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Circular Dichroism Studies on the Formation of Complexes of (–)-7-Chlorotetracycline with Vanadium, Chromium, and Cobalt

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Summary. The complex formation of the chiral antibiotic (–)-7-chlorotetracycline ((–)-*CTC*) with VO(IV), Cr(III), and Co(II) in methanol/water (80:20 v/v) was studied using circular dichroism. The ligand forms soluble complexes of the composition $[VO((-)\text{-}CTC)_3]^{2+}$, $[Cr((-)\text{-}CTC)(H_2O)_4]^{3+}$, and $[Co((-)\text{-}CTC)_2(H_2O)_2]^{2+}$ as predominat species in acidic solution ($pH = 3.2{-}4.9$). The CD spectra of the complex ions are interpreted and their formation constants are reported.

Keywords. Circular dichroism; (–)-7-Chlorotetracycline; Vanadium; Chromium; Cobalt; Complex; Stability constant.

Untersuchung der Komplexbildung von (–)-7-Chlortetracyclin mit Vanadium, Chrom und Cobalt mittels chiroptischer Methoden

Zusammenfassung. Die Komplexbildung des chiralen Antibiotikums (–)-7-Chlortetracyclin ((–)-*CTC*) mit VO(IV), Cr(III) und Co(II) in Methanol/Wasser (80:20 v/v) wurde mittels chiroptischer Methoden untersucht. In sauren Lösungen ($pH = 3.2{-}4.9$) bildet der Ligand vorwiegend Komplexe der Zusammensetzung $[VO((-)\text{-}CTC)_3)]^{2+}$, $[Cr((-)\text{-}CTC)\ (H_2O)_4]^{3+}$ und $[Co((-)\text{-}CTC)_2(H_2O)_2]^{2+}$. Die CD-Spektren der Komplexionen werden interpretiert und ihre Bildungskonstanten angegeben.

Introduction

Chiral antibiotics of the family of tetracyclines ((–)-tetracycline, (–)-7-chlorotetracycline, (–)-oxytetracycline, (–)-doxycycline, (–)-4-(dedimethylamine)tetracycline *etc.*) are versatile ligands and form complexes of different composition with metal ions [1–2]. However, isolated complexes of (–)-tetracycline are insoluble in water and many organic solvents. In solution, complexation of these antibiotics with different metal ions has been investigated by potentiometric and spectrophotometric methods [3–4]. The investigation of the complex forming ability of these drugs with transition metal ions in aqueous or non-aqueous solution using circular dichroism has been reported for a few systems only. In recent years, *Lamb et al.* [5] have utilized CD studies to investigate complexation of Ca(II), Mg(II), and Cu(II) ions with a number of (–)-tetracyclines in aqueous solution and

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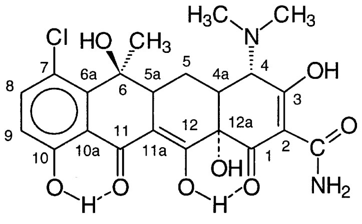
assigned metal-ligand bonding through different oxygen and nitrogen donor atoms. We have already reported on the formation of metal complexes of (–)-tetracycline ((–)-*TC*) in acidic aqueous solution [6]. In continuation of our research, we here report the formation of complexes of oxovanadium(IV), chromium(III), and cobalt(II) with (–)-7-chlorotetracycline ((–)-*CTC*, **1**) in methanol/water (80:20 v/v) at *pH* 3.2–4.9. In solution, $[VO((-)\text{-}CTC)_3]^{2+}$, $[Cr((-)\text{-}CTC)(H_2O)_4]^{3+}$, and $[Co((-)\text{-}CTC)_2(H_2O)_2]^{2+}$ have been identified as the predominant species. Their stability constants were determined and their CD spectra interpreted.



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Results and Discussion

The formation of complexes of VO(IV), Cr(III), and Co(II) with (–)-*CTC* in methanol/water is indicated by the development of well defined, characteristic CD bands upon mixing metal ion and ligand solutions. The CD spectra of these systems (Fig. 1) consist of 1–2 oppositely signed bands. The VO-(–)-*CTC* system exhibits a negative band at 17094 cm^{-1} with a positive component at 20000 cm^{-1}, Cr (III)-(–)-*CTC* gives rise to a positive band at 18182 cm^{-1}, and Co(II)-(–)-*CTC* shows a positive band at 18692 cm^{-1} and a negative band at 21505 cm^{-1}.

