Molybdenum(V) and Molybdenum(VI) Complexes with 8-Hydroxyquinoline-5-sulfonic Acid

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An investigation of molybdenum complexes with compounds structurally similar to flavin mononucleotide has led to a spectrophotometric study of molybdenum(V) and molybdenum(VI) complexes with 8-hydroxyquinoline-5-sulfonic acid in aqueous solution. These complexes have a molar ratio of one ligand to one metal ion over a wide range of concentrations and pH values. Molybdenum(V) is stabilized below pH 6 by the ligand against oxidation by atmospheric oxygen. Formation constants for both complexes have been calculated and found to be almost equal.

Recently, work in this Laboratory has revealed that a strong complex between flavin mononucleotide (FMN) and molybdenum(VI) exists in aqueous solution.[2] In an effort to determine which part of the FMN molecule may be involved in the complex, structurally similar ligands have been under investigation. Due to its similarity to FMN (when the isoalloxazine nucleus of FMN is in the enol form),[3] and to its water solubility, 8-hydroxyquinoline-5-sulfonic acid (HQSA) was chosen.

FMN   HQSA
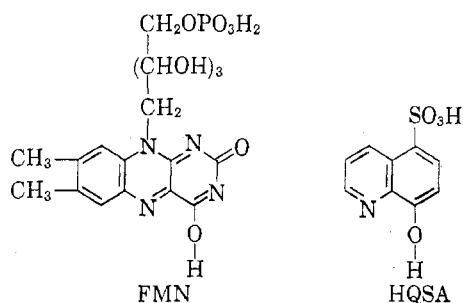
Molybdenum(VI) is precipitated from acidic solution by 8-hydroxyquinoline[4] and can be extracted into chloroform by this reagent.[5] The solid molybdenum(VI) complex has a ratio of two ligands per metal,[5] while the ratio in chloroform is unknown. It has been reported that molybdenum(V) forms a complex with 8-hydroxyquinoline,[6] but no information concerning its structure was given. In spite of its use as an analytical reagent for molybdenum, little is known of the complexes of 8-hydroxyquinoline with this metal in aqueous solution.

Experimental

Spectrophotometric measurements were made with two instruments: a Perkin–Elmer Model 4000-A recording spectrophotometer and a Beckman Model DU spectrophotometer, using cells of the appropriate path length. The Model DU was equipped with thermospacers which maintained the cell compartment at $25 \pm 1°$, while all solutions were kept in a thermostat at $25.0 \pm 0.1°$ for at least 30 min. before making measurements.

Molybdenum(V) and (VI) solutions were prepared and standardized from reagent grade sodium molybdate as described previously.[2] After recrystallization from water, HQSA was dried over phosphorus pentoxide and stored in a desiccator. Stock solutions were prepared by dissolving, with warming, the proper amounts in water. The HQSA was found by potentiometric titration to be 99.3% pure.

All buffer solutions were prepared from reagent grade chemicals.

Results

Molybdenum(VI).—In the pH range from 4–6, a yellow color is developed immediately when solutions of HQSA and sodium molybdate are mixed. A spectrum of the mixture at pH 5.00 indicates the complex has an absorption peak at 370 mμ. The ligand also absorbs slightly at this wave length. The effect of pH on the absorbance of the complex at this wave length is seen in Fig. 1. The complex has the greatest absorbance at pH 4.5. The greatest difference in absorbance between the complex and the ligand occurs at pH 5.00 and most subsequent work on this complex was done at this pH.

Figure 2 is a plot, at three different wave lengths, of mole fraction HQSA *vs.* absorbance,

(1) Abstracted from a thesis submitted by E.R.P. in partial fulfillment of the requirements for the M.S. degree at Utah State University, 1961.

(2) J. T. Spence and J. Tocatlian, *J. Am. Chem. Soc.*, **83**, 816 (1961).

(3) A. Albert, *Biochem. J.*, **54**, 646 (1953).

(4) G. Balenescu, *Z. anal. Chem.*, **83**, 470 (1931).

(5) C. H. R. Gentry and L. G. Sherrington, *Analyst*, **75**, 17 (1950).

