ChemPubSoc Europe

DOI: 10.1002/ejoc.201501557

EurJOC European Journal of Organic Chemistry

Communication

Chemoenzymatic Synthesis

Stereoselective Enzymatic Reduction of Ethyl Secodione: Preparation of a Key Intermediate for the Total Synthesis of Steroids

Martina Letizia Contente,[a] Francesco Molinari,[a] Immacolata Serra,[a] Andrea Pinto*[b] and Diego Romano*[a]

Abstract: Ethyl secodione (**1**) has been enantioselectively reduced by using different biocatalysts for the preparation of ethyl secol (13*R*, 17*S*)-**2a**. The recombinant ketoreductase KRED1-Pglu converted the substrate with the highest reaction rate and stereoselectivity (*ee* > 98 %), whereas whole cells of *Pichia minuta* CBS 1708 showed the highest productivity. Stereoselective reduction of **1** provides the key chiral precursor for the synthesis of a number of hormonal contraceptives (i.e., desogestrel, norgestrel, gestodene).

Introduction

Chemoenzymatic synthesis combines the flexibility of chemical synthesis and the high (stereo)selectivity often encountered with enzymatic transformations, being an effective approach for the synthesis of many drugs, including steroids.[1] Total synthesis of steroids has become widely applicable mostly due to the discovery by Ananchenko and Torgov of a straightforward process that makes the assembly of a steroidal A/B bicyclic core possible.[2,3] This strategy can be used for the synthesis of second-generation oral contraceptives, after reduction of the easily available ethyl secodione derivative **1**. Only 13-(*R*)-ethyl secols are useful for the synthesis of contraceptive hormones [i.e., desogestrel, levonorgestrel, gestodene (Figure 1)].[3]

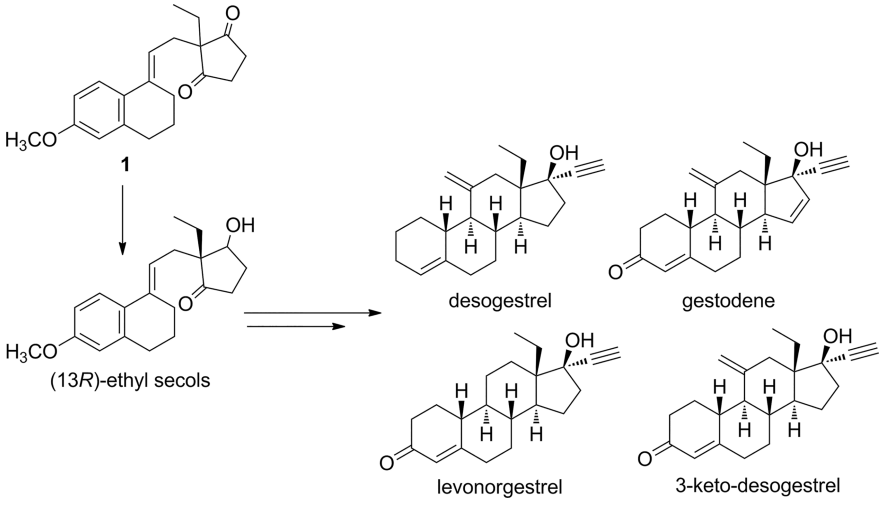
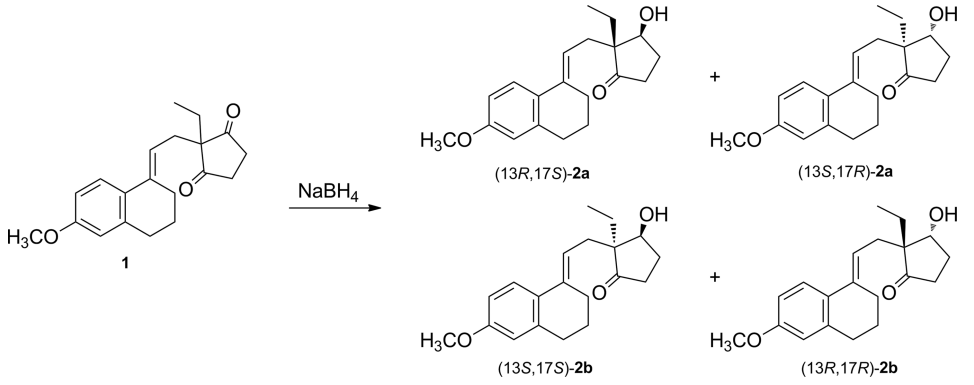


Figure 1. Second-generation oral contraceptives from ethyl secodione **1**.