The complexation in these systems depends on the *pH* value. The ligand in its hydrochloride form is soluble in slightly acidic medium, and solutions containing both components remain acidic during interaction. The CD bands in these systems remain unaltered upon *pH* changes. However, the band ellipticity is significantly affected as the *pH* of the system is raised. In VO(IV)-(–)-*CTC*, ΔOD at 17094 cm^{-1} increases up to $pH = 3.2$ and then rapidly decreases. The solution first becomes cloudy, and precipitation occurs when the *pH* reaches 3.5. Therefore, subsequent studies in this system were carried out at *pH* 3.2. In the Cr(III)-(–)-*CTC* system, ΔOD of the positive band at 18182 cm^{-1} gradually increased as the *pH* was raised to 4.8; it remained at a maximum between 4.8 and 5.0 and rapidly decreased beyond *pH* 5.0. The complex precipitated at $pH = 6.2$. Further studies on this system were carried out at *pH* 4.9. The band ellipticity at 18692 cm^{-1} in the Co(II)-(–)-*CTC* system gradually increased from *pH* 2.5 to 4.5, and precipitation started at $pH = 4.75$. This system was maintained at *pH* 4.4 during subsequent studies.

The complex formation in these systems also depends upon time. ΔOD of the *Cotton* band in the Cr(III)-(–)-*CTC* system gradually increased with time and achieved its maximum value after six hours. Afterwards, only negligible changes in the ellipticity of the band were observed, indicating that equilibrium conditions had been reached. In the case of VO(IV)-(–)-*CTC*, a maximum ellipticity was observed after two hours, whereas with Co(II)-(–)-*CTC* equilibrium was achieved in less

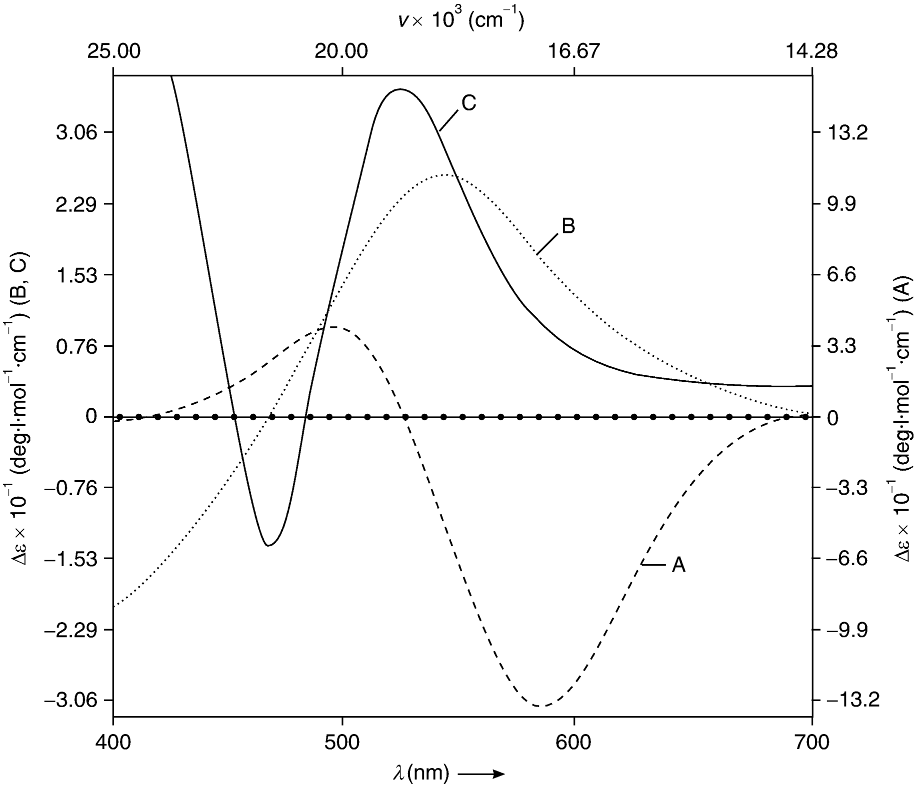

Fig. 1. Circular dichroism spectra of $[VO((–)\text{-}CTC)_3]^{2+}$ (A), $[Cr((–)\text{-}CTC)(H_2O)_4]^{3+}$ (B), and $[Co((–)\text{-}CTC)_2(H_2O)_2]^{2+}$ (C) formed in $MeOH/H_2O$

