CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF UTAH STATE UNIVERSITY, LOGAN, UTAH

Complexes of Cysteine with Molybdenum(V) and Molybdenum(VI)

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Spectrophotometric studies have shown that cysteine forms complexes in acidic solution with molybdenum(V) and molybdenum(VI). The molybdenum(VI) complex has a cysteine:metal ratio that varies from 3:1 to 1:1, depending upon concentration. Studies with compounds similar to cysteine have indicated that the ionized mercapto and carboxyl groups of cysteine are most likely involved in the complex. Molybdenum(V) forms a 1:1 complex with cysteine that is unstable to atmospheric oxidation. Formation constants for the complexes have been estimated. The implications for sulfhydryl enzymes are discussed.

The molybdo-flavoenzymes, xanthine oxidase and nitrate reductase, have been reported to be sulfhydryl enzymes.[2,3] In the course of an investigation in this Laboratory of the biological function of molybdenum it seemed of interest to study complexes of this metal with cysteine in aqueous solution, as a model for possible molybdenum–enzyme interaction.

It has been reported that thioglycollic acid (mercaptoacetic acid) forms complexes with both molybdenum(V) and (VI) and that this compound can be used as a reagent for the photometric determination of molybdenum.[4] It is also known that molybdenum forms complexes with various sulfhydryl compounds.[5,6] However, no reports have appeared concerning complexes with cysteine or cysteine-containing peptides.

Experimental

Spectrophotometric data were obtained with a Perkin-Elmer Model 4000-A recording spectrophotometer and Beckman Model DU and Model B spectrophotometers, using cells of the appropriate path length. A silica absorption cell, which could be evacuated, was joined to a specially constructed vessel for the molybdenum(V) measurements. This was necessary since molybdenum(V) is easily oxidized by the oxygen of the air unless kept in solutions 1 *M* or stronger in hydrogen ion. The proper amount of molybdenum(V) (in 3 *M* HCl), buffer, and cysteine were added to the vessel under nitrogen or helium. A sample then was drawn off into the evacuated cell by means of a stopcock and the closed cell removed from the vessel and placed in the spectrophotometer for absorption measurements. The absorbance of the sample in the cell was constant for 3 hr., indicating no oxidation during this period. All solutions were kept in a constant temperature bath at 25 ± 0.1° for at least 30 min. before measurement and the cell compartment of the Beckman DU spectrophotometer was equipped with thermospacers which kept the temperature in the cell compartment at 25 ± 1°.

Cysteine ethyl ester, β-mercaptopropionic acid, and β-mercaptoethylamine were slowly oxidized by Mo(VI) at pH 5, forming molybdenum blue. In order to minimize this reaction, solutions of these compounds and Mo(VI) were cooled to 0° immediately upon mixing and kept at this temperature until just before measurements were made. They then were placed in a constant temperature bath, warmed to 25°, and read immediately in the spectrophotometer. This procedure was unnecessary in the case of cysteine.

L-Cysteine, S-methylcysteine, L-cysteine ethyl ester hydrochloride, L-cystine, β-mercaptoethylamine hydrochloride, and L-alanine were purchased from Nutritional Biochemicals Corp. β-Mercaptopropionic acid was purchased from Eastman Kodak Co. Methyl β-mercaptopropionate was prepared according to the method of Schmidt and Grob.[7] All mercapto compounds were analyzed by the iodometric titration method of Neville and Gorin.[8] Deaerated solutions of these compounds were prepared just before use and kept under nitrogen. All other compounds were used without further purification.

Molybdenum(V) and (VI) solutions were prepared and standardized as described previously.[9] All buffers used were prepared from reagent grade chemicals.

