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STEROID DERIVATIVES*

MICROBIAL DEHYDROGENATION OF 3β-HYDROXY-Δ^5-PREGNENE DERIVATIVES

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THE first papers describing microbial dehydrogenation of 3β-hydroxy-Δ^5-unsaturated steroids to the corresponding Δ^4-3-ketosteroids appeared as early as 1938,[1,2] using the action of various microorganisms on simple derivatives of the cholestane,[3] androstane[1,4] or pregnane[2,5] series. Studying this process in more detail, we obtained interesting results with some strains of the genus Flavobacterium. Two strains of Flavobacterium (RIND 38/3 and RIND 39/2**) dehydrogenated in the expected manner 21-substituted 3β-hydroxy-Δ^5-pregnene derivatives leaving, surprisingly, the 21-acetoxy grouping quite intact. This result represents the first exception to the general experience that all microbial transformations are accompanied by hydrolytic cleavage of the ester groups.

The most striking example of this is the transformation, by the above microorganisms, of 21-acetoxy-Δ^5-pregnene-3β-ol-20-one (I, R_1 = H; R_2 = OAc) to 21-acetoxy-Δ^4-pregnene-3,20-dione (cortexone acetate) (II, R_1 = H; R_2 = OAc) in a smooth reaction without detectable by-product formation. In a

* Part XV. For Part XIV see Coll. Czech. Chem. Comm. 26, 1958 (1961).

** The numbering of the microorganisms collection of the Institute.

1 L. Mamoli and A. Vercellone, Ber. Dtsch. Chem. Ges. 71, 152, 154 (1938).

2 L. Mamoli, Ber. Dtsch. Chem. Ges. 72, 1863 (1939).

3 G.E. Turfitt, Biochem. J. 40, 79 (1946).

4 C. Arnaudi, Zentr. Bacteriol. Parasitenk., Abt. II 105, 352 (1942).

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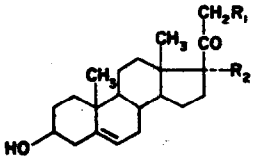
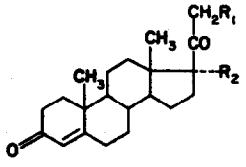
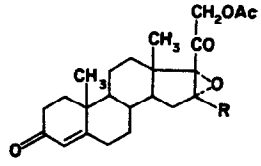
similar manner we obtained 16β-methyl-16α,17α-epoxy-21-acetoxyprogesterone (III, R = CH_3); 16α,17α-epoxy-21-acetoxyprogesterone (III, R = H), and 21-acetoxy-Δ^4-pregnene-17β-ol-3,20-dione (cortexolone acetate) (II, R_1 = OH; R_2 = OAc).



I



II



III

The absence of any proper hydrolytic enzyme system in the microorganisms employed was further proved by the fact that even as labile an ester group as formyl was not cleaved under the conditions of the transformation. For example, Δ^5-pregnene-3β,17α,21-triol-20-one 3-formate 21-acetate was regenerated without change from the fermentation wort after 24 hr.

For the transformation, the presence of a 21-oxygenated substituent is indispensable: none of the 21-unsubstituted derivatives tested (I, R = H) was transformed, although the 21-hydroxy derivatives, e.g. Δ^5-pregnene-3β,21-diol-20-one (I, R_1 = H; R_2 = OH) and Δ^5-pregnene-3β,17α,21-triol-20-one (I, R_1 = R_2 = OH), afforded the corresponding Δ^4-3-ketones as single products.

The following general experimental technique was used: the microorganisms were cultivated on glucose-yeast extract medium. The fermentation wort (1400 ml) of a similar composition was inoculated with the above

stationary culture, and after 16 hr propagation at 36° under aeration and stirring, 200 mg of the steroid tested was added, dissolved in 3 ml of dimethylformamide. After 5 hr fermentation under similar conditions, the wort was repeatedly extracted with dichloromethane, the solvent was removed by distillation in vacuo and the composition of the crude crystalline product was tested by thin layer chromatography,[6,7] and/or, after washing with petroleum ether and recrystallization, it was further characterized by mixed melting point with an authentic sample.

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[6] S. Heřmánek, V. Schwarz and Z. Čekan, Pharmazie 16, 566 (1961).

[7] V. Schwarz and K. Syhora, Coll. Czech. Chem. Comm. In press.