

and the 1,4 distance in **4** being less than in benzene. The effect is greater for the normal MINDO/3 calculations than for those with CI, as one would expect since the energies calculated for biradical species without CI are too large.<sup>17</sup> The geometry thus tends to approximate more closely to that of an equivalent non-radical structure (*e.g.*, **4** instead of **2**).

We also studied the energies of singlet **2** and **3** as a function of the distance between the "radical" centers. In the case of **2** only one minimum was found (Figure 1d, e, but with **3** there were two minima, one corresponding to **3** (Figure 1g, h, and the other to **5**. Thus **3** or **5** is predicted to exist in two isomeric forms with different C<sub>1</sub>C<sub>4</sub> distances. The second isomer is also predicted to be a distorted benzene (Figure 1j) with a very long transannular bond (1.667 Å) and little variation of bond lengths round the ring. The calculated  $\Delta H_f$  (152.8 kcal/mol) is much higher than that for **3** from which it is separated only by a low barrier (4.6 kcal/mol). In the transition state the C<sub>1</sub>C<sub>4</sub> distance is 1.85 Å.

All calculations<sup>8,10</sup> agree in predicting the singlet form of **1** to be the more stable. Those reported here differ, however, from previous ones<sup>8</sup> in predicting **2** to have a singlet ground state. Since this is true both with and without CI, the prediction seems very strong. The opposite conclusion reached in the earlier calculation could then well be due to use of an unrealistic geometry for the singlet.

In the case of **3** the calculations are more ambiguous, those without CI predicting the ground state to be a triplet and those with CI a singlet. It seems likely that the ground state is in fact a singlet but with a small singlet-triplet separation. The *ab initio* calculations<sup>8</sup> could well have led to the opposite conclusion through use of an incorrect geometry.

The most striking prediction from our calculations is that singlet **2** should be at least comparable in stability with singlet **1** (*cf.* Hess and Schaad<sup>18</sup>). This suggests that more consideration should be given to reaction mechanisms involving *m*-benzynes (*e.g.*, **2**) as intermediates.

**Acknowledgment.** This work was supported by the Air Force Office of Scientific Research through Contract F44620-71-C-0119 and by the Robert A. Welch Foundation through Grant F-126.

- (18) B. A. Hess and L. J. Schaad, *Tetrahedron Lett.*, 17 (1971).  
 (19) Robert A. Welch Postdoctoral Fellow.

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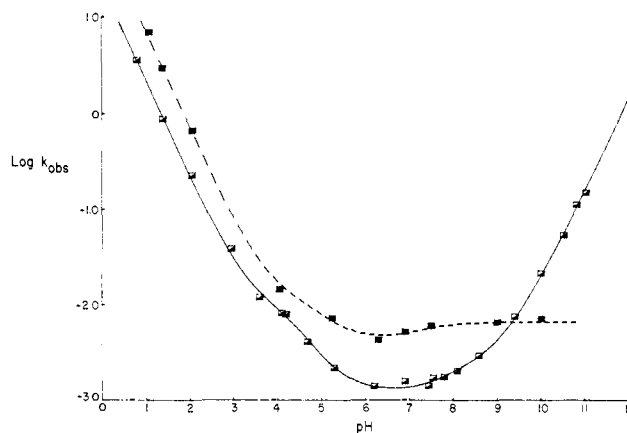
Received February 21, 1974

### A Kinetic Study of the Fate of a Covalent Intermediate of the Type Proposed to be Involved in Flavin Catalysis

Sir:

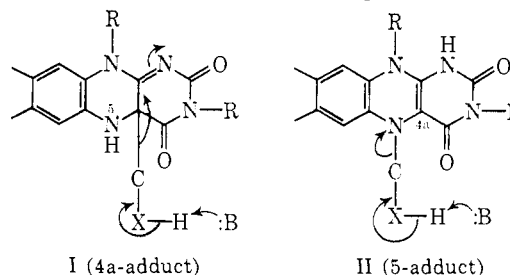
A non-free-radical<sup>1</sup> (dark) flavin oxo-reduction reaction not involving hydrogen transfer<sup>2,3</sup> from substrate to

