

SYNTHESIS, PROPERTIES, STRUCTURE AND BIOACTIVITY *IN VITRO* OF MONONUCLEAR COMPLEXES OF MANGANESE AND NICKEL WITH HYDROXYETHYLTRIBENZIMIDAZOLYLMETHYL ETHYLENE DIAMINE

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Abstract—Six novel mononuclear complexes, $\text{MnCl}_2\text{L}\cdot\text{H}_2\text{O}$, $\text{Mn}(\text{SCN})_2\text{L}\cdot 2\text{H}_2\text{O}$, $\text{Mn}(\text{ClO}_2)_2\text{L}\cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2\text{L}\cdot 2\text{H}_2\text{O}$, $\text{Ni}(\text{SCN})_2\text{L}\cdot\text{H}_2\text{O}$, $\text{Ni}(\text{ClO}_4)_2\text{L}$ ($\text{L} = \text{N}$ -hydroxyethyl- $\text{N},\text{N}',\text{N}'$ -tribenzimidazolymethyl ethylene diamine), have been synthesized and characterized. The UV-visible spectra of nickel complexes reveal that L is a weak field ligand. The ESR spectra indicate the asymmetric electronic environment of the manganese ion in its complex. The crystal and molecular structures of $\text{Ni}(\text{SCN})_2\text{L}\cdot\text{H}_2\text{O}$ were determined by X-ray diffraction analysis. The nickel ions have distorted octahedral symmetry. The preliminary results of bioassay of the complexes showed that monomanganeses have anti-tumour activities *in vitro*.

Benzimidazole-containing ligands have great importance in mimicking metalloproteins and metalloenzymes.¹ Most literature is mainly concentrated on the mimicking of oxygen carriers.² Transition metal complexes as useful therapeutic agents for the treatment of various diseases have been of great interest in recent years.³ Understanding the relationship between structure and reactivity is a fundamental problem and a challenge in chemistry and biochemistry. Manganese is of industrial and biological importance. The site where oxidation of water gives dioxygen in photosynthesis and the electron transfer reaction of mitochondrial superoxide dismutase both involve manganese

ions.^{4,5} The syntheses and characterization of manganese coordination compounds are useful towards the understanding of the structure and reactivity of manganese sites in biological systems. This paper reports syntheses and properties of some novel complexes of manganese and nickel with N -hydroxyethyl- $\text{N},\text{N}',\text{N}'$ -tribenzimidazolymethyl ethylene diamine (L) and the crystal structure of a nickel complex. The preliminary bioassay of the complexes shows that the manganese complexes have antitumour activities *in vitro*.

EXPERIMENTAL

Materials

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The ligand L was synthesized by the method described by Gomez-Romero.⁶ $\text{Mn}(\text{ClO}_4)_2\cdot$

$6\text{H}_2\text{O}$, $\text{Mn}(\text{SCN})_2$, $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{SCN})_2 \cdot 0.5\text{H}_2\text{O}$ were prepared by our laboratory. Solvents used for synthetic experiments and for spectroscopic measurements were of analytical grade available commercially.

Measurements

Elemental analyses of C, H, N were carried out using a Perkin–Elmer 240 analyser. The metal contents were determined by EDTA titration. Conductances were measured with a DDS-11A conductometer. IR spectra were recorded on an IR-480 spectrometer. Electronic spectra were recorded on a Shimadzu UV-240 spectrophotometer. Magnetic moments were measured using the Faraday method. The susceptibilities were corrected by Pascal's constants. X-band ESR spectra were recorded on a JES-FEIXG ESR apparatus. The single crystal structure was determined by an Enraf–Nonius CAD4 diffractometer.

Syntheses of complexes

$\text{MnCl}_2\text{L} \cdot \text{H}_2\text{O}$ (**1**). An ethanolic solution containing 2 mmol $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$ was added to a hot ethanolic solution containing 1 mmol L with stirring. The mixture was heated for 2 h and then kept at room temperature for several days. The crystalline precipitate was collected and washed with cool ethanol, dried *in vacuo* (P_2O_5) at room temperature. The product was light pink. Found: C, 52.3; H, 4.6; N, 17.1; Mn, 8.6. Calc. for $\text{C}_{28}\text{H}_{32}\text{N}_8\text{O}_2\text{MnCl}_2$: C, 52.6; H, 5.0; N, 17.6; Mn, 8.6%. Molar conductivity Λ_{M} (CH_3OH) = $109 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$, magnetic moment μ_{eff} = 5.91 B.M.

