Journal of Enzyme Inhibition and Medicinal Chemistry, 2002 VOL. 17 (2), pp. 87–91

Transition Metal Acetylsalicylates and Their Anti-inflammatory Activity

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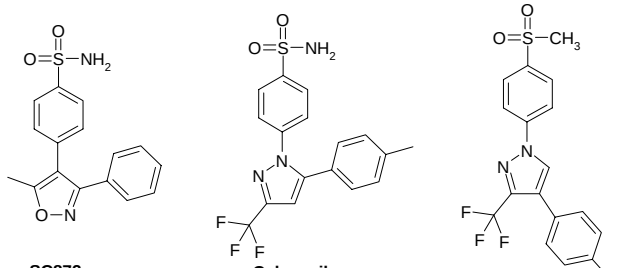
(Received 5 March 2002)

Mononuclear and binuclear transition metal [Co(II), Cu(II), Ni(II) and Zn(II)] acetylsalicylates of the type $[M(L)_2]$, $[M(L)_2Cl_2]$ and $[(M)_2(L)_4]$ have been prepared and characterized on the basis of their physical, spectral and analytical data. The complexes have been investigated in an *in vivo* animal model for anti-inflammatory activity and show a better effect and a more potent action than acetylsalicylic acid.

Keywords: Acetyl salicylic acid; Transition metal ion complex; Anti-inflammatory agent

INTRODUCTION

Aspirin (acetylsalicylic acid) has remained one of the most widely employed analgesic and anti-inflammatory agent for treating the majority of articular and musculoskeletal disorders.[1,2] It is absorbed as such and is rapidly hydrolyzed to acetic acid and salicylate by esterases in tissue and blood.[1–5] Epidemiological studies suggest that long-term use of acetylsalicylic acid at low dosage is associated with lower incidence of colon cancer.[1–5] It, also, irreversibly blocks the enzyme cyclooxygenase, COX (prostaglandin (PG) synthase) which catalyzes[3,4] the conversion of arachidonic acid to the endoperoxide that induces platelet aggregation and is known as a potential antithrombotic[5] drug. Acetylsalicylic acid is a non-selective COX inhibitor, acting on both isozymes COX-1 and COX-2.[1,2] It also interferes with the chemical mediators of the kallikrein system[6] that inhibits granulocyte adherence to damaged vasculature, stabilizes lycosome, and inhibits the migration of polymorphonuclear leukocytes into the site of inflammation and, therefore, is effectively used as an anti-inflammatory agent.



It has been demonstrated[7,8] that the use of acetylsalicylic acid, however, causes irritation to the digestive tract, and this is the reason why COX-2 specific inhibitors have recently been developed, such as valdecoxib **1**, celecoxib **2**, or SC125 **3**.[1,2] Such compounds only inhibit the inducible COX-2 isozyme which has been shown to be associated with inflammatory conditions, sparing the constitutive form (COX-1) which is responsible for the beneficial effects of the PGs.[1,2] Cu-acetylsalicylate, a copper complex of acetylsalicylic acid for the first time was used as a more active anti-inflammatory agent also showing lower ulcerogenicity and irritation in the digestive tract as compared to aspirin itself.[9,10] It was thus suggested that copper-acetylsalicylate might be

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ISSN 1475-6366 print/ISSN 1475-6374 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/14756360290030734

clinically used to treat arthritic and other degenerative changes.[5] In order to explore less ulcerogenic and more anti-inflammatory metal-acetylsalicylates, we have synthesized binuclear and mononuclear transition metal-acetylsalicylates of the types $[M_2L_4]$, $[ML_2]$ and $[ML_2Cl_2]$ where M = Co(II), Cu(II), Ni(II) or Zn(II) and L = acetylsalicylate anion and have studied their anti-inflammatory activity.

MATERIAL AND METHODS

All chemicals and solvents used were of Analar grade. Metal(II) salts were used as the chloride. Acetylsalicylic acid was obtained from Standpharm Pharmaceuticals. IR, spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. UV-Visible spectra were obtained on a Hitachi U-2000 double-beam spectrophotometer. Elemental analysis was done by Butterworth Laboratories by combustion (C and H) and gravimetrically for the metal ions. Conductances of the metal complexes were determined in DMF on a YSI-32 model conductometer. Melting points were recorded on a Gallenkamp apparatus and are not corrected.

