The calculated values of λ for the sorption process of lysine on the above column, using DSG-KRS-8p and KU-2×8 are 3.39 and 1.68, respectively, i.e., a regular sorption regime will take place ($\lambda > 1$).

Thus, the industrial experiments on the separation of lysine from the CL of Brevibacterium sp. 22 by means of ion exchange chromatography that have been carried out show that at high rates of filtration, the modified sorbent of the dispergel type ensures a high yield during the desorption and a higher yield of the amino acid in a crystalline state than KU-2×8 cation exchanger (41.4 and 17.8%, respectively). The time of the operating cycle of the column is shortened by 24-41%.

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TRANSFORMATION OF ANDROST-4-ENE-3,17-DIONE AND ANDROSTA-1,4-DIENE-3,17-DIONE BY MEANS OF Beauveria sp. II*

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We have earlier published the results of the microbiological hydroxylation of androst-4-ene-3,17-dione (I) by means of Rhizopus nigricans [2].

The aim of the present investigation was to study this process using a Beauveria sp. culture, which results in 11α-hydroxylation in the pregnane series [1, 9]. Compound I and androsta-1,4-diene-3,17-dione (II) were used as the substrates.

During the transformation of I, the expected 11α-hydroxy-androst-4-ene-3,17-dione (IV) was formed in a small amount. As the main product, a compound was isolated by crystallization, which, according to the IR spectral data, has two hydroxylic groups (3440 and 3320 cm^{-1}) and does not contain a carbonyl group in the five-membered ring. It has been concluded from these data that the Beauveria sp. fungus not only brings about 11α-hydroxylation, but also a reduction of the carbonyl group at C(17) and the compound obtained is 11α,17β-dihydroxyandrost-4-ene-3-one (III). This reaction is not characteristic

*Article I, see [2].

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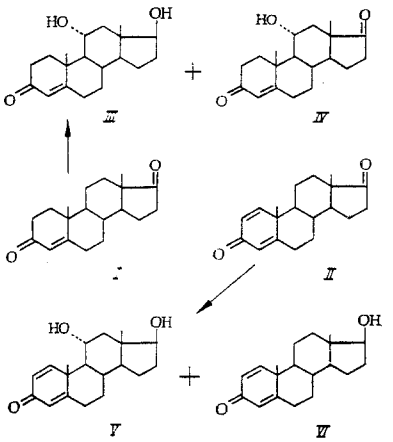
for Beauveria sp. According to the literature data, this process is affected by yeasts [5, 6].

During the transformation of II, a mixture of two products (V) and (VI) was also obtained. The compounds were separated by crystallization and chromatography of the mother liquor on a silica gel column. According to the IR spectrum data, the two compounds do not contain a carbonyl group at C(17); compound V has two hydroxyl groups, while compound VI has one. Based on these data, structures of 11α-17β-dihydroxyandrosta-1,4-dien-3-one and 17β-hydroxyandrosta-1,4-dien-3-one, were ascribed to compounds V and VI, respectively. In this case, the formation of 11α-hydroxyandrosta-1,4-diene-3,17-one is not observed, which is possibly due to the presence of a Δ^1-bond in the molecule of II.



The transforming ability of the Beauveria sp. mycelium was studied during its repeated application. For this, the mycelium is transferred after 24 h into a fresh medium for transformation of a new portion of the steroid. This operation is repeated four times. Glucose solution was used as the medium together with phosphate buffer.

In carrying out the transformation of I and II in a concentration of 1 g/liter in glucose solution, it was found that in the course of two passes, the culture liquid contained only III and V, respectively, whereby their amount increased in the second pass. In the third and fourth passes the amount of III and V decreased, and compounds IV and VI appeared in small amounts (Fig. 1a, 2a).

The repeated use of the mycelium in phosphate buffer during the transformation of I, gave in the first and second passes the same regularity as when glucose was used. In the third pass a sharp decrease in the amount of III was observed, and the initial I appeared (Fig. 1b). In the case of II, the decrease in V was observed also in the second pass, and unreacted II was left behind (Fig. 2b).

Thus, for the first time, the ability of Beauveria sp. to reduce the carbonyl group at C(17) simultaneously with the hydroxylation of compounds in the androstane series has been established. The obtained results indicate that in contrast with the phosphate buffer, during repeated use of mycelium, the glucose solution promotes the retention of the activity of the culture for four passes. The repeated use of the mycelium during the transformation of I, irrespective of the medium, leads to a sharp increase in the amount of compound III, but in the case of II, an increase in the formation of V is observed only on using a glucose solution.

EXPERIMENTAL

The chromatographic analysis of compounds I-VI and the monitoring of the course of the reaction were carried out on Silufol UV-254 plates (CSSR) in the $CHCl_3$–acetone–cyclohexane system (6:3:1), scanning was carried out in the UV spectrum, and the development by a 1% solution of vanillin in 10% $HClO_4$. Quantitative determinations were carried out on a SF-10 spectrophotometer at 240-242 nm. The IR spectra of the compounds were run on a "Perkin-Elmer 599" spectrophotometer (Sweden).

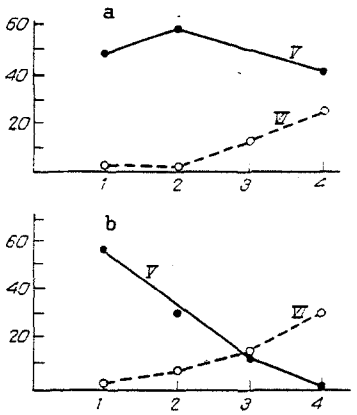


Fig. 1

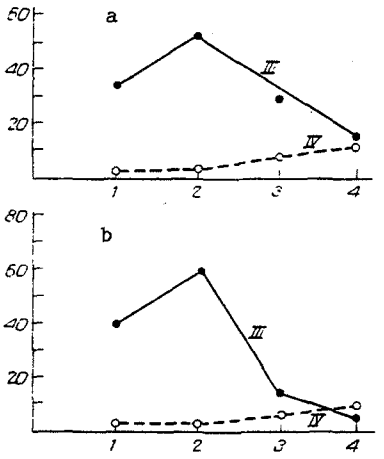


Fig. 2

Fig. 1. Ability of mycelium of Beauveria sp. to effect the repeated transformation of II. Here and in Fig. 2: a) a 0.5% solution of glucose, b) phosphate buffer. On ordinate – transformed steroid, %: on abscissa – number of passes.