$\text{Mn}(\text{SCN})_2\text{L} \cdot 2\text{H}_2\text{O}$ (**2**). This brown-red complex was obtained using a similar method for (**1**) by replacing $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$ with $\text{Mn}(\text{SCN})_2$. Found: C, 51.7; H, 4.7; N, 19.8; Mn, 7.9. Calc. for $\text{C}_{30}\text{H}_{34}\text{N}_{10}\text{O}_3\text{S}_2\text{Mn}$: C, 51.4; H, 4.9; N, 20.0; Mn, 7.8%. Λ_{M} = $97.7 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$, μ_{eff} = 5.96 B.M.

$\text{Mn}(\text{ClO}_4)_2\text{L} \cdot 2\text{H}_2\text{O}$ (**3**). An ethanolic solution containing 1 mmol L was added to an ethanolic solution containing 2 mmol $\text{Mn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$. The resulting solution was heated to reflux for 2 h and evaporated to about 5 ml. Then the solution was added to diethyl ether. The white precipitate was obtained, which was dissolved in ethanol and deposited with diethyl ether twice, then dried *in vacuo*. Found: C, 42.7; H, 3.8; N, 14.1; Mn, 7.0. Calc. for $\text{C}_{28}\text{H}_{34}\text{N}_8\text{O}_{11}\text{MnCl}_2$: C, 42.9; H, 4.3; N, 14.3; Mn, 7.0%. Λ_{M} = $146 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$, μ_{eff} = 5.78 B.M.

$\text{NiCl}_2\text{L} \cdot 2\text{H}_2\text{O}$ (**4**). The product was obtained by

a similar procedure to **1**. Found: C, 50.7; H, 4.7; N, 16.7; Ni, 9.3. Calc. for $\text{C}_{28}\text{H}_{34}\text{N}_8\text{O}_3\text{NiCl}_2$: C, 50.9; H, 5.1; N, 17.0; Ni, 8.9%. $\Lambda = 146 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$, μ_{eff} = 3.05 B.M.

$\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$ (**5**). The blue–purple complex was obtained using a similar method to **1** by replacing the MnCl_2 –ethanol with $\text{Ni}(\text{SCN})_2$ –ethanol–acetonitrile solution. Found: C, 52.9; H, 4.8; N, 21.4; Ni, 8.8. Calc. for $\text{C}_{30}\text{H}_{32}\text{N}_{10}\text{O}_2\text{S}_2\text{Ni}$: C, 52.4; H, 4.7; N, 20.4; Ni, 8.5%. Λ_{M} = $83.2 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$, μ_{eff} = 3.17 B.M.

$\text{Ni}(\text{ClO}_4)_2\text{L}$ (**6**). The blue–purple compound was prepared by a similar method to **3**. Found: C, 44.3; H, 3.9; N, 15.0; Ni, 7.8. Calc. for $\text{C}_{28}\text{H}_{30}\text{N}_8\text{O}_9\text{NiCl}_2$: C, 44.7; H, 4.0; N, 14.9; Ni, 7.8%. Λ_{M} = $146 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$, μ_{eff} 3.88 B.M.

Preparation, X-ray data collection, structure determination and refinement of a single crystal of $\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$

The dilute methanol solution of $\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$ was evaporated very slowly for about 6 months until a suitable size of single crystal for diffraction was obtained.

A transparent blue crystal of $\text{C}_{30}\text{H}_{32}\text{N}_{10}\text{NiO}_2\text{S}_2$, having approximate dimensions $0.2 \times 0.2 \times 0.3 \text{ mm}$ was mounted on a glass fibre in a random orientation. The determination of the unit cell and the data collection were performed with Mo- K_{α} radiation ($\lambda = 0.71073 \text{ \AA}$) on an Enraf–Nonius CAD4 diffractometer equipped with a graphite crystal monochromator. A total of 4506 independent reflections were collected in the range $2^\circ \leq \theta \leq 22^\circ$ by the ω – 2θ scan technique at $23 \pm 1^\circ\text{C}$ in which 2770 reflections with $[I \geq 3\sigma(I)]$ were considered to be observed and used in the succeeding refinement. A correction for Lp effects was applied to the data.

