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Biotransformation of dehydro-*epi*-androsterone by *Aspergillus parasiticus*: Metabolic evidences of BVMO activity

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ABSTRACT

The research on the synthesis of steroids and its derivatives is of high interest due to their clinical applications. A particular focus is given to molecules bearing a D-ring lactone like testolactone because of its bioactivity. The *Aspergillus* genus has been used to perform steroid biotransformations since it offers a toolbox of redox enzymes. In this work, the use of growing cells of *Aspergillus parasiticus* to study the bioconversion of dehydro-epi-androsterone (DHEA) is described, emphasizing the metabolic steps leading to D-ring lactonization products. It was observed that *A. parasiticus* is not only capable of transforming bicyclo[3.2.0]hept-2-en-6-one, the standard Baeyer-Villiger monooxygenase (BVMO) substrate, but also yielded testololactone and the homo-lactone 3β-hydroxy-17a-oxa-D-homoandrost-5-en-17-one from DHEA. Moreover, the biocatalyst degraded the lateral chain of cortisone by an oxidative route suggesting the action of a BVMO, thus providing enough metabolic evidences denoting the presence of BVMO activity in *A. parasiticus*. Furthermore, since excellent biotransformation rates were observed, *A. parasiticus* is a promising candidate for the production of bioactive lactone-based compounds of steroidal origin in larger scales.

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1. Introduction

Steroids are a large family of molecules widely distributed in Nature. They are structurally diverse solid alcohols sharing a common four-ring nucleus [1]. These molecules exhibit vital roles in animal, fungal and plant cells. Likewise, they are used to treat severe human health disorders such as cancer, diabetes and obesity [2,3], among others. In particular, the discovery of the antineoplasic properties of testolactone in 1962 [4] triggered the search for bioactive D-ring lactone-based compounds [5].

The generation of oxyfunctionalized steroidal products is a multistep, complex task to achieve by chemical means [6–8]. Alternatively, the use of microbial catalysts represents a simpler and greener strategy to perform regio- and stereoselective hydroxylations, regioselective C=C reductions and isomerizations and, to a lesser extent, C=O reductions and Baeyer-Villiger oxidation reactions on the D-ring and the lateral chain of these molecules [9–11]. In particular, the use of filamentous fungi to biotransform androstan- and pregnan-based steroids has been comprehensively documented [12,13]. Among them, *Fusarium* [14] species have been shown to display excellent dehydrogenation activities. On the other hand, strains from *Beauveria* [15], *Cunninghamella* [16], *Mucor* [17–19] as well as *Fusarium* [20] were reported to perform regio- and stereoselective hydroxylations. Recently, Wu and coworkers described the hydroxylation of dehydro-*epi*-androsterone (DHEA) by *Colletotrichum lini* [21]. Furthermore, the genus *Penicillium* [22,23] has been employed to carry out D-ring lactonizations. Nevertheless, *Aspergillus* strains might be the biocatalysts of choice to study the transformation profile of a particular steroidal molecule, since this genus is capable of performing all the aforementioned reactions, as extensively referred by several authors [24–28].

It is demonstrated that the *Aspergillus* genus provides a toolbox of redox enzymes [29,30]. Nowadays, the Baeyer-Villiger monooxygenase (BVMO) activity is one of the most studied from biomolecular as well as biotechnological perspectives [31–33]. In this context, the purpose of this work was to find *Aspergillus* strains capable of yielding steroidal D-ring lactonization products using DHEA as an androstan-derived model compound.

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2. Experimental

2.1. Chemicals and microorganisms

All the chemicals were purchased in analytical grade from Sigma Aldrich or Merck and were used without further purification. Culture media components were obtained from Britania. The *Aspergillus* strains used comprised *A. flavus, A. fumigatus* and *A. japonicus*, which were acquired from the collections of Universidad de Buenos Aires (Argentina), Universidad Nacional de Rio Cuarto (Argentina) and Colección de Cultivos Fúngicos IIB-INTECH (Argentina), respectively, as well as *A. candidus* and *A. parasiticus*, which were both obtained from Universidad Nacional del Litoral (Argentina). Microorganisms stored at 4 °C in Czapek Yeast (CY) agar slopes were inoculated to erlenmeyers containing CY liquid medium, which composition *per* 1 L distilled H_2O is 5 g yeast extract, 30 g sucrose, 1 g K_2HPO_3, 0.3 g $NaNO_3$, 0.5 g KCl, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.005 g $CuSO_4{\cdot}5H_2O$ and 0.01 g $ZnSO_4{\cdot}7H_2O$. Cultures were incubated at 28 °C and 150 rpm.

