TABLE I

PARTIAL PEPSIN AND ACID HYDROLYSIS OF PREPARATION E

Experiment	Dose, μg.	No. of rats	Average ascorbic acid depletion, mg./100 g. adrenals	Estimated ACTH potency, USP units/mg.
Starting material	0.05	22	84	30
Pepsin[a]	0.05	15	96	52
Acid[b]	0.03	16	132	(270)

[a] 100 mg. Preparation E and 1.25 mg. crystalline pepsin were dissolved in 50 cc. 0.01 *M* HCl and kept at 37° for 3 hr. [b] 1% solution of Preparation E in 0.2 *M* HCl was kept at 100° for 3 hr.

anode and cathode sections according to the ninhydrin-reactive areas. These sections were cut out and eluted with 0.1 *M* HCl for nitrogen determination and bioassay. Results are summarized in Table II; it is evident that part of the ACTH activity migrated to the cathode at *p*H 8 and stayed in the origins at *p*H 9 and 10, while at *p*H 12 it was the anodic fraction which possessed most of the hormonal potency. Thus, it may be concluded that the isoelectric point of the component with which the ACTH activity is associated is below that of the crystalline lysozyme and is located in the neighborhood of *p*H 9, indicating the basic nature of the hormone.

TABLE II

NITROGEN DISTRIBUTION AND BIOLOGIC ACTIVITY OF FRACTIONS OBTAINED FROM PAPER ELECTROPHORESIS OF ACTH PREPARATIONS AT VARIOUS *p*H

*p*H	Fractions from paper electrophoresis	Nitrogen distribution, %	ACTH potency, USP units/mg.
12	Origin	16	5
	Anode	84	7
10	Origin	84	40
	Anode	9	0
	Cathode	7	0
9	Origin	75	50
	Anode	11	0
	Cathode	14	0
8	Origin	36	30
	Anode	10	0
	Cathode	54	70

Further purification of Preparation E by various techniques is being investigated, and results will be reported in subsequent communications. The accompanying letter discusses the further purification of Preparation E by cellulose column chromatography.

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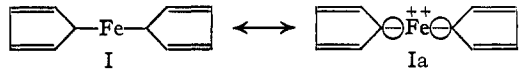
RECEIVED MARCH 22, 1952

THE STRUCTURE OF IRON *BIS*-CYCLOPENTADIENYL

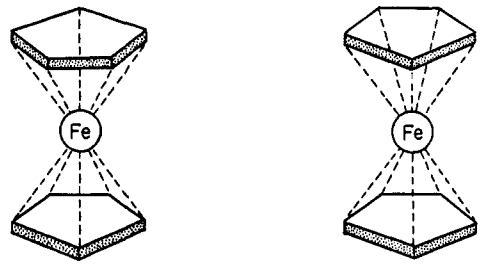
Sir:

In a recent publication, Kealy and Pauson[1] described the preparation of a compound, $C_{10}H_{10}Fe$, which was formulated as dicyclopentadienyl iron (I ⟷ Ia). This substance was found to be very stable toward acids and bases, and its isolation



I Ia

stands in striking contrast to the failure of previous investigators to prepare stable organo-iron compounds. These circumstances led us to consider whether an alternative to the structure (I ⟷ Ia) might be more nearly in accord with the unique character of Kealy and Pauson's compound. The equal unsaturation of each of the carbon atoms of the cyclopentadienyl anion suggested that two such units might form covalent bonds to ferrous iron symmetrically. The evidence summarized below provides strong support for the resulting structure (II).



II III

Iron biscyclopentadienyl is diamagnetic, with $\chi^{25°}_{mole} = -125 \times 10^{-6}$ cgsu. The infrared absorption spectrum contains in the 3–4μ region a single sharp band at 3.25μ, which indicates the presence in the compound of C–H bonds of only one type. The ultraviolet spectrum shows maxima at 326 mμ ($\epsilon = 50$) and 440 mμ ($\epsilon = 87$), and rising end absorption of moderate intensity ($\epsilon = 5250$ at 225 mμ). The dipole moment is effectively zero (0.05 *D*). The compound has detectable vapor pressure at 0° and resists pyrolysis at 470°.

Iron biscyclopentadienyl is readily oxidized to a blue cation $[Fe(C_6H_5)_2]^+$. The blue color observed by Kealy and Pauson to accompany dissolution of the iron compound in sulfuric or nitric acid is undoubtedly attributable to this change. Oxidation may be effected anodically, by air in the presence of acids, or by halogens, ferric chloride or ceric sulfate. Especially convenient are aqueous silver sulfate or *p*-benzoquinone in organic solvents in the presence of acids. The cation is reduced by stannous chloride. Polarographic studies indicate an oxidation–reduction potential of −0.59 v. The cation has been isolated as the crystalline tetrachlorogallate (*Anal.* Calcd. for $C_{10}H_{10}FeGaCl_4$: C, 30.25; H, 2.54; Fe, 14.02; Ga, 17.52; Cl, 35.70. Found: C, 30.47; H, 2.77; Fe, 13.97; Ga, 17.52; Cl, 35.76.) and the picrate (*Anal.* Calcd. for $C_{16}H_{12}N_3O_7Fe$: C, 46.35; H, 2.92; N, 10.13; Fe, 13.48. Found: C, 45.99; H, 3.27; N, 10.05; Fe, 13.43.). Most salts with common anions are readily soluble in water; however, a pale blue silicotungstate precipitates from acid solutions. The perchlorate in 1 *N* perchloric acid showed $\lambda\lambda_{max.}$ 253 mμ ($\epsilon = 13{,}300$) and 619 mμ ($\epsilon = 360$). For the picrate, $\chi^{25°}_{mol} = +2140 \times 10^{-6}$ cgsu.; the effective paramagnetic moment ($\mu_{eff} = 2.26$ B.M.) suggests the presence of one unpaired electron, as in the ferricyanide ion ($\mu_{eff} = 2.33$ B.M.).

(1) Kealy and Pauson, *Nature*, **168**, 1039 (1951).

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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RECEIVED MARCH 21, 1952

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).