Results

Molybdenum(VI).—In the pH range from 4–6, a deep yellow color is formed immediately when molybdenum(VI) is added to a solution of cysteine, indicating complex formation. Molybdenum(VI) solutions are colorless, but exhibit "end absorption," beginning at about 350 mμ in the concentration range used, with the absorbance increasing sharply with decreasing wave length. The spectrum of the complex shows the same kind of absorption, without any maximum, but shifted bathochromically. The yellow color is due to the long shoulder that extends into the visible region. At 400 mμ the absorbance of molybdenum(VI) is negligible, while that of the complex is sufficient for making measurements. This wave length was used for most measurements.

The effect of pH on the absorbance of the complex at 400 mμ is seen in Fig. 1. The rise of the absorbance below pH 4.6 is most likely due to a molybdenum(V)–molybdenum(VI) compound formed when reduction of some molybdenum(VI) by cysteine at this lower pH occurs. Ostrowetsky and Souchay have reported molybdenum(V)–molybdenum(VI) complexes that absorb at 450 mμ[10] and 325 mμ.[11] As the pH is lowered

(1) Abstracted from the thesis submitted by H. C. in partial fulfillment of the requirements of the M.S. degree at Utah State University, 1962. Journal paper No. 228, Utah State University Agricultural Experiment Station.

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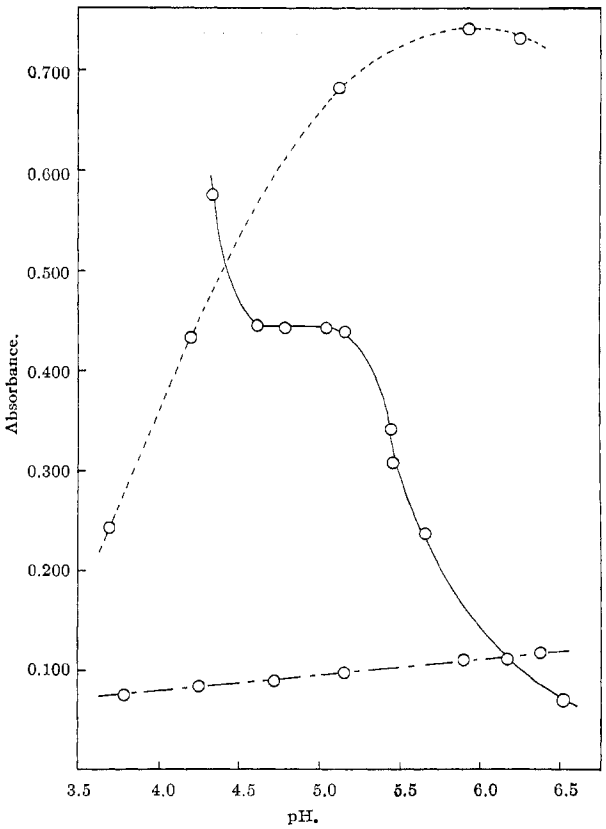

Fig. 1.—Effect of pH on absorbance of complexes: ——— 3.00 × 10^{-2} *M* cysteine + 1.00 × 10^{-2} *M* Mo(VI), 400 mμ, 1-cm. cells; – – – 1.00 × 10^{-2} *M* cysteine + 1.76 × 10^{-3} *M* Mo(V), 350 mμ, 0.2-cm. cells; — — — — 1.76 × 10^{-3} *M* Mo(V), 350 mμ, 0.2-cm. cells.

