Sagers, *et al.*,[7] on the basis of exchange experiments with glycine-2-C^{14} and FIG, have postulated the occurrence of a formimino transferring enzyme and the formation of formimino-THF. Miller and Waelsch[8] on the basis of kinetic experiments have suggested that 10-formimino-THF may be an intermediate in the formation of 10-formyl-THF from formiminoglutamic acid and THF.

(7) R. D. Sagers, J. V. Beck, W. Gruber and I. C. Gunsalus, THIS JOURNAL, **78**, 694 (1956).

(8) A. Miller and H. Waelsch, *Arch. Biochem. and Biophys.*, **63**, 263 (1956).

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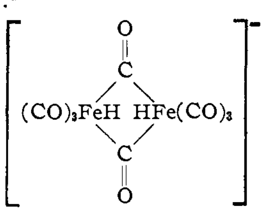
CHEMISTRY AND CATALYTIC PROPERTIES OF THE IRON PENTACARBONYL–AQUEOUS ALKALI SYSTEM

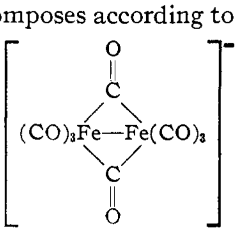
Sir:

Solutions obtained by treating $Fe(CO)_5$ with aqueous alkali have intriguing chemical and catalytic properties, which include the conversion of olefins to the next higher alcohols,[1] the reduction of nitrobenzene to aniline,[2] and acetylene to ethylene.[3] We have found that these properties can be explained and new reactions predicted on the basis of the existence of a dimeric ion (II), formed from two $[HFe(CO)_4]^-$ ions:[4]

$2[HFe(CO)_4]^- \longrightarrow$  (1)

I II

Complex II decomposes according to equation (2):

II $\longrightarrow$  $+ H_2$ (2)

III

In the presence of a hydrogen acceptor, II acts as a hydrogen donor (equation 3)

$$\text{II} + \text{acceptor} \longrightarrow \text{acceptor}\cdot H_2 + \text{III} \quad (3)$$

Evidence for the existence of II and III is based on these observations: (a) On standing, the light aqueous solution containing I becomes dark red, even in the absence of oxygen,[5] and slowly gives off hydrogen. Ether extraction yields a dark-red pyrophoric solid. The iron and sodium content of this solid indicates a mixture of $NaHFe_2(CO)_8$, (IV), and $H_2Fe_2(CO)_8$, (V), *i.e.*, the acid salt and free acid corresponding to III. (b) A freshly prepared solution of II absorbs at 4750 Å., while a solution of IV and V absorbs at 5350 Å. When the solution of II is allowed to stand the band at 4750 Å. gradually is replaced by the band at 5350 Å. (c) Acidification of an aqueous solution of the mixture of IV and V proceeds according to equation (4)[6]

$$[Fe_2(CO)_8]^= + 2H^+ \longrightarrow H_2 + \frac{2}{3}[Fe(CO)_4]_3 \quad (4)$$

III iron tetracarbonyl

(d) Equation 3 shows that reduction is achieved by transferring hydrogen from II to the substrate. The reducing properties of these solutions were previously attributed[7] to the oxidation-reduction potential

$$3[HFe(CO)_4]^- \longrightarrow [Fe(CO)_4]_3 + 3H^+ + 6e,\ E_0 = 0.35 \text{ volt} \quad (5)$$

However, there is no evidence that iron tetracarbonyl, $[Fe(CO)_4]_3$, is formed in alkaline solutions from $[HFe(CO)_4]^-$. The action of oxidizing agents (acceptors) on alkaline solutions containing $[HFe(CO)_4]^-$ ions always leads to dark-red solutions which yield the tetracarbonyl only on acidification.[8] The need for this acidification is not apparent from (5) but follows from (3) and (4).

In view of the structural resemblance between III and dicobalt octacarbonyl, we predicted that III, or solution in which III can be formed, should catalyze the isomerization of olefinic double bonds and the addition of carbon monoxide and hydrogen to olefins in a manner similar to dicobalt octacarbonyl.[9] These predictions proved to be correct. When 1-hexene was shaken with a solution containing $[HFe(CO)_4]^-$ ion for 24 hours at room temperature, 90% was isomerized to 2-hexene and 3-hexene. Treatment of excess cyclopentene with an aqueous solution containing $[HFe(CO)_4]^-$ ions at 155° and 160 atmospheres of CO yielded 33% of cyclopentanecarboxaldehyde. This is the first report of an iron catalyzed conversion of an olefin to the next higher aldehyde.

The conversion[1] of olefins to the next higher alcohols in the $Fe(CO)_5$-aqueous alkali system can now be explained as taking place in two steps, *i.e.*, formation of the next higher aldehyde followed by hydrogenation to the alcohol. That aldehydes are reduced by solutions that catalyze the conversion of olefins to alcohols was demonstrated by treating benzalde-

(1) W. Reppe and H. Vetter, *Ann.*, **582**, 133 (1953).