2.2. Microbial screening

To test the capability of the *Aspergillus* strains to transform the model BVMO substrate *rac*-bicyclo[3.2.0]hept-2-en-6-one (**1**), biocatalysts were prepared according to previous reports [31,34]. Then, **1** (2.5 mM final concentration) was added to 50 mL erlenmeyer flasks containing 500 mg wet cells (2.5% (*p*/*v*)) of an individual strain each in 20 mL of CY medium. Biotransformations were run by duplicate –mean values are given- and incubated at 28 °C and 150 rpm during 72 h. Controls without **1** and without biocatalyst were also run. Samples were taken as described in Section 2.3.1 and analyzed by GC-FID using a Clarus 500 instrument (Perkin Elmer) and pure analytic standards. Conversion was determined employing a Rt-βDEXse (Restek) chiral capillary column and calculated using relative areas of the substrate and the lactone-based products only. The enantiomeric excesses were calculated as done by Chen et al. [35]. See Supplementary Information for further details.

2.3. Biotransformation of steroids

Biocatalysts were prepared as referred above. In the analytical scale, 50 mL erlenmeyer flasks containing 500 mg wet cells (2.5% (*p*/*v*)) of *A. parasiticus* in 20 mL CY medium were used. To start the biotransformations, each substrate was dissolved in DMSO or DMF (1% (*v*/*v*) final concentration) and added to the erlenmeyer flasks. Biotransformations were incubated at 28 °C and 150 rpm and were run by duplicate, mean values are shown. Controls without biocatalyst and without substrate were also included. To perform time-course experiments, multiples batches were prepared and samples were taken by withdrawing batches and controls in duplicate at a time. In the semipreparative scale, the same procedure described for the analytical scale was followed, but using a biotransformation volume of 200 mL in a 500 mL erlenmeyer flask. A total of five batches were run and combined after 96 h.

2.3.1. Work-up and analysis

Each batch was filtered *in vacuo* to remove cells and then reaction mixtures were extracted three times with one volume of ethyl acetate. Organic layers were combined, dried with sodium sulfate, concentrated *in vacuo* and spotted on silica gel 60 F254 TLC plates (Merck) to qualitatively assess the substrate consumption and the appearance of biotransformation products. The chromatographic analysis was done using the mixture *n*-hexane: ethyl acetate as mobile phase and plates were revealed under UV light and by spraying the *p*-anisaldehyde reagent, as described elsewhere. To assess the biotransformation profile of each substrate, samples were analyzed by GC-FID using a Clarus 500 instrument (Perkin Elmer) using pure analytic standards. Conversion was determined using a 007 (methyl 5% phenyl silicone) capillary column (Quadrex) and calculated using relative areas. Product purifications were done by column chromatography on silica gel 60 (230-400 mesh, Merck). Mixtures of Hx: EtOAc were initially used as mobile phases, changing the composition from 100:0 to 30:70 by a 10% *per* column volume (V_c). Finally, two V_c of pure EtOAc followed by two V_c of EtOAc: MeOH (90:10) were employed. The identity of the isolated compounds was determined by NMR, HRMS-ESI and GC/LRMS-EI. The ^1H and ^{13}C NMR spectra were recorded on a AC-200 or AMX-400 spectrometer (at 200.13 or 400.16 MHz, and at 50.23 or 100.62 MHz, respectively) (Bruker) using $CDCl_3$ as solvent with TMS as internal standard. HRMS-ESI measurements were done on a microTOF-Q II (Bruker) coupled to a UPLC-DAD 1200 (Agilent) at a resolution of 5000 (5% valley definition), by 70 eV electron ionization, at an accelerating voltage of 8 kV. GC/LRMS-EI were performed at 70 eV using an ion trap (GCQ Plus) with MSn (Finnigan, Thermo-Quest), operated at a fundamental *rf*-drive of 1.03 MHz. Helium was used as the damping gas at an uncorrected gauge reading of $6{\cdot}10^{-5}$ Torr. An OV-5 (5% diphenyl 95% dimethylpolysiloxane) capillary column (OVS) was used. Compounds were analyzed by comparing their mass spectra with the NIST spectral library (Wiley). Structural data is given in the Supplementary Information.

3. Results and discussion

3.1. Screening of BVMO activity in Aspergillus strains

Considering previous reports in literature describing the potential of the *Aspergillus* genus as a biocatalyst to perform Baeyer-Villiger oxidations of the D-ring in several steroids [36–39], we selected a small collection of strains from this genus to screen for this enzymatic activity by using *rac*-bicyclo[3.2.0]hept-2-en-6-one (**1**) as model substrate [40]. Since redox cofactors are needed, we employed growing cell cultures in order to display self-sufficient transformations. Although all the strains assayed were capable to oxidize the model substrate **1** to the corresponding normal and/or abnormal lactones (Table 1, Supplementary Information), *A. parasiticus* exhibited the highest regio- and enantioselectivity by yielding mainly the normal (1*S*, 5*R*) and the abnormal (1*R*, 5*S*) lactones from the enantiomers (1*R*, 5*S*) and (1*S*, 5*R*) of substrate **1**, respectively (Fig. 1). Since dehydrogenase activity is ubiquitous and constitutive in these fungi, carbonyl reduction products were observed as well (data not shown). Because of these results, *A. parasiticus* was selected as the biocatalyst to perform the transformation of DHEA (**2**).

