Notes

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Spectroscopic and Catalytic Properties of Penicillamine-Molybdenum Complexes

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A new blue colored penicillamine–Mo complex was obtained at pH 9.2 under a reduction with sodium borohydride. This complex gives electron spin resonance signal which has high level of a monomeric Mo, and shows absorption maxima at 440 and 630 nm which could be assigned to d–d transitions arising from a $^4A_{2g}$ ground state. By an aeration, the Mo complex is oxidized to the yellow colored dioxo-bridged Mo^V complex. The catalytic activity of the penicillamine–Mo complexes has been investigated by the reduction of acetylene to ethylene and ethane in the presence of borohydride.

Keywords——penicillamine–Mo complex; Mo–enzyme model; acetylene reduction; active site; spectroscopy; catalyst

A special interest has been shown in chemical model systems which involve essential nitrogenase (N_2–ase)-system components, such as thiol compounds, molybdate, adenosine triphosphate (ATP), and iron. Especially, Mo^V complexes of cysteine,[2–6] 1-thioglycerol,[2,7] and glutathione[2,8] have been reported to be an interesting and functional model forN_2–ase. However, the great difference in the catalytic capability is quite apparent between native enzyme and the model complexes. It has been considered that the high catalytic activity of N_2–ase is attributed to high population of paramagnetic and catalytically active species. Accordingly, the Mo complexes which have high proportion of paramagnetic species, are expected to give high catalytic activity. As the Mo complex which gives electron spin resonance (ESR) signal having high level of a monomeric Mo was found in the penicillamine–Mo system, we report spectroscopic and catalytic properties of penicillamine–Mo complexes.

Experimental

Materials and Physical Measurements——DL-Penicillamine, L-cysteine, and ATP were purchased from Sigma Co. and $K_3Mo(CN)_8$ was prepared by the method of Furman and Miller.[9] All other reagents used were of commercial reagent grade. Infrared spectra were recorded as a KBr disk with a Hitachi spectrophotometer, Model 215, and ESR spectra were recorded on a JEOL ME-3X electron spin resonance spectrometer, calibrated with 2,2-diphenylpicrylhydrazyl(DPPH). Intensity (*i. e.* numbers of spins per Mo) was estimated by comparison with standard $K_3Mo(CN)_8$ solution. All physical measurements were carried out at 20°.

Preparation of Penicillamine–Mo Complexes——Sodium di-μ-oxo-bis[oxo-(DL-penicillaminato)molybdate (V)], $Na_2[Mo_2O_4\{(CH_3)_2C(S)CH(NH_2)CO_2\}_2]$ (II): Sodium molybdate (3.0 g) in water (3 ml) was added to DL-penicillamine (1.9 g) in water (2 ml). To the stirred solution sodium dithionite (1.0 g) in water (4 ml) was added. After 10 min orange crystals were filtered off, washed with ethanol, and recrystallized from an aqueous ethanol (1: 1). *Anal.* Calcd. for $C_{10}H_{18}O_8N_2S_2Mo_2Na_2$: C, 17.50; H, 4.11; N, 4.08. Found: C, 17.62;

1) Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto, 606, Japan.*
2) G.N. Schrauzer and P.A. Doemeny, *J. Am. Chem. Soc.*, **93**, 1608 (1971).
3) G.N. Schrauzer, P.A. Doemeny, G.W. Kiefer, and R.H. Frazier, *J. Am. Chem. Soc.*, **94**, 3604 (1972).
4) M. Ichikawa and S. Meshitsuka, *J. Am. Chem. Soc.*, **95**, 3411 (1973).
5) G.N. Schrauzer, G.W. Kiefer, K. Tano, and P.A. Doemeny, *J. Am. Chem. Soc.*, **96**, 641 (1974).
6) G.N. Schrauzer, *J. Less-Common Metals*, **36**, 475 (1974).
7) G.N. Schrauzer, G. Schesinger, and P.A. Doemeny, *J. Am. Chem. Soc.*, **93**, 1803 (1971).
8) D. Werner, S.A. Russell, and H.J. Evans, *Proc. Natl. Acad. Sci. (US)*, **70**, 339 (1973).
9) N.H. Furman and C.O. Miller, *Inorg. Syn.*, **3**, 160 (1950).

H, 3.79; N, 4.09. In lower yield, the complex (II) was also prepared by using an excess amount of penicillamine as the reducing agent.

