NISEB Journal Vol. 14, No. 1, March, 2014 1595-6938/2014
Printed in Nigeria (2014) Nigerian Society for Experimental Biology
http://www.nisebjournal.org

Synthesis and Characterization of Two Cobalt Complexes of Tetracyclines

Joshua A. Obaleye* and Olufunso O. Abosede

Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

ABSTRACT: *The pleitropic properties of tetracyclines, the biological role of cobalt coupled with the improved biological activity of doxycycline upon chelation by metal ions as well as the biological importance of bipyridine and phenathroline have prompted us to synthesize cobalt-tetracycline complex and mixed ligand cobalt complex of doxycycline with dipyridine quinoxaline (dpq) as ancillary ligand. The two complexes were characterized by electronic, FT-IR and elemental analysis. In both complexes tetracycline, doxycycline and dpq behave as bidendate ligands, dpq coordinating via the two diimine nitrogen atoms. Detailed structural analysis of the complexes by FT-IR and UV-Vis are presented.*

Keywords: Doxycycline; metal complex; cobalt; diimine; antibacterial.

Introduction

Tetracyclines form moderately stable complexes with metal ions usually acting as O,O-donor ligand [1] and have been used to remove iron from Fe^{III}_2-transferrin [2]. The bioavailability, activity and toxicity of tetracyclines have also been associated with metal coordination [3-5]. The complexing of iron to bleomycin [6] has been implicated in bleomycin-DNA interactions and the interaction of stable Fe^{III}-diimine complexes with DNA has also been documented. Co^{II} is a biologically relevant metal ion that can sometimes substitute for Mg^{II} in supporting nucleic acid folding and function in vitro [7].

Cobalt(II) is a key oxidation state, and the range of compounds extant is truly vast. Four coordinate tetrahedral and six-coordinate octahedral complexes, in particular, abound. Traditionally, the complexes of this d^7 paramagnetic ion are considered to be labile and ligand exchange facile.

Phenanthroline based ligands, such as dipyridine quinoxaline (dpq) with an extended planar quinoxaline moiety, can act as good bidentate ligands as well as good binders to DNA [11] . Since nucleic acids are targets of metal-based therapeutic agents [12], it is worth the task synthesizing a ternary complex of Co (II), dpq and an antibiotic such as tetracycline or doxycycline.

In view of the above, the redox activity and biological role of cobalt, the wide application of metal complexes in biology [8] and the use of metal-based compounds as chemotherapeutics [9-10] have enhanced us to synthesize and characterize homoleptic and heteroleptic cobalt complexes of tetracycline and doxycycline respectively. This work represents a subset of a larger effort concerned with utilizing metal complexes of tetracyclines for various applications. A brief listing of some applications of these complexes includes antibacterial, antimalarial, DNA binding, DNA nucleating and cytotoxic agents [8,10].

Materials and Methods

Materials and physical measurements

Doxycycline hyclate was a gift from Neimeth International Pharmaceuticals Plc, Nigeria and fresh solutions were used to ensure stability; 1,10-phenanthroline monohydrate was from S. D. Fine Chemicals Ltd., India. All other chemicals and reagents are of analytical grade and used without further purifications. UV/Vis spectra were recorded on a JENWAY 6405 UV/Vis. spectrophotometer. Infrared spectra were recorded in a range 4,000–400 cm^{-1} on Shimadzu FT-IR-8400 on samples pressed in KBr pellets. Elemental analyses were taken on Elementar Analysen Systeme Vario ® MICRO VI 6.2 GmbH. Melting points were taken on Jenway and were uncorrected.

The ligand dipyridine quinoxaline (dpq) [13] was prepared according to literature procedures.

Synthesis of complexes

$[CoTcCl_2(H_2O)_2].H_2O$ (1)

0.4822 g (1mM) of tetracycline hydrochloride was added to 8 ml of methanolic solution of 0.2382 g $CoCl_2.6H_2O$ (1 mM) turning the purple solution to yellow colour. The solution was stirred at room temperature for about seven hours and then allowed to evaporate slowly at room temperature. After two days, 0.7280 g of green solid was obtained and the crude product was purified by column chromatography. Pure product was obtained as a bright yellow solid by using alumina as the stationary phase and acetone-acetonitrile as eluent. Yield: 346 mg (55%). Melting point: 162-164 ^oC (decomposed). UV/Vis (MeOH): 279, 373, 394, 485. Calculated: C, 41.92; H, 5.12; N, 4.44. Found: C, 40.89; H, 4.81; N, 4.37

