Microbiological Transformation of Steroids

XV. Transformation of Steroid S (Reichstein) by *Absidia orchidis* 310

O. HANČ, A. ČAPEK and B. KAKÁČ

Research Institute for Pharmacy and Biochemistry, Praha 12

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The hydroxylation capacity of 28 different microbial species (a total of 64 strains) was tested in work described previously (Čapek & Hanč, 1961) and it was found that of this number as few as 10 species (19 strains) will hydroxylate steroid S (Reichstein) in the 11β-position. In all the strains of this series steroid S yielded more metabolites, the strain *Absidia orchidis* 310 being apparently relatively the most suitable. The enzymic system of this microorganism will transform steroid S into cortisol and into its 11α-epimer. Another oxidation product, cortisone, is also formed in traces.

The chosen strain of *Absidia orchidis* was then studied further, particularly with respect to the individual factors

![Diagram of steroid transformation](image-url)
affecting the rate of transformation of steroid S to a mixture of cortisol and its 11α-epimer.

Three methods were used for the analysis of the steroid metabolites: (1) spectrophotometry in the u.v. region, using extinction at 242 m\(\mu\). Cortisol, its 11α-epimer (epicortisol) as well as the original steroid S, show the same position of the maximum; the extinction values of cortisol and of its epimer are identical while the extinction of the S compound is relatively higher. (2) Polarography based on the reduction of the conjugated double bond \(O=\text{C}-\text{C}=\text{C}<\) (Eisenbrand & Picher, 1939). There is no difference in the polarographic behaviour of the three compounds. (3) Colorimetry was carried out using the Porter and Silber reaction (Porter & Silber, 1950) with phenylhydrazine in ethanolic solution. The method may only be applied to crude mixtures where both steroids (cortisol and epicortisol) are present in a ratio of 1 : 1, according to a calibration curve constructed for both steroids. The 11α-epimer of cortisol produces about 20\% more intense coloration under identical experimental conditions.

**MATERIALS AND METHODS**

**Microorganism.** The optimum conditions for the transformation of the steroid S to cortisol and its 11α-epimer were studied on the strain *Absidia orchidis* 310 (obtained from the Department of Fermentation Chemistry, University of Technical Science, Prague).

**Cultivation.** The strain *Absidia orchidis* 310 was grown under sterile conditions in 100 ml. medium D (white dextrin 3.0\%, corn-steep liquor 0.3\%—referred to dry weight, \((\text{NH}_4)_2\text{HPO}_4 0.3\%, \text{KCl} 0.04\%, \text{MgSO}_4 0.04\%, \text{FeSO}_4 0.001\%)\). The pH of the medium was adjusted to 6.2 prior to sterilization, using 10\% NaOH. The medium was placed in 750 ml. Erlenmeyer flasks on a reciprocal shaker (70 strokes/min., amplitude 6 cm.) at 27°C for 48 hr.

When the cultivation was terminated (the pH dropped to 4.2—4.5) the contents of 3 flasks were combined, the mycelium filtered off and remainder of the medium removed by washing the mycelium with 150 ml. physiological saline. The washed mycelium was immediately transferred to a flask (1000 ml. capacity) placed on a laboratory stirrer and the volume made up to 300 ml. with a phosphate buffer of known pH.

On account of the fact that the transformation of the steroid on the laboratory stirrer took place under non-sterile conditions, aqueous solution of chlortetracycline (100 \(\mu\)g. per 100 ml. medium) was added to each flask on the stirrer to prevent contamination during transformation.

**Transformation of the steroid.** The effect of pH, temperature, type and amount of solvents, concentration of added steroid S, number of revolutions of the stirrer and aeration, in the course of transformation of the steroid S was investigated. In studying these factors, a single factor was altered in each series. Every estimation was performed in three mutually independent experiments and the average of these three used for the evaluation.

**Analytical methods.** The course of transformation was evaluated qualitatively with the aid of paper chromatography (the presence of the original steroid S and the number and types of metabolites produced) and quantitatively by spectrophotometry after elution of the metabolites with ethanol from an undetected paper chromatogram (amounts of cortisol and 11α-epimer).

Whatman paper 4 impregnated with a mixture of formamide-ethanol (1 : 1) was used for chromatography. Chloroform served as the mobile phase. Samples for chromatography were removed in quantities corresponding to 1 mg. original steroid; they were extracted twice with chloroform, the extracts were combined