Metal Complexes of Anti-inflammatory Drugs. Part IV. Tenoxicam Complexes of Manganese(II), Iron(III), Cobalt(II), Nickel(II) and Copper(II)

A. BURY, A. E. UNDERHILL
Chemistry Department, University College of North Wales, Bangor, U.K.
D. R. KEMP, N. J. O'SHEA, J. P. SMITH and P. S. GOMM
Chemistry Department, Gresham's School, Holt, Norfolk, U.K.

(Received March 16, 1987)

Abstract

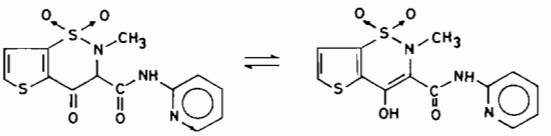
The preparation, spectroscopic and magnetic properties are reported for complexes of manganese(II), iron(III), cobalt(II), nickel(II) and copper(II) with the anti-inflammatory drug tenoxicam. In all the complexes studied the tenoxicam acts as a chelate monoanionic ligand with coordination involving the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

Introduction

Phagocytic cells release reactive oxygen species upon exposure to inflammatory stimuli [1]. During phagocytosis molecular oxygen is reduced initially to the superoxide radical anion $O_2^{\bullet -}$ and hydrogen peroxide H_2O_2 but if this is not removed by an efficient scavenger mechanism more potent oxygen species such as the hydroxyl radical $^{\bullet}OH$ and singlet oxygen ΔgO_2 result [2]. Oxygen derived free radicals are known to interact with various essential macromolecules and contribute to the tissue damage observed in inflammatory disorders [3]. Under normal circumstances, tissue damage is prevented by the stable intracellular enzyme superoxide dismutase, which efficiently scavenges the $O_2^{\bullet -}$ radical by catalysing its disproportionation to hydrogen peroxide and oxygen [4]. Eukaryotic cells contain two types of superoxide dismutase, a copper–zinc enzyme found in the cytosol [5] and a manganese enzyme found exclusively in the mitochondrial matrix of certain species while also present in the cytosol of other species [6].

Recent work suggests that oxygen radical production is inhibited not just by metalloproteins but also by free hydrated copper(II) ions [7] and low molecular mass copper(II) complexes [8, 9]. Copper complexes of a number of non-stereoidal anti-inflammatory drugs have been reported to be more potent anti-inflammatory agents and less ulcerogenic than the parent anti-inflammatory drug [10].

Although many non-steroidal anti-inflammatory agents are arylalkanoic acids [11] recent interest has focussed on a series of enolizable amides [12]. Tenoxicam (Ro12-0068; abbreviation TENOX) is a thienothiazine derivative of the oxicam class of non-steroidal anti-inflammatory drugs [13] that in animal models has shown both anti-inflammatory and analgesic activity [14].



Tenoxicam (TENOX)

In the present paper, we report on the preparation and properties of complexes formed between tenoxicam and some first row transition metal ions and examine the superoxide scavenging activity of these compounds.

Experimental

Tenoxicam was supplied by Hoffmann-La Roche (Basle) 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.).

0020-1693/87/$3.50

Preparation of Complexes

Tenoxicam (1.5 g, 1 mol equivalent) was dissolved in 0.1 M sodium hydroxide (45 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 a minimum to exclude excess hydroxide ions. The resulting pale yellow 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, followed by cold ethoxyethane and dried at 80 °C. 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 MSB1.

