Formation of Soluble Complexes of Oxovanadium(IV), Cobalt(II) and Nickel(II) with Antibiotic (-)-Tetracycline in Water

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Summary: The complex forming ability of (-)-tetracycline with VO(IV), Co(II) and Ni(II) has been examined through circular dichroism. At pH 3.65-4.0 in aqueous solution, the chiral antibiotic forms soluble complexes of varying composition: $[VO((-)-TC)_2H_2O]^{2+}$, $[Co((-)-TC)_3]^{2+}$ and $[Ni_2((-)-TC)(H_2O)_x]^{4+}$. The CD spectra of these complexes in aqueous solution enable interpretation of the electronic excitations to upper levels of the metal ions.

Introduction

Tetracyclines constitute an important group of antibiotics effective against various types of bacterial infections. These chiral molecules consist of a number of six membered fused ring systems along with substituents containing donor atoms including alternating keto-alcoholic oxygens. The keto- alcohol groups involve extensive hydrogen bonding and can participate in chelation with metal ions similar to those formed by tri- and tetraketones [1-2]. Tetracyclines form complexes of varying composition with VO(IV), Fe(II), Mn(II), Co(II), Ni(II), Cu(II), Pd(II), UO_2(VI) etc. [3]. However, isolated complexes of tetracyclines are insoluble in water and a number of organic solvents. This presents difficulties in assessing their antibacterial activity. Lambs *et al.*, [4] have studied circular dichroism (CD) and absorption spectra of Ca(II), Mg(II) and Cu(II) complexes of tetracycline, chlortetracycline and doxycycline ligands formed in aqueous solution.

These workers found that complexes of varying metal:ligand ratio, 2:1, 1:1 and 1:2 were formed under different pH conditions because of complex structure of the ligands. These workers identified metal ligand binding sites and proposed that Ca(II) and Mg(II) complexes should play a vital role in gastrointestinal absorption of these drugs. In solution, complexation of metal ions with chiral drugs may be determined by the development of CD bands in crystal field region of metals due to vicinal effect [5]. Optimum conditions of pH, time, and stoichiometry can be determined for complexation by following the CD response. In aqueous solution, studies on the complex forming ability of (-)-tetracyclines with transition metal ions are scarce and need to be explored further. We wish to report the formation of complexes of (-)-tetracycline (I) with VO(IV), Co(II) and Ni(II) ions' in aqueous solution. The optimum conditions of pH and

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stoichiometry for maximum complexation have been determined and the CD spectra of these complexes are interpreted.

I (-)-TC
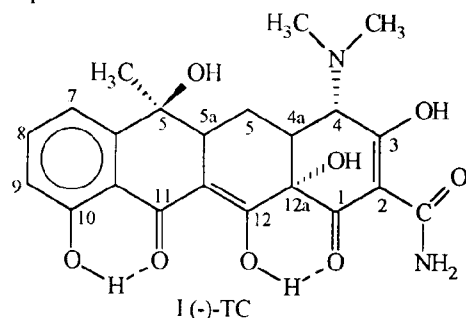
Results and Discussion

The interaction of (-)-tetracycline, (-)-TC, with VO(IV), Co(II) and Ni(II) ions in aqueous solution is indicated with the development of well defined CD bands in the visible region of the spectrum. A couplet of positive and negative bands occurs at 17,094 cm^{-1} and 20,409 cm^{-1} [VO((-)-TC)$_2$H$_2$O]$^{2-}$. A positive band is observed at 18,692 cm^{-1} for [Co((-)-TC)$_3$]$^{2+}$ and a weak negaive band occurs at 15,873 cm^{-1} for Ni(II) complex. The ligand, (-)-TC, was dissolved in dilute hydrochloric acid and pH of the mixed solution of (-)-TC and metals, remained acidic and complexation occurred in low pH conditions.

As pH of the mixed solutions was raised, CD band ellipticity gradually increased and precipitation of complexes occurred between pH 3.8-4.5 in these systems. Therefore, subsequent studies on [VO((-)-TC)$_2$H$_2$O]$^{2+}$ were carried out at pH 3.65 while [Co((-)-TC)$_3$]$^{2+}$ and [Ni$_2$(-)-(TC)(H$_2$O)$_x$]$^{n+}$ were maintained at pH 4.0 for detailed studies. The complexation in these systems is instantaneous and no change in spectra was observed even after keeping the solutions for 1-2 days.

