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Syntheses, Characterization and Anti-inflammatory Activity of a Benzamide Derivative and its Metal Chelates

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N-Phenylbenzamide-2,2′-dicarboxylic acid (PBDA) and its copper(II), nickel(II), cobalt (II), zinc(II) and manganese(II) chelates have been synthesized and characterized by their physical measurements, infrared and electronic spectra and magnetic moment data. In an acute anti-inflammatory test, the cobalt chelate was most active (31.1% inhibition) followed by the zinc and copper chelates, whereas the copper chelate (22.3% inhibition) was most active in an adjuvant arthritis test. Again the cobalt chelate was most active in the cotton-wad granuloma test. Gastric irritancy was markedly reduced after chelation by copper, followed in order by zinc, cobalt, manganese and nickel chelates. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

The pharmacotherapy of inflammation is characterized by an apparant abundance of drugs but a lack of truly effective and safe compound. The non-steroidal anti-inflammatory (AI) drugs (NSAIDs) at present available do not actually cure the problem and often have dangerous side-effects, viz. gastric irritation and ulceration, especially when in chronic use. This represents one of the major therapeutic challenges in the field of arthritic therapy.[1–3] However, this may be circumvented either by co-prescribing an anti-ulcer agent[1, 2] or by modification of the NSAID to reduce its propensity to cause gastro-intestinal ulceration. The former being costly, a better measure would be to modify the NSAID by a relatively inexpensive procedure.[4] An approach employing complexation of the NSAID with certain trace metals has partcular advantages as it may reduce ulcerogenicity and increase AI activity[2].

Despite the pioneering efforts of Rainsford,[1–4] Sorensen,[5–8] Whitehouse,[9–11] Bonta[12] and others in the field of trace metals and their complexes with NSAIDs for potential use as AI agents, the complexes could be used only with very limited success. Sorensen[5] observed that copper complexes participate in normal physiological or acute- and chronic-phase responses to inflammatory disease. They play a role in mediating copper-dependent biochemical processes which have a role in maintaining as well as in repairing the tissues and have fewer side-effects than existing NSAIDs. He postulated[13] that the copper complexes perhaps act through Cu–Zn superoxide dismutase or through copper-dependent lysyl oxidase, by modulating prostaglandin synthesis or by their lysosomal membrane permeability and their histamine activity. Rainsford[14] hypothesized that dietary and environmentally induced perturbations in the level of certain trace metals, especially as they influence the copper ion status, are contributory factors in the etiology of rheumatoid arthritis, osteoarthritis and related orthopathies. It is also documented that manganese is required for mucopolysaccharide synthesis[15] and plasma zinc levels decrease in patients with rheumatoid arthritis.[16]

Keeping this in view, and in search of safer and more effective NSAIDs, we have synthesized *N*-phenylbenzamide-2,-2′-dicarboxylic

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acid (PBDA) and its transition-metal chelates involving copper, zinc, manganese, nickel and cobalt.

EXPERIMENTAL

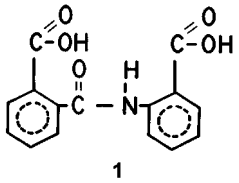
Chemistry

Synthesis of Ligand and Metal Chelates

N-Phenylbenzamide-2,2′-dicarboxylic acid (PBDA; compound **1**) was synthesized by the method reported earlier.[17] All the chemicals used were of AR grade and were purified further either by recrystallization or by distillation. The melting point of PBDA was 155 °C. The sodium salt of PBDA was prepared by dissolving it in an equimolar amount (0.02 M) of boiling sodium hydroxide solution. The hot solution was filtered and cooled in ice. The precipitated sodium salt was then filtered. The metal–PBDA chelates (**2**) were prepared by refluxing the sodium salt of PBDA (0.02 M in 100 ml) with the respective metal acetates (0.02 M in 100 ml) in a 1:1 ratio for 2 h. On concentrating and cooling the reaction mixture a coloured precipitate was obtained. It was filtered under suction and washed first with water, ethanol and finally with ether and dried over P_4O_{10} under vacuum.

Physical measurements

The synthesized ligand (PBDA) and metal chelates were analysed for C, H and N by microanalytical techniques and the metal contents in the chelates were estimated by the standard methods.[18] The molecular weight of the chelates was determined by cryoscopic methods in dimethyl sulphoxide (DMSO). The molar conductance of the chelates was measured on a Toshniwal conductivity bridge using DMSO as reference. The IR spectra were recorded using a Perkin-Elmer spectrophotometer, model 521. The UV–visible spectra were recorded on a Shimadzu double-beam spectrophotometer, model UV-150-02, using DMSO as reference. The magnetic moments were measured at room temperature by the Gouy's method using $CuSO_4 \cdot 5H_2O$ as calibrant. The values were corrected for diamagnetism by applying Pascals constant. Thermal studies were carried out to check for the presence of water molecules.