Superoxide Assay

The superoxide dismutase activity of the complexes [2] was assayed by its ability to inhibit the reduction of nitroblue tetrazolium (NBT) by an irradiated solution of methionine in the presence of riboflavin and oxygen at pH 8 (0.05 M phosphate buffer, adjusted to required pH with KOH) at 25 °C. Methionine, nitroblue tetrazolium and riboflavin were purchased from Sigma and sodium cyanide, potassium dihydrogen orthophosphate and potassium hydroxide from BDH. Absorbance was measured at 560 nm on a Pye Unicam SP8-100 spectrophotometer. Measurements were made in 3 cm^3 disposable cuvettes (Hughes and Hughes Ltd). Irradiation was carried out inside an aluminium foil lined box by two Philips 250 watt tungsten lamps at a distance of 5 cm from the cuvette which was inside a thermostated water jacket. The aqueous reaction mixture which contained 0.2 cm^3 DMSO was 1.2×10^{-6} M riboflavin, 0.02 M methionine, 5.6×10^{-4} M NBT, 2×10^{-5} M NaCN, and 0.05 M potassium phosphate at pH 8 and 25 °C. Irradiation for 6 min caused an increase in absorbance at 560 nm of 0.098. When the reaction was performed in the presence of 1.67×10^{-5} M of drug or metal complex (added in 0.2 cm^3 DMSO), this absorbance change was decreased. For example, in the presence of $Mn(TENOX)_2 \cdot 3H_2O$, the absorbance change was 0.019, corresponding to 20% of the increase in the absence of additive.

Results and Discussion

The microanalytical results show that the compleces are obtained as hydrates from solutions with the tenoxicam present as a mono-anionic ligand (Table I).

Infrared Spectra

Table II lists and compares the infrared spectra for free tenoxicam and the metal complexes. The bands in the drug spectrum are well reproduced with only minor shifts in the spectra of the complexes. The assignments of the various bands, without being rigorous, have been made by a direct comparison between the spectrum of coordinated tenoxicam and that of the free drug.

The ligand shows an absorption band centred at 3386 cm^{-1} characteristic of an O–H stretching vibration. The broadness of this band is indicative of hydrogen bonding and in the absence of hydration in the free ligand it is suggested that the most likely cause is intramolecular bonding involving the oxygen or nitrogen atoms of the secondary amide group. The enol tautomers of β-dicarbonyls exhibiting keto–enol tautomerism are known to be stabilized by strong intramolecular OHO hydrogen bonding [15]. All the metal complexes exhibit a broad absorption centred in the 3380–3430 cm^{-1} region. The drug is reacted as the enolate anion and as such no O–H vibration is expected in the complexes. Analysis of the complexes show them to be hydrated and it is

TABLE I. Analysis of Tenoxicam Complexes

	Found (%)			(Required (%)		
	C	H	N	C	H	N
$Mn(TENOX)_2 \cdot 3H_2O$	39.88	3.44	11.2	39.93	3.33	10.75
$Fe(TENOX)_3 \cdot 2H_2O$	42.63	2.97	11.85	42.52	3.09	11.45
$Co(TENOX)_2$	42.98	2.60	11.59	42.66	2.73	11.48
$Ni(TENOX)_2 \cdot 2H_2O$	40.00	3.31	11.20	40.67	3.13	10.95
$Cu(TENOX)_2 \cdot 2H_2O$	40.14	3.46	11.09	40.42	3.11	10.88

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

	O–H	N–H stretching	–NH⟩C–O stretching	SO_2 asymmetric stretching	SO_2 symmetric stretching	SO_2 scissoring	SO_2 wagging
TENOX	3386m,br	3130m	1630s	1330s	1146s	582m	528m
$Mn(TENOX)_2 \cdot 3H_2O$	3380s,br	3120w	1620s	1330s	1146s	590m	535m
$Fe(TENOX)_3 \cdot 2H_2O$	3387m,br	3120w	1600s	1310s	1155s		525w
$Co(TENOX)_2$	3390s,br	3120w	1616s	1332s	1150s	592m	540m
$Ni(TENOX)_2 \cdot 2H_2O$	3392s,br	3120w	1620s	1335s	1150s	592m	542m
$Cu(TENOX)_2 \cdot 2H_2O$	3430m,br	3120w	1620s	1345s	1155s	590w	550w

therefore suggested that hydrogen bonded water molecules could be responsible for the intensity and broadness of the absorption in this region.

The band observed at 1630 cm^{-1} in the free ligand is assigned to the carbonyl stretch of the secondary amide group –CO–NH–. This is lowered by 10–30 cm^{-1} in all the complexes. The N–H stretch observed in the ligand as a medium intensity band at 3130 cm^{-1} is seen in the complexes as a weak absorption centred at 3120 cm^{-1} but partially obscured by the broad bands due to hydrogen bonding.

