(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

RECEIVED FEBRUARY 3, 1954

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.

fully restored by the single addition of THFA. This reaction has been studied by incubating extracts with L-serine and 1-C^{14}-glycine under hydrogen for 1 hr. at 34°. Serine was quantitatively isolated from the deproteinated incubation mixture by chromatography on Dowex-50 columns and its radioactivity determined. The β-carbon of the L-serine provided the "C_1" unit for the conversion of 1-C^{14}-glycine to 1-C^{14}-serine. Treated[6] extracts did not introduce C^{14} into serine but after the addition of THFA produced an incorporation of glycine-C^{14} into serine 40 × that of untreated extracts and equal to that of untreated extracts + THFA. The addition of ATP, DPN, pyridoxal phosphate and homocysteine did not further increase the activity found in the serine. Folic acid and leucovorin were not active in substituting for THFA.

Addition of THFA alone to the inactivated[6] pigeon liver extracts also restored their ability to utilize formaldehyde for serine-β-carbon formation. This property has been studied by incubation of the extracts with C^{14}-formaldehyde and glycine for 1 hr. under hydrogen at 34° and determination of the radioactivity of the serine. Treated[6] pigeon liver extracts introduced very little formaldehyde-C^{14} into serine. The addition of THFA resulted in an incorporation of C^{14} into serine 36 × that of untreated extracts and equivalent to that of untreated extracts + THFA.

These results in which a rapid interconversion of serine and glycine and utilization of formaldehyde for serine-β-carbon formation has been stimulated in inactivated[6] pigeon liver extracts by the single addition of tetrahydrofolic acid, are consistent with a cofactor role of this substance in serine biosynthesis.

The utilization of formate for serine synthesis in inactivated[6] pigeon liver extracts was restored by the addition of THFA, ATP, DPN, glucose-6-phosphate, and Mn^{++} but not of THFA alone. Formate-C^{14} incorporated into serine, under these conditions, was thirteen times that of the untreated extracts and three times that of untreated extracts stimulated by homocysteine.[5] The radioactivity of the serine was as high as that which was obtained when THFA, ATP, DPN, glucose-6-phosphate and Mn^{++} were added to untreated pigeon liver extracts. Serine formation did not occur in the absence of ATP or of THFA but was not completely abolished by the omission of DPN or Mn^{++}. When glucose-6-phosphate alone was omitted, formate-C^{14} was rapidly incorporated into some substance (or substances) which was not serine, methionine, cystathionine, or purine, but may have been *citrovorum factor*.[7] When folic acid was substituted for THFA, the formate-C^{14} incorporated into serine by Dowex-1 treated-dialyzed extracts was one-fifth that obtained with THFA. These results are interpreted as suggesting that, in the presence of ATP, formate is incorporated into *citrovorum factor* which is subsequently reduced to N^5-hydroxymethyltetrahydrofolic acid by a DPN enzyme system. The ability of folic acid to substitute for THFA in this system indicates that DPN is also the cofactor of the reducing system involved in the conversion of folic acid to THFA.[8]

(7) This compound may also be identical with the intermediate of formate utilization for inosinic acid formation that has been obtained by G. R. Greenberg, private communication.

(8) Studies of formate utilization for purine formation by G. R. Greenberg also indicate the participation of DPN in folic acid reduction (private communication).

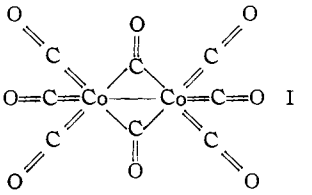
DEPARTMENT OF BIOCHEMISTRY
WESTERN RESERVE UNIVERSITY
SCHOOL OF MEDICINE
CLEVELAND, OHIO
ROY L. KISLIUK
WARWICK SAKAMI

RECEIVED FEBRUARY 4, 1954

A NEW TYPE OF METALLO-ORGANIC COMPLEX DERIVED FROM DICOBALT OCTACARBONYL AND ACETYLENES

Sir:

A recent investigation[1] showed that dicobalt octacarbonyl (I), like iron enneacarbonyl,[2] contains two types of carbonyl groups, *i.e.*, bridge and terminal carbonyl groups



I

It has now been found that the two bridge carbonyls in I can be replaced by acetylene and substituted acetylenes, such as $C_6H_5C{\equiv}CH$, $C_6H_5C{\equiv}CC_6H_5$, $CH_3(CH_2)_4C{\equiv}CH$, $CH_3CH_2C{\equiv}CCH_2CH_3$, $HOCH_2C{\equiv}CH$, $HOCH_2C{\equiv}CCH_2OH$, $HOOCC{\equiv}CC_6H_5$. The reaction proceeds smoothly and quantitatively at room temperature according to equation (1).

$$RC{\equiv}CR' + Co_2(CO)_8 \longrightarrow \underset{\text{II}}{RC_2R'Co_2(CO)_6} + 2\overline{CO} \quad (1)$$

The preparation of the diphenylacetylene complex is typical: a solution of 1.38 g. (4 millimoles) of dicobalt octacarbonyl in 5 ml. of petroleum ether (35–60°) is placed in an erlenmeyer flask provided with a mercury seal. A solution of 0.80 g. (4.5 millimoles) of diphenylacetylene in 15 ml. of petroleum ether is added to the flask, the mercury seal attached and the mixture allowed to stand two hours. Removal of the petroleum ether in a current of nitrogen at room temperature yields crude II (R = R′ = C_6H_5). In the case of $C_6H_5C{\equiv}CC_6H_5$, $HOCH_2C{\equiv}CH$, and $HOCH_2C{\equiv}CCH_2OH$ the reaction product (II) was purified by crystallization, while the product from $HC{\equiv}CH$ was purified by distillation.

II (R = R′ = C_6H_5), deep-purple crystals resembling iodine, m.p. 109.5–110.0° from methanol; sublimes at 90° (1 mm.). Calcd. for $C_{20}H_{10}O_6CO_2$: C, 51.75; H, 2.17; Co, 26.40; mol. wt., 464. Found: C, 51.61, H. 2.22; Co 25.6; mol. wt. (cryoscopic in cyclohexane), 463. The compound is diamagnetic ($\lambda = -0.3 \pm 0.3.10^{-6}$ c.g.s. units/g.) and has a dipole moment in benzene solution of 2.1 *D*.

II (R = CH_2OH, R′ = H), orange-red needles from petroleum ether (60–68°), m.p. 52.2–52.6°. Calcd. for $C_9H_4O_7Co_2$: C, 31.60; H, 1.18; Co, 34.49. Found: C, 31.65; H, 1.26; Co, 34.33.

(1) Unpublished work.

(2) R. K. Sheline and K. S. Pitzer, THIS JOURNAL, **72**, 1107 (1950).