METHODS OF SYNTHESIZING DRUGS AND THE TECHNOLOGY OF THEIR PRODUCTION

FLUOROSTEROIDS.

VI. MICROBIOLOGICAL 11β-HYDROXYLATION OF 6α-FLUORO-21-HYDROXY-16α,17α-ISOPROPYLIDENEDIOXYPREGN-4-ENE-3,20-DIONE

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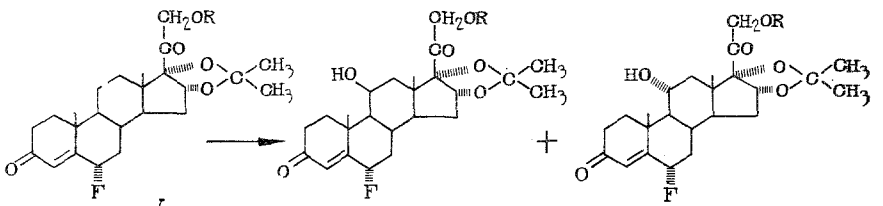
The introduction of a hydroxy group into position 11 of the steroid molecule by microbiological transformation is a common stage in the synthesis of corticosteroids. In the present paper we shall discuss a variant of the 11β-hydroxylation of a key product of the synthesis of 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (fluocinolone acetonide) — 6α-fluoro-21-hydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (Ib).



a) R=Ac
b) R=H

The following microorganisms are recommended in the literature for the introduction of an 11β-hydroxy group into compound (Ib): *Curvularia lunata*, which performs the process with a yield of 70% [1], *Cunninghamella blakesleeana*, with a yield of 50-75% [2, 3], and *Cunninghamella beinieri*, for which no yields or characteristics of the 11β-hydroxy compound are given [4].

We have studied three cultures of microorganisms: *Curvularia lunata*, *Cumminghamella blakesleeana*, and *Tieghemella orchidis*. A distinguishing feature of the hydroxylation process investigated is the very low solubility of the initial steroid (Ia). To increase the solubility and to raise the concentration of the initial compound in the culture liquid the compound with a free 21-hydroxy group (Ib) was subjected to transformation in the presence of a water-miscible solvent.

The reaction products were isolated and their structures were determined by converting the fermentation products into 21-acetoxy derivatives after 21-acetylation of these products. When *C. lunata* was used under the usual conditions for its transformation [5], only a weak capacity for the accumulation of a hydroxylated product was detected. More complete conversion was achieved by using an enriched medium in the transformation stage. However, in this case the newly formed product consisted of a nonsteroid compound with a molecular weight of 236 (according to mass spectroscopy).

When the process was performed with a culture of *C. blakesleeana*, the 11β-hydroxy compound (IIa) was isolated and characterized, its yield being 60%. In addition to this compound, the culture liquid contained its 11α epimer (IIIa). The ratio of 11α and 11β isomers in the culture liquid was 1:7. The orientation of the hydroxyls was confirmed by PMR spectroscopy.

When a culture of *T. orchidis*, which is used in the Soviet Union for obtaining hydrocortisone [6, 7], was studied, we obtained the best indices for the 11β-hydroxylation of fluorocortexolone acetonide (Ib): the ratio of α and β isomers in the culture liquid was

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1:11, and the reaction product isolated contained not more than 10% of the α isomer. The yield of desired product was 70% of theoretical. The method has been protected by an Inventor's Certificate [8]. Thus, as a result of the investigation performed, a culture of the microorganism *T. orchidis* has been selected for the 11β-hydroxylation of fluorocortexolone acetonide (Ib), this organism not having previously been used for fluorinated 16α,17α-acetonides.

EXPERIMENTAL

Cultures of *T. orchidis*, *C. blakesleeana*, and *C. lunata* were grown in 0.75-liter flasks on a circular shaking machine (220 rpm) at 26-28°C on a medium with the following composition: maize extract, in terms of dry matter, 1 g; peptone, 3 g; yeast autolysate, 3 g; monopotassium phosphate, 5 g; glucose, 30 g; mains water, 1000 ml; pH 6.8-7.2.

The seed material in an amount of 5% was transferred to a medium of the same composition, and subsequent growth was performed in a laboratory fermenter with a capacity of 7 liters with an operating stirrer (400 rpm) and a feed of air (0.5 liter/liter·min). In the case of the *C. lunata* culture, the initial steroid was added to the fermenter simultaneously with the seed material, while on working with the fungi *T. orchidis* and *C. blakesleeana* the steroid was added after the culture had grown in the fermenter for 24 h. The steroid was added in the form of a 2.5% solution in DMFA to give a concentration of 0.5 g/liter. The transformation was effected the same conditions as the growth of the cultures. The process was monitored by chromatographing the steroids isolated from a sample with methylene chloride on Silufol UV-254 plates in the methylene chloride–methanol–water (19:1:0.1) system. The process was terminated when the concentration of the initial steroid was no greater than 5%, the time of fermentation having then amounted to 18-24 h. The culture liquid was separated from the mycelium by filtration and was extracted with two volumes of ethyl acetate. The extract was evaporated *in vacuo*. The specific rotations were determined in chloroform, the IR spectrum of compounds in the form of mulls in paraffin oil were taken on Perkin-Elmer (USA) and IR-10 (GDR) spectrometers, and UV spectra on JNM-4H-100 and X4-100-A (JEOS) (Japan) spectrometers. Chemical shifts are given in the δ scale with tetramethylsilane as internal standard. Mass spectra were obtained on a Varian MAT-112 instrument (USA) (direct introduction of the sample into the source). Ionizing voltage 70 V (Varian MAT-L2, USA).