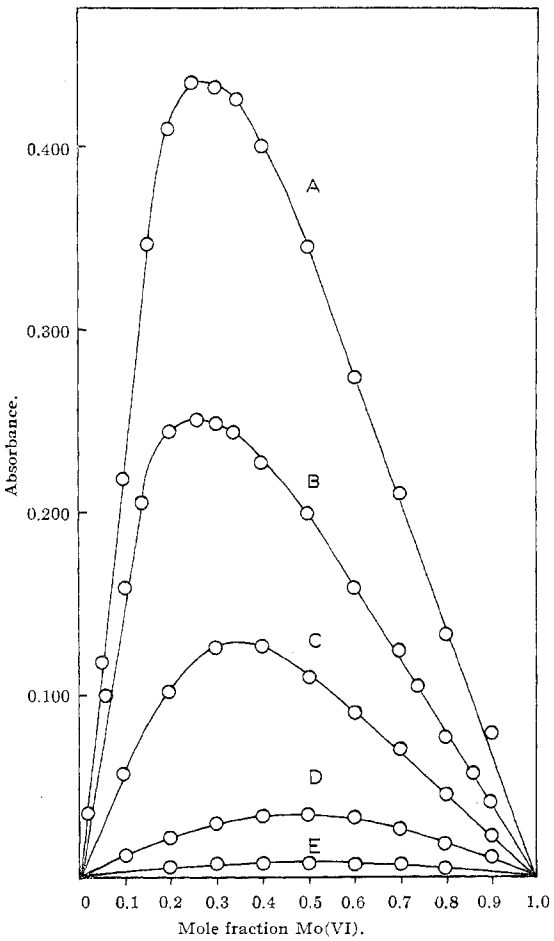

Fig. 2.—Job's method of continuous variations for Mo(VI)–cysteine complex. The sum of the Mo(VI) and cysteine concentrations is: A, 4.00 × 10^{-2} *M*; B, 3.00 × 10^{-2} *M*; C, 2.00 × 10^{-2} *M*; D, 1.00 × 10^{-2} *M*; E, 5.00 × 10^{-3} *M*. Absorbances at 400 mμ are plotted *vs.* mole fraction of Mo(VI) at pH 5.00 in 1.5 *M* acetate buffer; 1-cm. cells.

below 4, molybdenum blue is formed due to this reduction. As can be seen, little complex exists above pH 6.5. Because of this, useful measurements could be made only in the pH range 4.6–6.5.

Figure 2 is a Job's method plot for the molybdenum(VI) complex at 400 mμ and at five different total concentrations. It can be seen that the ratio of cysteine:molybdenum in the complex changes from 3:1 at 3.00 × 10^{-2} *M* and higher concentrations to 1:1 at 1.00 × 10^{-2} *M* and lower concentrations. Concentrations higher than 4.00 × 10^{-2} *M* could not be used due to the low solubility of cysteine at this pH. Similar plots made at other pH values within the useful range, and at wave lengths of 350 and 375 mμ, gave similar results.

Experiments involving compounds with structures similar to cysteine were carried out in order to determine which groups of the cysteine molecule are involved in complex formation. The results are found in Fig. 3. It can be seen that β-mercaptopropionic acid and cysteine ethyl ester both give 3:1 complexes with Mo(VI) under the same conditions as cysteine, but β-mercaptoethylamine gives only a weak 1:1 complex under these conditions.

No spectrophotometric evidence for complex formation between Mo(VI) and L-cystine, S-methylcysteine, methyl β-mercaptopropionate, or L-alanine was found. Furthermore, L-alanine was studied polarographically and polarimetrically to investigate the possibility of formation of non-absorbing complexes. Again, no indication of complex formation was obtained.

Molybdenum(V).—Solutions of molybdenum(V) are yellow-orange in color due to an absorption maximum at 298 mμ with a long shoulder sloping into the visible. When cysteine is added to solutions of molybdenum(V) at pH 3–6 the color becomes considerably more intense, indicative of complex formation. The spectrum of the complex is almost identical with that of molybdenum(V) except that the absorption is greatly increased. The largest difference in absorbance between molybdenum(V) and the complex occurs at 350 mμ and this wave length was used for measurements at a concentration of 4.60 × 10^{-3} *M*. Due to the high absorbance of the complex, a wave length of 425 mμ was used for the 4.00 × 10^{-2} *M* concentration.

The effect of pH on the absorbance of the complex at 350 mμ is seen in Fig. 1. No measurements were made above pH 6.2 because of the tendency of molybdenum(V) to precipitate as the hydroxide. It is apparent that the complex has a maximum absorbance between pH 5 and 6 and most measurements were made in this range.

