changed I (peak A) was identified by recrystallization with carrier from water to constant specific activity. The radioactivity in peak B could be volatilized by treatment with ammonia and acidification with formic acid, identifying it indirectly as II.[7] Peak D was shown to be hydantoin-5-propionic acid (IV) by recrystallization with carrier to constant specific activity from both ethanol–benzene and water and co-chromatography in six solvents with the synthetic compound.[8] Synthetic IV (Found: C, 41.69; H, 4.60; N, 16.29) prepared from L-glutamic acid[9] was eluted in the identical position as peak D. The dotted line in Fig. 1 is synthetic radioactive IV. Peaks A–D have 72, 1.1, 1.5 and 8.5% of the urinary radioactivity, respectively.

Incubation of I with rat liver slices forms small amounts of IV in the presence or absence of III; radioactive IV is present in monkey and human urine after intravenous C^{14} histidine.

The biochemical steps from I to IV have not yet been elucidated.

Acknowledgment.—The authors are grateful to Dr. Herbert Tabor for his helpful criticism.

(7) B. A. Borek and H. Waelsch, *J. Biol. Chem.*, **205**, 459 (1953).

(8) Synthetic IV sprayed with 0.1 *M* $AgNO_3$: 0.1 *M* NH_4OH (1:1) is white against a brown background. Radioautograph spots matched the outline and position of stained spots exactly. R_F values for benzene: 1-butanol:methanol:H_2O (1:1:2:1), 2-butanol:formic acid:H_2O (19:2:6), acetic acid:1-butanol:ethyl acetate:H_2O (1:1:1:1), ethanol:ether:H_2O:7.4 *N* NH_3 (4:5:1:0.1), 1-propanol:1 *N* acetic acid (3:1), 2-propanol:NH_3:H_2O (8:1:1) were 0.64, 0.67, 0.68, 0.13, 0.69, 0.07, respectively.

(9) H. D. Dakin, *Biochem. J.*, **13**, 398 (1919).

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MICROBIOLOGICAL TRANSFORMATIONS. III.[1] THE HYDROXYLATION OF STEROIDS AT C-9

Sir:

We wish to report the microbiological preparation and proof of structure of 9α-hydroxy-4-androstene-3,17-dione. This compound and the method used to establish its structure may help resolve the difficulties previously experienced in the formulation of 8- or 9-hydroxysteroids.[2]

Fermentation of 4-androstene-3,17-dione, by the methods previously described,[3] with a species of Nocardia (A20-10) isolated from soil produced 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione[1] and a monohydroxy-4-androstene-3,17-dione (I), m.p. 222–223.5°; $\lambda_{max.}^{methanol}$ 241 mμ (ϵ 16,100); $[\alpha]_D$ +181.7° ($CHCl_3$); λ_{max}^{KBr} 2.92 μ (—OH), 5.76 μ (17 C═O), 6.02 μ and 6.19 μ (3 C═O, Δ^4); (found: C, 75.21; H, 8.68). The hydroxy-4-androstene-3,17-dione (I) was recovered unchanged when treated with pyridine and acetic anhydride. Fermentation of I with a species of Arthobacter (B 20-178) that converts 4-androstene-3,17-dione to 1,4-androstadiene-3,17-dione in excellent yield, gave 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione. The latter compound was purified as its acetate, m.p. 143.5–146°, which proved to be identical in all respects (m.p., mixed m.p., and infrared) with the 9,10-seco-3-acetoxy-1,3,5(10)-androstatriene-9,17-dione reported previously.[1] Thus, the positions of the three oxygen atoms in I were established. The 9α-configuration was assigned to the new hydroxyl group because of its molecular rotatory contribution ($\Delta M_D^{OH—H} = -18$)[4] and because of the recent evidence that microbiologically introduced hydroxyl groups have the same configuration as the hydrogens replaced.[5]

In the aromatization-degradation of 4-androstene-3,17-dione it seems probable that this species of Nocardia[6] first hydroxylates at C-9 then introduces the Δ^1-double bond. This is just the opposite sequence originally found with *Pseudomonas.*[1] A paper chromatographic study of the fermentation of 9α-hydroxy-4-androstene-3,17-dione with *Pseudomonas* showed the formation of, at most, only trace quantities of phenolic material. With Pseudomonas the sequence in which the reactions occur seems to be limited.

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(1) Previous paper: R. M. Dodson and R. D. Muir, THIS JOURNAL, **80**, 5004 (1958). The numbers assigned to the organisms are our laboratory designations.

(2) (a) S. H. Eppstein, P. D. Meister, D. H. Peterson, H. C. Murray, H. M. Leigh Osborn, A. Weintraub, L. M. Reineke and R. C. Meeks, *ibid.*, **80**, 3382 (1958); (b) D. Stone, M. Hayano, R. I. Dorfman, O. Hechter, C. R. Robinson and C. Djerassi, *ibid.*, **77**, 3926 (1955).

(3) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, *ibid.*, **74**, 5933 (1952).

(4) The molecular rotatory contribution of the 9α-hydroxyl group in 3β-acetoxyergostan-9α-ol was −31. A. S. Hallsworth and H. B. Henbest, *J. Chem. Soc.*, 4604 (1957). The molecular rotatory contribution of the new (8 or 9) hydroxyl group in the steroids hydroxylated with *Helicostylum piriforme*, *Mucor parasiticus*, *Mucor griseocyanus* and *Neurospora crassa* (Ref. 2) indicates the probability of 9α, rather than 8β, hydroxylation. See: S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, "Vitamins and Hormones," Vol. XIV, 388 (1956), Academic Press, Inc., New York, N. Y. However, the specific rotation of the previously described 8β (or 9α)-hydroxy-4-androstene-3,17-dione,[2a] m.p. 214–217°, $[\alpha]_D$ +165° ($CHCl_3$), obtained via the hydroxylation of 11-deoxycortisol with *H. Piriforme*, does not agree with ours.

(5) (a) M. Hayano, M. Gut, R. I. Dorfman, O. K. Sebek and D. H. Peterson, THIS JOURNAL, **80**, 2336 (1958); (b) E. J. Corey, G. A. Gregoriou and D. H. Peterson, *ibid.*, **80**, 2338 (1958).

(6) We have isolated another strain of Nocardia (A20-9) which apparently follows the alternate sequence.

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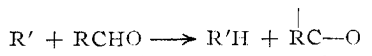
FREE RADICAL ADDITION OF CYCLOPENTANE AND CYCLOHEXANE TO FORMALDEHYDE

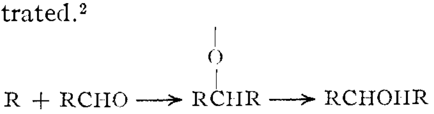
Sir:

Although alkyl free radical attack upon an aldehyde is most likely to result in hydrogen abstraction[1]



$$R' + RCHO \longrightarrow R'H + \overset{|}{R}C—O \qquad (1)$$

addition to the carbonyl group also has been demonstrated.[2]



$$R + RCHO \longrightarrow R\overset{\overset{|}{O}}{\overset{|}{C}}HR \longrightarrow RCHOHR \qquad (2)$$

Consideration of reaction (2) leads to the conclusion that it should be possible to bring about

(1) For summary, see Steacie, "Atomic and Free Radical Reactions," 2nd Ed., Reinhold Publishing Corp., New York, N. Y., 1954.

(2) F. F. Rust, F. H. Seubold and W. E. Vaughan, THIS JOURNAL, **70**, 4253 (1948).