生体関連金属錯体の薬化学的研究

STUDIES ON PHARMACEUTICAL CHEMISTRY OF BIOMIMETIC METAL COMPLEXES

I．ヒスタミン-H_2-アンタゴニスト・シメチジンの銅(Ⅰ,Ⅱ)錯体
COMPLEXES OF HISTAMINE H_2-ANTAGONIST CIMETIDINE WITH DIVALENT AND MONOVALENT COPPER IONS

II．アクシャル位にフェノール基の配位した大環状テトラアミン金属錯体
NOVEL SYNTHESIS AND COMPLEX PROPERTIES OF MACROCYCLIC TETRA-AMINE APPENDED WITH PHENOL AS AN AXIAL DONOR

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by

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A thesis submitted to the Graduate School of Pharmaceutical Sciences in Hiroshima University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Science.

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TOHRU KOIKE. **Complexes of Histamine H_2-Antagonist Cimetidine with Divalent and Monovalent Copper Ions,** and **Novel Synthesis and Complex Properties of Macrocyclic Tetra-amine Appended with Phenol as an Axial Donor.** (Under the direction of EIICHI KIMURA)

Abstracts

Chapter I. 1:1 and 1:2 complexation constants of histamine H_2-antagonist cimetidine (antiulcer drug) with Cu(II) ion have been determined by pH-metric technique. Relatively small stability constants with respect to those of biological ligands (e.g. amino acids, peptides) suggest cimetidine *in vivo* unbound to Cu(II) ion. However the Cu(I,II) redox potential E^o of +0.42 V *vs* NHE with cimetidine implies high chances of biological reductants such as ascorbic acid and hemoglobin. The E^o value is comparable to those of blue copper (type I) proteins and Cu-superoxide dismutase. Copper-cimetidine complexes exhibit the highest superoxide dismutase-like activity hitherto known to copper chelates with small molecular weight.

Chapter II. A novel annelation method is reported for the synthesis of the axial phenolate pendent cyclam [5-(2-hydroxyphenyl)-1,4,8,11-tetraazatetradecane] from coumarin and 1,9-diamino-3,7-diazanonane. This will be useful in the synthesis of a variety of macrocyclic

polyamine alkaloid analogues. The X-ray crystal structure of high-spin Ni^{II} complex with phenolate-pendant cyclam as mono-perchlorate salt is reported, with R factor of 0.047. The nickel is surrounded in square-pyramidal arrangement by equatorial cyclam nitrogens with mean Ni-N bond length of 2.07 Å and an axial phenolate with bond length of 2.02 Å. The present phenolate appended ligand can stabilize the high oxidative metal ions of Ni^{III} and Fe^{III}. The phenol-appendent cyclam Fe^{III} complex has similar visible absorptions to those of tyrosine co-ordinating Fe^{III} nonheme oxygenase.

要約

I ヒスタミン-H_2-アンタゴニスト・シメチジンの銅(I,II)錯体

ヒスタミン-H_2-アンタゴニストとして知られるシメチジンは、ラットやモルモットの中枢神経のイミダゾールレセプターに結合する。その結合が、銅(II)イオンの存在により増強されることや、アスコルビン酸などの還元剤がさらに銅(II)イオンの作用を強めることから、シメチジン－銅(II)あるいは銅(I) 錯体が、レセプター結合に関与する可能性が示唆されている。著者は、水溶液中でのシメチジンと銅イオンとの相互作用やシメチジンの銅錯体の化学的性質および薬理活性との関連について詳細に検討した。

水溶液中でシメチジン(L) は、銅(II)イオンと 1:1および2:1錯体形成する。錯生成定数 $K_{Cu(II)L}$および$K_{Cu(II)L2}$の値は、$3.02 \times 10^4 (M^{-1})$, $2.35 \times 10^4 (M^{-1})$である。一方、シメチジン-銅(I)錯体は、水溶液中では2:1以上の組成のものは確認されず、殆ど 1:1錯体として存在する。その錯生成定数 $K_{Cu(I)L}$は、銅(I)錯体としては異常な程大きく、$1.3 \times 10^9 (M^{-1})$である。この事実は、シメチジン－銅(I) 錯体がpH3以下の低いpHにおいても銅(I)イオンを解離することなく 安定に存在することを示唆している。

シメチジン-銅(II)錯体は、高い酸化還元電位,+0.42 V vs.NHEを持ち、比較的弱い還元剤（アスコルビン酸等）により容易に還元されて1:1-シメチジン－銅(I) 錯体になる。+0.42 V という電位は、牛のCu-スーパーオキサイドジスムターゼ(SOD)中の銅イオンや、ブルー銅(Type I)蛋白質の酸化還元電位に類似しており、生物無機化学的に見ても大変興味深い値である。

シメチジン－銅(I)錯体のSOD活性の測定を行なったところ、その活性は、牛-SODの1/50の活性で、これまで知られている低分子銅錯体の中で最も強いものであった。ペニシラミンやアセチルサリチル酸などの銅錯体は、牛-SODの1/100-1/1000の活性を持ち、

その結果強い抗炎症作用を表わすと考えられている。したがって、シメチジンの骨格をさらに化学修飾することにより、新しい抗炎症剤や、銅含有酵素モデル化合物の開発が期待される。

体内に分布する銅イオンの大部分は、二価イオンとしてアルブミンやアミノ酸などと結合している。銅(II)イオンのキャリヤーモデルとして確立されているグリシルグリシルヒスチジン (GGH) 及びシメチジンの銅(II)イオンに対する親和性を生理pHで比較すると、GGHの方が大きい。この事実は、シメチジン-銅(II)錯体として生体に投与しても、体内ですぐ銅(II)イオンを解離してしまうことを示唆する。しかし、アスコルビン酸等の生体還元剤が存在すると、シメチジンは銅(II)イオンを奪い安定な銅(I) 錯体を形成する。即ちシメチジンは、生体内で銅(I) 錯体として存在することが充分考えられる。脳レセプターにおいて銅(II)イオンおよびアスコルビン酸の添加によりシメチジン結合が強まるという事実は、シメチジン－銅(I) 錯体が結合活性種であることを示唆しているが、本研究は、化学的にシメチジンが銅(I) 錯体として生体内でも安定に存在しうることを証明したものである。

II アクシャル位にフェノール基の配位した大環状テトラアミン金属錯体

著者は、天然物として知られるクマリンを出発物質とする新合成法を開発し、フェノール基を持つ14員環テトラアミン(Phenol-cyclam)を合成した。その金属錯体は、N_4平面のアクシャル位にフェノール酸素が配位した構造を持っている。チロシン残基であるフェノールは、特に鉄イオンとの親和性が強く、プロトカテキュエート-3,4-ジオキシゲナーゼや、 カタラーゼなどの活性中心においても、鉄(III)イオンに配位している。 著者は、Phenol-cyclamの鉄(II,III)及びニッケル(II,III)錯体の物理化学的性質フェノールのアクシャル配位効果、及び生体金属酵素との関連性

について詳しく検討した。

Phenol-cyclamの構造は、大環状ポリアミンアルカロイドverbascenineなどのホモログである。 したがって、この合成法は、天然物アルカロイドあるいは、それらのホモログの合成という面からも大変有用性に富む。

Ni(II)-phenol-cyclamは、水溶液中でピンク色(λ max=520 nm, ε =10)を呈し、Ni(II)-high spin錯体(μ_B =2.90, at 35°C)である。フェノール酸素は、アクシャル配位に最適の位置にあることがX線解析の結果から分かった。その1:1-Ni(II)錯体生成定数は、7.0×10^{22} (M^{-1})である。Phenol-cyclam-Ni(II)錯体のフェノールのpK$_a$は、6.30である。その値は Phenol-cyclam のフェノールのpK$_a$ 8.86より小さい。すなわち、ニッケル錯体を形成することによってフェノールプロトンは、放出されやすくなっている。cyclam－Ni(II)錯体は、比較的容易に酸化されてNi(III) 錯体になる。その Ni(II/III)錯体の酸化還元電位は、+0.50 V vs.SCEである。 Phenol-cyclamの場合、フェノールプロトンの解離していない pH5以下では +0.50 V vs. SCEでcyclamのニッケル錯体と同じであるが、フェノラート酸素の配位した pH7以上では+0.35 V vs.SCEとより低い電位で酸化される。すなわち、アクシャル位にフェノラート基が配位することによりニッケルイオンは、三価の状態に酸化され易くなっている。ニッケルに配位したフェノラートアニオンの酸化電位は、+0.9 V vs.SCE である。フェノールの解離したPhenol-cyclam の酸化電位は、+0.5 V vs.SCE である。したがって、フェノール基は、ニッケル(III) イオンよって、逆に安定化されている。

フェノール基を持たない Cyclam は、水溶液中では安定な鉄錯体を形成することができない。一方、Phenol-cyclam は、pH7 付近でフェノールプロトンを解離して環内に鉄(II)イオンを取り込み、赤色の high spin－鉄(II)錯体 (μ_B=5.19, λ max=455 nm, ε =200)を形成する。その錯生成定数は、7.9×10^{14} (M^{-1}) である。この値を Cyclamと比較すると約10^6倍大く、フェノラート酸

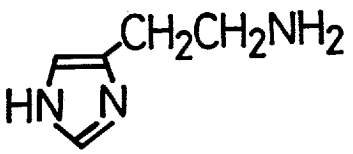
素が、鉄(II)イオンといかに大きな親和性を持っているかが分かる。 Fe(II)-Phenol-cyclam のII/III酸化還元電位は、-0.16V vs. SCE である。Phenol-cyclam の鉄(II)錯体は、容易に空気酸化されて赤色(λ max =480 nm, ε =2200)の鉄(III)錯体になる。その錯生成定数は、pH7において4.0×10^{26}(M^{-1})であり、鉄(II)錯体の場合よりもさらに大きい。 Phenol-cyclamの鉄(III) 錯体の分光光学的性質は、non-heme酵素であるプロトカテキュエート-3,4-ジオキシゲナーゼによく似ており、大変興味深い。したがって、Phenol-cyclam は、新しいタイプの鉄イオン捕捉剤や、フェノラートイオンの配位したオキシダーゼやカタラーゼの活性部位モデルとしてその応用が期待される。

CHAPTER I

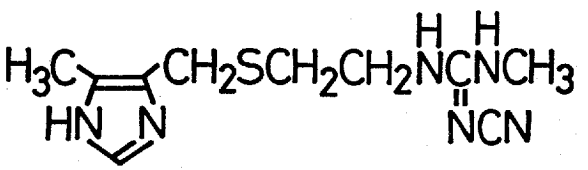
Complexes of Histamine H_2-Antagonist Cimetidine with Divalent and Monovalent Copper Ions

Introduction

Cimetidine (Tagamet®) is a potent histamine H_2-receptor antagonist, which inhibits excessive acid secretion caused by histamine, and currently is in world-wide clinical use for treatment of gastric ulcer.[1-3] The drug is taken orally, absorbed in the intestine and reaches H_2-receptors via the blood stream. Cimetidine, like histamine, is a potential chelating agent. Since micromolar levels of loosely bound Cu(II) ion are present in blood serum,[4] it may bind to the drug.



H_2-agonist
histamine



H_2-antagonist
cimetidine

Earlier, a polymeric 1:2 Cu(II)-cimetidine complex was isolated (as green crystals) from pH 7.0 aqueous solution for an X-ray analysis.[5] On the basis of ^{1}H and ^{13}C NMR studies of the 1:2 complex in aqueous solution, the binding sites of cimetidine were proposed to be the imidazole-N and nitrile-N donors.[5] A 1:1 Cu(II)-cimetidine complex is likely to coexist in the solution, but it has not been verified. Quantitative study on the copper complexes of cimetidine has not been reported until now. Especially interesting, from pharmacological point of view, is whether cimetidine can compete for Cu(II) ion against biological ligands such as serum albumin or amino acids.

Recently Cu(II) ion was demonstrated to dramatically increase the cimetidine binding to imidazole receptors located in rat brain.[6-8] Cu(II) ion has been implicated in the regulation of the cimetidine binding sites in the brain. Addition of ascorbic acid or dithiothreitol further enhances the cimetidine binding.[9] One hypothesis is that Cu(II) in the cimetidine complex may undergo reduction to Cu(I) to acts as a more potent binding promoter than Cu(II).[9] We therefore felt it imperative to determine the reduction potential of the Cu(II)-cimetidine complex. Our study has revealed that the Cu(I)-cimetidine complex is indeed generated in the presence of ascorbic acid. Its oxidation potential $\underline{E}^{o}$ of +0.42 V $\underline{vs}$ NHE is extremely high and more interestingly is nearly the same as the $\underline{E}^{o}$ value of bovine Cu-superoxide dismutase (SOD).[11,12] Then we have discovered that the copper-cimetidine complexes possess the superoxide dismutase-like

activity much stronger than any previously reported copper complex does.

Experimental Section

Materials. Cimetidine was purchased from Sigma Chem. and purified by recrystallization from MeOH/MeCN. A stock solution of Cu(II) ion was prepared from analytical grade $Cu(II)[ClO_4]_2$ and standardized by titration with disodium salt of ethylenediaminetetra-acetic acid (EDTA).[13] $Cu(I)[NCCH_3]_4ClO_4$ was freshly prepared according to the literature method.[14] Other chemicals employed were of analytical grade and were used without further purification.

Synthesis of 1:1 Cu(I)-Cimetidine Complex. Treatment of $Cu(I)[NCCH_3]_4ClO_4$ (16.4 mg) with two equivalent cimetidine (25.2 mg) in 10 ml of 2% CH_3CN/H_2O in Ar for one hour at 25 °C yields 1:1 Cu(I)-cimetidine complex as white precipitates (19.5 mg). Anal. Calcd for $C_{10}H_{16}N_6SCuClO_4 \cdot H_2O$: C, 27.72; H, 4.19; N, 19.39. Found: C, 27.92; H, 3.92; N, 19.49. The 1:2 Cu(II)-cimetidine complex was prepared as described in ref. 5.

Potentiometric Measurements. The formation constants of Cu(II)-cimetidine complexes were determined by potentiometric acid-base titration (an Orion Research 811 digital pH meter) of cimetidine with a carbonate-free 0.200 M NaOH solution in the presence of Cu(II) ion. The titration data was treated by a computer-aided Schwarzenbach method.[15] The temperature was maintained at 25.00 ± 0.05 °C and the ionic strength was adjusted to 0.20 M with $NaClO_4$. $-\log[H^+]$

Values were estimated with a correction of -0.13 pH unit to the pH meter readings.[16] All solutions were carefully protected from air by a stream of argon prepurified with an alkaline pyrogallol solution. The electrode system was calibrated with pH 7.00 and 4.01 buffer solutions and checked by the duplicate theoretical titration curves of 4.00×10^{-3} M $HClO_4$ with 0.200 M NaOH solution at 25°C and $\underline{I}$=0.20 M ($NaClO_4$).

Electrochemical Measurements. Cyclic voltammetry and dc polarography were performed with a Yanaco Polarographic Analyzer P-1100 system at 25.00 ± 0.05°C and $\underline{I}$=0.20 M ($NaClO_4$ or $NaNO_3$). A three-electrode system was employed: a 3-mm glassy carbon rod (grade GC-30, Tokai Electrode Company) or a Yanagimoto dropping mercury electrode as the working electrode, a mercury pool as the counter electrode, and a saturated calomel reference electrode (SCE) with a potential of +241 mV $\underline{vs}$ NHE at 25°C. The cyclic voltammograms with scan rates of 10-100 mVs^{-1} were evaluated graphically.