Although biocatalytic reduction of compound **1**, has been studied by industrial groups since the mid-sixties,[4] no detailed (stereo)chemical characterization of the reduction products of **1** has been reported in the literature. To this aim, compound **1** was reduced with $NaBH_4$ and the four possible stereoisomers of **2** were isolated and fully characterized (Scheme 1).

[a] *Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan*
Via Mangiagalli 25, 20133 Milano, Italy
E-mail: diego.romano@unimi.it
www.defens.unimi.it
[b] *Department of Pharmaceutical Sciences (DISFARM), University of Milan*
Via Mangiagalli 25, 20133 Milano, Italy
E-mail: andrea.pinto@unimi.it
www.disfarm.unimi.it
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under http://dx.doi.org/10.1002/ejoc.201501557.



Scheme 1. Monoreduction of ethyl secodione **1** with $NaBH_4$.

Results and Discussion

Firstly, *syn* racemate (±)-**2a** was separated from the *anti* racemate (±)-**2b** by semi-preparative silica HPLC. The relative stereochemistry of compounds (±)-**2a** and (±)-**2b** was initially deduced from the NOESY spectra.

Concerning derivative (±)-**2a**, the observation of NOESY correlations from H-17 to H-12 and H-11, and from H-18 to 17-OH, indicated that the 17-OH and ethyl group are in a *syn* configuration (Figure 2). On the other hand, for derivative (±)-**2b**, the observation of NOESY correlations from H-12 to 17-OH, and from H-18 to H-17, indicated that the 17-OH and ethyl group are in an *anti* configuration. The racemates (±)-**2a** and (±)-**2b** were, in turn, resolved into their pure enantiomers by semi-preparative chiral HPLC by using, in both cases, tris-(3,5-dimethylphenyl)carbamoyl amylose as the chiral stationary phase (see Supporting Information).

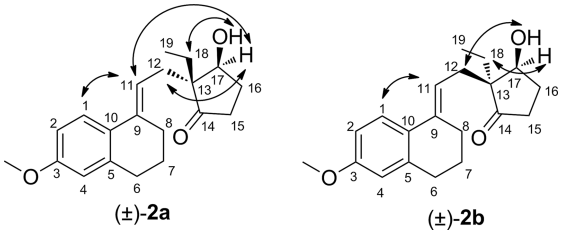


Figure 2. NOESY correlations in *syn* racemate (±)-**2a** and *anti* racemate (±)-**2b**.

We then succeeded in growing diffraction-quality crystals of one of the two enantiomers of **2b**. The relative stereochemistry was unequivocally determined as *syn* by accurate single-crystal X-ray experiments (Figure 3), confirming the results obtained by the NOESY technique (see Supporting Information for further details).

