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Abstract

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MICROBIOLOGICAL DEACETYLATION OF 21-ACETATES OF CORTICOSTEROIDS

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Microbiological transformations of steroids are attracting broad attention from researchers because of their enormous practical importance for the synthesis of cortisone and its analogues. At present, these transformations include oxidation–reduction processes involving carbonyl and hydroxyl groups, hydroxylation of the steroid nucleus and side chain, dehydration, cleavage of the side chain, and scission of steroid rings ([1]). In the course of the brilliant work of Peterson and co-workers it was found that hydroxylation of steroids containing acetoxy groups by means of imperfect fungi is accompanied by simultaneous deacetylation of these compounds ([2]). On the other hand, certain bacteria, for example *Corynebacterium simplex*, are capable of dehydrating 21-acetates of corticosteroids without saponification of the acetyl group ([3]).

In studying the microbiological dehydration of corticosteroids, we found that a culture isolated by us (VNIKhFI-1), which by its morphological and biochemical properties belongs to the group *Bacillus megatherium*, possesses the ability to deacetylate cortisone acetate to free cortisone. Further studies showed that this deacetylation is specific for 21-acetates of steroids containing a dioxyacetone chain (cortisone, Reichstein' s substance "S," 4,5 β-dihydrocortisone, Δ^1-dehydrocortisone). In the case of desoxycorticosterone acetate (a ketol chain instead of a dioxyacetone chain), deacetylation proceeds slowly and only partially. Acetoxy groups in positions 3β, 11α, and 17β are not saponified. Our strain causes no other transformations, such as hydroxylation, reduction, dehydration, etc., at least in the case of corticosteroids. In this respect, the recently published work of Herzog and co-workers ([4]) is of particular interest; they showed that the strain of *Bac. megatherium* isolated by them (Schering 41 ATCC No. 13368) is capable of introducing a 15β-hydroxyl group into Reichstein' s substance "S" and progesterone, and also of reducing the $\Delta^{1,2}$ double bond in dehydrocortisone and dehydrocortisol. We are currently studying the behavior of our strain toward steroids that do not contain acetoxy groups.

Experimental Part

Deacetylation using the VNIKhFI-1 strain was carried out in a medium consisting of yeast extract (0.5%) and glucose (0.5%). The experiments were conducted

in a glass fermenter equipped with a stirrer, an internal heater, and a tube for supplying sterile air. The temperature (28°) and the rate of air supply were regulated automatically. The ratio of air to medium volume was 1 : 1 per 1 min.

Under aseptic conditions, culture liquid containing a wash of strain VNIKhFI-1, grown for 48 h on agar matrices, is charged into the fermenter. Then an acetone solution of the steroid is added through a dropping funnel (45 ml of acetone and 8 l of culture liquid are taken per 1 g of the latter). The duration of the deacetylation process for various steroids varies from 24 to 48 h. The end of the reaction is determined by paper chromatography (formamide–benzene system).

The reaction product is extracted with chloroform. After the latter is distilled off, the residue is crystallized from the appropriate solvent. All substances obtained by deacetylation proved, according to IR spectroscopy data, to be identical with authentic samples of the corresponding steroids.

Cortisone acetate. On deacetylation of 1.00 g for 24-28 h, 0.81 g (90% of theory) of free cortisone is obtained, m.p. 226-227° (from alcohol). $[\alpha]_D^{20} = +215^\circ$ (0.5% solution in alcohol). $\lambda_{\max} = 238$ mμ, $\log \varepsilon = 4.406$.

Δ^1-Dehydrocortisone acetate (prednisone acetate). 1.00 g of the preparation is subjected to the fermentation process for 24-28 h. The yield of free Δ^1-dehydrocortisone (prednisone) is 0.65 g (73% of theory). M.p. 228.5-230° (from isopropyl alcohol), $[\alpha]_D^{20} = +178^\circ$ (1% solution in dioxane), $\lambda_{\max}$238 mμ, $\log \varepsilon = 4.193$.

Acetate of Reichstein' s substance "S" . The process is conducted for 24-28 h. From 1.00 g, 0.83 g (93% of theory) of the free hormone is obtained. M.p. 210-212° (from acetone), $[\alpha]_D^{20} = +124^\circ$ (1% solution in dioxane), $\lambda_{\max}$240 mμ, $\log \varepsilon = 4.24$.

4,5β-Dihydrocortisone acetate. 1.00 g of steroid is subjected to microbiological deacetylation for 48 h. 0.60 g (67% of theory) of dihydrocortisone is obtained. M.p. 227-229° (from butyl acetate), $[\alpha]_D^{20} = +88.8^\circ$ (1% solution in dioxane).

Deoxycorticosterone acetate. When the fermentation process is carried out with 1 g of the preparation, the substance obtained, according to paper chromatography data, is a mixture of deoxycorticosterone and its acetate. The isolated substance (0.5 g), on acetylation with acetic anhydride in pyridine, gives 0.6 g of deoxycorticosterone acetate. M.p. 155-156° (from acetone), $[\alpha]_D^{20} = +183^\circ$ (1% solution in chloroform), $\lambda_{\max} = 240$ mμ, $\log \varepsilon = 4.205$.

Δ^5-Pregnenol-3β-one-20 acetate was isolated unchanged upon fermentation for 48 h (yield 60%). M.p. 146-147°, $[\alpha]_D^{20} = +21.9$ (1% solution in alcohol).

11α-Hydroxyprogesterone acetate is recovered unchanged (yield 76%) when the fermentation process is carried out for 48 h. M.p. 173-175°,

$[\alpha]_D^{20} = +138^\circ$ (1% solution in acetone), $\lambda_{\max} = 240$ mμ, $\log \varepsilon = 4.20$.

Testosterone acetate and propionate also do not undergo microbiological deacetylation and are recovered after 48 h in 50 and 65% yield, respectively.

In all of the last four cases, no free oxy steroid was detected by paper chromatography.

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