Sodium di-μ-sulfide-bis[oxo-(DL-penicillaminato)molybdate (V)], $Na_2[Mo_2O_2S_2\{(CH_3)_2C(S)CH(NH_2)CO_2\}_2]$ (IV): Hydrogen sulfide was passed through a solution of the complex (II) (0.8 g) in water (20 ml) for 5 hr. The solution was evaporated to dryness and gave a dark orange solid. *Anal.* Calcd. for $C_{10}H_{18}O_6N_2S_4Mo_2Na_2$: C, 15.15; H, 3.65; N, 3.63. Found: C, 15.57; H, 3.55; N, 3.21.

A blue colored penicillamine–Mo^{III} complex (I) was obtained by reaction of penicillamine (0.1 M), sodium molybdate (0.01 M), and sodium borohydride (0.05 M) at pH 9.2. It was stable for several days in a fully deaerated Tunberg tube. This complex was also obtained by the reduction of the complex (II) with sodium borohydride in the presence of additional penicillamine. Samples for spectrophotometric and ESR analyses were removed with a gas-tight syringe and transferred to cells or ESR tubes under nitrogen gas.

Catalytic Reduction Experiments——Experiments with acetylene were carried out by the modified method of the published procedure.[2] Hydrocarbons were determined by gas chromatography using a Shimadzu gas chromatograph, Model GC-5A, equipped with 0.3 cm × 2 m column and flame ionization detector. The experimental conditions are given in the legends of the Table III.

Results and Discussion

Reaction of Penicillamine with Mo^{VI}

Figure 1 summarizes complex formation between penicillamine and Mo^{VI} ion. When sodium dithionite or excess penicillamine was used as reducing agent, the binuclear dioxo-bridged penicillamine-Mo^V complex (complex II) was produced. The compound was isolated and characterized by elemental analysis, infrared, electronic, and ESR spectra (see Tables I and II). An aqueous solution of the complex (II) showed an ESR signal which is attributed to a small amount of a monomeric species (complex III) in equilibrium with the complex (II). A similar observation has been reported in the cysteine–Mo^V system by Huang and Haight.[10] When an aqueous solution containing the complex(II) was treated with hydrogen sulfide, a dark solution resulted. From this solution, a red-orange crystalline material having the formula $Na_2Mo_2O_2S_2$ (penicillamine)$_2$(complex IV) was isolated. The complex was also obtained by the reaction of sodium sulfide to penicillamine–Mo^{VI} system. An aqueous solution of the complex (IV), however, showed no ESR signals. This result indicates that the penicillamine–Mo^V dimer species with sulfide-bridge does not convert to a paramagnetic monomer. We have already reported that the degree of dissociation of dimer having $Mo^VX_2Mo^V$ group follows to the order, $X{=}O \gg S \geqslant Se$.[11] When penicillamine–Mo^{VI} system was reduced by excess BH_4^- in neutral or alkaline solution, on the other hand, a bright blue colored species (complex I) was produced. The new complex gave ESR signal which has a high level of a monomeric Mo and is evidently different from that of paramagnetic penicillamine–Mo^V species (complex III). The complex (I) was very stable for several days in an anaerobic condition, but was unstable in an aerobic condition. As shown in Fig. 2A, the ESR signal of the complex (I) was decreased with time by an aeration. The visible absorption behavior also corresponds well to the change of ESR signal (see Fig. 2B). These observations suggest clearly that the blue colored complex (I) changes spontaneously to the yellow colored complex (II) under aerobic conditions. The assertion is supported by the fact that the complex (I) is produced by the reduction of the complex (II) with excess BH_4^- in the presence of additional penicillamine.

Spectroscopic Property of Penicillamine-Mo Complexes

Table I shows some selected infrared bands of the isolated complexes (II) and (IV). The disappearances of $\nu(NH_3^+)$ and $\nu(SH)$ at 3070 and 2570 cm^{-1} in free penicillamine indicate that the ligand coordinates through the thiol and amino groups in these Mo complexes. In addition, the difference (Δ) in energy between the antisymmetric and symmetric carboxylate stretching frequencies is indicative of the coordination of the carboxylate group. The X-ray

10) T.J. Huang and G.P. Haight, Jr., *J. Am. Chem. Soc.*, **92**, 2336 (1970).
11) Y. Sugiura, M. Kunishima, T. Kikuchi, and H. Tanaka, *J. Inorg. Nucl. Chem.*, **37**, 2399 (1975).