Spectrophotometric Measurements. UV spectra were measured with a Shimazu UV-200S spectrophotometer at 25.0 ± 0.1°C and $\underline{I}$=0.20 M ($NaClO_4$). IR spectra (KBr tablet) were obtained on a Shimazu IR-408 spectrophotometer.

Measurement of Superoxide Dismutase-like Activity. The superoxide dismutase-like activity was examined by a method of the xanthine-xanthineoxidase system of

Fridovich et al.[17] The assay is performed in 3 ml of 0.05 M potassium phosphate buffer at pH 7.8 containing 10^{-4} M EDTA in a 1-cm cuvette thermostated at 25.0 °C. The reaction mixture contained 1 x 10^{-5} M ferricytochrome c (Sigma Chem., Type-III), 1 x 10^{-4} M xanthine, 300 Sigma units of catalase (Sigma Chem., C-100) and sufficient xanthine oxidase (Sigma Chem.) to produce a rate of reduction ferricytochrome c (550 nm) at 0.025 absorbance unit per min. Under these conditions, the concentration of copper complexes required to halve the initial (till 5 min) rate is defined as IC_{50}. Since Cu(I)-cimetidine complex is very insoluble in aqueous solution, the saturated solution was prepared, and its concentration was estimated from copper content using an atomic absorption method with Shimazu AA-646 flame spectrophotometer.

Results and Interpretation

Copper(II) Complex Formation Constants. The potentiometric titration curves for the cimetidine(L)·$HClO_4$, 1:1 and 1:11 Cu(II)-cimetidine·$HClO_4$ are displayed in Fig.1 (a), (b), and (c), respectively. The mixed protonation constant $\underline{K}_L$ defined by equation (1) was calculated to be $10^{7.20}$ at 25 °C and $\underline{I}$=0.20 M ($NaClO_4$), which well agrees with the reported value of $10^{7.09}$ at 25°C and $\underline{I}$=0.1 M (NaCl).[18]

$$L + \underline{a}_{H^+} \rightleftharpoons HL^+, \quad \underline{K}_L = [HL^+]/([L]\cdot\underline{a}_{H^+}) \qquad (1)$$

In the latter stage of the Cu(II)-cimetidine titrations the green 1:2 complex[5] started to precipitate, see broken lines in Fig.1 (b) and (c). For equilibrium analysis, we have used the initial stage of the titration curve (c) [$0.1<\underline{a}<0.6$, where $\underline{a}$ is moles of base per moles of Cu(II) ion]. The data fit to simultaneous equilibria (2) and (3).

$$Cu^{II} + L \rightleftharpoons Cu^{II}\text{-}L, \quad \underline{K}Cu^{II}L = [Cu^{II}\text{-}L]/([Cu^{II}][L]) \qquad (2)$$

$$Cu^{II}\text{-}L + L \rightleftharpoons Cu^{II}\text{-}L_2, \quad \underline{K}Cu^{II}L_2 = [Cu^{II}\text{-}L_2]/([Cu^{II}\text{-}L][L]) \qquad (3)$$

The buffer pH region of (c) is less than 5 and hence, the hydrolysis of Cu^{II}_{aq} is negligible in light of $\underline{K}_{OH}$ = $[Cu(OH)^+]/([Cu^{II}][OH^-])$ = $10^{6.06}$ at 25°C.[19] The

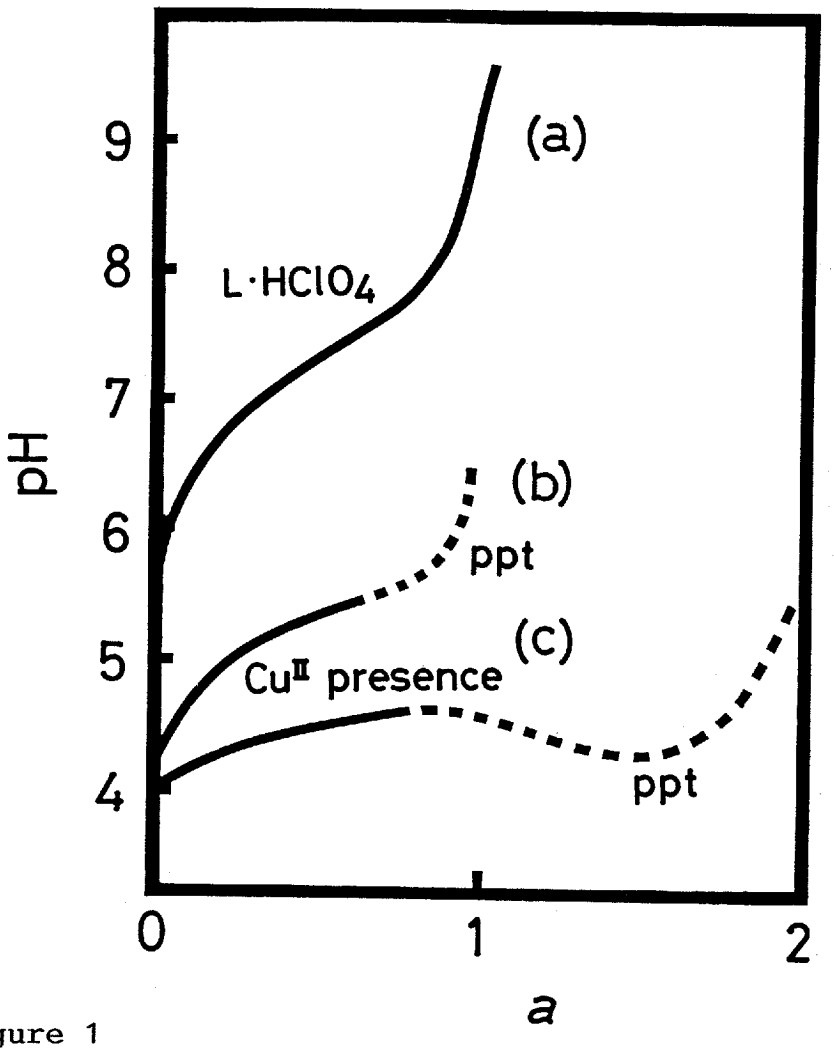


Figure 1

Potentiometric titration curves for cimetidine(L)·$HClO_4$ in the absence and presence of Cu(II) ion at 25°C and $\underline{I}$=0.2 M ($NaClO_4$): (a) [total L]=2.0 x 10^{-3} only, (b) [total L]= [total Cu(II)]= 2.0 x 10^{-3} (M), (c) [total L]=1.1 x 10^{-2} (M), and [total Cu(II)]=1.0 x 10^{-3} (M). $\underline{a}$ for (a) is mole of base per mole of cimetidine. $\underline{a}$ for (b) and (c) is mole of base per mole of Cu(II) ion. The broken lines indicate precipitation of the 1:2 Cu(II)-cimetidine complex.

calculated 1:1 and 1:2 Cu(II)-cimetidine complexes formation constants $\underline{K}_{Cu^{II}L}$ and $\underline{K}_{Cu^{II}L_2}$ at 25°C and $\underline{I}$=0.20 M ($NaClO_4$) are (3.02 ± 0.05) x 10^4 M^{-1} and (2.35 ± 0.05) x 10^4 M^{-1}, respectively.

Redox Properties. A typical polarogram of the 1:2 Cu(II)-cimetidine complex in aqueous solution at pH 7.8 [cimetidine was used ten times in excess to suppress the cimetidine dissociation to the 1:1 complex] showed two step reduction waves [Fig.2 (b)]. The first step at $\underline{E}_{1/2}$= +0.11 V *vs* SCE represents a reversible one electron reduction process for Cu(II)- to Cu(I)-complex, which was checked by the log plot method.[20] The second reduction step at $\underline{E}^2_{1/2}$= -0.32 V is non-reversible, which represents Cu(I)-complex to Cu^0-Hg. The cyclic voltammogram [Fig.2 (c)] corresponding to the first polarographic wave indicates a reversible redox process on a glassy carbon electrode. The midpoint potential $\underline{E}'_{1/2}$ between the anodic and cathodic peak is +0.13 V *vs* SCE. For reversibility test, we have measured the cyclic voltammogram ($\underline{E}'_{1/2}$= +0.16 V *vs* SCE) of Cu(II) with five equivalent cimetidine at pH 7.0 at the scan range of -0.20 V to +0.50 V *vs* SCE and the scan rates of 10 to 100 mVs^{-1}, and proved that: the cathodic and anodic peak separations are 58-65 mV; the ratio of the two peak heights is unity; and the peak heights are proportional to the square-roots of the scan rates.

The $\underline{E}_{1/2}$ values on the polarogram were found to shift to less positive potentials with an increase in ratio of [cimetidine] to [Cu^{II}]: +0.102 (12:1), +0.079

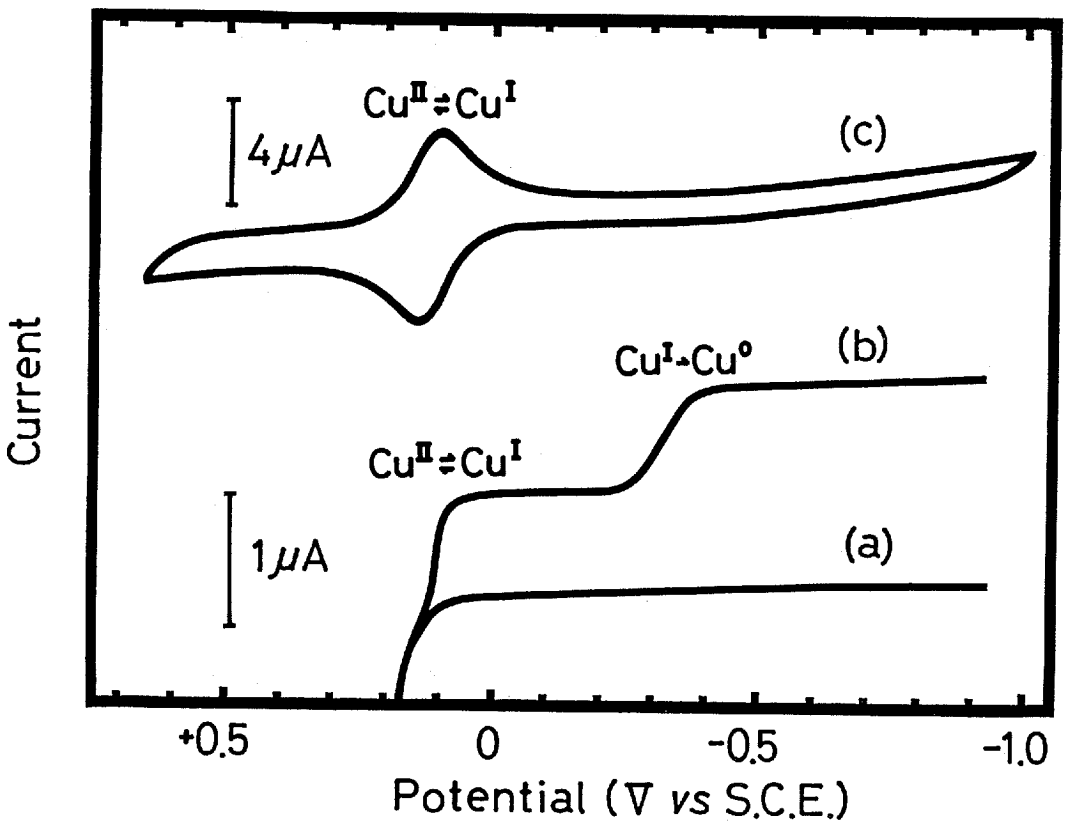


Figure 2

(a) Polarogram of 2.0×10^{-3} M cimetidine at pH 7.8, 25°C and $\underline{I}$=0.20 M ($NaNO_3$). (b) Polarogram of 2.0×10^{-4} M 1:2 Cu(II)-cimetidine complex in the presence of 2.0×10^{-3} M cimetidine at pH 7.8, 25°C and $\underline{I}$=0.20 M ($NaNO_3$). $\underline{E}_{1/2}$=+0.11, $\underline{E}^2_{1/2}$=-0.32 (V $\underline{vs}$. SCE).
(c) Cyclic voltammogram of 2.0×10^{-4} M 1:2 Cu(II)-cimetidine complex in the presence of 2.0×10^{-3} M cimetidine at pH 7.8, 25°C and $\underline{I}$=0.20 M ($NaClO_4$).
$\underline{E}'_{1/2}$= +0.13 (V $\underline{vs}$. SCE).

(22:1), +0.075 (29:1), +0.064 (42:1) [V vs SCE] at pH 7.8, 25.0°C, and $\underline{I}$=0.20 M ($NaNO_3$). The lowering of [cimetidine] to [Cu^{II}] raises the $\underline{E}_{1/2}$ value to ~+0.15 V vs SCE (at 5:1) where the Hg oxidation wave merges. These $\underline{E}_{1/2}$ values were used to calculate the following theoretical Cu(I)-cimetidine complex formation constant $\underline{K}_{Cu^{I}L}$.

Copper(I) Complex Formation Constants. The Cu(I)-cimetidine equilibria can be described by eqs.(4)-(6), which along with eqs.(2) and (3), are incorporated into the Nernst equation to obtain general formula eq. (7).

$$Cu^{I} + L \rightleftharpoons Cu^{I}\text{-}L, \qquad \underline{K}_{Cu^{I}L} \tag{4}$$

$$Cu^{I}\text{-}L + L \rightleftharpoons Cu^{I}\text{-}L_2, \qquad \underline{K}_{Cu^{I}L_2} \tag{5}$$

$$Cu^{I}\text{-}L_{n-1} + L \rightleftharpoons Cu^{I}\text{-}L_n, \qquad \underline{K}_{Cu^{I}L_n} \tag{6}$$

$$\underline{E}_{1/2} = \underline{E}_{Cu^{I},II} + \frac{RT}{F}\ln\left\{\frac{1 + \sum_{j=1}^{n}\prod_{i=1}^{j}(\underline{K}_{Cu^{I}L_i}(L))}{1 + \underline{K}_{Cu^{II}L}(L) + \underline{K}_{Cu^{II}L}\cdot\underline{K}_{Cu^{II}L_2}(L)^2}\right\} \tag{7}$$

Eq. (7) can be simplified to eq. (8) at [cimetidine] >> [Cu^{II}], pH >6 and 25°C, since the Cu(II)-complex is almost all in 1:2.

$$\underline{E}_{1/2} = \underline{E}_{Cu^{I},II} + 0.059 \log\left(\frac{1 + \sum_{j=1}^{n} \prod_{i=1}^{j} (\underline{K}_{Cu^{I}L_i}(L))}{\underline{K}_{Cu^{II}L} \cdot \underline{K}_{Cu^{II}L_2}(L)^2}\right) \quad (8)$$

The rearrangement of eq. (8) gives eq. (9).

$$\frac{\underline{K}_{Cu^{II}L} \cdot \underline{K}_{Cu^{II}L_2}(L)^2}{\mathrm{antilog}((\underline{E}_{Cu^{I},II} - \underline{E}_{1/2})/0.059)} - 1$$

$$= \underline{K}_{Cu^{I}L}(L)\left(1 + \sum_{j=2}^{n} \prod_{i=2}^{j} (\underline{K}_{Cu^{I}L_i}(L))\right)$$

$$= \underline{K}_{Cu^{I}L}(L)\{1 + A([L])\} \quad (9)$$

With displacement of -0.079 V vs SCE for the $\underline{E}_{Cu^{I},II}$ term[20] and the above values for $\underline{K}_{Cu^{II}L}$ and $\underline{K}_{Cu^{II}L_2}$, a plot of the left hand side against [L] of eq(9) gives a linear line with zero intercept (Fig.3), which indicates the A([L]) term to be negligibly small. That is, the 1:2 (or more) Cu^{I}-cimetidine complexes are virtually negligible under the reaction conditions. From the gradient of the linear line, we can obtain $\underline{K}_{Cu^{I}L}$ of $(1.3 \pm 0.2) \times 10^{9}$ M^{-1}.

