Metal Complexes of Anti-inflammatory Drugs. Part V. Meclofenamic Acid Complexes of Manganese(II), Iron(III), Cobalt(II), Nickel(II), Copper(II) and Zinc(II)

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Abstract

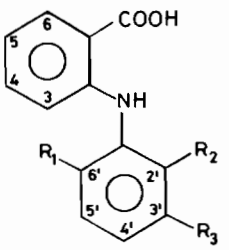
The preparation, spectroscopic and magnetic properties are reported for complexes of manganese(II), iron(III), cobalt(II), nickel(II), copper(II) and zinc(II) with the anti-inflammatory drug meclofenamic acid. In all the complexes studied the meclofenamic acid acts as a chelate monoanionic ligand with coordination involving the carboxylate oxygen atom and the nitrogen atom of the secondary amine group. The complexes appear to have an octahedral stereochemistry involving two chelate meclofenamate ligands in the case of divalent central metal ions and three chelate meclofenamate ligands in the iron(III) complex. The manganese(II) and copper(II) complexes exhibit marked superoxide dismutase activity in the nitro blue tetrazolium assay.

Introduction

Rheumatoid arthritis is characterised by extensive infiltration of activated polymorphonuclear leucocytes into the joint space [1]. Activated leucocytes produce superoxide radicals O_2^- by means of the enzyme NADPH oxidase located on the plasma membrane [2]. The function of the radical is to aid in the destruction of ingested microorganisms, although a significant fraction of the total superoxide produced escapes from the surface of the leucocyte [3]. Intracellular oxidative damage is controlled by the superoxide dismutases; a group of enzymes that are able to disproportionate superoxide radicals [4]. Extracellular concentration of the metalloprotein is low and it has been suggested that superoxide radicals may be involved in the depolymerisation of hyaluronic acid and general tissue damage that can accompany the inflammatory process [5].

The oxidative damage that may accompany superoxide production appears to result from the secondary generation of the hydroxyl radical $^{\bullet}OH$ via a metal catalyzed Haber-Weiss reaction [6]. Molecules capable of intercepting $O_2^{\bullet-}$ may prevent the formation of more reactive oxygen species and subsequent tissue damage. Superoxide dismutase has been shown to be an inefficient anti-inflammatory agent on intravenous administration due to its short half-life in extracellular fluid and rapid renal clearance [7]. Superoxide anion radical scavenging is not restricted to the metalloprotein superoxide dismutase. Many low molecular mass copper(II) compounds as well as free hydrated copper(II) and manganese(II) ions are known to bring about the decomposition of $O_2^{\bullet-}$ [8]. Recently, a number of investigations have focussed on the complexing of anti-inflammatory drugs to transition metal ions with a view to isolating anti-inflammatory agents with improved potency, less adverse side effects and longer residence time in extracellular fluid [9].

Non-steroidal anti-inflammatory agents may be characterised chemically as lipophilic, moderately strong organic acids. Although many such agents are arylalkanoic acids considerable attention has been directed towards the anti-inflammatory activity of the *N*-arylanthranilic acids (generic name fenamic acids) and the degree to which substitution can affect activity [10].



Meclofenamic acid

In general, substitution on the A-ring reduces activity whereas a large number of 2′,3′- and 2′,3′,6′-substituted *N*-arylanthranilic acids have high anti-

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TABLE I. Analysis of Meclofenamic Acid Complexes

	Found (%)			Required (%)		
	C	H	N	C	H	N
$Mn(MECLO)_2 \cdot 2H_2O$	49.71	3.00	3.98	49.34	3.52	4.11
$Fe(MECLO)_3 \cdot 2H_2O$	51.23	3.90	4.22	51.59	3.48	4.30
$Co(MECLO)_2$	51.63	3.50	4.20	51.78	3.08	4.32
$Ni(MECLO)_2 \cdot 5H_2O$	45.13	3.70	3.30	45.47	4.06	3.79
$Cu(MECLO)_2 \cdot 2H_2O$	48.41	3.38	3.68	48.73	3.48	4.06
$Zn(MECLO)_2 \cdot H_2O$	49.51	3.07	4.13	49.93	3.27	4.16

inflammatory activity. Three *N*-arylanthranilic acids, namely mefenamic acid ($R_1 = H$; $R_2 = R_3 = CH_3$), flufenamic acid ($R_1 = R_2 = H$; $R_3 = CF_3$) and meclofenamic acid ($R_1 = R_2 = Cl$; $R_3 = CH_3$) have attracted particular attention as anti-arthritis drugs.

