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).

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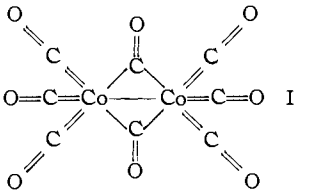
A NEW TYPE OF METALLO-ORGANIC COMPLEX DERIVED FROM DICOBALT OCTACARBONYL AND ACETYLENES

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.

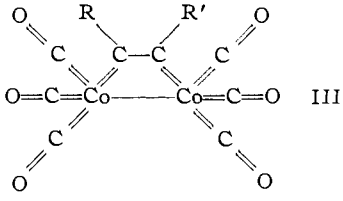
(2) R. K. Sheline and K. S. Pitzer, THIS JOURNAL, 72, 1107 (1950).

II (R = R′ = CH_2OH), light-orange-red needles from ethanol–water, m.p. *ca.* 135° with decomposition. Calcd. for $C_{10}H_6O_8Co_2$: C, 32.28; H, 1.63; Co, 31.69. Found: C, 32.24; H, 1.67; Co, 31.67.

II (R = R′ = H), a dark-red oil at room temperature; m.p. 13.0–13.6°; b.p. 64–66° (3.5–4 mm.) Calcd. for $C_8H_2O_6Co_2$: C, 30.80; H, 0.65. Found: C, 30.76; H, 0.62.

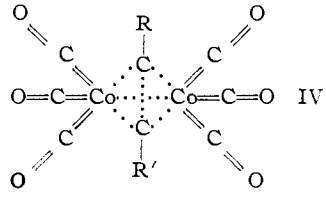
The infrared spectra of these compounds contain a characteristic group of three sharp bands at 2090, 2050 and 2025 cm.$^{-1}$, which are similar to the bands of the terminal carbonyl groups in dicobalt octacarbonyl. The 1859 cm.$^{-1}$ band corresponding to the bridge carbonyl group in I as well as the bands of the $-C\equiv C-$ group, are absent. The spectra of II (R = R′ = H), II (R = $CH_3(CH_2)_4$, R′ = H) and II (R = CH_2OH, R′ = H) contain a band at 3096 cm.$^{-1}$, characteristic of an ethylenic (or aromatic) carbon–hydrogen bond.

It is reasonable to assume that the C–C bond is either parallel to the Co–Co bond, such as in III



III

or perpendicular to the Co–Co bond, such as in IV.



IV

The analytical, spectroscopic, magnetic and dipole moment data, in conjunction with the high volatility and the solubility in organic solvents of II, are compatible with a structure such as III, where cobalt possesses a noble-gas configuration.

In IV the C–C Co–Co bonds may be either in the same plane (IVa) or in different planes (IVb). Structure IVa is very unlikely[3] in view of the dipole moment of II (R = R′ = C_6H_5). Neither the structure of IVa nor that of IVb can be represented readily in terms of localized bonds.

(3) Compound II (R = R′ = C_6H_5) has a molar polarization of 204 ± 4 at 30° and a calculated molecular fraction for the D sodium line of 121. For II (R = R′ = C_6H_5) to be symmetrical it would have to have an extremely high atomic polarization (*cf.* I. E. Coop and L. E. Sutton, *J. Chem. Soc.*, 1269 (1938)).

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RECEIVED FEBRUARY 6, 1954

A FORMYLATION COFACTOR[1]

Sir:

This paper reports the biosynthesis of a formylation cofactor and the transfer of its one-carbon group to a purine precursor. Formylation of IRMP-5[2,3,4] in pigeon liver extract may occur as

$$RH_4X + HCOOH + ATP \longrightarrow FRH_4X + ? \quad (1)$$
$$FRH_4X + IRMP\text{-}5 \longrightarrow RH_4X + IMP\text{-}5 \quad (2)$$

The over-all reaction exhibits almost an absolute requirement for IRMP-5, HCOOH, ATP, Mg^{++} and a cofactor. FRH_4X is a heat-stable cofactor obtained enzymatically from FRH_4 or from RH_4[5] and formate by ATP-dependent reactions. The following three experiments provide evidence for the above mechanism: (1) C^{14}-(formyl)-FRH_4X transformylated directly to IRMP-5 (Reaction 2) in a large pool of unlabeled formate to yield IMP-5 with a specific activity more than 10 times that of the formate. The C^{14}-activity of the FRH_4X was displaced by the unlabeled formate in the presence of ATP (Reaction 1) while without acceptor IRMP-5, FRH_4X lost no activity. (2) Purified FRH_4X formylated IRMP-5 in the absence of ATP, but FRH_4 alone was inactive. (3) An excess of FRH_4X obtained from FRH_4 plus ATP converted IRMP-5 completely to IMP-5 in the absence of formate. The resulting RH_4X accepted added C^{14}-formate to yield C^{14}-FRH_4X (paper chromatography) equivalent in quantity to 80% of the initial IRMP-5 (Reaction 1). Exchange between $HC^{14}OOH$ and IMP-5[6] was insignificant. Loss of X during transformylation (Reaction 2) is not excluded.

Table I shows that the synthesis of FRH_4X, which is measured by its cofactor effect on IMP-5 synthesis, depends on the presence of both ATP and FRH_4 during preincubation.

TABLE I

INTERACTION OF LEUCOVORIN AND ATP

Preincubation additions: 20 mg. lyophilized extract (acetone powder extracted with 0.05 *M* $KHCO_3$ and treated with Dowex-1 chloride), 2.5 μM·ATP, 7 μM, phosphoglycerate, 3 μM·$MgCl_2$, 0.5 mg. lyophilized muscle extract fraction,[7] 7.5 μM·$KHCO_3$, 0.2 μM·FRH_4, 8 μH·DL-homocysteine; vol., 0.6 ml.; time, 10 min.; temp., 38°; in air. After preincubation 0.36 μM·IRMP-5, 2 μM. C^{14}-formate and the omitted compounds were added and the reaction continued 5 min.; total vol. 1.0 ml.

Omitted in preincubation	None	FRH_4	ATP	FRH_4[a]	ATP[a]
Δ Amine, μM	0.262	0.084	0.057	0.024	0.002
C^{14} fixed, μM	.294	.116	.093	.010	.0

[a] Also omitted after preincubation.

IMP-5 synthesis is greatest when FRH_4X is preformed. One mole of diazotizable amine[8] disappears per mole of C^{14} formate fixed into IMP-5. On paper chromatograms FRH_4X appears as a blue-fluorescing compound.

(1) Aided by grants from the U. S. Public Health Service and Elisabeth Severance Prentiss Foundation.

(2) Abbreviations: IRMP-5, 5-amino-4-imidazolecarboxamide-5′-phosphoribotide; ATP, adenosine triphosphate; IMP-5, inosine-5′-phosphate; RH_2, 7,8-dihydrofolic acid; RH_4, 5,6,7,8-tetrahydrofolic acid; FRH_4, N-5-formyl-5,6,7,8-tetrahydrofolic acid (leucovorin); X, product of the interaction of a folic acid compound and ATP DPNH, reduced diphosphopyridine nucleotide.

(3) G. R. Greenberg, *Federation Proc.*, **12**, 211 (1953).

(4) G. R. Greenberg, *ibid.*, **12**, 651 (1953).

(5) B. L. O'Dell, *et al.*, THIS JOURNAL, **69**, 250 (1947).

(6) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).

(7) S. Ratner and A. Pappas, *ibid.*, **179**, 1183 (1949).

(8) J. M. Ravel, *et al.*, *ibid.*, **172**, 67 (1948). Diazotization was preceded by acetic anhydride treatment.