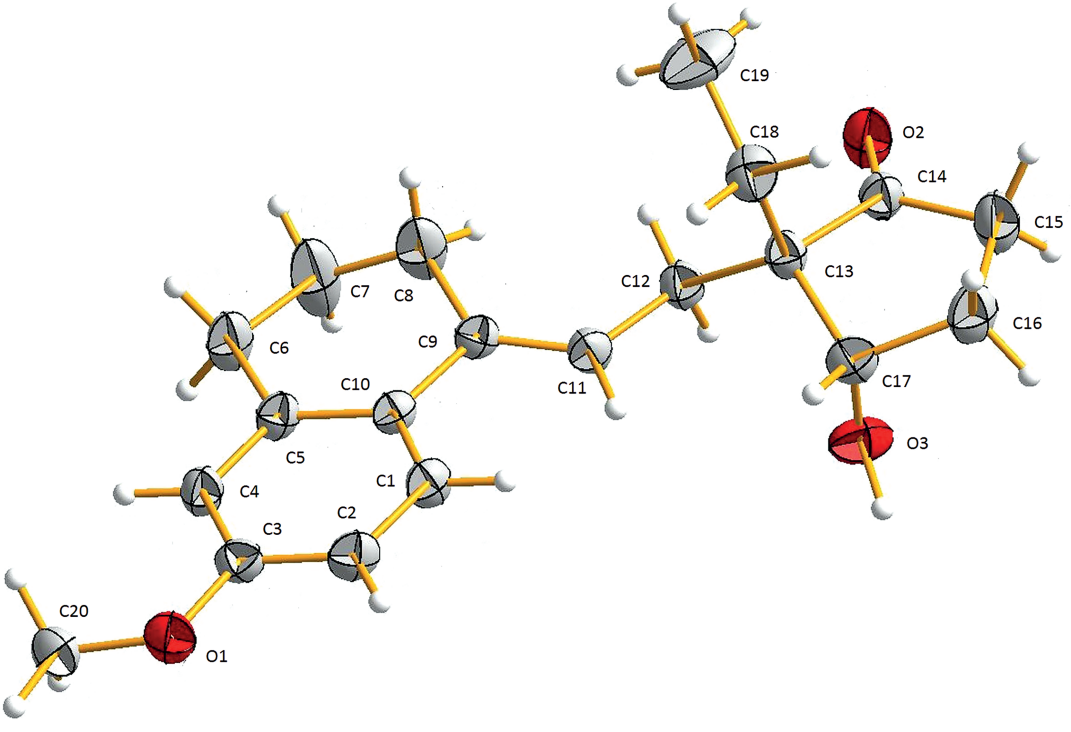


Figure 3. X-ray structure of the asymmetric unit of (–)-**2b** at room temperature. Thermal ellipsoids are drawn at the 30 % probability level.

To assign the absolute configuration of the four stereoisomers, we decided to follow Mosher's methodology. To this aim starting from enantiomerically pure (–)-**2a** and (–)-**2b**, (*R*)- and (*S*)-MTPA esters (MTPA = α-methoxy-α-trifluoromethylphenylacetic acid) of the alcohol at C-17 were prepared and the absolute configuration at C-17 was unambiguously assigned as 17*S* in both cases (see Supporting Information). Based upon the already assigned relative configuration, the absolute configuration of (–)-**2a** was established as 13*R*, 17*S* and the absolute configuration of (–)-**2b** was established as 13*S*, 17*S*.

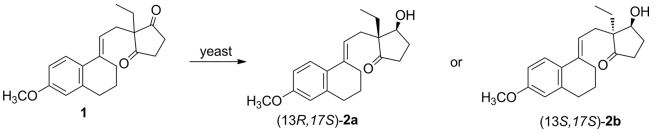
Corey and co-workers developed a method for the stereoselective reduction of methyl secodione (Torgov's diketone) by using oxazaborolidine catalysis, which furnishes enantiopure (13*R*, 17*R*)-methyl secol in 86 % yield with 99 % *ee* after recrystallization,[5] the preparation of which opened the route to a stereoselective version of Torgov's synthesis of estrone. Our attempts to apply Corey's method to the reduction of **1** gave poor results, with formation of (13*R*, 17*R*)-**2b** in low yields (25–30 %) and poor enantioselectivity (75 % *ee*).

Biocatalytic reduction (either by using isolated ketoreductases or ketoreductases bound to whole microbial cells) is an effective alternative for obtaining enantiopure compounds.[6] A recombinant ketoreductase (KRED1-Pglu), recently reported as an efficient stereoselective biocatalyst for the reduction of bulky diketones,[7] was employed for the reduction of **1**. KRED1-Pglu was used in the presence of a catalytic amount of $NADP^+$ and an enzyme-coupled system (glucose/glucose dehydrogenase, GDH) for the regeneration of the cofactor. Different parameters of the biotransformation (substrate and enzyme concentrations, pH, type and co-substrate concentrations) were optimized by using a Multisimplex, approach, previously used for biotransformations;[8] KRED1-Pglu under optimized conditions (0.05 M Tris-HCl at pH 8.0, 30 °C, 6.5 mM substrate concentration in the presence of 3.2 % EtOH and 2.5 % glucose) gave the desired stereoisomer (13*R*, 17*S*)-**2a** with outstanding stereoselectivity (> 98 % *ee*) and good reaction rates, but with moderate yields (65 % after 6 h). Notably, no further reduction to diol was observed even at prolonged times, indicating the total selectivity of KRED1-Pglu.

The relatively low yield observed with the recombinant ketoreductases, led us to screen various yeasts to find more productive biocatalysts. The screening was carried out with yeasts previously known for their ability to reduce structurally different ketones.[9–12] Table 1 gives the results of the biotransformation at the time of maximum production (only reactions with yields above 50 % are reported).

