perimental support to the postulate of orbital stabilization.[4] The susceptibility of the *mono* compound, 1.8 B.M., corresponds to one unpaired electron. This is not consistent with the theoretical values expected from the simple formula [FedipyCl_2] and may suggest a certain amount of metal-metal interaction. Detailed studies on these and similar compounds will be published later.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY FRED BASOLO
EVANSTON, ILLINOIS FRANK P. DWYER

RECEIVED JANUARY 13, 1954

THE OXYGEN-HYDROGEN PEROXIDE COUPLE AT THE DROPPING MERCURY ELECTRODE

Sir:

The first oxygen wave of the dropping mercury electrode (D.M.E.) arises from the two electron reduction of oxygen to hydrogen peroxide. Although this wave has universally been considered highly irreversible,[1] it was recently reported[2] that in unbuffered basic solutions hydrogen peroxide yields an anodic wave at the same potential. From this and other observations the investigators concluded that the oxygen–hydrogen peroxide reaction is completely reversible, but involves only one electron.

These conclusions are not consonant with the fact that H_2O_2 (or HO_2^-) and O_2 must be the diffusing species, and hence two electrons be involved in the over-all electrode process; and they conflict with the insensitivity of the oxygen half-wave potential ($E_{1/2}$) to changes in *p*H, as reported by Kolthoff and Miller.[3] In view of the fundamental importance of the oxygen wave in polarography, it was considered important to resolve this problem, and a preliminary report is provided in this Communication.

Polarograms of oxygen, hydrogen peroxide and their mixtures were run in buffered solutions, *p*H range 7.5–13, of ionic strength 0.15. In all these solutions, anodic and cathodic waves were observed, which in the less basic media exhibited the usual criteria of irreversibility—drawn-out shapes, non-linear "log plots," and in the case of the oxygen wave an $E_{1/2}$ which hardly varied with *p*H. In mixtures of hydrogen peroxide and oxygen the two waves joined without inflection; however, the $E_{1/2}$ shifted as the composition of the mixture was varied. This irreversible nature diminished as the alkalinity increased, until at *p*H 12 both anodic and cathodic waves gave identical $E_{1/2}$'s, and the log plot of both had the theoretical slope for a two-electron reaction.

At the point at which the electrolysis current in a H_2O_2–O_2 mixture crosses the residual current, the D.M.E. is functioning as a potentiometric null point detector. The "crossing point" potential will depend upon the bulk concentration as predicted by the Nernst equation, provided that we are dealing with the over-all reaction

$$O_2 + 2H^+ + 2e^- \rightleftarrows H_2O_2 \qquad (1)$$

for which the Nernst equation at 25° is

$$E = E^0 + 0.0296 \log (O_3)/(H_2O_2) - 0.0592\ pH \qquad (2)$$

Supposing that the absolute rate theory as recently applied to polarographic phenomena[4] is applicable here, it can be shown that these equations apply at the crossing point, however irreversible the waves may be.

In fact, Equation (2) was found to hold very well both with regard to the *p*H and the O_2–H_2O_2 ratio (corrected for the acid dissociation of H_2O_2) over the entire range investigated. The value E^0 = +0.70 v. ± 0.01 v. was found for the standard potential of the half-cell of Equation (1), in good agreement with Latimer's calculated value +0.682 v.[5]

These experimental results are also in good accord with the observations of Berl,[6] who found that the O_2–H_2O_2 couple was reversible at graphite and activated carbon electrodes in solutions of *p*H between 13 and 15, with an E^0 of +0.684 v.

In the range of *p*H studied the O_2–H_2O_2 couple at the D.M.E. evidently is of the transition (semi-reversible) type.[7] The situation is complicated by the probable presence of two steps[8] which may differ in degree of reversibility and dependence on *p*H. Analysis of the irreversible nature of the waves is continuing with the hope of elucidating the mechanism and kinetics of oxygen reduction. The combined results will be presented in a future publication.

(1) Kolthoff and Lingane, "Polarography," 2nd edition, Vol. II, Interscience Publishers, Inc., New York, N. Y., p. 552.

(2) S. Hacobian, *Australian Journal of Chemistry*, **6**, 211 (1953).

(3) I. M. Kolthoff and C. S. Miller, THIS JOURNAL, **63**, 1013 (1941).

(4) Tanford and Wawzonek, "Annual Reviews of Physical Chemistry," **3**, 247 (1952).

(5) W. M. Latimer, "Oxidation Potentials," 2nd edition, Prentice-Hall, Inc., New York, N. Y., p. 43.

(6) W. G. Berl, *J. Electrochem. Soc.*, **83**, 253 (1943).

(7) P. Delahay, THIS JOURNAL, **75**, 1430 (1953); M. Smutek, *Coll. Czech. Chem. Comm.*, **18**, 171 (1953).

(8) One of these may be the reversible one-electron step postulated by Hacobian, see ref. 2.

