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Study of the spectroscopic characteristics of methyl (ligand) cobaloximes and their antibacterial activity

N NAVANEETHA,[a] P A NAGARJUN[b] and S SATYANARAYANA[a,]*

[a]Department of Chemistry, and
[b]Department of Microbiology, Osmania University, Hyderabad 500 007
e-mail: ssnsirasani@yahoo.com

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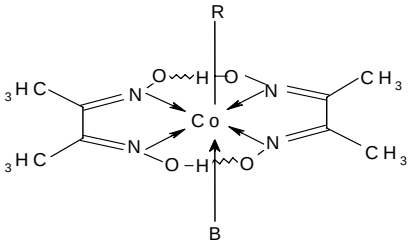
Abstract. Spectroscopic characterization (IR, NMR and electronic spectra) of methyl (ligand) cobaloxime was done, where ligand = pyrazole, dimethyl pyrazole, alanine and alanine methyl ester. The frequency changes in the IR spectra and shifts in the NMR were explained on the basis of basicity of the ligand, steric hindrance, HSAB principle and $d\pi$–$p\pi$ back-bonding from metal to ligand. Alanine and alanine methyl ester form more stable complexes than pyrazole and dimethyl pyrazole. Based on their IR and ^{1}H NMR spectra it is inferred that pyrazole and dimethylpyrazole bind to Co (III) via N–2 ring nitrogen, i.e. monodentate coordination.

Keywords. Cobaloximes; spectroscopic characterization; monodentate coordination.

1. Introduction

Discoveries on the structure and models of vitamin B_{12}[1,2] are assessed in the light of the advances in structural and spectroscopic methodologies. It is well known that organocobaloximes, classical models for coenzyme B_{12} represented as $RCo(DH)_2L$ (**1**, DH = mono anion of dimethyl glyoxime, R = alkyl,



Methyl(ligand)cobaloxime (**1**)
B = Py, DMPy, Ala and AME

L = neutral Lewis base) have been studied and reviewed in the last three decades[3] due to their use as catalysts[4] and as templates in organic synthesis.[5–13] However, the main interest still lies in their role as models of vitamin B_{12}, however, several additional structures have been reported including that of methylcobalamin[14,15] (MeCbl), which catalyses the conversion of homocystein to methionine[16,17] and ribonucleotide reductases.[18–20]

In the above reactions the key step is believed to be the homolytic cleavage of the Co–C bond,[21] which arises from the conformational changes in the enzyme which occurs upon substrate binding leading to a sterically strained adenosyl group. Spectroscopic studies on the model complexes help in establishing the basic relationship between structure and chemical properties. Kofod *et al*[22], characterized cobalt (III) compounds with classical ligands by spectroscopic techniques. Bhoopal[23] reported the equilibrium constants for the pH-dependent axial ligation of $RCo(DH)_2L$, where R = CH_3, C_2H_5 and L = Py, 4Me-Py, 4Eth-Py & $4NH_2Py$.

The systematic analysis of the structure–property relationship has furnished useful indications concerning the Co–C bond homolysis mechanism in the B_{12} coenzyme. Canpolat *et al*[24] reported that vicdioxime complexes of cobalt (III) complexes were the most active and may be promising for the development of new antibiotics.

2. Materials and methods

2.1 *Preparation of $CH_3Co(DH)_2L$*

$CH_3Co(DH)_2OH_2$ was prepared by the procedure of Brown *et al*[25]. All manipulations were performed

*For correspondence

Table 1. Antimicrobial activity of the Co complexes at 20 μg/ml*.

Complex	Bacterial species			
	E. coli	*K. pneumoniae*	*S. aureus*	*B. aureus*
$CH_3Co(DH)_2H_2O$	10	6	7	5
$CH_3Co(DH)_2Ala$	8	7	5	5
$CH_3Co(DH)_2AME$	8	8	9	–
$CH_3Co(DH)_2Py$	10	5	11	14
$CH_3Co(DH)_2DMPy$	5	–	–	–
Tetracycline	10–14			
Bacitracin	6–8			

*Values of zone of inhibition (mm, including the diameter of the disc)

Table 2. ^{1}H NMR and electronic spectral data$^{\$}$ of $CH_3Co(DH)_2L$*.