- (1) S. Shinkai, M. Brüstlein, R. F. Williams, and T. C. Bruice, to be submitted to *Biochemistry*.  
 (2) M. Brüstlein and T. C. Bruice, *J. Amer. Chem. Soc.*, **94**, 6548 (1972).  
 (3) S. Shinkai and T. C. Bruice, *J. Amer. Chem. Soc.*, **95**, 7526 (1973).



**Figure 1.** Log  $k_{\text{obs}}$  vs. pH rate profile for solvolysis of 4a-InH (—) and 4a-InMe (---): Solvent H<sub>2</sub>O (aerobic), 30°,  $\mu = 0.2$ . Rate constants in min<sup>-1</sup>. The E-2 elimination for 4a-InH may be seen in the slope of +1.

flavin would require the formation of a covalent intermediate. Based on chemical intuition<sup>4</sup> and photoreduction<sup>5</sup> studies the site of covalent adduct formation has been suggested to be the 4a- and 5-positions, I and II,



respectively. A 5-carbinolamine adduct has been suggested as an intermediate in the reaction of nitroethane anion with oxidized deuterated amino acid oxidase,<sup>6</sup> but this same adduct has been shown not to be an intermediate in the reduction of formaldehyde and ethyl pyruvate by reduced flavin.<sup>1,3</sup> No direct chemical evidence exists which establishes a 4a-adduct as an intermediate in a flavin catalyzed reaction though addition of SO<sub>3</sub><sup>2-</sup> to the 4a-position has been established for one isoalloxazine.<sup>7</sup>

In aqueous solution (30°,  $\mu = 0.2$  with KCl), the solvolysis of 4a-InH and 4a-InMe yields dihydroflavin (FH<sub>2</sub>) under anaerobic conditions and oxidized flavin (F<sub>ox</sub>) under aerobic conditions.<sup>8</sup> The pH-log  $k_{\text{obs}}$

- (4) G. A. Hamilton, *Progr. Bioorg. Chem.*, **1**, 83 (1971).  
 (5) P. Hemmerich and M. Schuman Jorns, "Enzymes: Structure and Functions," Vol. 29, C. Veeger, J. Drenth, and R. A. Oosterboon Ed., North Holland, Amsterdam, 1973, p 95.  
 (6) D. J. T. Porter, J. G. Voet, and H. J. Bright, *J. Biol. Chem.*, **248**, 4400 (1973).  
 (7) T. C. Bruice, L. Hevesi, and S. Shinkai, *Biochemistry*, **12**, 2083 (1973).  
 (8) 4a-InH ( $\lambda_{\text{max}}$  353 nm;  $\epsilon$  6200 M<sup>-1</sup> cm<sup>-1</sup> in acetonitrile) and 4a-InMe ( $\lambda_{\text{max}}$  355 nm;  $\epsilon$  5400 M<sup>-1</sup> cm<sup>-1</sup> in acetonitrile) were obtained (Ph.D. Dissertation, W. R. Knappe, University of Konstanz, 1971, Germany) by photolysis under argon of absolute acetonitrile solutions of 3-Me lumiflavin and 40-fold excesses of indole-3-acetic acid and 1-methylindole-3-acetic acid, respectively. Rate runs were initiated by introducing 0.1 ml of the acetonitrile stock solution into 3 ml of oxygenated or degassed water or 50% ethanol-water (v/v) and observing the increase in absorbance of F<sub>ox</sub> (441 nm) and decrease in absorbance of 4a-adducts (376 nm at low pH and 370 nm at high pH). 3-Hydroxymethylindole was identified as product at both acidic and basic pH under both anaerobic and aerobic conditions *via* chromatography on silica employing three different solvent systems by comparison of  $R_f$  values to that of the authentic compound and *via* reaction with *sym*-trinitrobenzene.

