7. A. Battersby and J. C. Robinson, J. Chem. Soc., 2076 (1956).
8. H. Hall and J. Brandt, J. Am. Chem. Soc., 80, 6420 (1958).
9. N. P. Dzyuba, Khim.-farm. Zh., No. 12, 39 (1971).
10. J. Welby, Sci. Tech. Pharm., 4, 141 (1975).
11. B. G. Yasnitskii, E. B. Dol'berg, and A. L. Spivak, Khim.-farm. Zh., No. 4, 78 (1979).

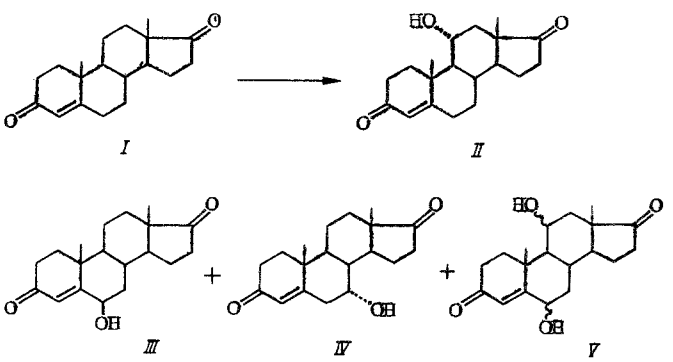
HYDROXYLATION OF ANDROST-4-ENE-3,17-DIONE BY *Rhizopus Nigricans*

K. N. Gabinskaya, V. I. Bayunova,
T. S. Kolyvanova, G. S. Grinenko,
and O. V. Messinova

UDC 615.357.453.012.6

One of the practically important microbiological transformations of steroidal compounds is hydroxylation. It can be applied to compounds of the androstane series [1].

Here we report a study of the conditions of the 11α-hydroxylation of androst-4-ene-3,17-dione (I) by *Rhizopus nigricans* including an examination of the reaction byproducts. We isolated the 7α-hydroxy derivative (IV), whose formation has not been reported in transformations with this fungus.



I II III IV V

We examined the hydroxylation of I by *R. nigricans* and *Tiegemella hyalospora* to select the transforming culture.

We subsequently used *R. nigricans* since it gave twice as much II.

To assess the effect of the mode of introduction of I on the course of the transformation we added it to the beer as a powder prepared with an agate mortar or as solutions in ethanol, acetone, and DMF. The amount of solvent was varied from 3-4% of the mass of the beer in the runs with ethanol and acetone to 0.4-1.4% when DMF was used. We got the best results by adding I in DMF solution with a DMF content in the beer of 1%. The other solvents inhibited the process.

We used medium A with peptone and medium B with cornsteep extract to find the most suitable medium for growing *R. nigricans*. Though the quantity of the biomass grown on medium A was twice that in medium B, we used the same quantity of the mycelium in the transformation. Subsequently we used medium A, since the yield of II was 50% greater than when the fungus was grown on medium B.

To select the transformation medium mycelium slurry was transferred to mains water or to 0.5% glucose solution. Hydrolysis in glucose solution was complete after 24 h (the final pH of the beer was 4.5) while in mains water the reaction had not ended after 46 h (pH 7.3). Hence we subsequently used mycelium slurry in glucose solution.

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 14, No. 1, pp. 76-79, January, 1980. Original article submitted July 4, 1979.

