The activity of a partially purified transketolase from *E. coli* was doubled by the addition of thiamine pyrophosphate (ThPP). No requirement for ThPP was found with a twice recrystallized preparation of the yeast enzyme, but extensive dialysis against a Versene–KCl solution caused nearly complete inactivation. Addition of magnesium chloride and of ThPP to the dialyzed enzyme restored the activity, as shown in Table II. The crystalline yeast enzyme shows no aldolase, triose isomerase or pentose isomerase activity.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY E. RACKER
YALE UNIVERSITY G. DE LA HABA
NEW HAVEN, CONNECTICUT I. G. LEDER

RECEIVED JANUARY 30, 1953

BIS-CYCLOPENTADIENYL DERIVATIVES OF SOME TRANSITION ELEMENTS

Sir:

The structure suggested[1] for bis-cyclopentadienyliron(II)[2] (ferrocene), in which the iron atom is symmetrically placed between two cyclopentadienyl rings, has been confirmed by X-ray crystal structure measurements.[1b,3] The original proposals[1] of this formulation were coupled with the suggestion that the electronic structure of the iron atom attains an inert gas configuration, and this idea could also be extended to the ruthenium analog $C_{10}H_{10}Ru$[4] and to the bis-cyclopentadienylcobalt(III) (cobalticinium) ion $[C_{10}H_{10}Co]^+$,[1b,5] which is isoelectronic with ferrocene.

In addition to the objection that a high negative charge would be placed on the central metal atom, the aromatic properties[6] of ferrocene make it seem most unlikely that all the π electrons of the cyclopentadienyl rings can be involved in the filling of the orbitals of the metal atom. More definite evidence against this view has now been obtained.

Bis-cyclopentadienylnickel(II) has been prepared by the reaction of cyclopentadienylmagnesium bromide with nickel(II) acetylacetonate. (*Anal.* Calcd. for $C_{10}H_{10}Ni$: C, 63.6; H, 5.3; Ni, 31.0. Found: C, 63.7; H, 5.5; Ni, 31.0). It forms dark green crystals from ligroin which decompose slowly even in absence of air and light; it sublimes above 130° but decomposes below the melting point. Solutions in an alcoholic supporting electrolyte show a polarographic anodic wave at −0.08 volt *versus* the saturated calomel electrode. The yellow solutions obtained by oxidation contain the bis-cyclopentadienylnickel(III) ion, $[C_{10}H_{10}Ni]^+$, and give precipitates with silicotungstic acid, Reinecke's salt, potassium triiodide, etc., as do the ferricinium,[1] ruthenicinium[4] and cobalticinium[5] ions. Aqueous solutions of the bis-cyclopentadienylnickel(III) ion are rather unstable, and decompose in a few minutes. An unstable dark brown crystalline picrate is obtained by mixing ether solutions of bis-cyclopentadienylnickel (II) and picric acid in presence of air (*Anal.* Calcd. for $C_{16}H_{12}N_3O_7Ni$: Ni, 14.1. Found Ni, 14.0).

On the above view, the nickel atom in bis-cyclopentadienylnickel(II) should have two electrons in excess of the krypton structure, which would be expected to occupy the 5s orbital. Magnetic susceptibility measurements show, however, that bis-cyclopentadienylnickel(II) has two unpaired electrons ($\chi^{25°}_{\text{mol.}} = +3440 \times 10^{-6}$ c.g.s.u. corrected for diamagnetic contribution, $\mu_{\text{eff}} = 2.88$ B.M.). In our view, this fact may be best accommodated by assuming that in these bis-cyclopentadienyl compounds, the metal ion utilizes three of the electrons from each of the two cyclopentadienyl anions, forming bonds involving the *s* and two *d* orbitals of the metal. In the case of Ni(II), the formation of $C_{10}H_{10}Ni$ would involve promotion of two electrons to the 4p orbitals, which must be singly occupied. Further, we have been unable to obtain a bis-cyclopentadienyl derivative of Cu(II), which has only one *d* orbital available, and cyclopentadienyl derivatives of the zinc group show properties of typical organo-metallic compounds. We have, however, been able to prepare bis-cyclopentadienyl compounds of titanium, zirconium and vanadium, where sufficient electrons are not available for completion of an inert gas configuration of the metal atom.