than half an hour. Therefore, VO(IV)-(–)-*CTC* and Cr(III)-(–)-*CTC* systems maintained at the appropriate *pH* were kept for 2 and 6 hours, respectively, before recording their CD spectra. The inert aquo complex of chromium(III) [7] reacts slowly with (–)-*CTC* and takes more time (6 hours) for completion compared to those of oxovanadium(IV) and cobalt(II) which are labile and fast reacting species.

The composition of the complex ions formed in $MeOH/H_2O$ solution was determined by varying the mole fractions of the components at optimum conditions of *pH* and time. It may be inferred from Fig. 3 (Experimental) that different number of ligand molecules coordinate with the metal ions, *i.e.* three molecules of (–)-*CTC* react with VO(IV) whereas Cr(III) and Co(II) coordinate with one and two ligands, respectively. It is also observed that the differential molar absorptivity ($\Delta\varepsilon$) at the respective CD band maxima follow the order $[VO((–)\text{-}CTC)_3]^{2+}>$ $[Co((–)\text{-}CTC)_2(H_2O)_2]^{2+}>[Cr((–)\text{-}CTC)(H_2O)_4]^{3+}$. This trend indicates that $\Delta\varepsilon$ depends on the number of chiral molecules coordinated with the metal ion.

The two nitrogen atoms of (–)-*CTC*, *i.e.* the tertiary amine attached to C-4 and the amide nitrogen at C-2 are poor donors due to crowding of methyl groups and the resonance effect of the carbonyl group, respectively. A possible mode of chelation through oxygen atoms at C-10 and C-11 may be ruled out because of the presence of the chiral centre C-12a outside the chelate which should result in extremely weak CD bands. Inspection of molecular models reveals that bidentate chelation through oxygen atoms attached to C-12 and C-1 or C-12 and C-12a incorporating asymmetric C-12a may produce stable complexes which should exhibit fairly well defined strong CD bands. It is therefore proposed that carbonyl and alcoholic oxygen atoms attached to C-1 and C-12 participate in chelation with metal ions. As complexation in these systems occurs in acidic medium, excess protons may be accepted by nitrogen atoms of the ligand keeping the complexes in solution. In case of $[VO((–)\text{-}CTC)_3]^{2+}$, one of the ligand coordinates as an ambidentate leading to an octahedral species, whereas in $[Cr((–)\text{-}CTC)(H_2O)_4]^{3+}$ and $[Co((–)\text{-}CTC)_2(H_2O)_2]^{2+}$ a different number of water molecules should be coordinated to maintain the same geometry. The actual symmetry of these species formed in MeOH/H_2O solution is very low (*i.e.* C_1) due to the presence of chiral centres in the ligand.

The molar differential absorptivity ($\Delta\varepsilon$) at the *Cotton* band maxima was determined by gradually increasing the concentration of the ligand while maintaining the metal ion concentration constant. The $\Delta\varepsilon$ values for each system were used to estimate the concentration of the components at equilibrium in a number of sample solutions of different concentration at optimum conditions and to calculate the formation constant of each system. The formation constants decrease in the order $[Co((–)\text{-}CTC)_2(H_2O)_2]^{2+}>[VO((–)\text{-}CTC)_3]^{2+}>[Cr((–)\text{-}CTC)(H_2O)_4]^{3+}$. The systems obey *Beer*'s law between concentrations of 2 and $20\times10^{-3}\,\mathrm{mol\cdot l^{-1}}$.