Complex	$(CH_3)^{\#}{}_4$	CH_3-Co	C-H-4	H-3/ Me-3	H-5/ Me-5	$-NH_2$/ –NH	–CH/ $-CH_3$¶	UV-Visible data		
								Peak I	Peak 2	Peak 3
$CH_3Co(DH)_2H_2O$	2·10	0·5	–	–	–	–	–	24183·8	27700·8	36024·5
$CH_3Co(DH)_2Py$	2·10	0·6	6·30	7·60(d)	7·40(d)	11·80	–	22805·0	27137·0	36563·1
$CH_3Co(DH)_2DMPy$	2·10	0·6	5·80(d)	2·5	2·3	10·6	–	22805·0	26773·8	36563·1
$CH_3Co(DH)_2Ala$	2·20	0·5	3·20	–	–	3·8	1·2	22805·0	26595·7	36231·9
$CH_3Co(DH)_2AME$	2·20	0·5	3·10	–	–	0·2	0·8	22675·7	26246·7	37950·0

$^{\$}$In ppm relative to tetra methyl silane
*L, Py = pyrazole, DMPy = dimethyl pyrazole, Ala = alanine, AME = alanine methylester
¶–CH/–CH_3 of the ligand

under minimal illumination due to photolability of the organo cobalt bond.[26] $[CH_3Co(DH)_2L]$ complexes were isolated by mixing 1 : 1 ratio of $CH_3Co(DH)_2OH_2$ and the base ligand(L) in methanol. This mixture was heated at 40–50°C by constant stirring for 1–2 h. Then the minimum amount of distilled water was added, the resulting precipitate of yellow powder was filtered, washed with distilled water, 95% methanol and ether and dried *in vacuo* (yields were 70–80%). ^{1}H NMR spectra were recorded on Varian Gemini 200 MHz NMR spectrometer. Samples were prepared by dissolving in $CDCl_3$ and DMSO-d_6. Infrared spectra were obtained on a Perkin–Elmer FTIR-1605 spectrometer using KBr pellets.

The antimicrobial activities of the compounds were determined in vitro using different microorganisms by the standard disc diffusion method.[27] The following bacteria were used: *Escherichia coli* (MTCC 1234), *Klebsiella pneumoniae* (MTCC 1234), *Staphylococcus aureus* (MTCC 1234) and *Bacillus subtilis* (MTCC 1234). Bacterial cultures were sub-cultured in nutrient broth medium and incubated at 37°C for 18 h and the logarithmic or exponential phase was achieved. Filter paper discs of 4 mm size were prepared by using Whatmann filter paper no. 1, and on to each of these discs a 5 μl of a solution of the complex in DMSO was added. At the end of the incubation period the zones of inhibition were measured (table 1).

3. Results and discussion

3.1 *Spectral analysis*

In this paper we report complexes of type $CH_3Co(DH)_2L$, where L = Py (pyrazole), DMPy (dimethyl pyrazole), Ala (alanine) and AME (alanine methyl ester). Electronic data (table 2) of the $CH_3Co(DH)_2L$ complexes show the most intense band in the highest energy region (~36,000 cm^{-1}) have been assigned to Co (III) $d\pi \rightarrow \pi^*$(DH) MLCT transition of the equatorial ligand. The lowest energy band (~22,000 cm^{-1}) has been assigned to a Co–C charge transfer, transition[28] due to R^- to Co (III) is a spin-allowed $^1A_1g \rightarrow {}^1T_1g$ transition,[29] and this band disappears or is drastically decreased in alkyl (ligand) cobaloximes due to σ donation by the ligand. The

Table 3. IR data for the methyl (ligand) cobaloxime.

Complex	DH					L	
	ν(CH$_3$)	ν(C=N)	N (NO)	ν(Co–N)	ν(Co–N)$^{\#}$	ν(C=N)	ν(–N–H)
$CH_3Co(DH)_2H_2O$	1360·8 1435·7	1557·9	1084·6 1225·6	508·8	–	–	–
$CH_3Co(DH)_2Py$	1372·5 1455·4	1556·2	1082·8 1229·0	513·8	–	573·4 2357·5	2907·5
$CH_3Co(DH)_2DMPy$	1381·6 1466·3	1566·6	1125·7 1232·4	517·2	–	614·5 2366·9	3130·8
$CH_3Co(DH)_2Ala$	1372·5 1425·4	1559·2	1091·7 1234·8	517·6	479·3	–	3280·5
$CH_3Co(DH)_2AME$	1371·7 1445·8	1573·9	1083·9 1230·3	511·6	450·0	–	3108·4

$^{\$}$Recorded as KBr discs and values in cm^{-1}
*L, Py = pyrazole, DMPy = dimethyl pyrazole, Ala = alanine and AME = alanine methyl ester
$^{\#}$ ν(Co–N), N of ligand

Table 4 MIC* of chemotherapeutic agent.