To get a support for this conclusion, we have examined direct interaction of Cu(I) ion with cimetidine (1:2 molar ratio) by a potentiometric acid-base titration method (Fig.4). Upon mixing $Cu(I)[NCCH_3]_4 \cdot ClO_4$ with cimetidine$\cdot HClO_4$ in aqueous solution, the colorless 1:1 Cu(I)-cimetidine complex immediately and

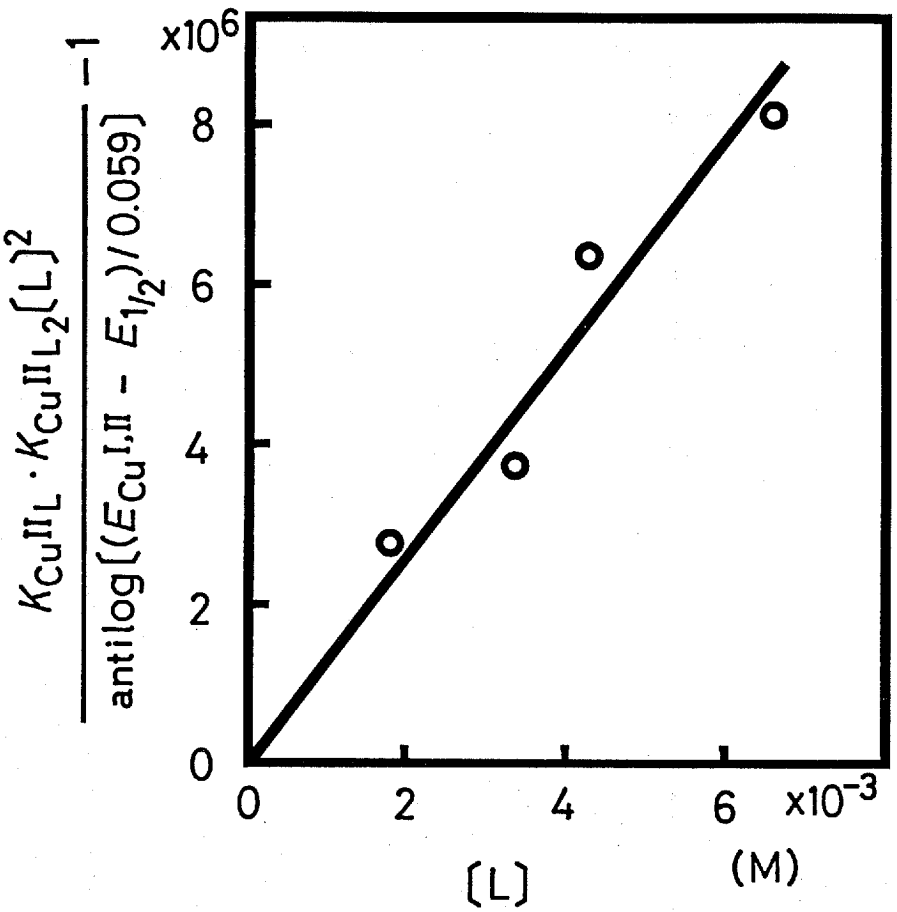


Figure 3.

Plots of equation (9). The $\underline{E}_{1/2}$ values used for the calculation were obtained by using the polarographic method at pH 7.8, 25°C, $\underline{I}$=0.20 M ($NaNO_3$), [total Cu(II)]=2.0 x 10^{-4} (M), and [total L]=2.4 x 10^{-3} to 8.4 x 10^{-3} (M).

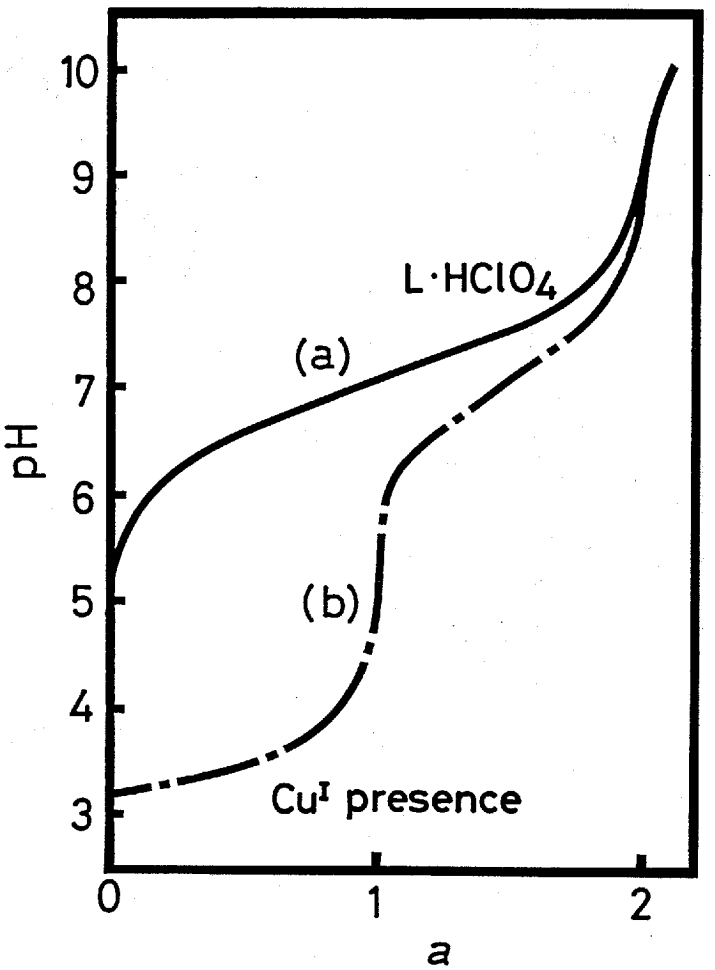


Figure 4.

Potentiometric titration curves for cimetidine(L)· $HClO_4$ in the absence and presence of Cu(I) ion in 2v/v% CH_3CN at 25°C and $\underline{I}$= 0.2 M ($NaClO_4$):
(a) [total L]=2.0 x 10^{-3} (M) only, (b) [total Cu(I)]=1.0 x 10^{-3}, and [total L]=2.0 x 10^{-3} (M). $\underline{a}$ is mole of base per mole of Cu(I) ion.

nearly quantitatively precipitated and at the same time one equivalent of H^+ was liberated [Fig.4 (b)]. Hence the titration curve until $\underline{a}$ = 1 overlaps with the titration curve of one equivalent H^+ in the presence of one equivalent cimetidine·H^+. The break at $\underline{a}$ = 1 (where all the liberated H^+ completes neutralization) and the subsequent buffer region until $\underline{a}$=2 where the uncomplexed one equivalent cimetidine·H^+ (pK_a = 7.20) is neutralized well illustrate the 1:1 Cu(I)-cimetidine equilibrium of eq. (4). The complex formation constant $\underline{K}_{Cu^{I}L}$ with Cu(I) is apparently larger than $\underline{K}_{Cu^{II}L}$ with Cu(II), as intuitively understood from the more depressed cimetidine buffer pH with Cu(I) [Fig.4 (b)] than with Cu(II) [Fig.1 (b)]. Because of the precipitation problem, we were unable to evaluate the $\underline{K}_{Cu^{I}L}$ value from the Fig.4 (b) titration data.

The theoretical Nernst equation (10) for the 1:1 complex formation constants $\underline{K}_{Cu^{I}L}$ and $\underline{K}_{Cu^{II}L}$ allows to assess a theoretical redox potential $\underline{E}^{o}(1:1)$ of +0.18 V $\underline{vs}$ SCE (at 25°C) for the 1:1 Cu(I,II)-cimetidine complexes.

$$\underline{E}^{\circ}(1:1) = \underline{E}_{Cu^{I,II}} + 0.059 \log \frac{\underline{K}_{Cu^{I}L}}{\underline{K}_{Cu^{II}L}} \qquad (10)$$

Isolation and Characterization of 1:1 Cu(I)-Cimetidine Complex. The $Cu^{I/II}$ redox potential (+0.18 V $\underline{vs}$ SCE or +0.42 V $\underline{vs}$ NHE) suggests appreciable stability of the Cu(I)-cimetidine complex with respect to the

Cu(II) complex. In fact, we have succeeded in isolating an analytically pure 1:1 Cu(I)-cimetidine complex [Cu(cimetidine)(ClO_4)·H_2O] (colorless) by treating Cu(I)[$NCCH_3$]$_4$(ClO_4)[15] with two equivalent cimetidine. The same species precipitated on chemically reducing 1:2 Cu(II)-cimetidine complex (green, water-soluble) with ascorbic acid or electrochemically applying 0 V vs SCE at pH 7 and 25°C. This is the first report of isolation of a stable Cu(I)-cimetidine complex. The nitrile absorption in the IR spectrum of 1:1 Cu(I)-cimetidine complex occurs as a strong singlet band at 2230 cm^{-1}, 30 cm^{-1} higher than that of the 1:2 Cu(II)-cimetidine complex.[5] The insufficient solubility of the cuprous complex in any solvent tested precluded its extensive characterization. Its maximum solubility in 0.05 M phosphate buffer at pH 7.8 and 25°C was estimated at 3.4 x 10^{-5} M by atomic absorption spectroscopic method. The colorless cuprous complex is gradually oxidized in air to green cupric complex at room temperature.

Superoxide Dismutase-like Activity of Cu(I)- and Cu(II)-cimetidine complexes. The IC_{50} of 1:1 Cu(I)-cimetidine complex was 4 x 10^{-7} M with additional presence of 2.0 x 10^{-5} M of cimetidine at 25°C and pH 7.8. Separately, we have confirmed that free cimetidine has no SOD-like activity. The IC_{50} of 1:2 Cu(II)-cimetidine complex was 4 x 10^{-6} M with presence of 4.0 x 10^{-5} M of cimetidine at 25°C and pH 7.8. In order to assess those values, we have measured IC_{50} of previous reported SOD-like complexes under the same

conditions: e.g. Cu(II)-[o-phenanthroline]$_2$,[21] 2 x 10^{-4} M; Cu(II)-glycylglycine,[22] 2 x 10^{-5} M; Cu(II)-[salicylate]$_2$,[23] 4 x 10^{-4} M; and Cu(II)-macrocyclic dioxo[12]N_4,[22] 5 x 10^{-4} M.

Discussion

Affinity of Cimetidine to Cu(II). Analysis of the pH-metric titration curve (c) in Fig. 1 before precipitation of the 1:2 Cu(II)-cimetidine complex has established the complexing equilibria of 1:1 (eq. 2) and 1:2 (eq. 3) in aqueous solution with formation constants $\underline{K}_{Cu^{II}L}$ = (3.02 ± 0.05) x 10^4 M^{-1} and $\underline{K}_{Cu^{II}L_2}$ = (2.35 ± 0.05) x 10^4 M^{-1}. Like an earlier report,[5] only the very insoluble (polymeric) 1:2 cupric complex was isolated from a mixture of Cu(II)$[ClO_4]_2$ and cimetidine in 1:1 to 1:11 molar ratio. We were unable to isolate the 1:1 complex. The coordination of cimetidine to Cu(II) in the 1:2 complex in solution will occur through the two imidazoles and two thioethers at equatorial site and weakly bound two cyanoguanidine (or H_2O) at axial positions (in crystals the cyano-N goes to an axial site of an adjacent molecule). For the 1:1 complex the three donors from a cimetidine may occupy three equatorial sites.

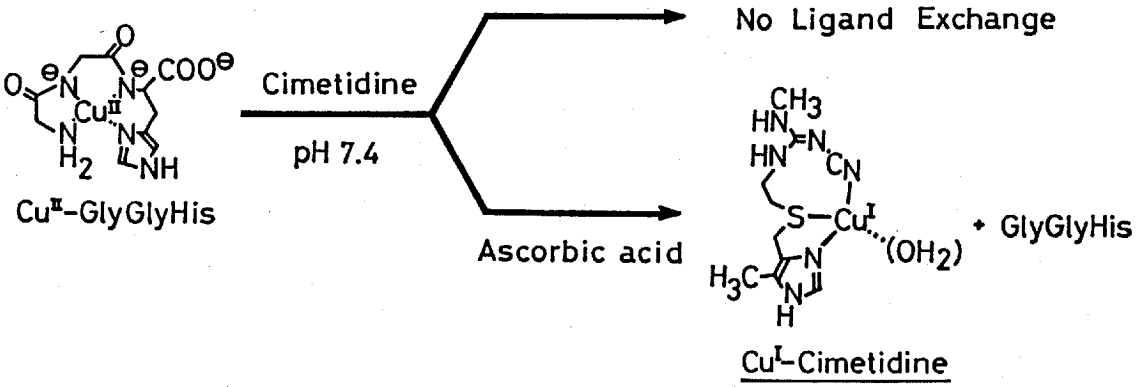
Apparently the cimetidine binding to Cu(II) ion is not so strong as those of relevant physiological bidentate histamine ($\underline{K}_{Cu^{II}L}$ = 3.8 x 10^9 M^{-1}, $\underline{K}_{Cu^{II}L_2}$ = 3.0 x 10^6 M^{-1}),[24] histidine ($\underline{K}_{Cu^{II}L}$ = 2.0 x 10^{10} M^{-1}, $\underline{K}_{Cu^{II}L_2}$ = 4.2 x 10^7 M^{-1}),[24] or other amino acids (*e.g.* with glycine; $\underline{K}_{Cu^{II}L}$ = 2.4 x 10^8 M^{-1}, $\underline{K}_{Cu^{II}L_2}$ = 7.4 x 10^6 M^{-1}).[25] Cimetidine-Cu is less stable than tetradentate glycylglycylhistidine (GGH)-Cu that is a biomimetic model of Cu(II)-binding to albumin,[26,27] whose 1:1 complex formation constant at pH 7.4 is calculated at 6.3 x 10^{12} M^{-1},[26] approximately 10^8 times

as great as the $\underline{K}_{Cu^{II}L}$ value of the cimetidine complex. This argument is supported by a qualitative test; addition of 10 times excess of cimetidine to Cu(II)-GGH complex (λ_{max} 525 nm) at pH 7.4 causes no visible absorption spectral change to the 1:2 Cu(II)-cimetidine (λ_{max} 606 nm), indicating no occurrence of the ligand exchange. These *in vitro* experiments taken together can exclude an earlier conception[5] that cimetidine might be bound with Cu(II) in our body.

