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Lipase-catalyzed preparation of corticosteroid 17α-esters endowed with antiandrogenic activity

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ABSTRACT

Several 17α-monoesters of cortexolone and its Δ^9-derivative are endowed with antiandrogenic activity. Their synthesis can be accomplished by means of a lipase-catalyzed chemoselective alcoholysis of the corresponding 17α,21-diesters.

1. Introduction

Recently, the antiandrogenic activity of a series of 17α-esters of cortexolone (also named Reichstein's substance S) **1** and Δ^9-cortexolone **2**, some of them known in the past only as anti-inflammatory or progestational compounds, has been reported. Particularly, among the screened esters **3a–d** and **4**, cortexolone 17α-propionate **3b** showed a potent antiandrogenic topical activity, in the absence of the systemic one,[1] whereas 17α-butanoate of Δ^9-derivative **4** displayed an almost exclusive systemic activity.[2]

Parent compounds **1** and **2** are devoid of antiandrogenic activity, the acylation of 17α-hydroxy group being then essential (Fig. 1).

The acylation of tertiary 17-hydroxy group can be achieved, according to a published method,[3] by acidic hydrolysis of 17,21-orthoester **5**, in turn prepared by reaction of the 17α,21-diol with a commercially available orthoester in the presence of *p*-toluensulfonic acid.[4] 21-Monoester **6**, derived from the well-known acyl transfer from 17α position to 21[5] is the main by-product, present up to 3%. 17→21 Migration is, also in vitro and in vivo, one of

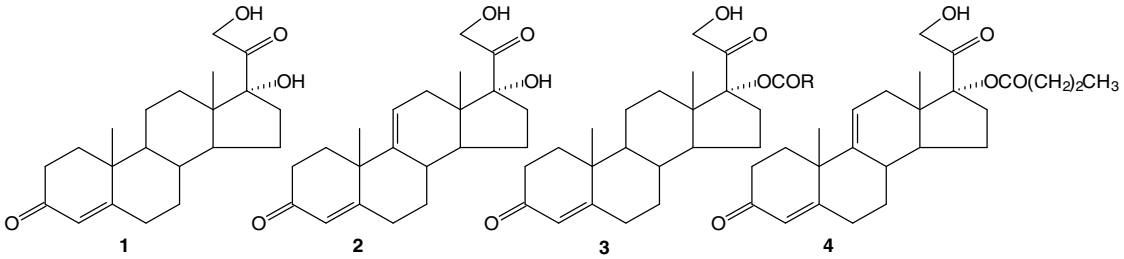

Figure 1.

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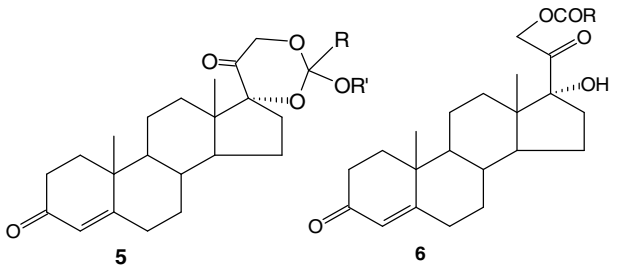


5 6

Figure 2.

the main metabolic transformations of 17-monoesters, leading to 21-monoesters devoid of antiandrogenic activity. In order to obtain, following the reported method,[3,4] 17α-monoesters **3** suitably pure for biological tests and pharmacological applications (>99%), a carefully and laborious purification procedure is usually necessary (Fig. 2).

The known properties of lipases[6,7] to maintain the activity in organic solvents, to selectively transform polyfunctional compounds and to require mild reaction conditions, prompted us to study a chemoenzymatic approach to the preparation of above esters **3** and **4**. Use of lipases for selective transformations of steroids is well documented and examples of acylation of hydroxy groups as well as of ester hydrolysis, for this class of compounds, are reported.[8,9]

Table 1
Lipase-catalyzed alcoholysis of **7b** to **3b**

Lipase	Solvent	Alcohol	Time (h)	Conversion[a] (%)
PPL	Toluene	*n*-Octanol	120	13
PFL	Chloroform	Methanol	24	0
CALB	Acetonitrile	*n*-Octanol	76	91
CALB	Toluene	*n*-Octanol	76	77
CCL	Acetonitrile	*n*-Butanol	96	91
CCL	Tetrahydrofuran	*n*-Butanol	96	86
CCL	Chloroform	*n*-Butanol	96	18
CCL	Toluene	Methanol	80	100
CCL	Toluene	Ethanol	80	86
CCL	Toluene	*n*-Butanol	24	100
CCL	Toluene	*n*-Octanol	24	100

[a] Evaluated from ^{1}H NMR spectra on the basis of the integration of H-21 signals of **3b** and **7b**. Signals due to H-21 of 21-monopropionate were not detectable.

