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Preparation and Characterization of Purple and Green Colored D-Cycloserine-Copper(II) Complexes

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The structure of cycloserine, a unique antibiotic substance [1, 2], suggests that the binding of metal ion might be an important chemical and pharmacological property of this compound. Although it has been reported that the ability of cycloserine is suppressed only by Cu(II) [3], except in the work of Neilands [4], a great deal about the metal-binding ability of cycloserine has not yet been studied in detail. During the investigations on solution chemistry and metal-binding property of cycloserine [5], we found that two types of complexes, green and purple in color, were formed in the reaction of cycloserine and Cu(II). The purple colored complex was isolated from buffer solution. This paper reports the preparation and characterization of these complexes.

D-Cycloserine, [D-4-amino-3-isoxazolidone], was a generous gift from Meiji-Seika, Ltd. The green colored complex was found to be 2:1 in the binding ratio of ligand to Cu(II) by molar ratio method, after three days following preparation in tris-HCl buffer solution (pH 6.99), and gradually changed its absorption maximum from 690 nm to 582 nm and its color to purple. The release of two protons from a molecule of cycloserine•hydrochloride was observed with potentiometric titration being 2:1 of the mixing ratio of ligand to Cu(II) and the solution became green during titration. The green colored solid complex has not yet been isolated in pure form.

After standing for a week the green colored solution, which was prepared at pH 6.99, precipitated a purple colored solid. The precipitate was collected, washed with water several times and dried *in vacuo* over P_2O_5 at room temperature. *Anal.* Calcd. for $C_6H_{10}N_4O_4Cu2H_2O$: C, 23.88; H, 4.68; N, 18.57, Found: C, 23.82; H, 4.74; N, 18.11. From the elemental analysis we conclude that in the purple colored complex, two cycloserine molecules bound around Cu(II), probably through oxygen and amino-nitrogen atoms. The optical spectrum of complex in solid state showed its maximum at 573 nm and the IR spectrum (KBr disk) indicated the coordination of amino group to metal ion.

Difference in the coordination mode between the green and the purple complexes was observed by CD and ESR spectrometry. The green complex showed a broad CD spectrum with negative sign at 720 nm, while the purple one with positive sign at 490 nm and 630 nm. Involvement of two nitrogen atoms at the metal-binding sites in the purple 2:1 cycloserine-Cu(II) complex was unequivocally demonstrated by ESR detection of the ligand hyperfine splitting (5 lines) due to equivalent nitrogen atoms (^{14}N, $I = 1$) at room temperature and at 77 K (Fig. 1, Table I). The tendency for $g_{\parallel}$ to increase and $A_{\parallel}$ to decrease

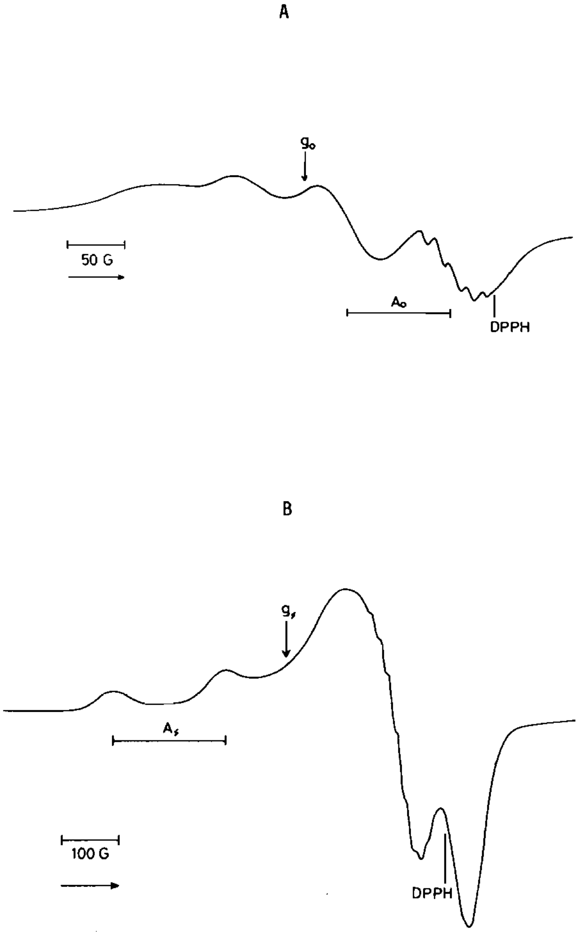

Fig. 1. ESR spectra of purple colored cycloserine-Cu(II) complex in tris-HCl buffer solution, pH 6.99, at 293 K (A) and at 77 K (B). The green colored complex was prepared from 50 m*M* D-cycloserine and 12.5 m*M* cupric chloride in 1 *M* Tris-HCl buffer solution, pH 6.99, and ESR spectra were recorded for the purple solution four days after preparation.

