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Transformation of Progesterone to 17α-Hydroxyprogesterone by *Trichoderma viride* Pers. ex Fries

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Introduction

The introduction of a hydroxyl with the *alpha* configuration at carbon-17 of progesterone by *Trichothecium roseum* has been reported by Meystre *et al.* (2) and by Meister *et al.*, cited by Peterson (4), also by Meister *et al.* (1) using a culture of *Cephalothecium roseum*. The present communication reports a similar transformation by *Trichoderma viride*.

Experimental and Results

The culture of *T. viride* was obtained from soil and purified by single conidial isolation. Five milliliters of vegetative mycelium developed in an edamine–cerelose–corn steep medium was used to inoculate 250-ml. Erlenmeyer flasks containing 50 ml. of the following medium autoclaved at 15 lb. pressure for 17 min.: edamine 20 g., cerelose 50 g., corn steep liquor 5 ml., and distilled water to 1 l. adjusted to pH 6.5 (3). Following sterilization and inoculation, the flasks of medium were incubated for 48 hr. at 28°C. on a rotary shaker moving at 220 r.p.m. and describing a circle 1½ in. in diameter. Each flask was then charged with 10 mg. of progesterone in 2½ ml. of propylene glycol. The flasks were incubated for an additional 48 hr. and the contents were pooled for extraction.

Three liters of pooled broth from 60 flasks was filtered with the aid of Supercel. The filtered broth was extracted with two 4-l. portions of ethyl acetate and the mycelia with 2 l. of the same solvent. The extracts were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml. of benzene and charged to a column containing 500 g. of silica gel.

Development of the column was carried out with: (*a*) 1 l. of benzene;

(*b*) 900 ml. of 5% ethyl acetate in benzene; (*c*) 1.2 l. of 20% ethyl acetate in benzene; and (*d*) 750 ml. of 50:50 ethyl acetate–benzene. Paper-strip chromatographic examination of the eluates revealed a monohydroxy product with mobility identical to 17α-OH progesterone in the 50:50 ethyl acetate–benzene fraction.

The fractions were combined and evaporated to dryness *in vacuo* on a steam bath. The residue was streaked on four sheets of Whatman #1 paper and developed with the system benzene–cyclohexane/propylene glycol–methanol for 6 hr. The bands opposite the 17α-OH progesterone control were cut out and eluted with methanol. The methanol solution was evaporated to dryness, the residue partitioned between water and chloroform, and the chloroform solution dried over sodium sulfate. The chloroform solution was evaporated to dryness and the residue taken up in ethyl acetate–petroleum ether. Upon cooling, the solution deposited a small crop of crystals, m.p. 210–214°C. Mixed melting point with 17α-OH progesterone gave no depression. The infrared spectrum of the microbial product was identical to that of 17α-OH progesterone. Quantitative paper strip chromatograms showed conversions of 20% with this organism.

Summary

The introduction of a 17α-hydroxyl group in progesterone by the microorganism *Trichoderma viride* has been demonstrated.

A method involving preparative paper-strip chromatography is described for isolating the steroid from the fermentation broth.

References

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