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Formation of Metformin Complexes with some Transition Metal Ions: their Biological Activity

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Abstract

Complexes of Co(II), Ni(II), Cu(II) and Zn(II) with metformin have been synthesized and characterized by elemental analysis, IR, electronic, ^1H NMR and EPR spectra, and conductance measurements, in order to throw more light on their structure and geometry. Also, the formation constants of the complexes have been evaluated by potentiometric techniques. The biological activity of the complexes formed has been tested and compared with metformin at different concentrations in order to obtain some quantitative information about their biological activity towards microorganisms.

Introduction

Metformin hydrochloride is a biguanide, a group of compounds which have great importance in clinical applications [1]. Metformin can produce a hypoglycaemic effect after total pancreatectomy and in complete absence of insulin [2]. Metformin lowers the blood sugar level to the minimum physiological limit and destroys malarial parasites by attraction. It is used as an antidiabetic, antimalarial and analgesic [3]. On the other hand, the biguanides are specific antimetabolites for microorganisms that inhibit folic acid metabolism [4]. Despite the importance of biguanides and their metal chelates, little attention has been paid to them by previous authors [5, 6].

The aim of our work was to synthesize the complexes of Co(II), Ni(II), Cu(II) and Zn(II) ions with metformin and to characterize them by elemental analysis, IR, electronic, ^1H NMR and EPR spectra, and conductance measurements, in order to throw more light on their structure and geometry. Also the biological activity of these complexes was tested against eight microorganisms and compared with metformin at different concentrations in order to obtain some quantitative information concerning the most effective concentration for biological activity towards microorganisms.

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Experimental

All compounds used in the present investigation were pure laboratory-grade chemicals from BDH. Metformin was an A.R. product from Abron.

Preparation of the Solid Complexes

0.004 mol of the ligand was dissolved in twice-distilled water. After complete dissolution of metformin, an ethanolic solution of the metal ion (0.002 mol) was added dropwise. Addition of (0.0038 mol) KOH, dissolved in 10 ml of distilled water, to the solution mixtures caused precipitation and various colour changes depending on the nature of the metal ion used: reddish-orange in the case of Co(II); yellow for Ni(II); violet for Cu(II); and colourless for Zn(II). After stirring the reaction mixture for *ca.* 3 h, the complexes thus obtained were filtered off, washed with ethanol then ether and dried *in vacuo*. The apparatus and working procedures were as described previously [7, 8].

Microbiological Measurements

The assay tests were carried out according to the diffusion method [9] and were repeated at different concentrations of metformin or the complexes under investigation.

Results and Discussion

Stoichiometry and Structure of the Complexes

On the basis of analytical data obtained (Table I), the prepared complexes were formulated as $[M(L)_2]$, where M = Co(II), Ni(II), Cu(II) and Zn^{2+}. The molar conductances of all complexes in DMF were

0020-1693/87/$3.50

TABLE I. Analytical and Spectroscopic Data for Co(II), Ni(II), Cu(II) and Zn(II)–Metformin Complexes

Complex	$[Co(L_2)]$	$[Ni(L_2)]$	$[Cu(L_2)]$	$[Zn(L_2)]$
Analytical Data (%)[a]				
C	30.48(30.1)	30.57(30.2)	30.04(29.80)	29.91(29.5)
H	6.34(6.0)	6.36(6.10)	6.26(5.9)	6.23(6.0)
N	44.44(44.1)	44.59(44.20)	43.82(43.5)	43.61(43.2)
M	18.73(18.2)	18.47(18.0)	19.85(19.4)	20.25(19.70)
Λ[b]	11.0	10.0	11.0	9.0
IR (cm^{-1})				
νNH_{III}	3280	3290	3270	3285
νNH	3390	3380	3380	3370
	3320	3320	3320	3320
νNH_{IV}	3260	3265	3260	3265
$\nu C{=}N$	1610	1610	1605	1610
$\nu M{-}N$	375	380	375	385
Electronic (nm)	825	780	740	380
EPR g value				
g_z	1.8174			
g_x	2.1080	d[c]	1.9994	d[c]
g_y	2.2400		1.825	
$\log K_1$	6.19	6.81	7.17	6.17
$\log K_2$	10.20	12.02	12.30	10.23

[a]calculated(found) (%). [b]A 10^{-3} M in DMF, $ohm^{-1}\ cm^{-2}\ mol^{-1}$. [c]d = diamagnetic.

less than 12.0 $ohm^{-1}\ cm^{-2}\ mol^{-1}$, indicating that they are non-electrolytes [10] and hence metformin behaves as a monobasic acid.

