12β-Hydroxylation of Lithocholic Acid using the Fungus *Helicostylum piriforme*

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Summary Lithocholic acid has been converted into 3α,12β-dihydroxy-5β-cholan-24-oic acid by the fungus *Helicostylum piriforme.*

ALTHOUGH the microbial hydroxylation of a variety of steroids is well documented,[1,2] little attention has been paid to the bile acids. Most of the studies[3] on microbial transformations of bile acids have concerned hydrolysis of the conjugate acids, oxidation of alcohols to ketones and the reverse reaction, dehydroxylation, nuclear dehydrogenation, aromatisation, and oxidative carbon–carbon bond cleavage; no instance of hydroxylation has been noted so far. We have carried out a preliminary screening and found that several steroid-hydroxylating fungi were able to hydroxylate chenodeoxy-, deoxy-, hyodeoxy-, and lithocholic acids in relatively high yields.

We now report the microbial hydroxylation of lithocholic acid. The fungus *Helicostylum piriforme* ATCC-8992 was cultivated for 3 days at 28 °C in a medium (pH 6·8—7·0) consisting of 3·5% glucose, 2% peptone, and 0·3% corn steep liquor in tap water on a rotary shaker. A wet mycelium harvested from the culture broth (100 ml) was suspended in deionized water (100 ml) and finely divided lithocholic acid (50 mg) was added to the suspension. The mixture was incubated for 2 days at 28 °C on the shaker. Extraction of the incubation mixture with AcOEt yielded a mixture of the hydroxylation products. The principal component was purified by column chromatography on silicic acid (Mallinckrodt 2847, Paris, Kentucky) and isolated by crystallization from AcOEt and then from acetone (*ca.* 40% yield based on starting material; m.p. 171·5—172·5 °C). This compound was identified as 3α,12β-dihydroxy-5β-cholan-24-oic acid by mixed m.p. and by comparison of its behaviour on t.l.c. and its i.r. spectrum with an authentic sample (m.p. 172·5—173·6 °C)[4] which was prepared from methyl 3α-ethoxycarbonyloxy-12-oxo-5β-cholan-24-oate[5] by $NaBH_4$ reduction followed by hydrolysis with MeOH–KOH.

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