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Baeyer-Villiger oxidation of DHEA, pregnenolone, and androstenedione by *Penicillium lilacinum* AM111

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ABSTRACT

The Baeyer-Villiger monooxygenase (BVMO) produced by *Penicillium lilacinum* AM111, in contrast to other enzymes of this group known in the literature, is able to process 3β-hydroxy-5-ene steroid substrates. Transformation of DHEA and pregnenolone yielded, as a sole or main product, 3β-hydroxy-17a-oxa-D-homo-androst-5-en-17-one, a new metabolite of these substrates; pregnenolone was transformed also to testololactone. Testololactone was the only product of oxidation of androstenedione by *P. lilacinum* AM111.

Investigations of the time evolution of reaction progress have indicated that the substrates stimulate activity of BVMO(s) of *P. lilacinum* AM111.

1. Introduction

Recent years have brought, along with intensive research on the role and biological activity of DHEA (3β-hydroxy-androst-5-en-17-one) and pregnenolone, many studies on their metabolism and the role and activity of the metabolites, including those which are not sex hormones [1–11]. DHEA derivatives with oxygen function at C-7, mainly 7α- and 7β-hydroxy-DHEA, were identified in many mammalian organs and tissues (e.g. brain, liver, skin) [1–8,12,13]. 7α-Hydroxy-DHEA was often found as the main metabolite product of microbiological transformations of DHEA [14–22].

In an effort to obtain new metabolites of DHEA and pregnenolone, different from any known derivatives with oxygen function at the C-7, screening tests were carried out and the strain *Penicillium lilacinum* AM111 was selected for further studies.

Literature reports indicate that the strains of genus Penicillium are able to carry out transformations of steroid substrates by means of reduction, hydroxylation or Baeyer-Villiger oxidation [23–32]. *Penicillium decumbens* strain has been used for the reduction of double bonds, particularly the conversion of 4-en-3-oxosteroids to 5α-3-ones [24,25]. In view of the fact that *P. decumbens* is one of only a few microorganisms known to perform this conversion, it occupies a central role in the metabolism of steroids. Some studies focused on 15α-hydroxylation of 3-ethyl-gone-4-en-3,17-dione catalyzed by *Penicillium raistrickii* [26,27]. Various strains of genus *Penicillium* carry out Baeyer-Villiger oxidation [26–32].

Baeyer-Villiger monooxygenases (BVMOs) are flavoenzymes that catalyze the Baeyer-Villiger reaction by insertion of an oxygen atom next to a keto function thus converting ketones to corresponding esters or lactones. BVMOs are produced by numerous bacteria (e.g. of the genera *Actinetobacter*,

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Arthobacter, *Nocardia*, *Rhodococcus*, *Streptomyces*) and fungi (e.g. of the genera *Aspergillus*, *Culvularia*, *Fusarium*, *Penicillium*) [33]. The strain *P. lilacinum* was the source of the first isolated BVMO responsible for oxidation of androstenedione to testololactone [28]. BVMOs carry out Baeyer-Villiger degradation of 17β-acetyl side chain of C-21 substrates and ring D oxidation of androstenedione [33]. Substrates of the reactions described above were usually 4-en-3-oxo steroids.

DHEA and pregnenolone were subjected to transformation by fungus *Penicillium citreo-viride*, which was shown to convert androstenedione and progesterone to testololactone [31]. In a culture of this strain, DHEA was oxidized to testololactone, while pregnenolone was not transformed.

2. Experimental

2.1. Materials

2.1.1. Substrates

DHEA, pregnenolone, androstenedione, and progesterone standard were purchased from Sigma–Aldrich Chemical Co. The microorganism *P. lilacinum* AM111 used in this study was obtained from the collection of the Institute of Biology and Botany, Medical University of Wrocław. Originally, the AM111 strain was isolated from synthetic fibres.