Bis-cyclopentadienyltitanium(IV) dibromide has been prepared by the reaction of excess cyclopentadienylmagnesium bromide with titanium tetrachloride in toluene solution. It forms dark red crystals, m.p. 240–243°, from toluene (*Anal.* Calcd. for $C_{10}H_{10}TiBr_2$: C, 35.6; H, 3.0; Ti, 14.2; Br, 47.3. Found: C, 36.0; H, 3.1; Ti, 14.3; Br, 47.3) and is diamagnetic ($\chi^{25°}_{\text{mol}} = -145 \times 10^{-6}$ c.g.s.u.). It is to some extent hydrolyzed by water, giving a yellow solution, which gives precipitation reactions similar to those of other bis-cyclopentadienyl metal ions. A crystalline picrate (m.p. 139–141°, explodes) has been isolated (*Anal.* Calcd. for $C_{22}H_{14}N_6O_{14}Ti$: Ti, 7.55. Found: Ti, 7.48). Aqueous perchlorate solutions show a polarographic cathodic wave at −0.44 volt *versus* the saturated calomel electrode. Controlled potential reduction, or reduction using a Jones reductor, produces a green solution containing the bis-cyclopentadienyltitanium(III) ion which shows a polarographic anodic wave at −0.44 volt.

The almost colorless bis-cyclopentadienylzirconium(IV) dibromide (*Anal.* Calcd. for $C_{10}H_{10}ZrBr_2$, C, 31.5; H, 2.7; Zr, 24.0; Br, 41.9. Found: C, 31.4; H, 2.7; Zr, 23.9; Br, 42.0) (m.p. 260° C. dec.) was prepared from zirconium tetrachloride and cyclopentadienylmagnesium bromide. Aqueous solutions of this compound show no polarographic reduction wave.

Vanadium tetrachloride reacts similarly, forming a dark green, rather unstable, ligroin soluble, bromide (*Anal.* Calcd. for $C_{10}H_{10}VBr_2$: V, 14.9; Br, 46.9. Found: V, 14.8; Br, 47.1) and a pale green, ligroin insoluble chloride (*Anal.* Calcd. for $C_{10}H_{10}VCl_2$: C, 47.6; H, 4.0; V, 20.2; Cl, 28.2. Found:

---

(1)(a) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2125 (1952); (b) E. O. Fischer and W. Pfab, *Z. Naturforschung*, **7B**, 377 (1952).

(2)(a) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951); (b) S. A. Miller, J. A. Tebboth and J. F. Tremayne, *J. Chem. Soc.*, 632 (1952).

(3) (a) P. F. Eiland and R. Pepinsky, THIS JOURNAL, **74**, 4971 (1952); (b) J. D. Dunitz and L. E. Orgel, *Nature*, **171**, 121 (1953).

(4) G. Wilkinson, THIS JOURNAL, **74**, 6146 (1952).

(5) G. Wilkinson, *ibid.*, **74**, 6148 (1952).

(6) R. B. Woodward, M. Rosenblum and M. C. Whiting, *ibid.*, **74**, 3458 (1952).

C, 47.2; H, 4.0; V, 20.3; Cl, 28.0). Bis-cyclopentadienylvanadium(IV) dichloride is soluble in chloroform, ethyl acetate and alcohol; it decomposes on heating above 250°. In water it forms a green unstable solution which gives the precipitation reactions typical of bis-cyclopentadienyl metal ions. A dark green picrate (*Anal.* Calcd. for $C_{22}H_{14}N_6O_{14}V$: N, 12.8; V, 7.6. Found: N, 12.8; V, 7.7) has been precipitated from this solution. Bis-cyclopentadienylvanadium(IV) dichloride is paramagnetic with one unpaired electron ($\chi_{\mathrm{mol}}^{23°\mathrm{C}} = +1600 \times 10^{-6}$ c.g.s.u., corrected for diamagnetic contribution; $\mu_{\mathrm{eff}} = 1.95$ B.M.).

The infrared absorption spectra of bis-cyclopentadienylnickel(II) and the bis-cyclopentadienyl dibromides of titanium, zirconium and vanadium are similar to those of ferrocene[1a] and ruthenocene.[4]

CHEMISTRY DEPARTMENT
HARVARD UNIVERSITY
CAMBRIDGE, MASS.

G. WILKINSON
P. L. PAUSON
J. M. BIRMINGHAM
F. A. COTTON

RECEIVED JANUARY 15, 1953

THE ACCUMULATION OF ACETYLMETHYLCARBINOL (3-HYDROXY-2-BUTANONE) BY ACETATE-REQUIRING MUTANTS OF *NEUROSPORA CRASSA*[1]

Sir:

The biological oxidation of pyruvate to acetate occurs in a number of steps, the first of which apparently includes decarboxylation of the pyruvate with the formation of a two-carbon–enzyme complex at the oxidation level of acetaldehyde.[2] This C_2-enzyme complex is then oxidized, as a complex, after which the oxidation product can be hydrolyzed to give acetic acid. Evidence for the first step has been obtained by Schweet, *et al.*,[3] who showed that enzyme preparations forming acetate can also form acetylmethylcarbinol (AMC-3-hydroxy-2-butanone) presumably by reaction of the C_2-enzyme complex at the acetaldehyde oxidation stage.