The mode of bonding between metformin and metal ions was deduced from the results of the following investigations.

(a) On comparing the IR spectra of the solid complexes with that of metformin, the following can be seen. (i) The three bands located at 3420, 3380 and 3340 cm^{-1} due to the ν(NH) of primary and secondary amines in positions V and III suffer obvious shifts to lower frequency by 20–70 cm^{-1} on complex formation. (ii) One of the two bands due to the imino groups located at 3310 and 3280 cm^{-1} disappears on complex formation, probably the one present at position II, which indicates that metformin is bonded to the metal ion through proton displacement to one NH group. The other imino group, probably that present at position IV, displays an obvious shift to lower frequency by 15–20 cm^{-1} on complex formation. (iii) The two new bands observed at 1610–1605 and 385–375 cm^{-1} (Table I) for all solid complexes, which are not present in the spectra of the free ligand, are assigned to ν(C=N) and ν(M–N), respectively.

(b) The reflectance spectra of the complexes under investigation exhibit a broad maximum with λ_{max} at 825, 780, 740 and 340 nm for Co(II), Ni(II), Cu(II) and Zn(II) complexes, respectively. These bands can be assigned to $d_{x^2-y^2} \rightarrow d_{yz}$, $^3A_{2g} \rightarrow {}^3T_g(F)$ and $^2E_g \rightarrow {}^2T_{2g}$ transitions for the first three complexes. These spectral patterns are characteristic of a square-planar configuration for Co(II), Ni(II) and Cu(II) complexes [11, 12]. The position of λ_{max} for the Zn(II) complex suggests a tetrahedral geometry around the Zn(II) ion [13].

(c) The ^1H NMR spectra for Ni(II) and Zn(II) complexes in DMSO-d6 indicate the disappearance of the signal due to the imine proton of the =N–H group (δ = 6.2 ppm) at position II. This confirms that the bonding of the metal ion to metformin takes place through displacement of one proton from the imine =NH group at position II. The other four signals observed in the spectra of metformin at 2.8, 5.3, 6.4 and 4.3 ppm, which can be assigned to $\delta(CH_3)N$ (I = 6H), $\delta_{NH\ III}$ (I = 1H), $\delta_{NH\ VI}$ (I = 1H) and $\delta_{NH_2\ V}$ (I = 2H), respectively, are shifted by 0.8–1.2 ppm downfield in the spectra of the complexes. The shift of the signals downfield is due to the deshielding effect of the central metal atom on the metformin protons as a result of coordination.

(d) X-band EPR spectra of Co(II) and Cu(II) complexes were obtained in the solid state at 300 K