$[CoDox_2dpq](ClO_4)_3.4H_2O$ (2)

Complex **2** was synthesized by heating dpq with an aqueous methanol solution of **$[CoDox_2(H_2O)_2](ClO_4)_3.6H_2O$** till the volume of the solution is reduced to about half. The resulting solution was allowed to stand and the precipitated orange solid filtered. The orange precipitate was washed with small amount of distilled water and dried in vacuum dessicator. Melting point: 208 ^oC (decomposed). UV/Vis (MeOH): 217, 234, 277, 373. Calculated: C, 44.93; H, 4.16; N, 7.23. Found: C, 45.70; H, 4.89; N, 5.38.

Results and Discussion

Two new cobalt complexes of tetracycline antibiotics have been synthesized and obtained in good yields. They are: binary Co-tetracycline and ternary Co-doxycycline-dipyridine quinoxaline (dpq). The structures of the complexes were elucidated by techniques such as electronic absorption, FT-IR, elemental analysis and electrospray mass spectroscopy. In the complexes, tetracycline or doxycycline behaved as bidentate ligand forming 1:1 metal-ligand complex i.e. tetracycline-Co^{II} (**1**) and Doxycycline-Co^{II}-dpq (**2**) (where dpq is dipyridine quinoxaline). The complexes were stable towards air and moisture. Complex **1** was soluble in water, methanol, ethanol DMF, DMSO and slightly soluble in acetonitrile while complex and most organic solvents while complex **2** was soluble in acetone, acetonitrile, methanol, DMF, DMSO but not in water.

The UV/Vis of the complexes in methanol showed evidence of coordination with shifts to longer wavelength of the absorption bands of tetracycline and doxycycline in both complexes. Intra-ligand absorptions in complex **2** appearing at 217 and 234 nm were due to the

* *Corresponding Author*

presence of dpq ligand in the complex and could be assigned to π-π* transition in the dpq ligand. Absorptions at 485 and 413 in **2** and **3** were due metal-to-ligand charge transfer. The difference and the narrow range of decomposition temperature of the complexes and the respective ligands also showed evidence for complexation and purity of the complexes.
In order to ascertain the coordination sites of doxycycline we have obtained the IR spectra of the complexes. The infrared spectra obtained were compared to that of the ligand and were assigned based on previous published reports [14-19].

For both complexes, the presence of υ(OH) and υ(NH_2) stretching of doxycycline moiety between 3348 cm^{-1} and 3400 cm^{-1} eliminated the possibility of amino and hydroxyl groups at ring A from participation in coordination. δNH_2 absorption of doxycycline at 1244 cm^{-1} was also present in both complexes appearing at 1231 and 1243 cm^{-1} in **1** and **2** respectively showing that NH_2 was not participating in coordination to metal ion. The absorptions due to the BCD chromophore which appeared at 1455 and 1393 cm^{-1} were unchanged in the complexes and further confirmed that coordination was not taking place at the BCD chromophore of doxycycline. The band at 847 cm^{-1} in complex **2** was due to C=N absorption of the dpq ligand in the complex. Metal ligand absorptions were observed at 535, 545 and 560 cm^{-1}. ClO_4 absorption in complex 2 was seen at 1035 cm^{-1}. Principal absorption bands of the complexes and doxycycline are summarized in Table 1.

Table 1: FT-IR spectra of doxycycline and the complexes

Dox	**$[CoDox_2dpq](ClO_4)_3.4H_2O$ (2)**	**$[CoTcCl_2(H_2O)_2].H_2O$ (1)**	**Assignment**
3452, 3331, 3290, 3217	**3400, broad**	**3348, broad**	**υ-N-H and υ-OH**
3010			**C-H aromatic**
2982, 2883, 2839			**C-H aliphatic**
1678, 1616			**Amide I C=O, absent in complexes and C=O of ring C**
1520	**1575, 1500**	**1581, 1532**	**Amide II absorption δNH_2 + υC-NH_2**
1458	**1455**	**1455**	**δ(COH)+ δ(CH_3) of**
		1393	**BCD Chromophore**
1332, 1311	**1323**	**1314**	
1244, 1219	**1243, 1216**	**1231**	**δNH_2 and υC-NH_2**
1174, 1130	**1170, 1091 br, str**	**1181, 1137**	**NO_3**
1041	**1035**	**1053**	**ClO_4^- and Cl^-**
995, 937	**995, 934**	**935**	
	847, 823, 805	**856, 821**	**C=N of diimine**
619	**620, 578**	**620, 593**	
547	**561, 544, 535**	**557, 547, 537, 525**	**Co-O**