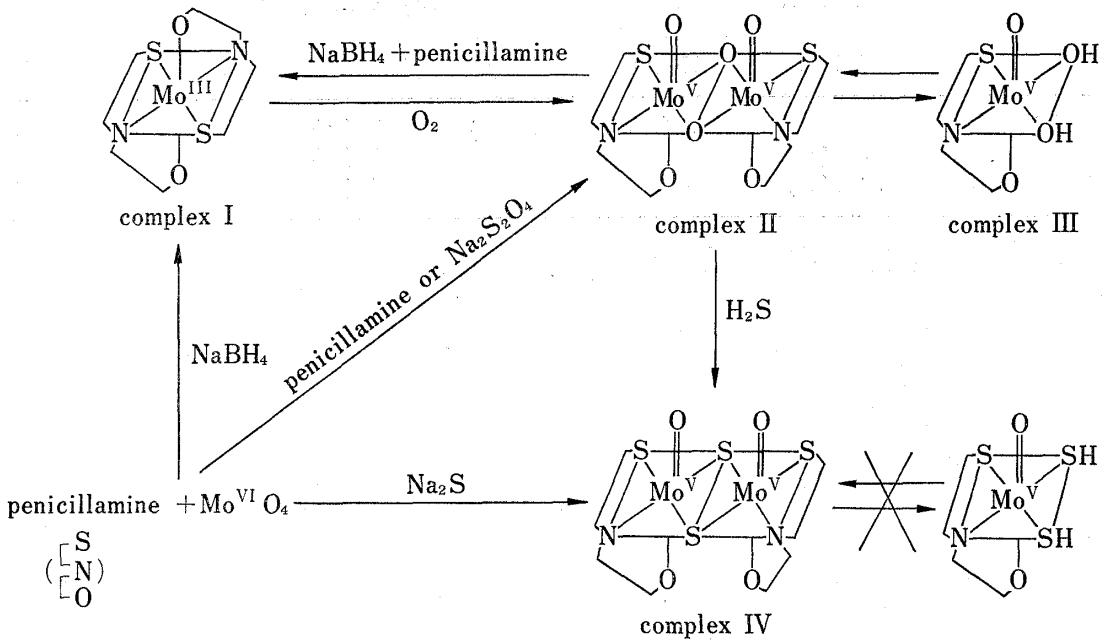

Fig. 1. Probable Reaction Scheme

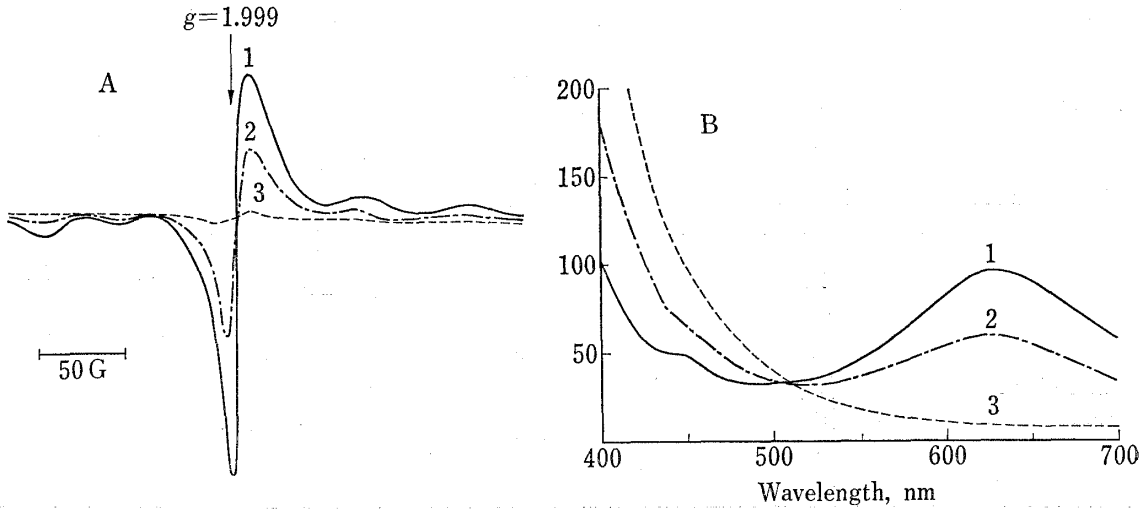

Fig. 2. ESR (A) and Visible(B) Spectral Evidences for Change of Complex(I) to Complex(II)