2. Results and discussion

The difficulty to selectively esterify the hindered 17α-tertiary alcohol, in the presence of the primary 21 one, suggested us to prepare a series of 17α,21-diesters by means of a reported method,[10] and to try to selectively remove the acyl group from the primary 21-alcohol, using a lipase. In fact traditional basic hydrolyses are not selective since, as reported by Gardi and Ercoli,[3b] mixtures of products are obtained. The desired diesters **7a**–**d**[11–14] and **8**[15] were obtained by reaction of **1** or **2**[16] with the suitable anhydride, in the presence of the corresponding acid and PTSA (about 70% yields).[10]

Table 2
CCL-catalyzed alcoholysis of **7a**, **7c** and **7d** in toluene

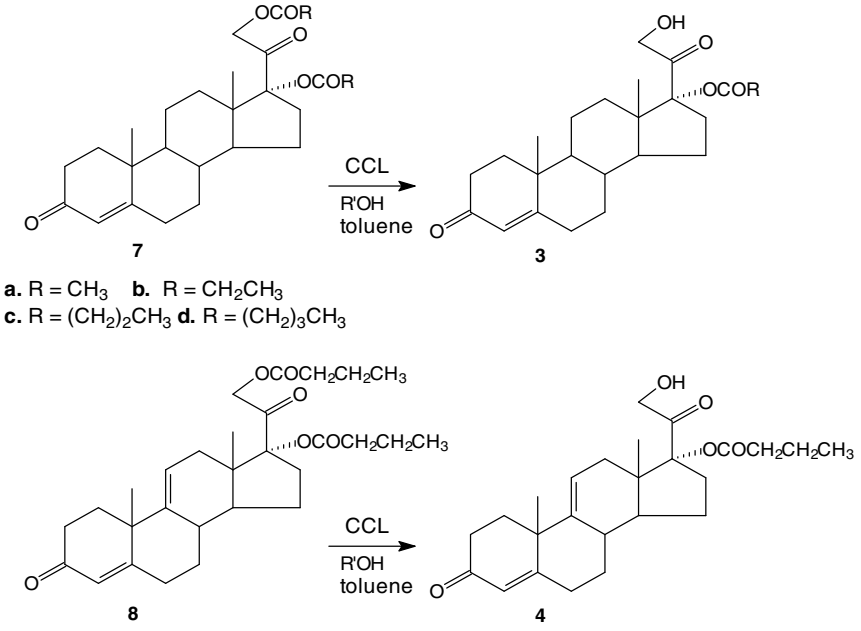
Substrate	*n*-Butanol		*n*-Octanol	
	Time (h)	Conversion[a] (%)	Time (h)	Conversion[a] (%)
7a	24	98	24	90
7c	74	98	24	98
7d	74	81	48	97

[a] Evaluated from ^{1}H NMR spectra on the basis of the integration of H-21 signals of **3a**, **3c**, **3d** and **7a**, **7c**, **7d**, respectively. Signals due to H-21 of cortexolone 21-monesters were not detectable.

Table 3
CCL-catalyzed alcoholysis of **8** in toluene

Alcohol	Time (h)	Conversion[a] (%)
Methanol	53	79
Ethanol	53	28
n-Propanol	53	100
n-Butanol	53	100

[a] Evaluated from ^{1}H NMR spectra on the basis of the integration of H-21 signals of **4** and **8**. Δ^9-Cortexolone 21-butyrate was not present.



Scheme 1.