Preparation of Metal Complex

Acetylsalicylic acid (0.72 g, 0.02 M) was dissolved in distilled water (100 ml) and sodium bicarbonate (0.084 g, 0.02 M) dissolved in 100 ml distilled water was added dropwise into this solution with constant stirring till a clear solution of aspirin sodium salt was obtained. For the preparation of mononuclear Co-acetylsalicylate complex having the composition $[ML_2Cl_2]$, an aqueous solution of $CoCl_26H_2O$ (0.23 g, 0.1 M) was added to the above solution of the aspirin sodium salt (10 ml, 0.2 M) with stirring. This solution was refluxed for 30 min. when a red precipitate was obtained on cooling, which was filtered, washed with ethanol and dried to give the title complex in 89% yield. For the preparation of other complexes having the composition, $[ML_2]$, $[ML_2Cl_2]$ and $[M_2L_4]$, the same method was used working with different molar ratios of the reactants.

Anti-inflammatory Activity

Kaolin paw oedema was induced, by a reported literature method[11] in male Wistar rats weighing 95–115 g, in groups of five animals. The complexes under investigation were administered orally in 5% Mulgophen (GAF, Manchester) in distilled water (0.2 ml per 100 g) 1 h before the kaolin oedema induction. The rats were dosed on a basis of weight of drug (mg) per body weight (kg) of animal. Oedema was evaluated 4 h after subplantar application of kaolin in 0.9% w/v sodium chloride solution. Inhibition of edema was evaluated by comparing the swelling obtained in treated animals with that in controls and was expressed in percentage inhibition (see Table II). Statistical significance was evaluated by use of the Student *t*-test.

RESULTS AND DISCUSSION

All the metal-acetylsalicylates were synthesized by refluxing an appropriate amount of the sodium salt of acetylsalicylic acid with the respective metal as their chlorides in water according to the required molar ratios of M:L (M = Co(II), Cu(II), Ni(II) or Zn(II) and L = sodium salt of acetylsalicylic acid). All the complexes were colored amorphous solids that decomposed above 200°C. The structures of these complexes were established with the help of their IR and electronic spectra and microanalytical data.

Both Cu(I)- and Cu(II)-acetylsalicylates have been used as potential anti-inflammatory agents.[12,13] In adjuvant arthritic rats following oral administration for 21 days, Cu(II)-acetylsalicylate acted as a mixed agonist-antagonist, showing agonist action at lower doses and antagonist action at higher doses. Sorenson investigated[5,12] the preliminary chronic toxic effects of Cu-acetylsalicylates by orally administring it to rats for 5 days a week, for 3 months period, and showed it to possess low toxicity. There have been reports[14–16] that the copper-acetylsalicylates although providing higher anti-inflammatory activity caused lower ulcerogenicity as compared to acetylsalicylic acid itself. In the present study, apart from preparation of the copper-acetylsalicylates, Co(II), Ni(II) and Zn(II) acetylsalicylates (**1**–**8**) have been obtained with two different stoichiometries, one having 1:2 (metal:acetylsalicylic acid, mononuclear) (Figures 1 and 2) and another 2:4 (metal: acetylsalicylic acid, binuclear) (Figure 3) in order to fully investigate the effect of metals other than copper coordinated to acetylsalicylic acid.

The significant IR spectral bands of metal complexes investigated here are listed in Table I. The IR spectra show that acetylsalicylic acid behaves in a bidentate manner, coordinating via the two oxygen atoms of the acidic group with the displacement of a hydrogen atom. This mode of chelation is supported by the disappearance of ν(COOH) and the appearance of a band at $\sim 1315\,cm^{-1}$, assignable[17] to ν(C–O), ν(C = O) shifts to lower frequency, indicating participation of this group in coordination. Also, appearance of bands at $\sim 420\,cm^{-1}$ were assigned to ν(M–O), confirming the coordination via oxygens of the acidic group in all the spectra of the metal complexes. A band at $345\,cm^{-1}$ assigned[18] to ν(M–Cl) in the spectra of only mononuclear Co(II), Ni(II) and Zn(II) complexes further indicated that

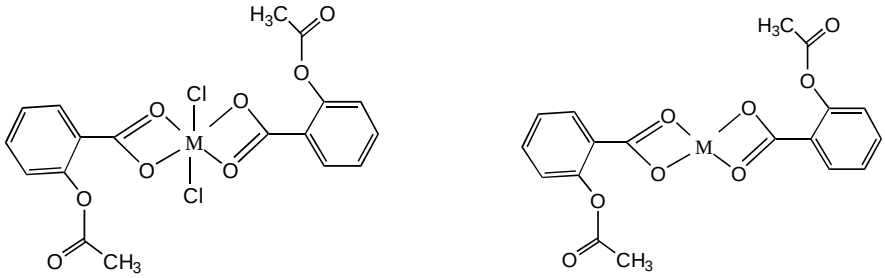

FIGURE 1 Proposed Structure of the Mononuclear Metal(II)-acetylsalicylate complexes.

two chlorides are coordinated to these metals showing them to be present in an octahedral geometry (Figure 1).