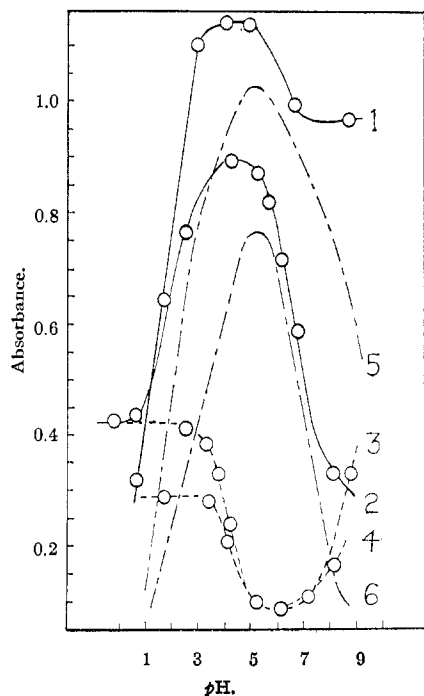(6) D. J. D. Nicholas and H. M. Stevens, *Nature*, **176**, 1066 (1955).

Fig. 1.—Variation of absorbance with pH: ———1, 1.754 × 10^{-4} M Mo(V) plus 1.754 × 10^{-4} M HQSA, 383 mμ; 2, 1.754 × 10^{-4} M Mo(VI) plus 1.754 × 10^{-4} M HQSA, 370 mμ; — — — —3, 1.754 × 10^{-4} M HQSA, 383 mμ; 4, 1.754 × 10^{-4} M HQSA, 370 mμ; — · —5, difference between curves 1 and 3; 6, difference between curves 2 and 4.
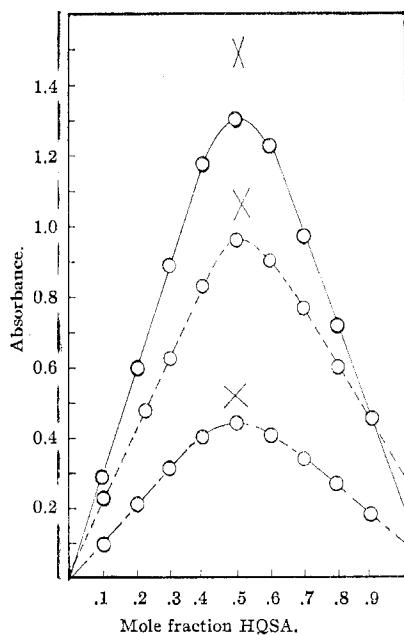

Fig. 2.—Job's method of continuous variations for Mo(VI)–HQSA complex. The sum of the Mo(VI) and the HQSA concentrations is 4.69 × 10^{-4} M. Absorbance is plotted $vs.$ mole fraction HQSA at pH 5.00 in 0.20 M acetate buffer: ——— 370 mμ; — — — 350 mμ; — · — 400 mμ.
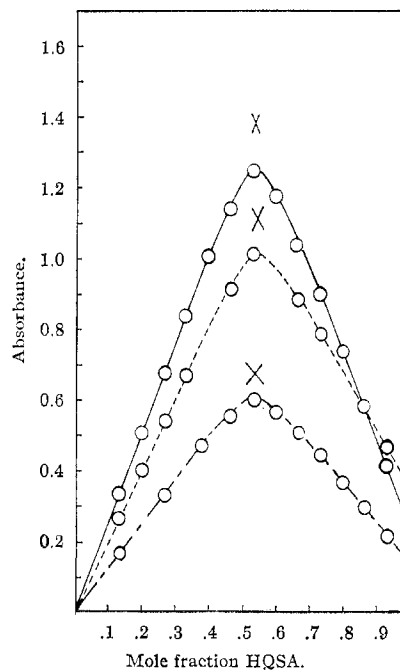

Fig. 3.—Job's method of continuous variations for Mo(V)–HQSA complex. The sum of the Mo(V) and the HQSA concentrations is 4.19 × 10^{-4} M. Absorbance is plotted $vs.$ mole fraction HQSA at pH 5.50 in 0.38 M acetate buffer: ——— 383 mμ; — — — 350 mμ; — · — 425 mμ.

according to the method of Job. This plot indicates that a one to one complex exists at all three wave lengths. The same ratio was obtained at three different total concentrations (3.18 × 10^{-3} M, 4.67 × 10^{-4} M, and 3.51 × 10^{-5} M) and at three pH values (2.68, 5.00, and 6.25).