Complex	MIC (μg)
$CH_3Co(DH)_2H_2O$	20
$CH_3Co(DH)_2Ala$	40
$CH_3Co(DH)_2AME$	20
$CH_3Co(DH)_2Py$	20
$CH_3Co(DH)_2DMPy$	80

*Minimum inhibitory concentration in μg/disc

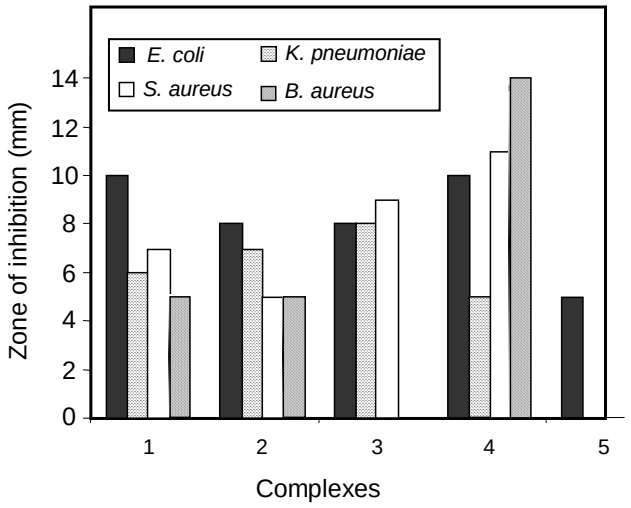


Figure 1. Bar graph showing the relative activity of the complexes.

$^1A_1g \rightarrow {}^1T_1g$ band is masked by the intense CT bands. Bands occurring at ~26,500 cm^{-1} are assigned to the L $\rightarrow$ Co (III), LMCT. The σDH $\rightarrow$ σ*Co (III) is masked by the intense short wavelength bands of alkyl (ligand) cobaloximes.[30] The Co–C CT band shifts to shorter wavelengths with decreasing electron-donating ability of the axial base.[31]

Marzilli *et al*[32], described the first application of near-IR excited Raman (near IR-FT Raman) spectroscopy to study photo-labile methyl cobaloxime. The disappearance of the peak at 3072 cm^{-1} and appearance of a new peak at 450 cm^{-1} υ(Co–N) indicates the formation of methyl(ligand)cobaloxime by replacing H_2O. The IR spectra of free ligands were compared with the IR spectra of their corresponding cobaloxime complexes in order to identify the diagnostic bands. For pyrazole and dimethyl pyrazole, monodentate coordination has been assigned through N–2, based on their IR spectra (table 3).

However, the definite relationship between frequency shifts and consecutive order of axial ligands was observed as mentioned, i.e. H_2O < pyrazole. In short, as the donating power of the base ligand increases, the υ(O–H…O) at ~1770 cm^{-1} and υ (C=N) at ~1570 cm^{-1} shifts to a lower wave number region, while υ(N–O) at ~1230 cm^{-1} and 1085 cm^{-1}, υ(Co–N) at ~510 cm^{-1} shift to higher one. These results can be interpreted as follows. The coordination of the more electron-donating base to Co atom causes increase in electron density in Co(III), which facilitates back-donation from Co(III) to the nitrogen atoms of dimethyl glyoximato ligands resulting in increase in electron densities in C=N and N–O bonds. The increase in electron density in N–O bonds leads to the stronger hydrogen bridges of O–H…O and higher frequency shifts of N–O stretching vibrations. The facilitated back-donation from the cobalt to nitrogen atoms of dimethyl glyoxime lowers the C=N stretching frequency.

Table.5. Formation constants for the axial ligation of $CH_3Co(DH)_2OH_2$ by bioactive ligands at 25°C in aqueous solution, ionic strength 1·0 M (KCl).

	R = CH_3			
pH	log K_{app}	log K_{app}	log K_{app}	log K_{app}
1·0	0·7314	–	–	–
1·5	1·2060	–	–	–
2·0	1·6326	–	–	–
2·5	1·9587	0·3363	–	–
3·0	2·1483	0·8201	–	–
3·5	2·2308	1·2740	–	–
4·0	2·2605	1·6535	–	–
4·5	2·2703	1·9064	–	–
5·0	–	2·0312	–	–
5·5	–	2·0768	–	–
6·0	–	2·0956	–	–
6·5	–	–	–	1·4022
7·0	–	–	–	1·8883
7·5	–	–	–	2·3454
8·0	–	–	1·7678	2·7310
8·5	–	–	2·2558	3·0759
9·0	–	–	2·7164	3·1234
9·5	–	–	3·1097	3·1748
10·0	–	–	3·3822	3·1924
10·5	–	–	3·5535	–
11·0	–	–	3·5895	–
11·5	–	–	3·5983	–
$K_{eq}(M^{-1})$	K_{Py} = 1884	K_{DMPy} = 127·01	K_{Ala} = 4041·3	K_{AME} = 1587·97