2.2. Conditions of cultivation and transformation

Fungi were incubated on 3% glucose and 1% aminobac, in 300 ml Erlenmeyer flasks with 100 ml of medium. After cultivation at 25 °C for three days on a rotary shaker, 20 mg of the substrate, dissolved in 1 ml of acetone, was added. Transformation of the substrate was carried out in 5 flasks under the same conditions. The reaction progress was monitored by TLC and GC. Each test was carried out in at least three independent, parallel experiments.

2.3. Isolation and identification of the products

The products of transformation were extracted from the mixtures three times with 20 ml of chloroform. Transformation products were separated by column chromatography on silica gel with ethyl acetate/methylene chloride/acetone (3:1:1) as eluent. TLC was carried out with Merck Kieselgel 60 F_{254} plates using the same eluent. In order to develop the image, the plates were sprayed with solution of methanol in concentrated sulphuric acid (1:1) and heated to 120 °C for 3 min. GC analysis was performed using Hewlett Packard 5890A Series II GC instrument (FID, carrier gas H_2 at flow rate of 2 ml min^{-1}) with a HP-1 column cross-linked Methyl Siloxane, 30 m × 0.53 mm × 1.5 μm film thickness. Applied temperature program: 190 °C/1 min, gradient 4 °C/min to 235 °C/5 min and 30 °C/min to 300 °C/3 min; injector and detector temperature was 300 °C. Retention times of the identified compounds are given in Table 1. Infrared spectra were recorded in KBr discs on a Mattson IR 300 Spectrometer. The NMR spectra were measured in $CDCl_3$ and recorded on a DRX 300 MHz Bruker Avance spectrometer with TMS as internal standard. Optical rotation measurements were carried out on Autopol IV automatic polarimeter (Rudolph).

2.4. Biotransformations of DHEA (1) by P. lilacinum AM111

After 36 h incubation of 100 mg DHEA in *P. lilacinum* AM111 culture, 92 mg of 3β-hydroxy-17a-oxa-D-homo-androst-5-en-17-one (**4**) have been isolated (Fig. 1).

2.4.1. 3β-Hydroxy-17a-oxa-D-homo-androst-5-en-17-one (4)

mp 227–230 °C; $[\alpha]_D^{20} = -93.9$ (c 0.1 in $CHCl_3$); IR ν_{max}(cm^{-1}): 3446, 1716, 1653, 1215; ^{1}H NMR: δ (ppm): 0.96 (s, 19-H_3), 1.30 (s, 18-H_3), 2.59 (m, 16α-H), 2.68 (m, 16β-H), 3.52 (m, W_h = 26.74 Hz, 3α-H), 5.33 (t, 6-H), ^{13}C NMR: δ (ppm): 171.5 (C-17), 140.6 (C-5), 120.8 (C-6), 71.5 (C-3), 83.2 (C-13), 49.0 (C-9), 46.7 (C-14), 41.9 (C-4), 38.9 (C-12), 36.9 (C-1), 36.6 (C-10), 34.4 (C-2), 31.5 (C-7),

Table 1 – Composition of crude mixture obtained in transformation by *P. lilacinum* AM111

Substrate	$R_{t\ (min)}$	Compounds present in mixture (%)[a]	Time of substrate incubation			
			6 (h)	12 (h)	24 (h)	30 (h)
DHEA (**1**)	4.38	DHEA (**1**)	93	66	14	–
	8.04	3β-Hydroxy-17a-oxa-D-homo-androst-5-en-17-one (**4**)	7	34	86	100
Androstenedione (**3**)	5.44	Androstenedione (**3**)	98	85	30	3
	9.40	Testololactone (**5**)	2	15	70	96
Pregnenolone (**2**)	6.43	Pregnenolone (**2**)	85	75	58	51
	7.80	Progesterone (**6**)[b]	15	9	–	–
	4.38	DHEA (**1**)[b]	–	5.5	7	2
	5.44	Androstenedione (**3**)[b]	–	9.5	12	7
	8.04	3β-Hydroxy-17a-oxa-D-homo-androst-5-en-17-one (4)	–	–	15	23
	9.40	Testololactone (**5**)	–	–	3	12

[a] Determined by GC analysis.
[b] Identified in GC and TLC on the basis of standards.