(2) German Patent to I. G. Farbenindustrie Akt.-Ges., 441,179 of January 18, 1925.

(3) H. W. Sternberg, R. A. Friedel, R. Markby and I. Wender, THIS JOURNAL, **78**, 3621 (1956).

(4) The presence of $[HFe(CO)_4]^-$ ions in solutions obtained by treating $Fe(CO)_5$ with aqueous alkali was shown by P. Krumholz and H. M. A. Stettiner, *ibid.*, **71**, 3035 (1949).

(5) These solutions were considered to be stable (see W. Hieber and F. Leutert, *Z. anorg. allgem. Chem.*, **204**, 145 (1932)) and the darkening was attributed to traces of oxygen or oxidizing agents. However, dimer formation accounts for the darkening in the absence of oxygen.

(6) Acidification of the monomeric $NaHFe(CO)_4$ and $Na_2Fe(CO)_4$ leads to an entirely different reaction. Iron hydrocarbonyl is liberated, which decomposes (see W. Hieber and H. Vetter, *Z. anorg. allgem. Chem.*, **212**, 145 (1933)) according to

$$2H_2Fe(CO)_4 \longrightarrow 2H_2 + Fe(CO)_5 + Fe(CO)_3$$

In this case 1 mole of hydrogen and 1/2 mole of $Fe(CO)_5$ and $Fe(CO)_3$ are obtained for each atom of iron present. No iron tetracarbonyl is formed.

(7) W. Hieber and W. Huebel, *Z. Elektrochem.*, **57**, 331 (1953).

(8) W. Hieber, *Z. anorg. allgem. Chem.*, **204**, 165 (1932).

(9) I. Wender, S. Metlin, S. Ergun, H. W. Sternberg and H. Greenfield, THIS JOURNAL, **78**, 5401 (1956).

hyde with a solution containing $[HFe(CO)_4]^-$ ion. Benzyl alcohol was obtained in 33% yield.

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INTERMEDIATE STEPS IN THE FORMYLATION OF TETRAHYDROFOLIC ACID BY FORMIMINOGLUTAMIC ACID IN RABBIT LIVER

Sir:

The involvement of folic acid in the metabolism of formimino compounds was first indicated by the accumulation of the histidine metabolite,[1] formiminoglutamic acid (FIGLU), in the urine of folic-deficient rats.[2] Subsequently, FIGLU was shown to be a suitable substrate for the formylation of folic acid[3a,b] and of THF in liver preparations, with the formation of 10-formyl-THF.[3c,4]

THF has also been shown to be a cofactor in the metabolism of formiminoglycine (FIG) in extracts of *Clostridium cylindrosporum* and *Clostridium acidiurici*[5a,b] with the formation of 10-formyl-THF.[5c] Rabinowitz and Pricer[6] have now demonstrated that three enzymatic steps are involved, and that 5-formimino-THF and 5,10-methenyl-THF are intermediates. In the present communication it is shown that these three enzymatic steps occur in the metabolism of formiminoglutamic acid in extracts of rabbit liver acetone powder:

$$\text{FIGLU} + \text{THF} \xrightarrow{\text{I}} [\text{5-Formimino-THF}] \xrightarrow{\text{II}} \text{5,10-methenyl-THF} \xrightarrow{\text{III}} \text{10-formyl-THF}$$

Enzyme I converts FIGLU and THF to 5-formimino-THF with essentially no change in the optical density at 355 mμ. Upon treatment with acid or enzyme II, 5,10-methenyl-THF is produced, with an increase in the optical density at this wave length (Table I, Fig. 1). By the action of enzyme III (Fig. 1) 5,10-methenyl-THF is converted to 10-formyl-THF, with the simultaneous decrease in the optical density at 355 mμ. Treatment of 10-formyl-THF with acid results in the non-enzymatic formation of 5,10-methenyl-THF.[7]

Enzyme I is readily separated from enzyme III by ammonium sulfate fractionation (enzyme I: 0-0.8M; enzyme III: 1.2-2M); it still contains enzyme II activity, even after 150-fold purification. Under the experimental conditions of Table I, however, the activity of enzyme II is sufficiently less than that of enzyme I to permit accumulation of the formimino-THF intermediate. Although this intermediate compound has not been characterized completely, it behaves like the 5-formimino-THF obtained from FIG + THF[6] in its conversion to 5,10-methenyl-THF when treated with enzyme II or with acid.[8]