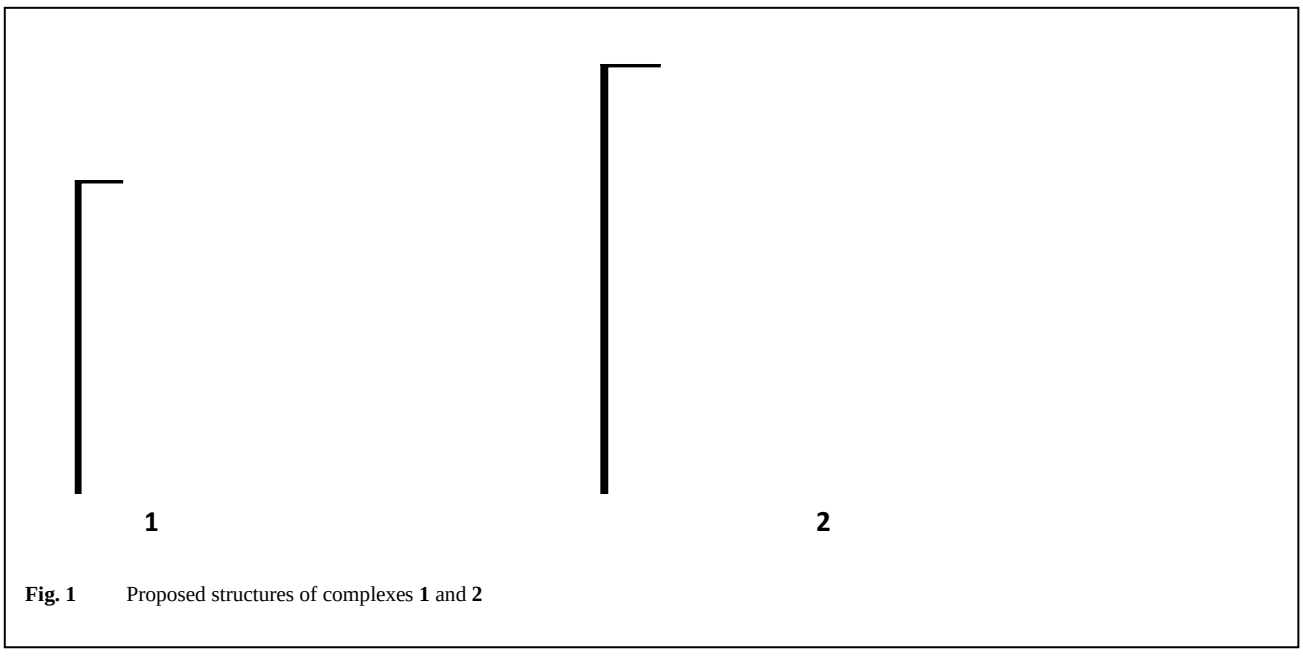

Fig. 1 Proposed structures of complexes **1** and **2**

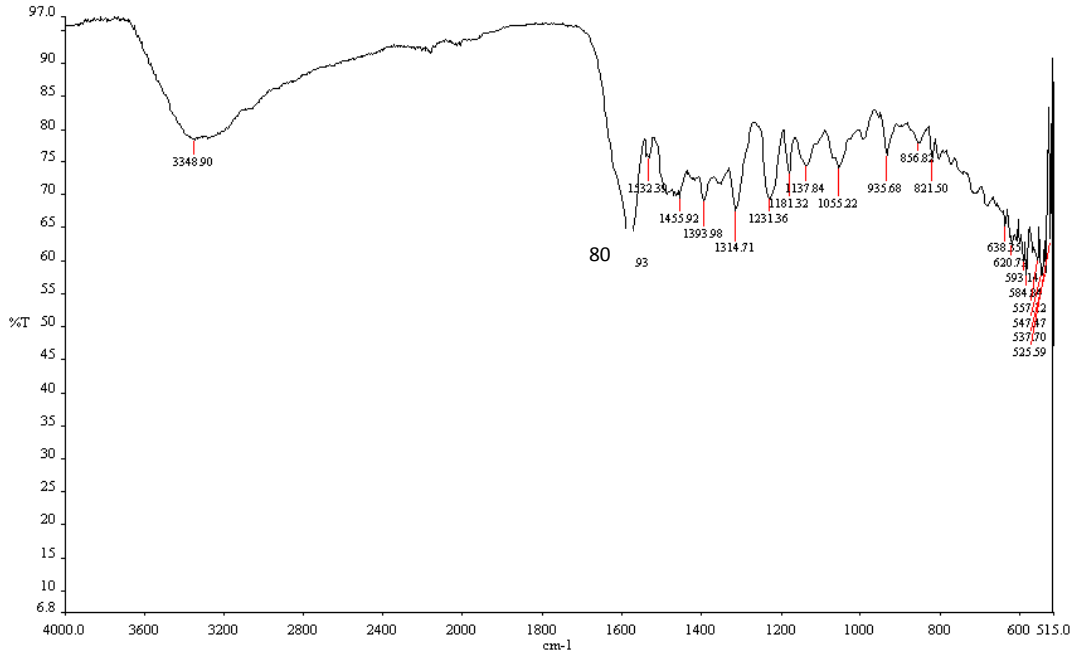

Fig. 2 FT-IR Spectra of $[CoTcCl_2(H_2O)_2].H_2O$

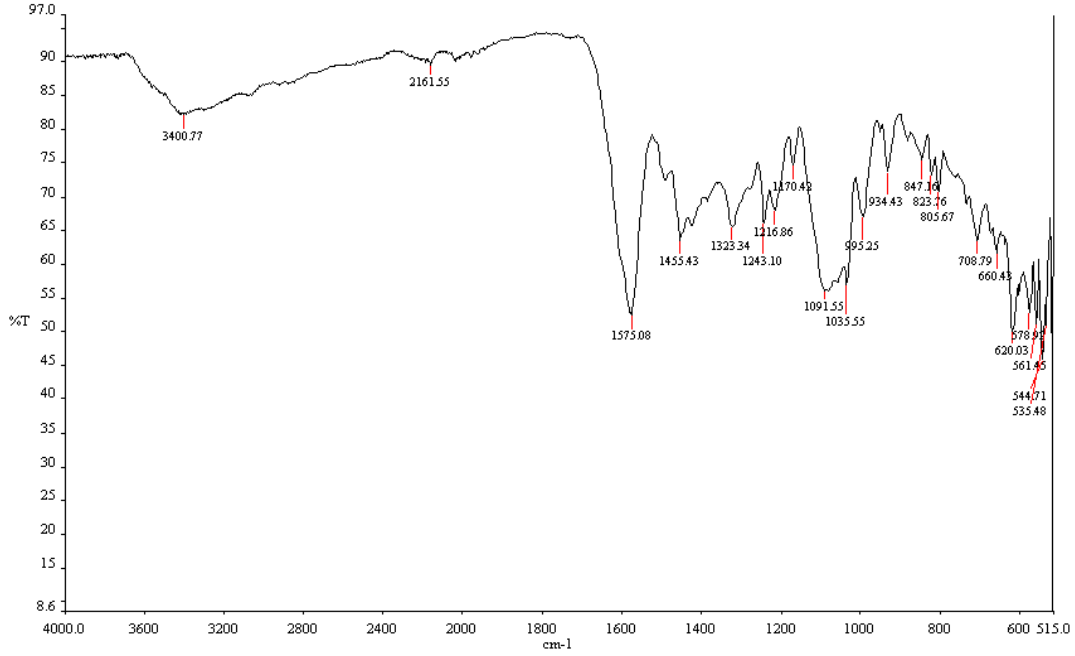

Fig. 3 FT-IR Spectra of $[CoDox_2dpq](ClO_4)_3.4H_2O$

References

1. Riaz M, Pilpel, NJ (1984) Pharm. Pharmacol., 36:153-156. Compleaxation of tetracyclines with metal ions in relation to photosensitization.
2. Grenier, D, Huot M-P, Mayrand D (2000) Antimicrob. Agents Chemother. 44:763-766. Iron-chelating activity of tetracyclines and its impact on the susceptibility of *Actinobacillus actinomycetemcomitans* to these antibiotics.
3. Pradines, B, Rogier C, Fusai T, Mosnier J, Daries W, Barret E, Parzy D (2001) Antimicrob. Agents Chemother. 45:1746-1750. In Vitro Activities of Antibiotics against *Plasmodium falciparum* are Inhibited by Iron.
4. Machado FC, Demicheli C, Gamier-Suillerot A and Beraldo H (1995) J. Inorg. Biochem. 6:163-173. Metal complexes of anhydrotetracycline. 2. Absorption and circular dichroism study of Mg(II), Al(III), and Fe(III) complexes. Possible influence of the Mg(II) complex on the toxic side effects of tetracycline.
5. Burns FR, Stack MS, Gray RD, Paterson CA (1989) Invest Ophthalmol Vis Sci 30: 1569–1575. Inhibition of purified collagenase from alkali-burned rabbit corneas.

* *Corresponding Author*