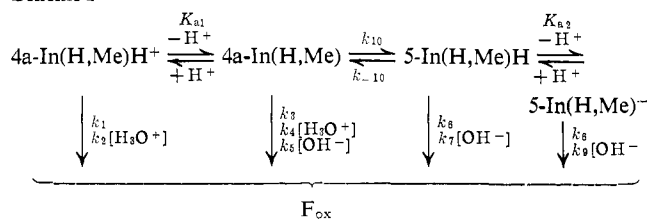
profiles for conversion of 4a-InH and 4a-InMe under aerobic conditions to  $F_{ox}$  are provided in Figure 1. The profiles were computer fitted to the data points employing the empirical eq 1 for 4a-InH and eq 2 for 4a-InMe.

$$k_{obsd} = \frac{(k_a + k_b a_H) a_H}{K_{a1} + a_H} + \frac{K_{a1}}{K_{a1} + a_H} \times \left[ k_c + \frac{k_d}{a_H} + \frac{k_e(k_f + a_H)(k_g + a_H)}{(k_2 + a_H)(k_i + a_H)} \right] \quad (1)$$

$$k_{obsd} = \frac{(k_a + k_b a_H) a_H}{K_{a1} + a_H} + \frac{K_{a1}}{K_{a1} + a_H} \times \left[ k_c + \frac{k_d + k_e a_H}{k_f + a_H} \right] \quad (2)$$

The mechanisms of Scheme I provide eq 3 and 4 which

#### Scheme I



are kinetically identical with the empirical expressions of eq 1 and 2, respectively ( $k_1$  and  $k_4$  and  $k_7$  and  $k_8$  represent kinetically identical paths). In the derivation of eq 3 and 4, steady state has been assumed for 5-In-

$$k_{obsd} = \frac{(k_1 + k_2 a_H) a_H}{k_{a1} + a_H} + \frac{K_{a1}}{K_{a1} + a_H} \left[ k_3 + \frac{k_5 K_w}{a_H} + \frac{k_{10}(k_8 a_H + k_7 K_w + k_8 K_{a2} + k_9 K_{a2} K_w / a_H)}{K_{a1}(k_{-10} + k_8 + k_9 K_w / a_H) + a_H(k_{-10} + k_6) + k_7 K_w} \right] \quad (3)$$

$$k_{obsd} = \frac{a_H(k_1 + k_2 a_H)}{K_{a1} + a_H} + \frac{K_{a1}}{K_{a1} + a_H} \times \left[ k_3 + \frac{k_{10}(k_6 a_H + k_8 K_{a2})}{k_{-10}(K_{a2} + a_H) + k_6 a_H + k_8 K_{a2}} \right] \quad (4)$$

(H,Me)H and 5-In(H,Me)<sup>-</sup> intermediates and the OH<sup>-</sup>-catalyzed paths through  $k_3$ ,  $k_7$ , and  $k_9$  are not allowed for 4a-InMe (see eq 1 vs. 2).<sup>9</sup> The values of  $pK_{a1} = 4.4$  and  $4.9$  are comparable to the  $pK_a$  of protonation on the 5-position obtained for 5-adducts (3.4)<sup>10</sup> and alkyl-substituted anilines (4.3–5.5)<sup>11</sup> while  $pK_{a2}$ 's = 8.1 are in the range of 7–8 previously reported for dissociation of the H–N<sub>1</sub> proton of N<sub>5</sub>-substituted 1,5-dihydroflavins.<sup>5</sup> The isomerization of the 4a- to 5-adducts of Scheme I has precedence in observations from Hemmerich's laboratory.<sup>12,13</sup> In Scheme I this reaction is spontaneous with equilibrium constants of  $K_2 = 5\text{-In(H,Me)}$

