Communications TO THE EDITOR

1-Hydroxylation of 9α-Fluorohydrocortisone

Sir:

Two reports of 1-hydroxylation of steroids induced by microorganisms have appeared recently. The first[1] relates to the production of 1α-hydroxy-4-androstene-3,17-dione and 1α-hydroxydehydroepiandrosterone with species of *Penicillium* and the second[2] to the 1ξ-hydroxylation of 4-pregnene-17α,21-diol-3,20-dione (Reichstein's Substance S) by *Rhizoctonia ferrugena*. In the latter communication reference was made to unidentified products arising from similar incubations with cortisone and hydrocortisone as substrates. We wish now to report on the formation of 1ξ-hydroxy-9α-fluorohydrocortisone upon the incubation of 9α-fluorohydrocortisone 21-acetate (I) with a species of *Streptomyces* (Merck collection number MA 320). The substrate I (80.0 g) was incubated for 72 hours with *Streptomyces sp.* in 400 l. of broth which had been pregrown for 48 hours in an Edamin-cerelose-cornsteep medium. The culture filtrate was extracted with ethyl acetate and the extract concentrated *in vacuo* at 45° to a viscous oil. The residue was triturated with petroleum ether (30–60°) to remove excess oils, dissolved in benzene:ethyl acetate (9:1) and charged to a column of Super-Cel saturated with water:methanol (1:1). Development of the column with benzene:ethyl acetate (9:1) brought the steroidal substrate off in the first fractions, the 1ξ-hydroxylated product in the middle fractions and 20-dihydro-9α-fluorohydrocortisone in the final fractions. Subsequent development with benzene:ethyl acetate (8:2) eluted a more polar product, 6β-hydroxy-9α-fluorohydrocortisone.

Combination of the middle fractions yielded 5.9 g. of crude 1ξ-hydroxy-9α-fluorohydrocortisone (II). Recrystallization first from acetone and then from methanol yielded 1.9 g. of white crystalline material in the first crop, m.p. 247–252°, λ_{max}^{MeOH} 237 mμ, $\epsilon^{\%}$ 425, λ_{max}^{Nujol} 2.9 μ (OH), 5.89 μ (20 carbonyl), 6.02 μ (α,β-unsaturated ketone). Calcd. for $C_{21}H_{29}O_6F$: C, 63.56; H, 7.31. Found: C, 63.94; H, 7.60. Homogeneity was also indicated by paper strip chromatography.

The isolated alcohol was treated with acetic anhydride in pyridine at room temperature for 16 hours to yield a diacetate, III, m.p. 218–221°C., λ_{max}^{MeOH} 238 mμ, $\epsilon^{\%}$ 343. Calcd. for $C_{25}H_{33}O_8F$: C, 62.50; H, 6.88. Found: C, 62.63; H, 7.14.

The identity of III was established by converting the diacetate to 1-dehydro-9α-fluorohydrocortisone 21-acetate IV. This was effected by refluxing a portion of the diacetate in glacial acetic acid for 1 hour. Paper strip chromatographic examination of the reaction mixture showed it to contain principally IV contaminated with traces of starting material. The solution was evaporated to dryness *in vacuo*, the residue chromatographed over acid-washed alumina and the eluted III, freed of starting material, was crystallized twice from acetone-Skellysolve B, m.p. 225–236°, λ_{max}^{MeOH} 238 mμ, $\epsilon^{\%}$ 358. Calcd. for $C_{23}H_{29}O_6F$: C, 65.64; H, 6.90. Found: C, 65.92; H, 6.97. Mixed melting point with an authentic sample gave no depression and the infrared spectra were identical. The original alcohol can be similarly converted to 1-dehydro-9α-fluorohydrocortisone. From this evidence it is clear that the fermentation product is 1ξ-hydroxy-9α-fluorohydrocortisone. The configuration of the 1-hydroxy group has not yet been established.

Other species of *Streptomyces* have been found to 1-hydroxylate 9α-fluorohydrocortisone. It is our impression that this is an ubiquitous transformation with *Streptomyce* cultures.

Surprisingly enough we found that I is virtually inactive in the liver glycogen and systemic granuloma assays. In the sodium metabolism test I appears to be less strongly active, in retaining Na^+, than the parent 1-desoxy compound.

Other substrates (hydrocortisone, cortisone, Reichstein's Substance S and progesterone) were incubated with the organisms and although transformation products were formed there was no evidence of 1-hydroxylation with these steroids.

MERCK SHARP AND DOHME RESEARCH LABORATORIES
DIVISION OF MERCK & CO., INC.
RAHWAY, N. J.

W. J. MCALEER
M. A. KOZLOWSKI
T. H. STOUDT
J. M. CHEMERDA

Received January 27, 1958

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(2) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog, and E. B. Hershberg, *J. Am. Chem. Soc.*, **79**, 3922 (1957).