N-(2',6'-dichloro-3'-methylphenyl)anthranilic acid (CI-583; approved name meclofenamic acid; abbreviation MECLO) has proved to be the most potent of the three in a variety of animal models and is currently under clinical investigation [11]. In this paper we report the preparation and properties of complexes formed between meclofenamic acid and some first row transition metal ions and examine the superoxide radical scavenging activity of these compounds.

Experimental

Meclofenamic acid was supplied by Warner-Lambert/Parke-Davis and was used without further purification. The metal salts were of Analar reagent grade with the exception of iron(III) chloride hexahydrate (B.D.H.).

Methionine, nitroblue tetrazolium and riboflavin were purchased from Sigma and sodium cyanide, potassium dihydrogen orthophosphate and potassium hydroxide from B.D.H.

Preparation of Complexes

Meclofenamic acid (1.0 g, 1 mol equivalent) was dissolved in 0.1 M sodium hydroxide (40 cm^3) by slow addition of the alkali with constant agitation and warming to $\leqslant 60$ °C. The volume of alkali was deliberately kept to the minimum to exclude excess hydroxide ions. The resulting solution was filtered to remove excess undissolved ligand and the filtrate was added slowly with stirring to a cold solution of the metal chloride (0.5 mol equivalent M(II) ions, 0.33 mol equivalent Fe(III) ions) in 20 cm^3 of distilled water. The resulting precipitate was filtered off under vacuum, washed with cold distilled water and dried. All the metal complexes were obtained in high yield (>90%) as microcrystalline powders.

Physical Measurements

Carbon, hydrogen and nitrogen analyses were performed in the microanalytical laboratory of the University College of North Wales, Bangor. The diffuse reflectance spectra were determined on a Beckmann DK-2A spectrophotometer fitted with a standard reflectance attachment. Infrared spectra (4000–200 cm^{-1}) were recorded on a Perkin-Elmer 580 spectrometer as caesium bromide discs. Room temperature magnetic susceptibility measurements were made on powdered samples using a Johnson Matthey Magnetic Susceptibility Balance MSBI.

The superoxide dismutase activity of the complexes was assayed by the nitroblue tetrazolium method [12] with absorbance measured on a Pye Unicam SP8-100 spectrophotometer.

Results and Discussion

The microanalytical results show the complexes to be hydrates with the drug present as a mono-anionic ligand (Table I).

Infrared Spectra

A partial assignment of the absorption bands observed for meclofenamic acid and the metal complexes is given in Table II. Meclofenamic acid possesses two potential donor sites: (i) carboxylate oxygen; (ii) secondary amine nitrogen. Due to the 1,2-position of these donor groups in the molecule six-membered chelate ring formation is possible on complexing.

The IR spectrum of the ligand shows an absorption at 3330 cm^{-1} characteristic of an N–H stretching vibration of an aromatic secondary amine group [13]. This band is retained in the complexes but is shifted by 10–30 cm^{-1}. The N–H deformation observed in the ligand at 1606 cm^{-1} as a weak absorption appears as a strong band in the complexes. The C–N stretching vibration is observed at 1320 cm^{-1} in the free ligand and as a stronger absorption at 1285 cm^{-1} in the divalent metal complexes [14].

The broadness of the OH stretching band at 3040 cm^{-1} and the out-of-plane OH deformation band at

TABLE II. IR Absorption Bands (cm^{-1})

	N–H stretching	N–H deformation	C–N stretching	O–H stretching	O–H deformation	C=O stretching	COO^- symmetric stretching
MECLO	3330m	1606w	1320m	3040m, br	915m, br	1655s	
$Mn(MECLO)_2 \cdot 2H_2O$	3330m	1612s	1285s				1395s, br
$Fe(MECLO)_3 \cdot 2H_2O$	3320s	1603m	1317m				1403m
$Co(MECLO)_2$	3300m	1610s	1285s				1390s, br
$Ni(MECLO)_2 \cdot 5H_2O$	3280m	1611s	1287s				1394s, br
$Cu(MECLO)_2 \cdot 2H_2O$	3310m	1612s	1287s				1395s, br
$Zn(MECLO)_2 \cdot H_2O$	3320m	1594s	1285s				1398s, br

TABLE III. Diffuse Reflectance Spectra[a] and Room Temperature Magnetic Moments

	μ_{eff} (BM)	Absorption bands (cm^{-1})		
$Mn(MECLO)_2 \cdot 2H_2O$	5.54			
$Fe(MECLO)_3 \cdot 2H_2O$	4.46			
$Co(MECLO)_2$	4.55	$\nu_1[^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)]$ 7273(0.32)	$\nu_2[^4T_{1g}(F) \rightarrow {}^4A_{2g}]$ 16260(sh, 0.37)	$\nu_3[^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)]$[b] 17094(0.39) 18349(sh, 0.35)
$Ni(MECLO)_2 \cdot 5H_2O$	3.40	$\nu_1[^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)]$ 8333(0.33)	$\nu_2[^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)]$[b] 13793(0.23)	
$Cu(MECLO)_2 \cdot 2H_2O$	2.17	11765(0.51)		

[a]Figures in parentheses represent intensity of the band on the arbitrary Beckmann scale. [b]For convenience pseudo-octahedral symmetry is assumed in assigning these bands.

