

The Microbial 1-Dehydrogenation of Some Δ^4 -3 Ketosteroids

The therapeutic value of 1-dehydrocortisone and 1-dehydrocortisol has been reported by Herzog *et al.*¹ The microbial conversion of Δ^4 -3 ketosteroids to the $\Delta^{1,4}$ dienone analog has been reported by Vischer *et al.*² using *Fusarium solani* and *Calonectria decora* with cortisone, corticosterone, 11-deoxycorticosterone, 17 α -hydroxy-11-deoxycorticosterone, and progesterone. Herzog *et al.*³ demonstrated the microbial 1-dehydrogenation of cortisone, cortisol, 9 α -fluorocortisone, corticosterone, 11-deoxycorticosterone, and 11-deoxy-17 α -hydroxycorticosterone with *Corynebacterium simplex* (ATCC 6946).

We wish to report on the selective microbial 1-dehydrogenation of a series of Δ^4 -3 ketosteroids, utilizing a bacterial culture belonging to the species *Bacillus sphaericus*.

The organism was grown aerobically for 24 hr. in 20 l. of medium of the following composition: 2% edamin,¹ 2% cerelese,² and 0.5% corn steep liquor. Four grams of the requisite Δ^4 -3 ketosteroid were then added in dimethylformamide solution and the fermentation was allowed to proceed an additional 24 to 48 hr.

The whole broth was extracted with ethyl acetate and the extract washed successively with concentrated sodium bicarbonate solution and water. The ethyl acetate solution was taken to dryness and the residue redissolved in 70% aqueous methanol. Following a petroleum ether extraction, the aqueous methanol solution was concentrated *in vacuo* to remove the methanol. An ethyl acetate extraction of the aqueous solution was made, the extract dried over anhydrous Na_2SO_4 , and subsequently treated with Nuchar C. The solution was concentrated to 50 ml. and the practically pure 1-dehydrosteroid allowed to crystallize out overnight.

The following 1-dehydrosteroids, all of which have previously been described,^{2, 3} were prepared: 1-dehydrocortisone, 1-dehydrocortisol, 1-dehydro-9 α -fluorocortisol, 1-dehydrocorticosterone, 1-dehydro-11-deoxycorticosterone, 1-dehydro-11-deoxy-17 α -hydroxycorticosterone, 1-dehydroprogesterone, and $\Delta^{1, 4}$ -androsteradiene-3,7-dione. In addition, the following new compounds were prepared:

1-dehydro-11 β -hydroxyprogesterone, m.p. 232–234°C., $\lambda_{\text{Max.}}^{\text{MeOH}}$ 243 μ ($\log \epsilon = 4.17$); $\lambda_{\text{Max.}}^{\text{Nujol}}$ 2.84, 2.99, 5.94, 6.02, 6.19, 6.23, 11.22, 11.29 μ .

1-dehydro-11-dehydrocorticosterone acetate,³ m.p. 190–193°C., $\lambda_{\text{Max.}}^{\text{MeOH}}$ 238 μ ($\log \epsilon = 4.20$); $\lambda_{\text{Max.}}^{\text{Nujol}}$ 5.27, 5.77, 5.88, 6.02, 6.15, 6.22, 8.1, 11.25 μ .

The $\Delta^{1, 4}$ -diene-3 ketosteroids have been found to possess some rather characteristic properties: (1) On paper chromatograms the mobility is 0.7–0.9 that of the Δ^4 -3 keto analog; (2) Infrared spectra always possess a band in the 11.2 μ region (4) and a triplet at 6.0–6.23 μ with slight variations; (3) Spectra in 95% sulfuric

¹ Enzymatic digest of lactalbumin obtained from Sheffield Farms, New York, N. Y.

² Commercial glucose obtained from Corn Products Refining Company, New York, N. Y.

³ Purification was best effected through the preparation of the acetate.

acid possess maxima between 258 and 265 $m\mu$ with the exception of 1-dehydro-11 β -hydroxyprogesterone; and (4) The polarographic half-wave potential is about -1.34 v. and has been quite useful in following the course of the microbial transformation.

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