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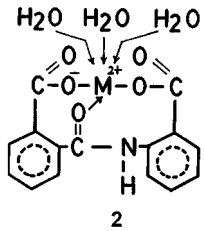

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Anti-inflammatory tests

Overnight-fasted male rats weighing between 100 and 140 g were arranged in three groups of eight each for each compound, one group serving as control. The drugs were administered subcutaneously as a suspension in saline containing 1.4% (w/v) of poly(vinyl alcohol). The doses used were 200 mg kg^{-1} for test compounds, 50 mg kg^{-1} for oxyphenbutazone and 35 mg kg^{-1} for ibuprofen.

Carrageenan-induced rat paw oedema test

For screening, the method of Winter *et al.*[19] was adopted. Oedema was induced by injecting 0.1 ml of a 1% carrageenan suspension in normal saline into the plantar aponeurosis of the right paw. The PBDA, metal chelate or oxyphenbutazone was given 1 h before the carrageenan challenge. The volume of the treated and untreated paw was measured before and at 1, 2, 3, 4 and 6 h after the carrageenan injection by a volume-differential meter (M 7101, Ugo Basile, Milan, Italy). The percentage inhibition in paw oedema was calculated.

Granuloma pouch test

Pellets of sterilized (air oven, 2 h) surgical cotton weighing 9.0 ± 1 mg were implanted in both the axillae and groins under light ether anaesthesia.[20] The drugs were given subcutaneously daily for six days from day 1 (i.e. the day of pellet implantation). The pellets were dissected out on the seventh day under light ether anaesthesia, kept separately in small glass vials, dried for two hours at 150 °C and weighed after cooling. The percentage inhibition of weight of granuloma was calculated.

Adjuvant arthritis test

Rats were injected with 0.1 ml of Freund's complete adjuvant (Difco) in the plantar aponeurosis.[21] The PBDA, metal chelates or ibuprofen was administered subcutaneously daily for 14 days. The volumes of the injected paw and uninjected paw were measured on days 0, 2, 4, 6, 8, 10, 12 and 14 using a water plathysmometer (M 7150, Ugo Basile, Milan, Italy).

Ulcerogenic effect in rats

Male rats (140 ± 10 g) that had fasted for 24 h were used having free access to water. The animals were divided into nine groups, each of 10 animals, one group serving as control. The test was carried out as reported by Dhawan and Srimal.[22] Test compounds and the reference standard (oxyphenbutazone) were administered orally as a suspension in water. The animals were sacrificed 6 h after administration of the drug and their stomachs were removed, opened carefully along the greater curvature, washed and examined under a binocular stereoscopic microscope (Meopta). The severity of the lesions appearing in the muscular portions were scored as follows: 0, normal; 1, haemorrhagic effusion; 2, mucosal ulceration (<2/3 area); 3, deep ulceration; 4, perforated ulcer.

The ulcerogenic index (UI) was calculated from UI = (ADU) × %RU)/100, where ADU and %RU were the average degree of ulceration and the percentage of rats with an ulcer, respectively.

RESULTS

Elemental analysis, molecular weight determination and conductance measurement

All the metal chelates were found to be thermally stable and insoluble in water. The low molar conductance values (0.8–1.9 $ohm^{-1}\ cm^2\ mol^{-1}$) indicated their non-electrolytic nature, due to charge neutralization of the metal ions with the ligand. The 1:1 metal ligand stoichiometry was deduced from their elemental analysis and molecular weight measurement data (Table 1).

Thermal studies

On gradual heating from room temperature, the hydrated metal complexes dehydrated competely within a temperature range of 115–185 °C. The complexes were found to be thermally stable and they undergo decomposition within a temperature range of 310–520 °C. The resultant final products were the metal oxides in all cases. The corresponding weight losses resulting from dehydration and decomposition were in agreement with the calculated values.

