TRANSFORMED STEROIDS

COMMUNICATION 81. PREPARATION OF $\Delta^{1,4}$-3-KETOSTEROIDS EMPLOYING IMMOBILIZED BACTERIAL CELLS

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A large number of papers, devoted to the preparation and application of immobilized enzymes, has appeared in recent years [1-3]. However, the isolation of some of the enzymes is associated with definite difficulties; a substantial number of the enzymes requires the participation of cofactors in the reaction. In view of this it is expedient at times to use immobilized bacterial cells to run enzymatic processes, in particular, for the microbiological transformation of steroids. The mycelium Curvularia lunata [4], embedded in polyacrylamide gel, was used for the 11β-hydroxylation of cortexolone; the immobilized hyphae retained their viability and grew in glucose solution. The immobilized cells of Mycobacterium globiforme 193 retained their viability for a long time and accomplished the processes of either 20β-reduction or 1,2-dehydrogenation, depending on the experimental conditions [5]. In the present paper we studied the ability of various immobilized cultures to effect the transformation of Δ^5-3β-hydroxy-, Δ^5-3β-acetoxy-, and Δ^4-3-ketosteroids with the formation of $\Delta^{1,4}$-3-keto compounds, and also the effect of the experimental conditions on the yield of the 1,2-dehydro compounds.

EXPERIMENTAL METHOD

The cultures of genera Arthrobacter, Mycobacterium, Nocardia were obtained from the Institute of Microbiology of the Academy of Sciences of the USSR and the Institute of the Biochemistry and Physiology of Microorganisms of the Academy of Sciences of the USSR. The cultures of Mycobacterium were stored on corn–glucose agar (corn extract 1%, agar–agar 2%, tap water, pH after sterilization 6.8–7.2). The cultures of Arthrobacter and Nocardia were stored on meat peptone agar. For experiment the needed culture was grown for 7 days on an agar medium at 28° C, after which the cells were rinsed from the agar with sterile water and the obtained suspension was sown with 150 ml of corn–glucose medium (without the agar–agar), poured into a 750-ml flask. All of the processes of growing and transformation were run at 28° on a shaker (200 turns/min). After growth for 48 h the cells were separated and rinsed from the medium by centrifuging at 3000 g. To the obtained deposit (0.4 g, here and subsequently based on the weight of the dry biomass) were added 18 ml of $^1/_{15}$ M phosphate buffer (pH 7.0), 3.3 ml of a solution of monomers, composed of a 45% solution of acrylamide, recrystallized from $CHCl_3$, and a 5% solution of N,N-methylenebis(acrylamide), and also 1.4 ml of a 5% $(NH_4)_2S_2O_8$ solution and 0.3 ml of a 5% N,N,N,N-tetramethylethylenediamine solution. Nitrogen was blown through the obtained mixture for 10 min, and the obtained gel was pressed through a sieve (15 mesh), after which the granules were elutriated in order to separate the smaller granules and then washed with distilled water until all of the unpolymerized cells had been removed. The granules were suspended in $^1/_{15}$ M phosphate buffer, pH 7.0 on the basis of 15 g of gel/100 ml of buffer. A solution of 50 mg of the steroid in 2 ml of ethanol was added to each 100 ml of this suspension. The absence of free cells in the buffer was checked for during the transformation process. The reaction course was checked by TLC, using Silufol and the system: 4:1 ether–hexane. When the starting steroid was absent in the test samples (based on the TLC data) the product was extracted by extracting the suspension with a triple volume of $CHCl_3$. The solvent was removed to give a crystalline product, in which the amount of steroids was determined quantitatively by GLC on a modernized LKhM-7A chromatograph using a 1.5 m × 3 mm glass column packed with 3% OV-17 deposited on Chromosorb W (80-100 mesh), a column temperature of 240°, a vaporizer temperature of 330°, and p_{N_2} = 1.2 atm.

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TABLE 1. 1,2-Dehydrogenation by Immobilized Cells of Various Cultures of Arthrobacter, Mycobacterium, and Nocardia

Culture	Duration of transformation, h	Total amount of isolated steroids, %	Yield, %* of Δ^4-3-ketone	Yield, %* of $\Delta^{1,4}$-3-ketone
Transformed steroid = 16 α, 17 α-isopropylidenedioxypregnenolone				
A. simplex 58	70	90	12	86
M. globiforme 193	40	92	5	78
M. hyalinum B-353	40	69	30	30
M. perrygosum B-850	40	64	36	58
N. erythropolis 271	24	90	34	63
Transformed steroid = progesterone				
M. globiforme 193	40	100	25	71
M.lacticolum B-854	40	100	64	4
M. mucosum 86	40	60	66	22
M. smegmatis B-817	40	100	59	41

*The relative amount of the Δ^4- and $\Delta^{1,4}$-3-ketones in the product isolated after reaction is based on the GLC data.

