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.

(1) S. Burstein, THIS JOURNAL, **78**, 1769 (1956).

(2) L. Axelrod, L. I. Miller and F. Herling, *J. Biol. Chem.*, **219**, 455 (1956).

(3) A. S. Meyer, M. Hayano, M. C. Lindberg, M. Gut and O. G. Rodgers, *Acta Endocrin.*, **18**, 148 (1955).

(4) F. W. Kahnt, R. Neher and A. Wettstein, *Helv. chim. acta*, **38**, 1237 (1955).

(4a) C. S. H. Eppstein, *et al.*, *Vitamins and Hormones* **14**, 339 (1956).

(5) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

(6) G. Rosenkranz, O. Mancera and F. Sondheimer, THIS JOURNAL, **77**, 145 (1955).

(7) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, *ibid.*, **75**, 4712 (1953).

CHEMICAL RESEARCH DEPARTMENT — HERSHEL L. HERZOG
DEPARTMENT OF INDUSTRIAL MICROBIOLOGY, SCHERING CORP., BLOOMFIELD, NEW JERSEY — MARGARET JEVNIK GENTLES, E. B. HERSHBERG
INSTITUTE OF MICROBIOLOGY, RUTGERS UNIVERSITY, NEW BRUNSWICK, NEW JERSEY — FERNANDO CARVAJAL, DAVID SUTTER, WILLIAM CHARNEY, CARL P. SCHAFFNER

RECEIVED JUNE 3, 1957

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

been observed.[1] In the preceding communication[2] we have described the first 2β-hydroxylation of Compound S with *Streptomyces* sp. We wish now to report the microbiological hydroxylation of 4-pregnene-17α,21-diol-3,20-dione (I, Reichstein's Compound S) in the 1ξ and 2β positions by *Rhizoctonia ferrugena* (CBS, Holland). The fermentation of I (3.0 g.) by *R. ferrugena* was carried out in a medium containing corn steep liquor, ammonium dihydrogen phosphate, yeast extract, dextrose, soybean oil and calcium carbonate. In the fermentation stage a forty-eight hour culture was incubated with the steroid substrate (introduced in ethanolic solution) on a rotary shaker for five days at 23°. The culture filtrate was extracted with chloroform and chloroform–methanol (4:1), and the extracts were concentrated to dryness in vacuo below room temperature. The residue was redissolved in dry, acid and alcohol-free chloroform and the solution was chromatographed on a silicic acid adsorption column. A methanol–chloroform gradient elution[3] was utilized to separate the two steroidal transformation products and residual substrate. The steroid substrate, I, was collected in the first fractions, the 2β-hydroxylated product, II, in the middle fractions and the 1-hydroxylated product, III, in the last fractions. Homogeneity was indicated by paper chromatography.

Crystallization of the middle fractions from chloroform–ether mixture afforded 101 mg. of 4-pregnene-2β,17α,21-triol-3,20-dione (II), m.p. 214–220° dec. Further recrystallization from acetone–hexane raised the m.p. to 220–222° dec. The infrared spectrum of II was identical with that of an authentic sample,[2] and the mixed m.p. showed no depression.

The crystallization of the last group of fractions (Product III) afforded 163 mg. of crystalline solid, m.p. 180–200° dec. Cautious recrystallization from acetone–hexane without application of heat, raised the m.p. to 193–207° dec. (with a transition of about 170°), $[\alpha]^{25}D$ +89 (dioxane) (corrected for acetone of solvation), λ_{max}^{MeOH} 241 mμ (ϵ = 16,500 —corrected for acetone of solvation), λ_{max}^{Nujol} 2.83 μ (OH), 5.81 μ (20-carbonyl), 6.04 μ (3-carbonyl), 6.18 μ (Δ^4) (one additional polymorphic variety has been observed.) Calcd. for $C_{21}H_{30}O_5 \cdot C_3H_6O$: C, 68.54; H, 8.63. Found: C, 68.41; H, 8.66.