Redox Potential of Cu(I,II)-Cimetidine. The theoretical redox potential of 1:1 Cu(I,II)-cimetidine complex is extremely high +0.42 V *vs* NHE at pH 7.8. Presence of higher concentration of cimetidine with respect to [Cu^{II}] has lowered the potential to +0.31 V *vs* NHE at 42:1. This is interpreted that the higher [cimetidine] makes the six coordinate 1:2 complex more favorable, which contributes to stabilization of Cu(II).

The equilibrium mixture of 1:1 and 1:2 Cu(II)-cimetidine complexes at pH 7.0 were readily and quantitatively reduced with ascorbic acid ($\underline{E}^{o}$ = +0.22 V *vs*. NHE at pH 7)[28] and dithiothreitol ($\underline{E}^{o}$ = -0.33 V *vs*. NHE at pH 7)[29] to the colorless 1:1 Cu(I)-cimetidine complex. Thus formed Cu(I) complex solution shows an identical cyclic voltammogram to the one obtained initially with Cu(II) complex ($\underline{E}'_{1/2}$ = +0.40 V *vs* NHE, at pH 7.0 and [cimetidine]/[Cu^{II}] = 5).

Most interestingly from physiological point of view, Cu(II) tightly bound to GGH at pH 7.4 is rapidly released to bind with cimetidine in the presence of



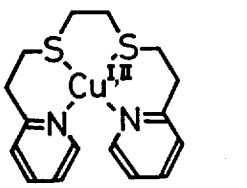
ascorbic acid (10 mM) (the half life time ca. 5 min, when [Cu(II)-GGH] = 1 mM and [cimetidine] = 2 mM) to the insoluble 1:1 Cu(I)-cimetidine complex. In our body, there are other biological reductants that possess less positive E^{o} values than $Cu^{II,I}$-cimetidine; e.g. hemoglobin (+0.17 V vs. NHE at pH 7), ubiquinone (+0.10 V at pH 7) and glutathione (-0.23 V at pH 7).[28] These facts may suggest that the administered cimetidine can interact with available copper ion and the resulting Cu(I)-cimetidine species precipitates on tissues. The present in vitro discovery might further be relevant to a pharmacological fact that cimetidine binding to "imidazole" receptors is greatly enhanced in the presence of Cu(II) ion and ascorbic acid.[9] Physiologically of further interest may be a fact that the Cu(I)-cimetidine complex is stable even at very low pH region, as illustrated in Fig.4 (b), which suggests that cimetidine could stay bound to cuprous complex in digestive organs. In this connection our preliminary test has revealed that the cimetidine activity to

inhibit the acid secretion in rats is dramatically enhanced by addition of copper ion. We are further pursuing experiments for the effects of copper ion on the cimetidine activities.

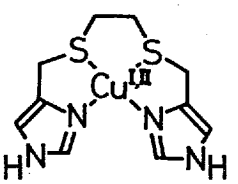
Cimetidine as a Biomimetic Ligand for Interconversion of Cu(I,II). The 1:1 Cu(I)-cimetidine complex formation constant $\underline{K}_{Cu^{I}L}$ of 1.3×10^{9} M^{-1}, which is greater than that for the 1:1 Cu(II)-cimetidine complex, is remarkably large for a Cu(I) complex in aqueous solution. We do not know of such a thermodynamically stable Cu(I) complex in aqueous solution with any of the previously reported ligands except for macrocyclic tetrathioethers.[30] All of the three donor groups of cimetidine; i.e. imidazole N, thioether S, and cyanoguanidine N, have π-donor characters and hence would prefer soft Cu(I) ion over hard Cu(II) ion. The IR spectrum of Cu(I)-cimetidine solid shows a strong $\nu_{C\equiv N}$ at 2230 cm^{-1}, 30 cm^{-1} higher than that for Cu(II)-$(cimetidine)_2$, suggesting a stronger -CN-Cu(I) bonding. Uncomplexed cimetidine has $\nu_{C\equiv N}$ at 2180 cm^{-1}. In tetrahedral $[Cu^{I}(NCCH_3)_4]ClO_4$ complex, $\nu_{C\equiv N}$ occurs at 2275 and 2300 cm^{-1}.[14] The unique electrochemical properties of cimetidine in favor of Cu(I) ion are obvious when its $\underline{E}^{o}$ value is compared with those of relevant ligand systems; tetraimidazole ($\underline{E}^{o}$ = +0.04 V vs. NHE),[31] tetraammonia ($\underline{E}^{o}$ = +0.02 V vs. NHE),[31] bis o-phenanthroline ($\underline{E}^{o}$ = +0.17 V vs. NHE).[32]

Recently, copper complexes containing thioether and heteroaromatic nitrogen ligands are attracting much

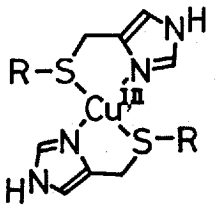
attention as models[33-37] for type I copper proteins (E^{o} = +0.2 ~ +1.0 V vs NHE[10]) having electron-transfer functions. Typical model structures are shown below.



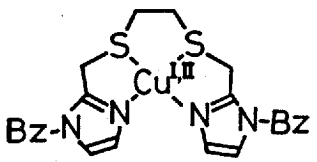
1[33]



2[34]



3[34]



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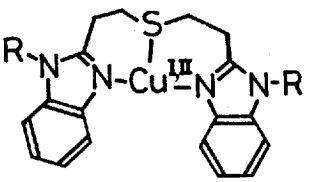
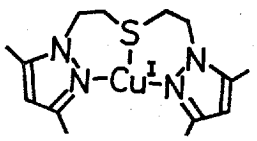


5[36]



6[37]

Their reported E^{o} values (vs NHE) are +0.9 V (for 1 in H_2O),[33] +0.47 V (for 2 in MeOH),[34] +0.61 V (for 3 with R=t-Butyl in MeOH).[34] Cimetidine has a similar structure to those, especially 3 with an additional cyanoguanidine. The electronic absorption bands of the possible square-planar (or octahedral) Cu(II)-(cimetidine)$_2$ complex (λ_{max} 344 and 615 nm in MeOH)[5] and of square-planar Cu(II) in 3 (R=t-Butyl, 340 and 624 nm in MeOH/EtOH)[33] are very close, indicating a similar ligand fields of cimetidine and 3. The more intense bands at ~340 nm for both are assigned to S→Cu charge transfer transitions.[5,34]

Superoxide Dismutase-like Activity by Cu-Cimetidine complexes. The $\underline{E}^{o}$ value of +0.42 V *vs.* NHE for Cu(I,II)-cimetidine is especially interesting in its similarity to the reported $\underline{E}^{o}$ value of +0.42 V[11] or +0.35 V[12] *vs.* NHE (at pH 7) for Cu-superoxide dismutase (Zn·Cu-SOD). We therefore have assayed the SOD-like activity of the Cu(I,II)-cimetidine complexes to compare with the previous, SOD-like complexes by the well-established xanthine-xanthine oxidase and ferricytochrome c system.[17] In our earlier experiments,[38] we have found parallel O_2^- scavenging activities of indirectly (from xanthine-xanthine oxidase) and directly (from KO_2) generated O_2^- by various copper complexes.

The assay result was very encouraging in that the 1:1 Cu(I)- and 1:2 Cu(II)-cimetidine complexes are extremely strong O_2^- scavengers, whose activities are much higher than any of the previously recognized SOD-like substances, Cu(II)-(*o*- phenanthroline)$_2$,[21] Cu(II)-glycylglycine,[22] Cu(II)-(salicylate)$_2$,[23] Cu(II)-macrocyclic polyamines.[38] Although the detailed reaction mechanism must await more precise experiments, we tentatively consider similar O_2^- dismutation reactions (11) and (12) as with SOD, in view of nearly the same $\underline{E}^{o}$ values for $Cu^{I/II}$.

$$\text{Cu(II)-cimetidine} + O_2^- \longrightarrow \text{Cu(I)-cimetidine} + O_2 \qquad (11)$$

$$\text{Cu(I)-cimetidine} + O_2^- + 2H^+ \longrightarrow \text{Cu(II)-cimetidine} + H_2O_2 \qquad (12)$$

The active species most likely is the 1:1 Cu-cimetidine species (the 1:2 Cu(II)-cimetidine complex should undergo prior dissociation to the 1:1 species), whose distinct features may be (i) cimetidine is a tridentate ligand and hence the remaining one coordination site is open to incoming O_2^-; (ii) cimetidine is a neutral ligand and hence the complexes bear positive net charges, ready to invite the attack of negative superoxide ion O_2^-; and (iii) the Cu(I) $\rightleftharpoons$ Cu(II) conversion is nearly reversible without decomposition of the complexes. In the Cu-SOD,[39] the Cu(II) in the active center is surrounded by four imidazole nitrogens and one H_2O that is to be displaced by the incoming O_2^-. For further advantage the Cu-SOD has Arg 141 (bearing a positive guanidinium cation, which help attract the negative O_2^- to the H_2O position.[40]

Recently, cimetidine has been reported to inhibit the oxidative metabolisms of steroid hormones,[41,42] drugs,[43-46] and other chemicals.[44] It was proposed that cimetidine directly binds at the sixth ligand position of cytochrome p-450.[47,48] Our present findings may invoke a new explanation that the active O_2 species formed on p-450 may be scavenged by the Cu-cimetidine complexes before they reach substrates. We are planning to test this hypothesis. In this regard, it may be recalled that Cu(II) complexes of tyrosine, salicylates, <u>etc</u> having the SOD-like activities are also reported to inhibit drug metabolisms by microsomal cytochrome p-450.[49,50] In light of the recent report[13] that a Cu(II) complex of 3,5-diisopropylsalicylate exhibiting a strong SOD-like activity is promising as

anti-inflammatory and anti-cancer agents, the copper-cimetidine complexes are certainly worthy of thorough pharmacological investigations.

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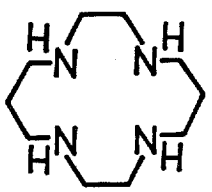
CHAPTER II

Novel Synthesis and Complex Properties of Macrocyclic Tetra-amines Appended with Phenol as an Axial Donor

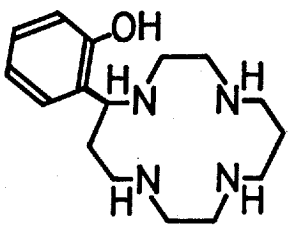
Introduction

The significance of macrocyclic polyamine ligands and their metal complexes is most obvious as it relates to such natural products as metalloporphyrins, vitamin B_{12} and metalloproteins. Cyclam 1 (1,4,8,11-tetra-azacyclotetradecane) is one of the most basic and well-known compound in the macrocyclic polyamines. The inclusion of metal ions (e.g. $Co^{II,III}$, Cu^{II}, $Ni^{II,III}$) within the saturated 14-membered macrocyclic tetra-amine cavities has been well documented.[1] However, limited efforts[2] have been made to attach axial donors



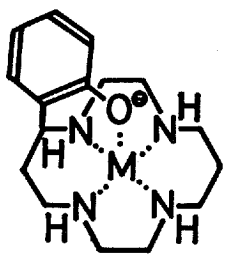
Cyclam

1



Phenol-cyclam

2



Phenol-cyclam-M Complex 3

M=$Ni^{II,III}$, $Fe^{II,III}$

that might dramatically affect the properties of the tetra-amine complex. The critical roles of the axial donors are most obvious in heme-iron systems of normal[3] or abnormal[4] hemoglobin and redox enzymes such as cytochrome P-450[5] and catalase.[6] Herein, I report a new one-step annelation method for a new tetra-amine macrocycle 2 bearing a potential phenolate donor for an axial position (Figure 1), and describe some of its novel complexing properties.

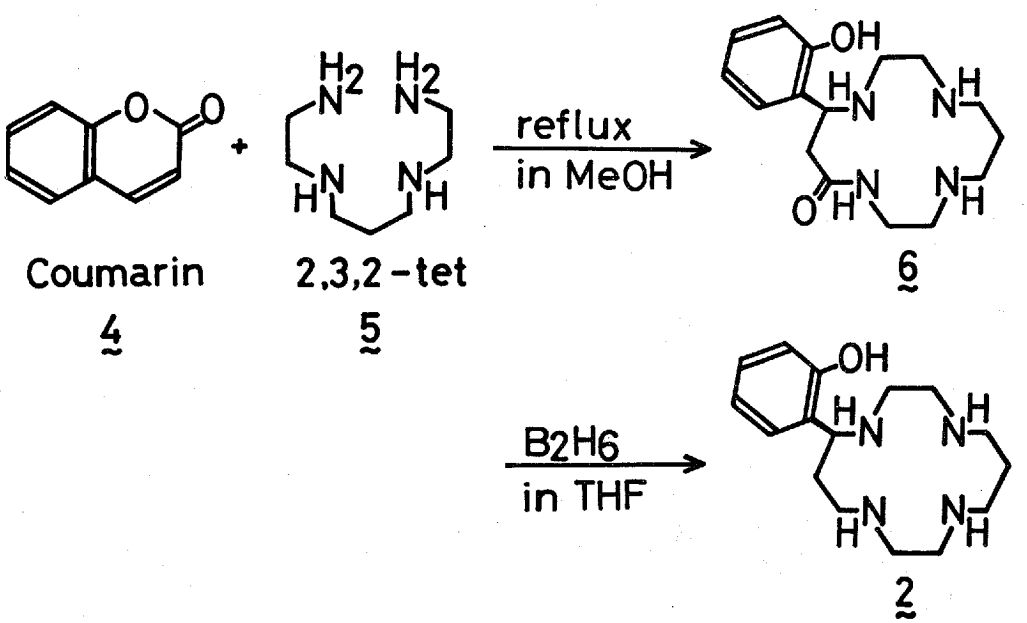


Figure 1. A novel "recycles" synthesis for phenol-pendent cyclam 2.

Experimental Section

Materials. A 10^{-2} M stock solution of Ni^{II} ion was prepared from analytical grade $Ni^{II}SO_4 \cdot 6H_2O$ and standardized by titration with disodium salt of ethylenediaminetetraacetic acid (EDTA).[7] Other chemicals employed were of analytical grade and were used without further purification.

