where A can be calculated from the equation

$$A = 16.75 + 8c. \tag{9}$$

Taking Eqs. (8) and (9) into account the yield of II can be determined from the equation

$$Y_{ab} = 8c + 0.44t + 10.15, \tag{10}$$

when t > 15 min, 0.7 ≤ c (moles/liter) ≤ 5.7, and Y_{ab} ≤ 100%, where c is the hydrochloric acid concentration, and t is the total time of the process in minutes.

According to Eq. (10), the complete hydrolysis of tannin in the presence of 10% hydrochloric acid (2.8 mole/liter) can be achieved in 2.5 h. Experimental verification showed that a yield of 96% is achieved for this period of time, which coincides quite well with the calculated value.

LITERATURE CITED

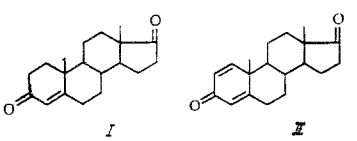
1. A. Blazhei and L. Shutyi, Phenolic Compounds of Plant Origin [Russian translation], Moscow (1977).
2. V. P. Georgievskii and L. M. Bobkova, Farmatsevtich. Zh., No. 4, 73-74 (1977).
3. C. K. Ingold, Theoretical Foundations of Organic Chemistry [Russian translation], Moscow (1973).

MICROBIOLOGICAL HYDROXYLATION OF ANDROST-4-ENE-3,17-DIONE AND ANDROSTA-1,4-DIENE-3,17-DIONE

T. S. Kolyvanova, V. I. Bayunova, K. N. Gabinskaya, Yu. N. Korobova, and G. S. Grinenko

UDC 615.357:577.175.624].012.6.07

We have previously studied the 11α-hydroxylation of androst-4-ene-3,17-dione (I) by means of Rhizopus nigricans [2], as well as the transformation of I and androsta-1,4-diene-3,17-dione (II) by means of a Beauveria sp. culture [1]. As a continuation of the research we investigated the possibility of the hydroxylation of I and II with Tieghemella hyalospora and Tieghemella orchidis cultures, which, respectively, bring about 11α- and 11β-hydroxylation in the pregnane series [3].



I II

The transformation of I and II by means of these cultures proceeds with the formation of a mixture of three hydroxylation products: 11α-hydroxyandrost-4-ene-3,17-dione (III), 11β-hydroxyandrost-4-ene-3,17-dione (IV), and 7α-hydroxyandrost-4-ene-3,17-dione (V) and 11α-hydroxyandrosta-1,4-diene-3,17-dione (VI), 11β-hydroxyandrosta-1,4-diene-3,17-dione (VII), and 7α-hydroxyandrosta-1,4-diene-3,17-dione (VIII) (Table 1).

In growing the T. hyalospora fungus we used three media in which glucose was the source of carbon, and the sources of nitrogen differed: corn extract (medium 60), a mixture of peptone and the extract (medium A), and all three components (medium P). The best results were obtained in media A and P.

S. Ordzhonikidze All-Union Institute of Pharmaceutical Chemistry, Moscow.

Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 23, No. 5, pp. 635-637, May, 1989. Original article submitted April 28, 1988.

TABLE 1. Yields of Products (%) of Hydroxylation of I and II by means of T. orchidis and T. hyalospora Cultures

Compound	T. orchidis			T. hyalospora		
	11β	11α	7α	11β	11α	7α
I	17,1	21,5	50,0	19,0	38,1	33,3
II	14,2	42,6	28,4	2,7	54,7	28,4

The steroids were introduced into the transformation medium in concentrations from 0.5 to 2 g/liter. Transformation does not occur at a II concentration above 0.7 g/liter, while the transformation of I is realized without a decrease in the yield of III when the concentration is increased to 2 g/liter. The introduction of I (2 g/liter) in fractional amounts does not promote an increase in the yield of III.

The ability of the T. hyalospora mycelium grown in medium A for the repeated carrying out of the hydroxylation of I and II was investigated. The above-noted mycelium was transferred to a solution of glucose or phosphate buffer containing the steroid. After each 24 h of transformation, the mycelium was washed with water and transferred to a fresh medium containing the steroid (0.7 g/liter). The operation was repeated four times.

It is known that in the 11α-hydroxylation of cortexolone acetate in glucose solution the activity of the culture is retained in the course of two passages [1]. However, in the androstane series we observed that under the same conditions the activity of the culture does not change in the course of four passages (Fig. 1). A decrease in the activity before two passages is observed only when a phosphate buffer is used.

The transformation of I (2 g/liter) and II (0.7 g/liter) by means of the T. hyalospora culture was carried out to accumulate the reaction products. The results are presented in Table 1.

The cultivation of the T. orchidis mycelium was carried out in media P and A. The best results were obtained when the latter medium was used, and it was therefore used in the subsequent research.

To optimize the growth of the mycelium the transformation was carried out with a biomass grown in the course of 24 and 48 h and with two inoculates (24 h each). The minimal activity was observed for the 24-h culture.