(9) The constants derived from the pH-log  $k_{obsd}$  profiles to fit eq 3 and 4 follow: for 4a-InH  $k_1 = 0$  when  $k_4 = 276 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_1 = 1 \times 10^{-2} \text{ min}^{-1}$  when  $k_4 = 0$ ,  $k_2 = 22 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_3 = 7.9 \times 10^{-4} \text{ min}^{-1}$ ,  $k_5 = 112 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_6 = 1.15 \times 10^{-4} \text{ min}^{-1}$ ,  $k_7 = 0$  when  $k_8 = 5.74 \times 10^{-4} \text{ min}^{-1}$ ,  $k_7 = 310 \text{ M}^{-1} \text{ min}^{-1}$  when  $k_8 = 0$ ,  $k_9 = 25 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_{10} = 3.5 \times 10^{-3} \text{ min}^{-1}$ ,  $k_{-10} = 8.0 \times 10^{-4} \text{ min}^{-1}$ ,  $pK_{a1} = 4.4$ ,  $pK_{a2} = 8.1$ ; for 4a-InMe  $k_1 = 0$  when  $k_4 = 954 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_1 = 1.2 \times 10^{-2} \text{ min}^{-1}$  when  $k_4 = 0$ ,  $k_2 = 71 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_3 = 1.5 \times 10^{-3} \text{ min}^{-1}$ ,  $k_5 = 8.0 \times 10^{-4} \text{ min}^{-1}$ ,  $k_6 = 9.7 \times 10^{-3} \text{ min}^{-1}$ ,  $k_{10} = 5.5 \times 10^{-3} \text{ min}^{-1}$ ,  $k_{-10} = 8.0 \times 10^{-4} \text{ min}^{-1}$ ,  $pK_{a1} = 4.9$ ,  $pK_{a2} = 8.1$ .

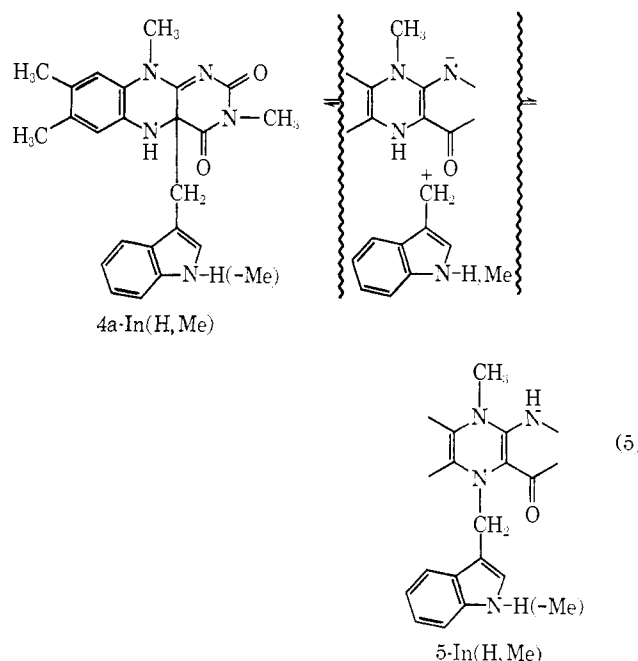
(10) W. R. Knappe and P. Hemmerich, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **13**, 293 (1971).

(11) H. C. Brown, D. H. McDaniel, and O. Häfliger in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955.

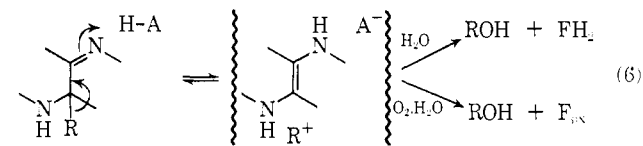
(12) W. Haas and P. Hemmerich, *Z. Naturforsch. B*, **27**, 1035 (1972).