Then, starting from 17α,21-dipropionate **7b**,[12] precursor of the monoester endowed with the highest topical antiandrogenic activity,[1] we screened several commercially available lipases under alcoholysis conditions in organic solvents; these experimental conditions were jugded more suitable, than the aqueous ones, to be used for lipophilic substrates as steroids. Lipases from *Pseudomonas fluorescens* (PFL) and from porcine pancreas (PPL) afforded negative results: PFL was able to transform only a little amount (10%) of starting material **7b** while no formation of monoester **3b** was observed, using PPL. Lipase from *Candida antarctica* B (CAL B) showed to selectively remove the 21-acyl group, 77 and 91% conversion to 17α-monoester **3b** being achieved in toluene or acetonitrile, respectively (see Table 1). Best results were observed when 17α,21-diester **7b** was treated with lipase from *Candida cylandracea* (CCL), with conversions depending on the chosen alcohol and the solvent: in fact, using butanol or octanol as the acyl acceptor and toluene as the solvent, a complete conversion was observed after 24 h,[17] and pure 17α-propionate **3b**[18] was recovered in >90% yields (Scheme 1). The CCL-catalyzed alcoholysis is less efficient if methanol or ethanol are used as acyl acceptors or if more polar solvents are chosen. The results are summarized in Table 1.

Also 17α,21-diacetate **7a**,[11] dibutyrate **7c**[13] and divalerate **7d**[14] were submitted to CCL-catalyzed alcoholysis in order to prepare the other biologically active monoesters. Selective hydrolysis of 21-ester was confirmed, 17α-monoesters **3a**,[19] **3c**[20] and **3d**[21] being obtained as unique products (Scheme 1); no relevant differences in the alcoholysis procedure were observed: only, the use of butanol showed a slower conversion rate of dibutyrate 7**c** and divalerate **7d**. The results are collected in Table 2.

Finally, we prepared Δ^9-cortexolone 17α-butyrate **4**, that is, the derivative endowed with a high systemic antiandrogenic activity.[2] Also in this case the CCL-catalyzed transformation of diester **8** was performed in toluene with different alcohols, comparing the conversion rate at the same time. Best results, like for dipropionate **7b**, were observed using butanol or octanol as acyl acceptors, a complete conversion of diester **8** to monoester **4**[22] being achieved after 53 h (Table 3, Scheme 1).

3. Conclusion

The CCL-catalyzed alcoholysis in organic solvents, selectively removing the 21-acyl group, allowed us to prepare 17α-monoesters **3a**–**d** and **4** in good yields and with the required purity (>99%), avoiding the 17→21 acyl migration.

Additionally, it is important to underline that CCL can be recycled at least four times, without sensible decrease of enzymatic activity: in fact the recovered enzyme from alcoholysis of **7b** to **3b** was reused, under the same reaction conditions (toluene as the solvent, butanol as the acyl acceptor and 24 h as the reaction time), and, after the fourth cycle, we observed only a lowered conversion from 100% to 94%.

Moreover, this chemoenzymatic approach represents a simple preparative method that could be used, after the suitable development, for a future scaling up (i.e., in a continuous packed-bed reactor) of the synthesis of cortexolone and Δ^9-cortexolone 17α-monoesters, recently recognized as antiandrogenic compounds, in good yields and purity.

