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TRANSFORMATION OF REICHSTEIN'S SUBSTANCE S TO PREDNISOLONE BY *PSEUDOMONAS*

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WE have reported previously[1] the microbiological conversion of Reichstein's Substance S (I) by *Pseudomonas* species into 1,4-pregnadiene-17α,20β,21-triol-3-one (II), hydrocortisone, 1,4-pregnadiene-17α,21-diol-3,20-dione and 4-pregnene-17α,20β,21-triol-3-one, thus disclosing the enzyme systems responsible for the three reactions, i.e. 1-dehydrogenation, 11β-hydroxylation and 20β-hydrogenation, to be present in the *Pseudomonas* species. From these findings the possibility was naturally anticipated that both the 1-dehydrogenation and the 11β-hydroxylation could be induced simultaneously in the same molecule of the substrate if the conditions for enzyme formation and for steroid transformation were properly selected. We now wish to record the successful microbial preparation of prednisolone from I, the realization of our anticipation.

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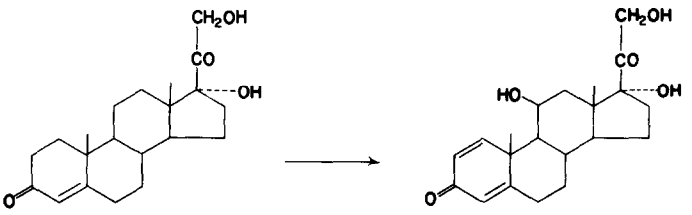
* With the co-operation of T. Kusunoki, K. Yoshino and H. Fujitani.

[1] H. Nawa, M. Uchibayashi, R. Takeda, I. Nakanishi, T. Kusaka, J. Terumichi, M. Uchida, M. Katsumata, K. Yoshino and H. Fujitani, Tetrahedron 4, 201 (1958).



Reichstein's Substance S (I) Prednisolone

Conditions for fermentation of Pseudomonas species 109, an organism identified as closely akin to Pseudomonas boreopolis,[1] were examined elaborately and as a result a trace of prednisolone together with II was detected as a transformation product of I on the paper chromatogram. The microorganism was then treated with ultra-violet ray (irradiation with a 30 W ultra-violet ray lamp at a distance of 54 cm for 1 - 5 min), and was incubated on a bouillon agar plate containing 0.1 per cent of I. Selection of the strain was conducted by paper-chromatographical examination of bioconversion products and a mutant could be isolated which showed an excellent ability of forming prednisolone.

The mutant of Pseudomonas sp. 109 thus obtained was incubated in a synthetic medium containing cornsteep-liquor, phosphates, sulphates, glycerol and urea, and fermentation of I was observed as described previously.[1] Extraction of the culture filtrate with ethyl acetate, followed by concentration of the extract afforded a crude steroid mixture which was

acetylated with acetic anhydride and pyridine and chromatographed on Florisil. Elution with a solvent pair of ether and acetone yielded the acetate of unchanged I and 21-acetoxy-1,4-pregnadiene-11β,17α-diol-3,20-dione (prednisolone acetate)[2] (m.p. 230°; υ^{Nujol} 3344, 3257, 1742, 1718, 1647, 1587, 1222 cm^{-1}; Found: C, 68.37; H, 7.38: Calc. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51), which was identified by comparison of paper chromatogram, melting point and infra-red spectrum with an authentic sample. The rate of transformation of the substrate I to prednisolone was 20 - 30 per cent.

The above finding clearly indicates that 1-dehydrogenation and 11β-hydroxylation were performed simultaneously be a single microorganism, and thus the one-step preparation of therapeutically valuable prednisolone from I was established for the first time. It is furthermore interesting to note that those enzymatic reactions can be conducted by microorganisms which belong to bacteria.

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[2] H. L. Herzog et al., Science 121, 176 (1955).