16β,18-Dihydroxylation of Oxygenated 5α-Androstanes with the Fungus *Leptoporus fissilis*

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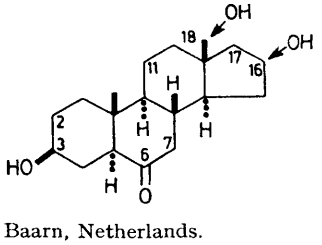
Summary Incubation of certain dioxygenated 5α-androstanes, notably 3,6-hydroxy-ketones, with *Leptoporus fissilis* gives 16β,18-dihydroxy- and 16β-monohydroxy-products in combined yields of over 50%.

MICROBIOLOGICAL hydroxylation of steroids at position 18 is uncommon, and previous reports appear to be confined to the following instances: 18-hydroxylation of androst-4-ene-3,17-dione,[1] 5α-androstane-2,7-dione,[2] and 11β,21-dihydroxypregn-4-ene-3,20-dione[3] with *Aspergillus niger*, *Wojnowicia graminis*, and *Corynespora cassiicola*, respectively, and 9α,18-dihydroxylation of androst-4-ene-3,17-dione[4] with *Cercospora melonis*. In recent work on the incubation (for 4 days under standard conditions[5] on a 2 g scale at a concentration of 40 mg/100 ml medium) of a series of dioxygenated 5α-androstanes with the fungus *Leptoporus fissilis*† we have obtained 16β- and 18-mono-



† Culture No. CBS 274·63 obtained from Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

TABLE

Substrate	Main product(s) (% yield)
3β-Hydroxy-5α-androstan-6-one	3β,16β,18-$(OH)_3$-6-one(39)[a] + 3β,16β-$(OH)_2$-6-one(31)
6β-Hydroxy-5α-androstan-3-one	6β,16β,18-$(OH)_3$-3-one(34)[b] + 6β,16β-$(OH)_2$-3-one(18)
5α-Androstane-3,6-dione	16β,18-$(OH)_2$-3,6-dione(5)[c] + 16β-OH-3,6-dione(12)
3β-Hydroxy-5α-androstan-7-one	3β,16β,18-$(OH)_3$-7-one(24)[d] + 3β,16β-$(OH)_2$-7-one(10)
3β-Hydroxy-5α-androstan-11-one	3β,16β,18-$(OH)_3$-11-one(24)[e]
5α-Androstane-7,17-dione	2β,17β-$(OH)_2$-7-one(24)

Constants of new compounds: [a] m.p. 243—245 °C, $[\alpha]_D$ −38° (MeOH); [b] (triacetate) 179—181 °C, −35° ($CHCl_3$); [c] 172—174 °C −9° ($CHCl_3$); [d] 218—220 °C, −58° (MeOH); [e] 182—183 °C, +34° (MeOH).

hydroxylated, and 16β,18-dihydroxylated derivatives, the yields and relative proportions of the products varying widely with the nature of the substrates. (The structures of the products were established by spectrometric examination of the hydroxy-compounds and the derived acetates, and by chemical inter-relationships.)

Hydroxylation at positions 16 and 18 occurs with substrates having one oxygen group in ring A and a second in ring B or ring C; with these androstanes the predominant processes are 16β,18-di- and 16β-mono-hydroxylation, the 18-monohydroxylated derivatives being formed only in low yield. The best directing effects[5] encountered so far with *L. fissilis* are those operating in 3,6-hydroxy-ketones, and a 'reverse effect,' observed with several fungi in our previous studies, is seen here in the 2-hydroxylation of 5α-androstane-7,17-dione. It might be assumed that the 16,18-dihydroxy-compounds arise by a consecutive process in which the free 16β-hydroxy-steroids are the first products. However, incubations of 3,16-dioxygenated androstanes (not reported here) suggest that this is too facile an interpretation and that 16,18-dihydroxylation occurs either directly or by a sequence in which the first formed product is hydroxylated further rather than being released into the medium.

(*Received, 3rd September* 1976; *Com.* 1016.)

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