (0.48 g, 1.32 mmol) was dissolved in the required solvent (30 mL). 1-Octanol (1.5 mL, 9.45 mmol) and CAL-B (2.4 g) were added. The mixture was kept at 30°C, under stirring, for the required time (see Table 1), monitoring the progress of the reaction by GLC. In the case of the alcoholysis carried out in toluene, the enzyme was removed by filtration and evaporation of solvents, at reduced pressure, afforded a mixture of 3β-acetate **5** and 3α-diol **2** that were separated by column chromatography (silica gel 1/10). By elution with hexane/ethyl acetate (8/2) 3β-acetate **5** (0.135 g) was obtained: endothermic peak of fusion (DSC) 153°C (dichloromethane/hexane); $[\alpha]_D$ +18 (*c* 1, chloroform); ^{1}H NMR δ 0.75 (d, 3H, 7α-CH_3), 0.86 (s, 3H, CH_3-18), 2.02 (s, 3H, CH_3CO), 2.55 (s, 1H, CH-21), 5.05 (m, 1H, CH-O); pure 3α-diol **2** (0.285 g) was recovered by elution with hexane/ethyl acetate (7/3).

Treatment of **5** with aqueous K_2CO_3 in methanol (as described for 3α-acetate **4**) afforded the pure 3β-diol in 90% yield.

Acknowledgements

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