MALLINCKRODT CHEMICAL LABORATORY
HARVARD UNIVERSITY DAVID M. H. KERN
CAMBRIDGE 38, MASS.

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9α-FLUORO DERIVATIVES OF CORTISONE AND HYDROCORTISONE

Sir:

In a recent communication[1] we have described a new group of derivatives of cortisone and hydrocortisone, in which the 9α-hydrogen atom is replaced by halogen. The main interest in this series of compounds derived from the fact that they possessed marked glucocorticoid activity, which in the case of the chloro derivatives exceeded by a factor of 4 that of the parent hormones. The finding that the activity was inversely proportional to the size of the halogen atom prompted the preparation of the last remaining members of this group, the 9α-fluoro derivatives, the description of which is the purpose of this communication.

9α-Fluorohydrocortisone acetate (I), m.p. 233–234°[2]; $[\alpha]^{23}D$ +123° (*c*, 0.64 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 238 mμ (ε = 16,800); $\lambda_{max.}^{Nujol}$ 2.94 μ, 3.03 μ (OH), 5.75 μ, 5.82 μ (acetylated side chain), 6.07 μ, 6.11 μ

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953).

(2) Occasionally samples began to melt at 205–208°, resolidified and eventually melted at 226–228°, probably due to polymorphism.

(Δ^4-3-ketone); (*Anal.* Calcd. for $C_{23}H_{31}O_6F$: C, 65.39; H, 7.39; F, 4.52. Found: C, 65.32; H, 7.26; F, 4.50) was obtained in about 50% yield when Δ^4-pregnen-9β,11β-oxido-21-ol-3,20-dione 21-acetate[1] (II) was treated with anhydrous hydrogen fluoride in alcohol-free chloroform at 0° for 4.5 hours. I possessed 10.7 ± 2.3 times the activity of cortisone acetate in the rat liver glycogen assay[3] which is in keeping with the relationship between activity and size of the halogen atom established for the other halogenated derivatives. Deacetylation of I with sodium methylate afforded 9α-fluorohydrocortisone, m.p. 260–262° (dec.); $[\alpha]^{23}D$ +139° (*c*, 0.55 in 95% alcohol); $\lambda_{max.}^{alc.}$ 239 mμ (ϵ = 17,600); $\lambda_{max.}^{Nujol}$ 3.01 μ (OH), 5.84 μ (20-carbonyl), 6.07 μ, 6.20 μ (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{21}H_{29}O_5F$: C, 66.30; H, 7.68. Found: C, 66.49; H, 8.22). Oxidation of I with chromic acid yielded 9α-fluorocortisone acetate (III), m.p. 254–255°; $[\alpha]^{23}D$ +155° (*c*, 0.45 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 234 mμ (ϵ = 17,000); $\lambda_{max.}^{Nujol}$ 2.86 μ (OH), 5.72 μ, 5.78 μ, 5.83 μ (11-ketone and acetylated side chain), 6.05 (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{23}H_{29}O_6F$: C, 65.70; H, 6.95. Found: C, 65.62; H, 7.19), which on deacetylation furnished the parent alcohol, m.p. 261–262°; $[\alpha]^{23}D$ +144° (*c*, 0.41 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 234 mμ (ϵ = 16,000); $\lambda_{max.}^{Nujol}$ 2.88 μ (OH), 5.87 μ (11- and 20-keto groups), 6.08 μ (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{21}H_{27}O_5F$: C, 66.65; H, 7.19. Found: C, 66.50; H, 6.98). III had 9.0 ± 2.7 times the activity of cortisone acetate.[3]

The reaction of II with hydrogen fluoride afforded in addition to I an isomer of II (10% yield) (IV), m.p. 259–262°; $[\alpha]^{23}D$ +272° (*c*, 0.53 in 95% alcohol); $\lambda_{max.}^{alc.}$ 239 mμ (ϵ = 20,200); $\lambda_{max.}^{Nujol}$ 2.93 μ, 3.03 μ (OH), 5.75 μ, 5.82 μ (acetylated side chain), 6.12 μ, 6.16 μ (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.60; H, 7.40), in which the epoxide group has been rearranged to form a double bond and a readily acylable hydroxyl group. Thus, IV on titration with perphthalic acid consumed one mole of peracid with the formation of an epoxide, m.p. 213–214° (dec.); $[\alpha]^{23}D$ +237° (*c*, 0.59 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 237 mμ (ϵ = 16,100); $\lambda_{max.}^{Nujol}$ 2.92 μ, 3.06 μ (OH), 5.73 μ, 5.80 μ (acetylated side chain), 6.16 μ (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: 66.03; H, 7.44), on treatment with propionic anhydride and pyridine at room temperature afforded a propionate, m.p. 261–264°[4]; $[\alpha]^{23}D$ +260° (*c*, 0.40 in $CHCl_3$), +243° (*c*, 0.52 in 95% alcohol); $\lambda_{max.}^{alc.}$ 238 mμ (ϵ = 18,300); $\lambda_{max.}^{Nujol}$ 3.05 μ (OH), 5.72 μ, 5.80 μ (propionyl and acetylated side chain), 6.07 μ, 6.11 μ (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{26}H_{34}O_7$: C, 68.10; H, 7.41. Found: C, 67.93; H, 7.36) and with mesyl chloride in pyridine at 0° formed a mesylate, m.p. 151–152° (dec.); $[\alpha]^{23}D$ +260° (*c*, 0.54 in $CHCl_3$), +238° (*c*, 0.35 in 95% alcohol); $\lambda_{max.}^{alc.}$ 237 mμ (ϵ = 17,800); $\lambda_{max.}^{Nujol}$ 3.06 μ (OH), 5.72 μ, 5.81 μ (acetylated side chain), 6.11 μ (Δ^4-3-ketone); (*Anal.* Calcd. for $C_{24}H_{32}O_8S$: C, 59.98; H, 6.71; S, 6.67. Found: C, 60.01; H, 6.73; S, 6.38). Additional data are required to permit a satisfactory structural assignment for IV.[5]