Figure 4 is a Job's method plot of the molybdenum-

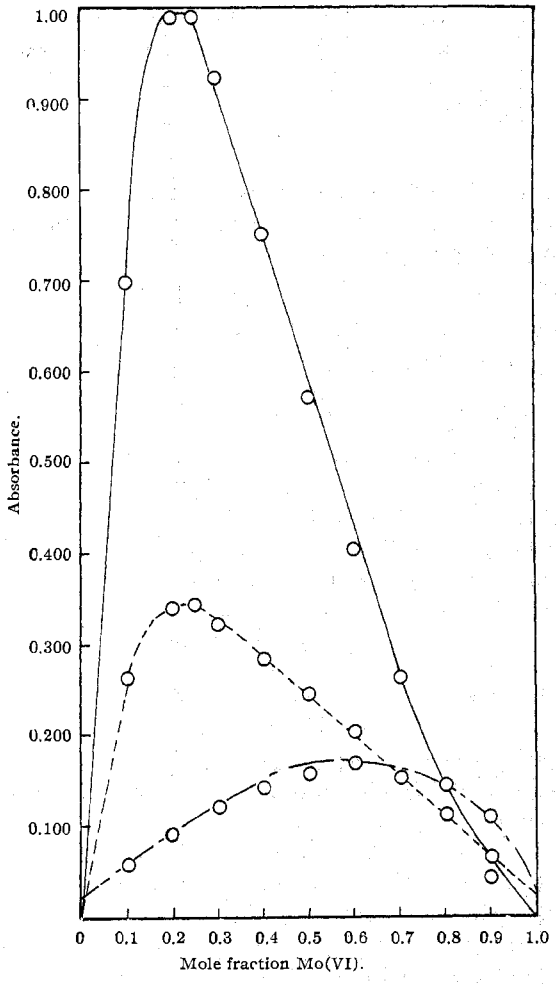

Fig. 3.—Job's method of continuous variations: ———, Mo(VI) + cysteine ethyl ester; ---, Mo(VI) + β-mercaptopropionic acid; — – — –, Mo(VI) + β-mercaptoethylamine. Absorbance at 400 mμ is plotted *vs.* mole fraction of Mo(VI) at pH 5.00 in 1.5 *M* acetate buffer. The sum of Mo(VI) and ligand concentrations is 4.00×10^{-2} *M*. Cysteine ethyl ester measured in 1-cm. cells; β-mercaptoethylamine and β-mercaptopropionic acid measured in 10-cm. cells.