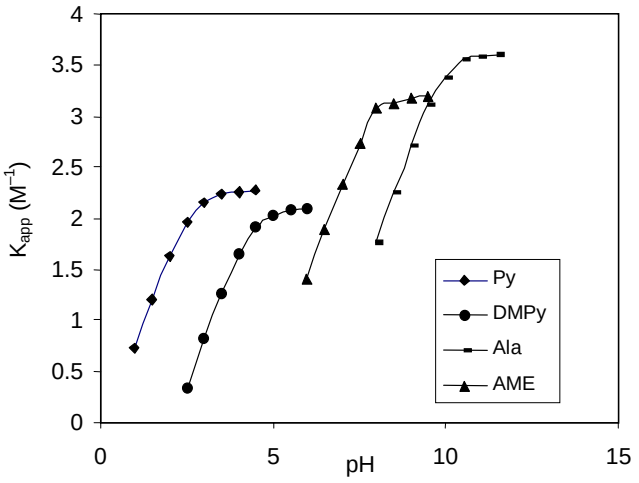


Figure 2. Dependence of apparent equilibrium constants (log K_{app}) on pH for the axial ligation of $CH_3Co(DH)_2OH_2$ by Py, DMPy, Ala and AME at 25°C in aqueous solution, ionic strength 1·0 M (KCl).

A strong electron-withdrawing ligand in the sixth position causes stronger interaction between the central metal and the fifth ligand in the *trans* position which could be caused by decrease in the cobalt → N (base) bond length. Donation of electrons from base to cobalt atom is thus facilitated which results in stronger back-donation from cobalt to the equatorial nitrogen atoms and hence an increase of electron density in the equatorial *bis*(dimethyl glyoximato) moiety. As the electron-donating power of ligand increases, the binding constant (K_{eq}) increases in the complexes. Figure 2 shows the pH dependence of the K_{eq}. Formation constants data for the substitution reaction of methyl(aquo)cobaloxime are given in table 5. This is also supported by our binding studies[33] of iodomethyl(aquo)cobaloxime and the formation constants (K_{eq}) follows the order

Ala > AME > Py > DMPy.

The ^{1}H NMR spectrum of $CH_3Co(DH)_2H_2O$ contain well resolved absorptions corresponding to the axial methyl and equatorial methyl groups of dimethyl glyoxime (DH). The sharp signals at 0·5 and 2·20 ppm integrating in the ratio 1 : 4 have been assigned to the *trans* CH_3 group (alkyl group *trans* to Co) and

the four CH_3 groups (of DMG) respectively. The ^1H NMR data (table 2) of methyl(ligand)cobaloxime show that *cis* and *trans* methyl groups are distinctly different.

In the free ligand (Py and DMPy) the H_3/CH_3 and H_5/CH_5 are averaged by rapid proton exchange and all the hydrogens are shifted upfield upon coordination. Pyrazole H_4 signal is at 6·1 ppm and it is slightly shifted upfield compared to the free ligand position because of the loss in aromaticity due to the withdrawal of electron density from N–2 to Co (III) and as a result ring protons experience a higher shielding effect. When pyrazole coordinates to Co (III), the C-3 and C-5 signals separates and gives two signals at 7·71 and 7·22 ppm respectively.

3.2 *Antibacterial activity*

From the zone of inhibition (table 1) test it has been found that when the agar plates were supplemented with antibiotics, e.g. tetracycline and bacitracin, the inhibited area was 10–14 mm and 6–8 mm respectively. When the same agar plates were supplemented with our complexes, it has been observed that *Klebsiella pneumoniae* and *Staphylococcus aureus* were sensitive to the complex as for tetracycline. The next species *Bacillus subtilis* lies in between and *Escherichia coli* is the least affected. In general, it is found that all the complexes exhibit inhibition against both gram-positive and gram-negative bacteria.

Among all the complexes tested, $CH_3Co(DH)_2$ (DMPy) is effective only with *Escherichia coli*. When compared to the antibiotic bacitracin, the cobaloximes were found to be more effective, and were less active than tetracycline. Compounds such as $CH_3Co(DH)_2(H_2O)$ and $CH_3Co(DH)_2(Py)$ were found to show activity equivalent to the antibiotic tetracycline against certain bacteria.

Initially at higher concentrations, the activity was determined and subsequently all these were further tested to determine the minimum growth inhibitory concentration for the growth of *E. coli* (table 4). The control MIC was 100 μg. From the above results it is seen that these complexes possess antibacterial activity.

