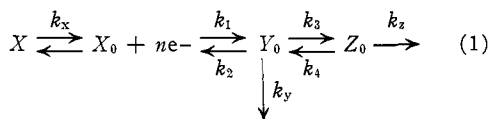


the electron transfer reaction is involved in a chemical reaction.

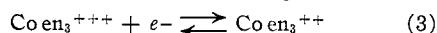
The theoretical part of this investigation is based upon the use of steady state^{2,3} and thermodynamic treatments. Consider the simplest possible case represented by the reaction scheme:



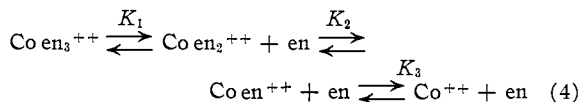
in which the concentrations of the species involved are denoted by X , Y and Z . The subscript 0 refers to surface concentrations. The various processes occurring at the electrode are considered as unimolecular rate processes, the rate constants are defined as heterogeneous constants. Application of the steady state treatment to the above mentioned scheme, on the assumptions that $i = nAF(X_0k_1 - Y_0k_2)$ and that $i_d = nAFXk_x$, leads to the following result

$$\frac{i_d - i}{i} = \frac{k_x}{k_1} \left[\frac{k_2k_4 + k_2k_3 + k_3k_2 + k_4k_y + k_yk_z}{k_4k_y + k_3k_2 + k_yk_z} \right] \quad (2)$$

This expression is valid whether or not the process is reversible. Depending upon the relative magnitude of the various rate constants (e.g., for the reversible case $k_1, k_2 \gg k_3, k_4, k_x, k_y, k_z$) and invoking the potential dependence of the electron transfer rate constants,⁴ equations of polarographic waves can be derived, which show log plots with varying degree of deviation from the reversible behavior. A suitable reaction for testing some of the implications of the theory was found in the system



The trisethylenediaminecobalt(III, II) couple in excess of ethylenediamine has been shown to be reversible potentiometrically by Bjerrum⁵ and polarographically by Grieb.⁶ The couple was found to show irreversible log plot slopes in the absence of the complexing agent in spite of the fact that the process was diffusion controlled, and that the electron transfer reaction was reversible, which was shown using alternating current polarography.⁷ Using the successive complex constants for the divalent form



as determined by Bjerrum⁵ and assuming that establishment of the various equilibria is instantaneous an expression for the current-voltage curve was derived

$$E_{d.e.} = E'_0 - \frac{RT}{F} \ln \alpha \cdot \frac{[\text{Co en}^{++}]_0}{[\text{Co en}_3^{++}]_0} \quad (5)$$

(2) K. B. Oldham, Ph.D. Thesis, Univ. of Manchester, England, 1952.

(3) M. G. Evans and N. S. Hush, *J. chim. phys.*, **49**, C 159 (1952).

(4) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 584.

(5) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941, p. 223.

(6) M. W. Grieb, Ph.D. Thesis, Univ. of Illinois, 1953.

(7) H. A. Laitinen and Pekka Kivalo, *THIS JOURNAL*, **75**, 2108 (1953).

where α is a function of the concentration of divalent complex. The predicted shift of the half-wave potential and change of the log plot slope were confirmed using a 50-fold concentration variation. It is believed that this type of polarographic wave is found in inorganic systems although it might be more numerous in organic polarography as pointed out by Kolthoff and Lingane.⁸

At a later date the details of this investigation, which is in progress, will be published.

(8) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1952, p. 266.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS
PEKKA KIVALO

RECEIVED APRIL 20, 1953

THE VIBRATIONAL SPECTRUM OF TETRACHLORODIBORINE

Sir:

While structural investigations in the field of boron chemistry have not been so numerous as might be desired there is now abundant evidence that the presence of two or more boron atoms in a molecule may result in its having an uncommon or even unique structure.^{1a,b,c,d} An interesting example is afforded by tetrachlorodiborane (B_2Cl_4) in which boron exhibits a normal tricovalence while being singly bonded to another boron atom. We have determined its infrared and Raman spectra and achieved a satisfactory assignment. The spectral data suggest, in agreement with the results of a recent electron diffraction study,² that B_2Cl_4 has the symmetry (V_d) of a non-planar ethylene model.

Approximately 4 ml. of liquid B_2Cl_4 was prepared by the method of Wartik, Moore and Schlesinger.³ Raman exposures were made with the liquid sample held at -35° . The infrared spectra were obtained with a Perkin-Elmer spectrometer (equipped with NaCl and KBr prisms) on about 5 mm. pressure of gaseous B_2Cl_4 at room temperature. The small amount of BCl_3 formed as a result of the instability of the compound at room temperature was corrected for by running blanks on pure BCl_3 .