915 cm^{-1} in the free drug spectrum is indicative of a hydrogen bonded carboxylic acid.

Strong O–H···N intramolecular hydrogen bonding has been observed previously in the infra-red spectra of aminobenzoic acids [15]. The absence of both the O–H stretch and deformation bands from the metal complex spectra indicate that the meclofenamic acid is attached to the metal centre as an anionic ligand via the carboxylate ion.

The strong band observed at 1655 cm^{-1} in the free ligand is assigned to the carbonyl stretch of the carboxylic acid group [16]. Ionisation of carboxylic acids results in equilibration of the two C–O bonds of the carboxylate group and disappearance of the characteristic carbonyl absorption. Two new bands are expected in the ranges 1610–1550 cm^{-1} and 1430–1300 cm^{-1} corresponding to the anti-symmetric and symmetric vibrations of the $-COO^-$ structure [14]. In the metal complexes of meclofenamic acid the strong carbonyl band disappears and is replaced by a strong, broad absorption at 1394 ± 4 cm^{-1} that is assigned to the symmetric vibration of the carboxylate group [17]. The anti-symmetric vibration of this group was not detected.

The absence of the O–H absorption bands and the replacement of the C=O vibration by a band assigned to the carboxylate group in the spectra of the metal complexes is indicative of metal attachment as an anionic ligand via the carboxylate oxygen atom. The retention of the secondary amine vibration with the observed shift to lower frequencies of the ν(N–H) and ν(C–N) bonds is consistent with metal attachment via the nitrogen atom of the amine group and the resultant formation of a six-membered chelate ring.

Electronic Spectra

Meclofenamic acid and all the complexes exhibit a moderately intense charge transfer band above 22000 cm^{-1}. The reflectance spectra of the $Zn(MECLO)_2 \cdot H_2O$ (d^{10}) complex, and of meclofenamic acid itself, in the region 4000–22000 cm^{-1} are quite similar and show only a number of low intensity bands that are considered to be infra-red overtones. The reflectance spectrum of the zinc(II) complex has been used as reference spectrum for the other complexes as the absorption bands are slightly more intense than in the free meclofenamic acid spectrum. The diffuse reflectance spectra for the $Mn(MECLO)_2 \cdot 2H_2O$ and $Fe(MECLO)_3 \cdot 2H_2O$ complexes are similar to that of the free ligand spectrum and to the spectrum of $Zn(MECLO)_2 \cdot H_2O$ except for

a pronounced shoulder at 21 050 cm^{-1} that forms part of the charge transfer band. This shoulder in the iron(III) spectrum is possibly a ligand → metal charge transfer band. The absence of appreciable absorption at lower energy than the charge transfer edge is consistent with a pseudo-octahedral environment around the metal ions. As these ions have a d^5 electron configuration, the ground state in the high spin complexes is 6S and since there are no other sextet energy levels, all d–d transitions are spin-forbidden and therefore extremely weak [18]. The observed magnetic moments of 4.46 BM (Fe^{3+} complex) and 5.54 BM (Mn^{2+} complex) are consistent with high spin complexes [19].

The complex, $Co(MECLO)_2$, has a magnetic moment of 4.55 BM which lies within the range normally observed for octahedral cobalt(II) complexes [19]. The reflectance spectrum consists of two low intensity absorption bands, the first a broad band at 7273 cm^{-1} and the second a more complex envelope centred at 17 094 cm^{-1} but incorporating a shoulder on either side of the main peak. The band at 7273 cm^{-1} is assigned (assuming pseudo-octahedral symmetry) to the $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(\nu_1)$ transition. The band system centred at 17 094 cm^{-1} probably arises from the $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(\nu_3)$ transition in octahedral symmetry which is known to be split in complexes of D_{4h} symmetry or less. The transition $^4T_{1g}(F) \rightarrow {}^4A_{2g}(\nu_2)$ in octahedral symmetry is spin allowed but known to be weak [20]. The ν_2 band is thought to be partially obscured by the ν_3 band, appearing as a shoulder at 16 260 cm^{-1} on the low energy side of the ν_3 absorption. In view of the fact that the microanalysis results indicate a cobalt(II) complex that is not hydrated, it is tentatively suggested that the octahedral stereochemistry around the cobalt(II) ion is completed by distant coordination of the 2′- or 6′-chlorine atom of the B ring of the ligand. Molecular models indicate that an $MO_2N_2Cl_2$ chromaphore is possible.