The absorption spectrum of $[VO((–)\text{-}CTC)_3]^{2+}$ consists of a single broad band at $19048\,\mathrm{cm^{-1}}$. The CD spectrum of this complex ion (Fig. 1) shows a couplet of oppositely signed bands, a negative band at $17094\,\mathrm{cm^{-1}}$ ($\Delta\varepsilon=1.32\,\mathrm{deg\cdot mol^{-1}\cdot l\cdot cm^{-1}}$), and a weaker positive band at $20000\,\mathrm{cm^{-1}}$ ($\Delta\varepsilon=0.42\,\mathrm{deg\cdot mol^{-1}\cdot l\cdot cm^{-1}}$) which may be assigned to the excitation of electrons to the $^2B_1$ and $^2A_1$ upper levels, respectively. These band assignments closely agree with those already reported for CD and absorption bands of vanadyl complexes [8].

The CD spectrum of $[Cr((–)\text{-}CTC)(H_2O)_4]^{3+}$ consists of a positive band at $18182\,\mathrm{cm^{-1}}$ ($\Delta\varepsilon=0.183\,\mathrm{deg\cdot mol^{-1}\cdot l\cdot cm^{-1}}$), its absorption spectrum of a broad band at $19120\,\mathrm{cm^{-1}}$. Both bands seem to originate from the same transition and may be assigned to $^4A_2\rightarrow{}^4A_1$ arising from a splitting of the $^4T_{2g}$ level in octahedral symmetry. This band assignment is very close to those already reported by others [9].

The CD spectrum of $[Co((–)\text{-}CTC)_2(H_2O)_2]^{2+}$ consists of a couplet of bands, a positive band at $18692\,\mathrm{cm^{-1}}$ ($\Delta\varepsilon=0.34\,\mathrm{deg\cdot mol^{-1}\cdot l\cdot cm^{-1}}$) and a negative band at $21505\,\mathrm{cm^{-1}}$ ($\Delta\varepsilon=0.19\,\mathrm{deg\cdot mol^{-1}\cdot l\cdot cm^{-1}}$). The absorption spectrum of this complex ion in MeOH/H_2O (80:20 v/v) at optimum conditions exhibits a broad band centered at $19417\,\mathrm{cm^{-1}}$ ($\varepsilon=500\,\mathrm{mol^{-1}\cdot l\cdot cm^{-1}}$) arising from a $^4T_{1g}\rightarrow{}^4T_{1g}(P)$ transition in an octahedral field. The couplet of CD bands at $18692\,\mathrm{cm^{-1}}$ and $21505\,\mathrm{cm^{-1}}$ seems to arise from excitation of electrons to $^4B_1$ and $^4B_2$ upper levels components of the $^4T_{1g}(P)$ state due to the lower symmetry of the complex ion. This band assignment is comparable with already reported absorption and CD spectra of a number of Co(II) complexes [10].

It is worth mentioning that the CD spectra of $[VO((+)\text{-}CCS)_3]^{2+}$, $[VO((+)\text{-}BP)_2\,(H_2O)]^{2+}$, and $[VO((–)\text{-}CTC)_3]^{2+}$ all have couplets of oppositely signed bands, the negative bands of which lie at lower frequency [11]. This indicates that asymmetric carbon atoms of the same absolute configuration participate in chelate formation in all these systems. Further, $[Co((+)\text{-}BP)_3]^{2+}$ and $[Co((-)\text{-}CTC)_2(H_2O)_2]^{2+}$ have couplets of CD bands with positive component at lower frequency, whereas $[Co((-)\text{-}TC)_3]^{2+}$ shows only a positive band in the same region, indicating that chiral atoms of the same absolute configuration should be involved.

It may be concluded from these studies that (–)-7-chlorotetracycline is capable of forming complexes of different composition with VO(IV), Cr(III), and Co(II) ions in methanol/water (80:20 v/v) between *pH* 3.2 and 4.9. Since similar conditions of *pH* prevail in the gastrointestinal track, presence of these metal ions in biological systems might effect the antibacterial activity of this drug.

Experimental

All reagents and solvents of analytical reagent grade were purchased from commercial sources and used without further purification. $VOSO_4 \cdot 3H_2O$, $CrCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, and (–)-7-chlorotetracycline hydrochloride (80% pure) were purchased from Aldrich, USA. The purity of (–)-*CTC* · HCl was ascertained from infrared and specific rotation measurements. Methanol was distilled before use; the mixed solvent was prepared from double distilled water.