The room temperature magnetic moment of the mononuclear solid Co(II) complex **1** was found to be of 4.6 B.M, indicative of three unpaired electrons in an octahedral environment.[19] The Cu(II) complex **3** displayed a μ_{eff} value of 1.5 B.M showing the presence of one unpaired electron for its square planar geometry and the Ni(II) complex **5** showed a μ_{eff} value of 3.1 B.M indicative of two unpaired electrons for their ideal octahedral configuration.[20] All binuclear complexes (**2**, **4** and **6**) were found to be diamagnetic. The unpaired electrons on the two metal ions interacted and coupled antiferromagnetically to produce a low-lying singlet level, behaving thus as diamagnetic solids.[21] The measured molar conductance values of all the complexes in DMF were found in the range of 7.8–8.2 $ohm^{-1}\,cm^2\,mol^{-1}$, showing their non-electrolytic nature.[22]

The electronic spectra of the mononuclear Co(II) chelate showed three bands at 29,965, 18,555 and 7850 cm^{-1} which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^4A_{2g}(F)$, and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transitions and are suggestive[23,24] of an octahedral geometry around the cobalt ion (Figure 1). The electronic spectra of the mononuclear Cu(II) complex showed two bands in the region of 28,635 and 16340 cm^{-1}. The low energy band may be assigned to Cu(II) in a square planar configuration (Figure 2) corresponding[25] to the transition $^2E_g \rightarrow T_{2g}$ and the other to the symmetry forbidden ligand → metal charge transfer band. The mononuclear Ni(II) complex exhibited three spin allowed bands at 29,265, 16,245 and 9420 cm^{-1} assignable respectively, to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(v_1)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(v_2)$, and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)(v_3)$ which are suggestive[26] of the octahedral geometry of Ni(II) (Figure 1). The electronic spectra of the mononuclear Zn(II) complex exhibited a high intensity band at 28,250 cm^{-1} assigned as a ligand → metal charge transfer band, and a band at 13,775 cm^{-1} due to transition $^2E_g \rightarrow {}^2T_{2g}$ in a distorted octahedral environment[27] (Figure 1).

All the binuclear complexes were found to be diamagnetic. This was mainly due to the strong metal-metal spin interactions[28] between them suggesting their square-planar 4-coordinate structure as shown in Figure 3. However, the electronic spectrum of the binuclear Co(II) complex exhibits characteristic bands at 28245 cm^{-1} due to a charge transfer $n \rightarrow \pi*$, 16385 cm^{-1} assigned[29] to spin orbital coupling and 14925 cm^{-1} to $^1A_{1g} \rightarrow {}^1T_{1g}$ compatible for a square-planar configuration,

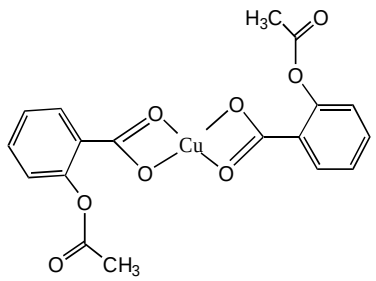

FIGURE 2 Proposed Structure of the Mononuclear Cu(II)-acetylsalicylate complex.

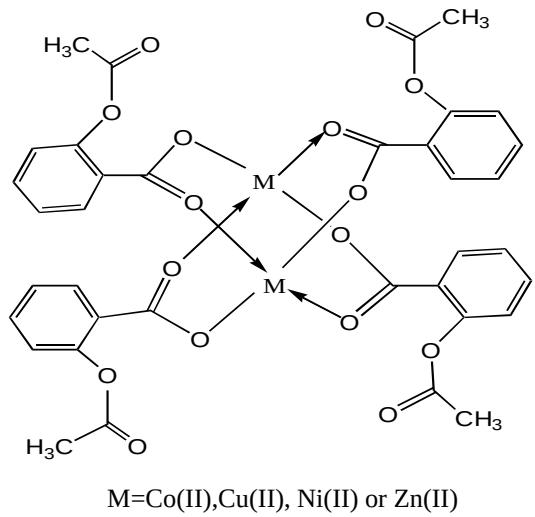

FIGURE 3 Proposed Structure of the Binuclear Metal(II)-acetylsalicylate derivatives.