Crystals are triclinic, space group $P\bar{1}$, with $a = 10.304$ (3), $b = 10.615$ (4), $c = 17.054$ (8) \AA , $\alpha = 105.79$ (4), $\beta = 96.80$ (4), $\gamma = 93.86$ (3) $^\circ$, $V = 1773$ (3) \AA^3 , $M_r = 687.47$, $Z = 2$, $D_x = 1.29 \text{ g cm}^{-3}$, $\mu = 7.01 \text{ cm}^{-1}$, $F(000) = 716$.

The structure was solved by the Direct Method (MULTAN 82). The position of nickel was located on an E-map. All the other non-hydrogen atoms were determined from successive differences Fourier syntheses.

The final refinement by full-matrix least-squares with anisotropic thermal parameters for non-hydrogen atoms covered with agreement factors of $R = \Sigma|\Delta F|/\Sigma|F_o| = 0.096$ and $R_w = [\Sigma w|\Delta F|^2/\Sigma|F_o|^2]^{1/2} = 0.100$ (unit weights for all observed reflections).⁷ The highest peak on the final difference Fourier map had a height of 0.91 e \AA^{-3} .

All calculations were performed on a PDP 11/44 computer using the SDP-PLUS program system.

RESULTS AND DISCUSSION

General properties

Elemental analyses are in agreement with monomeric formulae. The molar conductance values in methanol show that complexes **1**, **2**, **4** and **5** are 1 : 1 electrolytes, and **3** and **6** 2 : 1 electrolytes.⁸ Cl^- and SCN^- are partially coordinated to the metal ions. ClO_4^- is outside the coordination sphere. The magnetic moments of the complexes reveal that all the complexes are in high spin states.

IR spectra of all the complexes are similar and have the features of ligand L. The stretching vibrations of $\text{C}=\text{N}$ are at about 1450 and 1470 cm^{-1} in the complexes (free ligand L: 1430 cm^{-1}). The $\nu_{\text{C-H}}$ appearing at 1270 and $\delta_{\text{C-H}}$ ($\text{C}=\text{C}-\text{H}$) at 745 cm^{-1} remain about the same compared to those of the free ligand L. IR spectra indicate the formation of the complexes.

The $\nu_{\text{ClO}_4^-}$ is a strong broad absorption at about 1100 cm^{-1} . The ν_{SCN} absorptions are at 2050 and 840 cm^{-1} for compound **2**, and 2120, 2050 and 850 cm^{-1} for compound **5**. The ν_{SCN} indicates that the two SCN^- may be different in complexes **2** and **5**.

The structure of $\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$

Tables of final atomic positional and thermal parameters, full lists of bond lengths and angles, and equations of the least-squares plane have been deposited with the Cambridge Crystallographic Data Centre. The molecular structure of complex $\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$ is showed in Fig. 1. Some bond lengths and angles are given in Table 1.

In molecule $\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$, L is a pentadentate ligand. The five coordinated atoms are N(1), N(4), N(6) (benzimidazole), N(3), N(8) (amine). The OH of the hydroxyethyl group is not coordinated to nickel in the molecule. The sixth coordinated atom of nickel comes from a SCN^- . The six atoms around Ni^{2+} make up a distorted octahedral coordination sphere.

The distances of Ni—N in the molecule range from 2.006 to 2.183 Å (mean 2.092 Å). The shortest is $\text{SCN}-\text{Ni}$. Two amine Ni—N(3), Ni—N(8) are the largest.

The coordinated SCN^- in the molecule is basically linear with angle S—C—N 174.8°. The angle S—C—N in uncoordinated SCN^- is 148°. The bond S—C becomes longer when SCN^- is coordinated to Ni^{2+} . The difference between the two

SCN^- in the crystal explained the IR absorptions of complex **5** at 2120 and 2050 cm^{-1} .

Electronic spectra

The UV-visible spectra of the complexes in CH_3OH were recorded. There is no $d-d$ transition observed in the manganese complexes. Three bands of $d-d$ transitions appear in the nickel complexes.

The $d-d$ spectra of the mononickels are assigned as Table 2.¹⁰ Using the O_h model, the calculated ligand field parameters are also listed in Table 2.

The nephelauxetic effect parameter β reveals that the nickel complexes have about the same degree of covalency. L is a weak field ligand, which is in accordance with the magnetic moments of the complexes.