6. Loeb KE, Zalseki JM, Hess CD, Hecht SM, Solomon EI J. Am. Chem. Soc. (1998) 120: 1249-1259. Spectroscopic investigation of the metal ligation and reactivity of the ferrous active sites of bleomycin and bleomycin derivatives.
7. Shi H, Moore PB (2000) RNA 6:1091–1105. The crystal structure of yeast phenylalanine tRNA at 1.93A resolution: a classic structure revisited.
8. Haas KL and Franz KJ (2009) Chem. Rev. 109:4921–4960. Application of Metal Coordination Chemistry to Explore and Manipulate Cell Biology.
9. Goodwin KD, Lewis MA, Long EC, and Georgiadi MM (2008) PNAS, 5052-5056. Crystal structure of DNA-bound Co(III)·bleomycin B_2: Insights on intercalation and minor groove binding.
10. Bandow JE and Metzler-Nolte N (2009) ChemBioChem, 10:2847-2850. New ways of killing the beast: prospects for inorganic-organic hybrid nanomaterials as antibacterial agents.
11. Patra AK, Nethaji M and Chakravarty AR (2005) Dalton Trans 2798-2804. Red-light photosensitized cleavage of DNA by (L-lysine)(phenanthroline base)copper(II) complexes.
12. Jamieson E, Lippard SJ (1999) Chem. Rev. 99:2467–2498. Structure,recognition, and processing of cisplatin-DNA adducts.
13. Collins JG, Sleeman AD, Janice RAW, Greguric I. and Hambley TW (1998) Inorg. Chem. 37:3133. A ^{1}H NMR Study of the DNA Binding of Ruthenium(II) Polypyridyl Complexes.
14. Cheesbrough M (2006) District laboratory practice in tropical countries, Part 2, Cambridge University Press, Cambridge, UK, pp 137-150.
