Enzymatic Preparation of 1-Dehydro Steroids

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THE INTRODUCTION of 1,2-double bonds into steroids attracted considerable attention since the 1-dehydro derivatives of cortisone and cortisol, prednisone and prednisolone, showed increased antirheumatic and antiallergic activity and produced less undesirable side effects (1).

This communication describes a new, rapid, and efficient enzymatic method, suitable for the laboratory preparation of 1-dehydro steroids. We have found that Δ 4-steroidal 3-ketones are readily converted into the corresponding Δ 1,4-steroidal 3-ketones by incubating with cell-free extracts of *Nocardia restrictus* in the presence of an electron acceptor such as phenazine methosulfate. Several methods have been used for the introduction of 1,2-double bonds into steroids. Although satisfactory yields of 1-dehydro steroids are obtained using selenium dioxide (2), it is difficult to remove the residual selenium which is highly toxic. An additional double bond at the 6,7-position of steroids is often encountered using 2,3-dichloro-5,6-dicyanobenzoquinone (3) as the dehydrogenation reagent. Incubation of steroids such as testosterone or progesterone with microorganisms (4) often result in the complete oxidation of the steroid nucleus without the apparent accumulation of any 1-dehydro steroids. This enzymatic method thus offers certain advantages over these methods.

Experimental details are given for the preparation of 1,4-androstadiene-17β-ol-3-one and 1,4-pregnadiene-11β, 17α, 21-triol-3,20-dione to serve as model systems. The following 1-dehydro derivatives have also been prepared in this way, indicating the scope of this enzymatic reaction:

1,4-androstadiene-3,17-dione, 1,4-pregnadiene-3,-20-dione, 1,4-pregnadiene-17α,21-diol-3,11,20-trione, 1,4-pregnadiene-17α, 21-diol-3,20-dione, 9α-fluoro-1,4-pregnadiene-11β,17α,21 - triol - 3,20 - dione, 9α - fluoro-1,4-pregnadiene- 11β,16α,17α,21 - tetrol - 3,20-dione.

EXPERIMENTAL

Growth of the Organism.—The organism *Nocardia restrictus* was obtained from Dr. R. Gordon of the Institute of Microbiology, Rutgers University, and was used throughout this work.

Cells of this organism were grown on the following medium: corn steep liquor, 0.6%; ammonium dihydrogen phosphate, 0.3%; calcium carbonate, 0.25%; corn oil, 0.22%; yeast extract, 0.25%; glucose, 1%; and progesterone, 0.05%. After seventy-two hours of growth at 25°, the cells were harvested by centrifugation, washed with 0.3 *M* phosphate buffer (pH 7.0), and stored in a deep freeze.

Preparation of Cell-Free Extract.—Frozen cells (10 Gm.) of this organism were suspended in 50 ml. of 0.03 *M* trishydroxymethylaminomethane (Tris) buffer at pH 8.0 or pH 9.5, depending on the substrate used in the experiment; 30 Gm. of fine glass beads was added and the mixture was homogenized in a Vir-Tis homogenizer at maximum speed for twenty minutes, with cooling. The cell-debris and glass beads were removed by centrifugation at 1,000 × g for five minutes. The supernatant solution contained approximately 100 mg. of protein per ml. This solution was diluted fivefold with 0.03 *M* Tris buffer and was used for the preparation of 1-dehydro steroids.

1,4-Androstadiene-17β-ol-3-one.—To 100 mg. of testosterone dissolved in 3 ml. of dimethylformamide was added 50 mg. of phenazine methosulfate and 200 ml. of enzyme protein in 0.03 *M* Tris buffer, pH 9.5. The reaction mixture was incubated at 23° with stirring for three hours in the dark. The reaction was terminated by the addition of 2 *N* hydrochloric acid until strongly acidic, followed by Dowex 50, (H+) form, to remove the phenazine methosulfate. The mixture was filtered to remove the protein precipitate and the ion-exchange resin. The filtrate was then extracted three times with 50-ml. portions of chloroform. The chloroform extract was dried over sodium sulfate and concentrated to dryness. The crystalline residue weighed 78 mg. Recrystallization from ethyl acetate-hexane yielded a sample (64 mg.) which melted at 167–168°, $[\alpha]_D^{25} = +23°$ (c, 1.0, $CHCl_3$); $\lambda_{max.}^{alc.}$ 243 mμ (ε 16,100); $\lambda_{max.}^{Nujo}$ 2.96, 6.02, 6.18, and 6.24 μ; reported (5) m.p. 167–168°, $[\alpha]_D^{23} = +21°$ (c, 1.28, $CHCl_3$).

Anal.—Calcd. for $C_{19}H_{26}O_2$: C, 79.66; H, 9.15. Found: C, 79.55; H, 8.98.

1,4 - Pregnadiene - 11β,17α,21 - triol - 3,20-dione.—To 100 mg. of cortisol, dissolved in 4 ml. of dimethylformamide, was added 75 mg. of phenazine methosulfate and 200 ml. of enzyme protein in 0.03 *M* Tris buffer pH 8.0. The reaction mixture was incubated at 25° with stirring in the dark for twelve hours; the reaction was terminated by the addition of hydrochloric acid until strongly acidic to precipitate the proteins. Dowex 50 (H+) form, was then added to remove the phenazine methosulfate. The mixture was filtered and the filtrate extracted three times with 75-ml. portions of chloroform. The chloroform extract was dried over sodium sulfate and concentrated to dryness. The residue weighed 68 mg. Recrystallization from 95% ethanol gave a sample (56 mg.) which melted at 238–240°; $[\alpha]_D^{25} = +100°$ (c, 0.90, dioxane); $\lambda_{max.}^{alc.}$ 243 · mμ (ε 15,000); $\lambda_{max.}^{Nujol}$ 2.98, 5.82, 6.03, 6.18, and 6.24 μ; reported (6) m. p. 240–241°, $[\alpha]_D^{25} = +102°$ (dioxane).

Anal.—Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found: C, 70.12; H, 8.12.

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