5-(2-Hydroxyphenyl)-1,4,8,11-tetraazatetradecane, 2. Refluxing coumarin 4 (10 g, 68.5 mmol) and 1,9-diamino-3,7-diazanonane 5 (11 g, 68.7 mmol) in 1.5 L of dry MeOH for two weeks afforded 7-(2-hydroxyphenyl)-1,4,8,11-tetraazatetradecane-5-one 6 as its trihydrochloride (decomp. 185°C) in 20% yield (5.7 g), after purification by silica gel column chromatography (eluant CH_2Cl_2-MeOH-28%aq.NH_3, 100:5:1) and recrystallization from EtOH-HCl. Reduction of 6·3HCl (5.7 g) with B_2H_6 in tetrahydrofuran (THF) yielded a cyclam derivative 2 (2 g, 6.8 mmol). The total yield of 2 based on the starting coumarin was 10%. The product was purified by recrystallization from acetonitrile; m.p. 142-143°C; M^+ peak m/e 292 (M_r 292.43); ^1H NMR (in CCl_3D, 35°C, Me_4Si reference) δ 0-1.5 (br,1H, OH), 1.5-2.1 (br,4H,C-6,13), 2.3-3.2 (br,18H, C-2,3,7,9,10,12,14, N-1,4,8,11), 3.7-4.0 (dd,1H, C-5), 6.6-7.2 (br,4H, Ar) [Figure 2]; ^{13}C NMR (in CCl_3D, 22.5°C, Me_4Si reference) δ 157.8 (C-16), 127.9, 127.8 (C-18,20), 126.6 (C-15), 118.6 (C-17), 116.4 (C-19), 66.7 (C-5), 51.2, 50.9, 50.1, 49.6, 49.3, 49.2, 47.3 (C-2,3,7,9,10,12,14) 36.3 (C-6) 29.3 (C-13) [Figure 3].

Anal.Calcd for $C_{16}H_{28}N_4O$: C, 65.72; H, 9.65; N, 19.16. Found: C, 65.68; H, 9.24; N, 19.10.

$[Ni^{II}(\underset{\sim}{2}H_{-1})]ClO_4 \cdot H_2O$ or $\underset{\sim}{3}(M=Ni^{II})$. The phenol-pendent cyclam $\underset{\sim}{2}$ (146 mg, 0.5 mmol) and $Ni^{II}SO_4 \cdot 6H_2O$ (131 mg, 0.5 mmol) were dissolved in 25 ml of 0.5 M $NaClO_4$ aqueous solution at 50°C and the mixture was adjusted to pH 8 with 2 M NaOH solution for complexation. The resulting purple solution was filtered and the filtrate was stood for one week at room temperature to obtain purple crystals of $\underset{\sim}{3}(M=Ni^{II})$ (150 mg, 64 %). It was subjected to an X-ray analysis.

Spectrophotomeric measurements. ^1H and ^{13}C NMR spectra were obtained on a JOEL JNM-FX100S FT-NMR Spectrometer. UV spectra were measured with a Shimazu UV-200S spectrophotometer at 25.0 ± 0.1°C. The ESR spectra were recorded on a JES-FE1X spectrometer operating at 9300 MHz and equipped with a dual cavity. A small sample of Mn^{II} was placed in the reference cavity. Two spectra were recorded for each sample, wherein the field was swept alternatively in opposite direction and the average g values were taken. The g values were calculated by the approximation method of Knenbuhl.[8] The $g_{//}$ values are accurate to ±0.05 and the $g_{\perp}$ values to ±0.01.

Potentiometric Measurements. The formation constants of Ni^{II} and Fe^{II} complexes of $\underset{\sim}{3}$ were determined by a potentiometric acid-base titration method with an Orion Research 811 digital pH meter. The

titration data was treated by a Schwarzenbach method programed for NEC PC-9801 personal computer. All the measurements were performed at 25.00 ± 0.05°C and $\underline{I}$=0.1 M (adjusted with $NaClO_4$). Values of $-\log[H^+]$ were estimated by applying a correction of -0.08 pH unit to the pH readings.[9] All solutions were carefully protected from air by a stream of argon gas prepurified through an alkaline pyrogallol solution. The electrode system was calibrated with standard pH 6.86 and 4.01 buffer solutions and checked in duplicate by the theoretical titration curve for 4.00×10^{-3} M $HClO_4$ with carbonate-free 0.100 M NaOH under the same conditions.

Electrochemical Measurements. Cyclic voltammetry and dc polarography were performed with a Yanako Polarographic Analyzer P-1100 system at 25.00 ± 0.05°C. A three-electrode system was employed: a 3-mm glassy carbon rod (grade GC-30, Tokai Electrode Company) or a Yanagimoto P10-RE rotary glassy carbon disk electrode as the working electrode, a Pt-wire as the counter electrode, and a saturated calomel reference electrode (SCE). The cyclic voltammograms with scan rates of 10-100 mVs^{-1} and the dc polarograms with scan rates of 2-10 mVs^{-1} were evaluated graphically.

Crystallographic Study. The lattice constants and intensity data were obtained by the measurements on a Philips PW1100 diffractometer. The crystal data and the method of intensity measurement are summarized in Table 1. Structure determination and refinement process are also listed in Table 1.

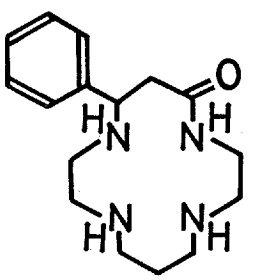
Table 1. Crystal data and summary of structure detetermination.

Compound	Phenol-pendent cyclam, 2	Ni^{II}-complex of 3
Chemical formula	$C_{16}H_{28}N_4O$	$C_{16}H_{27}N_4O.Ni$ $ClO_4 \cdot H_2O$
Formula weight	292.4	467.6
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/a$	$P2_1$
Cell dimensions		
a (Å)	15.335(8)	16.203(9)
b (Å)	8.535(5)	8.042(5)
c (Å)	13.331(7)	7.995(5)
β (°)	105.17(5)	104.02(5)
U ($Å^3$)	1684	1011
Z	4	2
D_{calc} (gcm^{-3})	1.153	1.536
μ for CuK_α (cm^{-1})	5.52	29.6

Crystal habit	Rhombic thick plate	Prism
Color	Colorless	Reddish purple
Specimen size (mm)	Block of ϕ =ca.0.3	0.1x0.2x0.4
Radiation	Graphite monochromated CuK_α	
Scan speed ($^\circ\theta\,min^{-1}$)	6	
Scan number of repeat	2 for I<3000c	
2θ range($^\circ$)	6 - 156	
No. of reflections		
Theoretical	3700	2280
Observed[I>2σ(I)]	2750	2286
In which symm. equiv. reflection	115	133
R for symm.equiv. reflection	0.026	0.023
Independent refl. (used for str.det.)	2635	2153
Phasing	Direct method	Heavy atom method
Refinement	Block-diagonal matrix least-squares method	
No. of heavier atom (anisotropic)	21	28
No. of hydrogen atom (isotropic)	28	29
Final R factor	0.061	0.047

Results and Discussion

Synthetic method. The novel feature of our synthetic method is the use of coumarin as a source of the phenol appendant and at the same time providing reactive sites for the addition of tetra-amine rings in a reaction that successively involves Michael addition followed by lactam formation. (Fig.1) This annelation principle can be extended to the preparation of the analogue 7 from methyl cinnamate,[10] which has a structural resemblance to the macrocyclic spermine alkaloid verbascenine 8 .[11] Thus, this annelations open a new synthetic route not only to metal chelating agents but also to various macrocyclic polyamine alkaloids and suggest a biosynthetic pathway to their ring cyclization.



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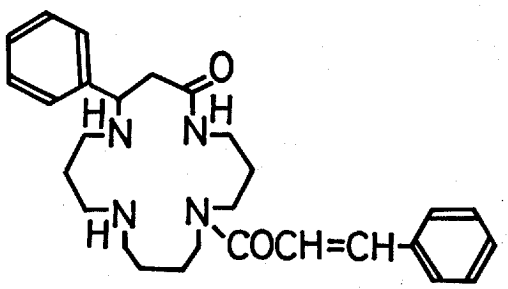

8

Properties of Phenol-pendent Cyclam 2. A significant effect of the directly bonded aromatic ring in 2 is that the motion of the phenolic group is frozen with the OH group held to the macrocyclic ring. The ^1H n.m.r. spectrum of 2 in CCl_3D shows an unusually high chemical shift for OH (δ0-1.5, H-D exchangeable) and a well-resolved doublet of doublets for the benzylic proton signal (δ 3.7-4.0) owing to couplings with the adjacent CH_2 protons (see Fig. 2), suggesting that there is an internal hydrogen bonding between the phenolic hydrogen and some of the tetra-amine nitrogens and that the resulting macrocyclic conformation is fixed.

The deprotonation constants pK_a of 2 were determined pH-metrically at 25°C and I=0.10 M ($NaClO_4$) to be 11.75, 10.84, 8.86, 2>, and 1>. The pK_a of 8.86 is assigned to the phenol group, which is confirmed spectrophotometrically (see Figure 4). In pH ~9 aqueous solution the deprotonated phenolate anion can shield the two undissociated protons in the macrocycle. This is concluded from their higher pK_a values than the corresponding ones (10.70 and 10.06) for 7. These H-bondings should certainly freeze the flexible macrocyclic conformation (Figure 5).

The crystal structure of the phenol-pendent cyclam 2 is shown in Figure 6. The two imino hydrogen atoms, HN_4 and HN_{11} (those attached to N_4 and N_{11}, respectively), turn inside the ring and hydrogen-bond with N_1 and N_8. The phenol hydroxy group also forms intramolecular hydrogen bond to N_4 with bond length 2.16 Å.

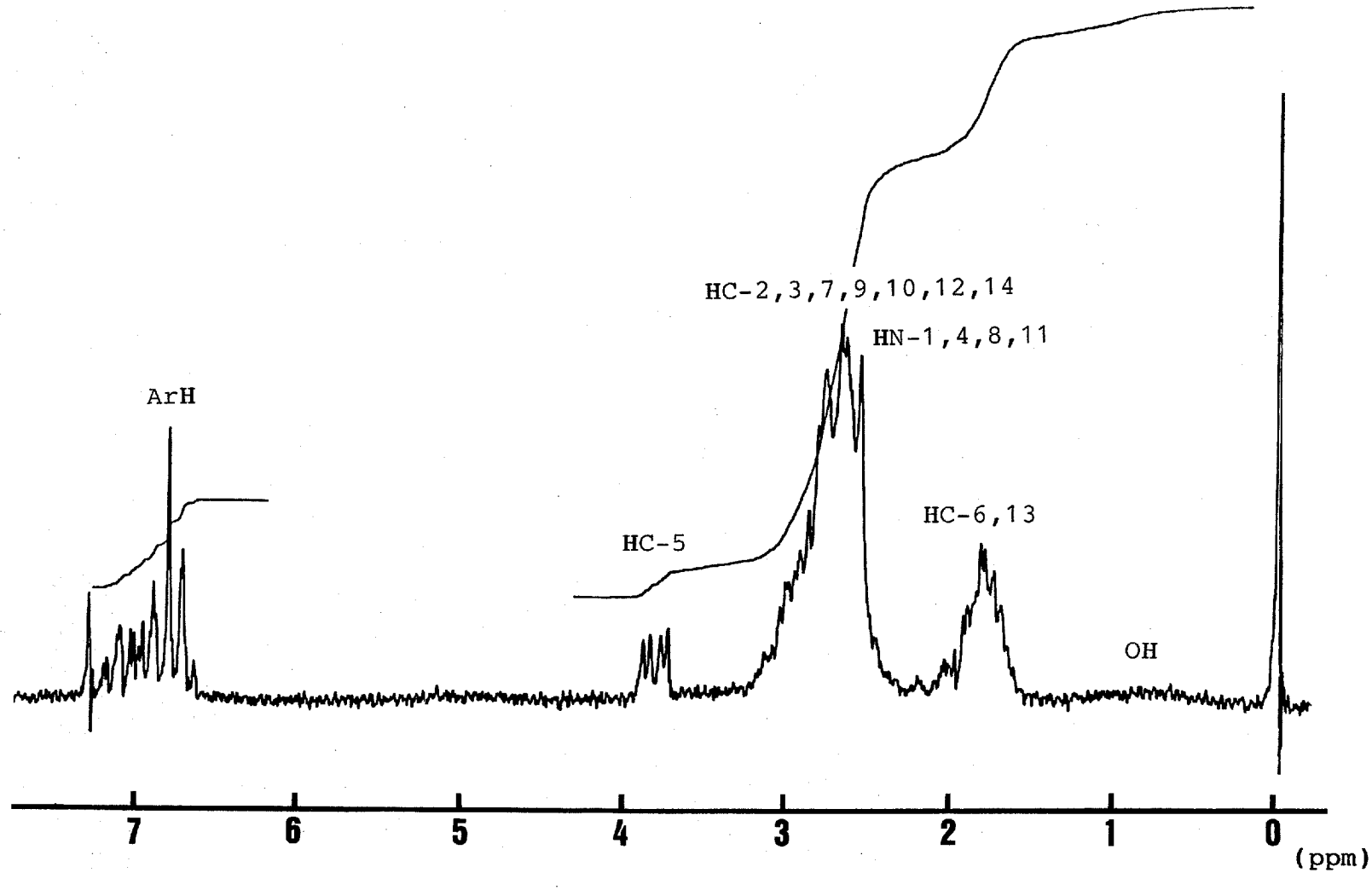

Figure 2. ^{1}H NMR spectrum of 2 in CCl_3D at 35°C.

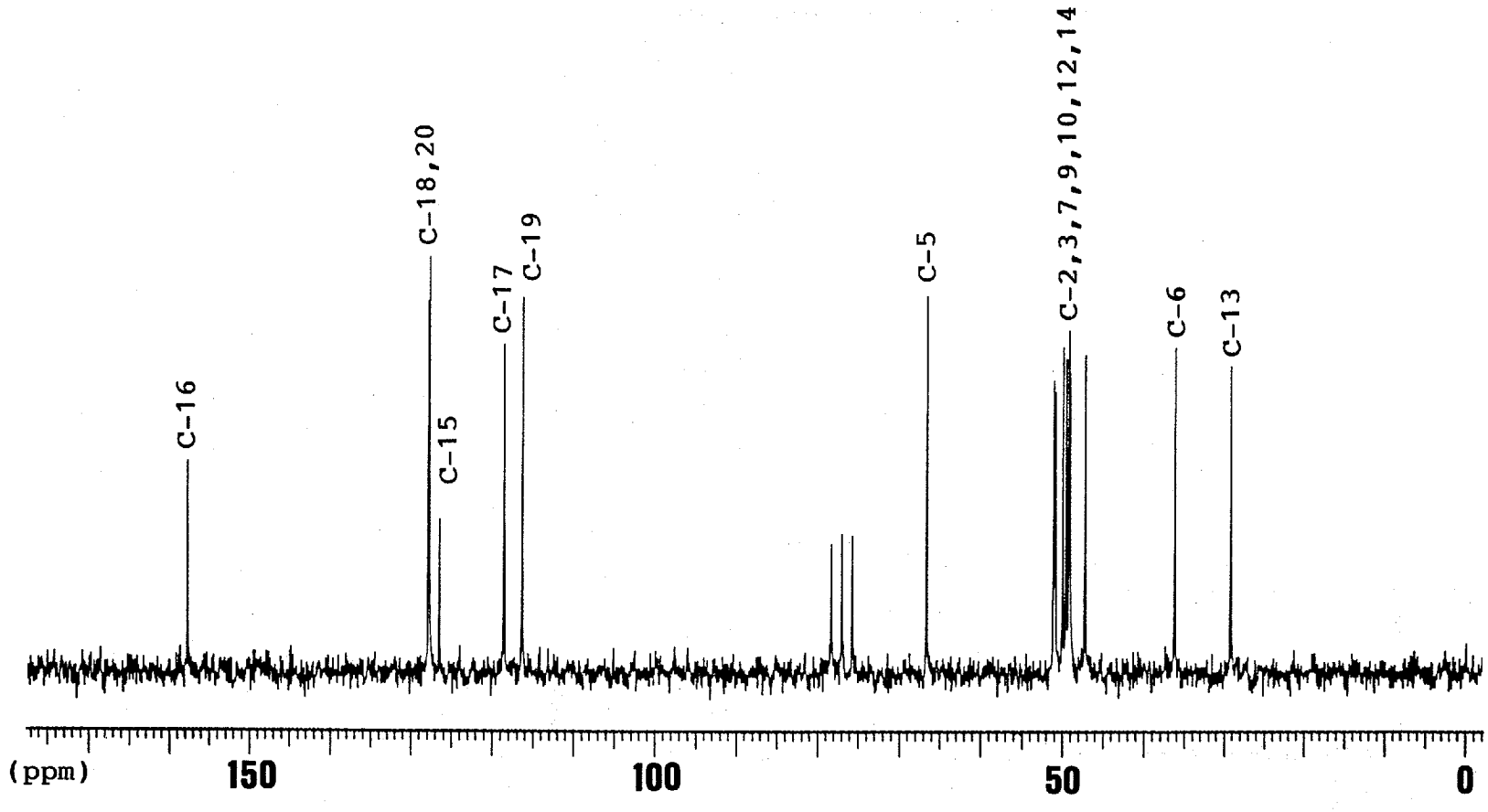

Figure 3. ^{13}C NMR spectrum of **2** in CCL_3D at 22.5°C.