Fig. 2. Ability of mycelium of Beauveria sp. to effect the repeated transformation of I.

Cultivation of Culture and Carrying out the Transformation. A Beauveria sp. culture from the collection of the All-Union Scientific Chemical Pharmaceutical Institute was sustained on a brewing agar. The mycelium was cultivated on a medium of the following composition: glucose – 2%, peptone – 0.5%, yeast autolysate – 0.3%, potassium dihydrophosphate – 0.5%, pH 5.6. Portions of 4 ml of an aqueous suspension of the fungus spores were introduced into 750 ml flasks containing 100 ml of the medium. After 48 h of growth, 5% of the inoculation material was introduced into flasks containing fresh medium. The transformation was carried out with a 24 h old mycelium, washed out of the culture medium in a concentration of 6 g/liter in 100 ml of phosphate buffer (pH 6.2) or in 100 ml of a 0.5% solution of glucose. The cultivation of the mycelium and the transformation stage were carried out on a shaking device (200 cycles/min) at 28°C. A 100 mg portion of I or II in 1 ml of 10% solution of $CaCl_2$ in MeOH (concentration 1 g/liter) was introduced into the medium for transformation.

On repeated use of the mycelium, after 24 h of the transformation, the biomass was filtered from the medium and was transferred into fresh medium containing a new portion of the steroid. The transformation products were determined in the filtrate. Four passes were carried out.

To isolate the transformation products, expanded scale experiments were carried out: 19 flasks with 100 ml of the buffer and 100 mg of I (1.9 g of I) and 6 flasks with 100 mg of II (0.6 g of II, example 1); 15 flasks with 100 ml of 0.5% solution of glucose and 100 mg of II (1.5 g of II, example 2). After 24 h of transformation, the culture liquid was separated from the mycelium and extracted with CH_2Cl_2. The latter was washed with water to a neutral reaction, dried and evaporated.

11α,17β-Dihydroxyandrost-4-en-3-one (III) and 11α-Dihydroxyandrost-4-ene-3,17 dione (IV). The solution obtained after the evaporation (~20 ml) was boiled with carbon. The carbon was filtered, and the solvent evaporated to dryness. The oily residue (1.9 g) was dissolved in 10 ml of a mixture of benzene and $CHCl_3$ (1:1) and the solution was deposited on a column with 5 g of silica gel (40 × 100 μm). Elution with chloroform gave 0.08 g (4.2%) of I, mp 162-164°C. Literature data [7]: mp 165-166°C. Then 0.16 g (8%) of IV were eluted, mp 223-225°C. According to the literature data [2], mp 226-227°C. By further elution with chloroform, 1.54 g (76.2%) of III were obtained, mp 175-176°C. IR spectrum, ν_{max}, cm^{-1}: 3440, 3320 (OH), 1655 (C=O), 1600 (C=O). According to the literature data [8], mp 180-182°C.

11α,17β-Dihydroxyandrosta-1,4-dien-3-one (V) and 17β-hydroxyandrosta-1,4-dien-3-one (VI).

Example 1. The oily residue obtained after evaporation (0.6 g) was ground with a mixture of ether and methanol (10:1). The precipitate was filtered and washed with ether. Yield 0.4 g (62.7%) of V, mp 177-178°C. IR spectrum, ν_{max}, cm^{-1}: 3420, 3220 (OH), 1655 (C=O), 1620, 1600 (C=C). According to the literature data [3], mp 183-185°C.

The mother liquor was evaporated to dryness, and the residue (0.2 g) was dissolved in a mixture of benzene and $CHCl_3$ (1:1) and chromatographed on 2 g of silica gel. Elution with the above mixture gave 0.08 g (12.4%) of VI, mp 170-171°C. Literature data [4]: mp 172-173°C. Further elution with chloroform gave 0.04 g (6.3%) of V, mp 175-176°C.

Example 2. The oily residue obtained after evaporation of the solvent (1.5 g) was dissolved in a mixture of benzene and $CHCl_3$ (1:1) and the solution was chromatographed on 15 g of silica gel. Elution with the above mixture gave 0.58 g (38%) of VI, mp 170-172°C. Further elution with chloroform gave 0.74 g (46%) of V, mp 181-182°C.

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REDUCTION OF NITRO-SUBSTITUTED BENZO-2,13-THIADIAZOLES BY IMMOBILIZED CELLS OF E. coli

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Nitro-substituted benzo-2,1-3-thiadiazoles have herbicidal, fungicidal, and insecticidal activity, are plant growth regulators, and their amino derivatives are used as complex forming agents in chemistry, and have been studied as potential radioprotectors in medicine [1-2].

In connection with the study of the metabolism of nitro derivatives of benzo-2,1,3-thiadiazoles in the organism, and also in development methods for the synthesis of the corresponding amines, it has been shown previously that during their microbiological reduction by E. coli cells, amines are formed as the sole product [3].

Considering this and the high yields of the amino derivatives, we examined the immobilization of the cells of E. coli in polyacrylamide and Carrageen gels (PAG and CG) as a route for developing biocatalysts for reduction of nitro groups.

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