(13) W. H. Walker, P. Hemmerich, and V. Massey, *Eur. J. Biochem.*, **13**, 258 (1970).

Me)/4a-In(H,Me) = 4.4 and 6.9 respectively. Thus, in contrast to the  $\text{SO}_3^{2-}$ -addition to 10-(2',6'-dimethylphenyl)-3-methylisoalloxazine-6,8-disulfonate,<sup>7</sup> the 5-adduct is more thermodynamically stable, but importantly the equilibrium constants are close to unity. This isomerization undoubtedly occurs *via* a tight ion pair (eq 5). Comparison of the pH dependence of the



conversion of 4a-InH to  $F_{ox}$  (aerobic) and  $FH_2$  (anaerobic, argon atmosphere) in 50/50 ethanol-water (v/v),<sup>14</sup> revealed that under anaerobic conditions the pH-log  $k_{obsd}$  profile below pH 3 was depressed by about 0.9 log units, the spontaneous terms were undetectable, and the HO<sup>-</sup>-catalyzed conversion of 4a-InH to  $FH_2$  or  $F_{ox}$  possessed the same rate constants. The acid catalyzed conversions to  $FH_2$  and  $F_{ox}$  may logically be considered to proceed *via* a carbonium ion pair (eq 6). The log of

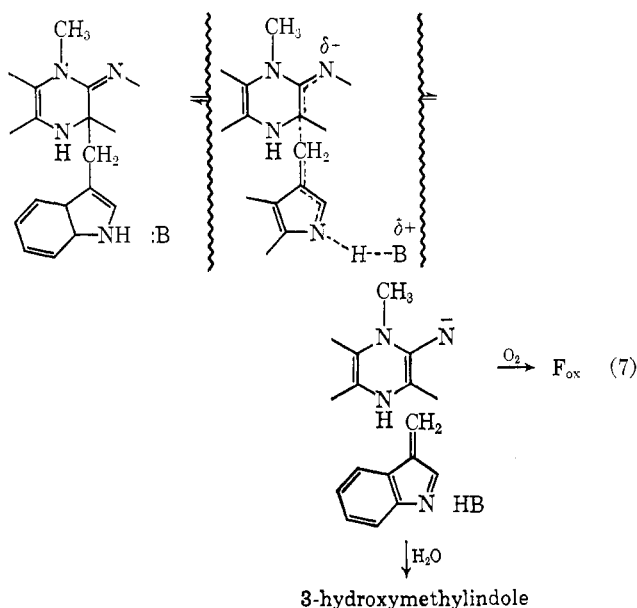


the second-order rate constants for  $\text{H}_3\text{O}^+$ ,  $\text{HCOOH}$ , and  $\text{CH}_3\text{COOH}$  catalysis (aerobic,  $\text{H}_2\text{O}$ ) describe a linear relationship (deviation = 0.0) of slope -0.5 when plotted *vs.*  $pK_a$  of the acids<sup>13</sup> establishing general acid catalysis as indicated in eq 6. The spontaneous terms  $k_1$ ,  $k_3$ ,  $k_6$ , and  $k_8$  (Scheme I) represent direct oxidation. The rate ratio for spontaneous oxidations ( $k_3$ ) of 4a-adducts (4a-InH/4a-InMe = 0.53) dictates little charge on the indole nitrogen and, therefore, little carbonium ion character (possibly direct  $\text{O}_2$  oxidation of substrate). In contrast, the ratio of rate constants for spontaneous ( $k_6$ ) oxidation of neutral 5-adducts (5-InH/5-InMe = 0.14) dictates a more significant (+) charge delocalization to the indole nitrogen and therefore greater carbonium ion character in the transition state (oxidation of the ion pair by  $\text{O}_2$ ).

(14) Because of the sparing solubility of 3-methyl-1,5-dihydroflavin in  $\text{H}_2\text{O}$  these comparative studies necessitated a mixed solvent.