3.2. Biotransformation of dehydro-epi-androsterone (DHEA)

When the biotransformation of **2** was assayed in analytical scale (100 mg), five products were detected (Fig. 2) by GC/ LRMS-EI and NMR analyses (see Supplementary Information). Among them, three compounds are supposed to directly derive from the substrate, being androstenediol (**3**) –a product of the reduction of the carbonyl in the D-ring-, androstenedione (**4**) –obtained by the oxidation of hydroxyl in position 3 and the subsequent isomerization of the C=C from C5 to C4- and the homo-lactone 3β-hydroxy-17a-oxa-D-homoandrost-5-en-17-one (**5**), produced after a D-ring lactonization reaction. Supported in literature reports, it is possible to propose that these molecules were obtained by the action of a dehydrogenase, a 3β-HSD/isomerase (E_2) and a BVMO

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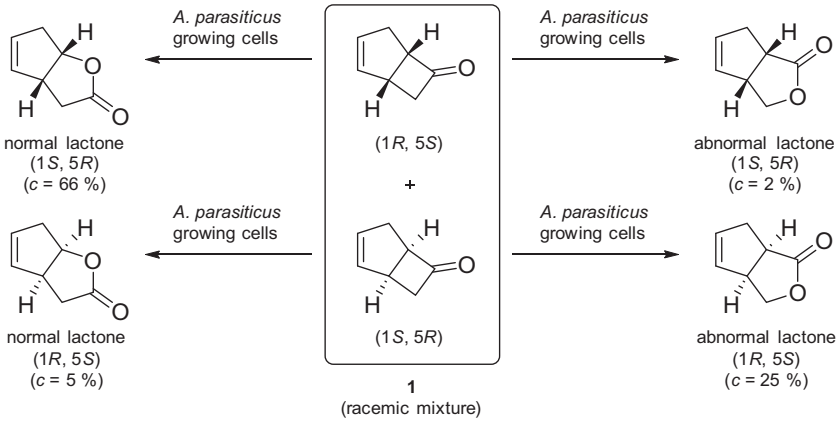

Fig. 1. Biotransformation of *rac*-bicyclo[3.2.0]hept-2-en-6-one (**1**) by *A. parasiticus* growing cells. Conversions (*c*) were determined by GC-FID.

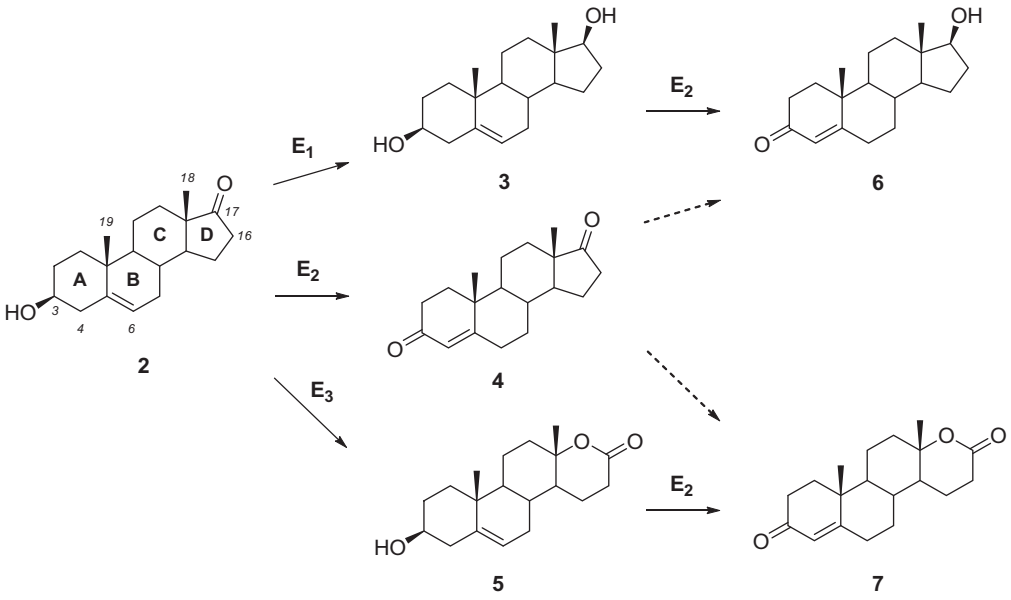

Fig. 2. Biotransformation route of DHEA (**2**) by *A. parasiticus* growing cells. The reaction sequences are given from time-course experiments (solid arrows). Enzymes E_1 to E_3 are proposed to catalyze each step on the basis of literature reports. Dashed arrows are given for possible although not detected reactions. E_1: dehydrogenase, E_2: 3β-HSD/isomerase, E_3: BVMO.