Molybdenum(V).—At a hydrogen ion concentration lower than 1 M, molybdenum(V) is oxidized to molybdenum(VI) by atmospheric oxygen. When solutions of molybdenum(V) and excess HQSA are mixed at pH 5.00 a deep yellow color is formed, indicating complex formation. This complex has an absorption peak at 383 mμ and its absorbance is stable for 24 hr. After this time, the absorbance begins to decrease slowly due to oxidation of the molybdenum(V). Figure 1 shows that the absorbance of the complex is a maximum at pH 4.5 and the difference in absorbance between the complex and the ligand is greatest at pH 5.30.

A Job's plot, Fig. 3, at three different wave lengths, indicates the complex exists in a one to one ratio. The same results were obtained at different concentrations and pH, similar to the molybdenum(VI) complex. It was found that the absorbance of solutions equimolar in

molybdenum(V) and HQSA remained constant for at least 4 hr., which allowed calculation of formation constants from the Job's method plot.

Calculations.—HQSA exists in one of four forms, dependent on pH

$$H_3L^+ \quad pK_1 = 1.3 \quad H_2L \quad pK_2 = 4.104 \quad HL^- \quad pK_3 = 8.750 \quad L^-$$
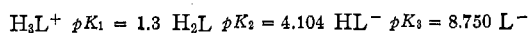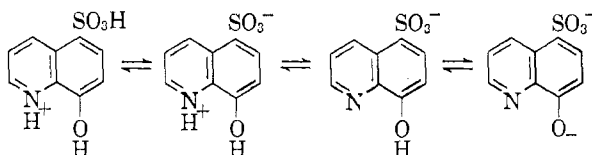
At the pH of interest (5.00–5.50) HQSA exists primarily as HL^-, with a small amount of H_2L and negligible quantities of the other species present.[7] When solutions of HQSA and molybdenum(VI), each initially at pH 5.00, were mixed, a substantial drop in pH occurred, indicating that the species complexing with molybdenum is L^-.

In order to calculate a formation constant, the concentration of L^- in equilibrium with the complex must be known. Since this species is present in such small amount at the pH of interest, its concentration must be calculated using the third dissociation constant for HQSA. This in turn requires that the concentration of HL^- and H_2L in equilibrium with the complex be known. Furthermore, since both of these species contribute to the absorbance of the solution, their extinction coefficients also are necessary. The extinction coefficient for H_2L, ϵ_1, was determined at pH 2.70 with solutions containing ligand only. At this pH, the concentration of this species is a maximum, and the concentrations of the other species are very small, as determined from calculations using the dissociation constants of HQSA. Values for ϵ_1 of 2510 at 370 mμ and 1710 at 383 mμ were obtained.

The concentration of HL^- is a maximum at pH 6.42 and its extinction coefficient, ϵ_2, was measured at this pH at wave lengths of 370 and 383 mμ. Surprisingly, ϵ_2 was found to be concentration dependent, varying from 5200 at $1.054 \times 10^{-5} M$ to 293 at $6.29 \times 10^{-4} M$. This can be seen in Fig. 4, which is a plot of ϵ vs. wave length at different concentrations of HQSA alone. It is evident that one species, absorbing at 370 mμ, is being converted to another species, absorbing at 308 mμ, as the concentration increases. This is confirmed by the presence of an
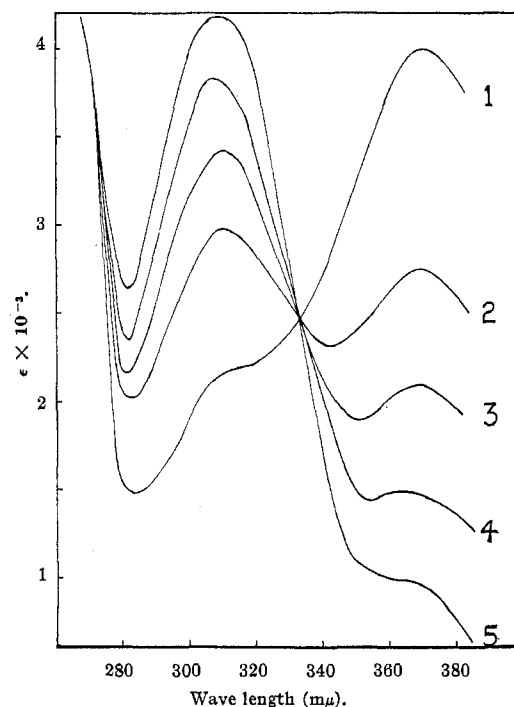
(7) R. Nasanen, A. Ekman, and E. Uusitalo, *Acta Chem. Scand.*, **8**, 112 (1954).