Additional evidence relating to the initial stages of pyruvate oxidation has been obtained using a series of mutants of *Neurospora crassa* which require acetate for growth. These mutants are deficient in their ability to oxidize pyruvic acid.[4] The acetate-requiring strains 50–6[4], S34, S48, S48+sp, S210[5] accumulate a volatile substance giving a positive Voges–Proskauer reaction on standing. This substance has been identified as AMC on the basis of the following criteria: (a) distillates of media in which acetate mutants have grown give a positive Voges–Proskauer reaction; (b) no reaction is observed on treating distillates of media with hydroxylamine and nickelous chloride but a heavy red precipitate characteristic of nickel dimethylglyoxime is obtained after oxidation with ferric chloride[6] and redistillation; (c) the 2,4-dinitrophenylhydrazones of the substances, obtained by the method of Green, *et al.*,[7] from distillates of the culture media of S48 and S48+sp. (grown six days in 20 ml. of minimal medium[8] containing 20 mg. of acetic acid as the sodium salt), gave decomposition points similar to that reported for the dinitrophenylhydrazone of diacetyl (317°, reported 315°) and there was no depression of the decomposition point on mixing the derivative from S48+sp with dinitrophenylhydrazone prepared from an authentic sample of diacetyl.

Neurospora metabolizes pyruvate via a carboxylase as well as by an oxidative system.[4] This carboxylase reaction is involved in ethanol production. That the carboxylase system is not involved in the production of AMC by acetate mutants is indicated by Table I. Acetate requiring strains with both high and low carboxylase activities accumulate large amounts of AMC. Acetate independent strains do not accumulate significant amounts of AMC regardless of their alcohol production or carboxylase activity.

TABLE I

ACCUMULATION OF ACETYLMETHYLCARBINOL BY MUTANTS OF *Neurospora*

Strains grown 4 days on minimal medium[8] containing 20 mg. of acetic acid as the sodium salt. AMC was determined by the method of Westerfeld, *et al.*,[9] alcohol was determined by the method of Friedmann and Klass.[10] Carboxylase was assayed as the μl. of CO_2 evolved in 10 minutes at *p*H 5.3 from 9×10^{-2} *M* pyruvate at 37°. The extract used for carboxylase assay was the supernatant fraction of a mycelial homogenate.

	Strain			
	Acetate-requiring		Acetate-independent	
	S48	S48+sp	8a	50-8
Dry weight, mg.	38.3	28.3	71.2	87.6
Acetylmethylcarbinol accumulated g./mg., dry wt.	175	450	0.9	0.2
Ethanol accumulated mg./mg. dry wt.	0.64	0.07	1.25	0.32
Carboxylase activity μl. CO_2/10 min./mg. N	156	16	285	101

The acetate requiring mutants of *Neurospora* are apparently blocked in pyruvic acid oxidation after the formation of a C_2-enzyme complex at the acetaldehyde oxidation stage. As a result of the block preventing oxidative metabolism of the complex, the complex reacts with a C_2 compound at the acetaldehyde oxidation stage or with pyruvate to give AMC. This mechanism is consistent with, and supports, present ideas of the initial stages of pyruvate oxidation. Since at least three genetically different acetate requiring mutants accumulate AMC further studies of the biochemical differences among these mutants can be expected to help elucidate the details of pyruvate oxidation.

DEPARTMENT OF ZOOLOGY
SYRACUSE UNIVERSITY
SYRACUSE 10, NEW YORK

BERNARD S. STRAUSS

RECEIVED JANUARY 14, 1953

---

(1) Supported by contract AT(30-1)-1138 between Syracuse University and the Atomic Energy Commission.

(2) S. Ochoa, *Physiol. Rev.*, **31**, 56 (1951).

(3) R. S. Schweet, M. Fuld, K. Cheslock and M. H. Paul, "Phosphorus Metabolism," Vol. 1, Johns Hopkins Press, Baltimore, Md., p. 246; M. I. Dolin and I. C. Gunsalus, *J. Bact.*, **62**, 199 (1951).

(4) B. S. Strauss, *Arch. Biochem. Biophys.*, **36**, 33 (1952).

(5) J. Lein and P. S. Lein, *Proc. Nat. Acad. Sci.*, **36**, 44 (1952). The author thanks these workers for making their strains available.

(6) E. Stotz and J. Raborg, *J. Biol. Chem.*, **150**, 25 (1943).

(7) D. E. Green, W. W. Westerfeld, B. Vennesland and W. E. Knox, *ibid.*, **145**, 69 (1942).

(8) G. W. Beadle and E. L. Tatum, *Am. J. Bot.*, **32**, 678 (1945).

(9) W. W. Westerfeld, *J. Biol. Chem.*, **161**, 495 (1945).

(10) T. E. Friedemann and R. Klass, *ibid.*, **115**, 47 (1936).