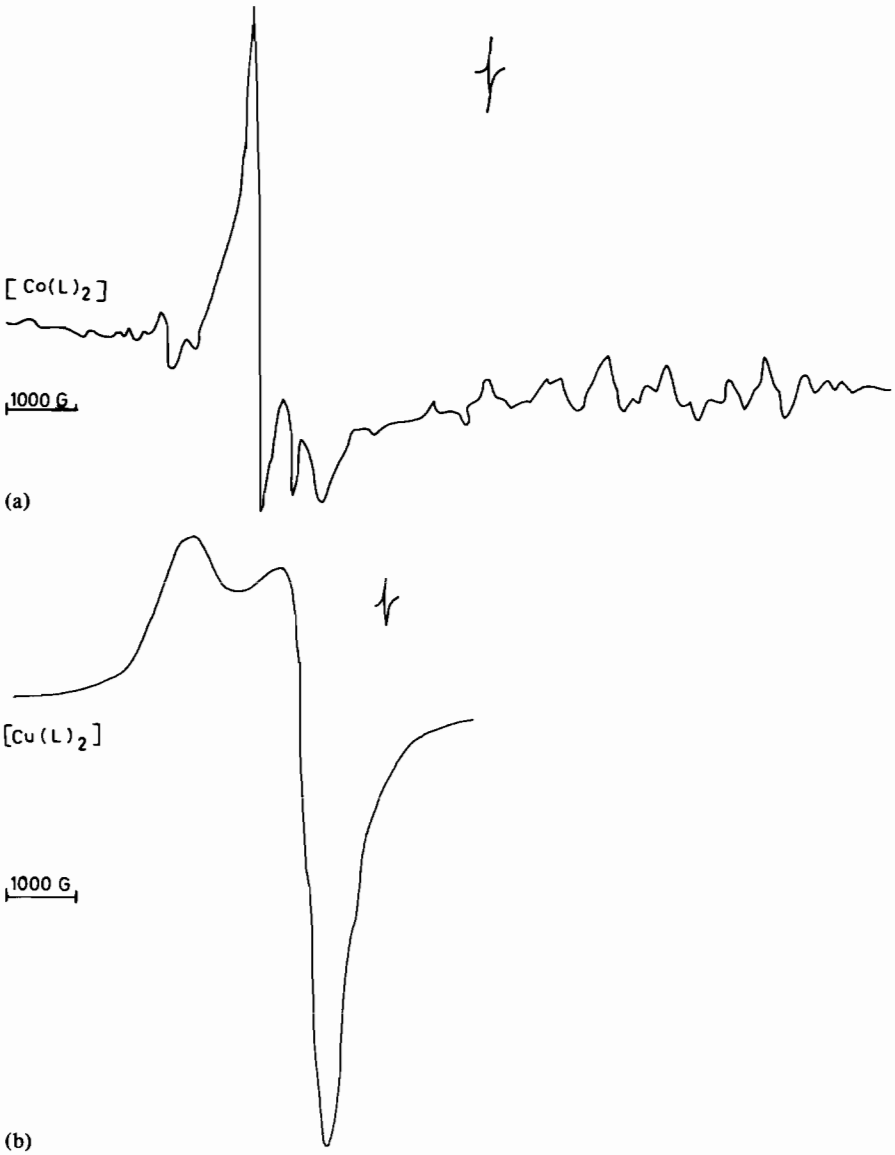
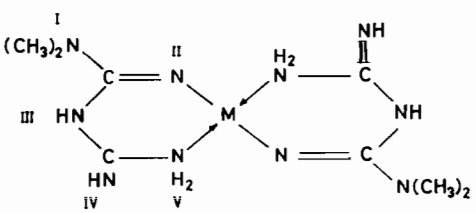


Fig. 1. EPR spectra of (a) $[Co(L_2)]$ and (b) $[Cu(L_2)]$ complexes.

and 77 K in order to elucidate the nature of the coordination around the Co(II) and Cu(II) ions. Figure 1 shows the EPR spectra of $[Co(L_2)]$ and $[Cu(L_2)]$ complexes at 77 K, the EPR spectra of Cu(II) complexes at 300 K is found to resemble that obtained at 77 K, whereas the Co(II) complex is EPR silent at room temperature. The shape of the EPR signals and the pattern of the g values (Table I) are very similar to square-planar Co(II) and Cu(II) complexes obtained by previous authors [14, 15]. Therefore one may suggest a square-planar geometry around the Co(II) or Cu(II) ion.

Based on knowledge gained from the present investigation, the bonding of the metal(II) ion to metformin can be formulated as follows:



Conditional Stability Constants

Since the formation of Co(II), Ni(II), Cu(II) and Zn(II) complexes with metformin is associated with the liberation of H^+ ions from the ligand, it is possible to apply the pH-metric method for the determination of the composition and conditional stability constant of the complexes formed [16].