Solutions

Stock solution of (–)-7-chlorotetracycline hydrochloride

1.61 g (2.5 mmol) of the ligand was shaken with 70 ml of methanol/water (80:20 v/v) until all solid particles were dissolved. The solution was diluted to 100 ml, thoroughly mixed, and stored in the dark.

Metal salt solutions

Metal salt solutions were obtained by dissolving appropriate amounts of $VOSO_4 \cdot 3H_2O$, $CrCl_3 \cdot 6H_2O$, or $CoCl_2 \cdot 6H_2O$ in the mixed solvent and diluting to 100 ml to give 0.01–0.025 *M* solutions.

Background electrolyte solution

A 0.5 *M* solution of $NaClO_4$ was obtained by dissolving 6.122 g of $NaClO_4$ in 100 ml of mixed solvent.

Solutions of required concentrations (0.01–0.02 *M*) were obtained by mixing appropriate volumes of stock solutions of (–)-*CTC*, metal ions, and background electrolyte in a 1:3:1 ratio and diluting with mixed solvent.

Solutions for complexation studies

The effect of *pH* upon complexation was studied in a series of solutions obtained by mixing 1.00 ml of metal ion solution with 3.00 ml of ligand stock solution and adjusting the *pH* value with dilute (0.05 M) solutions of NaOH or HCl. The samples were diluted to 10 ml and kept in the dark for 1–6 h before recording their CD spectra. The differential optical density (ΔOD) at the CD band maxima was plotted as a function of *pH* (Fig. 2). From these plots, the optimum *pH* value for each system was selected and maintained during subsequent studies.

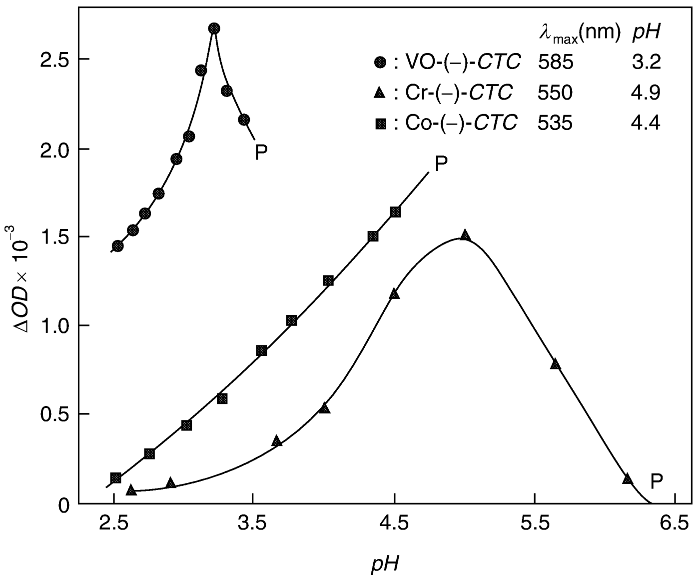

Fig. 2. Effect of *pH* upon the differential optical density at the *Cotton* band maxima of metal-(–)-*CTC* systems (P: precipitation)