The concentration of molybdenum in the complex was 0.01M and the numbers on each spectrum indicate time after aeration to complex(I): (1), 0(blue); (2) 90 (green); (3), 180 min (yellow). conditions of ESR spectroscopy: microwave power, 4 mW; frequency, 9.08 GHz; modulation amplitude, 10 G; time constance, 1.0 sec; temperature, 20°; pH 9.2

crystallography of the cysteine–Mo^{V} dimer with dioxo-bridge shows that each Mo atom is bonded to one terminal and two bridging oxygen atoms and that cysteine acts as a terdentate ligand.[12]

Table II summarizes the visible and ESR absorption characteristics of the penicillamine–Mo complexes. The complex (II) shows an absorption peak at 310 nm and the complex (IV) at 300 and 460 nm. The result is in harmony with the observation by Kay and Mitchell[13] that the Mo^{V} complexes of sulfur-donor ligands have a strong peak at 26000—30000 cm^{-1} and the sulfur-bridged Mo^{V} complexes have an additional peak or shoulder at 20000—24000 cm^{-1}. In contrast with these Mo^{V} complexes, the complex (I) gave absorption maxima at 440 and

12) J.R. Knox and K. Prout, *Acta Crystallogr.*, **B25**, 1857 (1969).
13) A. Kay and P.C.H. Mitchell, *J. Chem. Soc.* (*A*), **1970**, 2421.

Compound	Frequencies (cm^{-1}) $\nu(NH_2)$	$\nu(NH_3^+)$	$\nu(SH)$	$\nu(COO^-)$ *asym*	$\nu(COO^-)$ *sym*	Δ
Penicillamine	—	3070(s)	2570(m)	1590(s)	1400(s)	190
Complex II	3400(s)	—	—	1615(s)	1395(s)	220
Complex IV	3420(s)	—	—	1620(s)	1380(s)	240

intensity designations: s, strong; m, medium.
The "Δ" represents the difference in energy between the antisymmetric and symmetric carboxylate stretching frequencies.

630 nm with low extinction coefficient which could be assigned tentatively to d–d transitions. The electronic spectrum of the complex(I) is very similar to those of the penicillamine–Cr^{III} [420(ε=90) and 550(80) nm] and the penicillamine–W^{III} [440(20) and 580(100) nm] complexes, but not to those of the oxo-bridged Mo^{III} complexes reported by Mitchell and Scarle.[14] It seemed reasonable to consider, therefore, that these electronic bands are d–d transitions arising from a $^4A_{2g}$ ground state of d^3 ion in an octahedral field. The complex (I) gave a higher *g*-value than that of the complex (III) as seen in Table II. Generally, Mo^{III} complexes have higher *g*-value and lower *a*-value than those of Mo^V complexes with similar sulfur-donor ligands. It has been presented that a series of well-characterized Mo^{III}–sulfur complexes have *g*-value at 1.990—2.005.[14] Another characteristic ESR feature of the complex (I) was the high proportion of Mo in the form of a paramagnetic, presumably mononuclear species. The ESR absorption of the complex (I) was approximately 100 times as intense as that of the complex (III). The recent ESR measurements for nitrate reductase from *E. coli* revealed a Mo^V signal at g= 1.988 which on reduction is quantitatively converted to a postulated Mo^{III} species with $g_\perp$ at 2.008 and $g_{||}$ at 2.032.[15] On the basis of these results, we would like to assign tentatively the complex (I) to Mo^{III} species.

Table II. Electronic Spectral Data and ESR Parameters of Penicillamine–Mo Complexes

Complex	Visible spectra abs. max., nm (ε)[a]	g_{av}	ESR signals[b] a(G)	ΔH(G)	Electron spin / Mo (%)
Complex I	440(30) 630(90)	1.999	33	14	~100
Complex II	310(12000)		no ESR signal		
Complex III	not determined	1.977	not resolved	13	<1
Complex IV	300(8000) 460(750)		no ESR signal		

a) The ε represents molar extinction coefficient per molybdenum.
b) The g_{av}, *a* and ΔH represent average *g*-value, *a*-value (^{95,97}Mo-hyperfine splitting) and line-width of major absorption, respectively.