The stoichiometry of the complex ions formed in aqueous solution was determined by varying mole fractions of the ligand and metal ions. It was found that different number of the ligands coordinate with each of the metal ions. Oxo-vanadium (IV) reacted with two molecules of (-)-TC, Co(II) reacted with three molecules while two Ni(II) ions coordinated with one molecule of the ligand

The ligand has a number of potential donor oxygen atoms attached to C-1, C-3, C-6, C-10, C-11, C-12 and C-12a. It has carbonyl oxygens at C-1, C-11 and alternate alcoholic oxygens attached at C-10 and C-12 involving extensive hydrogen bonding similar to that observed in keto-enol tautomers of polyketones. The two nitrogen atoms, a tertiary amine attached to C-4 and an amide at C-2 position, are very poor donors due to crowding methyl groups on the tertiary amine and inductive effect on the amide. The ligand has a number of chiral centres, C-4, C-4a, C-6 and C-12a, which contribute to its optical activity. The CD bands of these complexes formed in aqueous solution are fairly strong and well defined, indicating that asymmetric carbon atoms are part of the chelate formed. The asymmetric centres C-4a and C-6 of the ligand are not likely to be associated with complexation because bonding in this region cannot lead to chelation. However bonding through oxygen donors attached to C-12 and C-1 or C-12 and C-12a incorporate the C-12a asymmetric center of the chelate. Another possible mode of bonding with the metal through nitrogen of the tertiary amine and the alcoholic oxygen attached to C-3 involving asymmetric C-4 center is less probable due to crowding methyl groups. Further metal ligand binding through its amide and alcoholic oxygen donors may be ruled out on the basis that chiral atoms of the ligand remain out side the chelate and very weak bands should be observed. In all of these bonding positions, protons attached to alcoholic oxygens are fairly acidic and remain attached to the ligand due to acidic medium. These protons are easily lost as pH of the system is raised, leading to precipitation of neutral complexes. It is therefore, proposed that (-)-TC is bound to the metal ions through its oxygen atoms attached with C-1 and C-12 incorporating asymmetric C-12a in the chelate. The metal ions in [VO(-)-(TC)$_2$H$_2$O]$^{2+}$ and [Co(-)-(TC)$_3$]$^{2+}$ have octahedral environments. In the case of [Ni$_2$((-)-TC)(H$_2$O)$_x$]$^{4+}$ ion, the second metal ion may be coordinated through the oxygen donors of C-10 and C-11. The relatively weak CD band in this complex ion indicates that asymmetric centres of the ligand are not involved in second chelate formed by Ni(II). The complexes, [VO(-)-(TC)$_2$H$_2$O]$^{2+}$, [Co(-)-(TC)$_3$]$^{2+}$ and [Ni$_2$((-)-(TC)(H$_2$O)$_x$]$^{4+}$ are low symmetry (C$_1$) species due to coordination of chiral ligand.

In aqueous solution, the absorption spectrum of $[VO((-)-TC)_2H_2O]^{2+}$ consists of a broad band at 19,960 cm^{-1} (ε 299 mol^{-1} L cm^{-1}) while its CD consists of a couplet of a negative band at 17,094 cm^{-1} ($\Delta\varepsilon$ 0.70 mol^{-1} L cm^{-1}) and a positive band at 20,408 cm^{-1} ($\Delta\varepsilon$ 0.20 mol^{-1} L cm^{-1}). This couplet arises from excitation of electrons to the split levels of triply degenerate state of the absorption band at 19,960 cm^{-1}. Therefore the couplet at 17,094 cm^{-1} and 20,408 cm^{-1} for $[VO((-)-TC)_2H_2O]^{2+}$ may arise from excitation of electrons to $^2B_1$ and $^2A_1$ states respectively. These band assignments agree well with earlier reported spectra of a number of low symmetry VO(IV) complexes [6].

The absorption spectrum of $[Co((-)-TC)_3]^{2+}$ consists of a broad crystal field band at 20,833 cm^{-1} (ε, 181.0 mol^{-1} L cm^{-1}) and a number of very intense charge transfer bands at 27, 933 cm^{-1}, 36,765 cm^{-1} and 40, 323 cm^{-1}. The CD spectrum of this ion consists of only a single positive band at 18,692 cm^{-1} ($\Delta\varepsilon$ 1.2 mol^{-1} L cm^{-1}). This band may be assigned to the $^4A_1 \rightarrow {}^4B_1$ transition ($^4B_1$ arising from the $^4T_{1g}(P)$ state of an octahedral environment). A number of earlier workers have assigned CD bands between, 178,150-21,400 cm^{-1} for low symmetry Co(II) complexes to a similar transition [7].

The CD spectrum of $[Ni_2((-)-TC)(H_2O)_x]^{4+}$ consists of a single negative band at 15,873 cm^{-1} ($\Delta\varepsilon$ 0.02 mole^{-1} L cm^{-1}). This band is relatively weak and broad indicating that either asymmetric centres are present outside the chelate or chelate is not formed. The proposed bonding sites of two nickel atoms with (-)-TC indicate that one Ni(II) does not incorporate an asymmetric centre in the chelate. The negative Cotton band at 15,873 cm^{-1} in $[Ni_2((-)TC)(H_2O)_x]^{4+}$ seems to originate from electronic excitation to the $^3A_1$ component of $^3T_{2g}$ upper level in an octahedral symmetry. This assignment is in accordance with the earlier reported spectra of a number of Ni(II) complexes [8].