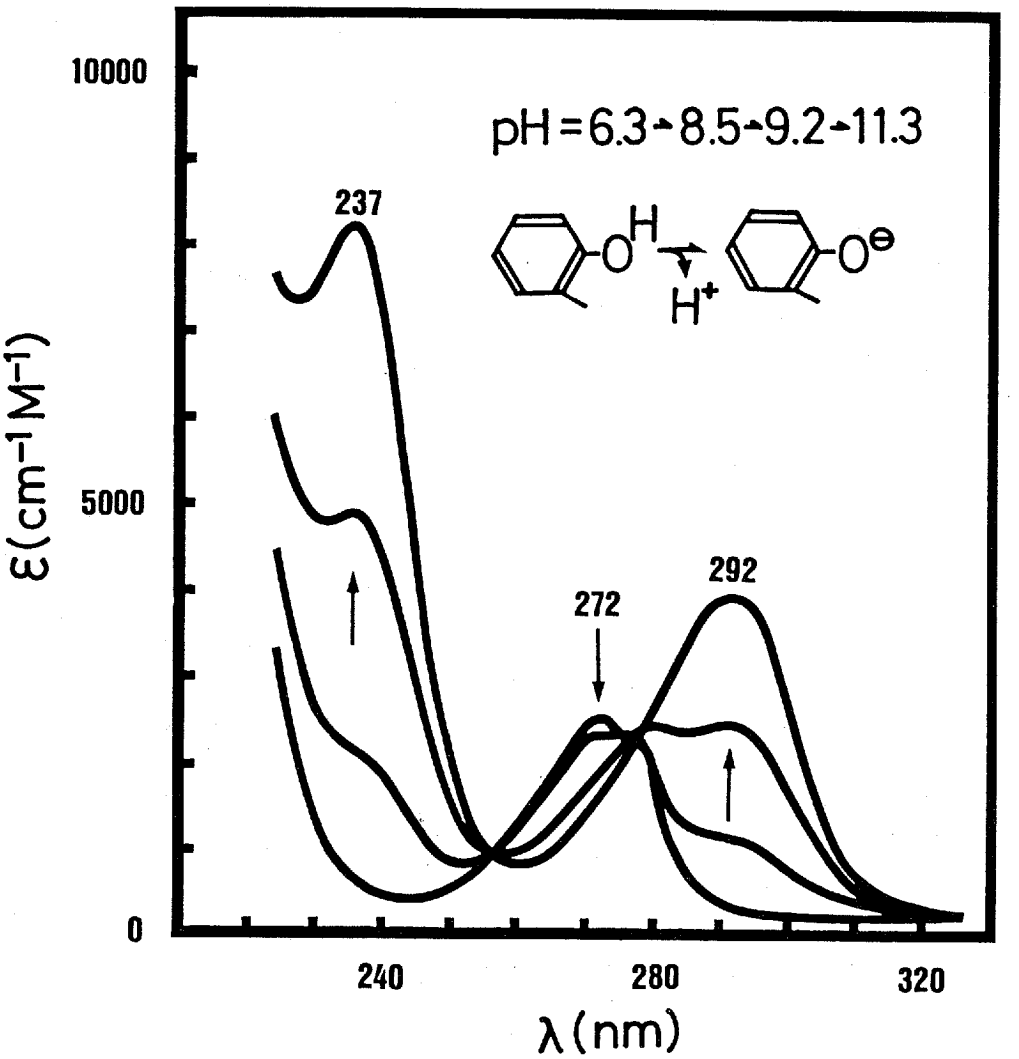

Figure 4. UV absorption spectra of 2 in 0.1 M $NaClO_4$ at 25°C.

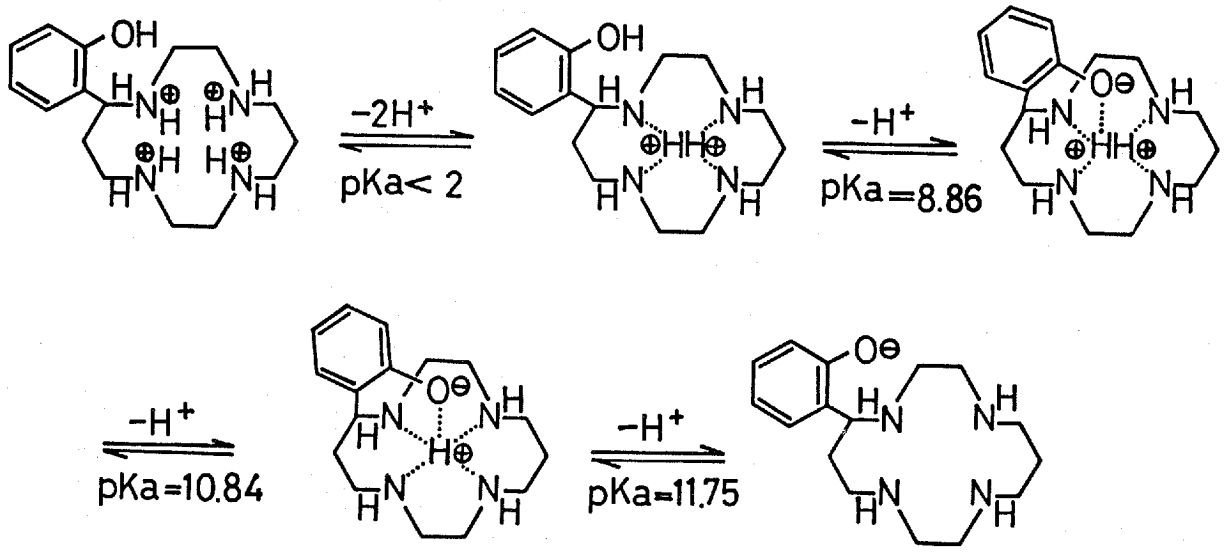

Figure 5. Deprotonation of $\underset{\sim}{2}\cdot 4HClO_4$.

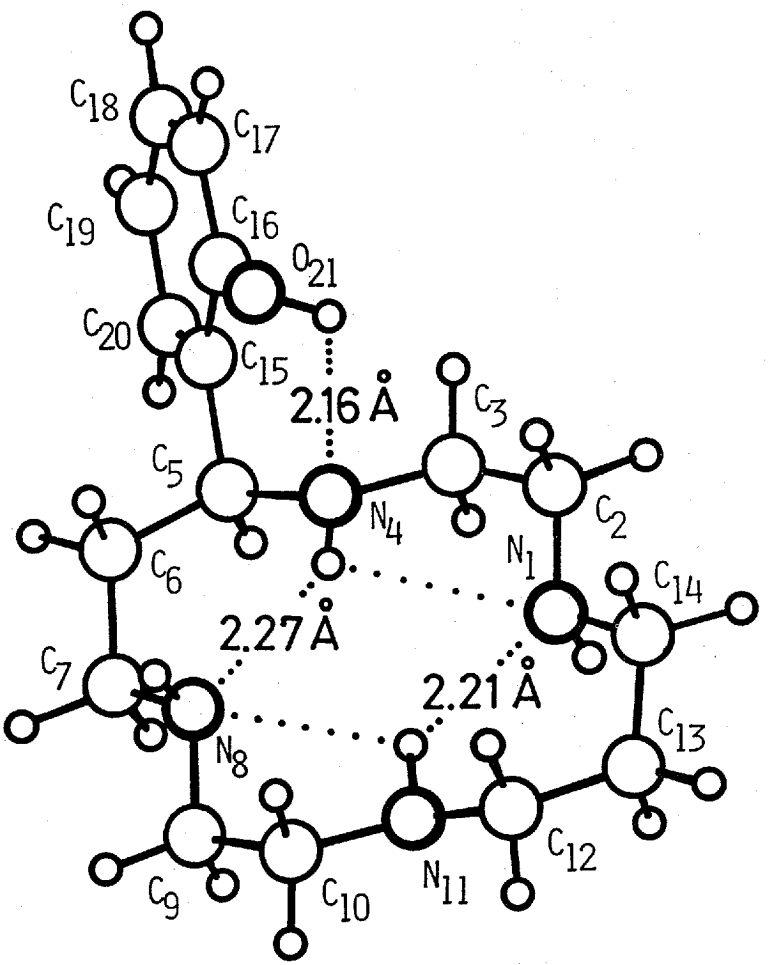

Figure 6. The structure of phenol-pendent cyclam 2.

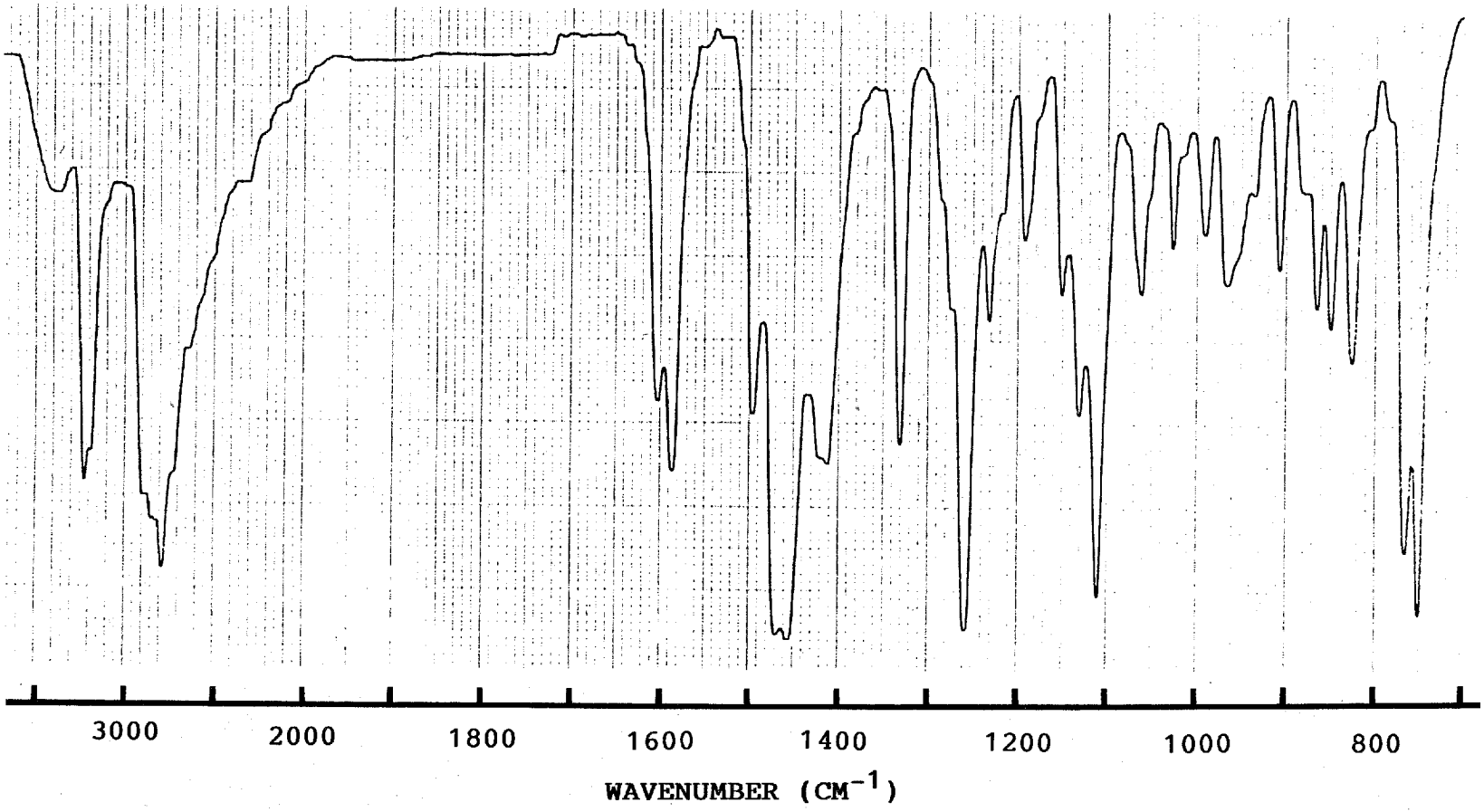

Figure 7. IR spectrum of 2 in KBr.

Ni^{II} Complex of 3. The structure of the axial phenolate-pendant cyclam complex 3 is resolved by the present X-ray crystal study. The correlation of high-spin Ni^{II} ion size with the best-fit ring cavity or with the conformation of the macrocyclic tetraamine ligands has been amply investigated.[12-16] However, the present structural parameters of cyclam complex affected by the apical phenolate coordination are the first of this kind to be reported. The crystal data of the Ni^{II} complex of 3 (formula $C_{16}H_{27}N_4ONi\cdot ClO_4\cdot H_2O$) are monoclinic, space group is $P2_1$, a=16.203(9) Å, b=8.042(5) Å, c=7.995(5) Å, β=104.02(5)°, Z=2, D=1.536 gcm^{-3}. The structure was solved by the heavy-atom method and refined by block-diagonal matrix least-squares method to an R value of 0.047. Dispersion corrections for C,O,N,Cl, and Ni atoms were applied taking a set of atomic coordinates which gave smaller R value. Absorption corrections were not applied. The resulting molecular structure is shown in Figure 8.