Six strains (*Pichia etchellsii* CBS 2011, *Pichia glucozyma* CBS 5766, *Pichia minuta* CBS 1708, *Rhodotorula glutinis* NRRL-Y1587, *Rhodotorula rubra* MIM 146, *Saccharomyces cerevisiae* CEN.PK113-7D) gave monoreduction of **1** with yields higher than 70 %. *P. minuta* CBS 1708 and *S. cerevisiae* CEN.PK113-7D afforded the desired stereoisomer (13*R*, 17*S*)-**2a** with *ee* ≥ 90 %; different stereoselectivity was detected with *P. glucozyma* CBS 5766 and *R. glutinis* NRRL-Y1587, giving (13*S*, 17*S*)-**2b** with a high *ee* value. Notably, all the biocatalysts employed (recombinant KRED1-Pglu and whole cells) followed Prelog's rule, reducing the 17-carbonyl to give the corresponding (*S*)-alcohol. The

Table 1. Reduction of ethyl secodione **1** by different yeasts.



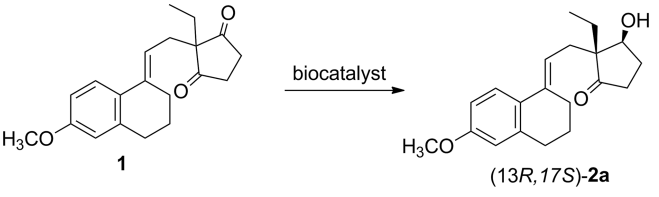
Yeasts[a]	Product	*ee*[b] [%]	m.c. [%]	Time [h]
Pichia anomala DBVPG 2873	(13*R*,17*S*)-**2a**	20	65	24
P. capsulata CBS 1993	(13*R*,17*S*)-**2a**	51	62	96
P. etchellsii CBS 2011	(13*R*,17*S*)-**2a**	50	75	24
P. glucozyma CBS 5766	(13*S*,17*S*)-**2b**	94	85	2
P. minuta CBS 1708	(13*R*,17*S*)-**2a**	90	80	24
Rhodotorula glutinis NRRL 1587	(13*S*,17*S*)-**2b**	90	78	24
R. rubra MIM146	(13*S*,17*S*)-**2b**	76	92	24
S. cerevisiae CEN.PK113-7D	(13*R*,17*S*)-**2a**	96	81	48

[a] To compare yields, all biotransformations were performed with the same optical density of yeast culture (OD_{600}/mL = 60). [b] Determined by analytical chiral HPLC (details in the Supporting Information); in all the biotransformations *de* was > 98 %.

biotransformation with *P. minuta* CBS 1708 and *S. cerevisiae* CEN.PK113-7D were further optimized by evaluating different parameters (temperature, pH, substrate and cells concentration, and co-substrate type and concentration).

Table 2 reports the comparison between the results obtained in the biotransformation of **1** accomplished on a 1 liter scale with the best-performing biocatalysts (recombinant KR or yeasts) used under optimized conditions. Although *S. cerevisiae* and the recombinant ketoreductase furnished the highest stereoselectivity, *P. minuta* resulted in the formation of the highest amounts of enantiomerically pure (13*R*, 17*S*)-**2a** after a single crystallization from diisopropyl ether (DIPE). Actually, *P. minuta* allowed us to perform the biotransformation at substrate concentrations up to 4.7 g/L (15 mM) with > 95 % yield, whereas *S. cerevisiae* and KRED1-Pglu provided high yields only at much lower concentrations.

Table 2. Optimized reduction of **1** into (13*R*, 17*S*)-**2a**.



Biocatalyst	*ee* [%]	m.c[a] [%]	Isolated yield [%][b]	Time [h]
P. minuta[c]	92	> 95	73	24
S. cerevisiae[d]	> 98	> 95	87	24
KRED1-Pglu[e]	> 98	65	56	8

[a] Analytical yield determined by HPLC. [b] After crystallization from DIPE. [c] Conditions: 0.1 M phosphate buffer at pH 7.2, 30 °C, 15 mM substrate concentration in the presence of 3.2 % EtOH and 25 $g_{dry\ weight}$/L of yeast cells. [d] Conditions: 0.1 M phosphate buffer at pH 6.5, 30 °C, 10 mM substrate concentration in the presence of 3.5 % EtOH and 30 $g_{dry\ weight}$/L of yeast cells. [e] Conditions: 0.05 M Tris-HCl buffer at pH 8.0, 28 °C, 6.5 mM substrate concentration in the presence of 3.2 % EtOH, 2.5 % glucose, KRED1-Pglu and GDH.

Conclusions

In summary, we used different biocatalysts (yeasts or recombinant ketoreductase) for chemo- and stereoselective carbonyl reduction of secodione derivative **1**. This approach is a simple and complementary strategy for the enzymatic generation of optically pure intermediates to be used for the synthesis of commercially relevant steroids.

