PRODUCTION OF $3\beta,17\alpha,21$-TRIHYDROXY-16α-METHYL-Δ^5-PREGNEN-20-ONE WITH THE AID OF Bacillus megaterium

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The deacylating capacity of Bacillus megaterium with respect to a series of steroid compounds containing a Δ^4-3-oxo grouping or saturated A and B rings has been reported previously [1-3]. For the present work with B. megaterium as the substrate we used a steroid containing a Δ^5-3-hydroxy grouping. The examples of the microbial deacetylation of such substrates mentioned in the literature relate to cultures simultaneously converting the Δ^5-3-hydroxy group into a Δ^4-3-oxo group [4-8]. The transformation performed with B. megaterium of 21-acetoxy-$3\beta,17\alpha$-dihydroxy-16α-methyl-Δ^5-pregnen-20-one gave the possibility of obtaining free $3\beta,17\alpha,21$-trihydroxy-16α-methyl-Δ^5-pregnen-20-one with a yield of 74.4% of theoretical.

A culture of Bacillus megaterium VNIKhFI-1 was grown for 48 h on sloped maize-glucose agar, after which the spores were washed with water (5 ml per test tube). A suspension (0.5 ml) was mixed with 200 ml of a medium having the following composition: 30 g of glucose, 20 g of maize extract, 6 g of peptone, and 5 g of monopotassium phosphate, pH 6.5. At the same time as the seed material, 50 mg of 21-acetoxy-$3\beta,17\alpha$-dihydroxy-16α-methyl-Δ^5-pregnen-20-one in 2 ml of ethanol was added. The transformation was performed in 750-ml flasks on a shaking machine at 28°C.

The course of the transformation was monitored by the TLC method. After 44 h, the transformation was complete. The culture liquid (four liters) was extracted three times with methylene chloride. The extract was evaporated to dryness. The biomass was separated from the steroid by boiling the mixture twice with isopropanol, followed by treatment with activated carbon. The isopropanol was distilled off in vacuum to dryness. The oily residue was triturated with ether. This yielded 0.85 g of a crystalline product which was recrystallized from acetone, giving 0.68 g of $3\beta,17\alpha,21$-trihydroxy-16α-methyl-Δ^5-pregnen-20-one [9] with mp 208-210°C, $[\alpha]_D^{20}$ $-62°$ (dimethylformamide), R_f 0.13-0.15 in the chloroform–acetone–cyclohexane (60 : 10 : 10) system on Silufol, the spots being revealed with 10% phosphomolybdic acid; λ_{max}^{Nujol} 3380 cm^{-1} (OH), 1700 cm^{-1} (20-CO). NMR spectrum (100 MHz, C_5D_5N, internal standard HMDS, δ, ppm): $H_{C_{18}}$ 0.80; $H_{C_{19}}$ 0.92; $H_{C_{16}}$ 0.98; $H_{C_{21}}$ 4.06; H_{C_6} 5.28.

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