

Novel Microbial Transformation of Deoxycholic Acid

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Summary. Deoxycholic acid has been converted into 12 ξ -hydroxyandrosta-1,4-diene-3,17-dione by *Pseudomonas* sp. NCIB 10590.

ALTHOUGH the microbial side-chain degradation of neutral steroids is well documented, relatively little attention has been paid to the bile acids. Most of the studies^{1,2} on bile

acids have concerned dehydroxylation, nuclear dehydrogenation, and aromatisation; very few instances of side-chain degradation have been noted. Nagasawa³ has demonstrated the conversion of lithocholic acid into androsta-1,4-diene-3,17-dione by *Arthrobacter simplex* and Hayakawa² and Severina⁴ have observed partial side-chain degradation of bile acids by *Streptomyces gelaticus*, *Corynebacterium equi* and *Mycobacterium mucosum*. The conversion of deoxycholic acid into a 17-oxo steroid has not been previously recorded.

We have isolated, from animal faeces a *Pseudomonas* sp. NCIB 10590 in aerobic culture, on a medium containing 0.1% w/v deoxycholic acid as the sole carbon source. Extraction of this culture with CH₂Cl₂ 12 h after inoculation yielded a mixture of neutral and acidic components. The principal component of the neutral fraction was purified by column chromatography on Kieselgel 60, 70—230 mesh ASTM (E. Merck, Darmstadt) and isolated by crystallisation from CH₂Cl₂-MeOH, m.p. 213—214°, (10% yield based on starting material). This compound was assigned the structure 12 ξ -hydroxyandrosta-1,4-diene-3,17-dione from the following observations.

G.l.c.-mass spectral analysis using a column of 3% OV-1 on 100/120 mesh Gas-Chrom Q at 240° gave a single major peak, the mass spectrum of which showed an intense molecular ion at m/e 300 (40%). The base peak at m/e 122 suggested a 1,4-dien-3-one structure for ring A.⁵ Spectroscopic properties: λ_{\max} 244 nm (ϵ_{\max} 19,140); ν_{\max} (KBr disc) 3495 (12-OH, H-bonded), 1740 (17-C=O), and 1660, 1618, and 1600 (1,4-dien-3-one); ¹H n.m.r. spectrum τ 3.05 (1H, *d*, *J* 20.0 Hz, 1-H), 3.82 (1H, *d* showing further splitting, *J* 20.0 Hz, 2-H), 3.98 (1H, *s*, slight splitting, 4-H), 6.30, (1H, 4-line *m*, 12-H), 7.04 (1H, *s*, OH), 7.50—7.70 (2H, *m*, 16-CH₂), 8.77 (3H, *s*), and 9.01 (3H, *s*, 18- and 19-Me). The product of Jones' oxidation showed a molecular ion at m/e 298 with the base peak remaining at m/e 122.

Further evidence for the suggested transformation was provided by an analogous conversion. *Pseudomonas* sp. NCIB 10590 also grew on 0.1% w/v lithocholic acid medium and yielded androsta-1,4-diene-3,17-dione as the principal neutral product which was identified by t.l.c. and g.l.c.-m.s. comparison with the authentic compound.

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⁴ L. O. Severina, I. V. Torgov, G. K. Skrjabin, V. I. Zaretskii, N. S. Wulfson, and I. B. Papernaja, *Tetrahedron*, 1969, **25**, 5617.

⁵ H. Budziewicz, 'Biochemical Applications in Mass Spectrometry,' ed. G. R. Waller, Wiley-Interscience, New York, 1972, ch. 10, p. 267.