4. Conclusions

Methyl(aquo)cobaloxime reacts with Py, DMPy, Ala, AME and forms stable methyl(ligand)cobaloxime whose stability has been explained on the basis of basicity of the ligand, steric hindrance, HSAB principle and $d\pi$–$p\pi$ back-bonding. Py and DMPy bind to Co (III) through the N-2 of the ring and Ala and AME through N of NH_2 group. The reported cobaloximes possess a broad range antibiotic activity and show promise for the development of new antibiotics.

References

1. Hodgkin D C, Lindsey J, Sparks R A, Trueblood K N and White J G 1962 *Proc. R. Soc.* **A266** 494
2. Brink-Shoemaker C, Cruickshank D W J, Hodgkin D C, Kamper M J and Pilling D 1964 *Proc. R. Soc.* **A278** 1
3. Bresciani-Pahor N, Forcolin M, Marzilli L G, Randaccio L, Summers M F and Toscano P J 1985 *Coord. Chem. Rev.* **63** 1
4. Nemeth S and Simandi L 1982 *J. Mol. Catal.* **12** 87
5. Giese B 1986 *Radicals in organic synthesis formation of carbon–carbon bond* (eds) (Oxford: Pergamon)
6. Scheffold R, Rytez G and Walder L 1983 *Transition metals in organic synthesis* (ed.) R Scheffold (Chichester: Wiley) vol. 3
7. Ghosh A K and Chen Y 1995 *Tetrahedron Lett.* 505
8. Wright M and Welker M E 1996 *J. Org. Chem.* **61** 133
9. Gupta B D, Singh V, Qanungo K, Vijaikanth V and Sengar R S 1999 *J. Organomet. Chem.* **582** 279
10. Gupta B D, Dixit V and Das J 1999 *J. Organomet. Chem.* **572** 49
11. Brown T, Dronsfield A, Jablonski A and Wilkinson A S 1996 *Tetrahedron Lett.* **37** 5413
12. Gill G B, Pattenden G and Raon G A 1996 *Tetrahedron Lett.* **37** 9369
13. Gage J L and Branchaud B P 1997 *Tetrahedron Lett.* **40** 7007
14. Halpern J 1985 *Science* **227** 869
15. Glusker J P B_{12} (ed.) D Dolphin (New York: Wiley) vol 1, pp 23–106, vol 2
16. Mathews R G and Drummond J T 1990 *Chem. Rev.* **90** 1275
17. Drennan C L, Dixon M M, Hoover D M, Jarret J T, Goulding C W, Mathews R G and Ludwig M L 1998 *Vit. B_{12} & B_{12} proteins* (eds) B Krautler, D Arigoni and B T Goldings (Weinheim: Wiley-VCH) p. 133
18. Stubbe J, Licht S, Gerfen G and Booker S 1998 *Vit B_{12} and B_{12} proteins* (eds) B Krautler, D Arigoni and B T Goldings (Weinheim: Wiley-VCH) p. 320, and ref. therein
19. Licht S, Booker S and Stubbe J 1999 *Biochemistry* **38** 1221
20. Licht S, Gerfen G and Stubbe J 1996 *Science* **271** 477
21. Cregan A G, Brasch N E and Eldik R V 2001 *Inorg. Chem.* **40** 1430
22. Kofod P, Harris P and Larsen S 1997 *Inorg. Chem.* **36** 2258
23. Bhoopal M and Satyanarayana S 2004 *J. Indian Chem. Soc.* **A43** 1409

24. Canpolat E and Kaya M 2004 *Turk. J. Chem.* **28** 235
25. Brown K L 1986 *Organometallic syntheses* (eds) R B King and J Eisch (Amsterdam: Elsevier) vol 3, p. 186
26. Brown K L and Kallen R G 1972 *J. Am. Chem. Soc.* **94** 1894
27. Drew W L, Barry A L, O'Toole R and Sherris J C 1972 *Appl. Environ. Microbiol.* **24** 240
28. Lever A B P 1968 *Inorganic electronic spectroscopy* (Amsterdam: Elsevier)
29. Halpern J, Palmer R A and Blakley L M 1966 *J. Am. Chem. Soc.* **88** 2897
30. Yamano Y, Masuda I and Shimura K 1972 *Bull. Chem. Soc. Jpn.* **44** 1581
31. Radhakrishna Reddy M, Mohana Raju K and Hussain Reddy K 1996 *Indian J. Chem.* **A35** 677
32. Nie S, Marzilli L G and Yu N T 1989 *J. Am. Chem. Soc.* **111** 9256
33. Navaneetha N and Satyanarayana S 2005 *Indian J. Chem.* **A44** 1191