(E_3) [41–43], respectively. Then, compounds **3** and **5** were further metabolized to yield testosterone (**6**) and testololactone (**7**), respectively. Again, a 3β-HSD/isomerase activity might have led to these structures. Noteworthy, product **6** could have also been obtained from compound 4 by the reduction of the carbonyl group at position 17, as determined by Cvelbar et al. [44]. In addition, the steroid **7** could have been produced from the D-ring lactonization of the same molecule [36]. Besides, testosterone (**6**) would have yielded testololactone (**7**) *via* **4**, as reported by Yildirim et al. using cultures of *A. terreus* [39] and *A. tamarii* [26]. However, from time-course experiments the occurrence of these transformations was not observed under the assayed conditions (Table 2, Supplementary Information). Our results are in agreement with early findings of Mostafa et al. [36], which showed *A. parasiticus* is capable of oxidizing progesterone to **6** and **7**. Nevertheless, it should be noted that, as far as we are concerned, the biotransformation profile of DHEA by *A. parasiticus* as described herein was not reported previously. It should be addressed that during the preparation of this manuscript, the first version of the genome of *A. parasiticus* SU-1 was released (genome ID: ASM95608v1). By performing a preliminary *in silico* homology searching for BVMO-encoding sequences, we found at least 17 open reading frames as putative BVMOs, thus supporting our considerations based on metabolic observations.

It is worthy to highlight that when the biotransformation of **2** was performed in semipreparative scale –five times larger than the analytical scale, 500 mg DHEA-, the homo-lactone **5** was detected as the only product (166 mg, 33% isolated yield after column chromatography). The different results obtained from one scale to the other could be attributed to a variation in the molecular oxygen availability, as observed by Kolek and coworkers employing *P. lilacinum* as biocatalyst under similar conditions [45]. Specifically, a more extensive surface between the biocatalyst and the reaction medium might have considerably eased the O_2 mass transfer, due to cells may be increased in number but not in size. Moreover, since no further conversion of product **5** to **7** was evidenced it would be possible to assume that the isomerization activities, as well as the reductive ones, might have been suppressed by an increase in the molecular oxygen availability in the cells. Hence, the metabolic fate of **2** could have been routed to the BVMO-catalyzed D-ring lactonization by this effect.

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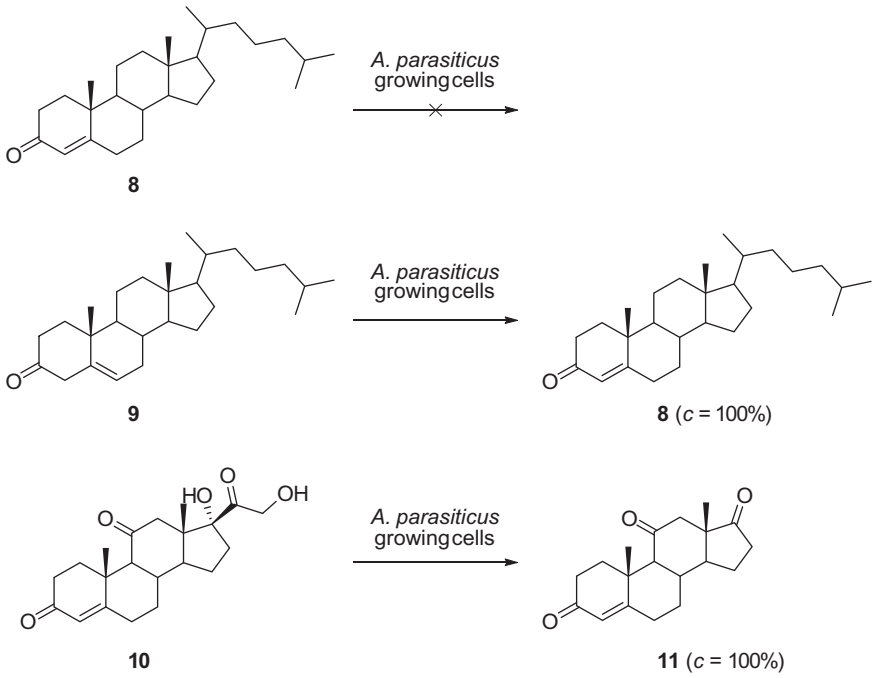

Fig. 3. Transformation of 4-cholesten-3-one (**8**), 5-cholesten-3-one (**9**) and cortisone (**10**) biocatalyzed by *A. parasiticus* growing cells. Conversions (*c*) as well as product identities were determined by GC/EI-LRMS.