Experimental Section

General Procedure for the Preparative Asymmetric Reduction with Recombinant Enzyme: The biotransformation using KRED1-Pglu was carried out by addition of substrate (6.5 mM) dissolved in ethanol (32 mL) to a solution containing KRED1-Pglu (20 mU/mL), GDH (1 U/mL), $NADP^+$ (0.1 mM), and glucose (25 mM) in 50 mM Tris/HCl buffer pH 8.0 (1 L). The reaction mixture was stirred at 28 °C for 6 h and then extracted with EtOAc (600 mL); the aqueous phase was extracted twice more with EtOAc (500 mL). The organic phases were collected and dried with Na_2SO_4 and the solvent was evaporated. The crude residues were crystallized with DIPE.

General Procedure for the Preparative Asymmetric Reduction with Whole Cells of Yeasts: The biotransformation using *Pichia minuta* and *Saccharomyces cerevisiae* was carried out by addition of the substrate as an ethanolic solution (32 mL of ethanol in the case of *P. minuta*, 35 mL of ethanol in the case of *S. cerevisiae*). The biotransformation was kept under reciprocal shaking (150 rpm) at 30 °C. When the reaction was over, EtOAc (600 mL) was added and the resulting mixture was shaken and centrifuged; the aqueous phase was extracted twice more with EtOAc (500 mL). The organic phases were collected and dried with Na_2SO_4 and the solvent was evaporated. The crude residues were crystallized with DIPE.

CCDC 1437502 (for **2b**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Supporting Information (see footnote on the first page of this article): See Supporting Information for all the details about the preparation of the biocatalysts, stereochemical analysis and analytical data of the products.

Acknowledgments

We would like to thank Dr. Roberto Lenna for providing us with the substrates and for his precious help with the analytical section.

Keywords: Enzymatic reduction · Yeast · Steroids · Hormones · Enzymes

[1] J. D. Mortison, D. H. Sherman, *J. Org. Chem.* **2010**, *75*, 7041–7051.
[2] S. N. Ananchenko, I. V. Torgov, *Tetrahedron Lett.* **1963**, *4*, 1553–1558.
[3] A. S. Chapelon, D. Moraleda, R. Rodriguez, C. Ollivier, M. Santelli, *Tetrahedron* **2007**, *63*, 11511–11616.
[4] H. Kosmol, K. Kieslich, R. Vossing, H. J. Koch, K. Petzoldt, H. Gibian, *Justus Liebigs Ann. Chem.* **1967**, *701*, 199–205.
[5] Y. Y. Yeung, R. J. Chein, E. J. Corey, *J. Am. Chem. Soc.* **2007**, *129*, 10346–10347.
[6] a) F. Hollmann, I. W. C. E. Arends, D. Holtmann, *Green Chem.* **2011**, *13*, 2285–2314; b) Y. Ni, J. H. Xu, *Biotechnol. Adv.* **2012**, *30*, 1279–1288; c) M. Hall, A. S. Bommarius, *Chem. Rev.* **2011**, *111*, 4088–4110; d) D. Monti, G. Ottolina, G. Carrea, S. Riva, *Chem. Rev.* **2011**, *111*, 4111–4140.
[7] M. L. Contente, I. Serra, M. Brambilla, I. Eberini, E. Gianazza, V. De Vitis, F. Molinari, P. Zambelli, D. Romano, *Appl. Microbiol. Biotechnol.* **2016**, *100*, 193–201.
[8] D. Romano, R. Gandolfi, S. Guglielmetti, F. Molinari, *Food Chem.* **2011**, *124*, 1096–1098.
[9] D. Romano, V. Ferrario, D. Mora, R. Lenna, F. Molinari, *Steroids* **2008**, *73*, 112–115.
[10] P. Hoyos, G. Sansottera, M. Fernández, F. Molinari, J. V. Sinisterra, A. R. Alcántara, *Tetrahedron* **2008**, *64*, 7929–7936.
[11] S. M. Husain, T. Stillger, P. Dünkelmann, M. Lödige, L. Walter, E. Breitling, M. Pohl, M. Bürchner, I. Krossing, M. Müller, D. Romano, F. Molinari, *Adv. Synth. Catal.* **2011**, *353*, 2359–2362.
[12] M. L. Contente, F. Molinari, P. Zambelli, V. De Vitis, R. Gandolfi, A. Pinto, D. Romano, *Tetrahedron Lett.* **2014**, *55*, 7051–7053.

Received: December 11, 2015
Published Online: February 18, 2016