TABLE II. Antimicrobiological Potential of Metformin and its Complexes

Microorganism	$[Co(L)_2]$ concentration (g/L)				$[Ni(L)_2]$ concentration (g/L)		$[Cu(L)_2]$ concentration (g/L)				$[Zn(L)_2]$ concentration (g/L)				L concentration (g/L)			
	10^{-2}	10^{-3}	10^{-4}	10^{-6}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-4}	10^{-8}	10^{-2}	10^{-3}	10^{-4}	10^{-8}	10^{-2}	10^{-3}	10^{-4}	10^{-8}
Kelebsiella aerogenes (I)	1.7	1.6			1.6	1.4	1.7	1.7	2.2	1.7	1.8	1.6	1.6	1.6	2.2	2.0	1.8	1.4
Sarcina lutea (II)							2.3	2.0							2.2	2.0	2.2	
Bacillus cereus (III)	2.0	1.5	2.0	2.9	1.6	2.3	1.3	1.8			1.8	1.9	1.8	1.2	1.8	1.4		
Staphyloccocus aureus (IV)	3.0	2.0					2.5	2.0			3.0	2.0	1.8	1.6	3.0	2.8	2.0	2.2
Escherichia coli (V)	2.2	2.1			2.0	2.1	2.0	1.7			2.2				1.6	2.0		
Candida albicans (VI)																		
Salmonella typhimurum (VII)	2.3	2.7	2.7	2.8	2.0	3.3	2.0	1.8	1.6		2.8	2.0			2.1	2.0		
Mycobacterium phlei (VIII)																		

The pH titration curves of the Co(II), Ni(II), Cu(II) and Zn(II) complexes under investigation are S-shaped; the volume of alkali consumed in the titration corresponds to the liberation of one H^+ ion per metal ion on complex formation. The titration curves for the Zn(II) complex exhibit inflection within the pH range 7.3–8.2 which can be ascribed to the formation of different types of complexes, probably of the hydroxo type.

The formation curves obtained for the different complexes investigated show that two types of chelates are formed having stoichiometric ratios 1:1 and 1:2 (M^{2+}:ligand). The conditional stability constants of the two types of complexes, log K_1 and log K_2, were then determined by the method of Bjerrum [17] and Albert [18], applying the least-squares method. The stability of the complexes increases in the order: Cu(II) > Ni(II) > Co(II) > Zn(II). This runs parallel with the order found by Irving and Williams [19].

Biological Activity

The results gained from testing metformin and the complexes under investigation against 8 microorganisms (Table II) reveal the following.

(i) Metformin is biologically active towards microorganisms **I**–**V** and **VII**, and is biologically inactive towards **VI** and **VII**: The biological activity for **IV** is independent of the concentration within the range 10^{-2}–10^{-8} g.

(ii) The $[Co(L_2)]$ complex is biologically active towards microorganisms **I**, **III**, **IV**, **V** and **VII**, and biologically inactive towards **II**, **VI** and **VII**. The complex shows higher activity towards **VI** and **VII** than does metformin itself at all concentrations in the range 10^{-2}–10^{-8} g, while the complex is only sensitive at the concentration range 10^{-2}–10^{-3} g to **I**, **IV** and **V**.

(iii) The $[Ni(L_2)]$ complex is biologically active towards microorganisms **I**, **III**, **V** and **VII**, and inactive towards **I**, **IV**, **VI** and **VII**. The biological activity is observed for the concentration range 10^{-2}–10^{-3} g.

(iv) The $[Cu(L_2)]$ complex shows activity towards microorganism **I** at all concentrations in the range 10^{-2}–10^{-3} g and towards **II**, **IV**, **V** and **VII** in the concentration range 10^{-2}–10^{-3} M, while for **VI** and **VIII** it is biologically inactive.

(v) The $[Zn(L_2)]$ complex shows high activity towards **I**, **IV** and **IV** at all concentrations from 10^{-2}–10^{-8} g, whereas for **V** and **VII** it is only sensitive within the concentration range 10^{-2}–10^{-3} g. The complex is inactive towards **II**, **VI** and **VII**.

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