The observed frequencies and their present interpretation are given in Table I. The frequency to be associated with the b_1 torsional mode cannot yet be estimated. The b_2 deformation is assigned the value 445 cm.^{-1} as deduced from combination bands. The a_1 B-B stretch appears as a polarized doublet in the Raman spectrum as a result of the $\text{B}^{10}\text{-B}^{11}$, $\text{B}^{11}\text{-B}^{11}$ isotopic shift. The observed intensity ratio accords with the natural isotopic distribution of boron. A fuller discussion of the assignment, together with the results of a normal-coordinate, force-constant treatment, will be given elsewhere.

(1) (a) R. C. Lord and E. Nielsen, *J. Chem. Phys.*, **19**, 1 (1951); (b) K. Hedberg, M. E. Jones and V. Schomaker, *Proc. N. A. S.*, **38**, 678 (1952); (c) J. S. Kasper, C. M. Lucht and D. Harker, *THIS JOURNAL*, **70**, 881 (1948); (d) W. J. Dulmage and W. N. Lipscomb, *Acta Cryst.*, **5**, 260 (1952).

(2) Private communication from Dr. K. Hedberg.

(3) T. Wartik, R. Moore and H. I. Schlesinger, *THIS JOURNAL*, **74**, 3265 (1949).

TABLE I
THE INFRARED AND RAMAN SPECTRA AND THEIR INTERPRE-
TATION FOR TETRACHLORODIBORINE

| Raman (cm. ⁻¹) | Infrared (cm. ⁻¹) | Intensity and polarization | Interpretation |
|----------------------------|-------------------------------|----------------------------|--|
| 107 | .. | m | $\nu_9(e; \beta\text{-BCl}_2)$ |
| 177 | .. | m | $\nu_8(e; \beta\text{-BCl}_2)$ |
| 291 | .. | w,p | $\nu_3(a_1; \delta\text{-BCl}_2)$ |
| 347 | .. | vw | $2 \times 177(e) = 354(A_1 + B_1 + B_2)$ |
| 401 | .. | vs,p | $\nu_2(a_1; \nu\text{-BCl})$ |
| 550 | .. | vvw | $107(e) + 445(b_2) = 552(E);$ $2 \times 291(a_1) = 582(A_1)$ |
| .. | 622 | w | $177(e) + 445(b_2) = 622(E);$ $730(b_2) - 107(e) = 623(E)$ |
| .. | 689 | w | $107(e) + 2 \times 291(a_1) = 689(E)$ |
| .. | 720 | s | |
| .. | 729 | vs | $\nu_6(b_2; \nu\text{-BCl})$ |
| .. | 739 | vs | |
| .. | 746 | s | $291(a_1) + 445(b_2) = 736(B_2)^*$ |
| 750 | .. | vvw | $291(a_1) + 445(b_2) = 736(B_2)^*$ |
| .. | 760 | vw | $177(e) + 2 \times 291(a_1) = 759(E)$ |
| .. | 821 | ms | $107(e) + 729(b_2) = 836(E)$ |
| 900 | .. | w | $2 \times 445(b_2) = 890(A_1);$ $177(e) + 729(b_2) = 906(E)$ |
| .. | 920 | vvs | $\nu_7(e; \nu\text{-BCl})$ |
| 927 | .. | w | $\nu_7(e; \nu\text{-BCl})$ |
| 948 | .. | w | $107(e) + 401(a_1) + 445(b_2) =$ $953(E); 2 \times 107(e) + 729(b_2) =$ $943(A_1 + A_2 + B_1 + B_2)$ |
| .. | 1029 | w | $291(a_1) + 729(b_2) = 1020(B_2);$ $107(e) + 920(e) = 1027(A_1 + A_2 + B_1 + B_2)$ |
| .. | 1057 | w | $177(e) + 2 \times 445(b_2) = 1067(E)$ |
| .. | 1090 | w | $177(e) + 920(e) = 1097(A_1 + A_2 + B_1 + B_2)$ |
| 1123 | .. | w,p | $\nu_1(a_1; \nu\text{-B}^{11}\text{B}^{11})$ |
| 1150 | .. | w,p | $\nu_1(a_1; \nu\text{-B}^{11}\text{B}^{10})$ |
| .. | 1232 | w | $107(e) + 1123(a_1) = 1230(E)$ |
| 1259 | 1259 | vw | $107(e) + 1150(a_1) = 1257(E)$ |
| .. | 1324 | w | $401(a_1) + 920(e) = 1321(E)$ |
| .. | 1362 | vw | $445(b_2) + 920(e) = 1365(E)$ |
| .. | 2058 | w | $920(e) + 1123(a_1) = 2043(E);$ $920(e) + 1150(a_1) = 2070(E)$ |

* Shifted by Fermi resonance with ν_5 fundamental.

