Microbial Aromatization of Androsta-1,4,7-Triene-3,17-Dione

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Several microorganisms transform androsta-1,4,7-triene-3,17-dione into equilin and equilenin, presumably via a 19-hydroxylated intermediate.

Aromatization of the steroid molecule through microbial C-1 dehydrogenation of 19-nor-steroids was first reported by Levy and Talalay (3). Dodson and Muir (1) and Sih and Rahim (5) found the same reaction to occur with 19-OH steroids. Microbial 19-hydroxylation of Δ1-19-methyl steroids should lead to the same end products and is the object of the present note. 19-Hydroxylation of steroids remains a very rare microbial reaction. It was first described by Hasegawa and Takahashi (2) and Nishikawa and Hagiwara (4), who used Cotticiium sasakii to transform Reichstein's compound S into 17α,19,21-trihydroxyepregnen-4-ene-3,20-dione. Takahashi (6) also found that Pellicularia filamentosa var. microsclerotia transformed 17α,20α,21-trihydroxyepregnen-4-en-3-one into a mixture of 17α,19,20α,21-tetrahydroxyepregnen-4-en-3-one and 11β,17α,20α,21-tetrahydroxyepregnen-4-en-3-one. Microbial 19-hydroxylation of several other steroids of the pregnene series has been reported in the U.S. patent literature (T. Hasegawa et al., U.S. Patent 2,966,444, Dec. 27, 1960; G. M. Shull, U.S. Patent 3,039,926, June 19, 1962; G. M. Shull, U.S. Patent 3,040,038, June 19, 1962). All microorganisms recorded in these patents to perform this reaction are basidiomycetes of the family Corticiaceae.

In the search for a chemical synthesis of estrogens, several steroid intermediates were prepared and used as substrates in a microbiological screening program for organisms capable of hydroxylating carbon 19. We are reporting here the microbial transformation of androsta-1,4,7-triene-3,17-dione (I) into equilin (II) and equilenin (III). This transformation is probably brought about through 19-hydroxylation followed by a reverse aldol reaction (Fig. 1). Results indicate that the capacity to aromatize is widespread among families, orders, and even classes of microbes. Of the Actinomycetales, the following species transformed I into II and III: Streptomyces fradiae ATCC 10745, S. microflavus NRRL 3261, and S. rimosus NRRL 3259; Nocardia asteroides ATCC 8674, N. corallina ATCC 13259, and N. erythropolis ATCC 17895. In the Eubacterales, the following species yielded positive results: Bacillus coagulans NRRL B-3257, Bacterium cycloexodans ATCC 12673, Corynebacterium simplex ATCC 19140, and Micrococcus flavus NRRL B-3258. Absidia coerulea ATCC 1359b and Cunninghamamella elegans ATCC 10028a (both of the order Mucorales) and Aspergillus sclerotiorum NRRL 415 were also active.

Bacteria (including nocardiae and streptomycetes) were inoculated into a medium containing (g/liter): glucose, 1.5; yeast extract (Difco), 1.5; peptone (Difco), 5.0; K2HPO4, 3.5 (pH 7.0). Fungi were grown in a medium containing (g/liter): Cereolose (technical grade of glucose), 50; Edamine (Sheffield Co.), 20; corn steep liquor, 3 (pH 5.8). Inoculated flasks (50 ml of medium per 250-ml Erlenmeyer flask) were incubated at 24 to 28 C on a rotary shaker (240 rev/min, 2-inch stroke) for 16 to 32 hr, the steroidal substrate was added as an ethanolic solution, and the incubation was continued for an additional 36 to 48 hr. At the end of the incubation, the whole broth was acidified to pH 2 with 1 N H2SO4 and extracted exhaustively with chloroform. The organic extract was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was dissolved in a small volume of ethanol, and the solution was spotted on thin layers of Silica Gel G plates which were
developed in benzene-isopropanol (85:15); I, II, and III served as standards. Compounds were visualized by spraying plates with p-nitrobenzene diazonium fluoroborate (1% in 50% acetic acid). For quantitative analysis, the estrogentic compounds were purified by thick-layer chromatography on Silica Gel G plates in a solvent system containing benzene-ethyl acetate (7:3) and analyzed by gas liquid chromatography (G. Schilling, personal communication). The yields varied between 1 to 2% of II and 2 to 3% of III. The ratios of the two estrogens did not change with various incubation times.

All attempts to transform androsta-1,4-diene-3,17-dione into estrone with the above-mentioned organisms were unsuccessful.

LITERATURE CITED