In agreement with previous work on N-substituted sulphonamides [16], we assign the asymmetric $-SO_2-$ stretching vibration to strong bands in the range 1310–1345 cm^{-1} and the symmetric stretching vibration to the range 1146–1150 cm^{-1}. Two $-SO_2-$ group deformations have been reported at 610–499 cm^{-1} and 570–465 cm^{-1} [17]. In the present study, the scissoring (higher frequency) and wagging vibrations have been assigned to the ranges 592–582 cm^{-1} and 550–525 cm^{-1} respectively. The weak bands at 1095 ± 5 cm^{-1} and 917 ± 3 cm^{-1} have been assigned to the C_{th}–S and S–N stretching vibrations in agreement with previous measurements on 2-thiophenesulphonamides [18].

By comparing the IR spectra of the tenoxicam complex with the spectral data of the metal complexes of the structurally related drug isoxicam [19], it is clear that in the present complexes, tenoxicam acts as a mono-anionic ligand bonded through the enolate oxygen atom. It is likely that a second metal ligand bond is formed through the carbonyl oxygen atom of the amide group forming a six-membered chelate ring.

Electronic Properties

The reflectance spectrum of the ligand exhibits a number of low intensity absorption bands, that are considered to be infrared overtones, together with a shoulder at 23 250 cm^{-1} that forms part of the charge transfer band. The diffuse reflectance spectra for the $Mn(TENOX)_2 \cdot 3H_2O$ and $Fe(TENOX)_3 \cdot 2H_2O$ complexes are similar to that of the free ligand spectrum except for a very low intensity absorption in the 6750 cm^{-1} region that is assignable to an infrared overtone of water molecules. The absence of appreciable absorption at lower energy than the charge transfer edge is consistent with a pseudo-octahedral environment about the metal ions. All d–d transitions for an octahedral d^5 ion are spin forbidden and therefore any absorption bands will be extremely weak [20]. The magnetic moments (6.25 BM (Fe^{3+} complex); 5.99 BM (Mn^{2+} complex)) are consistent with high spin complexes [21].

The low intensities and postions of the absorption bands in the reflectance spectra of $Co(TENOX)_2$, $Ni(TENOX)_2 \cdot 2H_2O$ and $Cu(TENOX)_2 \cdot 2H_2O$ are indicative of an essentially pseudo-octahedral stereochemistry (Table III). The magnetic moment of 4.61 BM for the $Co(TENOX)_2$ complex does not distinguish between tetrahedral or octahedral cobalt(II). The reflectance spectrum consists of a single broad, low intensity absorption band centred at 9740 cm^{-1} and a low intensity shoulder at 17 880 cm^{-1} that forms part of the charge transfer edge. Three spin-allowed transitions: $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(\nu_1)$: $^4T_{1g}(F) \rightarrow {}^4A_{2g}(\nu_2)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(\nu_3)$ are expected for octahedral cobalt(II). However, it has been pointed out that the transition $^4T_{1g}(F) \rightarrow {}^4A_{2g}$ corresponds to a two-electron jump and as such will have a much lower oscillator strength than the other two bands and will be much weaker [22]. The band at 9740 cm^{-1} has therefore been assigned to the ν_1 transition and the shoulder at 17 880 cm^{-1} to the ν_3 transition. 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 is completed by distant coordination of the pyridine nitrogen atoms. Molecular models indicate that a CoO_4N_2 chromaphore is possible.

The magnetic moment of the $Ni(TENOX)_2 \cdot 2H_2O$ complex, $\mu_{eff} = 3.20$ BM, is near the top of the range normally observed for octahedral nickel(II) complexes but Lever has shown that tetragonal complexes may have moments as high 3.5 BM [23]. The intensity and position of the reflectance spectrum, however, suggests pseudo-octahedral symmetry for the nickel(II) ion. Three bands are ex-

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

	μ_{eff} (BM)	Absrotpion bands (cm^{-1})	
$Mn(TENOX)_2 \cdot 3H_2O$	5.99		
$Fe(TENOX)_3 \cdot 2H_2O$	6.25		
$Co(TENOX)_2$	4.61	$\nu_1[^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)]$ 9740(0.20)	$\nu_3[^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)]$ [b] 17880(0.30)
$Ni(TENOX)_2 \cdot 2H_2O$	3.20	$\nu_1[^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)]$ 9170(0.19)	$\nu_2[^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)]$ [b] 16950(0.21)
$Cu(TENOX)_2 \cdot 2H_2O$	1.94	16080(sh, 0.63)	

[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.