The five-coordinate, square pyramidal coordination geometry around nickel is evident. The atoms N_1, N_4, N_8, and N_{11} are coplanar, and the nickel stays in this plane[17]. The phenolate oxygen O_{21} is almost at the apex of the pyramid with the very short apical Ni-O_{21} bond distance 2.015(5) Å. The previous octahedral high-spin Ni^{II}(cyclam)X_2 complexes showed longer Ni-X axial bonds(2.492 Å for X=Cl[18], 2.169 Å for X=NO_3[16]). More significantly, the axial Ni-O_{21} bond length is shorter than the equatorial Ni-N bond distances ranging 2.051(5)~2.078(5) Å. By contrast, the other axial Ni-O_1(of perchlorate) distance is very long at 2.402(7) Å,

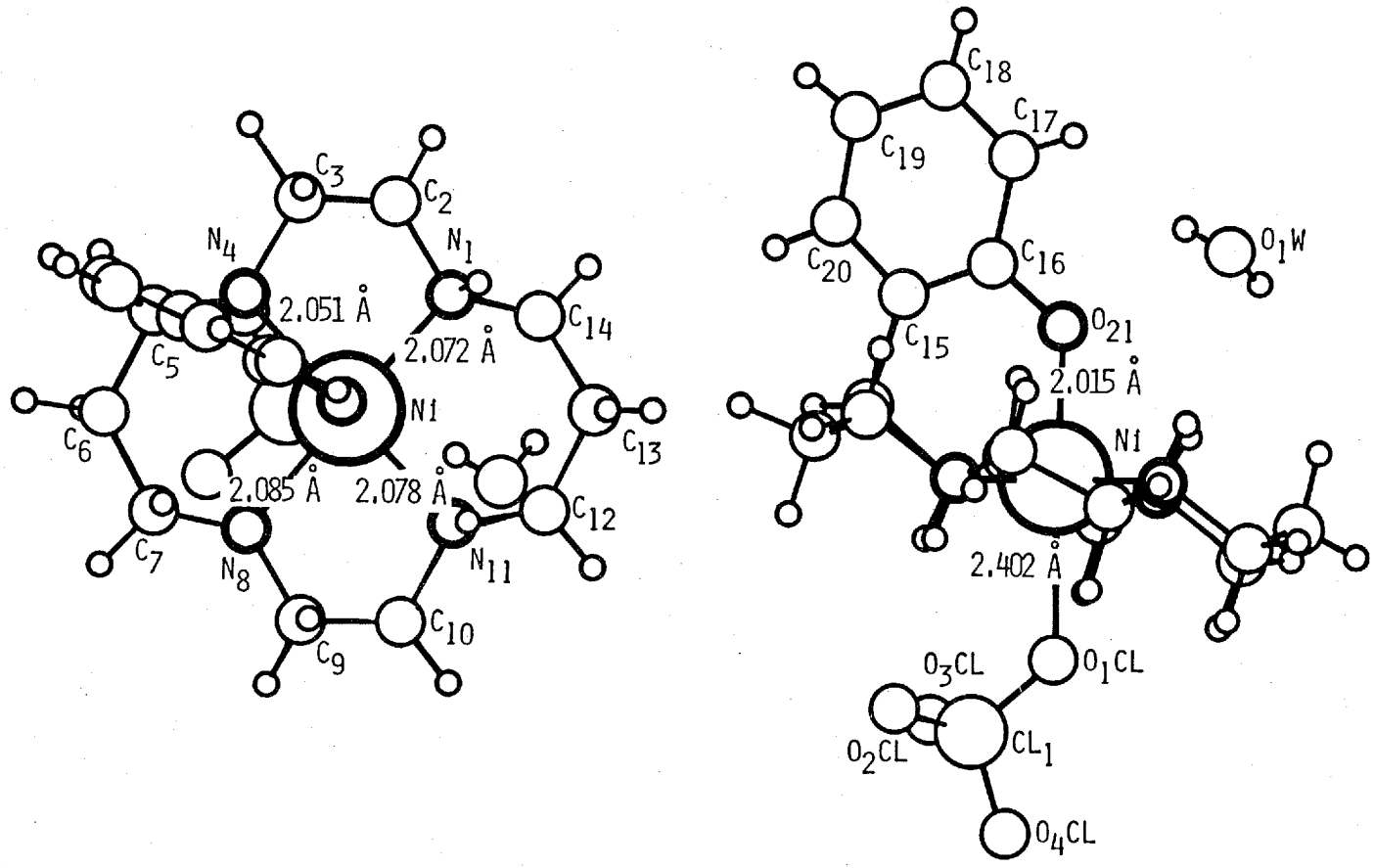

Figure 8. Two views of the structure of Ni^{II}-phenolate-pendent cyclam·ClO_4·H_2O: (left)up-side view;(right)side-on view.

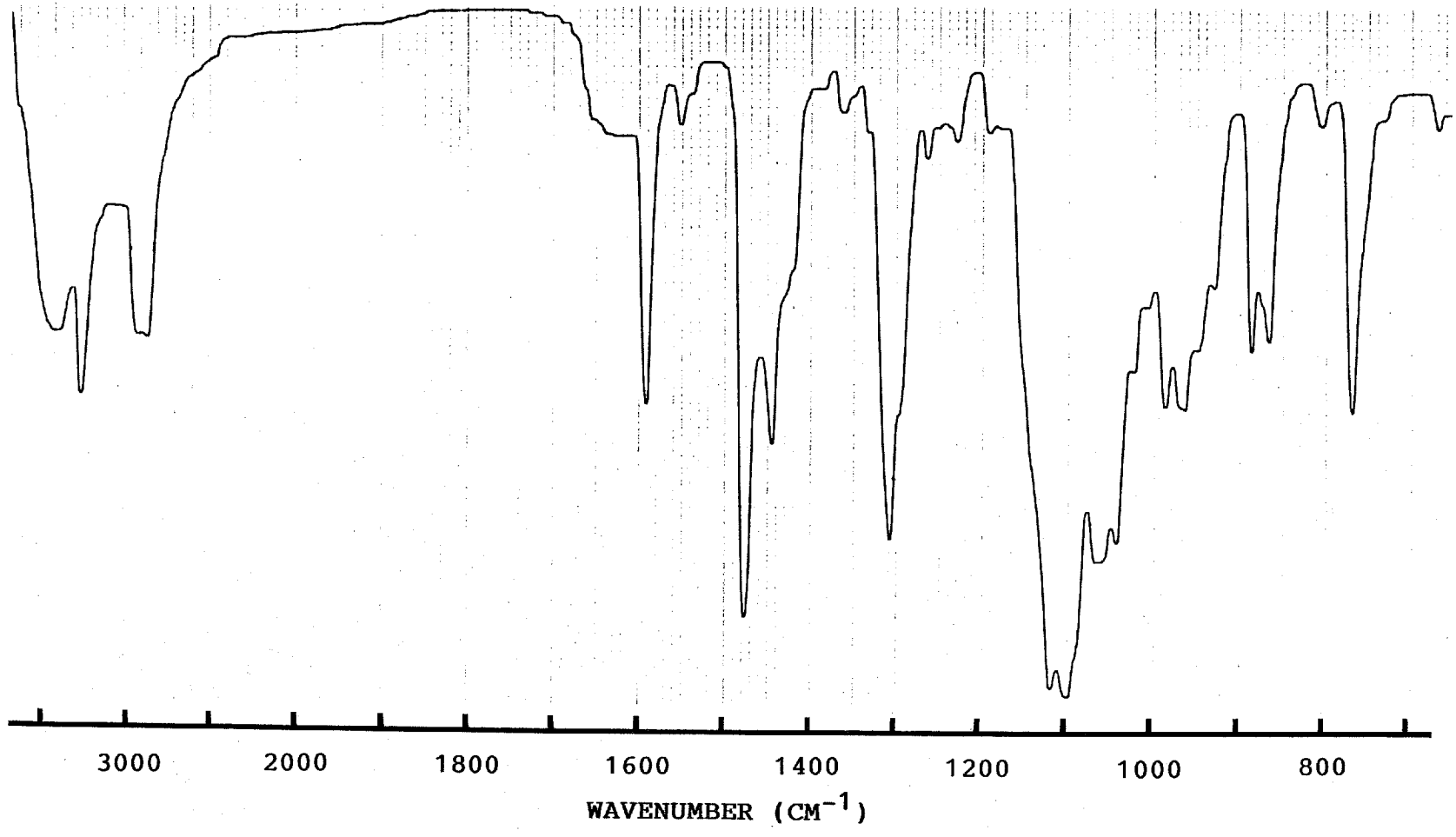

Figure 9. IR spectrum of Ni^{II}-phenolate-pendent cyclam·ClO_4·H_2O.

indicating a very weak coordinate bond between them. This can be viewed as being a result of the phenolate trans influence. The observed Ni-N bond distances of ~2.07 Å well indicate high-spin Ni^{II} in an octahedral complex of the 14-membered macrocyclic tetraamine to be in high spin state.[16] In the reported, octahedral high-spin Ni^{II}(cyclam)X_2 complexes (X=Cl[18] or NO_3[16]), the Ni-N bond lengths are a little shorter 2.05~2.06 Å, which were considered to be affected by the steric lengthening of the Ni-X bonds.

The cyclam moiety in 3 takes the normal trans-III conformation (i.e. the 1,3-diaminopropane rings have a chair conformation) as in the Ni^{II}-cyclam (without the pendant) complex.[16,18] The axial nickel-oxygen-aromatic carbon bond ($Ni-O_{21}-C_{16}$) angle of 127.2(5)° is another interesting structural feature. The Dreiding model of 3 indicates that the observed trans-III configuration with $Ni-O_{21}$ bond distance of 2.0 Å and the $Ni-O_{21}-C_{16}$ angle of 127°is the least strained structure. This fact in turn suggests that the short axial $Ni-O_{21}$ bond and the coplanar Ni position in the N_4 plane are determined mostly by the ligand steric requirement. The strong axial interaction by the phenolate should contribute to fix Ni^{II} in high-spin state.[2]

From the pH-metric titration of 2 in the presence of one equivalent of Ni^{II} at 25°C and I=0.1 M ($NaClO_4$), the 1:1 complexation constant $K(Ni^{II}-2H_{-1})$ (=$[Ni^{II}-2H_{-1}] / [Ni^{II}][2H_{-1}]$) was determined to be 7.0×10^{22} M^{-1}. This value is almost the same as that of the Ni^{II}-cyclam (1.6×10^{22} M^{-1})[19], where $Ni^{II}-2H_{-1}$ denotes the

phenolate form complex 3. In aqueous solution the Ni^{II} [in 3 (λmax 520 nm, ϵ 10)] remains high-spin at μ_{eff}=2.90 μ_B [by Evans method[20] at 35°C and I=0.1 M ($NaClO_4$)]. Without the axial phenolate, Ni^{II} (in complex of 1)is in high-spin and low-spin equilibrium with the μ_{eff} being lowered to 2.35 μ_B[21].

It is of interest to add that the protonation of the coordinated phenolate ion in 3 starts to occur below pH 6 to yield Ni^{II}-2, its pK_a being potentiometrically determined to be 6.30 at 25°C and I=0.1 M ($NaClO_4$). Upon protonation, the pendent phenol loses the coordinating ability with Ni^{II}. The complex Ni^{II}-2 exhibits the $Ni^{III/II}$ redox potential of +0.50 V vs SCE [0.5 M Na_2SO_4, pH=5.2, 25°C, first oxidation step of Figure 10 (c)], the same value as Ni^{II}-cyclam[22]. On the other hand, the Ni^{II}-phenolate-pendant cyclam 3 shows a significantly lowered redox potential +0.35 V vs SCE [0.5 M Na_2SO_4, pH=7.5, 25°C, first oxidation step of Fig. 10 (b), and Fig. 10 (a)] for $Ni^{III/II}$. Since addition of ten times excess of phenol to Ni^{II}-cyclam under the same conditions does not change the $Ni^{II/III}$ redox potential, this is concluded to be the effect of the directly (intramolecularly) bonded phenolate anion. The 3(M=Ni^{III}) was obtained by oxidizing 3(M=Ni^{II}) with ammonium peroxodisulfate or by electrochemical oxidation at +0.5 V vs. SCE at pH 8 and 25°C. The 3(M=Ni^{III}) complex has intense charge transfer absorptions [λ max 318 nm, ϵ 7 x 10^3 and 290 nm, ϵ 9 x 10^3)]. The ESR spectrum of the 3(Ni^{III}) in frozen solution at 77 K with $g_\perp$=2.18 and $g_{//}$=2.03 is similar to that found for Ni^{III}-cyclam.[22] Another

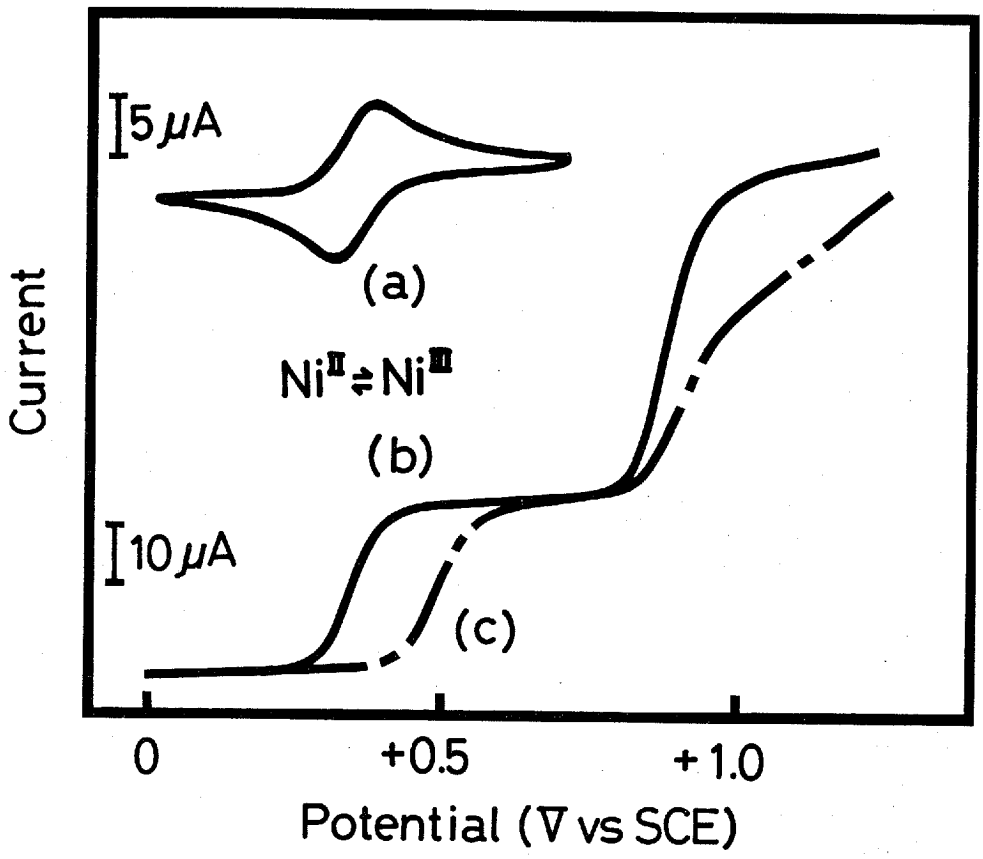

Figure 10.

