In view of the unique character of the iron compound, and of the inherent difficulties in the precise formulation of the covalent complexes of the transition metals, particularly those with unsaturated hydrocarbons, detailed proposals with respect to the electronic structure of iron biscyclopentadienyl would be premature. However, it may be noted, that the number of electrons available (but not necessarily used) for iron to carbon binding, is eighteen (five π electrons for each cyclopentadienyl unit, plus the eight electrons of the iron atom). Thus the effective atomic number of the central iron atom is thirty-six (krypton structure) as in the ferrocyanide ion and in iron pentacarbonyl. Details of hybridization will determine the precise geometry of the molecule. For example, while the compound is formulated above as a pentagonal anti-prism, a prismatic structure (III) such as might result from split d^3p^2 plane pentagonal bonding, is not excluded.

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MICROBIOLOGICAL HYDROXYLATION OF PROGESTERONE

Sir:

The dehydrogenation of steroid alcohols by bacteria and the reduction of steroid ketones by yeasts has been studied in great detail by Mamoli, Vercellone, Butenandt and their collaborators.[1] Trufitt[2] has effected oxidative ring cleavage and removal of the steroidal side chain by the use of *Proactinomyces* species. We wish to report a novel type of microbiological oxidation, *viz.*, the introduction of one or more hydroxyl groups into the intact steroid nucleus by an unidentified actinomycete[3] in submerged culture.

In the example reported here progesterone (0.25 g./l.) was used as the substrate in a simple medium containing glycine, glutamate, soybean oil and inorganic salts. Media containing soybean meal, dried brewers' yeast or cornsteep liquor could be substituted for the above. After incubation for three days at 25° the culture was filtered and the oxidized steroids recovered from the filtrate by chloroform extraction followed by distribution between 80% methanol and hexane. The crystalline residue (8.7 g., from 17.5 g. of progesterone) from the alcoholic phase was chromatographed on magnesium silicate-celite and yielded three hitherto undescribed derivatives of progesterone. The major product eluted with chloroform-benzene 1:1 and also obtained directly by recrystallization of the above residue was 16α-hydroxyprogesterone (I), m.p. 225–226°; $[\alpha]^{23}D$ +158° (*c*, 0.65 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 239 mμ (ϵ = 17,000); $\lambda_{max.}^{Nujol}$ 3.04μ (OH); 5.90μ (20-keto); 6.02 and 6.20μ (3-keto, $\Delta^{4,5}$). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 76.61; H, 9.36, Monoacetate, m.p. 134–135°; $[\alpha]^{22}D$ +107° (*c*, 0.33 in $CHCl_3$). *Anal.* Calcd. for acetyl: 11.6. Found: acetyl, 11.6. The position of the hydroxyl group in this substance followed from its conversion into the known Δ^{16}-dehydroprogesterone[4] (m.p. 190–191.5°[5]; $[\alpha]^{23}D$ +134.5° (*c*, 0.90 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 240 mμ (ϵ = 28,400) by means of aluminum butylate. The latter reaction has its analogy in the conversion of allopregnanetriol-3β,16α,20β (Marrian's triol) to Δ^{16}-allopregnenedione-3,20 by means of aluminum isopropylate.[6] The contributions to the molecular rotation made by the 16-hydroxyl group in the free ($[M]_D^{OH-H}$ = −82°) and in the acetylated form ($[M]_D^{OAc-H}$ = −205°) strongly suggest the α-orientation for that group.[7]

Preceding I in the chromatogram was pregnanol-16α-dione-3,20 (II) present in small amount only, m.p. 199–200°; $[\alpha]^{23}D$ +90.5° (*c*, 0.82 in $CHCl_3$)[8]; $\lambda_{max.}^{alc.}$ 284 mμ (ϵ = 65). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.72. Found: C, 76.12; H, 9.73. The latter on treatment with aluminum *t*-butylate yielded Δ^{16}-pregnenedione-3,20, m.p. 196–198°; $[\alpha]D$ +83°; $\lambda_{max.}^{alc.}$ 239 mμ (ϵ = 9100).[9]

A third substance present in small amount was eluted with chloroform–acetone 3:1, m.p. 215.5–16.5°; $[\alpha]^{24}D$ −39° (*c*, 0.76 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 243 mμ (ϵ = 14,400). It had the composition of a dihydroxyprogesterone. *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 73.09; H, 8.68.

(1) For a review on this subject see the chapter by F. G. Fischer, "Biochemical Oxidations and Reductions" in Newer Methods of Preparative Organic Chemistry, Interscience Publishers, Inc., New York, N. Y., 1948.

(2) G. E. Turfitt, *Biochem. J.*, **42**, 376 (1948).

(3) The culture carries the designation MD-2428 in our own collection.

(4) A. Butenandt and J. Schmidt-Thomé, *Ber.*, **72**, 182 (1939); *cf.* also D. K. Fukushima and T. Gallagher, THIS JOURNAL, **73**, 196 (1951).

(5) A mixture melting point of this material with an authentic sample of Δ^{16}-dehydroprogesterone kindly supplied by Dr. Carl Djerassi (m.p. 188–190° after three recrystallizations) showed no depression.

(6) R. E. Marker and D. L. Turner, THIS JOURNAL, **62**, 2541 (1940).

(7) The average values for $[M]_D^{16\alpha-OH-H}$ and for $[M]_D^{16\alpha-OAc-H}$ are −64° and −284°, respectively, while those for 16β-substituted derivatives are +38° and +98°, respectively. *Cf.* H. Hirschmann and F. Hirschmann, *J. Biol. Chem.*, **184**, 259 (1950), and D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **73**, 196 (1951).

(8) The value for $[M]_D^{I-II}$ (+221°) is in good agreement with that for $[M]_D$ Δ^4-cholestenone-coprostanone (+203°).

(9) A. Butenandt, L. Mamoli and A. Heuser, *Ber.*, **72**, 1616 (1939).

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HYDRODEXTRAN

Sir:

A solution of degraded dextran has been shown to be an effective blood volume extender in clinical studies.[1] Occasional side reactions in man following dextran infusions have been observed.[2] At the present time, specifications for clinical dextran require a weight average molecular weight by light scattering of 75,000 ± 25,000 with the upper 5 to 10% having a weight average molecular weight not exceeding 200,000 and the lower 5 to 10% having a weight average molecular weight above 25,000.[3] In the usual preparation of clinical dextran, native

(1) B. Ingelman, *Upsala Läkarefören Förh.*, **54**, 107 (1949).

(2) Unpublished results reported to National Research Council, Subcommittee on Shock.

(3) U. S. Military Medical Purchase Description, 1-161-890, May 24 (1951).