It was established that the T. orchidis mycelium also retains 11α-hydroxylating activity in the course of four passages in the transformation of I and II (0.7 g/liter) in glucose solution (Fig. 2).

The transformation of I (1.5 g/liter) and II (0.7 g/liter) by means of the T. orchidis culture was carried out to accumulate the reaction products. The results obtained (Table 1) make it possible to conclude that 11α-hydroxy compound VI is formed as the principal product in the transformation of II, while 7α-hydroxy compound V predominates in the transformation of I. Compound V was obtained in the hydroxylation of I by means of the Absidia orchidis culture [7].

Thus, for the first time we have carried out the transformation of I and II by means of T. hyalospora and T. orchidis cultures. In contrast to the results of 11-hydroxylation of pregnanes, high selectivity and stereoselectivity are not observed in the case of androstanes. Primarily the 11α-hydroxy compound, together with a significant amount of the 7α-hydroxy compound, is formed in the transformation of II by both cultures, while the transformation of I by means of T. hyalospora gives the 11α- and 7α-hydroxy compounds in approximately equal amounts, whereas the transformation of I by means of T. orchidis gives the 7α-hydroxy derivative as the principal product. In addition, we ascertained the possibility of the repeated use of T. hyalospora and T. orchidis mycelia in glucose solution without a substantial change in their 11α-hydroxylating activities.

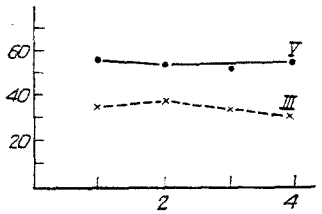


Fig. 1

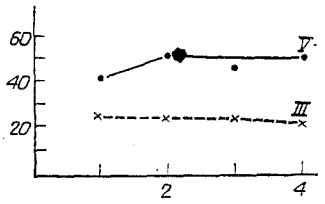


Fig. 2

Fig. 1. Capacity of the Tieghemella hyalospora mycelium for repeated carrying out of 11α-hydroxylation of I and II in glucose solution. Here and in Fig. 2 the number of passages is plotted along the axis of the abscissa, while the amount of converted steroid (in percent) is plotted along the axis of the ordinate.

Fig. 2. Capacity of the Tieghemella orchidis mycelium for repeated carrying out of 11α-hydroxylation of I and II in glucose solution.

EXPERIMENTAL

The chromatographic analysis of I-VIII and monitoring of the course of the reactions were carried out on Silufol UV-254 plates (Czechoslovakian SSR) in a CH_2Cl_2–acetone–cyclohexane system (6:3:1) with twofold chromatographing, scanning in UV light, and development with a 1% solution of vanillin in 10% $HClO_4$. Quantitative determination was carried out with an SF-10 spectrophotometer at 240-242 nm. The PMR spectra of the compounds were recorded with a Varian XL-200 spectrometer (West Germany) with tetramethylsilane (TMS) as the internal standard.

Growth of the Cultures and Carrying Out the Transformations. The Tieghemella hyalospora and Tieghemella orchidis cultures were maintained in test tubes with must agar. The mycelia were grown in the following media. Medium A: 2% glucose, 0.5% peptone, 0.3% autolyzate, 0.5% potassium dihydrophosphate, pH 5.6. Medium P: 3% glucose, 1% corn extract, 0.3% peptone, 0.3% yeast autolyzate, 0.5% potassium dihydrophosphate, pH 6.9-7.2. Medium 60: 5% glucose, 9.6% corn extract, pH 5.2.

A 4-ml sample of an aqueous suspension of the spores was introduced into a 750-ml flask containing 100 ml of the medium. The 24-h mycelium (the other growth time is indicated in the text) was removed from the medium by filtration, washed with 100 ml of water, and transferred to 100 ml of 0.5% glucose solution (use of a phosphate buffer, pH 6.2). The growth of the mycelium and the transformation step were carried out with a rocker (200 rpm) at 28°C. Compounds I and II were introduced into a 10% solution of $CaCl_2$ in MeOH in 100 mg amounts per milliliter. The use of other charges is indicated in the text.

In the case of repeated use of the mycelium after each 24 h of the transformation the biomass was removed from the medium by filtration, washed with water, and transferred to a fresh medium with a new portion of the steroid. The transformation products in the filtrate were determined.

Consolidated experiments were set up to isolate the transformation products: with T. orchidis 20 flasks, each with 70 ml of II (1.4 g of II, example 1), and 14 flasks with 150 mg of I in each (2.1 g of I, example 1); with T. hyalospora 20 flasks, each with 70 mg of II (1.4 mg of II, example 2), and three flasks with 200 mg of I in each (0.6 g, example 2). After 24 h of transformation, the culture liquid was separated from the mycelium and extracted with CH_2Cl_2. The extract was washed with water until the wash water was neutral, dried, and evaporated.

