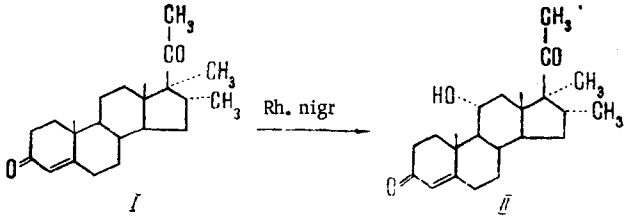
MICROBIOLOGICAL HYDROXYLATION of 16α,17α-DIMETHYLPROGESTERONE

L. S. Morozova, K. N. Gabinskaya, and G. S. Grinenko

UDC 547.92+547.55

In synthesizing corticosteroids containing methyl groups on carbon atoms 16 and 17, we have studied a microbiological method of hydroxylating 16α,17α-dimethylprogesterone (I) [1]. For this purpose we investigated the strain of Rhizopus nigricans VNIKhFI-7 used previously in the 11α-hydroxylation of progesterone [2, 3] and of 16α-methylprogesterone [4]. The optimum conditions for the cultivation of this fungus and the transformation of steroids have been selected [5].



I II

On performing the transformation of 16α,17α-dimethylprogesterone under the optimum conditions found for progesterone and for 16α-methylprogesterone, it was established that the rate of 11α-hydroxylation depends on the presence of methyl groups in ring D. It decreases in the following sequence: progesterone → 16α-methylprogesterone → 16α,17α-dimethylprogesterone. This is possibly connected with the increase in the hydrophobic properties of the steroids.

By the hydroxylation of (I) and chromatography on Al_2O_3 we isolated 11α-hydroxy-16α,17α-dimethylprogesterone (II) with a yield of 61%. Its structure was established spectroscopically. In its IR spectrum, a band characteristic for the hydroxy group and absent from the spectrum of the initial compound (I) appeared at 3490 cm^{-1}. The NMR spectrum of compound (II) showed singlets of protons of angular methyl groups at 0.72 and 1.01 ppm; a doublet of protons of the C_{16} methyl group at 0.84 ppm and a singlet of the protons of the C_{17} methyl group at 1.26 ppm; a singlet of the $COCH_3$ group at C_{21}, 2.06 ppm; and a singlet of the proton at C_4 – 5.67 ppm. The chemical shift of the proton at a hydroxy group – 3.99 ppm – shows that hydroxylation took place at C_{11}, since according to literature [6], shifts in the 4.3-4.4-ppm regions are observed for protons on the 6α, 6β, 11α, and 15α carbon atoms, a shift of 3.58 ppm for the 7α position, and one of 4.05 ppm for the 11β proton. The signal of the proton at C_{11} appears in the form of a broad multiplet (half-width 12 Hz) due to two large diaxial interactions (C_9 and C_{12}, J 10 Hz) and a small axial-equatorial interaction (C_{12}, J 5 Hz) [6]. The nature of the splitting of this signal confirms the axial positions of the proton at C_{11} and, correspondingly, the equatorial position of the hydroxy group (11α).

EXPERIMENTAL

The NMR spectra were taken on a JNM-4H-100/100 MHz instrument (in $CDCl_3$ with tetramethylsilane as standard); the UV spectra were taken in 96% ethanol; the IR spectra were recorded in the form of mulls in paraffin oil; and the specific rotations (c 1) were determined in chloroform.

The microbiological process consisted in the growing of the culture and the stage of transforming the steroid. The culture was maintained on wort agar. The mycelium was grown on a synthetic medium

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemicals. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 46-48, January-February, 1973. Original article submitted May 15, 1972.

with the following composition (%): glucose – 1, ammonium nitrate – 0.3, monopotassium phosphate – 0.3, magnesium sulfate – 0.1. The pH of the medium was 5.0.

Flasks with a capacity of 750 ml each containing 100 ml of the medium were inoculated with 2 ml of the aqueous suspension of fungal spores. The growth of the mycelium and the transformation process were performed on a shaking machine at 28°C. The transformation was performed with 24-hour mycelium washed free from medium in a 0.5% solution of glucose, pH 5.0. Each of 17 flasks was charged with 90 mg of previously ground 16α,17α-dimethylprogesterone (total amount 1.53 g; particle size 6 μ) and a 0.005% solution of Tween 80. The transformation process lasted 48 h.

The course of the transformation was monitored by paper chromatography, for which purpose the product from 10 ml of the culture liquid was twice extracted with 20 ml of chloroform. The extract was concentrated and the steroids were separated in the heptane–ethyl acetate–methanol–water (2.5 : 2.5 : 4 : 1) system. Quantitative determinations of the steroids were performed spectrophotometrically on an SF-4 instrument. According to quantitative chromatography, the chloroform extract contained 14-16% of the initial (I), 61-68% of 11α-hydroxy-16α,17α-dimethylprogesterone, and 7-8% of dihydroxy compound.

The steroid was extracted from the culture liquid with chloroform three times, and after the chloroform extract had been evaporated to dryness a residue was obtained (1.73 g of resinous solid) which was dissolved in 15 ml of benzene and transferred to a column containing 80 g of alumina (activity grade II). Elution was performed with mixtures of benzene and ether with gradually increasing concentrations of ether. The fractions were examined by chromatography on Silufol plates in the benzene–methanol (20 : 1.5) system. The corresponding fractions of the eluate were distilled in vacuum, giving successively 0.3 g (15%) of the initial (I), 0.98 g (61%) of the 11α-hydroxy compound (II), and 0.3 g of a mixture of (II) and a dihydroxy compound the structure of which is being determined. The 11α-hydroxy-16α,17α-dimethylprogesterone, $C_{23}H_{34}O_3$, had mp 185-187°C (methylene chloride, ether), $[\alpha]_D^{20}$ +73°, λ_{max} 242 nm (log ε 4.187). IR spectrum, cm^{-1}: 3490 (OH), 1696 and 1665 (CO and C=C–CO), and 1615 (C=C). NMR spectrum, ppm: 0.72 (18–CH_3), 1.01 (19–CH_3), 0.84 J 7.5 Hz (16–CH_3), 1.26 (17–CH_3), 2.05 ($COCH_3$), 5.67 (4–H) and 3.99 J 12 Hz (11–H).

SUMMARY

1. The 11α-hydroxylation of 16α,17α-dimethylprogesterone has been effected by the strain of Rhizopus nigricans VNIKhFI-7 with a yield of hydroxy derivative of 61%.

2. In the transformation an unidentified dihydroxy compound is formed together with the 11α-hydroxy-16α,17α-dimethylprogesterone.

LITERATURE CITED

1. V. I. Maksimov, F. A. Lur'i, and L. S. Morozova, Zh. Obshch. Khim., 33, 1666 (1963).
2. N. N. Suvorov, Med. Prom. SSSR, No. 3, 22 (1956).
3. A. G. Timofeeva, O. S. Madaeva, E. G. Gusakova, N. F. Kovylkina, N. I. Men'shova, and V. M. Novikova, Izv. Akad. Nauk SSSR, Ser. Biol., No. 6, 712 (1958).
4. W. P. Schneider, F. H. Lincoln, G. B. Spero, H. C. Muffay, and I. L. Thomson, J. Amer. Chem. Soc., 81, 3167 (1959).
5. V. M. Ryzhkova, K. N. Gabinskaya, V. F. Shner, and N. N. Suvorov, Mikrobiol. Prom. SSSR, No. 2, 23 (1970).
6. N. Bhacca and D. Williams, Applications of NMR Spectroscopy in Organic Chemistry, Holden-Day, San Francisco (1964).