TABLE IV. Superoxide Assay

Additive	% Absorbance increase at 560 nm compared with blank
TENOX	79
$Mn(TENOX)_2 \cdot 3H_2O$	19
$Fe(TENOX)_3 \cdot 2H_2O$	78
$Co(TENOX)_2$	57
$Ni(TENOX)_2 \cdot 2H_2O$	74
$Cu(TENOX)_2 \cdot 2H_2O$	2

pected for octahedral nickel(II) complexes and are assigned, assuming pseudo-Oh symmetry, in increasing order of energy as: $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$; $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$. The low intensity bands observed at 9170 cm^{-1} and 16950 cm^{-1} are therefore assigned to the ν_1 and ν_2 transitions respectively with the ν_3 band obscured by the charge transfer edge.

The reflectance spectrum of $Cu(TENOX)_2 \cdot 2H_2O$ consists of a broad, low intensity shoulder centred at 16080 cm^{-1} that forms part of the charge transfer band. The ^2E_g and $^2T_{2g}$ states of the octahedral copper(II) ion (d^9) split under the influence of a tetragonal distortion and the distortion can be such as to cause the three transitions $^2B_1 \rightarrow {}^2B_2$; $^2B_1 \rightarrow {}^2E$ and $^2B_1 \rightarrow {}^2A_1$ to remain unresolved in the spectrum [24]. In the absence of any other bands in the $Cu(TENOX)_2 \cdot 2H_2O$ spectrum, it is concluded that all three transitions lie within the single broad envelope centred at 16080 cm^{-1}. This assignment is in aggreement with the general observation that copper(II) d–d transitions are normally close in energy [25]. The magnetic moment of 1.94 BM falls within the range normally observed for mononuclear complexes having no appreciable interaction between copper(II) centres.

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 by an irradiated solution of methionine in the presence of riboflavin and oxygen at pH 8 and 25 °C as described earlier. Four assays were performed for each additive and the results are set out in Table IV. It is evident from these superoxide scavenging data that the Fe(III) and Ni(II) complexes of TENOX exhibit the same acitivity as the free drug whilst the Co(II) complex is only marginally more active. However, the $Mn(TENOX)_2 \cdot 3H_2O$ and $Cu(TENOX)_2 \cdot 2H_2O$ complex exhibit a marked increased superoxide dismutase activity compared with the parent drug molecule. The results support earlier conclusions that superoxide anion radical scavenging is not restricted to the metalloprotein superoxide dismutase. Many low molecular mass copper(II) compounds as well as free hydrated Cu^{2+} and Mn^{2+} ions are known to bring about the breakdown of $O_2^{\cdot -}$ [9, 26]. It is often assumed that electron transfers between copper(II) and superoxide anion radicals occurs through direct binding [27]. The site proposed for $O_2^{\cdot -}$ attachment is the axial site of copper(II) complexes and the mechanism of the interaction is:

$$Cu^{2+} + O_2^{\cdot -} \rightleftharpoons Cu^{+} + O_2$$

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

Due to the high rate constant of copper(II) mediated superoxide dismutation a fast exchange of coordinated water is considered essential to allow the binding of the $O_2^{\cdot -}$ radical [28]. We believe that in the tetragonally distorted octahedral complex $Cu(TENOX)_2 \cdot 2H_2O$, the water molecules are *trans*, weakly bonded ligands that are readily substituted by $O_2^{\cdot -}$.

Acknowledgements

We would like to thank Hoffmann-La Roche (Basle) for the supply of tenoxicam and The Royal Society for a Research in Schools award (to P.S.G.). One of us (P.S.G.) would like to thank the Headmaster and Governors of Gresham's School for the provision of research facilities.

