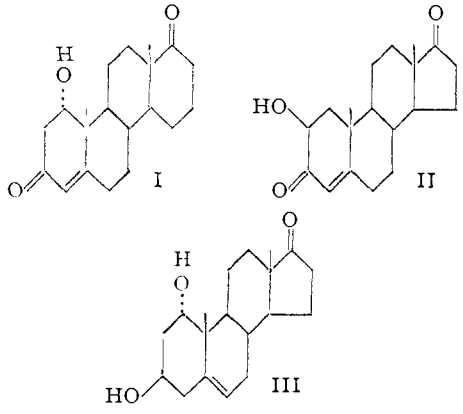
MICROBIOLOGICAL HYDROXYLATION OF C_{19}-STEROIDS AT POSITIONS C-1 AND C-2

Sir:

Extensive studies[1] have shown that steroids can be hydroxylated by microörganisms at positions 6β, 7α, 7β, 8- and/or 9-, 10, 11α, 11β, 14α, 15α, 15β, 16α, 17α and 21. We now wish to report the preparation of 1α-hydroxy-4-androstene-3,17-dione (I); m.p. 218–221°; $[\alpha]_D$ +184° ($CHCl_3$); $\lambda_{max}^{methanol}$ 240 mμ (15,000); (found: C, 75.32; H, 8.46); and 2β-hydroxy-4-androstene-3,17-dione (II); m.p. 143–145°; $[\alpha]_D$ −36.8° ($CHCl_3$); $\lambda_{max}^{methanol}$ 242 mμ (14,200); (found: C, 75.80; H, 8.71); by the microbiological action of a species of *Penicillium*, isolated from local soil, on 4-androstene-3,17-dione. By subjecting dehydroepiandrosterone to the same oxidative fermentation, we obtained, besides I, 1α-hydroxydehydroepiandrosterone (III); m.p. 275–277.5°; $[\alpha]_D$ +10.6° ($CHCl_3$); (found: C, 74.92; H, 9.21). The hydroxylated steroids were prepared by the fermentation and extraction techniques previously described,[2] and, in the case of the hydroxylated androstenediones, were purified by chromatography on silica gel. 1α-Hydroxydehydroepiandrosterone (III) was readily obtained by direct crystallization on concentration of the methylene chloride extract.



The structure of II was indicated by the contribution of the new hydroxyl to the molecular rotation of the compound (ΔM_D −658),[3a] by a positive "blue tetrazolium" test, and by the characteristic change of ultraviolet spectra with time in 0.1 *N* methanolic potassium hydroxide.[4] The structure was established by converting II to its acetate, m.p. 157–158°; $[\alpha]_D$ −5.9° ($CHCl_3$); $\lambda_{max}^{methanol}$ 243 mμ (15,300); (found: C, 73.47, H; 8.04); and epimerizing II acetate, by heating with anhydrous potassium acetate in glacial acetic acid,[5] to the known 2α-acetoxy-4-androstene-3,17-dione,[3b] m.p. 209–211°; $[\alpha]_D$ +146.8° ($CHCl_3$); $\lambda_{max}^{methanol}$ 239.5 mμ (15,500).

The structure of I was established by the conversion of I acetate, m.p. 112.5–113.5°; $[\alpha]_D$ +191.7° ($CHCl_3$); $\lambda_{max}^{methanol}$ 239.5 mμ (16,500); (found: C, 72.71; H, 8.07); by means of dilute methanolic sodium hydroxide to 1,4-androstadiene-3,17-dione,[6] m.p. and mixed m.p. 139–140°. Comparison of the infrared spectrum with that of an authentic sample confirmed identity. Oppenauer oxidation of III to 1,4-androstadiene-3,17-dione[6] established the structure of III.

The configuration of the 1-hydroxyl group in both I and III was initially assigned on the basis of molecular rotatory contributions.[7] The structure of III was definitively confirmed by its catalytic reduction to 1α,3β-dihydroxyandrostan-17-one (IV), m.p. 202–203.5°; $[\alpha]_D$ +88.2° ($CHCl_3$); (found: C, 74.32; H, 9.74); followed by reduction of IV with sodium borohydride to 1α,3β,17β-androstanetriol (V), m.p. 238–239°; $[\alpha]_D$ +20.2° ($CHCl_3$); (found: C, 73.99; H, 10.47). This compound (V) was identical in all respects with that prepared by Benn, Colton, and Pappo[8] by the reduction of 1α,2α-oxidoandrostane-3,17-dione.

Reduction of III with sodium borohydride or lithium aluminum hydride gave 5-androstene-1α,3β,17β-triol (VI), m.p. 212–213°; $[\alpha]_D$ −54.8° ($CHCl_3$); (found: C, 74.41; H, 10.22); triacetate, m.p. 179–181°; (found: C, 69.11; H, 8.24). Cautious acetylation of III produced 1α-hydroxy-3β-acetoxy-5-androsten-17-one (VII), m.p. 243–244°; $[\alpha]_D$ −6.7° ($CHCl_3$); (found: C, 72.58; H, 8.83); which was oxidized with chromium trioxide in pyridine to 3β-acetoxy-5-androstene-1,17-dione (VIII), m.p. 156–157.5°; $[\alpha]_D$ +40.7° ($CHCl_3$); (found: C, 73.27; H, 8.27). The reduction of VIII with sodium borohydride yielded VI and 5-androstene-1β,3β,17β-triol (IX), m.p. 270–278°; triacetate, m.p. 147.5–148.5°; $[\alpha]_D$ −32.4° ($CHCl_3$); (found: C, 69.11; H, 8.54). Compound IX and its triacetate were identical in all respects (m.p., mixed m.p., and infrared) with the corresponding triol and triacetate obtained from Ruscogenin by Benn, Colton, and Pappo.[8]

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MICROBIOLOGICAL TRANSFORMATION OF STEROIDS. 2β-HYDROXYLATION

Sir:

It has become increasingly evident that enzymatic hydroxylations of steroid occurring in the mammalian tissues will inevitably find their counterparts in microbially-induced transformations of the same or similar substrates. Of the hydroxylation processes occurring in mammals the