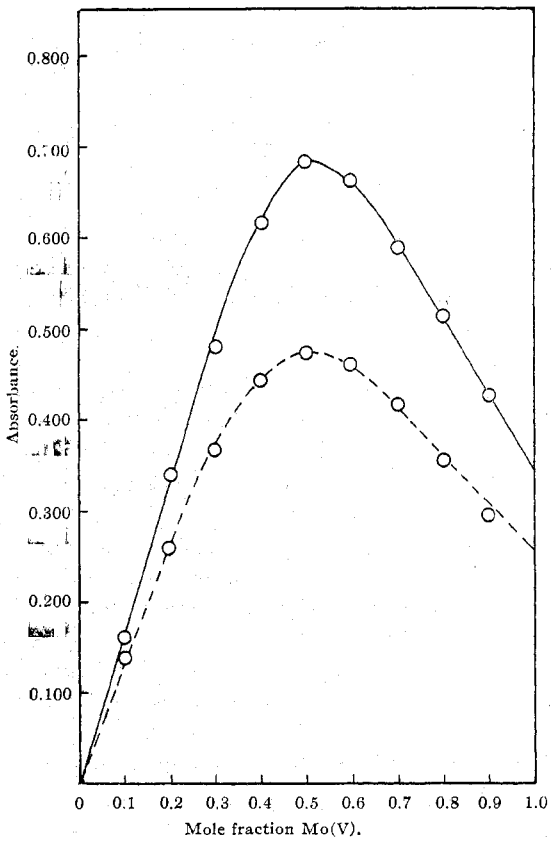

Fig. 4.—Job's method of continuous variations for Mo(V)–cysteine complex: ——— Mo(V) + cysteine concentrations = 4.00×10^{-2} *M*, absorbance at 425 mμ is plotted *vs.* mole fraction of Mo(V) at pH 5.45 in 3 *M* acetate buffer, 0.2-cm. cells; --- Mo(V) + cysteine concentrations = 4.60×10^{-3} *M*, absorbance at 350 mμ is plotted *vs.* mole fraction of Mo(V) at pH 5.45 in 1.5 *M* acetate buffer, 0.2-cm. cells

(V)–cysteine complex at 350 and 425 mμ. It is clear that a 1:1 complex is formed. Similar results were obtained at other wave lengths and at pH 4.60, indicating only a 1:1 complex is formed under these conditions. The weakness of the complex was evident upon opening the system to the atmosphere. The absorbance at 350 mμ decreased rapidly as the molybdenum(V) was oxidized to molybdenum(VI). This is in marked contrast to the behavior of molybdenum(V) complexes with EDTA[12] and 8-hydroxyquinoline-5-sulfonic acid,[13] which are stable to oxidation for long periods.

Discussion

Molybdenum(VI).—The data indicate that Mo(VI) forms various complexes with cysteine in the pH range 4–6. The ratio of cysteine to molybdenum is extremely sensitive to concentration, varying from 3:1 to 1:1 over a limited concentration range. This variation is somewhat unusual; however, it may be due to an influence on the state of polymerization of molybdate, which is known to vary with concentration in acidic solution.[14]

It is of considerable interest to know which functional groups of cysteine are involved in complex formation. In the 3:1 complex, cysteine is most likely bidentate. This is substantiated by the results with β-mercaptopropionic acid, which forms a complex, and its methyl ester, which does not, indicating that two groups are necessary. There are, therefore, three possibilities for chelation: (1) the amino and the carboxy groups, (2) the amino and mercapto groups, and (3) the carboxy and the mercapto groups. The experiments with alanine, which gives no evidence for complex formation, rule out the first possibility. Since no complexes are formed with S-methylcysteine or cystine, the ionized mercapto group is almost certainly involved. The results with β-mercaptopropionic acid and cysteine ethyl ester, both of which complex in a manner identical with that of cysteine, indicates both possibilities (2) and (3) may be correct. However, the results with β-mercaptoethylamine, which forms only a weak 1:1 complex, seem to contradict those with cysteine ethyl

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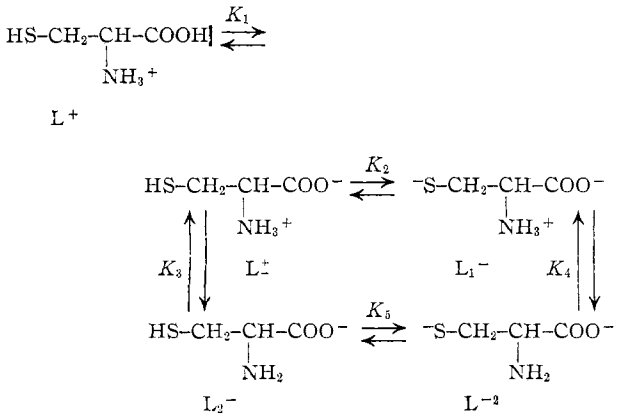
TABLE I
ACID DISSOCIATION CONSTANTS