TABLE 2. 1,2-Dehydrogenation of 16α,17α-Isopropylidenedioxypregnenolone by Immobilized and Not Immobilized Cells (Control)

Culture	Transformation method	Duration of transformation, h	Yield, % of Δ^4-3-ketone	Yield, % of $\Delta^{1,4}$-3-ketone
Mycobacterium globiforme 193	Immobilized cells	40	8,5	70,1
	Control	40	9,6	60,0
Arthrobacter simplex 58	Immobilized cells	72	7,2	76,4
	Control	72	6,0	51,0

TABLE 3. Transformation by Immobilized Cells of Mycobacterium globiforme 193

Steroid	Time of transformation, h	Total amount of isolated steroids, %	Yield, % $\Delta^{1,4}$-3-ketone	Yield, % Δ^4-3-ketone
16α-Methylcortexolone	60	88	68	—
17α-Methylandrostenediol	24	90	85	—
Pregnenolone	20	78	80	11
Pregnenolone 3-acetate	65	87	70	28
Progesterone	40	96	75	23
16α, 17α-Isopropylidenedioxypregnenolone	40	92	78	5

DISCUSSION OF RESULTS

The cells of the various species of genus Arthrobacter, Mycobacterium, and Nocardia, which affected the transformation of Δ^5-3β-acetoxy-, Δ^5-3β-hydroxy-, and Δ^4-3-ketosteroids to $\Delta^{1,4}$-3-keto compounds, were embedded in 7% polyacrylamide gel with a relative content of 7% crosslinking agent. The cells that are capable of 1,2-dehydrogenation do not lose this ability after immobilization (Table 1). The total amount of steroids isolated after transformation reaches 90-100%. As a result, the intensity of the complete cleavage of the steroid molecule decreases in the 1,2-dehydrogenation process. In Table 2 are given the comparative data that were obtained in the transformation of 16α,17α-isopropylidenedioxypregn-5-en-3β-ol-20-one (16α, 17α-isopropylidenedioxypregnenolone) by the immobilized and not immobilized cells (control). We took the same amount of biomass for the control as for the immobilization process (0.5 g/18 ml of buffer). The yield of the $\Delta^{1,4}$-3-ketone when using the immobilized cells was substantially higher (see Table 2).

The amount of gel, suspended in a definite volume of the buffer, the amount of biomass, embedded in the gel, and the duration of transformation all exert an important effect on the yield of the $\Delta^{1,4}$-3-ketones in the transformation by the immobilized cells. For the dehydrogenation of the pregnenolone or progesterone we

used gel suspensions with a concentration of 100, 165, 200, 230, and 250 g/liter. For the immobilization we took 0.4 g of the biomass per 18 ml of buffer solution. For both steroids the optimum dehydrogenation course was observed at 165-200 g/liter. At a concentration of 100 g/liter the dehydrogenating agent is too dilute and the course of the transformation is retarded. At a gel concentration of 230 g/liter and higher the amount of the $\Delta^{1,4}$-3-ketone decreases due to the formation of a secondary product (apparently the 20β-hydroxy derivative of the 1,2-dehydroprogesterone). This compound is also formed in those cases where more than 0.4 g of the biomass was taken. Thus, in the experiments with 0.7 g of the biomass (per 18 ml of buffer), with a concentration of 230 g/liter for the gel suspension, the yield of the postulated 20β-hydroxy derivative of the 1,2-dehydroprogesterone was 100% after 72 h. The selective 20β-reduction of Δ^4- and $\Delta^{1,4}$-3-ketosteroids when the amount of biomass in the granules is increased was also mentioned in [5]. The use of the optimum conditions (biomass 0.4 g, gel concentration 165 g/liter) enabled us to employ the immobilized cells of Mycobacterium globiforme to effect the hydrolysis of the 3-acetoxy group, as well as the oxidation of Δ^5-3β-hydroxysteroids to the Δ^4-3-keto compounds and their 1,2-dehydrogenation (Table 3).

CONCLUSIONS

1. It was shown that it is theoretically possible to transform Δ^5-3β-hydroxy-, Δ^5-3β-acetoxy-, and Δ^4-3-ketosteroids to $\Delta^{1,4}$-3-ketosteroids employing the immobilized cells of various species of bacteria.

2. The immobilization of the bacterial cells in polyacrylamide gel reduces the rate of the complete cleavage of the steroids and in this way increases the yield of the 1,2-dehydro compounds.

3. The $\Delta^{1,4}$-3-ketosteroids were obtained in 68-85% yield by employing the immobilized cells of Mycobacterium globiforme 193.

LITERATURE CITED

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