0091-150X/80/1401-0063$07.50

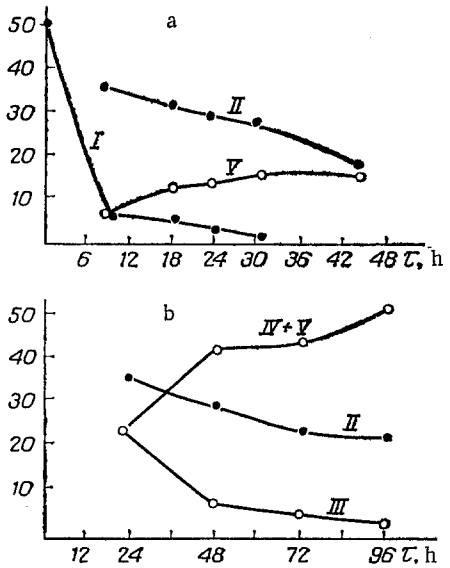


Fig. 1. Variation in the content (mg per 100 ml of medium) of the products of transformation of I (II, III, IV, and V) by the fungus *R. nigricans* in the beer as a function of reaction time (h) with initial concentrations of I of a) 0.5 g/liter and b) 1.0 g/liter.

We examined the effect of the quantity of the mycelium used for the transformation of I on the formation of II. We found that change in the biomass from 0.12 to 0.78 g per 100 ml of medium did not affect the quantity of II formed. When the quantity of biomass was increased to 1.1 g per 100 ml it began to inhibit the process. Consequently the amount of biomass used for the hydroxylation was not more than 0.6 g per 100 ml.

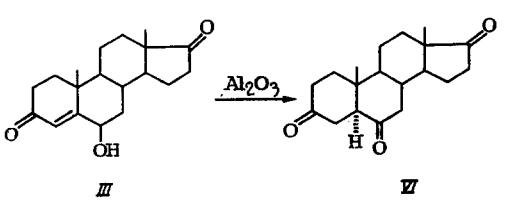
We followed the variation in the concentration of the transformation products of I as a function of the reaction time with an initial concentration of I in the beer of 0.5 and 1 g/liter. Our results are shown in Fig. 1. The maximum quantity of II was derived when unreacted I was still present in the beer. When the reaction time was extended the quantity of II began to diminish. In addition V, the possible secondary hydroxylation product of II, began to accumulate in the beer.

We detected in the reaction mixture (the concentration of I was 1 g/liter) after 24 h in addition to II and V compounds III and IV, which are new byproducts of the transformation of I. The concentrations of II and III were reduced by further extension of the reaction time, since the concentration of IV increased. The concentration of IV + V was twice that of II 96 h after the start of the reaction. Thus an increase in the initial concentration of I from 0.5 to 1.0 g/liter led to the appearance of hydroxylation byproducts in the beer.

We carried out extended tests in a fermentor to isolate the reaction products in quantity and identify them. We isolated androst-4-en-11α-ol-3,17-dione (II), in 62% yield, and the reaction byproducts androst-4-en-6β-ol-3,17-dione (III) and androst-4-en-7α-ol-3,17-dione (IV).

We isolated the transformation product II from the reaction mixture by crystallization. Its constants were identical to literature data [1].

Attempted separation by column chromatography on aluminum oxide of compounds III and IV, which were present in the mother liquor in the ratio 1:1 (by thin-layer chromatography, TLC), revealed the susceptibility of III to rearrangement to a less polar compound. Since this compound does not absorb in the UV and its IR spectrum contained only bands at 1695, 1715, and 1730 cm^{-1} due to the stretching modes of the three carbonyl groups, we assign it the structure of androstane-3,6,7-trione (VI).



Mass spectrometric determination of the molecular weight supported structure VI. The rearrangement of III to VI is consistent with earlier work [2].

Compound (IV) was eluted with chloroform. We verified its structure from the IR spectrum and by comparison with literature data [3].

We detected only a small quantity of the more polar compound V by TLC. Consequently we did not isolate it but its polarity suggests that it has two hydroxyl groups.

EXPERIMENTAL

Chromatographic analysis of compounds II-V was carried out on Silufol UV-254 plates in the system chloroform–acetone–cyclohexane (6:3:1) with visualization by 1% vanillin solution in 10% aqueous perchloric acid. Measurements of $[\alpha]_D^{20}$ were made on an SF-8 spectrophotometer; quantitative determinations were carried out with an SF-10 spectrophotometer.