References

1 B. M. Babior, *N. Eng. J. Med., 298*, 721 (1978).
2 B. Halliwell and J. M. C. Gutteridge, *Biochem. J., 219*, 1 (1984).
3 J. M. McCord, *Science, 185*, 529 (1974).
4 J. A. Fee, J. Piesach and W. B. Mims, *J. Biol. Chem., 256*, 1910 (1981).
5 J. M. McCord and I. Fridovich, *J. Biol. Chem., 244*, 6049 (1969).
6 F. S. Archibald and I. Fridovich, *J. Bacteriol., 146*, 928 (1981).
7 W. H. Betts, L. G. Cleland and M. H. Whitehouse, 'Inflammatory Diseases and Copper', Humana Press, Clifton, N.J., 1982 pp. 553–564.
8 M. Younes and U. Weser, *Biochem. Biophys. Res. Commun., 78*, 1247 (1977).
9 R. Brigelius, R. Spottl, W. Bors, E. Lengfelder, M. Saran and U. Weser, *FEBS Lett., 47*, 72 (1974).
10 D. H. Brown, W. E. Smith, J. W. Teape and A. J. Lewis, *J. Med. Chem., 23*, 729 (1980).
11 J. G. Lombardino, *Annu. Rep. Med. Chem., 13*, 167 (1978).
12 J. G. Lombardino, E. H. Wiseman and W. M. McLamore, *J. Med. Chem., 14*, 1171 (1971).
13 E. H. Wiseman and J. G. Lombardino, *Eur. J. Rheumatol. Inflamm., 4*, 280 (1981).
14 K. M. Strub, L. Aeppli, A. Daum and R. K. M. Müller, *XVth Int. Cong. Rheumatol. (Paris)*, 1981, Abstract 376.
15 J. Emsley, *Struc. Bonding (Berlin), 57*, 147 (1984).
16 M. Goldstein, M. A. Russell and H. A. Willis, *Spectrochim. Acta, Part A, 25*, 1275 (1969).
17 W. R. Feairheller and J. E. Katon, *Spectrochim. Acta, 20*, 1099 (1964); G. Malewski and H. J. Weigmann, *Spectrochim. Acta, 18*, 725 (1962); H. J. Weigmann and G. Malewski, *Spectrochim. Acta, 22*, 1045 (1966).
18 A. Arcoria, E. Maccarone, G. Musumarra and G. A. Tomaselli, *Spectrochim. Acta, Part A, 30*, 611 (1974).
19 D. O. Harrison, R. Thomas, A. E. Underhill, J. K. Fletcher, P. S. Gomm and F. Hollway, *Polyhedron, 4(4)*, 681 (1985).
20 C. K. Jørgensen, 'Absorption Spectra and Chemical Bonding in Complexes', Addison-Wesley, Reading, Mass, 1962.
21 B. N. Figgis and J. Lewis, *Prog. Inorg. Chem., 6*, 37 (1964).
22 S. Koide, *Philos. Mag., 4*, 243 (1959).
23 A. B. P. Lever, *Inorg. Chem., 4*, 763 (1965).
24 E. Konig and H. L. Schlafer, *Z. Phys. Chem., 26*, 371 (1960).
25 O. G. Holmes and D. S. McClure, *J. Chem. Phys., 26*, 1686 (1957); D. W. Smith, *Inorg. Chem., 5*, 2236 (1966).
26 K. I. Fong, P. B. McCay, J. L. Poyer, B. B. Keele and H. Misra, *J. Biol. Chem., 248*, 7792 (1973); J. Lumsden and D. O. Hall, *Biochem. Biophys. Res. Commun., 64*, 595 (1975).
27 J. A. Fee, in H. Sigel (ed.), 'Metal Ions in Biological Systems', Vol. 13, Marcel Dekker, New York, 1981, p. 259; A. Rigo, P. Viglino and G. Rotilio, *Biochem. Biophys. Res. Commun., 63*, 1013 (1975).
28 N. Boden, M. C. Holmes and P. F. Knowles, *Biochem. Biophys. Res. Commun., 57*, 845 (1974).
29 C. Beauchamp and I. Fridovich, *Anal. Biochem., 44*, 276 (1971).