ESR spectra

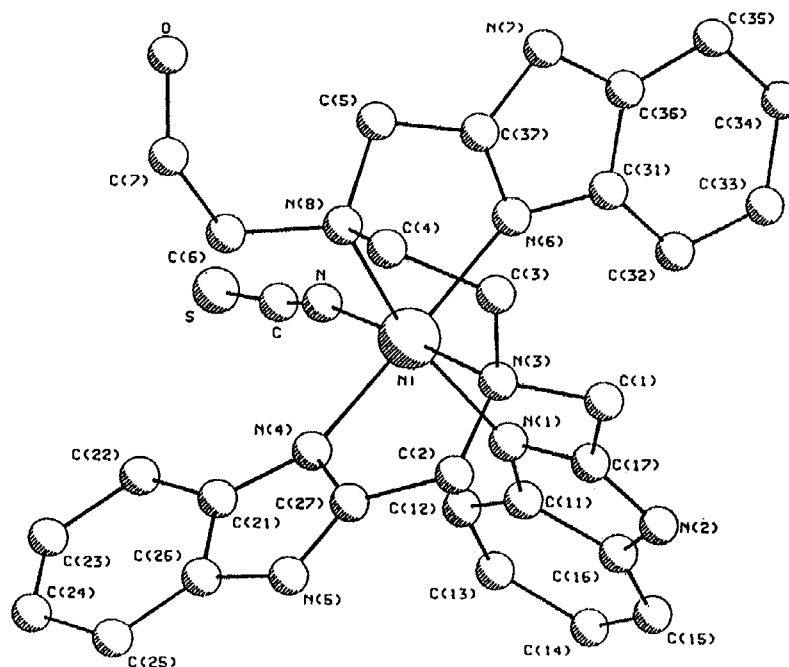
The powder ESR spectra of compounds **1**, **2**, **3** and **5** at room temperature were recorded. The effective g value (from ESR) for compound **5** is 2.253 which is in the range of that of octahedral nickel compounds. This is also in accordance with the structure and magnetic moment.¹¹

The ESR spectra of the three manganese complexes are showed in Fig. 2. They are indicative of the asymmetric electronic environment of the manganese in its complex and the complexes are significantly distorted from octahedral symmetry. For complexes with strong axially symmetric electric field $\lambda = 0$ ($\lambda = E/D$, $D \neq 0$ and $E = 0$, where D and E are the axial and rhombic zero-field splitting parameters, respectively), effective g values are predicted at $g = 2$ and 6.¹² The absence of a single strong resonance at $g = 6$ indicates that the complex does not satisfy a strong axially symmetric electronic environment with $D \geq 0.2$. A rhombic distortion from octahedral geometry can be deduced for the complex.¹³ The effective g values are 5.9 and 2.03 for complex **2** and 5.14 and 2.03 for complex **1**.

The highly distorted electronic environment for **3** is demonstrated by the many features of the spectrum $g = 13.2$, 4.45, 2.84 and 1.54.¹⁴ A more detailed analysis of the spectrum is not possible at the present time.

Antitumour activities *in vitro*

The antitumour activities *in vitro* were determined using the MTT method.¹⁵ The carcinoma used were L1210 murine leukaemia, human H60 leukaemia cells, human hepatocellular carcinoma Bel-7402, ESCL human carcinoma of the oeso-

Fig. 1. Perspective view of the $\text{Ni}(\text{SCN})_2\text{L} \cdot \text{H}_2\text{O}$.Table 1. Selected bond distances (\AA) and angles ($^\circ$)^a

Ni—N	2.006(5)	Ni—N(4)	2.070(5)
Ni—N(1)	2.045(6)	Ni—N(6)	2.089(5)
Ni—N(3)	2.160(5)	Ni—N(8)	2.183(6)
S—C	1.660(7)	S(40)—C(41)	1.49(2)
N—C	1.21(1)	C(41)—N(42)	1.11(1)
N—Ni—N(1)	100.1(2)	N(1)—Ni—N(8)	161.5(2)
N—Ni—N(3)	178.0(2)	N(3)—Ni—N(4)	81.0(2)
N—Ni—N(4)	97.1(3)	N(3)—Ni—N(6)	88.9(2)
N—Ni—N(6)	93.2(2)	N(3)—Ni—N(8)	83.0(2)
N—Ni—N(8)	97.1(2)	N(4)—Ni—N(6)	167.1(2)
N(1)—Ni—N(3)	80.0(2)	N(4)—Ni—N(8)	90.5(2)
N(1)—Ni—N(4)	93.9(2)	N(6)—Ni—N(8)	80.4(2)
N(1)—Ni—N(6)	92.2(2)	Ni—N—C	165.8(6)

^aNumbers in parentheses are estimated standard deviations in the least significant digits.

