Novel Microbial Transformations of Androst-4-ene-3,17-dione by a *Mucor* sp.

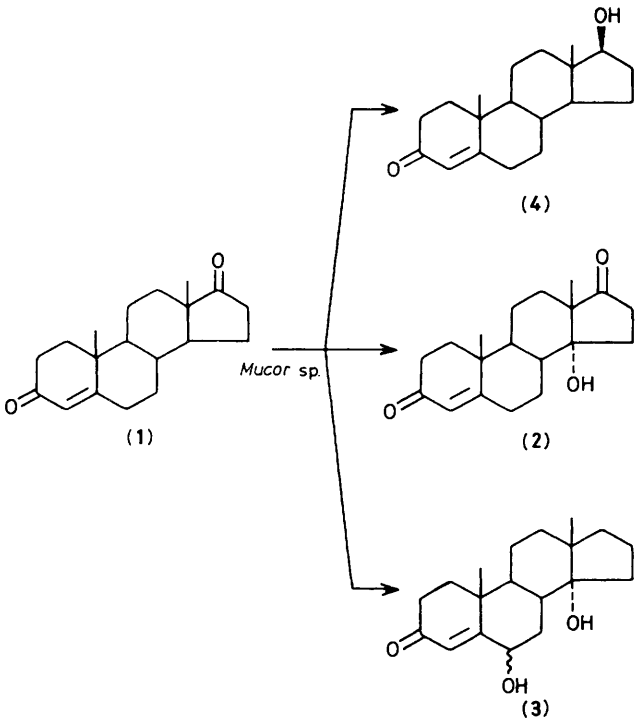
Madhava K. Madyastha* and Khadar Valli

Department of Organic Chemistry, Bio-organic Section Indian Institute of Science, Bangalore-560012, India

An efficient reduction of the 17-carbonyl group in a steroid skeleton to a methylene group has been observed for the first time during the microbial conversion of androst-4-ene-3,17-dione (**1**) and 14α-hydroxyandrost-4-ene-3,17-dione (**2**) into 6,14α-dihydroxyandrost-4-ene-3-one (**3**).

Many fungi have been used in the preparation of steroid hormones and their precursors, and reactions effected by fungal systems include: introduction of a double bond, conversion of a ketone into an alcohol, hydrolysis of an ester to an alcohol, reduction of a double bond, ring A aromatization, and hydroxylation.[1]

Organisms of the order *Mucorales* are known to exhibit a variety of steroid transforming activities.[2–4] We have recently isolated a fungal strain belonging to the genus *Mucor* which has been shown to effect preparatively useful conversions of (**1**). The most intriguing aspect of this organism is its unique ability to convert the 17-carbonyl group of (**1**) into a methylene group efficiently. This type of reduction mediated by a microbial system, a process similar to the non-enzymatic Clemmensen reduction of ketones, has not been observed before.



Fermentation† of (1) with *Mucor* sp. using standard procedures[5] resulted in the formation of a major metabolite (3) accounting for approximately 65% of the total transformation products formed, accompanied by small amounts of metabolites (2) and (4). Nearly 75% of (1) was metabolized in 24 h. Incubation of (2) with *Mucor* sp. resulted in high yields of the metabolite (3). The metabolites (2), (3), and (4) have been characterised spectroscopically.

Metabolites isolated from the culture broth were chromatographed on a silica gel column using 5—20% ethyl acetate in chloroform and purified individually on silica gel G coated plates with benzene–propan-2-ol (7:1) as solvent system. The minor metabolites (2) and (4) were identified as 14α-hydroxyandrost-4-ene-3,17-dione and testosterone respectively by comparison (i.r., n.m.r., mass spectra; t.l.c.) with authentic‡ samples.

The spectroscopic properties of the major metabolite (3) and its monoacetyl derivative§ indicated that it was the hitherto unknown 6,14-α-dihydroxyandrost-4-ene-3-one, the configuration at C-6 being uncertain. The ^{1}H n.m.r. spectrum of (3) suggested the presence of a proton on an allylic carbon atom bearing a hydroxy group, and the mass spectrum indicated that two hydroxy groups were present. The i.r. and ^{13}C n.m.r. spectra clearly showed the absence of a 17-oxo-group. The c.i. mass spectrum of the acetyl derivative suggested the presence of a tertiary hydroxy group. Treatment of (3) with toluene-*p*-sulphonic acid led to a linearly conjugated dienone system (λ_{max} 280 nm). These data, and the microbial conversion of (2) into (3), showed that the hydroxy groups had been introduced at C-6 and C-14 in (3).

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† Details of the fermentation conditions will be described in a full paper.

‡ An authentic sample of 14α-hydroxyandrost-4-ene-3,17-dione was kindly supplied by Prof. D. N. Kirk, M.R.C. Steroid Reference Collection, Westfield College, London.

§ (3): m.p. 174—175 °C (from CH_2Cl_2), $[\alpha]_D^{25}$ 148° ($CHCl_3$); λ_{max} ($CHCl_3$) 240 nm (α,β-unsaturated ketone); ν_{max} (Nujol) 3420—3360 (OH); 1660 and 1610 cm^{-1} (α,β-unsaturated ketone); δ (^{1}H; $CDCl_3$) 0.92 (3H, s, 18-H$_3$), 1.22 (3H, s, 19-H$_3$), 4.30 (1H, diffuse t, sharp t on D_2O exchange, 6-H), and 5.71 (1H, s, 4-H); *m/z* 304 (M^+), 286, and 268. Acetyl derivative: m.p. 195 °C (from CH_2Cl_2); ν_{max} (Nujol) 3420 (OH); 1720 (OAc); 1660 and 1610 cm^{-1} (α,β-unsaturated ketone); δ (^{1}H; $CDCl_3$) 0.95 (3H, s, 18-H$_3$), 1.20 (3H, s, 19-H$_3$), 2.06 (3H, s, OAc), 5.16 (1H, m, 6-H), and 5.72 (1H, s, 4-H); *m/z* [chemical ionisation (c.i.) with NH_3] 347 ($M^+ + 1$) and 329.