We should like to thank Professor D. H. Rank for making available the spectroscopic equipment used in this study, and the Department of the Air Force and the Navy Bureau of Aeronautics for sponsorship of the investigation.

DEPARTMENT OF CHEMISTRY
THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE PENNSYLVANIA, AND
THE NATIONAL BUREAU OF STANDARDS
WASHINGTON 25, D. C.

M. J. LINEVSKY
E. R. SHULL
D. E. MANN
THOMAS WARTIK

RECEIVED JUNE 5, 1953

BUTYRYL CoA-DEHYDROGENASE, A CUPRO-FLAVO- PROTEIN

Sir:

Butyryl CoA dehydrogenase, previously shown to be a flavoprotein of vivid green color,¹ contains

(1) H. Beinert, R. Bock, D. S. Goldman, D. E. Green, H. R. Mahler, S. Mil, P. O. Stanaly and S. J. Wakil, unpublished.

copper as part of its prosthetic group. This identification rests on the following observations: (1) a solution of the enzyme of highest purity isolated from a preparative electrophoresis run, and shown to be homogeneous in the analytical ultracentrifuge, was dialyzed exhaustively against 10^{-4} M tris-hydroxymethyl-aminomethane buffer of pH 8.0. Part of the preparation was then denatured by boiling for three minutes and the supernatant flavin solution, the denatured protein and the original preparation were examined by arc and spark spectroscopy.² The results summarized in Table I clearly indicate the presence of copper tightly bound to the enzyme protein. (2) Aliquots of two preparations shown to be approximately 22% and 98% pure by analytical electrophoresis (in 0.1 M tris-hydroxymethyl-aminomethane buffer of pH 8.0), and containing 4.7×10^{-5} and 2.6×10^{-5} M flavin,³ respectively, were washed in platinum crucibles for 1 hour at 1000° F. Manometric copper determination with internal standards⁴ showed the presence of 6.9 γ and 3.2 γ of cupric ion per ml. in the two preparations. Thus the mole ratios Cu⁺⁺/flavin were 2.23/1 and 1.90/1 or approximately equal to 2, at two purity levels of the dehydrogenase.

TABLE I
SPECTROSCOPIC METAL DETERMINATION

| | Cu ^a | Mg ^a |
|------------------------------|-----------------|-----------------|
| Buffer before dialysis | — | — |
| Buffer after dialysis | — | — |
| Enzyme after dialysis | ++ | + |
| Supernatant of boiled enzyme | — | + |
| Residue of boiled enzyme | ++ | — |

^a Only metals found present, all others, specifically Fe, Zn, Mn, Mo, Co, Ca, absent in all samples.

(3) The four-banded spectrum of the enzyme (λ_{\max} at 265, 355, 432.5 and 685 m μ) undergoes the following transformations: (a) on treatment with butyryl CoA the peaks at 355, 432.5 and 685 m μ all disappear and the extinctions at these wave lengths are reduced to 0.86, 0.47 and 0.45 times their original value; (b) after dialysis against 5×10^{-3} M KCN,⁵ buffered at pH 7.5, for 36 hours, the flavin peaks at 265, 355 and 432.5 are unchanged in both position and extinction while the peak at 685 m μ disappears.

(4) The cyanide-treated enzyme can still be reduced by butyryl CoA as shown by changes in the flavin region (390–480 m μ) of the spectrum, and is capable of catalyzing the interaction between butyryl CoA and dyes such as 2,6-dichlorophenolindophenol, a two-electron change. However, when the enzyme is tested with oxidizing agents mediating one-electron changes such as ferricytochrome c

(2) We are indebted to Mr. Rubin Shapiro, Department of Chemistry, University of Wisconsin for performing the spectroscopic analyses.

(3) Flavin was determined by assuming a molecular extinction coefficient $\epsilon = 10.6 \times 10^3$ for flavoproteins at their visible maximum (O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150 (1938)). The value so found, was in good agreement with the amount of flavin liberated by boiling the enzyme preparations and calculated assuming an extinction coefficient at 450 m μ $\epsilon = 11.3 \times 10^3$ (E. Dimant, D. R. Sanadi and F. M. Huennekens, *THIS JOURNAL*, **74**, 5440 (1952)).

(4) O. Warburg and H. A. Krebs, *Biochem. Z.*, **190**, 143 (1927); O. Warburg, *ibid.*, **187**, 255 (1927).

(5) F. Kubowitz, *Biochem. Z.*, **292**, 221 (1937); **299**, 32 (1939).