(15) Second-order rate constants for  $\text{H}_3\text{O}^+ = 22 \text{ M}^{-1} \text{ min}^{-1}$ , for  $\text{HCOOH} = 3.53 \times 10^{-2} \text{ M}^{-1} \text{ min}^{-1}$  and for  $\text{CH}_3\text{COOH} = 1.21 \times 10^{-2} \text{ M}^{-1} \text{ min}^{-1}$  based on buffer dilution plots at three concentrations at each of three pH values.

Since the HO<sup>-</sup> catalyzed conversion of 4a-InH to both F<sub>ox</sub> and FH<sub>2</sub> (not seen with 4a-InMe) is dependent upon the same rate constant, the mechanism can only represent an E-2 elimination to provide FH<sub>2</sub> (eq 7). The



log of the second-order rate constants for HO<sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and HPO<sub>4</sub><sup>2-</sup> dictate a Brønsted line (deviation = 0.0) of slope +0.5<sup>16</sup> in accord with general-base catalysis as in eq 7.

These results suggest that in some flavin oxo-reduction reactions: (1) formation of a 5-adduct may lead to a 4a-adduct (dependent upon the carbonium ion stability of the migrating group<sup>3</sup>) prior to base catalyzed formation of oxidized substrate and FH<sub>2</sub>, (2) formation of FH<sub>2</sub> from 5- and (appropriate) 4a-adducts is general-acid and general-base catalyzed, (3) formation of either a 4a- or 5-adduct may lead to oxidized substrate without reduction of F<sub>ox</sub>, and (4) the stabilities of the 4a- and 5-adduct are such that, as intermediates, they should be observable, particularly in a model reaction. The elimination reaction of eq 7 represents an experimental verification of the feasibility of the Hamilton<sup>4</sup> mechanism for flavin catalysis in appropriate cases.<sup>3</sup>

**Acknowledgment.** This work was supported by a grant from the National Science Foundation. We would like to thank the CNRS of France and NATO for partial support and travel grant to D.C.

(16) Second-order rate constants for HO<sup>-</sup> = 112 M<sup>-1</sup> min<sup>-1</sup>, for CO<sub>3</sub><sup>2-</sup> = 1.88 × 10<sup>-1</sup> M<sup>-1</sup> min<sup>-1</sup>, and for HPO<sub>4</sub><sup>2-</sup> = 5.1 × 10<sup>-3</sup> M<sup>-1</sup> min<sup>-1</sup>.

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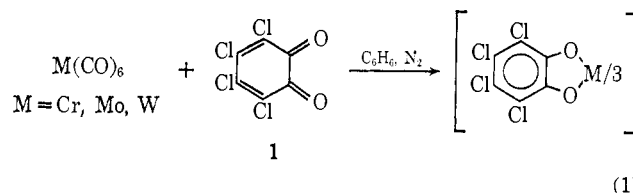
### Neutral Tris(*o*-benzoquinone) Complexes of Chromium, Molybdenum, and Tungsten

Sir:

Considerable attention has been directed at the coordination properties of bidentate ligands which form

unsaturated five-membered chelate rings with metal ions.<sup>1</sup> Interest in these systems stems, in part, from the facile electrochemical properties observed for such complexes. A large majority of the ligands used in these studies have contained nitrogen and sulfur donor atoms. In an effort to investigate the chemical and structural properties of various related oxygen donor ligands, we have begun a study of simple binary metal-*o*-benzoquinone complexes.