The preparation of fluorocortexolone acetonide (Ia) has been described previously [9].

Saponification of the 21-Acetoxy Group of Fluorocortexolone Acetonide. In a current of inert gas, a solution of 7 g of caustic potash in 0.13 liter of methanol was added to a suspension of 78 g of (Ia) in 0.22 liter of methanol and 0.33 liter of methylene chloride. The suspension dissolved 5-10 min after the addition of the alkali (monitoring by TLC; Silufol, chloroform–methanol (100:1)). After the end of the reaction the solution was neutralized with acetic acid to pH 7.0, the methylene chloride was distilled off *in vacuo*, and the residue was treated with 1 liter of water. The resulting precipitate was filtered off. This gave 60 g of (Ib), mp 267.5-268.5°C (from methylene chloride and hexane); $[\alpha]_D$ +120°C. UV spectrum, λ_{max} (log ε): 236 nm (4.16).

Transformation of 6α-Fluoro-21-hydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (Ib) by a Culture of *C. lunata*. To the culture liquid was added 2.5 g of (Ib). After the end of the extraction process and evaporation of the solvent, the residue was triturated with ether and the resulting precipitate was filtered off, giving 0.8 g of a product with a molecular weight of 236 (mass spectrum).

Transformation of 6α-Fluoro-21-hydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (Ib) by a Culture of *C. blakesleeana*. To the fermenter was added 5 g of (Ib) and after the end of the fermentation process, extraction, and evaporation of the extractant, the residue was dissolved in 120 ml of glacial acetic acid and the solution was treated with 4.8 ml of acetic anhydride and 2.5 g of barium acetate. After 24 h, the reaction mixture was poured into 2 liters of water. The precipitate that deposited was filtered off, washed with water, and dried. This gave 4.2 g of a substance which, according to TLC, contained two compounds, with R_f 0.21 and 0.55. The mixture was dissolved in 30 ml of ethylene chloride and the solution was passed through a layer of alumina in a column 2.5 cm high and

4 cm in diameter. The filtrate was evaporated *in vacuo*, giving 3.1 g of the 11 β-hydroxy compound (IIa), (R_f 0.55), mp 266-267°C (from a mixture of methylene chloride and methanol); $[\alpha]_D$ +138°C. UV spectrum, λ_{max} (log ε): 236-238 nm (4.7). PMR spectrum, $CDCl_3$, ppm: 0.86 (18-CH_3); 1.17 (19-CH_3); 1.1 and 1.36 [$C(CH_3)_2$]; 2.25 (-$OCOCH_3$); 4.4 (11-H); 4.86 (AB system -CH_2-21); 4.94 (H-16); 4.96 and 5.45 (H-6, J 50 Hz); 5.95 (H-4). According to the literature [10]: mp 261-263°C; $[\alpha]_D$ +135°; λ_{max} 236-238 nm, log ε 4.18. The alumina was washed with a mixture of ethanol and methylene chloride (2:1). The solvent was evaporated off *in vacuo* and the residue was crystallized from a mixture of acetone and hexane, giving 0.15 g of the 11α-hydroxy compound (IIIa). (R_f 0.21) mp 290-290.5°C; $[\alpha]_D$ +92°. PMR spectra ($CDCl_3$, ppm): 0.67 (18-CH_3); 1.20 and 1.45 [$C(CH_3)_2$]: 1.29 (19-CH_3); 2.13 (-$OOCH_3$); 3.89 (H-11); 4.85 (-CH_2-21); 4.8 and 5.3 (H-6, J 50 Hz); 6.02 (H-4). According to the literature [11]: mp 293-295°C; $[\alpha]_D$ +88°.

Transformation of 6-Fluoro-21-hydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (Ib) by a Culture of *T. orchidis*. To the culture liquid was added 2.5 g of (Ib). After the performance of the extraction process and the evaporation of the solvent, the residue was dissolved in 15 ml of glacial acetic acid and 0.87 g of barium acetate was added. The reaction mixture was left at room temperature for 20 h and was then poured into 150 ml of water. The precipitate was filtered off, giving 2.23 g (70%) of (IIa), mp 267-268°C (from a mixture of methylene chloride and methanol), $[\alpha]_D$ +132°. UV spectrum, λ_{max} (log ε): 236-238 nm (4.17). According to the literature [10]: mp 261-263°C; $[\alpha]_D$ +135°; λ_{max} 236-238 nm, log ε 4.18.

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