Fig. 4.—Variation with concentration of the extinction coefficient of HQSA at pH 5.13 in 0.20 M acetate buffer. ϵ is plotted vs. wave length (mμ) for different concentrations: 1, $1.173 \times 10^{-5} M$; 2, $2.346 \times 10^{-5} M$; 3, $3.52 \times 10^{-5} M$; 4, $5.87 \times 10^{-5} M$; 5, $1.173 \times 10^{-5} M$.

isosbestic point at 333 mμ. A pH of 5.13 was used for this plot since it is comparable to that at which the complexes were studied. At this pH the main species present still is HL^-, and the contribution to the total absorbance by the H_2L present is small.

Because of this variation of ϵ_2 with concentration, it was necessary to use a method of successive approximations to determine the concentration of HL^- in equilibrium with the complex. Using the Job's method plot, the tangents to the two straight line portions of the curve were extended until they intersected. The absorbance at the intersection, A_T, is the theoretical absorbance to be expected if the complex was completely undissociated. The actual observed absorbance at this mole fraction of molybdenum, A, is due to the complex, HL^- and H_2L. (At this pH, the contribution of H_3L^+ and L^- is negligible.) As a first approximation, the observed absorbance was assumed to be due only to the complex. If the total molybdenum (or HQSA) concentration is B, then

$$[H_2L] + [HL^-] = B(1 - A/A_T) \qquad (1)$$

The relationship between $[H_2L]$ and $[HL^-]$ is

given by the second dissociation constant for HQSA[7]

$$\frac{[HL^-][H^+]}{[H_2L]} = K_2 = 7.79 \times 10^{-5} \quad (2)$$

Solving for $[HL^-]$ and $[H_2L]$ gives

$$[HL^-] = \frac{B(A_T - A)K_2}{A_T([H^+] + K_2)} \quad (3)$$

$$[H_2L] = B(1 - A/A_T) - [HL^-] \quad (4)$$

These concentrations of HL^- and H_2L are only approximations, however, since HL^- and H_2L do contribute to the total absorbance. To obtain a better value of the free ligand concentration, a plot of the extinction coefficient of HL^-, ϵ_2, *vs.* concentration was made at pH 6.42 and at wave lengths of 370 and 383 mμ. Using the value of $[HL^-]$ calculated above, the value of ϵ_2 corresponding to this concentration was obtained from this plot. Using this value of ϵ_2 and the calculated values of $[HL^-]$ and $[H_2L]$, the absorbance, A_L, due to the total free ligand in equilibrium with the complex was calculated from Beer's law

$$A_L = \epsilon_1[H_2L] + \epsilon_2[HL^-] \quad (5)$$

This absorbance, A_L, was subtracted from the original observed absorbance, A, giving a better estimate of the actual absorbance due to the complex alone. This corrected value of the absorbance of the complex then was used in equation 1 and a new value of $[HL^-]$ and A_L was obtained using equations 2 through 5. This process was repeated until two successive values of the concentration of HL^- agreed. This required four approximations before a constant value of $[HL^-]$ was obtained.

Using the values of $[HL^-]$ and $[H_2L]$ thus calculated the concentrations of the complex (C), the free metal anion (Mo), and the free ligand

species (L^-) were found from the equations

$$[C] = B - [H_2L] - [HL^-] \quad (6)$$

$$[Mo] = [H_2L] + [HL^-] \quad (7)$$

$$\frac{[H^+][L^-]}{[HL^-]} = K_3 = 1.778 \times 10^{-9}$$

(third dissociation constant of HQSA[7]) (8)

The formation constant then was calculated with the equation

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

The logs of the formation constants were found to be

Mo(V) complex: log K = 8.2, pH 5.50,
0.38 M acetate buffer

Mo(VI) complex: log K = 8.6, pH 5.00,
0.20 M acetate buffer

Discussion

The results indicate that HQSA forms strong one to one complexes with both molybdenum(V) and (VI) over a wide range of concentrations and pH values. Furthermore, formation constants calculated for the two oxidation states are very nearly the same.

It seems unlikely that molybdenum interacts with the isoalloxazine nucleus of FMN in a manner similar to the interaction with HQSA, since no change in the visible or ultraviolet spectrum of FMN was observed in the presence of molybdenum(V) or (VI).[2] The work reported here seems to support the conclusion that the site of complex formation of molybdenum(VI) with FMN is the side chain.[2]