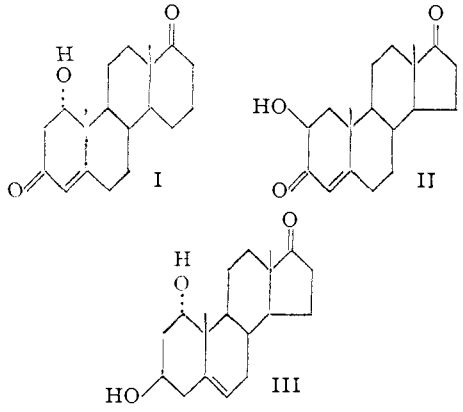
MICROBIOLOGICAL HYDROXYLATION OF C_{19}-STEROIDS AT POSITIONS C-1 AND C-2

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

reactions at 2α[1], 2β[2], 6α[3], 18-[4] and 19-[4] have not as yet been duplicated by incubations with microorganisms.[4a]

We wish to report that microbiological 2β-hydroxylation of 4-pregnene-17α,21-diol-3,20-dione (I, Reichstein's Compound S) has now been achieved with the aid of several unidentified *Streptomyces* species isolated from soil (Schering collection numbers FC7-206, FC6-53S, DS 81-B). Incubation of Compound S (600 mg.) in a peptone–soybean meal–yeast extract–cerelose medium with a 72-hour growth culture of *Streptomyces* sp. DS-81-B with rotary shaking at 28° for 48 hours afforded, after chloroform extraction and chromatography, 35 mg. of 4-pregnene-2β,17α,21-triol-3,20-dione (II), m.p. 215–220° dec. Further recrystallization from acetone–hexane raised the m.p. to 225.5–228° dec. and gave II with the following constants: $[\alpha]^{25}D$ −58° (dioxane), λ_{max}^{MeOH} 243 mμ (ϵ = 14,500), λ_{max}^{Nujol} 3.01 μ (OH), 5.81 μ (20-carbonyl), 5.94 μ (3-carbonyl) and 6.18 μ (Δ^4); three hydroxyl groups by integration of the OH band. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.81; H, 8.76. A polymorphic modification of II exists which possesses an altered infrared spectrum, λ_{max}^{Nujol} 2.87 μ (OH), 5.82 μ (20-carbonyl), 5.89 μ and 6.02 μ (3-carbonyl) and 6.20 μ (Δ^4). The latter polymorph is converted to the former by recrystallization from acetone–hexane and seeding with the former. The infrared spectra of the two forms differed in considerable detail from the 6β, 11α, 11β, 15α and 15β-hydroxy derivatives of I. The 2,21-diacetate of II, prepared with acetic anhydride and pyridine melts at 218–219°, $[\alpha]^{25}D$ +9° (dioxane), λ_{max}^{MeOH} 244 mμ (ϵ = 16,200), λ_{max}^{Nujol} 2.73, 2.83, 2.97 μ (OH), 5.71 μ (acetate carbonyl), 5.78 μ (20 carbonyl), 5.95 μ (3-carbonyl), 6.16 μ (Δ^4), 8.06 μ and 8.25 μ (C–O–C of acetate). Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 66.99; H, 7.74. The 21-monoacetate of II, prepared with one equivalent of acetic anhydride in pyridine solution, melted at 234–235°, $[\alpha]^{25}D$ −24° (dioxane), λ_{max}^{MeOH} 243 mμ (ϵ = 14,300), $\lambda_{max}^{CHBr_3}$ 2.86 μ (OH), 5.71 μ (acetate carbonyl), 5.76 μ (20-carbonyl), 5.95 μ (3-carbonyl), 6.17 μ (Δ^4) and 8.12 μ (C–O–C of acetate). Calcd. for $C_{23}H_{30}O_6$; C, 68.29; H, 7.97. Found: C, 68.00; H, 7.63.

The structure of II has been assigned on the basis of the following arguments and experiments. Appearance of the conjugated carbonyl band at 5.94 μ is interpreted as indicating a hydrogen bond interaction between the carbonyl at 3- and an hydroxyl group on an adjacent carbon atom. This is further substantiated by the observed interaction of the neighboring acetate with the 3-carbonyl in the 2,21-diacetate of II as well. The observed ultraviolet maximum of II excludes 4- as a site for neighboring hydroxyl since enols of α,β-diketones absorb in the region of 280 mμ. Measurement of the ultraviolet absorption in alkaline solution, according to Meyer,[5] after heating at 60° for 4 hours, afforded a curve which corresponded exactly in location of maxima with the highly characteristic curve from 2α-hydroxy-4-androstene-3,17-dione.[5] The rotation of the 2,21-diacetate of II differs markedly from that reported for 4-pregnene-2α,17α,21-triol-3,20-dione 2,21-diacetate[6] ($[\alpha]^{25}D$ +122° (chloroform), m.p. 200–202°, 215–217° (polymorphs)). In like fashion II contrasts with the 4-pregnene-2α,17α,21-triol-3,20-dione, $[\alpha]^{25}D$ +130° (chloroform), m.p. 219–221°. Since 2β-hydroxyl reaches equilibrium with 2α-hydroxyl in mildly alkaline solution,[7] it is obvious that the same alkaline ultraviolet spectrum must result for both configurations in a given pair of 2-hydroxy-3-keto-Δ^4-steroids.[5] Hence, II must contain a 2β-hydroxyl group.

The assignment is corroborated by the fact that the predicted shift in molecular rotation (ΔM-581)[7] based on the only 2β-hydroxy-3-keto-Δ^4-steroid known previously, 2β-hydroxytestosterone 2,17-diacetate[7] (2β-hydroxytestosterone itself[2] is characterized incompletely), is in reasonable agreement with the observed values of the shift for 21-acetate of I *vs.* 2,21-diacetate of II (ΔM-500). This is especially noteworthy since the 2β-hydroxyl group contributes much more strongly to the levorotation of 3-keto-Δ^4-steroids than does any other hydroxyl group.

It is known that hydroxyl groups at 2- or 6- in 3-keto-Δ^4-steroids can be removed reductively by mild treatment with zinc and acetic acid, affording the parent steroid.[7] Treatment according to this method converted II 2,21-diacetate into I 21-acetate, indistinguishable from an authentic sample by infrared comparison.

We hope to report a more complete proof of structure, relating II with 2β-hydroxytestosterone by degradation, at a later date. The chemistry of the 2β-hydroxyl group, heretofore a rather inaccessible function, is being studied.

We wish to acknowledge the helpful advice of Dr. André Meyer in the interpretation of the alkaline ultraviolet spectra.

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TRANSFORMATION OF STEROIDS BY FUNGI. INTRODUCTION OF 1ξ AND 2β-HYDROXYL GROUPS INTO REICHSTEIN'S COMPOUND S.

Sir:

Heretofore, no unambiguous microbiologically induced 1-hydroxylation of steroid substrates has