Culture Growth and Transformation Procedure. Cultures of *R. nigricans* and *T. hyalospora* from the collection of the All-Union Scientific Research Pharmaceutical Chemistry Institute were maintained on wort agar. The mycelium was grown on medium A (glucose 2%, peptone 0.5%, yeast autolyzate 0.3%, potassium dihydrogen phosphate 0.5%, pH 5.6). For comparison we used medium B (glucose 1%, cornsteep extract 1%, pH 5.0). An aqueous suspension (2 ml) of spores was added to a 250-ml flask containing the medium (100 ml). The transformation was carried out with mycelium slurry in 0.5% glucose solution. The mycelium was grown and the transformation was carried out on a shaker (200 rev/min) at 28°C. Substrate I was added to the flask in solution in 1 or 2 ml of DMF on the basis of 50 or 100 mg per 100 ml of medium. The transformation products were extracted from the beer with two portions of chloroform, separated on plates, and determined spectrophotometrically at 242 nm.

When the run was carried out in a fermentor, the inoculum of *R. nigricans* was grown in flasks on a shaker. The 24-h culture (600 ml) was transferred to a fermentor containing medium A (6 liter). The transformation was carried out with 20-h mycelium slurry in 0.5% glucose solution. The mycelium was grown and the transformation was carried out with a running stirrer (600 rev/min) with aeration (0.6 liter/h), and at a temperature of 28°C. Compound I was added in DMF solution (60 ml) on the basis of 3 g (0.0105 mole) per 6 liters of medium. After 28 h of the transformation the mycelium was filtered from the beer and washed. The mycelium contained I (TLC). The beer and washings were extracted with chloroform (3.5 liters + 1.5 liters + 1.5 liters). The chloroform extracts were washed with water until neutral, dried, and evaporated.

Androst-4-en-11α-ol-3,17-dione (II). The oily residue (2.6 g) derived after evaporation of the chloroform was refluxed twice with hexane (10 ml). The precipitate was filtered off and washed on the filter with hexane to give I and II (95 g, 61.7%), mp 226-227°C (ethyl acetate–methylene chloride), $[\alpha]_D^{20}$ + 164°C (c 1, chloroform). Literature [1] mp 225-227°C (ethyl acetate), $[\alpha]_D^{20}$ + 162° (c 1.4, chloroform).

Androst-4-en-7α-ol-3,17-dione (IV) and Androstane-3,6,7-trione (VI). The mother liquor remaining after the isolation of II from two fermentations was evaporated. The gummy residue (1.3 g) was dissolved in benzene and applied to a column of aluminum oxide (130 g). After 72 h the column was eluted with chloroform to give VI (0.08 g, 6%), mp 190-192°C, $[\alpha]_D^{20}$ + 65° (c 1, chloroform). Found: molecular weight 302 (mass spectrometry). $C_{19}H_{26}O_3$. Calculated: molecular weight 302.4. Literature [2] mp 191-193°C, $[\alpha]_D^{20}$ + 67°.

Further elution with chloroform gave IV (0.1 g, 8%), mp 244-245°C (from methanol), $[\alpha]_D^{20}$ + 161° (c 0.7, chloroform), λ_{max} 244 nm, log ε 4.21. IR spectrum, ν: 1660, 1735 (C=O), 1610 (C=C), and 3420 (–OH) cm^{-1}. Literature [3] mp 241-244°C, $[\alpha]_D^{20}$ + 169°, λ_{max} 243 nm, log ε 4.24.

LITERATURE CITED

1. S. H. Eppstein, P. D. Meister, et al., J. Am. Chem. Soc., 76, 3174 (1964).
2. L. Kornel and K. Motohashi, Steroids, 6, 9 (1965).
3. K. Singh, S. N. Sehgal, and C. Vézina, Can. J. Microbiol., 13, 1271 (1967).