Table 2. Ligand field parameters for the nickel complexes^a

Compound	$\nu_1(\text{cm}^{-1})^b$	$\nu_2(\text{cm}^{-1})$	$\nu_3(\text{cm}^{-1})$	$\Delta(\text{cm}^{-1})^c$	$B(\text{cm}^{-1})$	β
4	12700	17200	27500	10900	801	0.77
5	12700	17400	28200	10900	862	0.83
6	12800	17100	28200	10600	891	0.86

^a $\nu_1 = {}^3A_2 \rightarrow {}^3T_2$; $\nu_2 = {}^3A_2 \rightarrow {}^3T_1$; $\nu_3 = {}^3A_2 \rightarrow {}^3T_1$ (P).

^b ${}^3A_2 \rightarrow {}^1E$

^c $\nu_1 = \Delta$. $\beta = B/B_0$; $B_0 = 1040 \text{ cm}^{-1}$.

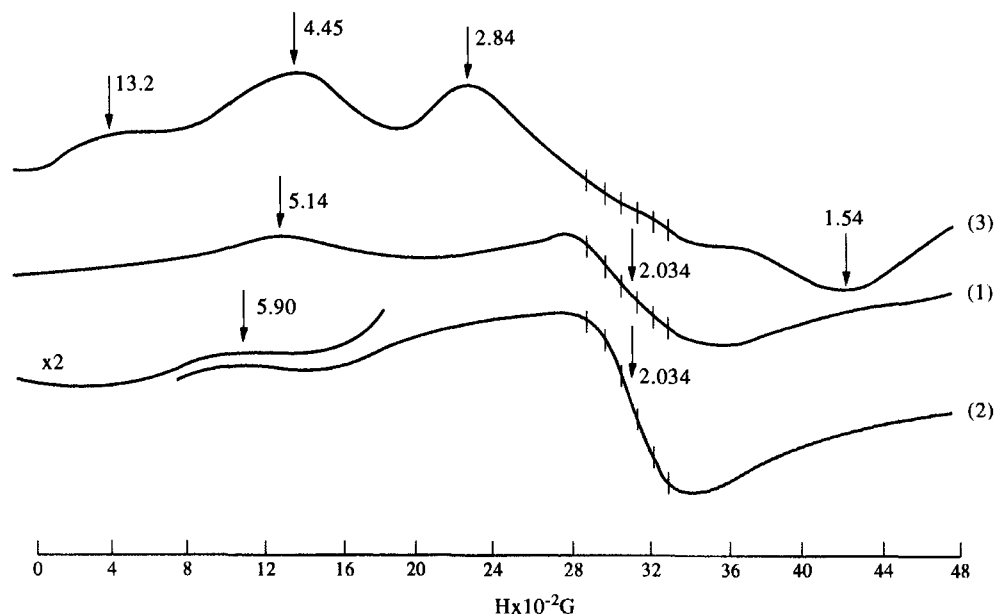


Fig. 2. Powder ESR spectra of manganese complexes at room temperature.

phagus, human nasopharyngeal carcinoma KB, human carcinoma ventriculi BGC-823. The results of the preliminary bioassay are shown in Table 3.

The result indicates that the manganese complexes have antitumour activities *in vitro*, but the nickel complexes have no activity. The counterions influence the IC_{50} (the concentration of the complex when the carcinoma cell were inhibited up to 50%). The detailed results are not available at the present time.

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7. The quality of the crystal is not very high, further improving the R factor is not possible. (The single crystal is very difficult to obtain and readily disintegrates when separated from the parent liquid.)

Table 3. Antitumour activities *in vitro*

Complex	Bel-7402	L1210	IC_{50} ($\mu\text{g} \times \text{ml}^{-1}$)		ESCL	BGC-823
			HL60	KB		
1	16.758	7.945	—	—	—	13.042
2	9.332	5.673	22.808	8.814	8.300	—
3	8.206	~5	16.871	~5	7.905	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—

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