The identity of III was established in the following manner. Integration of the hydroxyl bands of the infrared spectrum confirmed the presence of three hydroxyl groups. Measurement of the ultraviolet spectrum of III in alkaline solution[4] showed a shift in the maximum to 245 mμ but no peak in the 380 mμ region after two hours at 60°. This is consistent with the conversion of a 1-hydroxy-3-keto-Δ^4-steroid into a $\Delta^{1,4}$-diene-3-keto-steroid, which transformation would be expected to occur under these conditions. Also the ultraviolet spectrum of III was strikingly different from that of a 2-hydroxy-3-keto-Δ^4-steroid in alkaline solution.[2,4] The presence of the dienone among the products from the alkaline treatment of III was further substantiated by the measurement of the polarographic reduction potential of the solution.[5] A shift in the half-wave potential from 1.46 volts (III) to 1.31 volts (after alkaline treatment) corresponds well to the shifts observed for cortisone *vs.* prednisone (0.16 volt) and cortisol *vs.* prednisolone (0.17 volt). Furthermore, pyrolysis of a sample of III at the melting point for ten minutes afforded a mixture of products whose infrared spectrum contained the characteristic $\Delta^{1,4}$ diene-3-one bands.

Finally, a solution of III in glacial acetic acid was refluxed for one hour. Upon concentration of the solution *in vacuo*, chromatography of the residue on Florisil and recrystallization from acetone–hexane there was obtained 1,4-pregnadiene-17α,21-diol-3,20-dione, m.p. 237–240°, whose infrared spectrum was identical with that of an authentic sample.[6] From this evidence we conclude that the structure of III is clearly 4-pregnene-1ξ,17α,21-diol-3,20-dione. The configuration of the 1-hydroxyl group remains to be fixed.

With cortisol, cortisone, corticosterone, desoxycorticosterone, progesterone and testosterone transformations occur upon incubation in the presence of *R. ferrugena* as determined by paper chromatographic examination of the product. The structure of the transformation products in these instances remains to be elucidated.

Other species of *Rhizoctonia* have effected the transformation of I into cortisol, cortisone and 11-epicortisol; a more detailed account of these findings will be published subsequently. Additional conversion products obtained with organisms of this genus are now under investigation.

We wish to make the following acknowledgments. From the Schering Corporation we acknowledge the helpful advice of Drs. André Meyer and Alexander Nussbaum, the assistance of Dr. P. Kabasakalian and Mr. J. McGlotten who carried out the polarographic reductions, of Dr. J. Y. Chen who interpreted the infrared spectra, and of Mrs. M. J. Gentles. From the Institute of Microbiology we acknowledge the technical assistance of Mr. A. Chernov. We also wish to thank Dr. C. M. Haenseler for his helpful advice and for various cultures of *Rhizoctonia*.

(1) S. Kushinsky (Abstracts of Papers, 131st Meeting of the Am. Chem. Soc., Miami, Florida, April 7–12, 1957, p. 36-O) mentions the isolation of a 1- or 2-hydroxy intermediate from conversion of 19-nortestosterone 17-acetate to estradiol 17-acetate by the action of *Corynebacterium simplex*.

(2) H. L. Herzog, M. J. Gentles, F. Carvajal, D. Sutter, W. Charney, E. B. Hershberg and C. P. Schaffner, THIS JOURNAL, **79**, 3921 (1957).

(3) T. K. Lakshmanan and S. Lieberman, *Arch. Biochem. Biophys.*, **53**, 258 (1954).

(4) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

(5) P. Kabasakalian, S. DeLorenzo and J. McGlotten, *Anal. Chem.*, **28**, 1669 (1956).

(6) A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, THIS JOURNAL, **77**, 4184 (1955).

INSTITUTE OF MICROBIOLOGY — GEORGE GREENSPAN
RUTGERS UNIVERSITY — CARL P. SCHAFFNER
NEW BRUNSWICK, N. J. — WILLIAM CHARNEY
SCHERING CORPORATION — HERSHEL L. HERZOG
BLOOMFIELD, NEW JERSEY — E. B. HERSHBERG

RECEIVED JUNE 3, 1957

CYCLOPENTENE-3,5-DIONE

Sir:

The synthesis of many substituted and unsaturated cyclopentane derivatives of theoretical