Table III. Yields of Ethylene and Ethane produced from Acetylene with Penicillamine–Mo Complexes

System	C_2H_4 (μmol)	C_2H_6 (μmol)	C_2H_4:C_2H_6
Complex (I)+$NaBH_4$	34.4	7.8	4.4
Complex (II)+$NaBH_4$	123.0	23.5	5.2
Complex (IV)+$NaBH_4$	44.3	4.5	9.9
Complex (II)+$NaBH_4$+ATP	162.4	37.4	4.3
Complex (IV)	0	0	0

test system: 0.029 M of complex, 0.15 M of $NaBH_4$, 1 atm of acetylene, 0.029 M of ATP; 3.5 ml reaction volume; 60 min reaction time at 20° and pH 9.2 in Tunberg tube

14) P.C.H. Mitchell and R.D. Scarle, *J. Chem. Soc.* (Dalton), **1975**, 110.
15) D.V. Dervartanian and P. Forget, *Biochim. Biophys. Acta*, **379**, 74 (1975).

Catalytic Property of Penicillamine-Mo Complexes

Table III presents the results of the reduction of acetylene with the penicillamine–Mo complexes and sodium borohydride system. Indeed, these Mo complexes catalyze the reduction of the substrate to ethylene and ethane, and the reaction was absolutely dependent upon the addition of the complex, the substrate, and borohydride. The reaction of the complex (II) system was enhanced 1.5 fold by ATP. The low activity of the complex (IV) is attributed to less readiness of conversion to catalytically active monomeric form. Unfortunately, the catalytic activity of the complex (I) was low against expectation, nevertheless the high population of paramagnetic species. It is presumed that the low activity of the complex (I) is due to lack of efficient residual coordination sites of Mo for the substrate.

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Synthetic Antibacterials. VII.[1] N-(1,8-Naphthyridin-7-yl)-methylenamine Derivatives

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Treatment of ethyl 1,4-dihydro-1-ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylate (**1**) with selenium dioxide afforded a mixture of ethyl 1,4-dihydro-1-ethyl-7-formyl-4-oxo-1,8-naphthyridine-3-carboxylate (**2**) and 1,4-dihydro-1-ethyl-7-formyl-4-oxo-1,8-naphthyridine-3-carboxylic acid (**3**). Condensation of **2** or **3** with respective amines provided the corresponding N-(1,4-dihydro-3-ethoxycarbonyl-1-ethyl-4-oxo-1,8-naphthyridin-7-yl)methylenamine (**4**—**15**) and N-(3-carboxy-1,4-dihydro-1-ethyl-4-oxo-1,8-naphthyridin-7-yl)methylenamine (**16**—**27**), respectively. These compounds were tested for *in vitro* antibacterial activity.

Keywords——N-(1,8-naphthyridin-7-yl)methylenamines; *in vitro* antibacterial activity; selenium dioxide oxidation; ethyl 1,4-dihydro-1-ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylate; ethyl 1,4-dihydro-1-ethyl-7-formyl-4-oxo-1,8-naphthyridine-3-carboxylate; 1,4-dihydro-1-ethyl-7-formyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

In the previous papers[1,3a–c] of this series, we have synthesized a number of 1,8-naphthyridine and pyrido[2,3-*d*]pyrimidine derivatives carrying a vinylene group and found that certain compounds exhibit potent *in vitro* activity against various microorganisms. In connection with these findings, it became desirable to synthesize a series of 1,8-naphthyridine derivatives having an azomethine group at the position 7 to pursue their antibacterial activity since the trivalent nitrogen atom (–N=) and the –CH= group have successfully been interchanged in many bioisosteric systems.[4]

1) Part VI: S. Nishigaki, N. Mizushima, and K. Senga, *Chem. Pharm. Bull.* (Tokyo), 24, 1658 (1976).
2) Location: *35, Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.*
3) *a*) S. Nishigaki, F. Yoneda, K. Ogiwara, T. Naito, R. Dohmori, S. Kadoya, Y. Tanaka, and I. Takamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1827 (1969); *b*) S. Nishigaki, N. Mizushima, F. Yoneda, and H. Takahashi, *J. Med. Chem.*, **14**, 638 (1971); *c*) S. Nishigaki, K. Ogiwara, S. Fukazawa, M. Ichiba, N. Mizushima, and F. Yoneda, *J. Med. Chem.*, **15**, 731 (1972).
4) A. Burger, "Medicinal Chemistry," Part I, ed. by A. Burger, Wiley-Interscience, New York, 1970, p. 64, and references cited therein.