MICROBIOLOGICAL SYNTHESIS OF 17β-HYDROXYANDROSTA-1,4-DIEN-3-ONE

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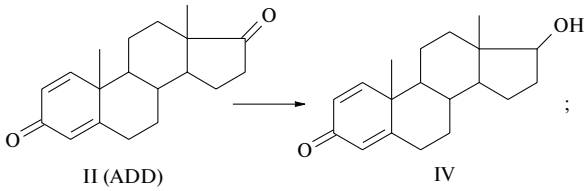
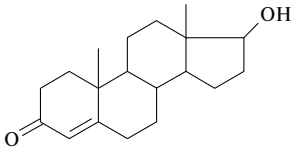
Many drugs possessing steroidal structures are obtained from sterols, which are first microbiologically transformed into androst-4-en-3,17-dione (I, AD) and androsta-1,4-dien-3,17-dione (II, ADD) and then chemically converted into target compounds (e.g., III and IV).



II (ADD) IV



III

The process of microbiological degradation of sterols was studied in sufficient detail [1]. It should be noted that investigations of the synthesis of compounds I and II (selecting strains, media, biotransformation conditions, etc.) were accompanied by studying the chemical stability of these products and the possibility of converting them microbiologically into some other compounds [2, 3].

The early papers reported that cultures of *Myc. sp.* NRRL B 3805 and *Myc. sp.* NRRL 3683 transformed AD, albeit in insignificant amounts, into 6α-hydroxy-AD; moreover, the *Myc. sp.* NRRL 3683 culture produced an estrone as well. At present, the fact of reduction of the 17-keto groups in 3,17-diketones by *Myc. sp.* is reliably established [3 – 5]. The process of the 17-keto group reduction was found to depend on the presence of a 1,2-double bond in the initial substrate. The formation of testosterone (III) from ADD is related to the simultaneous reduction of 17-keto groups and 1,2-double bonds by the *Myc. sp.* NRRL B 3683 culture [5]. Investigation of the analogous process with *Myc. sp.* BKM Ac 1815D showed the absence of 17β-reductase activity with respect to 1,2-reduced steroids (e.g., AD) and the presence of such activity with respect to 1,2-dehydrosteroids (such as ADD) [3].

We report on the results of investigation of the process of AD and ADD reduction by *Myc. vaccae* and *Myc. sp.* No. 22 cultures, which transform sterols into AD and ADD. We have established that these strains only weakly modify AD: 98% of the initial amount was isolated after a 24 h incubation.

In contrast to the cases described in [3, 5], *Myc. vaccae* and *Myc. sp.* No. 22 cultures only reduce 17-keto groups in ADD, while not modifying the 1,2-double bond. As a result, we obtained 1,2-dehydrotestosterone (IV) with a yield of 80% (relative to the cultural liquid) for both strains. In order to evaluate the transformation results, the cultural liquid (together with biomass) was extracted with ethyl acetate. Then ethyl acetate was evaporated and the residue was analyzed by HPLC. Dehydrotestosterone IV was isolated by chromatography and identified based on the results of spectroscopic measurements and published data.

During the experiments, we selected optimum conditions for the microbiological transformation process. It was found that optimum conditions for *Myc. sp.* No. 22 are provided by medium 1 (%): glucose, 1; microbe fermentation agent (MFA), 2; potassium dihydrophosphate, 0.5; ammonium hydrophosphate, 0.3; magnesium sulfate, 0.02 (pH 7.4). For *Myc. vaccae*, the optimum conditions are provided by medium 4 (%): glucose, 1; soybean meal, 1; citric acid, 0.22; ammonium hydrophosphate, 0.15; magnesium sulfate, 0.02; iron sulfate, 0.005 (pH 7.0). Somewhat less favorable were medium 2 (glucose, 0.3; MFA, 1; peptone, 0.3; pH 6.4) and medium 3 (glucose, 0.3; MFA, 1; potassium hydrophosphate, 0.18; sodium hydrophosphate, 0.4; sodium chloride, 0.2; pH 7.3). The results of our experiments are summarized in

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TABLE 1. Microbiological Synthesis of 17β-Hydroxyandrosta-1,4-dien-3-one Using *Myc. vaccae* and *Myc. sp.* No. 22 Cultures