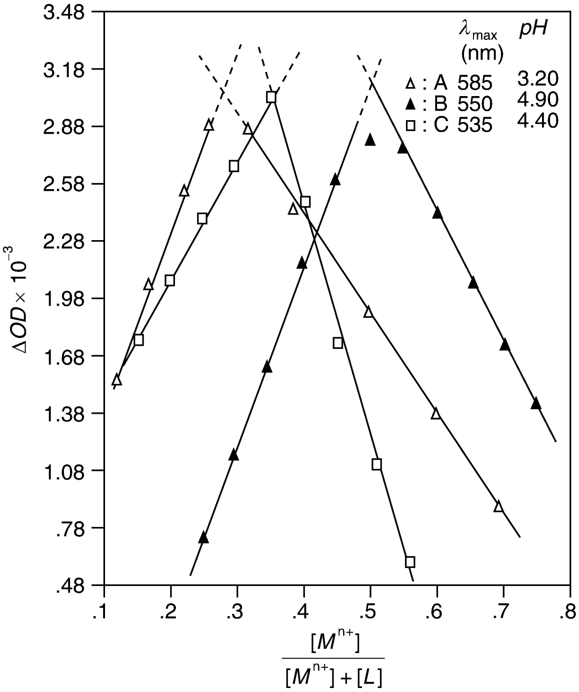

Fig. 3. Mole ratio plots for the determination of the stoichiometry of metal complexes of (–)-*CTC* in methanol/water; A: VO(IV)-(–)-*CTC*, B: Cr(III)-(–)-*CTC*, C: Co(II)-(–)-*CTC*; M = metal, L = ligand

Table 1. Optimum conditions of *pH* and time, stability constants, and absorption and CD spectra of complexes of (–)-7-chlorotetracycline in methanol/water (80:20 v/v)

Complex ion (concentration ($\mathrm{mol \cdot l^{-1}}$))	*pH*	Time to attain maximum CD (h)	Stability constant (logβ)	Absorption spectra		Cd Spectra		
				ν ($\mathrm{cm^{-1}}$)	ε	ν ($\mathrm{cm^{-1}}$)	$\Delta\varepsilon$	Band assignment
$[\mathrm{VO}((–)\text{-}CTC)_3]^{2+}$	3.2	2.0	5.90±0.44	19048	308	17094,	−1.32	$^2B_2 \rightarrow {}^2B_1$
(0.0048)						20000	+0.42	$^2B_2 \rightarrow {}^2A_1$
$[\mathrm{Cr}((–)\text{-}CTC)(H_2O)_4]^{3+}$	4.9	6.0	2.25±0.27	19120	950	18182	+0.18	$^4A_2 \rightarrow {}^4A_1$ ($^4T_{2g}$)
(0.018)								
$[\mathrm{Co}((–)\text{-}CTC)_2(H_2O)_2]^{2+}$	4.4	0.5	6.22±0.40	19417	500	18692,	+0.34	$^4A_2 \rightarrow {}^4B_1$ ($^4T_{1g}$ (P))
(0.014)						21505	−0.19	$^4A_2 \rightarrow {}^4B_2$($^4T_{1g}$ (P))

The stoichiometry of the complexes formed was determined by varying the mole fraction of metal and ligand in a series of samples prepared by mixing different volumes (1–9 ml) of equimolar stock solutions of metal ions and (–)-*CTC*, adjusting their *pH* value, and diluting to 25 ml. The sample solutions were kept in the dark for 1–6 h; then CD spectra were recorded. A plot of ΔOD at the CD band maximum *vs.* mole fraction of the metal ion was prepared for each system. The stoichiometry of the complex ions was determined from the points of intersection of two straight lines as shown in Fig. 3.

A series of solutions was prepared by mixing 1 ml of metal ion stock solution with different volumes (0.66–6.66 ml) of ligand solution. The ionic concentration was maintained at 0.025 *M* with $NaClO_4$. The samples were brought to the appropriate *pH* value and kept in the dark for 0.5–6 h before recording their CD spectra. The CD band ellipticity was plotted as a function of ligand concentration. From these plots, $\Delta\varepsilon$ at λ_{max} were determined for each system. The $\Delta\varepsilon$ values were subsequently used to estimate equilibrium concentrations of complex ions, free metal ion, and uncomplexed ligand in a number of sample solutions of varying concentrations ($2–20 \times 10^{-3}$ *M*). The stability constants (logβ) are reported and statistically analyzed. The optimum *pH* values, time, stoichiometry, stability constants, absorption, and CD spectra of these complexes are reported in Table 1.

Instrumentation

Circular dichroism spectra were recorded on a Jasco-20A spectropolarimeter using a 10 mm cylindrical quartz cell. The baseline of the spectra was obtained from a pure (–)-*CTC* · HCl solution maintained at the appropriate *pH* value in the mixed solvent. Absorption spectra were measured on a Shimadzu model UV-265 spectrophotometer using a pair of 10 mm quartz cuvettes. *pH* measurements were made on a Horiba model F-8 digital *pH* meter equipped with a combination electrode system.

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