15. Silva PP, Guerra W, Silveira JN, Ferreira AMC, Bortolotto T, Fischer FL, Terenzi H, Neves A, and Pereira-Maia EC (2011), Inorg. Chem. 50:6414–6424. Two New Ternary Complexes of Copper(II) with Tetracycline or Doxycycline and 1,10-Phenanthroline and Their Potential as Antitumoral: Cytotoxicity and DNA Cleavage.
16. Chartone-Souza E, Loyola TL, Bucciarelli-Rodriguez M, Menezes MA, Rey NA, Pereira-Maia EC (2005) J. Inorg. Biochem. 95:1001–1008. Synthesis and characterization of a tetracycline-platinum (II) complex active against resistant bacteria.
17. de Paula FCS, Carvalho S, Duarte HA, Paniago EB, Mangrich AS, Pereira-Maia EC (1999) J. Inorg. Biochem.76:221–230. A physicochemical study of the tetracycline coordination to oxovanadium(IV).
18. Mikulski CM, Fleming J, Fleming D (1988) Inorg. Chim. Acta 144:9–16. Chelates of Tetracycline with First Row Transition Metal Perchlorates.
19. Guerra W, Azevedo EA, Monteiro ARS, Bucciarelli-Rodriguez M, E Chartone-Souza, Nascimento AMA, Fontes APS, Moyec LL, Pereira-Maia EC (2005) J. Inorg. Biochem, 99:2348–2354. Synthesis, characterization, and antibacterial activity of three palladium(II) complexes of tetracyclines.
20. Obaleye JA, Adeyemi OG, Balogun EA (2001 Int. J. Chem. 11 (2): 101-106. Some metal tetracycline complexes: Synthesis, Charcterization and their effects against malaria parasite.