Culture	Medium (growth and transformation)	Substrate load, g/liter	Cultural liquid composition, % (HPLC data)			
			I (AD)	II (ADD)	III	IV
Myc. vaccae	4	1.5	1	8.0	6.2	80.0
	2	1.5	0.8	4.0	5.3	34.4
Myc. sp No. 22	3	2.0	0.8	6.0	6.2	52.8
	1	2.0	0.3	11.8	8.3	80.3
	2	2.5	3.0	10.0	8.6	64.0

Table 1. The low content of steroids observed after ADD transformation by *Myc. vaccae* in medium No. 2 can be explained by the degradation of compound IV.

Optimum media for the growth of transforming cultures are the same as those used for the transformation process. The amount of inoculated material in the medium must amount to 30%. We have also studied the role of the method of substrate introduction and its concentration in the cultural liquid (see Table 1), as well as of the aeration conditions and transformation duration. It was established that a useful factor is an additive of 10% calcium chloride in methanol (0.6 – 1 ml per 100 ml of substrate). The optimum time of transformation is 24 h; a further increase in the time is accompanied by the reverse process, whereby product IV is oxidized to ADD.

Thus, our investigation determined optimum conditions for the microbiological synthesis of dehydrotestosterone using *Myc. vaccae* and *Myc. sp.* No. 22 cultures with a yield of up to 60 – 65%. Dehydrotestosterone and its esters are used in medicine as anabolic preparations. These products are available from foreign companies under the trade names of boldenone, vebonol, parenabol, and boldenone undecylate.

EXPERIMENTAL PART

The UV spectra were measured on a Specord M-80 spectrophotometer as chloroform solutions. The UV spectra were recorded on a Specord UV-VIS spectrophotometer (Germany). The HPLC analyses were performed with a Gilson chromatograph equipped with a Silasorb C18 (10 μm) column (4.0 × 250 mm) and an UV detector (λ = 254 nm). The measurements were conducted at room temperature using a MeOH–H_2O (70 : 30) mobile phase at a flow velocity of 0.8 ml/min; the sample volume was 20 μl and the elution times (min): ADD, 5.8; IV, 7.1; AD, 8.0; III, 10.

Culture growth and substrate transformation process. The *Myc. vaccae* and *Myc. sp.* No. 22 species (from collection of the Bioengineering Center, Russian Academy of Sciences, Moscow) were maintained on corn – glucose agar. The biomass was grown on media 1 – 4 with compositions indicated above.

The transformation products from the *Myc. sp.* No. 22 culture were isolated as follows: medium 1 was loaded with 2.0 g/liter II and incubated for 24 h. After transformation, the content of six flasks (0.25 × 6 = 1.5 g) was combined. The biomass was separated from cultural liquid and treated with acetone. Then acetone was evaporated and the oily residue triturated with ether, which yielded 0.90 g of 17β-hydroxyandrosta-1,4-dien-3-one (IV) with an admixture (~5%) of III; yield, 60%. The cultural liquid contains II with an admixture of I, which can be isolated by extracting with ethyl acetate.

For identification of the individual products, the above experiment (6 × 250 ml) was used to obtain 2.2 g of the product. According to HPLC data, this product contains 5% II, 51% IV, 0.7% I, 4.25% III, and 39% biomass. This corresponds to the product (IV) obtained with a yield of 80% (relative to the cultural liquid). The product was purified by chromatography on 50 g silica gel eluted stepwise with benzene and benzene – ether (5 and 10%) mixtures. Insignificant amounts of I and III, as well as of residual II, allows the product to be identified by TLC and HPLC with reference to the standard samples [7 – 9].

The main target product is obtained from the silica gel column in an amount of 0.5 g; m.p., 172 – 173°C; λ_{max}, 243 nm (ε, 15,700); IR spectrum in chloroform (ν_{max}, cm^{-1}): 3616 (OH), 1662 (C=O), 1636, 1602 (C=C). Published data [7]: m.p., 167 – 168°C; λ_{max}, 243 nm (ε, 16,100).

17β-Hydroxyandrosta-1,4-dien-3-one (IV) obtained from II using *Myc. vaccae* culture was identified by a similar method.

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