Infrared spectra

The important infrared bands of *N*-phenylbenzamide-2,2′-dicarboxylic acid and its metal chelates have been discussed. The amide (I) band appeared at 1735 cm^{-1} in the case of the free ligand (PBDA). A lowering in this band

Table 1 Elemental analysis, molar conductance, magnetic moment and molecular weight of ligands and metal chelates

Compound	Colour	Analysis: Found (Calcd) C	H	N	M	Molar conductance (Ohm^{-1} ($cm^2\ mol^{-1}$)	Molecular weight: Found (Calculated)
PBDA	Dull white	63.21 (63.16)	3.85 (3.88)	4.94 (4.91)	— —	—	278 (285)
$Cu(PBDA)\cdot 3H_2O$	Blue	44.89 (44.95)	3.80 (3.77)	3.51 (3.49)	15.78 (15.85)	1.25 400	389 (400)
$Ni(PBDA)\cdot 3H_2O$	Light green	45.51 (45.49)	3.78 (3.82)	3.56 (3.53)	14.71 (14.82)	0.87	385 (396)
$CO(PBDA)\cdot 3H_2O$	Violet	45.50 (44.47)	3.84 (3.81)	3.49 (3.54)	14.81 (14.87)	1.12	383 (396)
$Zn(PBDA)\cdot 3H_2O$	White	44.69 (44.74)	3.71 (3.75)	3.50 (3.48)	16.19 (16.23)	0.95	392 (402)
$Mn(PBDA)\cdot 3H_2O$	Light grey	45.96 (45.93)	3.79 (3.85)	3.54 (3.57)	13.97 (14.01)	1.77	388 (392)

($\sim 25\ cm^{-1}$) is observed in the case of metal chelates, indicating co-ordination through the carbonyl oxygen atom of the amide group.[23] A slight lowering of the position of the amide II band supports the above finding. The NH band appearing at $\sim 3350\ cm^{-1}$ and the free ligand remain unaffected into metal complexes. This clearly indicates that the imine nitrogen is not taking part in co-ordination. The carboxylic group stretching vibrations are found at $1680\ cm^{-1}$; this is lowered ($\sim 20\ cm^{-1}$) in the case of metal complexes, showing co-ordination through the carboxylic group. The appearance of new bands at 3550–3450 cm^{-1} and around $1570\ cm^{-1}$ indicates the antisymmetric and symmetric ν–(OH) stretching and H–OH bending modes. It also suggests the presence of water molecules in the chelates. Co-ordination of the metal through the oxygen donor sites of the ligand was further confirmed by the appearance of new bands in the region 500–440 cm^{-1}, which could be assigned to respective M–O frequencies.

UV–visible spectra and magnetic moment data

The magnetic moments of the copper(II), nickel(II), cobalt(II) and manganese(II) complexes were calculated from corrected magnetic susceptibility values and UV–visible spectral data are given in Table 2. The magnetic moment data suggest an octahedral environment around the metal ion.[26] The UV–visible spectrum of the copper chelate shows only a band around $13986\ cm^{-1}$, probably due to the $^2E_g \rightarrow {}^2T_{2g}$ transition.[27] Three bands at 9716, 16420 and $25000\ cm^{-1}$ in the spectrum of the nickel chelate are due to three spin-allowed transitions from $^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F), and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) respectively in the octahedral environments. In the cobalt chelate three bands observed at 8549, 16339 and $21505\ cm^{-1}$ may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^4A_{2g}$ (F) and $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) transitions respectively, which are suggestive of octahedral geometry around the cobalt ion.[28] The spectrum of the manganese chelate exhibits three bands at 16313, 24570 and $29585\ cm^{-1}$ due to the transitions $^6A_{1g} \rightarrow {}^4T_{1g}$ (G), $^6A_{1g} \rightarrow {}^4E_g$, $^4A_{1g}$ (G) and $^6A_{1g} \rightarrow {}^4E_g$ (D) respectively, suggesting octahedral geometry.

Anti-inflammatory studies

Anti-inflammatory studies indicate that PBDA inhibited only 18.6%, 12.1% and 9.6% inflammation in carrageenan-induced paw oedema, adjuvant-induced arthritis and cotton-pellet granuloma tests respectively (Table 3). However, the cobalt chelate was most effective in acute and sub-acute inflammatory tests (34.1% and 16.2% respectively) whereas the copper chelate was most active in the chronic inflammatory test. The nickel and manganese chelates were ineffective in all the three tests.

Ulcerogenic activity

The ulcerogenic potential of the compounds is reported in Table 4. It was found that ulcerogenic potential of all the chelates was less than 50 mg kg^{-1} of phenylbutazone. However, copper and zinc complexes had marked decrease in gastric irritancy compared with PBDA and other chelates.