The positions and low intensity of the absorption bands in the reflectance spectra for $Ni(MECLO)_2 \cdot 5H_2O$ suggest a pseudo-octahedral stereochemistry around the nickel(II) ion [21]. The reflectance spectrum consists of two broad, low intensity absorption bands centred at 8333 cm^{-1} and 13 793 cm^{-1} together with a low intensity shoulder at 23 122 cm^{-1} that forms part of the charge transfer edge. Three bands are expected for octahedral Ni(II) complexes. On the basis of pseudo-octahedral symmetry the band at 8333 cm^{-1} is assigned to the $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$ transition and that at 13 793 cm^{-1} to the $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ transition. The $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$ transition is often obscured by the charge transfer band and on this basis the weak shoulder at 23 122 cm^{-1} is tentatively assigned to this transition. The room temperature magnetic moment of μ_{eff} = 3.40 BM is at the top of the range normally observed for octahedral nickel(II) complexes but it has been shown that tetragonal complexes may have moments as high as 3.5 BM [22].

The reflectance spectra of $Cu(MECLO)_2 \cdot 2H_2O$ consists of a broad, moderately intense absorption with a maximum at 11 765 cm^{-1} and a pronounced shoulder at 22 222 cm^{-1} that forms part of the charge transfer peak. The spectrum is similar to those reported for copper compounds in which the copper-(II) ion is in an octahedral environment with a very large tetragonal distortion [23]. For a tetragonally distorted octahedral copper(II) complex the ^2E_g and $^2T_{2g}$ states in O_h symmetry split, resulting in the three spin-allowed transitions $^2B_1 \rightarrow {}^2B_2$, $^2B_1 \rightarrow {}^2E$ and $^2B_1 \rightarrow {}^2A_1$. The distortion can be such as to cause the three transitions to remain unresolved or only partially resolved in the spectrum [24]. The position and intensity of the absorption bands indicate that the copper(II) ion in the $Cu(MECLO)_2 \cdot 2H_2O$ complex is in just such a distorted octahedral environment. The magnetic moment of μ_{eff} = 2.17 BM for this complex indicates the absence of any interaction between the metal atoms.

Superoxide Assay

The superoxide dismutase activity of the drug and the metal complexes were assayed by their ability to inhibit the reduction of nitroblue tetrazolium as reported previously [12]. The superoxide scavenging data (Table IV) indicates that the Fe(III), Ni(II) and Zn(II) complexes are only marginally more active than the free drug, whilst the Co(II) complex is moderately active. The $Mn(MECLO)_2 \cdot 2H_2O$ and $Cu(MECLO)_2 \cdot 2H_2O$ complexes exhibit a greatly increased superoxide dismutase activity compared with the parent drug molecule.

TABLE IV. Superoxide Assay

Additive	Absorbance increase at 560 nm compared with blank (%)
MECLO	87
$Mn(MECLO)_2 \cdot 2H_2O$	8
$Fe(MECLO)_3 \cdot 2H_2O$	71
$Co(MECLO)_2$	47
$Ni(MECLO)_2 \cdot 5H_2O$	61
$Cu(MECLO)_2 \cdot 2H_2O$	0
$Zn(MECLO)_2 \cdot H_2O$	65

Naturally occurring superoxide dismutases are metalloproteins containing copper-zinc, manganese or iron as the prosthetic group [25]. The enzymes are able to scavenge superoxide radical anions to produce oxygen and hydrogen peroxide.

$$2O_2^{-\cdot} + 2H^+ \longrightarrow H_2O_2 + O_2$$

The mechanism believed to be operating in the metalloproteins involves a one-electron reduction of a metal ion by superoxide followed by re-oxidation of the reduced metal ion by a second superoxide anion.

$$E\text{–}M^{n+} + O_2^{-\cdot} = E\text{–}M^{(n-1)+} + O_2$$

$$E\text{–}M^{(n-1)+} + O_2^{-\cdot} + 2H^+ = E\text{–}M^{n+} + H_2O_2$$

(E = Enzyme)

Metal complexes that can undergo such redox cycling are likely to function as superoxide scavengers. It is assumed that electron transfer between the metal centre and superoxide anion radicals occurs through direct binding [26]. A fast exchange of coordinated water and limited steric hindrance to the approach of the superoxide anion are considered essential requirements for the successful binding of the $O_2^{-\cdot}$ radical [27]. We consider that both these requirements are satisfied in the tetragonally distorted complexes $Cu(MECLO)_2 \cdot 2H_2O$ and $Mn(MECLO)_2 \cdot 2H_2O$.

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