Production of 16 α -Hydroxydehydroepiandrosterone-3-succinate by *Streptomyces roseochromogenes*

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In the previous paper¹⁾ we reported that *Streptomyces roseochromogenes* hydroxylated pregnenolone-3-succinate to 16 α -hydroxypregnenolone and 16 α -hydroxypregnenolone-3-succinate. The ability of *Streptomyces* to hydroxylate at carbon 16 of a steroid nucleus is well known, but as far as we know there are few reports on the microbial 16 α -hydroxylation of dehydroepiandrosterone and its derivatives.^{2,3)} This paper concerns on the microbial transformation of dehydroepiandrosterone-3-succinate into 16 α -hydroxydehydroepiandrosterone-3-succinate. By incubation of dehydroepiandrosterone-3-succinate with *S. roseochromogenes* IFO 13080 we obtained 16 α -hydroxydehydroepiandrosterone and 16 α -hydroxydehydroepiandrosterone-3-succinate in yields of about 15% and 10%, respectively.

The experimental methods were described previously.¹⁾ The culture broth was combined and centrifuged at 3000 rpm for 10 min to remove cells. The supernatant was adjusted to pH 6.0 with 1 N HCl and was extracted twice with equal portions of ethyl acetate, and the extracts were concentrated to dryness *in vacuo*. A small amount of the oily residue thus obtained was subjected to thin-layer chromatography, developed with chloroform-methanol (9:1). Two

main products giving a positive blue-tetrazolium test were found: one product (A) showed a mobility similar to 16 α -hydroxydehydroepiandrosterone, the other product (B) showed a lower mobility than the substrate. This oily residue (one g from 600 mg of substrate) was dissolved in benzene and subjected to chromatography on a silica gel column, being eluted with various proportions of ethyl acetate in benzene. Approximately 250 mg of a mixture of product A and the substrate were eluted with 15% ethyl acetate in

FIG. 1. Infrared Absorption Spectra of A: Authentic 16 α -Hydroxydehydroepiandrosterone, B: Product A and C: Hydrolyzate of Product B.

FIG. 2. Infrared Absorption Spectrum of Product B.

FIG. 3. NMR Spectrum of Product B.

benzene, and approximately 100 mg of crude product B were eluted with 20% ethyl acetate in benzene. Product A was isolated by preparative thin-layer chromatography and recrystallized from acetone-ether: mp 183°C (90 mg). Its IR spectrum was identical with that of authentic 16 α -hydroxydehydroepiandrosterone (Sigma Chem. Co.) (Fig. 1). Product B was purified as follows: 100 mg of crude B were suspended in 10 ml of distilled water, pH was adjusted to 9.0 with 8% aqueous sodium bicarbonate, and the mixture was extracted several times with ethyl acetate. The aqueous layer was taken and adjusted to pH 3.0 with 1N HCl, and extracted with ethyl acetate. The solvent was evaporated *in vacuo* and product B was recrystallized from acetone-ether: mp 211~218°C (53 mg). Figure 2 shows the IR spectrum of product B. Incubation of product B with Diastase (Dainihon-Seiyaku, Osaka) formed a compound whose IR spectrum was identical with that of 16 α -hydroxydehydroepiandrosterone (Fig. 1). The NMR spectrum of product B showed signals of steroidal angular methyl protons at δ 0.89 (C₁₈: 3H, singlet) and δ 0.97 (C₁₉: 3H, singlet), steroidal proton at δ 4.65 (C₁₆: 1H, triplet) and four succinate protons at δ 2.90 (4H, singlet) (Fig. 3). These spectra together with the result obtained by incubation

with Diastase supported the conclusion that product B is 16 α -hydroxydehydroepiandrosterone-3-succinate.

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