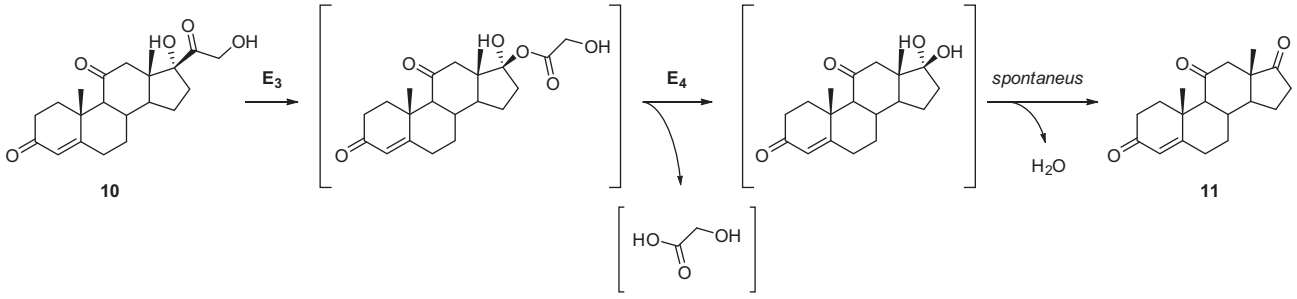

Fig. 4. Suggested metabolic pathway in *A. parasiticus* for the biotransformation of cortisone (**10**) to adrenosterone (**11**). Compounds between brackets were not detected. E_3: BVMO, E_4: hydrolase.

It should be emphasized that although the comprehensively reported whole-cell biotransformations of steroids represent a strong evidence of the presence of BVMOs in fungi [23,36,46] there is no register in literature up to date of an isolated fungal BMVO acting on this class of compounds. The available recombinant fungal enzymes, CAMO [47], $BVMO_{Af1}$ [48] and those from *Aspergillus flavus* [49] are not capable of transforming them. For this reason, these results represent a starting point to perform the cloning and expression of new fungal BVMOs and its further biochemical and biocatalytic characterization.

3.3. Biotransformation of other oxo-functionalized steroids susceptible of BMVO transformation

To further assess the biocatalytic performance of *A. parasiticus*, three other steroids structurally linked to **2** and susceptible of BMVO transformation were also tested as substrates in analytical scale (Fig. 3). The use of 4-cholesten-3-one (**8**) did not yield biotransformation products. On the other hand, when 5-cholesten-3-one (**9**) was selected, compound **8** was quantitatively formed, thus showing the occurrence of isomerization activity. Finally, andrenosterone (**11**) –also known as the Reichstein's substance G [50]- was produced with complete conversion when cortisone (**10**) was employed as substrate. The structures of the obtained compounds were analyzed by GC/LRMS-EI (see Supplementary Information). Interestingly, *A. parasiticus* did not exhibit BVMO neither reductive activities on carbonyl groups in rings A and/or C in any of the assayed molecules. However, the biocatalyst was capable of transforming the lateral chain of **10** in an oxidative fashion so as to yield **11**, as aforementioned. Noticeably, the pathway leading to this molecule could involve an initial step that may be catalyzed by a BVMO, in agreement with early observations made by Brannon and coworkers for the bioconversion of progesterone to testololactone by *A. tamarii* [51]. A possible mechanistic interpretation of the route leading from compound **10** to product **11** by *A. parasiticus* is given in Fig. 4.

4. Conclusion

In initial experiments, we detected a novel biocatalyst from the *Aspergillus* genus, named *A. parasiticus*, that transformed

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rac-bicyclo[3.2.0]hept-2-en-6-one –the standard substrate to assess BVMO activity- with good regio- and stereoselectivity. Furthermore, this fungus was capable of catalyzing the D-ring lactonization of DHEA with excellent rates. Moreover, the lateral chain degradation pathway of cortisone exhibited by the biocatalyst may suggest a BMVO enzyme is involved. These results provide enough metabolic evidences to assume the occurrence of BVMO activity in *A. parasiticus*. Therefore, this work may lead to the cloning and expression of new fungal BVMOs and to its further biochemical characterization. Furthermore, from a biotechnological focus, up scaling procedures are feasible since the developed biotransformations are not only simple and green, but foremost efficient.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2016.03.018.