TABLE I Physical, spectral and analytical data for the metal(II)-acetylsalicylates complexes

Acetylsalicylic acid/complex	M.P (°C)	IR (cm^{-1})	λ_{max} (cm^{-1})	Calculated (found) %		
				C	H	M
Acetylsalicylic acid	–	1680 (COOH)	–	–	–	–
$[Co(L)_2Cl_2]$ $C_{18}H_{14}CoO_8Cl_2$ [487.8]	205–207	1595 (C = O), 1315 (C–O), 418 (M–O), 345 (M–Cl)	29965, 18555, 7850	44.3 (44.8)	2.9 (3.1)	12.1 (12.4)
$[(Co)_2(L)_4]$ $C_{36}H_{28}Co_2O_{16}$ [833.9]	211–213	1595 (C = O), 1318 (C–O), 420 (M–O)	28245, 16385, 14925	51.8 (52.2)	3.4 (3.2)	14.1 (14.5)
$[Cu(L)_2]$ $C_{18}H_{14}CuO_8$ [421.5]	202–204	1590 (C = O), 1310 (C–O), 415 (M–O)	28635, 16380, 14920	51.2 (51.3)	3.3 (3.5)	15.1 (14.9)
$[(Cu)_2(L)_4]$ $C_{36}H_{28}Cu_2O_{16}$ [843.1]	208–210	1595 (C = O), 1310 (C–O), 415 (M–O)	27655, 16390	51.2 (51.5)	3.3 (3.7)	15.1 (15.5)
$[Ni(L)_2Cl_2]$ $C_{18}H_{14}NiO_8Cl_2$ [487.6]	203–205	1595 (C = O), 1315 (C–O), 420 (M–O), 345 (M–Cl)	29265, 16245, 9420	44.3 (44.8)	2.9 (2.6)	12.0 (12.3)
$[(Ni)_2(L)_4]$ $C_{36}H_{28}Ni_2O_{16}$ [833.4]	212–214	1595 (C = O), 1315 (C–O), 420 (M–O)	27275, 19610	51.8 (51.5)	3.4 (3.3)	14.1 (14.2)
$[Zn(L)_2Cl_2]$ $C_{18}H_{14}ZnO_8Cl_2$ [494.3]	206–208	1595 (C = O), 1318 (C–O), 420 (M–O), 345 (M–Cl)	28250, 13775	43.7 (43.9)	2.8 (3.1)	13.2 (12.9)
$[(Zn)_2(L)_4]$ $C_{36}H_{28}Zn_2O_{16}$ [846.8]	210–212	1595 (C = O), 1318 (C–O), 420 (M–O)	27260, 18385	51.0 (5.12)	3.3 (3.5)	15.4 (15.7)

TABLE II Anti-inflammatory results for acetylsalicylic acid and some of its metal complexes reported here

Complex (M:L)	Dose ($mg\,kg^{-1}$)	Inhibition of oedema (%)
Acetylsalicylic acid	50	33
Co-Asp (1:2)	50	55
Co-Asp (2:4)	50	58
Cu-Asp (1:2)	50	90
Cu-Asp (2:4)	50	95
Ni-Asp (1:2)	50	52
Ni-Asp (2:4)	50	57
Zn-Asp (1:2)	50	60
Zn-Asp (2:4)	50	68

respectively. The binuclear Cu(II) complex showed two absorption bands at 16390 and 27655 cm^{-1} due to a d–d transition in square-planar geometry[30] and a charge transfer, respectively. The electronic spectrum of the binuclear Ni(II) complex exhibited a characteristic band at 19610 cm^{-1} corresponding to the $^1A_{1g} \rightarrow {}^1A_{2g}$ transition in a square-planar geomtry[31] and a band at 27275 cm^{-1} which was assigned to the charge transfer, respectively. The diamagnetic binuclear Zn(II) complex also showed bands at 27260, 18385 cm^{-1} assigned[28,29] to the charge transfer and $^1E_g \rightarrow {}^1T_{2g}$ transition respectively for a square-planar geometry (Figure 3).

Anti-inflammatory Studies

The *in vivo* anti-inflammatory results (Table II) showed that the metal chelates reported here certainly showed more anti-inflammatory activity against paw oedema induced in rats, as compared to the free ligand. These studies are in line with the results already reported by Sorensen regarding the anti-inflammatory properties of copper-acetylsalicylate **3**.[3] Extending these studies,[3] bi- as well as mononuclear Co(II), Ni(II) and Zn(II) metal-acetylsalicylates were investigated in the present work, it being found that binuclear Co(II), Ni(II) and Zn(II) metal-acetylsalicylates showed 35–37% less anti-inflammatory activity as compared to the parent compound **3**. It was also found that binuclear complexes showed more activity than the mononuclear acetylsalicylates. This may be due to the more induced metalloenzyme effect. In future reports we shall investigate whether metal complexes of COX-2 specific inhibitors are more potent as compared to the free ligands as anti-inflammatory agents.

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