Compound	pK_1	pK_2	pK_3	pK_4	pK_5
L-Cysteine[15]	2.00	8.53	8.86	10.36	10.03
L-Cysteine ethyl ester[15]		7.45	6.77	8.41	9.09
β-Mercaptopropionic acid[16]	4.33	10.54			
β-Mercaptoethylamine[15]		8.35		10.81	

ester; both of these have only a mercapto and an amino group available for complexing. The difference in behavior between these two compounds is very likely due to the differences in acid dissociation constants, as seen in Table I. It is clear that at pH 5, the concentration of the species of cysteine ethyl ester which can complex ($^-S—CH_2—CH(NH_2)—C(=O)—OCHC_2H_3$) is considerably greater than the concentration of the complexing form of β-mercaptoethylamine ($^-S–CH_2–CH_2–NH_2$), and is of the same order of magnitude as that for the corresponding form of β-mercaptopropionic acid. Apparently, Mo(VI) complexes with the ionized mercapto group and either the free amino or ionized carboxy group, with both complexes having the same order of stability. This conclusion is confirmed by a comparison of the estimated formation constants (see below).

Cysteine exists in five forms in solution, depending on the pH[15]



At the pH of interest (4–6) the predominant species is the zwitterion, $L^{\pm}$. However, the experiments with similar compounds referred to above indicates that the main form that complexes is L_1^-, since the complex involves the ionized mercapto group and either the free amino or ionized carboxy group, and L_1^- is present in considerably larger amounts in this pH range than L^{-2}. There, is, of course, a small amount of L^{-2} complex in equilibrium with the L_1^- complex.

The formation constant for the 3:1 cysteine complex was *estimated* from the continuous variations plots in the following way: The linear part of the curve, from mole fraction Mo(VI) = 0 to mole fraction Mo(VI) = 0.1, was extended until it intersected a vertical line drawn through mole fraction Mo(VI) = 0.25 (3:1 complex). The absorbance corresponding to this point is the hypothetical absorbance, A_m, the complex would have if it was completely undissociated. The concentration of the complex, [C], is then given by the ratio of the actual absorbance, A, to the hypothetical absorbance

$$[C] = A/A_m \cdot M_t \quad (1)$$

where M_t is the total metal ion concentration. The concentration of the free metal ion, [M], then is obtained from the equation

$$M_t = [M] + [C] \quad (2)$$

The total uncombined ligand (L) ($L = L^{\pm} + L_1^- + L_2^- + L^{-2}$) is obtained from the equation

$$L_t = [L] + 3[C] \quad (3)$$

where L_t is the total ligand concentration. The concentration of free ligand in the L_1^- form then was calculated from the dissociation constants of cysteine in Table I. Finally, the formation constant was calculated at total concentrations of 0.03 and 0.04 M from the equation

$$K = [C]/[M][L_1^-]^3 \quad (4)$$

In order to obtain some measure of the precision of the results, it would be desirable to have continuous variation plots at higher total concentrations. Due to the limited solubility of cysteine this was not possible. However, since the complex is somewhat more soluble than cysteine, solutions containing cysteine and Mo(VI) in a 3:1 ratio were prepared having total concentrations (Mo(VI) + cysteine) of 0.05 and 0.06 M and their absorbance measured. The theoretical absorbances (α_m) of these solutions, if the complex was completely undissociated, were calculated using Beer's law. The molar absorptivity for the complex was obtained from the theoretical absorbance of the complex on the 0.04 M continuous variation plot. This allowed the calculation of two more values of the formation constant. The result of these four calculations gives a value for log $K_{Mo(VI)}$ and its standard deviation of 18 ± 1. It is clear that the results indicate only the order of magnitude of the constant.

This rather large uncertainty may mean that the solutions contain some lower complexes in addition to the 3:1 complex. In addition, the complexes may very possibly be polymerized. Nevertheless, the values give an estimate of the relative stabilities of the complexes.

Formation constants for β-mercaptopropionic acid and cysteine ethyl ester complexes were estimated in the same way; log $K_{Mo(VI)}$ for both complexes was found to be 23.

The formation constants for β-mercaptopropionic acid and cysteine ethyl ester indicate that the affinity of Mo(VI) for the ionized carboxy and amino groups is very similar. The greater value of the formation constant for β-mercaptopropionic acid, as compared with that for cysteine, probably reflects the presence of the charged amino group in cysteine, which would reduce the availability of the electrons of the carboxy and mercapto groups for bond formation with the metal ion.