References

[1] H.N. Bhatti, R.A. Khera, Biological transformations of steroidal compounds: a review, Steroids 77 (2012) 1267–1290, http://dx.doi.org/10.1016/j.steroids.2012.07.018.
[2] N. Richter, R.C. Simon, W. Kroutil, J.M. Wardd, H.C. Hailes, Synthesis of pharmaceutically relevant 17-α-amino steroids using an ω-transaminase, Chem. Commun. 50 (2014) 6098–6100, http://dx.doi.org/10.1039/c3cc49080g.
[3] M. Garrido, E. Bratoeff, M. García-Lorenzana, Y. Heuze, J. Soriano, N. Valencia, et al., Biological evaluation of androstene derivatives, Arch. Pharm. Chem. Life Sci. 346 (2013) 62–70, http://dx.doi.org/10.1002/ardp.201200335.
[4] A. Segaloff, J.B. Weeth, K.K. Meyer, E.L. Rongone, M.E. Cunningham, Hormonal therapy in cancer of the breast. XIX. Effect of oral administration of Δ1-testololactone on clinical course and hormonal excretion, Cancer 15 (1962) 633–635.
[5] R.T. Blickenstaff, Antitumor Steroids, Academic Press Inc., San Diego, 1992.
[6] P.B. Reese, Remote functionalization reactions in steroids, Steroids 66 (2001) 481–497.
[7] V. Dobricic, B. Markovic, N. Milenkovic, V. Savic, V. Jacevic, N. Rancic, et al., Design, synthesis, and local anti-inflammatory activity of 17β-carboxamide derivatives of glucocorticoids, Arch. Pharm. Chem. Life Sci. 347 (2014) 1–12, http://dx.doi.org/10.1002/ardp.201400165.
[8] Y. Huang, M. Liu, L. Meng, P. Feng, Y. Guo, M. Ying, et al., Synthesis and antitumor evaluation of novel hybrids of phenylsulfonylfuroxan and epiandrosterone/dehydroepiandrosterone derivatives, Steroids 101 (2015) 7–14, http://dx.doi.org/10.1016/j.steroids.2015.05.003.
[9] N. Nassiri-Koopaei, M.A. Faramarzi, Recent developments in the fungal transformation of steroids, Biocatal. Biotransform. 33 (2015) 1–28, http://dx.doi.org/10.3109/10242422.2015.1022533.
[10] M. Petrusma, R. van der Geize, L. Dijkhuizen, 3-Ketosteroid 9α-hydroxylase enzymes: Rieske non-heme monooxygenases essential for bacterial steroid degradation, Antonie Van Leeuwenhoek 106 (2014) 157–172, http://dx.doi.org/10.1007/s10482-014-0188-2.
[11] A. Swizdor, T. Kolek, A. Panek, N. Milecka, Selective modifications of steroids performed by oxidative enzymes, Curr. Org. Chem. (2012) 2551–2582, http://dx.doi.org/10.2174/138527212804004625.
[12] P.C. Peart, W.F. Reynold, P.B. Reese, The facile bioconversion of testosterone by alginate-immobilised filamentous fungi, J. Mol. Catal. B Enzym. 95 (2013) 70–81, http://dx.doi.org/10.1016/j.molcatb.2013.05.025.
[13] V.V. Kollerov, V.V. Fokina, G.V. Sukhodolskaya, A.A. Shutov, M.V. Donova, 11β-Hydroxylation of 6α-fluoro-16α-methyl-deoxycorticosterone 21-acetate by filamentous fungi, Appl. Biochem. Microbiol. 51 (2015) 169–179, http://dx.doi.org/10.1134/S0003683815020106.
[14] S. Ghasemi, R. Kheyrabadi, Z. Habibi, Microbial transformation of hydrocortisone by two fungal species *Fusarium fujikuroi* PTCC 5144 and *Rhizomucor pusillus* PTCC 5134, Biocatal. Biotransform. 32 (2014) 168–172, http://dx.doi.org/10.3109/10242422.2014.913581.
[15] A. Swizdor, T. Kolek, A. Panek, A. Bialonska, Microbial Baeyer-Villiger oxidation of steroidal ketones using Beauveria bassiana: Presence of an 11α-hydroxyl group essential to generation of D-homo lactones, Biochim. Biophys. Acta 1811 (2011) 253–262.
[16] C. Yang, H. Fan, Y. Yuan, J. Gao, Microbial transformation of pregnane-3β,16β,20-triol by *Cunninghamella echinulata*, Chin. J. Chem. 31 (2013) 127–131, http://dx.doi.org/10.1002/cjoc.201201080.
[17] Y. Wang, D. Sun, Z. Chen, H. Ruanb, W. Ge, Biotransformation of 3β-hydroxy-5-en-steroids by *Mucor silvaticus*, Biocatal. Biotransform. 31 (2013) 168–174, http://dx.doi.org/10.3109/10242422.2013.813490.
[18] S.P. Kolet, S. Niloferjahan, S. Haldar, R. Gonnade, H.V. Thulasiram, Biocatalyst mediated production of 6β,11α-dihydroxy derivatives of 4-ene-3-one steroids, Steroids 78 (2013) 1152–1158, http://dx.doi.org/10.1016/j.steroids.2013.08.004.
[19] Silva E. de Oliveira, N.A. Jacometti Cardoso Furtado, J. Aleu, Collado I. González, Non-terpenoid biotransformations by *Mucor* species, Phytochem. Rev. 14 (2015) 745–764, http://dx.doi.org/10.1007/s11101-014-9374-0.
[20] H. Zhang, J. Ren, Y. Wang, C. Sheng, Q. Wu, A. Diao, et al., Effective multi-step functional biotransformations of steroids by a newly isolated *Fusarium oxysporum* SC1301, Tetrahedron 69 (2013) 184–189, http://dx.doi.org/10.1016/j.tet.2012.10.047.