(3) M. L. Pabst, R. Sheppard and M. H. Kuizenga, *Endocrinology*, **41**, 55 (1947). We are indebted to Drs. A. Borman and F. M. Singer for the liver glycogen assay data. A detailed account of these and other assay results will be published elsewhere.

(4) A mixture of IV and its propionate melted at 236–252°.

(5) Although the above data are not sufficient to establish the structure of IV, certain structural possibilities can be ruled out on the basis of the available evidence. Thus, the reactivity of the new hydroxyl group toward acylating agents appears to exclude the presence of an 11β-hydroxyl in a conventional ring C-saturated steroid. The allylic Δ^9-11β-ol structure, which for steric reasons might be expected to be susceptible to acylation (or any other allylic structure) is considered unlikely, since (1) IV is resistant to oxidation with manganese dioxide (*cf.* F. Sondheimer, C. Amendolla and G. Rosenkranz, THIS JOURNAL, **75**, 5930 (1953)), and (2) the changes in molecular rotation attending propionylation (+20°) and mesylation (+49°) of IV are considerably smaller than those produced by acylation of allylic alcohols (*cf.* W. Klyne, *Helv. Chim. Acta*, **35**, 1224 (1952)). Rotational data likewise serve to exclude the *a priori* less likely Δ^7-11β-ol structure, inasmuch as the average contribution of the 7,8-double bond in two $\Delta^{4,7}$-3-ketones is −304° (*cf.* R. Antonucci, S. Bernstein, R. Lenhard, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1369 (1952)) as compared to a value of +516° for the difference between IV and hydrocortisone acetate. Among the more likely possibilities being considered at the moment are structures arising from II by a Wagner–Meerwein type rearrangement involving C_9.

THE SQUIBB INSTITUTE
FOR MEDICAL RESEARCH JOSEF FRIED
NEW BRUNSWICK, NEW JERSEY EMILY F. SABO

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THE STIMULATION OF SERINE BIOSYNTHESIS IN PIGEON LIVER EXTRACTS BY TETRAHYDROFOLIC ACID[1]

Sir:

Serine biosynthesis has been postulated to occur by a process resembling the Mannich reaction[2,3]: the α-carbon of glycine, activated by Schiff base formation between glycine and pyridoxal phosphate, combines with a condensation product of formaldehyde and tetrahydrofolic acid (THFA), N^5-hydroxymethyltetrahydrofolic acid. Serine is produced by hydrolytic cleavage of the resulting product.

Studies have been reported indicating that pyridoxal phosphate participates in serine biosynthesis.[3,4] In the present communication, experimental evidence supporting a cofactor role of THFA in this reaction is described.

Pigeon liver extracts prepared as described by Berg[5] interconvert glycine and serine

$$CH_2OHCHNH_2COOH \rightleftharpoons \text{"}C_1\text{"} + CH_2NH_2COOH$$

Formate and formaldehyde are utilized for the formation of the "C_1" unit. These properties are lost on treatment of the liver extracts with Dowex-1 (chloride).

The ability of inactivated[6] pigeon liver extracts to interconvert serine and glycine has now been

(1) This investigation has been supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) W. Sakami, American Chemical Society Symposium on 1-Carbon Compounds, Chicago, September, 1953.

(3) In the Mannich reaction formaldehyde couples either ammonia, or a primary or secondary amine with an atom possessing an active hydrogen.

(4) S. Deodhar and W. Sakami, *Federation Proc.*, **12**, 195 (1953).

(5) P. Berg, *J. Biol. Chem.*, **205**, 145 (1953).

(6) Extracts treated with Dowex-1 (chloride) and dialyzed for 15–16 hr. against flowing 0.1 *M* K-phosphate buffer, *p*H 7.5.