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Molybdenum(V).—Since molybdenum(V) absorbs at 425 and 350 mμ, the formation constant for the 1:1 molybdenum(V)–cysteine complex was calculated in the following manner: At the maximum point on the Job's method plot the total absorbance (A_T) is due to the complex [C] and the free molybdenum(V) species (M)

$$A_T = \epsilon_C[C]b + \epsilon_M[M]b \quad (5)$$

where ϵ_C is the molar absorptivity for the complex. This was obtained from the Job's method plot at low mole fractions of Mo(V) where essentially all the metal ion is in the form of the complex. ϵ_M is the molar absorptivity for Mo(V). This was obtained from the plot of [Mo(V)] *vs.* absorbance. b is the path length.

Equations can be written for the total concentration M_T of molybdenum(V) and the total concentration L_T of cysteine at this point.

$$M_T = [M] + [C] \quad (6)$$

$$L_T = [L] + [C] \quad (7)$$

where [L] = total uncombined ligand concentration. Combining eq. 5, 6, and 7 and solving for [C] gives

$$[C] = \frac{A_T - \epsilon_M M_T b}{b(\epsilon_C - \epsilon_M)} \quad (8)$$

Using the values of [C], [M], and [L] thus obtained, and the dissociation constants for cysteine, the formation constant was calculated with eq. 9.

$$K = \frac{[MoL]}{[Mo][L_i^-]} \quad (9)$$

The value for $\log K_{Mo(V)}$ was found to be 6.0 ± 0.1.

In conclusion, the 3:1 complex with molybdenum(VI) involves the ionized mercapto group and most likely the carboxyl group. In addition, complexes of similar stability are formed with related compounds involving the mercapto and amino groups. Thus, it would appear that molybdenum(VI) could complex with cysteine-containing enzymes, perhaps binding between the mercapto group and a free carboxyl or amino group. Further work involving small peptides to determine this is planned.

In view of the weakness of the molybdenum(V)–cysteine complex and its ease of oxidation it is unlikely that interaction of molybdenum(V) and sulfhydryl enzymes in this manner is of any biological significance.

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Infrared Spectra and Structures of Some Metal Hexacarbonyl Derivatives[1]

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The carbonyl stretching frequencies for a variety of substituted metal hexacarbonyls are reported, and the type of bonding to be expected in such complexes is discussed briefly. Arguments are given to show that $W(CO)_4(CH_3CN)_2$ and $W(CO)_3(CH_3CN)_3$ exist as the *cis* and symmetrical isomers, respectively, and that the acetonitrile ligands are attached to the metal by coördinate bonding through the nitrogen rather than by π-bonding through the CN group.

Introduction

When solutions of metal hexacarbonyls (M = Cr, Mo, W) in non-aromatic solvents are exposed to ultraviolet radiation, color changes, usually to yellow, are observed. In most cases these changes have been found to be caused by the formation of new complexes in which one or more solvent molecules have replaced carbonyl groups. Several such complexes have been isolated, characterized, and their infrared spectra recorded.[2–5] The formation of such complexes is believed to proceed through the stepwise replacement of carbonyl groups after the initial photolytic formation of $M(CO)_5$.[2,6–8] Little has been done, however, with respect to the determination of the structures of complexes of this nature, although both the stereochemistry and the nature of the bonding between the solvent molecules and the metal atom where either coördinate covalent or π-bonding is possible, *e.g.*, with acetonitrile, would be of considerable interest. This paper will explore some of these problems using infrared spectroscopy as the principal tool.

In general it has been found that solvent molecule-substituted metal hexacarbonyls are somewhat unstable and that irradiated solutions containing the complexes exhibit a marked tendency to shift back to the original reactants after the irradiation is stopped.[2] Such properties render isolation of many complexes imprac-

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