Voltammograms of Ni^{II}-2 and -$2H_{-1}$ complexes
(a) Cyclic voltammogram of 1 mM Ni^{II}-$2H_{-1}$ at pH 7.5, 25°C and I=1.5 M (Na_2SO_4). Scan rate = 50 mVs^{-1}. $E_{1/2}(Ni^{II/III})$= +0.35 (V vs. SCE).
(b) Polarogram of 1 mM Ni^{II}-$2H_{-1}$ under the same condition of (a). Scan rate is 10 mVs^{-1} at 2000 rpm. $E_{1/2}(Ni^{II/III})$= +0.35 (V vs. SCE).
(c) Polarogram of 1 mM Ni^{II}-2 at pH 5.2, 25°C and I=1.5 M (Na_2SO_4). Scan rate is 10 mVs^{-1} at 2000 rpm. $E_{1/2}(Ni^{II/III})$= +0.50 (V vs. SCE).

interesting fact is that upon coordination with nickel in 3(M=Ni^{III}), the phenolate becomes robust toward oxidation, as shown by a shift of the phenolate oxidation potential from ~+0.5 V of uncoordinated 2 at 25°C and pH 10 to ~+0.9 V vs SCE of 3(M=Ni^{III}) [second oxidation step of Fig. 10 (b)].

Iron complexes. In the co-ordination of 2 with metal ions, an unusual characteristic of the axial phenolate donor is the ability to dissolve an equimolar amount of insoluble $Fe(OH)_3$ and form a red 1:1 complex (confirmed by atomic absorption spectroscopic measurement) at neutral pH in aqueous solution. No other saturated macrocyclic polyamine [e.g. cyclam] has succeeded in taking up solid Fe^{III} into aqueous solutions. From the pH-metric titration of 2 in the presence of one equivalent of $Fe^{II}SO_4$ under Ar [25°C, I=0.10 M ($NaClO_4$)], we have determined $K(Fe^{II}\text{-}2H_{-1})$ (=$[Fe^{II}\text{-}2H_{-1}]/[Fe^{II}][2H_{-1}]$) value of 7.9×10^{14} M^{-1}, where $Fe^{II}\text{-}2H_{-1}$ denotes the phenolate form complex 3(M=Fe^{II}) (Figure 11). The phenol-free tetra-amine 1 is known to yield a six-coordinate, low-spin Fe^{II} complex in nonaqueous solutions, which is subject to rapid oxidation in aqueous solution, resulting in Fe^{III} oxide precipitation. The yellow 3(M=Fe^{II}) complex in aqueous solution (λ_{max} 455 nm, ε 200 at pH7.4) is high-spin, $\mu_{eff} = 5.19 \mu_B$ at 35°C by Evans method,[20] and is oxidized immediately in air to give red (turning purple on acidifying) 3(M=Fe^{III}) complex solution [λ_{max} 480 (555) nm, 2200 (2300) at pH7.4 (4.3)]. An identical Fe^{III} complex was obtained by mixing Fe^{III} with 2 or by

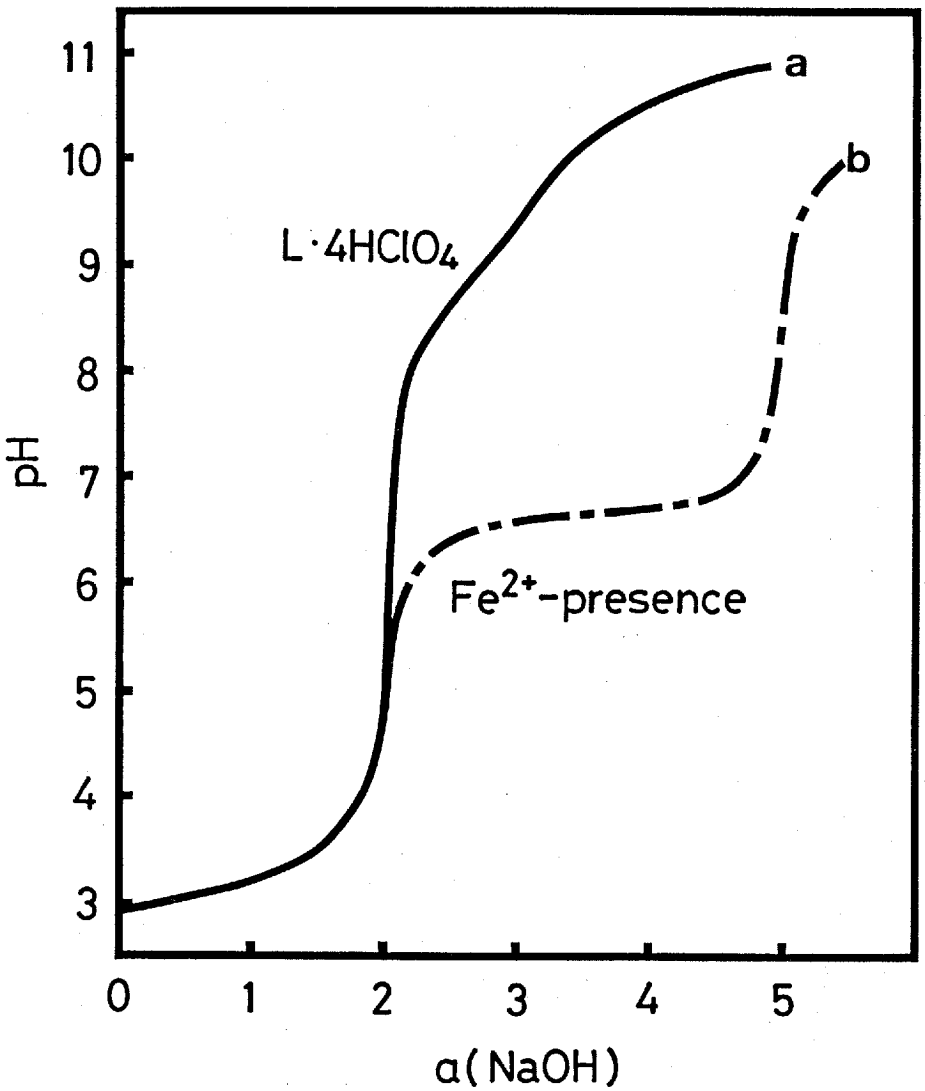

Figure 11.

Titration curves for 2 at 25°C and I=0.1 M ($NaClO_4$); a is moles of NaOH per mole of ligand.
Curve (a); 1mM 2·4$HClO_4$ alone.
Curve (b); 1mM 2·4$HClO_4$ plus 1mM $FeSO_4$.

electrochemical oxidation (at 0 V vs. SCE) of the $\underset{\sim}{3}(M=Fe^{II})$ complex in aqueous solution. The red $\underset{\sim}{3}(M=Fe^{III})$ complex can be reduced again to the $\underset{\sim}{3}(M=Fe^{II})$ with $Na_2S_2O_4$ or by electrochemical reduction at -0.5 V vs. SCE. The $\underset{\sim}{3}(M=Fe^{II})$ complex shows a quasi-reversible (one electron) cyclic voltammogram which enabled us to determine the redox potentials for $Fe^{III/II}$ as -0.16 V vs. S.C.E. (non-buffered at 7 < pH < 9) (Figure 12). The separations of anodic and cathodic peaks are less than 90 mV and the peak height ratios are unity. On the basis of the reversible $Fe^{III/II}$ redox behaviour, we calculated the conditional constant $\underset{\sim}{K}'(Fe^{III}\text{-}\underset{\sim}{2}H_{-1})$ $(=[Fe^{III}\text{-}\underset{\sim}{2}H_{-1}]/[Fe^{III}][\underset{\sim}{2}H_{-1}])$ at pH 7.0 to be 4.0×10^{26} M^{-1}. Evidently, the phenolate interaction should contribute to stabilization of the Fe^{III} state with respect to the Fe^{II} state. This is understood by comparing its redox potential with those for phenolate-free polyamine-Fe complexes: e.g. the 16-membered saturated penta-amine macrocyclic complex (-0.04 V)[23] or hemoglobin (-0.07 V at pH 7).[24] However, the value of -0.16 V is higher than those for Fe^{III}-carriers such as a mugineic acid (-0.34 V),[25] microbial hydroxamates (-0.59 to -0.69 V),[25] and enterobactin (-0.99 V).[26]

In conclusion, the present phenolate appended ligands may provide new types of macrocyclic $Ni^{II,III}$ and $Fe^{II,III}$-sequestering agents and simplified models for the study of phenolate co-ordinating effects in tyrosine co-ordinating Fe^{III} nonheme oxygenases (having similar visible absorptions)[27] or axial phenolate co-

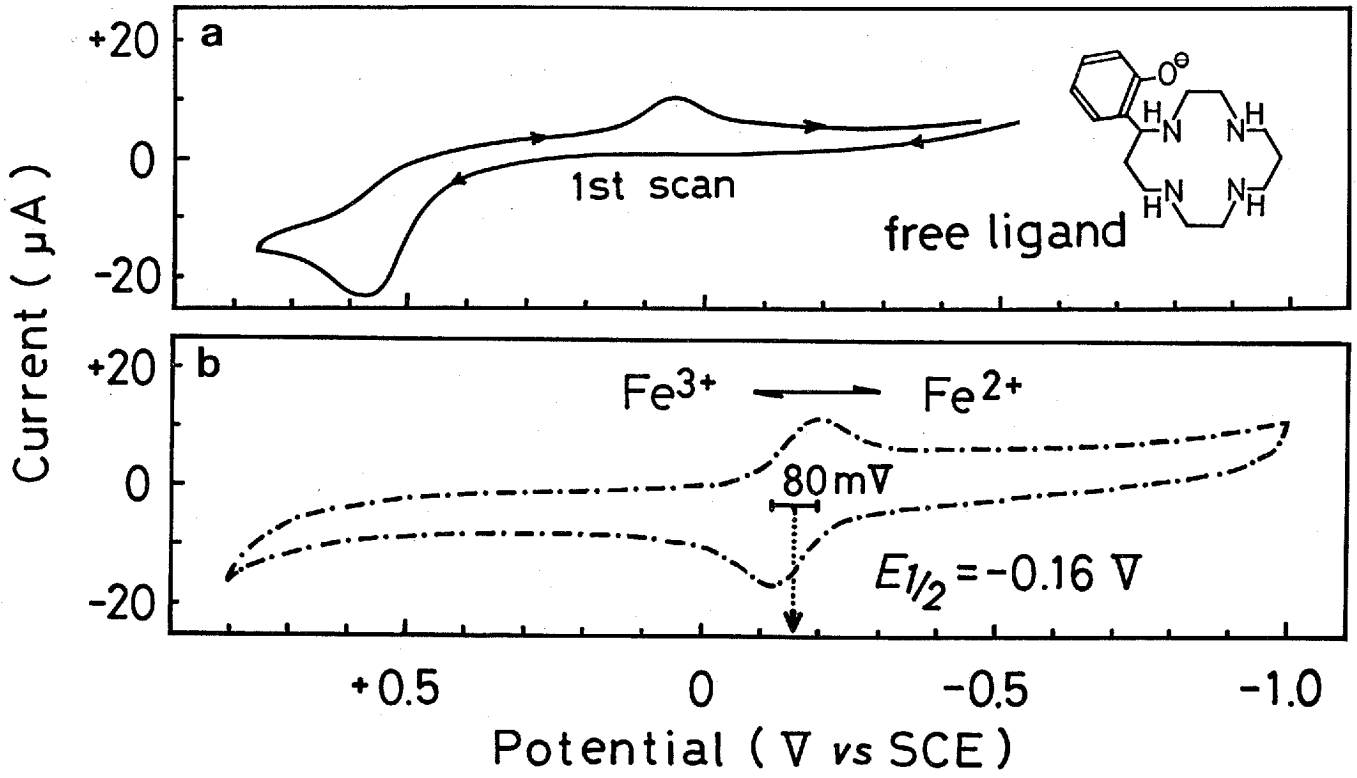

Figure 12. Cyclic voltammograms of $\underset{\sim}{2}H_{-1}$ and Fe^{II}-$\underset{\sim}{2}H_{-1}$.
(a) Cyclic voltammogram of 1 mM $\underset{\sim}{2}H_{-1}$ at pH10.5, 25°C and $\underline{I}$=0.1 M ($NaClO_4$).
(b) Cyclic voltammogram of 1 mM Fe^{II}-$\underset{\sim}{2}H_{-1}$ at pH 7.0, 25°C and $\underline{I}$=0.1 M ($NaClO_4$).
$\underline{E}_{1/2}(Fe^{II/III})$=-0.16 V $\underline{vs}$. SCE.

ordinating hemes (having low $Fe^{III,II}$ redox potentials).[6] Furthermore, the simplicity and versatility of the annelation method will be useful in the synthesis of a variety of spermine and spermidine alkaloid analogues.

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Conclusions

Chapter I. Complexes of Histamine H_2-Antagonist Cimetidine with Divalent and Monovalent Copper Ions

The histamine H_2-antagonist cimetidine (L) forms 1:1 and 2:1 complexes with Cu(II) ion in aqueous solutions. The complexation constants $\underline{K}_{Cu^{II}L} = (3.02 \pm 0.05) \times 10^4\ M^{-1}$ and $\underline{K}_{Cu^{II}L_2} = (2.35 \pm 0.05) \times 10^4\ M^{-1}$ are determined by the pH-metric titration method. By comparison with greater $\underline{K}_{Cu^{II}L}$ and $\underline{K}_{Cu^{II}L_2}$ values of other biological ligands such as histamine and peptides, it is concluded that cimetidine is unlikely to bind with Cu(II) *in vivo*. The polarographic mesurements have estimated the Cu(I,II) redox potential $\underline{E}^{\circ}$ of +0.42 V *vs.* NHE for the 1:1 cimetidine complex, which implies likelihood of physiological reduction with ascorbic acid or hemoglobin. Indeed, a stable 1:1 Cu(I)-cimetidine complex has been isolated (as very insoluble solid) on treatment of Cu(II)-cimetidine complexes with ascorbic acid. The stability of Cu(I)-cimetidine complex is enormous in that it can survive in the presence of biological ligands. These results may indicate an important role of copper ion in the pharmacological activities of cimetidine as cuprous-cimetidine complexes in our body.

Since its $\underline{E}^{\circ}$ value of +0.42 V *vs.* NHE is comparable to those of blue copper (type I) proteins and Cu-superoxide dismutase, cimetidine is promising as a new biomimetic ligand for interconversion of Cu(I) and Cu(II). The Cu-cimetidine complexes exhibit higher

superoxide dismutase-like activity than any previous complex, suggesting great biochemical and drug potentials of cimetidine as copper complexes.

Chapter II. Novel Synthesis and Complex Properties of Macrocyclic Tetra-amine Appended with Phenol as an Axial Donor

A novel, one-pot annelation method that "recycles" coumarin with linear tetra-amine, 1,9-diamino-3,7-diazanonan is discovered for the synthesis of a phenol-pendent 14-membered macrocyclic tetra-amine (cyclam). The simplicity and versatility of the annelation method will be useful in the synthesis of a variety of macrocyclic polyamine alkaloid analogues. The pendent phenolate strongly co-ordinetes, as an intramolecular axial donor, with metal ions incorporated in the tetra-amine macrocycle below. This is proved by an X-ray crystal analysis of the Ni(II) complex. One remarkable effect of the axial phenolate co-ordination is that this ligand can sequester Fe(III) in aqueous solution. Another effect is the stabilization of unusual oxidation state of Ni(III) in the N_4 macrocycle. The Fe^{III} complex is appropriate for the study of phenolate co-ordinating effects in tyrosine co-ordineting Fe^{III} nonheme oxygenase or axial phenolate co-ordinating hemes.

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