It is evident from the proceeding discussion that VO(IV), Co(II) and Ni(II) ions instantaneously react with (-)-tetracycline in acidic medium to form soluble complexes of different composition. Since gastrointestinal fluid is also acidic, it is expected that trace amounts of these metals in the fluid effectively bind the antibiotic and cause their easy absorption in the system. Further, antibacterial efficiency of (-)-tetracycline may be drastically affected if administered in presence of trace amounts of transition metal ions in biological systems.

The CD spectra of these systems may be effectively utilized to investigate metal ligand interactions and Cotton bands may be assigned to electronic transitions to unidimensional levels arising due to lower symmetry of these complex ions.

Experimental

Materials

All chemicals of analytical reagent grade were obtained from commercial sources. The chiral drug, (-)-Tetracycline as free base was purchased from Fluka biochemica, Switzerland and stored at 4°C in a refrigerator. The purity of (-)-TC was checked by its infrared and specific rotation measurements.

All solutions were prepared in double distilled water. On account of instability of (-)-TC in aqueous media, fresh solutions were prepared every day and kept in the dark. Standardized solutions of HCl/NaOH (0.1M) were used to impose specific pH values on experimental samples.

Solutions

(a) Stock solution of (-)-Tetracycline

An accurately weighed 4.44 g (0.01 mole) of (-)-TC was suspended in a small volume (25 ml) of water and carefully titrated with a standard (0.1M) solution of HCl with shaking till all the solid particles were completely dissolved. The solution was diluted to 100 ml. The contents were thoroughly mixed and kept in dark.

(b) Metal salt solutions

Calculated amounts of hydrated metal chlorides or sulfates were dissolved in water and diluted to 100 ml so as to form 0.1 mol L^{-1} solution. The contents were thoroughly mixed and kept for measurements.

(c) Buffer solutions

A buffer of pH 4.0 was prepared by mixing 80 ml of 0.2M acetic acid and 20 ml of 0.2M sodium
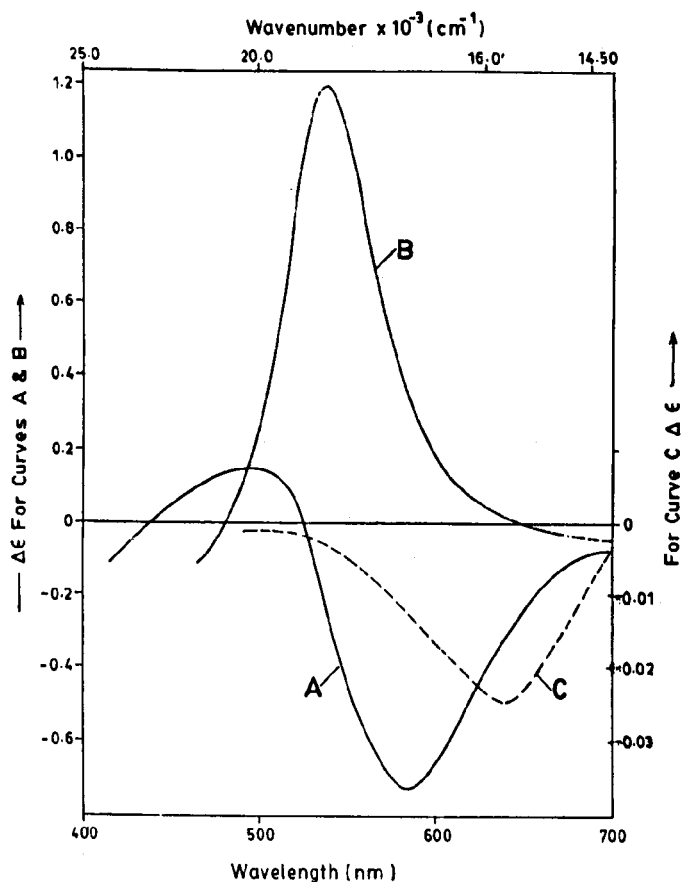
Fig. 1: Circular dichroism spectra of (A) [VO((-)-TC)₂H₂O]²⁺ at pH 3.65, (B) |Co((-)-TC)₃]²⁺ at pH 4.0 and
(C) [Ni₂(-)-TC)(H₂O)ₓ]⁴⁺ at pH 4.0 formed in aqueous solution.