Table 2 UV–visible and magnetic moment data of metal chelates

Compound	Octahedral geometry (B and maxima, cm^{-1})	Transition	Magnetic moment (B.M. at 303 K)
$Cu(PBDA)\cdot 3H_2O$	13 986	$^2E_g \rightarrow {}^2T_{2g}$	1.98
$Ni(PBDA)\cdot 3H_2O$	9 716	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F)	3.11
	16 420	$^3A_{2g} \rightarrow {}^3T_{1g}$ (F)	
	25 000	$^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	
$Co(PBDA)\cdot 3H_2O$	8 549	$^4T_{1g} \rightarrow {}^4T_{2g}$ (F)	5.02
	16 339	$^4T_{1g} \rightarrow {}^4A_{2g}$ (F)	
	21 505	$^4T_{1g} \rightarrow {}^4T_{1g}$ (P)	
$Mn(PBDA)\cdot 3H_2O$	16 313	$^6A_{1g} \rightarrow {}^4T_{1g}$ (G)	4.99
	24 570	$^6A_{1g} \rightarrow {}^4E_g\ {}^4T_{1g}$ (G)	
	29 585	$^6A_{1g} \rightarrow {}^4E_g$ (D)	

Table 3 Anti-inflammatory activity[a]

Compound (dose, mg kg^{-1})	Inhibition (%)		
	Carrageenan oedema	Adjuvant arthritis (non-estab.)	Cotton-wad granuloma
PBDA (200)	18.6	12.1	9.6
PBDA–Cu (200)	20.1	22.3	10.2
PBDA–Zn (200)	24.5	15.1	12.7
PBDA–Co (200)	34.1	18.7	16.2
Oxyphenbutazone (50)	57.2	—	35.6
Ibuprofen (34)	—	34.9	—

[a] Number of animals in each group is 8.

DISCUSSION

Many reports have documented a rise in the concentration of circulating copper ions including the fraction that is exchangeable and not protein-bound, with a concomitant decrease in circulating zinc and iron levels during the inflammatory process. However, it is not clear that the mobilization/sequestration of metal ions, reflected by changes in their plasma levels, serves to defend the host more efficiently from the effects of inflammation,[29] as drugs like D-penicillamine and salicylates can equally well mobilize copper, zinc and iron from their respective storage compartments or withdraw them from circulation and accelerate their excretion. Also, it should not be forgotten that a metal complex may be an efficient mode of delivering a particular ligand, the metal being either a carrier or providing a targeting mechanism. Therefore, it is difficult to assign an unambiguous function to the responses which affect the status and reactivity of several metal ions.

In an attempt to investigate these problems we have synthesized numerous ligands and their transition-metal chelates. We have studied PBDA and its chelates with copper, zinc, cobalt, nickel and manganese for their physico-chemical properties and their anti-inflammatory as well as gastric irritant effects in different animal models. As the ligand and the complexes were insoluble in water, the compounds were administered subcutaneously as a suspension in saline containing 1.4% polyvinyl alcohol. Sorenson[5] observed that copper complexes of some anti-inflammatory and non-anti-inflammatory ligands produce greater anti-inflammatory effect in several models of inflammation and show less gastric irritation than the parent compounds. Our results also seem to confirm that the copper complex of PBDA is a more effective AI compound and shows much less gastric irritation, compared with PBDA. Similarly it was reported that zinc sulphate can be used in the treatment of rheumatoid arthritis[16] and can markedly inhibit gastric ulceration.[30] The results of our study seem to agree with these findings, as the zinc complex was approx. 30% more active than the ligand in all three sets of experiments.

Table 4 Ulcerogenic potential

Compound (Dose, mg kg^{-1} p.o.)	ADU (%)	RU	UI
PBDA (200)	2.5	90	2.25
PBDA–Cu (200)	1.0	50	0.50
PBDA–Zn (200)	1.0	70	0.70
PBDA–Mn (200)	2.0	80	1.60
PBDA–Ni (200)	2.0	90	1.80
PBDA–Co (200)	1.5	80	1.20
Oxyphenbutazone (50)	2.0	100	2.00
Oxyphenbutazone (100)	4.0	100	4.0

The nickel complexes have been shown to produce mucous effusion and thus act as gastroprotectants.[31] However, in our study the nickel complex was less gastroprotectant than all the other metal complexes, but more protective than PBDA. Our laboratory has already reported the AI and gastroprotectant activity of some cobalt chelates[32, 33] and this is further confirmed in this study. However, the pharmacology of transition metals and metal complexes deserve more extensive bioinvestigation, and the belief that, in general, metal ions and their complexes are very toxic requires reconsideration. As the metal

chelates are generally insoluble, the problem can be overcome by using delivery systems such as transcutaneous presentation of uncharged metal complexes or slow release implants such as osmotic pumps or intramuscular depots of slowly degrading materials, either as resin-bound complexes or as complexes entrapped in liposomes.