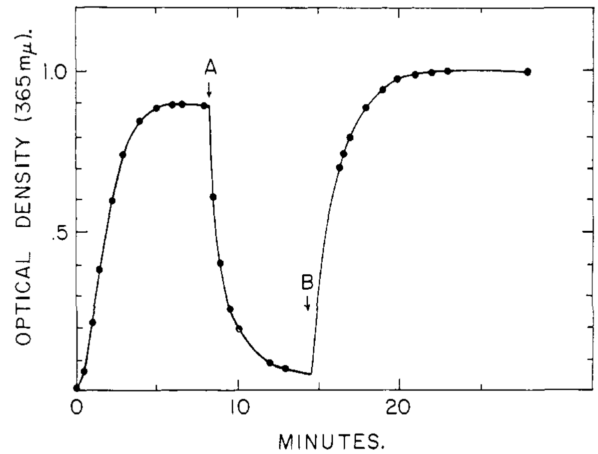

Fig. 1.—The incubation mixture contained 5 μmoles of Na FIGLU, 0.16 μmole of *dl*-THF, 10 μmoles of phosphate buffer (*p*H 7.2), 60 μmoles of mercaptoethanol, 300 γ of liver enzyme (I + II), and water in a total volume of 1 ml. The optical density was measured directly against a blank cell without FIGLU (light path, 1 cm.). At A, 300 γ of enzyme III were added; at B perchloric acid was added to a final concentration of 2.3%. Control experiments without FIGLU or without enzyme showed essentially no changes in O.D. 365 mμ. The optical density obtained represents approximately a 70% yield, assuming that only one optical isomer is active; the low yield is probably due to impurities in the THF preparation used. The rate of formation of 5,10-methenyl-THF when acid was added at B is similar to the rate found with synthetic 10-formyl-THF.

Enzyme I from liver will react with formiminoglutamic acid, but not with formiminoglycine, while the transferase from *C. cylindrosporum* will react with formiminoglycine, but not with formiminoglutamic acid. 5-Formimino-THF, formed by either enzyme, is converted to 5,10-methenyl-THF by enzyme II from liver or from *C. cylindrosporum.* The evidence that this compound is 5,10-methenyl-THF is based on the similarity of the enzymatic product and synthetic[9] 5,10-methenyl-THF in spectral characteristics in neutral and acid *p*H (absorption maxima: 355 mμ and 350 mμ,[7] respectively), in their conversion to 10-formyl-THF by en-

(1) For references on the structure of FIGLU, and on its role in histidine metabolism, see B. Borek and H. Waelsch, THIS JOURNAL, **75**, 1772 (1953); *J. Biol. Chem.*, **205**, 459 (1953); H. Tabor and A. H. Mehler, *ibid.*, **210**, 559 (1954).

(2) H. Bakerman, M. Silverman and F. S. Daft, *ibid.*, **188**, 117 (1951); M. Silverman, *et al.*, *ibid.*, **194**, 815 (1952); H. Tabor, M. Silverman, A. H. Mehler, F. S. Daft and H. Bauer, THIS JOURNAL, **75**, 756 (1953); J. F. Seegmiller, M. Silverman, H. Tabor and A. H. Mehler, *ibid.*, **76**, 6025 (1954).

(3) (a) K. Slavik and V. Matoulkova, *Coll. Czechoslov. Chem. Commun.*, **17**, 1032 (1954); (b) A. Miller and H. Waelsch, *Biochim. Biophys. Acta*, **17**, 278 (1955); (c) A. Miller and H. Waelsch, *Arch. Biochem. Biophys.*, **63**, 263 (1956).

(4) Abbreviations are the same as used in the accompanying paper.[6]

(5) (a) R. D. Sagers, J. V. Beck, W. Gruber and I. C. Gunsalus, THIS JOURNAL, **78**, 694 (1956); (b) J. C. Rabinowitz and W. E. Pricer, Jr., *ibid.*, **78**, 1513 (1956); (c) J. C. Rabinowitz and W. E. Pricer, Jr., *ibid.*, **78**, 4176 (1956).

(6) J. C. Rabinowitz and W. E. Pricer, Jr., *ibid.*, **78**, 5702 (1956).

(7) M. May, *et al.*, *ibid.*, **73**, 3067 (1951); D. B. Cosulich, *et al.*, *ibid.*, **74**, 3252 (1952).

(8) The rate of conversion of the enzymatically-formed 5-formimino-THF to 5,10-methenyl-THF in acid (2.3% perchloric acid, 25°) is approximately 25% in 4 minutes and 50% in 10 minutes. This is similar to the rate of cyclization observed with 5-formyl-THF; the cyclization of 10-formyl-THF in acid occurs more rapidly, and is essentially complete in 8 minutes under these conditions.

(9) Synthetic 5,10-methenyl-THF was prepared[7] by incubating 5-formyl-THF in a 0.1 *N* HCl-0.05 *M* mercaptoethanol mixture. We wish to thank Dr. Harry Broquist of the Lederle Laboratories for a generous gift of 5-formyl-THF (calcium leucovorin).