[21] Y. Wu, H. Li, X.-M. Zhang, J.-S. Gonga, Z.-M. Rao, J.-S. Shi, Efficient hydroxylation of functionalized steroids by *Colletotrichum lini* ST-1, J. Mol. Catal. B Enzym. 120 (2015) 111–118, http://dx.doi.org/10.1016/j.molcatb.2015.07.003.
[22] A. Bartmanska, J. Dmochowska-Gladysz, E. Huszcza, Steroids' transformations in *Penicillium notatum* culture, Steroids 70 (2005) 193–198, http://dx.doi.org/10.1016/j.steroids.2004.11.011.
[23] B. Yang, Y. Wang, X. Chen, J. Feng, Q. Wu, D. Zhu, et al., Biotransformations of steroids to testololactone by a multifunctional strain *Penicillium simplicissimum* WY134-2, Tetrahedron 70 (2014) 41–46, http://dx.doi.org/10.1016/j.tet.2013.11.039.
[24] M.A. Faramarzi, M.T. Yazdi, M. Amini, F.A. Mohseni, G. Zarrini, A. Amani, A. Shafiee, Microbial production of testosterone and testololactone in the culture of *Aspergillus terreus*, World J. Microb. Biotechnol. 20 (2004) 657–660, http://dx.doi.org/10.1007/s11274-004-1003-4.
[25] A.C. Hunter, C. Collins, H.T. Dodd, C. Dedi, S.-J. Koussoroplis, Transformation of a series of saturated isomeric steroidal diols by *Aspergillus tamarii* KITA reveals a precise stereochemical requirement for entrance into the lactonization pathway, J. Steroid Biochem. Mol. Biol. 122 (2010) 352–358, http://dx.doi.org/10.1016/j.jsbmb.2010.08.010.
[26] K. Yildirim, A. Uzuner, E.Y. Gulcuoglu, Baeyer-Villiger oxidation of some steroids by *Aspergillus tamarii* MRC 72400, Collect. Czech. Chem. Commun. 76 (2011) 743–754, http://dx.doi.org/10.1135/cccc2011008.
[27] K. Yildirim, Microbial hydroxylation of some steroids by *Aspergillus wentii* MRC 200316, Collect. Czech. Chem. Commun. 75 (2010) 1273–1281, http://dx.doi.org/10.1135/cccc2010112.
[28] M. Shuhong, H. Boyuan, W. Na, H.U. Xiaojie, G. Zhijiang, L. Yanqing, et al., 11α hydroxylation of 16α, 17-epoxyprogesterone in biphasic ionic liquid/water system by *Aspergillus ochraceus*, J. Chem. Technol. Biotechnol. 88 (2012) 287–292, http://dx.doi.org/10.1002/jctb.3828.
[29] M.A. Palazzolo, M.L. Mascotti, E.S. Lewkowicz, M. Kurina-Sanz, Self-sufficient redox biotransformation of lignin-related benzoic acids with *Aspergillus flavus*, J. Ind. Microb. Biotechnol. 42 (2015) 1581–1589, http://dx.doi.org/10.1007/s10295-015-1696-4.
[30] I.A. Parshikov, J.B. Sutherland, Biotransformation of steroids and flavonoids by cultures of *Aspergillus niger*, Appl. Biochem. Biotechnol. 176 (2015) 903–923, http://dx.doi.org/10.1007/s12010-015-1619-x.
[31] M.L. Mascotti, A.A. Orden, F.R. Bisogno, G. de Gonzalo, M. Kurina-Sanz, *Aspergillus genus* as a source of new catalysts for sulfide oxidation, J. Mol. Catal. B Enzym. 82 (2012) 32–36, http://dx.doi.org/10.1016/j.molcatb.2012.05.003.
[32] R.D. Ceccoli, D.A. Bianchi, D.V. Rial, Flavoprotein monooxygenases for oxidative biocatalysis: recombinant expression in microbial hosts and applications, Front Microbiol 5 (2014) 1–14, http://dx.doi.org/10.3389/fmicb.2014.00025.
[33] D. Gamenara, G. Seoane, P. Saenz Mendez, P. Dominguez de Maria, Redox Biocatalysis: Fundamentals and Applications, John Wiley & Sons, Hoboken, 2012.
[34] F.R. Bisogno, M.L. Mascotti, C. Sanchez, F. Garibotto, F. Giannini, M. Kurina-Sanz, R. Enriz, Structure–antifungal activity relationship of cinnamic acid derivatives, J. Agric. Food Chem. 55 (2007) 10635–10640, http://dx.doi.org/10.1021/jf0729098.
[35] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, Quantitative analyses of biochemical kinetic resolutions of enantiomers, J. Am. Chem. Soc. 104 (1982) 1294–1299, http://dx.doi.org/10.1021/ja00389a064.
[36] M.E. Mostafa, A.A. Zohri, Progesterone side-chain degradation by some species of *Aspergillus flavus* group, Folia Microbiol. 45 (2000) 243–247, http://dx.doi.org/10.1007/BF02908952.
[37] A.C. Hunter, J. Elsom, L. Ross, R. Barrett, Ring-B functionalized androst-4-en-3-ones and ring-C substituted pregn-4-en-3-ones undergo differential transformation in *Aspergillus tamarii* KITA: Ring-A transformation with all C-6 substituted steroids and ring-D transformation with C-11 substituents, Biochim. Biophys. Acta 1761 (2006) 360–366, http://dx.doi.org/10.1016/j.bbalip.2006.02.011.
[38] A.C. Hunter, S. Patel, C. Dedi, H.T. Dodd, R.A. Bryce, Metabolic fate of 3α,5-cycloandrostanes in the endogenous lactonization pathway of *Aspergillus*