Table-1: Optimum conditions, absorption and circular dichroism spectra of complexes of
(-)-tetracycline formed in aqueous solution

Complexe ion	pH	M:L Ratio	Absorption spectra		Circular dichroism spectra		Band Assignments
			v (cm⁻¹)	ε L.mol⁻¹ cm⁻¹	v (cm⁻¹)	$\Delta\varepsilon$ L.mol⁻¹ cm⁻¹	
[VO((-)-TC)₂H₂O]²⁺	3.65	1:2	19,960	299.0	17,094	−0.73	²B₂ → ²B₁
					20,408	+0.16	²B₂ → ²A₁
[Co((-)-TC)₃]²⁺	4.0	1:3	20,833	181.1	18,692	+1.2	⁴A₁ → ⁴B₁(⁴T₁g(P))
			27,933	3073.0			
			36,765	3758.0			
			40,323	2790.0			
[Ni₂((-)-TC)(H₂O)ₓ]⁴⁺	4.0	2:1	15,823	8.0	15,873	−0.025	³A₂ → ³A₁ (³T₂g)
			21,367	61.0			
			23,256	266.4			
			27,624	9750.0			

acetate. Another buffer solution of pH 3.72 was
obtained by mixing 90 ml of 0.2M acetic acid with
10 ml of 0.2M sodium acetate.

(d) Solutions for complexation studies

The sample solutions for complexation
studies, determining optimum pH conditions,

stoichiometery and determining concentration limits were prepared according to the procedures described earlier [7-9].

.These solution systems were maintained at appropriate pH with suitable buffer solution in proper molar proportions for recording their CD and absorption spectra. The CD spectra of these complex ions formed in aqueous solution is shown in Fig. 1 and data on optimum conditions and spectra are given in Table-1.

(e) Instrumentation

The pH measurements were carried out on Horiba F-8 pH meter equipped with a combination electrode system. The circular dichroism spectra were recorded with the help of a Jasco - J 20A spectropolarimeter using 10 mm cylinderical cells. The base line of these spectra were recorded using (-)-TC solution. The solution absorption spectra were measured on Shimadzu UV-265 spectrophotometer using matched pair of 10 mm quartz cuvettes.

References

1. (a) R.L. Lintvedt, M.D. Glick, B.C. Tomolonvic and D.P. Gavel, *Inorg. Chem.*, 15, 1633 (1976).
    (b) R.L. Lintvedt, B.A. Schoenfelner and K.A. Rupp. *Inorg. Chem.*, 25, 2704 (1986).
    (c) R.L. Lintvedt, M.J. Heeg, N. Ahmad and M.D. Glick, *Inorg. Chem.*, 21, 2350 (1982).
2. (a) D.E. Fenton, J.R. Tate, U. Casellato, S. Tambirini, P.A. Vigato and M. Vidali, *Inorg. Chim. Acta.*, 83, 23 (1984).

(b) R. L. Lintvedt, B.A. Schoenfelner, C. Ceccarelli and M.D. Glick, *Inorg. Chem.*, 23, 2867 (1984).
3. (a) W.A. Baker, Jr. and P.M. Brown, *J. Am. Chem. Soc.*, 88, 1314 (1966).
    (b) N. Ahmad, M. Jamil and C. Munir, *J. Chem. Soc. Pak.*, 12, 168 (1990).
    (c) C.M. Mikulski, J. Fleming, D. Fleming, N.M. Karayanuis and M. Nicholas, *Inorg. Chim. Acta.*, 144, 9 (1988).
4. (a) L. Lambs, B.D. Reverend, H. Kozlowski and G. Berthon, *Inorg. Chem.*, 27, 3001 (1988).
    (b) M. Jezowska-Bojezuk, L. Lambs, H. Kizlowski and G. Berthon, *Inorg. Chem.*, 32, 428 (1993).
5. (a) P. Salvadori, G. Rosini and C. Bartueci, *J. Am. Chem. Soc.*, 106, 2439 (1984).
    (b) Y. Fajii, Y. Yaslikama, M. Syoji and H. Shinohana, *Bull. Chem. Soc. Jpn.*, 63, 138 (1990).
6. (a) D.J. Robbin, J.J. Stillman and A.J. Thompson, *J. Chem. Soc.*, 813 (1974).
    (b) D. Collison, B. Gahan, C.D. Garner and F.E. Mabbs, *J. Chem. Soc. Dalton*, 667 (1980).
7. (a) N. Ahmad, C. Munir and M. Jamil, *J. Chem. Soc. Pak.*, 17, 149 (1995).
    (b) N. Ahmad, J. Safdar and C. Munir, *Turkish J. Chem.*, 20, 214 (1996).
8. (a) A.B.P. Lever, I.M. Walker and P.J. McCarthy, *Spectry Lett.*, 12, 739 (1979).
    (b) N. Ahmad, C. Munir, M. Jamil and S. Rauf, *J. Chem. Soc. Pak.*, 15, 190 (1993).
9. N. Ahmad, A. Khalique and C. Munir, *J. Chem. Soc. Pak.*, 19, 259 (1997).