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Abstract

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Microbiological 20α-Reduction of Ketosteroids by Means of *Bacillus megatherium*

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It had previously been established that the strain VNIHFI-1 isolated by us is capable of carrying out selective deacetylation of 21-acetates of corticosteroids ([1]). A thorough morphological and biochemical study of this microorganism showed that, systematically, it is indeed *Bacillus megatherium*. Since there are indications in the literature of the ability of various strains of the latter to carry out 15 β-hydroxylation of steroids ([2,3]), we decided to study the relation of strain VNIHFI-1 to steroids that do not contain a 21-acetoxy group. This was all the more interesting because even in the case of the latter, when a nutrient medium rich in nitrogen was used, formation of more polar substances was observed alongside deacetylation, according to paper-chromatography data. As the object of the study, 16α,17α-oxidoprogesterone (I) was chosen, giving the maximum yield of the biotransformation product. Upon incubation of (I) with a growing culture of *Bac. megatherium* VNIHFI-1, it is possible to obtain substance (II) of composition $C_{21}H_{30}O_3$, forming a monoacetate (IIa). The ultraviolet spectrum of (II) showed that the Δ^4-3-keto unsaturated grouping in ring A remained unaffected. The data of quantitative infrared spectroscopy (determination of the frequencies and integral intensities of the carbonyl and hydroxyl groups of II and IIa ([4])), as well as the presence in the spectra of II and IIa of frequencies characteristic of the Δ^4-3-keto unsaturated grouping and of the α-oxide ring, clearly showed that in the course of the microbiological transformation of (I) reduction of the 20-keto group occurred (see Table 1), which was also confirmed by reverse oxidation of (II) with chromic acid to the starting oxidoprogesterone (I). It remained to settle the question of the configuration of the

Table 1

No. of substance	Substance	$\nu_{\max}$ $C_{16}=O$, cm^{-1}	Crystals in Vaseline oil: $\nu_{\max}$ $C_{17}-O-C(=O)-CH_3$, cm^{-1}	Crystals in Vaseline oil: $\nu_{\max}$ $C=O$, cm^{-1}	Solution in dioxane: $\nu_{\max}$ OH, cm^{-1}	Solution in dioxane: $A\cdot 10^{-4}$, $l\cdot cm^{-2}$	Solution in dioxane: $\nu_{\max}$ $C=O$, cm^{-1}	Solution in dioxane: acet., mol^{-1}	Solution in CCl_4: $\nu_{\max}$ OH, cm^{-1}	Solution in CCl_4: $A\cdot 10^{-4}$, $l\cdot cm^{-2}$	Solution in CH_2Cl_2: $\nu_{\max}$ OH, cm^{-1}	Solution in CH_2Cl_2: $A\cdot 10^{-4}$, $l\cdot cm^{-2}$
II	$16\alpha,17\alpha$-oxido-Δ^4-pregnenol-20α-one-3	873	1668		3427	1678	3.8		3577	3627	3568	3652
III	$16\alpha,17\alpha$-oxido-Δ^4-pregnenol-20β-one-3	874	1669		3434	1679	3.7		3592	3626	3580	3619
IIa	Acetate of $16\alpha,17\alpha$-oxido-Δ^4-pregnenol-20α-one-3	843	1669	1733		1677	3.8	1739	2.9			
IIIa	Acetate of $16\alpha,17\alpha$-oxido-Δ^4-pregnenol-20β-one-3	851	1672	1735		1680	3.8	1742	3.0			

20-hydroxyl group (II). Substance (II) differed in its constants from $16\alpha,17\alpha$-

oxido-Δ^4-pregnenol-20β-one-3 (III), obtained according to the data of Camerino et al. ([5]) by reduction of oxidoprogesterone (I) with sodium borohydride. The acetates of these substances IIa and IIIa are also different.

Thus, *Bac.* megatherium VNIKhFI-1 carries out the microbiological reduction of 16α,17α-oxidoprogesterone (I) to 16α,17α-oxido-Δ^4-pregnenol-20α-ol-3 (II):

$$\text{(III)} \xleftarrow{\text{NaBH}_4} \text{(I)} \underset{\text{CrO}_3}{\overset{\textit{B. megath.}}{\rightleftharpoons}} \text{(II)}$$

The possibility of microbiological 20α-reduction is of great interest, since, as a rule, in processes of microbiological transformation 20β-hydroxy compounds are formed. 20α-Reduction has apparently been observed only in the case of the yeast *Rhodotorula longissima* ([6]), although it is quite characteristic of enzyme systems of higher animals ([7]).

Experimental part

IR spectra were obtained with a UR-10 instrument; UV spectra (in alcohol) with an SF-4 spectrophotometer.

16α,17α-Oxido-Δ^4-pregnenol-20α-ol-3 (II). A culture of *Bac.* megatherium VNIKhFI-1 is grown on corn-glucose agar for 2 days at 37°. The fermentation process is conducted for 48 h in a fermenter (stirring rate, 260 rpm; air supply, 1 liter per 1 liter of medium per minute; temperature, 37°) in a medium containing 0.2% corn extract, 0.2% ammonium succinate, and 1.5% glucose. The starting 16α,17α-oxidoprogesterone is introduced as a solution of 1 g of (I) in 50 ml of acetone into 8 liters of culture medium. The process is monitored by paper chromatography in the formamide–benzene system. The transformation product is extracted with chloroform. Yield of (II), 0.7 g. M.p. 242.5-245° (from isopropyl alcohol); R_f 0.53; $[\alpha]_D^{20}$ + 114° (C = 1, $CHCl_3$); $\lambda_{\max}$ = 240 mμ ($\log\varepsilon$ = 4.22).

Found, %:	C 76.42; H 9.40
$C_{21}H_{30}O_3$. Calculated, %:	C 76.33; H 9.15

To a solution of 0.10 g of II in 5 ml of acetic acid, 0.05 g of CrO_3 in 1 ml of the same acid is added. After 12 h the excess oxidant is destroyed with Na_2SO_3. As a result of the usual workup, 0.07 g of 16α,17α-oxidoprogesterone (I), m.p. 202-203°, is obtained.

Acetate of 16α,17α-oxido-Δ^4-pregnenol-20α-ol-3 (IIa) is obtained by acetylating 0.59 g of II with acetic anhydride (1.2 ml) in pyridine (6 ml) for 16 h at room temperature. Yield 0.52 g, m.p. 228.5-229.5° (from CH_3OH), $[\alpha]_D^{20}$ + 78° (C = 1, $CHCl_3$); $\lambda_{\max}$ = 240 mμ ($\log\varepsilon$ = 4.17).

Found, %:	C 74.27; H 8.47
$C_{23}H_{32}O_4$. Calculated, %:	C 74.13; H 8.66

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Note: Figure translations are in progress. See original paper for figures.