tamarii KITA, Phytochemistry 119 (2015) 19–25, http://dx.doi.org/10.1016/j.phytochem.2015.09.003.

[39] K. Yildirim, A. Uzuner, E.Y. Gulcuoglu, Biotransformation of some steroids by *Aspergillus terreus* MRC 200365, Collect. Czech. Chem. Commun. 75 (2010) 665–673, http://dx.doi.org/10.1135/cccc2009545.

[40] N.M. Kamerbeek, D.B. Janssen, W.J.H. van Berkel, M.W. Fraaije, Baeyer-Villiger monooxygenases, an emerging family of flavin-dependent biocatalysts, Adv. Synth. Catal. 345 (2003) 667–678, http://dx.doi.org/10.1002/adsc.200303014.

[41] T. Kolek, A. Szpineter, A. Swizdor, Studies on Baeyer-Villiger oxidation of steroids: DHEA and pregnenolone D-lactonization pathways in Penicillium camemberti AM83, Steroids 74 (2009) 859–862, http://dx.doi.org/10.1016/j.steroids.2009.05.007.

[42] L.-H. Huang, J. Li, G. Xu, X.-H. Zhang, Y.-G. Wang, Y.-L. Yin, H.-M. Liu, Biotransformation of dehydroepiandrosterone (DHEA) with *Penicillium griseopurpureum* Smith and *Penicillium glabrum* (Wehmer) Westling, Steroids 75 (2010) 1039–1046, http://dx.doi.org/10.1016/j.steroids.2010.06.008.

[43] A. Swizdor, Baeyer-Villiger oxidation of some C19 steroids by *Penicillium lanosocoeruleum*, Molecules 18 (2013) 13812–13822, http://dx.doi.org/10.3390/molecules181113812.

[44] D. Cvelbar, V. Zist, K. Kobal, D. Zigon, M. Zakelj-Mavri, Steroid toxicity and detoxification in ascomycetous fungi, Chem. Biol. Interact. 202 (2013) 243–258, http://dx.doi.org/10.1016/j.cbi.2012.11.025.

[45] T. Kolek, A. Szpineter, A. Swizdor, Baeyer-Villiger oxidation of DHEA, pregnenolone, and androstenedione by *Penicillium lilacinum* AM111, Steroids 73 (2008) 1441–1445, http://dx.doi.org/10.1016/j.steroids.2008.07.008.

[46] A.S. Lamm, A.R.M. Chen, W.F. Reynolds, P.B. Reese, Steroid hydroxylation by *Whetzelinia sclerotiorum*, *Phanerochaete chrysosporium* and *Mucor plumbeus*, Steroids 72 (2007) 713–722, http://dx.doi.org/10.1016/j.steroids.2007.05.008.

[47] F. Leipold, R. Wardenga, U.W.T. Bornscheuer, Cloning, expression and characterization of a eukaryotic cycloalkanone monooxygenase from *Cylindrocarpon radicicola* ATCC 11011, Appl. Microbiol. Biotechnol. 94 (2012) 705–717, http://dx.doi.org/10.1007/s00253-011-3670-z.

[48] M.L. Mascotti, M. Juri Ayub, H. Dudek, M. Kurina-Sanz, M.W. Fraaije, Cloning, overexpression and biocatalytic exploration of a novel Baeyer-Villiger monooxygenase from *Aspergillus fumigatus* Af293, AMB Express 14 (2013) 1–10, http://dx.doi.org/10.1186/2191-0855-3-33.

[49] F.M. Ferroni, M.S. Smit, D.J. Opperman, Functional divergence between closely related Baeyer-Villiger monooxygenases from *Aspergillus flavus*, J. Mol. Catal. B Enzym. 107 (2014) 47–54, http://dx.doi.org/10.1016/j.molcatb.2014.05.015.

[50] F. DeVenuto, U. Westphal, Metabolism of cortisol by subcellular fractions of the rat liver, Biochim. Biophys. Acta 54 (1961) 294–303. PMID 13885873.

[51] D.R. Brannon, F.W. Parrish, B.J. Wiley, L. Long Jr., Microbial transformation of a series of androgens with *Aspergillus tamarii*, J. Org. Chem. 32 (1967) 1521–1527. PMID 6041431.

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