Reflux of *o*-tetrachlorobenzoquinone (1) with carbonyls of Cr, Mo, and W in benzene yields diamagnetic, neutral tris complexes of the metals according to reaction 1.<sup>2</sup> The complexes are only slightly soluble in



benzene and may be isolated from the reaction mixture by reducing the volume of the cooled solution.<sup>3</sup> In solid they are moderately air stable<sup>4</sup> and are highly colored: red-purple for Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub>, violet and brown for the Mo and W complexes. Infrared spectra show no bands above 1550 cm<sup>-1</sup> consistent with oxygen-bonded, reduced *o*-benzoquinone ligands.<sup>5</sup> Spectral bands for Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> are sharp and agree with spectra reported previously for chelated *o*-tetrachlorobenzoquinone complexes.<sup>6</sup> Spectra for Mo(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> and its W analog contain broader bands which in some cases are split into two distinct sets. Electronic spectra of the complexes show similar trends.<sup>7</sup> The spectrum of the Cr complex is well resolved while the Mo and W complexes show only large broad bands. The electrochemical properties of all three complexes have been examined using cyclic voltammetry.<sup>8</sup> The Mo and W complexes undergo one electron reduction reactions irreversibly while the Cr complex undergoes three reversible oxidation steps at +0.12, +0.54, and +0.88 V, with reduction of Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub><sup>3+</sup> occurring at +0.07, +0.41, and +0.77 V. Attempts are currently underway to examine each member of this series.

The complex Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> is clearly different from its Mo and W analogs and also from related 1,2-dithiolene

(1) (a) J. A. McCleverty, *Progr. Inorg. Chem.*, **10**, 49 (1968); (b) G. N. Schrauzer, *Accounts Chem. Res.*, **2**, 72 (1969).

(2) A reaction time of about 36 hr is required for the Cr complex. The Mo and W complexes require about 12 hr.

(3) *Anal.* Calcd for Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub>·4C<sub>6</sub>H<sub>6</sub>: C, 45.7; H, 2.2; Cl, 38.6; Cr, 4.7. Found: C, 45.8; H, 2.2; Cl, 38.9; Cr, 4.5. Calcd for Mo<sub>2</sub>(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub>·3C<sub>6</sub>H<sub>6</sub>: C, 34.1; H, 1.0; Cl, 44.8; Mo, 10.1. Found: C, 33.2; H, 1.1; Cl, 44.6; Mo, 10.4. Calcd for W<sub>2</sub>(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub>·3C<sub>6</sub>H<sub>6</sub>: C, 31.2; H, 0.9; Cl, 41.0; W, 17.7. Found: C, 28.2; H, 0.7; Cl, 41.2; W, 16.7. The presence of solvent of crystallization has been verified by mass spectra.

(4) The Cr complex is quite stable in solid or in solution. The Mo and W complexes decompose in air over the period of a few weeks as solid samples.

(5) Principal infrared bands (cm<sup>-1</sup>): Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> 1440, 1330, 1270, 1245, 1210, 983, 813, 792; Mo<sub>2</sub>(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> 1505, 1355, 1200, 960, 815, 800, 777, 757; W<sub>2</sub>(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> 1530, 1375, 1250, 980, 825, 805, 795, 773.

(6) Y. S. Sohn and A. L. Balch, *J. Amer. Chem. Soc.*, **94**, 1144 (1972).

(7) Electronic spectra (λ<sub>max</sub>, nm (ε); measured in dichloromethane): Cr(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> 1190 (1100), 1000 (1600), 765 (5600), 525 (17,600), 495 (11,400) (sh), 460 (5900) (sh), 313 (14,300) (sh); Mo<sub>2</sub>(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> 730 (6800), 560 (5000) (sh), 510 (4400) (sh), 290 (12,000) (sh); W<sub>2</sub>(O<sub>2</sub>C<sub>6</sub>Cl<sub>4</sub>)<sub>3</sub> 620 (1600) (sh), 560 (2300) (sh), 405 (4200), 290 (23,300) (sh).

(8) Cyclic voltammetry performed in dichloromethane with (C<sub>4</sub>H<sub>9</sub>)N-ClO<sub>4</sub> as supporting electrolyte vs. Ag/AgCl reference electrode.