REFERENCES

1. K. D. Rainsford, *Ali. Pharmacol. Ther.* **25**, 43 (1988).
2. S. Szabo, W. F. Spill and K. D. Rainsford. *Med. Toxicol.* **4**, 77 (1989).
3. K. D. Rainsford, *Drug Invest.* **2** (Suppl. 4), 3 (1990).
4. K. D. Rainsford, *Acta Phys. Hung.* **72**, 261 (1989).
5. J. R. J. Sorenson, *J. Med. Chem.* **19**, 135 (1976).
6. J. R. J. Sorenson, Development of copper complexes for potential therapeutic use. In: *Trace Elements in Pathogenesis and Treatment of Inflammation*, Rainsford, K. D., Brune, K. and Whitehouse, M. W. (eds), Birkhauser Verlag, Basel, 1981, pp. 305–326.
7. J. R. J. Sorenson, The anti-inflammatory activity of some copper chelates. In: *Trace Substances in Environmental Health* VIII, Hemphill, D. D. (ed.), University of Missouri Press, Columbia, 1974, pp. 305–311.
8. J. R. J. Sorenson, *Inflammation* **1**, 317 (1976).
9. M. W. Whitehouse, *Agents Action* **6**, 201 (1976).
10. M. W. Whitehouse and W. R. Walker, *Med. J. Aust.* **1**, 938 (1977).
11. M. W. Whitehouse, L. Field, C. W. Denke and R. Ryall, *Scand. J. Rheumatol.* **4**, Suppl. 8, Abstr. no. 183 (1975).
12. I. L. Bonta, Acta Physiol. Pharmacol. Neerl. **15**, 188, 1969.
13. J. R. J. Sorenson, The anti-inflammatory activities of copper complexes. In: Metal ions in Biological Systems, Siegel, H. (ed.), Marcel Dekker, New York, 1982, pp. 77–124.
14. K. D. Rainsford, Environmental metal ion perturbations, especially as they affect copper status, are a factor in the etiology of arthritic conditions. An hypothesis in: *Inflammatory Disease and Copper*, Sorenson, J. R. J. (ed.), Humana Press, Clifton, NJ 1982, pp. 137–143.
15. R. M. Leach, *Fed. Proc.* **30**, 991 (1971).
16. P. A. Simkin, Treatment of rheumatoid arthritis with oral zinc sulphate. In: *Trace Elements in Pathogenesis and Treatment of Inflammation*, Rainsford, K. D., Brune, K., and Whitehouse, M. W. (eds.), Birkhauser Verlag, Basel, 1981, pp. 587–596.
17. R. Nagar, *Main Group Metal Chem.* **12**, 201 (1989).
18. A. I. Vogel, *A Text Book of Quantitative Inorganic Analysis*, Longman Green, London, 1971.
19. C. A. Winter, E. A. Risley and G. W. Nuss, *Proc. Soc. Expl. Biol.* **111**, 544 (1962).
20. R. Meier, W. Schuler and P. Desaulles, *Experientia* **6**, 469 (1950).
21. B. B. Neubould, *Br. J. Pharmacol.* **21**, 127, 1963.
22. B. N. Dhawan and R. C. Srimal, *Br. J. Pharmacol.* **49**, 64 (1973).
23. R. K. Agarwal, *J. Ind. Chem. Soc.* **65**, 448 (1988).
24. L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, John Wiley, New York, 1971.
25. J. R. Ferrero, *Low-frequency Vibrations of Inorganic and Co-ordination Compounds*, John Wiley, New York, 1971.
26. B. N. Figgis and J. Lewis, *Prog. Inorg. Chem.* **6**, 37 (1964).
27. A. B. P. Lever, *Inorganic Electronic Spectroscopy*, Elsevier, Amsterdam, 1984.
28. B. N. Figgis, *Introduction to Ligand Fields*, Wiley Eastern, New Delhi, 1976.
29. D. D. Perrin and M. W. Whitehouse, Metal ion therapy: some fundamental considerations. In: *Trace Elements in Pathogenesis and Treatment of Inflammation*, Rainsford, K. D., Brune, K., and Whitehouse, M. W. (eds.), Birkhauser Verlag, Basel, 1981, pp. 261–290.
30. C. J. Pfeiffer, C. C. Cho, A. Cheema and D. Saltman, *Eur. J. Pharmacol.* **61**, 347 (1980).
31. K. D. Rainsford and M. W. Whitehouse, *Experientia* **32**, 1172 (1976).
32. R. Nagar and G. Mohan, *J. Inorg. Biochem.* **42**, 9 (1991).
33. G. Mohan and R. Nagar, *Indian Drugs* **31**, 414 (1994).