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TABLE 1. ESR Parameters of Cycloserine-Cu(II) Complexes.[a]

Complex	g_0	$g_\parallel$	$g_\perp$	A_0[b]	$A_\parallel$[b]	$A_\perp$[b]	N-hfs[c]
Green Complex	2.109	2.251	2.038	5.91	17.13	0.30	–
Purple Complex	2.110	2.192	2.069	9.06	20.16	3.51	2N: $A_{iso}(^{14}N) = 13.6$ $A_{77\,K}(^{14}N) = 15.5$

[a] The $g_\perp$- and $A_\perp$-values were calculated with the relations, $g_0 = (g_\parallel + 2g_\perp)/3$ and $A_0 = (A_\parallel + 2A_\perp)/3$, using set of g_0- and $g_\perp$- and A_0- and $A_\parallel$-values, respectively, obtained from the ESR spectra measured at 293 K and 77 K. [b] Measured in MK. [c] Nitrogen nuclear hyperfine structure measured in Gauss.

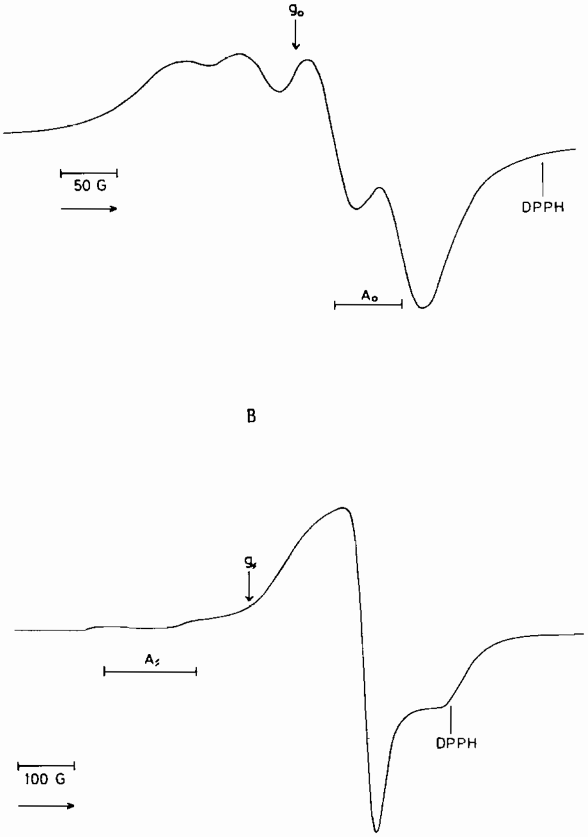

Fig. 2. ESR spectra of green cycloserine-Cu(II) complex in tris-HCl buffer solution, pH 6.99, at 293 K (A) and at 77 K (B). The green colored complex was prepared under the same conditions as Fig. 1, and ESR spectra were recorded immediately after the preparation.

with increase in the degree of tetrahedral distortion from square planar configuration has been reported for structurally well-characterized complexes of Cu(II) [6]. Based on this result, the purple cycloserine-Cu(II) complex having CuN_2O_2 center is almost in square planar coordination geometry.

In the green complex, the coordination of amino-nitrogen to Cu(II) was ruled out from ESR spectrometry at room temperature and 77 K (Fig. 2). Therefore, we assumed that two cycloserine molecules coordinate to Cu(II) through oxygens and the other ligand to metal ion might be oxygens of coordinating water. The set of $g_\parallel$-$A_\parallel$ values close to the CuO_4 center in non-tetrahedrally distortion [6].

From these data we proposed that the cycloserine–Cu(II) complex, (purple in color) has two coordination sites with Cu(II), the amino-nitrogen and oxygen, forming a tetra-coordinated square planar configuration and that the green colored complex has two coordination sites, oxygens of cycloserine and water, forming also square planar geometry (Fig. 3).

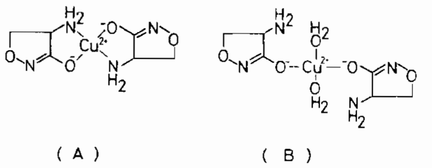

Fig. 3. Possible coordination modes of cycloserine–Cu(II) purple (A) and green colored (B) complexes.

The mechanism of formation of the purple complex from the green one in the buffer solution, pH 6.99, depending on the reaction time, is unresolved at present and further investigations are in progress to establish this point.

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