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11. *Cortexolone, 17α,21-diacetate* **7a**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.71 (br s, 1H, H-4), 4.86 (d, 1H, H-21, *J* 16.5 Hz), 4.61 (d, 1H, H-21, *J* 16.5 Hz), 2.14 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 1.17 (s, 3H, 19CH_3), 0.73 (s, 3H, 18CH_3).
12. *Cortexolone, 17α,21-dipropionate* **7b**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.77 (br s, 1H, H-4), 4.93 (d, 1H, H-21, *J* 16.6 Hz), 4.65 (d, 1H, H-21, *J* 16.6 Hz), 1.22 (s, 3H, 19CH_3), 1.16–1.22 (t + t, 6H, CH_3), 0.79 (s, 3H, 18CH_3).
13. *Cortexolone, 17α,21-dibutyrate* **7c**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.77 (br s, 1H, H-4), 4.92 (d, 1H, H-21, *J* 16.6 Hz), 4.64 (d, 1H, H-21, *J* 16.6 Hz), 1.21 (s, 3H, 19CH_3), 0.97–1.04 (t + t, 6H, CH_3), 0.78 (s, 3H, 18CH_3).
14. *Cortexolone, 17α,21-divalerate* **7d**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.77 (br s, 1H, H-4), 4.92 (d, 1H, H-21, *J* 16.5 Hz), 4.63 (d, 1H, H-21, *J* 16.5 Hz), 1.22 (s, 3H, 19CH_3), 0.92-0.97 (t + t, 6H, CH_3), 0.79 (s, 3H, 18CH_3).
15. *Δ^9-Cortexolone, 17α,21-dibutyrate* **8**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.78 (br s, 1H, H-4), 5.59 (m, 1H, H-11), 4.94 (d, 1H, H-21, *J* 16.6 Hz), 4.66 (d, 1H, H-21, *J* 16.6 Hz), 1.36 (s, 3H, 19CH_3), 0.96–1.03 (t + t, 6H, CH_3), 0.74 (s, 3H, 18CH_3).
16. Cortexolone **1** is commercially available (Aldrich); its Δ^9-derivative was prepared according a reported procedure starting from cortisol: Chamberlin, E. M.; Tristram, E. W.; Utne, T.; Chemerda, J. M. *J. Org. Chem.* **1960**, *25*, 295.
17. *Typical procedure of CCL-catalyzed alcoholysis*: To a solution of cortexolone, 17α,21-dipropionate **7b** (0.5 g, 1.09 mmol) in toluene (35 mL), *n*-butanol (0.4 g, 5.45 mmol) and CCL (23 g, 3.86 U/mg, FLUKA) were added. The mixture was kept at 30 °C under stirring, monitoring the reaction progress by TLC ($PhCH_3$/AcOEt) until starting material disappearance (24 h). The enzyme was removed by filtration on a Celite pad. After evaporation of solvent at reduced pressure pure cortexolone, 17α-propionate **3b** was recovered (0.420 g, 96%). Crystallization from isopropylether afforded **3b** with the required purity for biological test (>99% by HPLC).
18. *Cortexolone, 17α-propionate* **3b**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.78 (br s, 1H, H-4), 4.32 (dd, 1H, H-21, *J* 18.3 and 4.9 Hz), 4.25 (dd, 1H, H-21, *J* 18.3 and 4.9 Hz), 1.22 (s, 3H, CH_3-19), 1.17 (t, 3H, CH_3, *J* 7.6 Hz), 0.72 (s, 3H, CH_3-18). Mp 133 °C (*t*-butylmethylether).
19. *Cortexolone, 17α-acetate* **3a**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.76 (br s, 1H, H-4), 4.20–4.35 (d + d, 2H, H-21), 2.10 (s, 3H, CH_3CO), 1.21 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18). Mp 195 °C (acetone/diethylether).
20. *Cortexolone, 17α-butyrate* **3c**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.78 (br s, 1H, H-4), 4.32 (dd, 1H, H-21, *J* 18.0 and 4.5 Hz), 4.26 (dd, 1H, H-21, *J* 18.0 and 4.5 Hz), 1.22 (s, 3H, CH_3-19), 0.99 (t, 3H, CH_3, *J* 7.5 Hz), 0.71 (s, 3H, CH_3-18). Mp 135 °C (isopropylether).
21. *Cortexolone, 17α-valerate* **3d**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.77 (br s, 1H, H-4), 4.31 (dd, 1H, H-21, *J* 18.1 and 4.5 Hz), 4.26 (dd, 1H, H-21, *J* 18.1 and 4.5 Hz), 1.21 (s, 3H, CH_3-19), 0.94 (t, 3H, CH_3, *J* 7.2 Hz), 0.72 (s, 3H, CH_3-18). Mp 114 °C (isopropylether).
22. *Δ^9-Cortexolone, 17α-butyrate* **4**: ^{1}H NMR (500 MHz, $CDCl_3$): selected data δ 5.77 (br s, 1H, H-4), 5.57 (m, 1H, H-11), 4.29 (dd, 1H, H-21, *J* 18.0 and 4.5 Hz), 4.24 (dd, 1H, H-21, *J* 18.0 and 4.5 Hz), 1.37 (s, 3H, CH_3-19), 0.98 (t, 3H, CH_3, *J* 7.5 Hz), 0.66 (s, 3H, CH_3-18). Mp 136 °C (acetone/hexane).