11α-Hydroxyandrost-4-ene-3,17-dione (III), 11β-Hydroxyandrost-4-ene-3,17-dione (IV), and 7α-Hydroxyandrost-4-ene-3,17-dione (V)

Example 1. Transformation of I by means of T. orchidis. The oily residue (2.1 g) obtained after evaporation of the solvent was dissolved in a mixture of $CHCl_3$ and benzene (1:1), and the mixture was applied to a column packed with 105 g of silica gel. Elution with the indicated mixture gave 0.38 g (17.1%) of IV with mp 189-191°C (mp 200°C [6]).

Elution with $CHCl_3$ then gave 0.48 g (21.5%) of III with mp 223-225°C. PMR spectrum ($CDCl_3$), δ, ppm: 0.99 s (3H, 19-CH_3), 1.37 s (3H, 18-CH_3), 4.20 m (11-H), 6.14 s (4-H). Elution with $CHCl_3$ also gave 1.1 g (50%) of V with mp 241-243°C. PMR spectrum ($CDCl_3$), δ, ppm: 0.98 s (3H, 19-CH_3), 1.24 s (3H, 18-CH_3), 4.12 s (7-H), 5.85 s (4-H). According to the literature data, III had mp 226-227°C, and V had mp 244-245°C.

Example 2. Transformation of I by means of T. hyalospora. The oily residue (0.6 g) obtained after evaporation of the solvent was dissolved in a mixture of $CHCl_3$ and benzene (1:1), and the solution was applied to a column packed with 30 g of silica gel. Elution with the indicated mixture gave 0.12 g (19%) of IV with mp 198-200°C. Elution with $CHCl_3$ then gave 0.24 g (38.1%) of III, with mp 224-226°C, and 0.21 g (33.3%) of V with mp 243-244.5°C.

11α-Hydroxyandrosta-1,4-diene-3,17-dione (VI), 11β-Hydroxyandrosta-1,4-diene-3,17-dione (VII), and 7α-Hydroxyandrosta-1,4-diene-3,17-dione (VIII)

Example 1. Transformation of II by means of T. orchidis. The oily residue (1.4 g) obtained after evaporation of the solvent was dissolved in a mixture of $CHCl_3$ and benzene (1:1), and the solution was applied to a column packed with 70 g of silica gel. Elution with the indicated mixture gave 0.06 g (4.3%) of II, with mp 138-140°C (mp 139.5-140.5°C [8]), and 0.21 g (14.2%) of VII with mp 175-176°C (mp 176-179°C [5]). Elution with $CHCl_3$ then gave 0.63 g (42.6%) of VI with mp 202-204°C (mp 207-208°C [4]). PMR spectrum ($CDCl_3$), δ, ppm: 0.98 s (3H, 19-CH_3), 1.30 s (3H, 18-CH_3), 3.59 s (11-H), 6.11 s (4-H), 6.18 dd (2-H, J_1 = 10.16 Hz, J_2 = 1.91 Hz), 7.05 d (1-H, J = 10.19 Hz). Elution with $CHCl_3$ also gave 0.42 g (28.4%) of VIII with mp 260-265°C (dec.) (mp 238-291°C (dec.) [9]). PMR spectrum ($CDCl_3$), δ, ppm: 0.94 s (3H, 19-CH_3), 1.26 s (3H, 18-CH_3), 4.17 broad s (7-H), 6.17 s (4-H), 6.24 dd (2-H, J_1 = 10.12 Hz, J_2 = 1.91 Hz), 7.06 d (1-H, J = 10.19 Hz).

Example 2. Transformation of II by means of T. hyalospora. The oily residue (1.4 g) obtained after evaporation of the solvent was dissolved in a mixture of $CHCl_3$ and benzene (1:1), and the solution was chromatographed on 70 g of silica gel. Elution with the indicated mixture gave 0.04 g (2.7%) VII with mp 174-175.5°C. Elution with $CHCl_3$ gave 0.81 g (54.7%) of VI, with mp 205-206°C, and 0.42 g (28.4%) of VIII with mp 259.263°C (dec.).

LITERATURE CITED

1. K. N. Gabinskaya, A. A. Shpingis, and V. M. Ryzhkova, Mikrobiologiya, 40, No. 3, 448-454 (1971).
2. K. N. Gabinskaya, V. I. Bayunova, T. S. Kolyvanova, et al., Khim.-farm. Zh., No. 1, 76-79 (1980).
3. L. K. Garcia-Rodriguez, K. N. Gabinskaya, L. A. Krasnova, et al., ibid., No. 4, 66-69 (1977).
4. West German Patent No. 2940285; Chem. Abstr., 95, 95484j (1981).
5. W. Charney, A. Nobile, C. Federbush, et al., Tetrahedron, 18, 591-596 (1962).
6. M. E. Herr and F. W. Heyl, J. Am. Chem. Soc., 75, 5927-5930 (1953).
7. K. Kieslich, Microb. Enzym. Bioconvers. (London), 5, 369-465 (1980).
8. M. Kocor and M. Maczka, Bull. Acad. Pol. Sci., Ser. Sci. Chim., 14, 347-351 (1966).
9. R. G. Park, Steroids, 38, 383-395 (1981).